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# XVIII INTERNATIONAL SILAGE CONFERENCE 2018

**24-26 July 2018**

Bonn, Germany

UNIVERSITÄT BONN

LfL
Bavarian State Research Center for Agriculture

DLG

# Proceedings of the
# XVIII International Silage Conference


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Edited by

K. Gerlach and K.-H. Südekum

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## Welcome Letter

Distinguished Delegates, Ladies and Gentlemen, Dear Colleagues and Friends,

It gives me immense pleasure to extend to you all a very warm welcome on behalf of the Organising Committee of the XVIII International Silage Conference (XVIII ISC 2018) and to say how grateful we are to the members of the International Silage Conference Continuation Group who have accepted our invitation to host this conference for the first time in Germany, here in Bonn. It is an opportune time to renew and intensify contacts and discuss problems of mutual interest with delegates from countries all over the world.

It is gratifying to note that the agenda of the conference covers a wide range of very interesting items relating to the science of silage production and utilization. Silage production will remain a key part in animal production systems across tropical, subtropical and temperate regions. This demands continuing approaches for production of high quality silages involving improvements in management practise to minimise losses and maximise the preservation of the inherent feeding value of the parent crop. Fermented substrates other than silage for ruminants also play a more prominent role nowadays and we are pleased to see that this is also reflected in contributions to this conference. No matter how much we can do by ourselves on the national level, whether it be research, development or extension, it is never enough. In a spirit of true cooperation, we must join in action-oriented efforts to address the challenges and solve the problems that beset plant materials between harvest and utilisation by animals and people.

Generous sponsorships from many industrial companies have recognized that the XVIII ISC 2018 is an important venue to present and discuss scientific and technological progress in silage research across the world. Our Organising Committee members are committed to provide maximum hospitality. Please feel free to ask questions to committee members. We are here to serve you. Enjoy your participation in the XVIII ISC 2018.

In conclusion, I wish you every success in interchange of ideas and a very pleasant stay in Germany.

On behalf of the Organising Committee

Karl-Heinz Südekum

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# The future of ensiling: challenges and opportunities

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**Summary**

This paper looks at the challenges and opportunities that lie ahead for ensiling, from crop to feed-out from the silo or pile. Challenges are coming from a wide spectrum. Faster harvest rates are making it more difficult to achieve target densities in bunker and pile silos. Larger harvest equipment is increasing soil compaction and rural road issues. As farm sizes grow, old silos are either overfilled, creating safety issues, or temporary piles are placed on bare ground permitting soil contamination. Mycotoxins and the development of various pathogens in silages are still a problem. Global warming may affect the forage crops grown and crop characteristics as well as rates of silage fermentation and aerobic deterioration. In Europe, the future is unclear as to the impact of using silage as an input to bio-refineries. Silage analysis is challenged by how to take good samples safely and knowing what components truly predict the nutritional value of silage to livestock.

The future holds many opportunities for both ensiling and silage research. Robotic harvesting will release more labour for silo packing, and there are opportunities to develop tools to estimate silage density during filling. Total mixed ration (TMR) silages should allow more by-products in rations. The opportunity to develop silage additives that either improve silage hygiene or increase nutrient availability to livestock appears promising. Prediction of the onset of aerobic deterioration with quick tests for lactate-assimilating yeasts or silage temperatures seems possible. Metabolomics and metabonomics, in addition to all of the microbiome tools that are in development, put us at the cusp of being able to see which microorganisms are active in the silo and rumen and what compounds, particularly the minor ones, they are producing. This could lead to all kinds of advances including reduced microbial toxins in silage, better silage hygiene and improved utilisation of silages by livestock.

## Introduction

Developments in silage conservation technologies in the past five decades, reviewed by Wilkinson and Rinne (2017), have led to increased speed of physical and microbiological processes that have accompanied the increased size of livestock production units. In the 1960s, forage harvesters equipped with flail or flywheel chopping mechanisms were capable of harvesting about 10 tonnes of fresh crop per hour (MAFF 1961). Today, self-propelled machines can harvest more than 350 tonnes of fresh crop per hour (Marsh 2013) and the limiting factor to the speed of ensiling has moved from field to silo or pile.

A critical control point in the ensiling process occurs at the time of harvest when the subsequent fermentation can be influenced by extent of chopping and by addition of chemical and/or biological inoculant additives. For example, addition of formic acid reduces crop pH immediately, restricts extent of fermentation and reduces risk of undesirable metabolic end-products in silage (Wilson and Wilkins 1973). Inoculation with lactic acid bacteria (LAB) at levels equal to, or exceeding the indigenous population (e.g. $10^6$ colony forming units (cfu)/g fresh crop) accelerates the speed of decrease in crop pH in the initial period of ensilage (Pitt and Leibensperger, 1987). Biological inoculation is now the predominant technology employed to influence the silage fermentation.

Meta-analysis of 130 peer-reviewed papers published since 1996 on the effect of inoculation of crops with homolactic and facultative heterolactic LAB revealed improved fermentation quality (assessed as

increased lactate and reduced silage pH, acetate and ammonia nitrogen) in legumes, temperate perennial grasses and tropical grasses, but no effect of inoculation on the fermentation of whole-crop maize, sorghum or sugarcane. Inoculation increased dry matter (DM) recovery in those crops in which fermentation quality was improved. There was no effect of inoculation on aerobic stability or *in vitro* DM digestibility (Oliviera et al. 2017). In a separate meta-analysis of 31 peer-reviewed papers, inoculation increased the yield of dairy cows by 0.37 kg milk/cow/day, and the inoculant effect on milk yield was not affected by forage type, inoculant species or level of production (Oliviera et al. 2017).

The demand for high-quality silage to provide conserved forage feeds for livestock is growing in importance globally as competition increases for limited arable (i.e. cultivatable) land on which to produce food for the human population together with animal feed, especially for monogastric livestock that currently receive a high proportion of their diet as human-edible cereal grain and pulse seeds (Wilkinson and Lee 2017). Biogas units are a recent growth sector for silage production, many linked to industrial units with little or no land of their own and reliant on local arable land as their main source of forage, grown on contract to a prescribed crop specification.

In this review, we highlight some challenges and opportunities for the future of ensiling, from crop to feed-out from the silo or pile, in the hope that research will continue to be stimulated to develop novel approaches to improving silage quality and its contribution to meeting the total nutrient requirements of productive livestock.

## Challenges

*Mechanisation*

Have we reached the limit to forage harvester size and chopping capacity? Heavier machines increase the risk of compaction of clay-based soils and erosion of calcareous and sandy soils. Road transportation of harvesters, trucks and trailers from farm to farm is more problematic with larger equipment than with smaller machinery. Transport is an increasing cost element in ensiling as greater quantities of harvested crop are hauled longer distances from field to silo.

Rapid crop harvesting can compromise silo packing efficiency. In a survey of 149 farm bunker silos filled with whole-crop maize, only 36% were considered to have been packed correctly to a crop density of more than 240 kg DM/$m^3$ (Andrieu and Demey 2015).

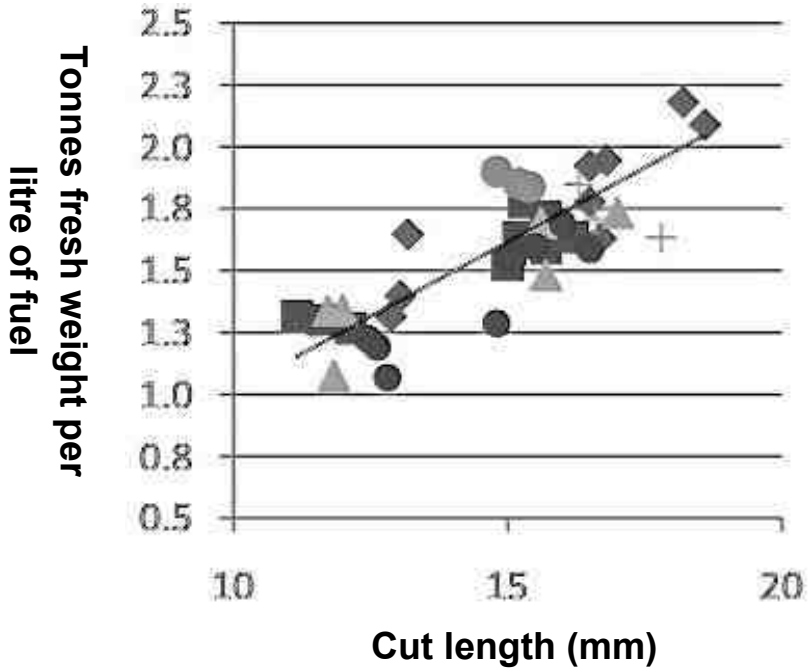
Short chopping and kernel processing are characteristics of best-practice in whole-crop maize harvesting, and for silage destined for biogas production there is a perceived requirement for very short chopping to between 4 and 12 mm theoretical length of cut (TLC, Claas 2018). However, the recent trend with crops ensiled for livestock is towards increased forage particle size at ensiling. For example, with whole-crop maize a chopping system has been introduced involving a longer TLC, 26 to 30 mm, compared to conventional 19 mm, by removing half the knives in the chopping cylinder. At the same time, kernel processing rolls are set with a conventional 2 to 3 mm gap but with a greater roll-speed differential - 32% compared to 21% - to produce ‘shredlage’ (Claas, 2018). This new approach has been found to improve starch digestibility and milk production compared to conventional kernel processing (Vanderwerff et al. 2015).

The amount of crop harvested per unit of fuel consumed is directly related to length of chopped forage particles. In a three-year comparison of six different self-propelled forage harvesters operated in forage maize crops, all set to chop at 16 mm TLC, Marsh (2013) found that the quantity of crop harvested per litre of fuel consumption increased by 0.12 tonnes per millimetre increase in measured particle cut length (Figure 1).

Self-propelled forage harvesters are expensive items of specialised machinery that cannot be afforded by many livestock farmers. We estimate that about 40% of all silage harvested in developed countries is custom-harvested by specialist contractors who travel from farm to farm with their equipment during the crop harvesting season. Waiting for a contractor to arrive can be a stressful experience. Mechanical breakdowns and periods of wet weather can delay harvest, compromising crop and silage quality. To avoid delays, reduce risk of delayed harvest, and decrease cost per tonne of crop ensiled,

alternative harvesting approaches are available, at least for perennial grass and legume crops, including trailed self-loading/chopping forage wagons that can out-perform the standard forage harvester in terms of both fuel and labour per tonne of crop harvested (Frost and Binnie 2005, Brownell et al. 2012).

**Figure 1**. Effect of particle cut length on weight of fresh whole-crop maize harvested per litre of fuel consumed by self-propelled harvesters (Marsh 2013).

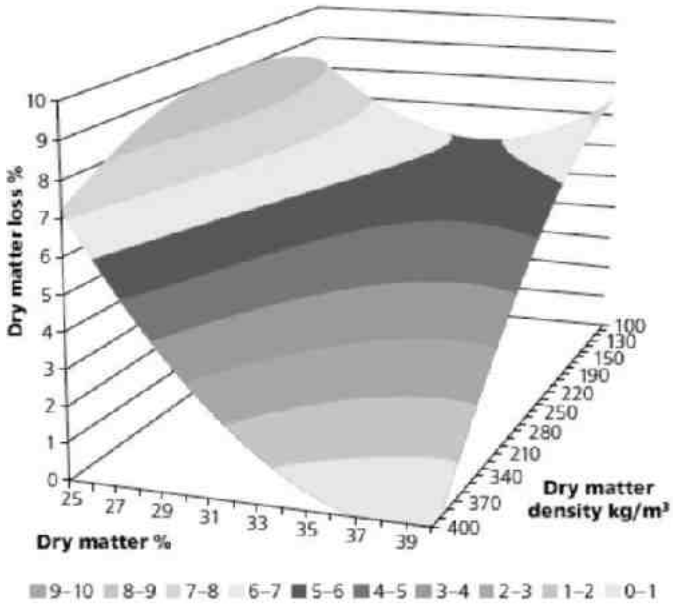



Wrapped bales have some advantages over forage harvesters. Bales can be made on land with steeper slopes than are suitable for larger machinery and can be wrapped immediately or very soon after the bale has been formed. Baled silage can be transported from farm to farm. However, speed of harvesting is slower for balers than for forage harvesters and the quantity of plastic film used per hectare is five times higher for bales than for clamp silage (Forristal and O'Kiely 2005).

*Silos*

Loss of DM as carbon dioxide produced from crop respiration and fermentation in crops stored in oxygen-limiting tower silos is 5 to 8% of crop DM ensiled (Rotz et al. 2003). Similar low levels of loss may be expected from crops stored in large bunker silos at densities in excess of 250 kg DM/$m^3$ (Figure 2) where the highest losses occurred in wetter crops (<30% DM), possibly due to more extensive fermentation and loss of liquid effluent or leachate. Density had the greatest influence on loss at high DM levels (>35% DM), most likely due to oxidative DM loss.

**Figure 2** Effect of maize silage dry matter and density on dry matter loss during storage in bunker silos (Griswold et al., 2010).




Many silos are too old and too small to meet the needs of the increasing size of livestock units, with the result that bunker silos are either over-filled or temporary drive-over piles are made in fields, with greater risk of soil contamination during filling and feed-out. Bunker wall strength is challenged when silos are packed with heavy equipment and operator safety is put at risk when silos are filled above wall height. Lateral hydraulic pressures on bunker silo walls are less with drier crops (>35% DM), than with wetter crops due to lower silage fresh weight density (Savoie and Jofriet 2003), but greater packing tractor weight is required with drier crops to achieve the target silage density (Holmes and Bolsen 2009), putting additional pressure on walls.

*Hazards to animal and human health*

Silage fermentation is only partially controlled. Undesirable microorganisms and their toxins can be present in silage when it is consumed by the animal due to contamination of the crop pre-harvest, during storage and during feed-out. Potential hazards to human, animal and food safety from silage have been reviewed by Driehuis (2013) and Driehuis et al. (2018). The major microbiological hazards include *Clostridium botulinum*, *Bacillus cereus*, *Listeria monocytogenes*, Shiga-toxin producing *Escherichia coli* (STEC), *Mycobacterium bovis*, mycotoxins and pathogenic protozoa such as *Cryptosporidium parvum*. The challenge for future research is to determine the specific crop and ensiling conditions in which pathogenic microorganisms can develop and to link those conditions to signs of clinical disease in livestock. In other words, we know these undesirable organisms can be identified in silages, we also know about their likely effects on the animal, but do we know when livestock are suffering from a specific disease? For example, the incidence of mycotoxin contamination of maize silage can be very high (Cogan et al., 2017) and effects of mycotoxins on animal cells and body tissues have been documented (Fink-Gremmels, 2008), but on-farm diagnosis of clinical mycotoxicosis is difficult. There is a clear need for novel animal-based diagnostics that are non-invasive, rapid and sensitive.

*Global warming*

According to estimates (FAO 2013), the livestock supply chain accounts for 14.5% of anthropogenic greenhouse gas (GHG) emissions on a $CO_2$-equivalent basis. Approximately two-thirds is attributable to beef and dairy cattle production. While there is considerable commitment across the world to reduce GHG emissions, the demand for livestock products is growing, particularly in developing countries. FAO projects that the worldwide per capita consumption of meat and milk/dairy products in 2050 will be 26 and 19% higher, respectively, than 2005/2007 levels (FAO 2012). Together with population growth, the growth in demand for livestock products will place more pressure on the dairy and beef industries to reduce GHG emissions. A life-cycle assessment of dairy production worldwide found substantial differences in GHG emissions by region (1.3 to 7.5 kg $CO_2$-equivalent/kg of fat- and protein-corrected milk) with the lowest values in industrialised regions such as Europe and North America (FAO 2010). This suggests that adoption of current practices in developed countries, e.g. ensiling high-quality crops to increase output per animal and dilute maintenance, may be useful in mitigating GHG emissions per unit of product worldwide. Even so, research to find novel ways of reducing GHG emissions in ruminant production is important.

In FAO's life-cycle assessment of dairy production, methane accounted for 52% of GHG emissions in both developed and developing countries. In developed countries, nitrogen oxide ($NO_x$) emissions from fertiliser and manure accounted for 27% and $CO_2$ from all farm sources 21%. In developing countries, $NO_x$ emissions were 38% and $CO_2$ 10% (FAO, 2010). Differences in methods of crop storage did not appear to be specifically addressed in that analysis. The direct contribution of silage to global warming, although substantially less than its indirect contribution via livestock enteric fermentation, is through emissions to the atmosphere of $CO_2$, volatile organic compounds (VOC) and $NO_x$. VOC such as alkenes, ethyl esters (e.g. ethyl acetate and ethyl lactate) and carbonyl compounds (aldehydes) are potent environmental pollutants because they are ozone precursors (Mitloehner et al. 2009). Weiss et al. (2016) and Brüning et al. (2017) found that elevated concentrations of VOC were associated with delayed silo sealing which was accompanied by increased ethanol and yeast counts. Research has shown little effect of inoculation of crops with homofermentative lactic acid bacteria on VOC formation. Addition of *L. buchneri* produced a limited effect on VOC levels, probably due to the slow growth of the organism and limited metabolism of lactate to acetate and 1,2-propanediol. In contrast, additions of sodium benzoate and potassium sorbate gave substantial reductions in VOC in a range of silages (Weiss 2016, Weiss et al. 2016).

Global warming may influence the silage crops grown in many regions as environmental temperatures increase and water availability decreases. Maximum leaf photosynthetic capacity is reached in temperate ($C_3$) grasses (e.g. *Lolium* spp.) at about 27 $^{o}$C (Robson et al. 1988) whilst $C_4$ grasses (e.g. *Zea mays*) show superiority over $C_3$ grasses at higher temperatures (Orsenigo et al. 1997). Simulations of temperature and rainfall climate changes in semi-humid temperate grassland over a 100-year period revealed advantages of mixed $C_3$/$C_4$ grasses over $C_3$ species alone, mainly due to the superiority of $C_4$ grasses in growth and water use efficiency in the summer months (Seastedt et al. 1994).

Global warming is expected to affect crop characteristics. Increased atmospheric $CO_2$ levels, in combination with higher temperature and water stress may prove beneficial to levels of fermentable substrates in silage crops. In a study of the effect of increased $CO_2$ level (620 ppm vs. 392 ppm), elevated temperature (+3 $^{o}$C) and water deficit on the composition of temperate grasses and legumes, AbdElgaward et al. (2014) found that the imposed climate extreme was reflected in increased non-structural carbohydrates and phenolics in all species, increased lignin in legumes and decreased tannins in grasses. There were no effects of the extreme climate on structural carbohydrate, protein, lipid or mineral levels.

A final concern regarding global warming are direct effects on ensiling. If crops are harvested at higher ambient temperatures, one would expect faster fermentations in the silo. However, there are limits. Most LAB species found in silages have optimum temperatures for growth around 30 ºC and do not grow at 45 ºC (McDonald et al. 1991). So, LAB at 40 ºC may ferment sugars slower than at 35 ºC. Another worry is that clostridia generally have a higher optimum growth temperature, typically 37 ºC,

and will grow at 45 ºC. Clostridial fermentations may therefore become more prevalent in silage crops limited by low water-soluble carbohydrates. Maillard reactions (the polymerisation of sugars and amino acids to form acid detergent insoluble nitrogen) occur at temperatures above 35 ºC. As oxygen is introduced to the silage, higher silage temperature will increase the movement of oxygen into the silage mass and increase the growth rates of spoilage microorganisms (Pitt and Muck 1993). However, yeasts that initiate spoilage typically have optimum growth temperatures in the 30s, and their growth rate drops precipitously above 40 ºC. So, silages above 40 ºC may spoil aerobically via a different pattern than what we have come to expect. Most laboratory silage research has been undertaken in temperate regions at temperatures between 20 and 25 $^{o}$C. Therefore, current knowledge is dominated by conditions optimal for growth of lactic acid bacteria, rather than extreme bacterial growth environments. In future, especially in tropical areas, research should be directed at studying patterns of fermentation and aerobic spoilage at temperatures above 40 ºC in anticipation of these conditions becoming more common.

*Silage bio-refineries*

The introduction of the combine harvester revolutionised grain harvesting. Previously, cereal crops were harvested as whole-crop forages, and stored for several weeks prior to threshing – separation of grain from the remainder of the plant. The current practice of ensiling forages as whole plants introduces the opportunity for fractionation of grasses either prior to, or after storage to produce a range of products, some of which may be stored as liquid for further refining and use in human foods, pharmaceuticals, or as chemical feedstocks for industrial use (McEniry and O'Kiely 2014; Schwarz et al. 2016). Similarly, there are efforts to harvest lucerne leaves separately from stems, processing the leaves for possible industrial uses while harvesting the stems for livestock feed (Digman et al. 2013). However, it is likely that the substantial investment in bio-refining infrastructure will only occur if significant value can be added to forage crops, for example by upgrading amino acids from bio-refined silage (Kromus et al. 2004).

*Silage analysis*

Are we sampling silage correctly? In a large study, St-Pierre and Weiss (2015) found that sampling error was the biggest source of variation in maize silage composition in a single silo. The authors advised that to avoid risk of human injury or death due to feed-face avalanche, multiple handfuls of silage should be sampled from different bucket-loads before they are put into the mixer wagon. The handfuls should be placed into a small bucket, mixed thoroughly and sub-sampled into a plastic bag to give two or three samples for shipment to the laboratory. Then the results should be averaged, and the mean data used for diet or biogas feedstock formulation only until the next set of results becomes available. Rolling averages based on historical analyses should not be created.

More than twenty years ago, Weinberg and Muck (1996) commented that the causes of observed animal responses to silage inoculation were unclear. They noted that recorded changes in fermentation pattern and in DM recovery were inadequate to explain significant animal responses and speculated that inoculation might be influencing DM digestibility through a probiotic effect on the rumen microbial population. The recent meta-analysis of LAB inoculants (Oliviera et al. 2017) provides further indirect evidence for this in that increases in milk yield from inoculation were not affected by crop type and inoculation had little or no effect on silage fermentation in maize, sorghum and sugarcane silages. It appears that the effects of silage inoculation on the cow are related to fermentation in the rumen with ruminal *in vitro* studies suggesting increased rumen microbial biomass (Contreras-Govea et al. 2011) or reduced methane production (Jalc et al. 2009) as possible reasons for increases in milk yield. These studies raise several challenging questions – are we measuring the most appropriate parameters in conventional silage analysis? Are we assessing current parameters accurately, especially predictions of degradability, by scanning fresh material with near infra-red reflectance spectrometers? What components are we missing that might be especially useful to biogas plants and bio-refineries, e.g., predicted specific methane yield?

**Opportunities**

*Advances in crop harvesting and ensiling*

Global positioning systems and field maps along with sensors, cameras and actuators on tractors, harvesters and implements are relieving the operator from routine driving and implement control in the field. Fendt (2017) recently announced field testing of MARS (Multiple Agricultural Robot Swarms), small battery-operated robotic units for planting maize and other crops that will allow planting 24 hours per day, minimise soil compaction and reduce $CO_2$ emissions.

Automated filling of a truck or trailer from the forage harvester has become available, reducing losses. All of these developments improve the precision and efficiency of field operations without affecting manpower for forage harvesting. However, we are at the brink of a revolution in agriculture much like that in cars and trucks – driverless vehicles. Imagine trucks without drivers carrying forage between the field and bunker, increasing the manpower available to pack the silo.

There are opportunities at the bunker or pile to improve silage density. There is a need for means to estimate silage density during filling so that the packing crew is provided frequent feedback and can make adjustments while the silo is being filled. Probes to estimate density have not been promising because of the variability between crops and the variability in compression characteristics due to maturity, DM, particle size, etc. For farms that weigh all loads as they arrive at the silo or pile, density could be calculated during filling if the volume of the packed crop could be estimated. With today's laser measuring tools, perhaps estimates of volume will soon be practical. Further in the future, it may be possible to pack bunkers and piles robotically. This would allow more packing vehicles to operate simultaneously and safely to achieve a high density. Perhaps there will be improvements in how loads of forage are distributed across the surface to achieve thin layers.

Re-usable bunker or pile covers that can be deployed and removed easily seem a feasible opportunity to reduce the environmental impact of ensiling. Currently there is a manufacturer of covers for bunkers in The Netherlands that fits this description (Agridek 2018), but can costs be reduced and the solution made more scalable? Less likely are edible films sprayed on exposed surfaces or sprayed on the open face to reduce oxygen penetration during silo emptying.

*TMR silages*

Cattle and other ruminants are often fed by-products such as brewers' grains, distillers' grains, beet and citrus pulp and waste vegetables. Many of these products are wet, which limits how far they can be transported unless dried. Nishino et al. (2003) investigated ensiling wet brewers' grains with lucerne hay, dried beet pulp, maize, wheat bran and molasses to create an ensiled total mixed ration (TMR). The TMR ensiled well and had greater aerobic stability than the brewers' grains ensiled alone. Nishino's group has studied other by-products as well as studying the LAB community in various mixtures with and without inoculation using various LAB species. This area warrants additional research. From the practical perspective, large agricultural cooperatives may be able to produce TMR silages for small farms using by-products to reduce feed costs. Large farms that purchase by-product feeds may be able to create their own TMR silages that are more aerobically stable than the separate commodities, minimizing their losses. On the scientific side, there is much to be done to find optimum mixtures and to understand how and why these mixtures are aerobically stable. The latter may lead to improved inoculants or chemicals for conventional silages.

*Additives to improve silage hygiene and increase nutrient availability*

In the 20$^{th}$ century, silage additives were used largely to ensure a fermentation dominated by LAB and/or improve aerobic stability. The inoculants sometimes increased nutrient availability to the ruminant by mechanisms not yet understood, and silage hygiene was improved indirectly by the dominance of the LAB. Formic and other acids reduced pH to give the epiphytic LAB an advantage over clostridia and enterobacteria, indirectly improving silage nutrient availability. Certain chemical

additives provided direct means of improving silage hygiene: nitrites to inhibit clostridia and propionates, acetates, sorbates and benzoates to inhibit fungi.

Direct inhibition of clostridia by bacteriocins produced by LAB has been a focus of a number of laboratories in the 21st century (Muck et al. 2018). At present, the bacteriocins investigated have not been completely satisfactory, either failing to inhibit a wide range of clostridial species or inhibiting beneficial LAB. Nevertheless, this is an important area of research.

Reduction of mycotoxins is another potential opportunity for additives. Good silage management can prevent mycotoxin production in the silo, but growing season conditions can result in a crop with high levels of mycotoxins at harvest. Are there microorganisms or enzymes that can detoxify mycotoxins during silo storage? Could binding agents or other chemicals be applied at ensiling to reduce the effects of mycotoxins at feeding?

There are various opportunities to improve nutrient availability using additives. Enzymes to improve fibre digestibility have been the most investigated. However, the cellulase/hemicellulase combinations of the 20th century typically broke down fibre that was digestible in the rumen, providing little benefit to the cow (Muck et al., 2018) and sometimes increasing effluent production. A new approach was the *L. buchneri* strain that produces ferulic acid esterase, an enzyme capable of breaking ester linkages between sugars and ferulic acid in cell walls (Nsereko et al. 2008). These ester linkages in grasses are some of the key ones limiting cell wall digestion. While this inoculant approach has had variable success (Muck et al. 2018), the concept is important: to hydrolyse ether and ester cell wall bonds in the silo that are difficult for rumen microorganisms to attack.

In ryegrass (*Lolium* spp.) and lucerne (alfalfa, *Medicago sativa*), proteolysis, i.e., the breakdown of true protein to soluble non-protein N, may limit the efficient utilisation of the N from the silage if the total diet does not account for the soluble N in the silage. Tannins found in many legumes or the polyphenol oxidase system found in red clover have the potential to be additives for the future to reduce proteolysis in ryegrass or lucerne and increase the proportion of N in the diet that goes to milk or meat (Muck et al. 2018).

Starch availability is an issue in maize silage as the starch granules are held in a protein matrix that inhibits ruminal starch digestion (Hoffman et al. 2011). This protein matrix slowly breaks down in the silo so that the starch becomes more available with increasing time in the silo. Recent studies with acidic proteases applied at ensiling look promising for breaking the protein matrix more rapidly and increasing starch digestion, but more research is needed (Muck et al. 2018).

*Early indicators of aerobic instability*

It would be useful if we could provide producers with a tool that provides a warning that a silage is aerobically unstable. Ideally one would like to know if lactate-assimilating yeast counts are above $10^5$ cfu/g silage, which has traditionally been the sign that a silage is likely to spoil within 24 h. However, we know that sending samples to a forage testing laboratory for yeast counts is fraught with issues to obtain an accurate assessment. Quick tests for human pathogens have been developed. Is it possible to do something similar for yeasts, allowing for immediate on-farm analysis?

We know that once yeast populations reach approximately $10^7$ cfu/g both silage pH and temperature begin to increase. Borreani and Tabacco (2010) in making measurements on the feed-out faces of commercial maize bunker silos in Italy found that the difference in temperature 200 mm behind the face minus the temperature at 400 mm behind the middle of the face was positively correlated with pH, yeast and mould counts. Are their findings valid in countries with cooler and warmer climates? Working at the face to probe for temperature is hazardous due to the risk of injury or suffocation if an avalanche occurs suddenly. However, would it be possible to develop a safe system to probe for temperature at 200 and 400 mm behind the face?

*Metabolomics and metabonomics*

There is currently an opportunity to use new technology to accumulate much information about what happens during ensiling and how a silage is fermented and digested in the cow. Some of this research may be more descriptive in nature rather than driven by hypothesis. Metabolomic and metabonomic approaches are now being applied to silage research. Metabolomic research, based on mass spectrometry, involves systematic assessments of the metabolic products of silage fermentations. Initial work using single molecule analysis in conjunction with real time sequencing technology has revealed 280 substances and 102 different metabolites in ensiled *Medicago sativa* together with changes in microbial composition during the ensiling process (Guo et al. 2018). Relative concentrations of metabolites at 90 days, where there were significant differences among the 3 treatments (untreated, inoculated with *L. plantarum* or *L. buchneri*), are shown in Table 1.

**Table 1**. Relative concentration using GC-MS of major metabolites in *Medicago sativa* silages without or with inoculation with *L. plantarum* or *L. buchneri* after 90 d ensiling (Guo et al. 2018).

| | **Relative Concentration** | | | **Fold-Changes**[1] | | |
|---|---|---|---|---|---|---|
| Metabolite | Control | *L. plantarum* | *L. buchneri* | P/C | B/C | P/B |
| 2,3-Butanediol | 87 | 171 | 340 | * | * | * |
| 2-Aminobutyric acid | 61.5 | 12.5 | 25.1 | * | * | * |
| 4-Aminobutyric acid | 112 | 169 | 272 | | * | |
| Benzoic acid | 43.9 | 56.9 | 55.8 | * | | |
| Adenine | 2.6 | 26.2 | 21.0 | * | * | |
| α-Hydroxyisobutyric acid | 3.51 | 0.77 | 1.03 | * | * | |
| Aminomalonic acid | 5.39 | 8.81 | 10.33 | | * | |
| Arabitol | 4.36 | 6.21 | 21.43 | | * | * |
| Cadaverine | 192 | 22 | 101 | * | * | * |
| Erythritol | 10.4 | 10.5 | 39.1 | | * | * |
| Glycerol | 190 | 184 | 301 | | * | * |
| Inositol | 31.7 | 42.0 | 40.9 | * | | |
| Ketomalonic acid | 0.46 | 4.05 | 1.77 | * | * | * |
| Malonic acid | 2.15 | 10.89 | 7.68 | * | * | * |
| Mannitol | 0.43 | 0.47 | 3.94 | | * | * |
| Phenethylamine | 3.62 | 0.27 | 1.16 | * | * | * |
| Threitol | 1.51 | 1.99 | 3.24 | * | * | * |
| Lactic acid | 132 | 167 | 120 | * | | * |
| Succinic acid | 158 | 83 | 112 | * | * | * |
| Trans-Ferulic acid | 3.91 | 1.69 | 5.34 | | | * |
| Threonine | 41.3 | 64.6 | 74.0 | * | * | * |
| Tyrosine | 3.23 | 16.76 | 98.42 | * | * | * |
| Valine | 162 | 172 | 220 | | * | * |
| Ornithine | 28.8 | 44.4 | 65.8 | | * | |
| Lysine | 12.2 | 38.3 | 50.2 | * | * | * |
| β-Alanine | 1.65 | 2.12 | 4.04 | | * | |
| Aspartic acid | 140 | 290 | 311 | * | * | |
| Glutamic acid | 76.9 | 39.4 | 28.6 | * | * | |

[1]Significance of $\log_2(X/Y)$ where X and Y are the relative concentrations of the treatments: C, control; B, *L. buchneri*; P, *L. plantarum*. * $P < 0.05$.

Many of the compounds in Table 1 are amino acids and amines in addition to acids and polyols. It is interesting that some of these compounds vary much more on a relative basis by treatment than standard fermentation products. Guo et al. (2018) also identified the microbial species at 14, 30, 60 and 90 days of ensiling. *Lactobacillus plantarum* dominated at all times in the *L. plantarum* treatment whereas *L. buchneri* was dominant only at 14 and 30 days in the *L. buchneri* treatment. While these results are very interesting, the combination of metabolomic data with microbiome data is needed within the first few days of ensiling i.e. before day 14, when the majority of chemical changes are occurring in the silo, to help identify the roles of different microbial species.

Metabonomic research comprises quantitative measurement of metabolic responses in the animal, in the rumen, or in the silage microflora, to changes associated with external environmental factors including disease stimuli such as mycotoxins in silage. In a current research project on mycotoxicosis

(Bovmycotox 2017), samples of animal cells, tissues and body fluids are analysed by hydrogen nuclear magnetic resonance ($^1$H NMR), mass spectroscopy, and high-performance liquid chromatography to determine metabolite profiles, pattern recognition and metabolite identification.

By focusing on the silage microflora or on the animal itself, these new techniques of metabolomics and metabonomics may provide a better understanding, for example, how homolactic LAB change silage fermentation and later improve digestion and utilisation of the diet in the rumen and may lead to inoculants that are more effective in improving animal productivity. Overall, the metabolomic and metabonomic tools that are becoming available put the research community at the cusp of being able to unlock interactions that are occurring in the silo and in the animal. We need to find out not only which microbial species are active and thriving at various times but also what they are producing and how those compounds are influencing the microbial community and the animal itself. Potentially these technologies will open up new opportunities to improve silages in the silo, inhibit detrimental microorganisms, enhance rumen microbial activity and improve animal health.

*Artificial intelligence and machine learning*

At present the silage fermentation is understood incompletely, and there is an opportunity to apply developments in artificial intelligence and machine learning to gain a deeper insight into the crop and silage microbiome. This work requires a multidisciplinary approach to mining relevant data from the world's scientific literature and creating algorithms to allow machine learning to identify the most important factors affecting the silage microbiome and natural environments or management interventions that encourage desirable and discourage undesirable microflora. This may be the key to gaining insights across studies from metabolomic, metabonomic and microbiome research.

Although there are examples of big data applications in smart farming (e.g. biometric sensing and tracking livestock movements), artificial intelligence in agriculture appears to be dominated by conceptualisation. Big data applications, although predominantly at the level of business analytics, can play an influential role in benchmarking best practice silage production, sensor deployment, and predictive modelling to manage crop failure risk and improve efficiency of silage use by livestock. A recent review of the state of the art of big data applications in smart farming and key issues (Wolfert et al. 2017) did not mention silage, despite algorithms for optimising silage making being in existence for twenty years (Parsons 1998). There is clearly an opportunity for some innovative research on this topic.

**Ensiling in 2050**

What might the future of ensiling hold? The opportunities discussed above will drive changes in the crops and by-products we use for ensiling, the methods we employ in the ensiling process itself, and the ways in which we assess the composition and quality of the ensiled products. Here are some changes that we think have the possibility of becoming true in 30 years:

1. Drone or satellite information on crop quality variation in the field at the time of harvest – mycotoxins, LAB populations, sugar, DM as well as nutritive characteristics such as neutral detergent fibre, crude protein and starch.
2. Robotic harvesting of forages using drone information to potentially separate the crop (e.g., high/low mycotoxins, high/low DM, etc.) and possibly change the type or amount of additive used. Harvest the driest forage first allowing the wetter parts of the field to dry more or specifically directing wetter or poorer quality forage to a separate silo or pile.
3. Robotic transport of forage from the field to the silo. This will free up labour to control the filling and packing of silos and piles. Because drivers would not be needed, smaller trucks could be used with less impact on soils and roads.
4. Equipment to receive forage from the field and distribute it onto the surface of a bunker or pile in thin layers so that tractors on silos can be solely for packing, improving safety. May also make robotic packing possible if forage does not need to be distributed by a packing tractor.

5. Silage additives that consistently improve fibre and nutrient availability to livestock compared to that of the harvested crop.
6. Silage additives that reduce or eliminate mycotoxins and other microbial toxins that enter the silo on the harvested crop.
7. Silo and pile covers that can be re-used for many years.
8. Robotic emptying of bunkers or piles, keeping a smooth face. Through sensors on the device it can recognize and divert spoiled silage so that it is not incorporated in the TMR.

The extent to which these changes are realised will depend on the rate of progress in research and development, driven by new environmental legislation and supported by producers in their demands for increased efficiency and higher quality silages. There is no doubt, however, that some of the changes are occurring right now and that the silage we make in 2050 will be quite different to what we make today.

## Conclusions

Increases in efficiency at one stage in the ensiling process can produce challenges at subsequent stages. For example, increased speed of harvest can lead to low silage density due to inadequate packing. Improved fermentation quality can reduce aerobic stability. These challenges create research opportunities. Progress has been made but we still have some way to go to be confident we can provide producers with ensiling systems that meet all their needs fully.

Automated approaches, especially the application of robotics and artificial intelligence to mechanisation offer new opportunities for improving efficiency in ensiling by enabling farm staff to concentrate on fine-tuning different stages of ensiling instead of being engaged in repetitive work.

Plant, microbial and animal responses to external perturbations can be inexplicable by conventional methods of analytical investigation, and there is now a great opportunity to apply new molecular technologies to solving the challenge of why some silages under-perform.

Ensiling is such an important global means of preserving crops that it deserves continued, sustained, multidisciplinary research to help users realise its full potential. The goal is to achieve greater knowledge of the relative importance of the many factors that contribute to low quality silage as well as those that contribute to high quality material, with the overall objective of being able to include greater levels of top quality silage in diet formulations for high-producing livestock and in feedstocks for biogas and bio-refineries.

## References

AbdElgawad, H., Pershev, D., Zinta, G., Van den Ende, W., Janssens, I.A. & Asard, H. (2014) Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated $CO_2$: A comparison of fructan and non-fructan accumulators. PlosOne, 9, (3), e92044. Accessed 23 January 2018 from: https://doi.org/10.1371/journal.pone.0092044.

Agridek (2018) Oplossingen (Solutions). Accessed 14 February 2018 from https://agridek.nl/oplossingen/.

Andrieu, B & Demey, V. (2015) On-farm corn silage investigation: multi-analysis on silage practices, silage quality and its effect on density. In: Daniel, J.L.P, Morais, G., Junges, D. and Nussio, L.G. (eds) Proceedings of the XVII International Silage Conference, Piracicaba, Sao Paulo, Brazil, 1-3 July 2015, pp. 310-311.

Borreani, G. & Tabacco, E. (2010) The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science, 93, 2620-2629.

Bovmycotox (2017) Metabonomics typical strategy. Accessed 31 December 2017 from http://www.bovmycotox.co.uk/the-problem/metabonomics/.

Brownell, D.K., Liu, J., Hilton, J.W., Richard, T.L. & Macafee, B.R. (2012) Evaluation of two harvesting systems for herbaceous biomass harvesting. Transactions of ASABE, 55, 1651-1658.

Brüning, D., Gerlach, K., Weiss, K. & Südekum, K-H. (2017) Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. Grass and Forage Science. Published online 17 April 2017. DOI: 10.1111/gfs.12288.

Claas. (2018) Claas corncracker expertise. Accessed 3 January 2018 from http://www.claas.co.uk/blueprint/servlet/blob/818332/515f66b8ae34fbabdb74cc718bbc1c6b/262813-dataRaw.pdf.

Cogan, T., Hawkey, R., Higgie, E., Lee, M.R.F., Mee, E., Parfitt, D., Roderick, S., Walker N., Ward P. and Wilkinson J.M. (2016) Silage and total mixed ration hygienic quality on commercial farms: implications for animal production. Grass and Forage Science, 72, 601-613. DOI: 10.1111/gfs.12265.

Contreras-Govea, F.E., Muck, R.E., Mertens, D.R. & Weimer. P.J. (2011) Microbial inoculant effects on silage and in vitro ruminal fermentation, and microbial biomass estimation for alfalfa, bmr corn, and corn silages. Animal Feed Science and Technology, 163, 2-10.

Digman, M.F., Runge, T.M., Shinners, K.J. & Hatfield, R.D. (2013) Wet fractionation for improved utilization of alfalfa leaves. Biological Engineering Transactions, 6, 29-42.

Driehuis, F. (2013) Silage and the safety and quality of dairy foods: a review. Agricultural and Food Science, 22, 16-34.

Driehuis, F., Wilkinson, J.M., Jiang, Y., Ogunade, I. & Adesogan, A.T. (2018) Animal and human health risks from silage. Journal of Dairy Science (Accepted 11 November 2017).

FAO (2010) Greenhouse gas emissions from the dairy sector: A life cycle assessment. Food and Agriculture Organization of the United Nations, Animal Production and Health Division, Rome, Italy. Accessed 6 February 2018 from http://www.fao.org/docrep/012/k7930e/k7930e00.pdf.

FAO (2012) World agriculture towards 2030/2050: the 2012 revision. ESA Working Paper No. 12-03, Food and Agriculture Organization of the United Nations, Rome, Italy. Accessed 6 February 2018 from http://www.fao.org/3/a-ap106e.pdf.

FAO (2013) Tackling climate change through livestock-A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations, Rome, Italy. Accessed 6 February 2018 from http://www.fao.org/3/a-i3437e.pdf.

Fendt (2017) MARS: Robot system for planting and accurate documentation. Accessed 23 January 2018 from https://www.fendt.com//int/fendt-mars.html.

Fink-Gremmels, J. (2008) The role of mycotoxins in the health and performance of dairy cows. The Veterinary Record, 176, 84-92.

Forristal, P.D. & O'Kiely, P. (2005) Update on technologies of producing and feeding silage. In: Park, R.S and Stronge, M.D. (eds) Silage Production and Utilisation, Proceedings of the XIV International Silage Conference, Belfast, Northern Ireland, 3-6 July 2005, pp.83-96.

Frost, J.P. and Binnie, R.C. (2005) The effect of silage harvester type on harvesting efficiency. In: Park, R.S and Stronge, M.D. (eds) Silage Production and Utilisation, Proceedings of the XIV International Silage Conference, Belfast, Northern Ireland, 3-6 July 2005, p.191.

Griswold, K.E., Craig P.H., Graybill, J.S. & Dinh, S.K. (2010) Relating dry matter density to dry matter loss within corn silage bunker silos. Journal of Dairy Science 93(E-Suppl.1), 620.

Guo, X.S., Ke, W.C. Ding, W.R., Ding, L.M., Xu, D.M., Wang, W.W., Zhang, P. & Yang, F.Y. (2018) Profiling of metabolome and bacterial community dynamics in ensiled Medicago sativa inoculated without or with Lactobacillus plantarum or Lactobacillus buchneri. Scientific Reports. Published online 18 January 2018. DOI:10.1038/s41598-017-18348-0.

Hoffman, P.C., Esser, N.M., Shaver, R.D., Coblentz, W.K., Scott, M.P., Bodnar, A.L., Schmidt, R.J. & Charley, R.C. (2011) Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. Journal of Dairy Science, 94, 2465-2474.

Holmes, B.J. & Bolsen, K.K. (2009) What's new in silage management? In: Broderick G.A., Adesogan, A.C., Bocher, L.W., Bolsen, K.K., Contreras-Govea, F.E., Harrison, J.H. and Muck, R.E. (eds) Proceedings of the XV International Silage Conference, pp. 61-76. Madison, Wisconsin, USA.

Jalc, D., Laukova, A., Varadyova, Z., Homolka, P. & Koukolova, V. (2009) Effect of inoculated grass silage on rumen fermentation and lipid metabolism in an artificial rumen (RUSITEC). Animal Feed Science and Technology, 151, 55-64.

Kromus, S., Wachter, B., Koschuh, W., Mandl, M., Krotscheck, C & Narodoslawsky, M. (2004) The green biorefinery Austria – development of an integrated system for green biomass utilization. Chemical and Biochemical Engineering Quarterly, 18, 7-12.

McDonald, P., Henderson, A.R. and Heron, S.J.E. (1991) Biochemistry of Silage. 2nd ed., Chalcombe Publications, Marlow, UK.

McEniry, J. & O'Kiely, P. (2014) Developments in grass/forage-based biorefineries. In: K. W. Waldron (ed) Advances in Biorefineries – Biomass and Waste Supply Chain Exploitation. Woodhead Publishing Series in Energy No. 53. pp. 335-363.

MAFF (1961) Silage, Bulletin No. 37. Ministry of Agriculture, Fisheries and Food, London, Her Majesty's Stationery Office.

Marsh, B. (2013) A comparison of fuel usage and harvest capacity in self-propelled forage harvesters. International Journal of Agricultural and Biosystems Engineering, 7, 649-654.

Mitloehner, F.M., Malkina, I.L., Kumar, A & Green, P.G. (2009) Volatile organic compounds emitted from dairy silages and other feeds. In: Broderick G. A., Adesogan, A.C., Bocher, L.W., Bolsen, K.K., Contreras-Govea, F.E., Harrison, J.H. and Muck, R.E. (eds) Proceedings of the XV International Silage Conference, pp. 15-26. Madison, Wisconsin, USA.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C. & Kung, L. Jr. (2018) Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science, (Accepted 1 December 2017).

Nishino, N., Harada, H. & Sakaguchi, E. (2003) Evaluation of fermentation and aerobic stability of wet brewers' grains ensiled alone or in combination with various feeds as a total mixed ration. Journal of the Science of Food and Agriculture, 83, 557-563.

Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K. & Harman, B.R. (2008) Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Animal Feed Science and Technology, 145, 122-135.

Oliviera, A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.P., Arriola, K.G., Jiang, Y., Kim, D., Li, M., Conçalves, C.M., Vyas, D. & Adesogan, A.T. (2017) Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. Journal of Dairy Science, 100, 4587-4603.

Orsenigo, M., Patrignani, G. & Rascio, N. (1997) Ecophysiology of $C_3$, $C_4$ and CAM plants. In: M. Pessarakii (ed) Handbook of Photosynthesis, Second Edition, Boka Raton, Taylor & Francis CRC Press, pp 1-25.

Parsons, D.J. (1998) Optimising silage harvesting plans in a grass and grazing simulation using the revised simplex method and a genetic algorithm. Agricultural Systems, 56, 29-44.

Pitt, R.E. & Leibensperger, R.Y. (1987) The effectiveness of silage inoculants: A systems approach. Agricultural Systems, 25, 27-49.

Pitt, R.E. & Muck, R.E. (1993) A diffusion model of aerobic deterioration at the exposed face of bunker silos. Journal of Agricultural Engineering Research, 55, 11-26.

Robson, M.J., Ryle, G.J.A. & Woledge, J. (1988) The grass plant – its form and function. In: Jones, M.B. & Lazenby, A. (eds) The Grass Crop pp. 25-83. Dortrecht, Springer Nature. https://doi.org/10.1007/978-94-009-1187-1.

Rotz, C.A., Ford, S.A. & Buckmaster, D.R. (2003) Silages in farming systems. In: Buxton, D.R., Muck, R.E. & Harrison, J.H. (eds) Silage Science and Technology, pp. 505-546. Madison, WI, USA: Agronomy Publication 42, American Society of Agronomy.

Savoie, P. & Jofriet, J.C. (2003) Silage storage. In: Buxton, D.R., Muck, R.E. & Harrison, J.H. (eds) Silage Science and Technology, pp. 405-467. Madison, WI, USA: Agronomy Publication 42, American Society of Agronomy.

Schwarz, D., Dörrstein, J, Kugler, S., Schieder, D., Zollfrank, C. & Sieber, V. (2016) Integrated biorefinery concept for grass silage using a combination of adapted pulping methods for advanced saccharification and extraction of lignin. Bioresource Technology, 216, 462-470.

Seastedt, T.R., Coxwell, C.C. Ojima, D.S. & Parton, W.J. (1994) Importance of photosynthetic pathways, management, and climate on plant production and soil carbon of semihumid temperate grasslands. Ecological Applications, 4, 344-354.

St-Pierre, N.R. & Weiss, W.P. (2015) Partitioning variation in nutrient composition data of common feeds and mixed diets on commercial dairy farms. Journal of Dairy Science, 98, 5004-5015.

Vanderwerff, L.M., Ferraretto, L.F. & Shaver, R.D. (2015) Brown midrib corn shredlage in diets for high-producing dairy cows. Journal of Dairy Science, 98, 5642-5652.

Weinberg, Z.G. & Muck, R.E. (1996) New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiology Reviews, 19, 53-68.

Weiss, K. (2016) Volatile organic compounds (VOC) in silages – effects of management factors on their formation. In: Rajčáková, L. (ed.) Proceedings of the 17th International Conference Forage Conservation, pp. 27-38, Slovak Republic.

Weiss, K., Kroschewski, B. & Auerbach, H. (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. Journal of Dairy Science, 99, 8053-8069.

Wilkinson, J.M. & Lee, M.R.F. (2017) Use of human-edible animal feeds by ruminant livestock. Animal. Published online 12 September 2017. DOI:10.1017/S175173111700218X.

Wilkinson, J.M. **&** Rinne, M. (2017) Highlights of progress in silage conservation and future perspectives. Grass and Forage Science. Published online 26 October 2017. DOI: 10.1111/gfs.12327.

Wilson, R.F. & Wilkins, R.J. (1973) Formic acid as a silage additive for wet crops of cocksfoot and lucerne, Journal of Agricultural Science, Cambridge, 80, 225-231.

Wolfert, S., Ge, L., Verdouw, C. & Bogaardt, M-J. (2017) Big Data in Smart Farming – A review. Agricultural Systems, 153, 69-80.

# Application of opto-chemical sensing technology for measuring oxygen in the gas atmosphere of grass-silage during fermentation and under aerobic stress conditions

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**Keywords:** aerobic stability, grass silage, monitoring, opto-chemical sensing technology, oxygen

**Introduction** The degree of oxygen availability determines the life conditions of microorganisms, the type of fermentation of conserved forage plants and the stability of a fermented silage. Conventional oxygen monitoring in silage typically entails invasive sampling (Green et al. 2012). Alternatively, $O_2$-levels may be estimated by statistical modelling based on the $O_2$-consumption of the involved microorganisms (Williams et al. 1994). With the introduction of opto-chemical sensing technology, a non-invasive and non-destructive method for monitoring oxygen is available for a few years now (Resch et al. 2017, Tscherner et al. 2016). In the presented study, we adapted an opto-chemical sensing scheme to the specific demands of $O_2$-monitoring in round baled grass silage under practical conditions.

**Materials and Methods** A round bale (diameter 120 cm, compaction 288 kg DM $m^{-3}$, baler type "Krone Comprima V150 XC") was prepared from forage of a highly wilted grass-clover mixture (DM 475 g FM $kg^{-1}$; 52% grass, 46% legumes, 2% herbs, 4$^{th}$ cut of the season on September 15$^{th}$, 2016). Screen-printed and calibrated sensor patches were affixed to the binding net at the circumference of the bale before wrapping for non-invasive monitoring of the oxygen levels right beneath the stretch film. After pre-stretching (70% rate) of a transparent 25 µm PE-film, the bale was wrapped with six layers of film (wrapper type "Göweil G2020"). Transparency or at least translucency of the stretch film which, in addition, needs to be devoid of auto-fluorescence is essential to enable a non-invasive optical readout of the sensor patches which was accomplished through six layers of film using an opto-electronic setup (modified OEM-components developed by JOANNEUM RESEARCH) tailored for the experiment. In an alternative implementation, opto-chemical oxygen sensors were positioned at the end of light-guiding fibers in the vicinity of an additional temperature sensor to form an "in-depth probe head" (development by JOANNEUM RESEARCH tailored for the experiment). Fiber, wiring and probe head were protected by a 10 mm tube of stainless steel and an end-cap of sintered porous metal. The thus formed probes were inserted into the bale at different depths (10, 50, 90 cm) and sealed against the stretch film using an adhesive mass. During fermentation (59 days) and experimentally induced aerobic stress upon partial removal of the stretch film (14 days), oxygen levels were continuously recorded (interval 30 s.) at three of the sensing positions. The majority of the installed sensors were monitored manually on a regular basis in intervals of 3 hrs - 10 days, depending on the dynamics of the fermentation using an additional opto-electronic hand-held module (commercialised by "TecSense" on the basis of developments by JOANNEUM RESEARCH).

**Results** This globally first reported study on the applicability of opto-chemical oxygen sensors in silage media provided plausible data for the oxygen levels during the experiment, including the initial phase right after bale wrapping as well as two weeks of aerobic stress conditions at the end. Upon wrapping, the oxygen inside the bale was consumed almost completely within 95 minutes and levels remained well below 1% as long as the stretch film was left intact. Partial removal of the film after 59 days of fermentation (Nov. 14$^{th}$, 2016) resulted in an ingress of oxygen into the bale. The increase rate of the oxygen was found to be strongly dependent on the respective sensor position relative to the opening in the stretch film: comparably fast ingress of oxygen near the opening opposed a delayed,

gradual increase of oxygen levels in the bale's core (Figure 1). During the experiment, temperature monitoring resulted in plausible and climatically induced decreasing values (25 to 0 °C). At the bale's surface, time-of-the-day-dependent fluctuations of ambient temperature had a much more pronounced effect than in the core. After the trial, the performance of the oxygen sensors was evaluated by re-calibration.

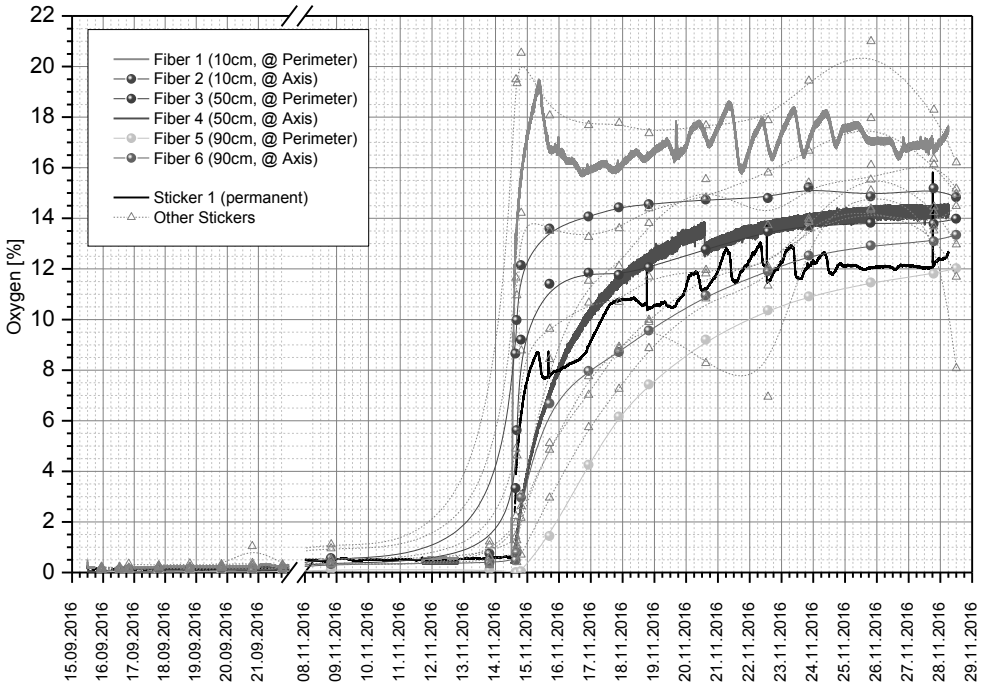


**Figure 1**. The progress of the oxygen levels in a round baled silage, monitored by means of opto-chemical sensing in different positions. Data were recorded from the time of wrapping until two weeks after partial film removal.

**Conclusions** Opto-chemical sensing technology appears well suited for oxygen monitoring in silage systems for R&D purposes (preserving jars, laboratory barrels etc.) as well as in practice (bales, clamps, bunkers etc.) and may thus develop into a powerful tool in the field of fermentation research and forage quality monitoring.

**References**

Green, O., Bartzanas, T., Løkke, M.M., Bochtis, D.D., Sørensen, C.G., Jørgensen, O.J., & Tortajada, V.G. (2012) Spatial and temporal variation of temperature and oxygen concentration inside silage stacks. Biosystems Engineering, 111, 155-165.

Resch, R., Schweiger, M., Huber, G., & Tscherner, M. (2017) Anwendung von opto-chemischen Verfahren zur Messung der Sauerstoffkonzentration in der Gärgasatmosphäre von Grassilage während der Gärphase sowie unter Luftstress nach Siloöffnung, Abschlussbericht der wissenschaftlichen Tätigkeit "Silagesensor", Nr. 3641 (DaFNE 101073), HBLFA Raumberg-Gumpenstein, 24 p.

Tscherner, M., Resch, R., Schweiger, M., & Köstler, S. (2016) Permanent oxygen monitoring in the fermentation atmosphere of grass silage using optochemical sensing technology. Sensing in Food and Agriculture, Cambridge UK, 29.-30. November 2016.

Williams, A.G., Lowe, J.F., & Rees, D.V.H. (1994) The effect of oxygen concentration on changes in the microbial population, temperature and dry-matter content in grass silage. Grass and Forage Science, 49, 183-191.

# Can lucerne silage replace grass silage in Nordic climate?

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**Keywords:** Medicago sativa L., milk production, overwintering, protein supplementation

**Introduction** Lucerne (Medicago sativa L.) is not a common species in northern climates because of its poor overwintering. Recently introduced new varieties offer a possibility to use lucerne as an alternative silage raw material to grasses. The reason for including lucerne in ration formulation is the expected increase both in intake and milk production. Lucerne can also supplement low N content forages such as maize or whole-crop silage. The first objective of the experiment was to determine the effects of replacing grass silage with lucerne silage on milk production and nutrient utilization of dairy cows. The second objective was to study if the inclusion of lucerne can replace protein supplementation. An additional objective of this study was to test the overwintering of lucerne in Finland.

**Material and Methods** Second cut grass silage and second cut lucerne silage were prepared on 9 and 16 August 2017, respectively. Lucerne was sown (21 kg/ha) in 2016 as a seed mixture containing varieties Live, Nexus and Plato. Grass silage was a mixture of timothy and meadow fescue (varieties Nuutti and Inkeri, 20 kg/ha). Both silages were preserved in round bales using a formic-acid-based additive at 5 l/tn. The treatments were Control (grass silage + rapeseed meal supplement), LucerneNormal (grass silage + lucerne silage + rapeseed meal supplement) and LucerneLow (grass silage + lucerne silage + lowered rapeseed meal supplement). The lucerne and grass silages were mixed in the ration at 40:60 on a dry matter basis. The diets were fed as TMR ad libitum. The proportion of rapeseed meal in the concentrate was 20% in Control and LucerneNormal, and 15% in LucerneLow. The average concentrate proportion in the diets was 46% on DM basis. The experiment was conducted using 48 dairy cows in a change-over design with two periods. The average milk yield at the beginning of the experiment was 34.8 kg (SD 7.67 kg) per day and the cows were, on average, 138 days in milk (SD 83.8).The cows were divided into four blocks according to parity and lactation stage. The statistical model included treatment, period and the block as fixed variables and animal as a random variable. The differences between LS means were tested using contrasts: Control *vs* lucerne diets and LucerneLow *vs* LucerneNormal.

**Results and Discussion** The lucerne mixture overwintered relatively well. The total lucerne dry matter yield was 4200 kg DM/ha which was about half of an average grass silage yield at Luke Maaninka experimental farm. The reasons for low yield were the cool weather in summer and the use of herbicide (Harmony, tifensulfuron-metyl), which damaged the lucerne vegetation after the first cut. The DM yield of lucerne was 30% lower compared with the results reported by Bélanger et al. (2014) with two cuts. Taking an additional third cut would increase the yield, but it is not recommendable because of risk for overwintering (Bélanger et al. 1999). The energy content of lucerne was low (10.0 MJ ME/kg DM) compared with grass (10.6 MJ ME/kg DM), whereas the crude protein content of lucerne was high (221 g/kg DM) compared with grass (166 g/kg DM). The average DM intake of lucerne diets was on average 15% higher compared to grass silage diet (Table 1). This is in agreement with earlier results reported using leguminous plants (Huhtanen et al. 2007). Rapeseed meal supplementation has been reported to increase DM intake (Huhtanen et al. 2007) which can be seen in a higher DM intake of LucerneNormal compared with LucerneLow, but the effect was not statistically significant due to a small difference in rapeseed supplementation between lucerne diets. Increased intake with lucerne diets resulted in increased milk yield. Milk protein and fat concentrations did not differ between treatments, but milk urea concentration was higher with LucerneNormal compared with LucerneLow and Control. High milk urea is linked with increased N excretion in urine (Castillo et al. 2000).

The DM intake of Control was low taking account the production level of the cows leading to a clearly negative energy balance. The moderate fermentation quality of second cut grass silage is one reason for this. Maintaining milk yield with a negative energy balance is possible in short term experiments, but the expected milk yield in long term might be lower compared to the results in this experiment. The inclusion of lucerne silages improved the energy balance close to zero. Thus, the low energy content of lucerne was compensated by increased intake.

**Table 1**.The effect of treatments on feed intake, milk production and nutrient utilization.

| | Control | LucerneNormal | LucerneLow | sem[1] | C1[2] | C2[3] |
|---|---|---|---|---|---|---|
| Intake, kg DM[4]/d | | | | | | |
| Silage | 10.9 | 12.5 | 12.3 | 0.20 | <0.001 | 0.25 |
| Concentrate | 9.0 | 10.6 | 10.3 | 0.16 | <0.001 | 0.09 |
| Total | 19.9 | 23.1 | 22.6 | 0.36 | <0.001 | 0.16 |
| ME[5], MJ | 216 | 247 | 243 | 3.5 | <0.001 | 0.18 |
| Milk, kg/d | 29.3 | 31.3 | 30.5 | 0.57 | <0.001 | 0.06 |
| ECM[6], kg/d | 32.5 | 34.9 | 33.8 | 0.63 | <0.001 | 0.08 |
| Nutrient utilization | | | | | | |
| ME, MJ/kg ECM | 4.59 | 5.13 | 5.22 | 0.106 | <0.001 | 0.46 |
| kg ECM/kg DM | 1.64 | 1.51 | 1.49 | 0.027 | <0.001 | 0.39 |
| ME balance, MJ/d | -19 | -2 | -0.4 | 3.4 | <0.001 | 0.65 |
| Milk N/N intake | 0.322 | 0.285 | 0.279 | 0.0005 | <0.001 | 0.27 |

[1]standard error of mean, [2]Control *vs* lucerne diets, [3]LucerneNormal *vs* LucerneLow
[4]dry matter, [5]metabolizable energy, [6]energy corrected milk

The high N content of lucerne is not recommendable from the environmental point of view and protein supplementation increases the nitrogen content of the diet further. There was a tendency for milk yield to increase with LucerneNormal compared with LucerneLow. The additional amount of rapeseed meal between these diets was 0.5 kg/d so the supplementation was economically profitable On the other hand, the high nitrogen content of the diet increases both excreted urine N and ammonia emissions (EMEP/EEA 2016). A hypothesis of the experiment was that the inclusion of Lucerne in the grass silage diet would reduce the use of rapeseed meal in the diet. Numerically, LucerneLow produced more milk compared with Control. Thus, if the target is only to maintain the production level with a grass silage based diet, the use of Lucerne can decrease the use of rapeseed meal.

**Conclusion** Lucerne was not a recommendable alternative for grass if high forage yields are targeted. However, partial replacement of grass with lucerne increased intake, milk yield and energy balance demonstrating the nutritional advantages of lucerne compared with sole grass silage. Lucerne can partially replace the protein supplementation with grass silage based diets if the target is to maintain milk production. A protein supplemented lucerne diet is economically reasonable if the target is to maximize economic output at the expense of environmental emissions.

**References**

Bélanger, G., Kunelius, T., McKenzie, D., Papadopoulos, Y., Thomas, B., McRae, K., & Fillmore, S. & Christie, B. (1999) Fall cutting management affects yield and persistence of alfalfa in Atlantic Canada. Canadian Journal of Plant Sciences, 79, 57-63.

Bélanger, G., Castonguay, Y. & Lajeunesse, J. (2014) Benefits of mixing timothy with alfalfa for forage yield, nutritive value, and weed suppression in northern environments. Canadian Journal of Plant Sciences, 94, 51-60.

Castillo, A., Kebreab, E., Beever, D. & France, J. (2000) A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. Journal of Animal and Feed Sciences, 9, 1-32.

EMEP/EEA. (2016) EMEP/EEA air pollutant emission inventory Guidebook 2016. 3.B Manure management. Copenhagen, European Environment Agency. EEA Report No 21/2016.

Huhtanen, P., Rinne, M. & Nousiainen, J. (2017) Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal, 1, 758-770.

# Carbon absorption in silages: a novel approach in silage microbiology

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**Keywords:** carbon dioxide, $CO_2$, internal pressure, maize, microbiology

**Introduction** In the last few years our team has made efforts for developing a method to evaluate the dynamics of gas production in silages, by collecting and measuring all gases produced during the fermentation (Schmidt et al. 2012). This system consists of a PVC silo attached to a graduated chamber immersed in water. From a completely closed system, we have surprisingly detected that a new phase of negative pressure inside the silos starts one to two weeks after sealing (Souza et al. 2015). This effect has been detected throughout the trials we have performed with maize silage (n=7), even under a controlled-temperature room. From this unintentional discover, we have designed a simple trial to start studying this effect. This trial aimed to evaluate the pressure inside the silos as well as the carbon absorption when feeding the silos with carbon dioxide ($CO_2$). The hypothesis is that a homoacetogenic acetyl-CoA pathway performed by bacteria occurs in maize silages which can lead to carbon absorption under an anaerobic metabolism. Findings in the literature support this idea.

**Material and Methods** Eight PVC silos (8.8 L) were filled (196 kg DM $m^{-3}$) with chopped maize forage (332 g $kg^{-1}$ DM), closed with a proper cap and completely sealed with a liquid rubber glue, on April 08, 2016. A previous test to block the escape of gases was performed. Silos were stored in a controlled-temperature room (24±1 ºC) for 5 months. In the cap of each silo a silicon tube attached to a 3-way valve was inserted. A device composed of a low-density polyethylene chamber (1 L) was attached to the valve in each silo to collect and measure the volume of all gases produced during the fermentation. The internal pressure inside the silos was assessed using a mercury column manometer attached to the valve, with no escape of gases (Figure 1).

**Figure 1**. Details of the silos, gas collector device, and manometer (photos: Patrick Schmidt).

Once the silos stopped the gas production and started showing negative pressure, four of the eight silos were weekly fed with pure $CO_2$. The polyethylene chamber was filled up with $CO_2$ and then the valve was opened in order to balance the pressure inside the silo. The $CO_2$ was not forced inside the silo. The volume of $CO_2$ absorbed by the silo was recorded. The other four silos remained closed and their internal pressure continued being measured. After 147 days of storage, silos were opened and samples were taken for pH measuring. Descriptive statistics of the average data is presented.

**Results** Silos showed gas production until 11 days after closing (3235±388 mL $kg^{-1}$ DM). Twelve days after, a negative pressure (-0,5 mm Hg) inside the silos was detected. The silos that were kept closed showed increase of the negative pressure until 101 days after sealing (-43±2.6 mm Hg), and the value lasted until the opening (-44±2.8 mm Hg) (Figure 2a). The $CO_2$ was made available for the silos 28 days after sealing, and they absorbed 225±52 mL. Four days after, another 387±59 mL of $CO_2$

were absorbed. A total of 19 supplies of $CO_2$ were made during the trial, and silos have absorbed 5590±2492 mL of $CO_2$ during the whole trial (Figure 2b). One replicate stopped absorption in the first month, probably related to sealing failure. At the opening, the average pH of the silages was 3.76±0.03 (kept closed) and 3.63±0.02 ($CO_2$).

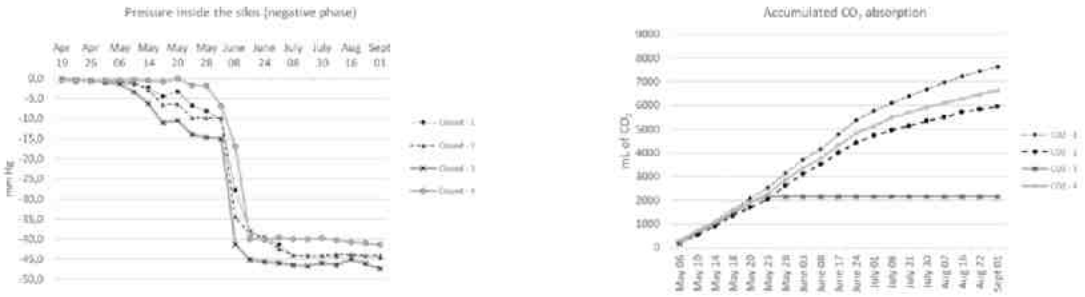


**Figure 2**. Negative pressure inside the silos that did not receive $CO_2$ (a); Accumulated $CO_2$ absorption by the silos where $CO_2$ was made available (b).

**Discussion** Although never described in silages, the homoacetogenic pathway of Acetyl-CoA, where an anaerobic bacteria is capable of reducing $CO_2$ to acetate, has been a long time known (Wood and Ljungdahl 1991). Also referred as the Wood-Ljungdahl pathway, this energy-generating process is described for 19 bacterial genera and has been postulated as the first autotrophic process of the planet (Drake and Küsel 2003). Homoacetogenic bacteria have been intensively researched in recent years by pharmaceutical and petrochemical area. This biotechnology allows the use of gaseous residues for the production of fuels and organic acids, in bioreactors (Liew at al. 2016). The silage environment (anaerobiosis, pH and temperature) is prone for the development of these worldwide spread microorganisms. In the future, silos can be used as a bioreactor fixing pollutant gases from farm activities (CO, $CO_2$, $N_2O$) into high quality nutritive compounds of feed. A series of studies will be necessary for the practical application of this technology in silage science.

**Conclusion** Maize silage is able for absorbing and fixing $CO_2$ by the Wood-Ljungdahl pathway.

**References**

Drake, H.L. & Küsel, K. (2003) How the diverse physiologic potentials of acetogens determine their in situ realities. In: Ljungdahl, L. G., Adams, M. W. Barton, L. L., Ferry, J. G. & Johnson, M. K. (eds.). Biochemistry and physiology of anaerobic bacteria. New York, USA: Springer. p. 171-190.

Liew, F.M., Martin, M.E., Tappel, R.C., Heijstra, B.D., Mihalcea, C. & Köpke, M. (2016) Gas fermentation – A flexible platform for commercial scale production of low-carbon-fuels and chemicals from waste and renewable feedstocks. Frontiers in Microbiology, 7, 1-28.

Schmidt, P., Novinski, C.N., Carneiro, E.W. & Bayer, C. (2012) Greenhouse gas emissions from fermentation of corn silage. In: Kuoppala, K., Rinne, M., & Vanhatalo, A. (eds). Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 448-449.

Souza, C.M., Bach, B.C., Novinski, C.O., Strack, M.C., Silva, E P.A., Pereira, L.M. & Schmidt, P. (2015) Does the silage absorb air during its fermentation? A lab trial on maize silages added with natamycin. In: Daniel, J. L. P, Morais, G., Junges, D., & Nussio, L. G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 350-351.

Wood, H.G. & Ljungdahl, L.G. (1991) Autotrophic character of the acetogenic bacteria. In: Shively, J.M., & Barton, L.L. (eds.). Variations in Autotrophic Life. San Diego, USA: Academic Press. p. 201-250.

# Cutting herbage PM or AM and subsequent effects on silage quality

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**Keywords:** cutting time, grass silage, silage quality, water soluble carbohydrates

**Introduction** Cutting time influences the contents of water soluble carbohydrates (WSC), where herbage cut in the evening normally has higher WSC contents than herbage cut in the morning (Berthiaume et al. 2012; Pelletier et al. 2010). An experiment was conducted to investigate the effect of cutting time on the fermentation quality and aerobic stability of silage.

**Material and Methods** Part of the first growth of a grass-dominated ley (A) and a grass–clover-mixture ley (B) was cut in the evening (7 p.m.) and the rest on the following morning (9 a.m.) on 16 and 17 May 2017, respectively. The forage was wilted to 40% dry matter (DM), chopped and ensiled during the afternoon of 17 May 2017 in laboratory silos with a capacity of 1.5 L. For each treatment, three samples were taken and analysed. After a storage time of 93 days, the silos were opened. The DM content was determined by oven drying (15 h at 60°C and 3 h at 105°C), and the nutrient contents were analysed by near-infrared spectroscopy. Additionally, in the silages, the fermentation parameters (pH, acids, ethanol and ammonia) were analysed, and the aerobic stability was investigated. Data were analysed using analysis of variance (Systat 13).

**Results and Discussion** The DM contents of herbage cut in the evening from leys A and B were higher than that of the herbage cut on the following morning from the same ley (Table 1). For ley A, the WSC contents of the herbage decreased during the night, but this was not the case for the herbage of ley B. The herbage of ley A had less ash and crude protein and more fibres (neutral detergent fibre [NDF] and acid detergent fibre [ADF]) compared to that of ley B, which had a higher proportion of clover.

**Table 1.** Dry matter (DM) content and nutrients in the herbage before cutting

| | | Ley A | | Ley B | | SE | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PM | AM | PM | AM | | Ley | Time | L x T |
| DM | % | 23.0 | 21.1 | 18.6 | 17.9 | 0.15 | *** | *** | * |
| Ash | g/kg DM | 77 | 77 | 91 | 95 | 1.7 | *** | ns | ns |
| CP | g/kg DM | 106 | 99 | 144 | 139 | 2.3 | *** | ns | ns |
| NDF | g/kg DM | 458 | 493 | 414 | 433 | 5.5 | *** | ** | ns |
| ADF | g/kg DM | 237 | 262 | 224 | 234 | 3.3 | ** | ** | ns |
| WSC | g/kg DM | 242 | 214 | 179 | 191 | 5.6 | ** | ns | * |

SE: standard error; PM: evening; AM: morning; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; WSC: water soluble carbohydrates

**Table 2.** Dry matter (DM) content and nutrients in the herbage at ensiling

| | | Ley A | | Ley B | | SE | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PM | AM | PM | AM | | Ley | Time | L x T |
| DM | % | 45.2 | 40.4 | 40.8 | 37.2 | 0.98 | * | * | ns |
| Ash | g/kg DM | 74 | 75 | 88 | 86 | 1.4 | *** | ns | ns |
| CP | g/kg DM | 104 | 101 | 144 | 142 | 0.9 | *** | ns | ns |
| NDF | g/kg DM | 459 | 464 | 413 | 412 | 1.9 | *** | ** | ns |
| ADF | g/kg DM | 244 | 249 | 228 | 232 | 1.2 | *** | * | ns |
| WSC | g/kg DM | 277 | 254 | 213 | 203 | 2.9 | *** | ** | ns |

SE: standard error; PM: evening; AM: morning; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; WSC: water soluble carbohydrates

At ensiling, the DM contents were higher in the herbage of ley A than that of ley B. In addition, the nutrient contents were influenced by the type of ley (Table 2). For the cutting time, WSC, NDF and ADF were influenced significantly.

The pH, lactic acid, acetic acid and ethanol in the silages were influenced by the type of ley and the cutting time (Table 3). Higher pH values and lower volatile fatty acid contents in the silages cut in the afternoon were also found by Berthiaume et al. (2012). All silages showed a good fermentation quality. The Deutsche Landwirtschafts-Gesellschaft (DLG) points varied between 90 and 93, and they were influenced by the cutting time.

The silage of the morning cuts had a better aerobic stability than the silage of the evening cuts (Table 3). The reasons for these differences could be the lower DM content and higher acetic acid content. The extent to which the microbiological quality of the silage was responsible for the aerobic stability was not investigated.

**Table 3.** Dry matter (DM), fermentation parameters and aerobic stability of the silages

| | | Ley A | | Ley B | | SE | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PM | AM | PM | AM | | Ley | Time | L x T |
| DM | % | 43.6 | 39.6 | 39.5 | 34.8 | 0.77 | ** | ** | ns |
| pH | g/kg DM | 5.5 | 4.8 | 5.2 | 4.7 | 0.04 | * | *** | ns |
| Lactic acid. | g/kg DM | 23 | 46 | 48 | 76 | 2.0 | *** | *** | ns |
| Acetic acid | g/kg DM | 7 | 14 | 13 | 22 | 0.8 | *** | *** | ns |
| Butyric acid | g/kg DM | 1 | 2 | 2 | 2 | 0.1 | ** | ns | ns |
| Ethanol | g/kg DM | 29 | 7 | 9 | 8 | 1.5 | ** | ** | ** |
| $NH_3$-N/N | % | 5.8 | 6.9 | 11.2 | 11.1 | 0.27 | *** | ns | ns |
| DLG | points | 90 | 91 | 90 | 93 | 0.5 | ns | * | ns |
| Aerobic stability | days | 4.9 | 10.4 | 5.9 | 13.3 | 0.70 | ns | *** | ns |

SE: standard error; PM: evening; AM: morning; $NH_3$-N/N: ammonia-N content of total N; DLG: Deutsche Landwirtschafts-Gesellschaft

**Conclusion** In the fresh herbage, the WSC contents of the herbage were higher in the evening than in the morning for the grass-dominated, but not for the grass–clover-mixture ley. At ensiling, both leys demonstrated higher WSC contents in the herbage cut in the evening. The pH, lactic acid and acetic acid were influenced by the cutting time and type of ley, but only the cutting time influenced aerobic stability. The lower DM-content and higher acetic acid content were partly responsible for this results.

**References**

Berthiaume, R., Cino, A., Lafrenière, C., Fortin, J., Gariépe, C, Mandell, I. & Faucitano, L. (2012) Growth, feed efficiency, carcass quality and consumer perception of beef cattle fed PM vs AM cut grass or a red clover-grass mixture. Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland. pp. 166-167.

Pelletier, S., Tremblay, G.F., Bélanger, G., Bertrand, A., Castonguay, Y., Pageau, D. & Drapeau, R. (2010) Forage nonstructural carbohydrates and nutritive value as affected by time of cutting and species. Agronomy Journal 102, 1388-1398.

# Development of a preservative for moist hay to extend the hay baling window

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**Keywords:** additive, inhibition of spoilage, moist hay, nutritive value, preservation

**Introduction** It is becoming increasingly difficult to produce high-quality hay, because the hay rarely dries evenly on the field. Especially high mass coverage and unstable weather conditions make it almost impossible to uniformly dry to higher than 85% dry matter. Often it is the stem nodes that are not sufficiently dry. After harvesting, residual moisture returns to the stems and leaves. This benefits certain microorganisms, especially moulds. Their growth reduces quality and feed hygiene. External signs of microbial activity include the rise in the temperature in the stored hay. In order to counteract such spoilage processes, the use of preservatives is gaining increasing attention. As a rule, products based on propionic acid are used for this purpose. However, their use is not entirely unproblematic. In addition to relatively high application rates, the risk of corrosion in the recovery technology, the need for a special acid-resistant dosing technology and transport restrictions on possible application restrictions make its use difficult. The aim of the investigations was therefore to develop a user-friendly alternative product based on neutral salts to the classic acid-based products.

**Materials and Methods** In the laboratory trial the relation between heating and microbial development and biochemical changes were studied. For this trial German Grazing Grass *(Lolium perenne)* was harvested in 2015, dried and pressed into 25 kg square bales as moist hay. The target value for the residual moisture was 22%. In addition to the untreated control (T1), the moist hay was treated with a special formulation of three anti-fungal chemicals (T3) (potassium sorbate, sodium benzoate and sodium propionate, produced by Danstar Ferment (Zug, Switzerland) with different individual modes of action. This salt mixture was applied at 250 g / t, solved in 1 litre water. Propionic acid (T2) (99.8%, 4.5 l / t) was used for positive control. The moist hay was examined at the beginning of each test (Table 1, composite sample) and after 30 and 100 days of storage (sampling of 5 individual bales). During storage, the temperature development in the bales was recorded by means of a data logger. As the bales were stored, in such a way that the formed heat could escape easily, a temperature of 45 ° C was never exceeded. Furthermore, individual bales (n=5) of each treatment were examined after 30 and 100 days of storage and the dry matter content, feed value and hygiene status were determined.

**Table 1**. Nutritional and microbial parameters of grass prior to baling

| Parameter | Unit | Value |
|---|---|---|
| Dry matter | % FM[1] | 78.0 |
| Crude ash | % DM[2] | 9.7 |
| Crude protein | % DM | 8.3 |
| Crude fibre | % DM | 28.2 |
| Neutral detergent fibre | % DM | 58.1 |
| Acid detergent fibre | % DM | 29.8 |
| Lactic acid bacteria | Log CFU[3]/g FM | 4.67 |
| Yeasts | Log CFU/g FM | 5.86 |
| Moulds | Log CFU/g FM | 6.18 |
| Enterobacteria | Log CFU/g FM | 4.96 |

[1]Fresh material, [2]Dry matter, [3]Colony-forming unit

**Results and discussion: Raw material (Table 1):** The target residual moisture content of 22% was achieved. With regard to the hygiene status (mould count) the values, according to the VDLUFA-orientation values of 1.500.000 CFU/g were achieved. **Temperature development (Figure 1):** During storage, there is a distinct warming in the untreated control, which was a clear indication of microbial spoilage processes. On the other hand, no increase in temperature was observed in all the replicates of moist hay treated with preservatives. **Hygiene status (Table 2):** Microbial spoilage quickly began in the untreated control. The mould count remained unchanged and high (after 30 days log 5,90 or after 100 days log 5,60 CFU/g). In contrast, the number of moulds found in both preserved varieties decreased, with the largest decrease in the neutral salts mixture variant, with 100 days of storage; the number of moulds decreased here to 44,668 CFU/g. **DM-content and further parameters (Table 2):** Compared to untreated control, all preservative treated bales had a better feed value and higher energy densities. Thus, the digestibility of the organic matter in the bales, preserved with the new formulation, was 2.1% higher than the control, and the net energy was 0.12 MJ NEL/kg DM higher. Spoilage and loss of nutrients could be safely avoided. As expected, there was a further increase in dry matter content in the bales stored during the entire storage period.

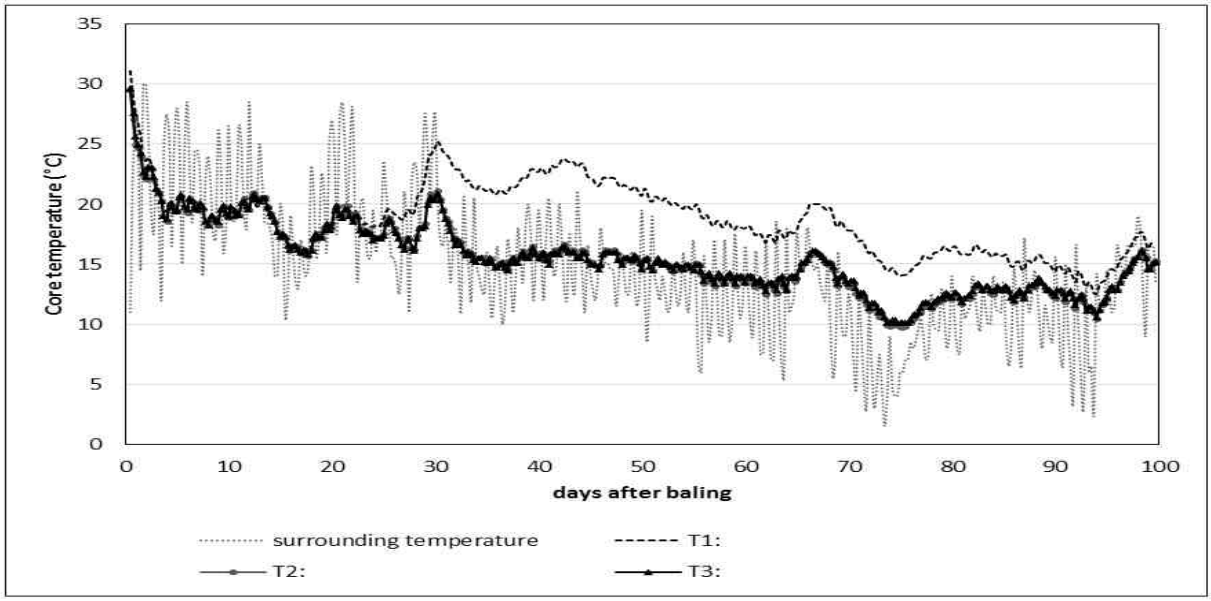


**Figure 1**. Bale temperature profile (5 replicates)

**Table 2**. Cumulative temperature, hygiene status and nutritional value after 30 and 100 days of storage (5 replicates) (P<0,05)

| | Cumulative temperature °C | Moulds Log CFU/g FM | DM % | dOS[1] % | NEL MJ/kg DM |
|---|---|---|---|---|---|
| 30 days storage | | | | | |
| T1 | 2422 ± 62 | 5,90 | 83,1 | 60,3 | 4,57 |
| T2 | 2223 ± 54 | 5,13 | 85,4 | 61,1 | 4,61 |
| T3 | 2242 ± 66 | 5,62 | 85,0 | 63,7 | 4,75 |
| 100 days storage | | | | | |
| T1 | 7458 ± 198 | 5,60 | 84,0 | 60,1 | 4,54 |
| T2 | 6134 ± 40 | 4,85 | 84,2 | 60,8 | 4,59 |
| T3 | 6168 ± 22 | 4,65 | 84,1 | 62,2 | 4,66 |

[1]Digestibility of organic matter

**Conclusions** With the help of preservatives, moist hay can be stored safely and loss-free. Heating and spoilage are prevented and quality is ensured. With the new product formulation based on neutral salts, a user-friendly alternative to the acid-based preservatives for moist hay is now available.

# Evaluation of a new aerobic preservation solution for high moisture hay

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**Keywords:** aerobic preservation, chitinase, moist hay, Pediococcus pentosaceus,

**Introduction** Arbitrating between the ideal nutritional content of grass hay and moisture content at baling for optimal preservation usually go opposite directions. Knowing that leaf separation from stems is a major cause of dry matter (DM) quality decrease in hay, many producers prefer to harvest at a higher moisture content to preserve nutrients concentrated in the leaves. Difficulty to anticipate weather conditions is also driving the decision to cut at a lower than optimal DM content too. Recent results about hay inoculants (Jin et al. 2017) demonstrated an effect on heating mitigation and digestibility enhancement of moist alfalfa hay inoculated with a combination of *Pediococcus pentosaceus* (PP) and chitinase (Ch). The objective of this study was to assess the effectiveness of this new technology on European grass and lucerne hays harvested at more than 20% moisture.

**Materials and Methods** Grass (*Lolium multiflorum*) and lucerne (*Medicago sativa*) were harvested at 66.6 and 68.1% DM respectively in 450 kg rectangular bales. The experimental treatment (PP+Ch), was compared to a negative control (C), propionic acid (PA) and low moisture hay (LMH, 79.9% DM). Five bales per treatment were harvested. Application rate was 2L/t for C (water), PP+Ch ($10^{12}$ CFU/kg PP NCIMB 12674 + chitinase (LANiHay01) at a minimum of 6 unit of chitinase activity/g, i.e. 1.5L/t) and 5L/t for PA. Bales were moved to a common storage site and stacked in piles of 2 or 3 bales within treatment under the shed of a farm building. Piles were spaced by a minimum of 60cm to allow sampling and air circulation.., A temperature probe (Thermo-Tracer 21G, Oceasoft, France) was manually inserted in the core of the bale for 120d of recording. At days 0, 15, 30, 60, 120 and 240 of storage, each bale was mechanically drilled and samples dried in a forced air oven at 55°C for 48 hours to determine the dry matter content. In addition, in vitro digestibility of aNDFom fraction (IVNDFD) was determined after 24h and 240h for each forage, as well as 48h for Lucerne, and 72h for Grass according to Palmonari et al. (2017). Each sample was analyzed in duplicate, in two different in vitro incubations. For each time point, two replicates of blank sample were run. Blanks were prepared as described above but without the addition of any sample, and then treated as the other samples. The two IVNDFD digestibility assays started within 10 days of each other. For all analysis, sample preparation was the same, as were the donor cows and their diet. Digestibility at any given time point was calculated as:

IVNDFD, % aNDFom = [1 – (aNDFom*r* – aNDFom*b*) / aNDFom*i*] *100

where aNDFom*r* is the residual aNDFom, aNDFom*b* is the blank correction, and aNDFom*i* represents the initial NDF. All the described terms are expressed in grams. Statistical analysis was conducted via ANOVA using the JMP-12 software (SAS Institute Inc., Cary NC). Least squared means were compared using the Tukey adjustment, setting significance level at $P < 0.05$.

**Results** Average temperature (°C) was similar among treatments over 120d but some differences occurred within the first 30 d: Grass 50, 50, 42, 49 °C; Lucerne: 57, 49, 46, 50 °C for C, PP+Ch, PA and LMH respectively. Recorded temperatures were the highest for C in Lucerne only ($P < 0.05$). The 24 h IVNDFD was not affected by treatments after 30 d in grass hay, but PP+Ch enhanced ($P < 0.05$) 72 h IVNDFD compared to C from 60 d of storage (Table 1) until 240 d(50.9 vs 59.5%, for C vs PP+Ch). For Lucerne, 24 h IVNDFD was improved ($P < 0.05$) for PP+Ch after 4 months of storage (Table 1), and more digestible fibre was also preserved after 48 h with highest IVNDFD for PP+Ch ($P < 0.05$), i.e. + 13-15% NDFD over C after 60-240 d days of storage. Interestingly, 240 h IVNDFD was still greater ($P < 0.05$) for PP+Ch than C in both forages, which was also better than PA and LMH

starting after 60 d of storage. Sugars were already consumed by the microflora after 15 d, and ash content was constant across treatments.

**Table 1**. In vitro NDF digestibility (IVNDFD) at 24, 48, 72 or 240 h and 60, 120 or 240 d of storage of grass or lucerne hay.

| Grass hay | C[1] | PP+Ch | PA | LMH | SEM |
|---|---|---|---|---|---|
| aNDFom, 60d | 61.2 [a] | 55.5 [b] | 61.3 [a] | 55.4 [b] | ? |
| 72h IVNDFD, 60d | 46.7[a] | 54.9 [b] | 47.3 [a] | 47.1 [a] | 1.5 |
| aNDFom, 120d | 62.2 | 60.2 | 61.9 | 57.0 | ? |
| 240h IVNDFD, 30d | 60.28 [a] | 65.31 [b] | 61.96 [a] | 58.49 [a] | 2.01 |

| Lucerne hay | C | PP+Ch | PA | LMH | SEM |
|---|---|---|---|---|---|
| aNDFom, 120d | 46.7 | 41.7 | 41.6 | 41.0 | ? |
| 24h IVNDFD, 120d | 25.8 [a] | 30.6 [b] | 25.8 [a] | 29.2 [ab] | 1.6 |
| aNDFom, 240d | 48.0 [a] | 41.7 [b] | 42.9 [b] | 39.6 [b] | |
| 240h IVNDFD, 240d | 46.8 [a] | 53.1 [b] | 49.2 [c] | 50.0 [c] | 1.5 |

[1]C = negative control; PP+Ch = *P. pentosaceus* NCIMB 12674 + chitinase; PA = propionic acid; LMH = low moisture hay. Different letters in superscripts within the same row represent statistical difference at $P < 0.05$.

**Discussion** Preservation of moist hay with a specific hay inoculant looks encouraging since nutritional value can be optimized. Indeed, even if actual chemical composition was not affected by the type of inoculation or moisture content, fibre digestibility was improved with the new “inoculant”. This feature is highlighted by the higher values obtained even against traditional preservatives, such as propionic acid, or good dry hay. Moreover, considering that fibre in grasses is slower digested than in legumes, even if their overall digestibility is greater, the increase of slowly digestible NDF with PP+Ch after 72 h is emphasized by the differences observed in the indigestible fibre fraction after 240 h incubation (-26.6% vs C) at 60 d of storage. It suggests that undesirable fermentations inside the bale were prevented with PP+Ch since a greater fibre fraction was still available after 72 h. PP+Ch is likely to have modified hay microbiome toward a reduced activity of fibrolytic bacteria and fungi, but it still has to be documented. On the other hand, Lucerne digestibility was improved even at 24 h, which represents the rapidly digestible fibre fraction. Interestingly, PA behaved similarly to C, suggesting an impact of propionic acid on the hay microbial inoculum. However, temperature rise was constantly reduced by PA for both forages, whereas PP+Ch was more effective on Lucerne.

**Conclusions** Results of this study suggest that moist hay can be well preserved with an inoculant made of *Pediococcus pentosaceus* NCIMB 12674 with a chitinase enzyme, already after 30 d of storage. Moreover, the treatment positively impacted in vitro fibre digestibility, leading to an improved nutritional value of the forage. Further, PP+Ch mitigates heating process for Lucerne.

**References**

Jin L, Chevaux E., McAllister T., Baah J., Drouin P. & Wang Y. (2018) Impact of *Pediococcus pentosaceus* and *Pichia anomala* in combination with chitinase on the preservation of high moisture alfalfa hay. Grass and Forage Science, DOI: 10.1111/gfs.12351.

Palmonari A., Canestrari G., Bonfante E., Fustini M., Mammi L. & Formigoni A. (2017) Technical note: In vitro digestibility of amylase-treated, ash-corrected neutral detergent fibre, with addition of sodium sulfite, at 240 hours with or without rumen fluid reinoculation. J. Dairy Sci. DOI: 10.3168/jds.2016-11368.

# Extension of the biological relevance of the Rostock Fermentation Test by curve fitting and interpretation

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**Keywords:** biological interpretation, curve fitting, ensilability, Rostock Fermentation Test

**Introduction** The Rostock Fermentation Test (RFT) has been established as quick method to assess the ensilability of plant material (Pieper et al. 1989) and the fermentation performance of lactic acid bacteria (LAB) as silage additives (Zierenberg 2000). RFT pictures particularly the beginning of the fermentation process with a reliable accuracy (Pieper et al. 2017). In Germany, the RFT is also used for the re-evaluation of biological silage additives with quality labels of the German Agricultural Society (Richter et al. 2010). Criterion is the pH decline during simulated onset of lactic acid fermentation with cluster analysis for the comparison of treatments. Modelling of the process dynamics has not been applied so far and was the objective of this study. The hypothesis was that a mathematical model fitted to the pH decline during RFT reflects biological principles and that curve interpretation furthermore allows a more sophisticated investigation of the biological process and distinction between treatments.

**Material and Methods** 2,420 RFT records from own studies and the literature (Briest 1994, Acosta Aragon 2004, Schmidt 2007, Hoedtke and Zeyner 2011, Gefrom 2012, Pieper et al. 2017, Hoedtke and Zeyner, unpublished) with different plant material (grasses, legumes, grass-clover-mixture, wild plants, maize silage, cereal and legume grains) and a multitude of experimental variations (osmolality, additives) were used for curve fitting and interpretation via NLMIXED for non-linear models (SAS).

**Results and Discussion** The following two non-linear functions have been selected providing superior convergence with distinct curve variables allowing predictions and biological interpretations:

Logistic function $$y(t) = a_0\left(1 - \frac{a}{1+\exp\left(\frac{t-b}{c}\right)}\right)$$

Gompertz function $$y(t) = a_0\left(1 - a \cdot \exp\left(-\exp\left(\frac{b-t}{c}\right)\right)\right)$$

The biological interpretation of the **logistic function** is the following:

$a_0$ = pH value at the beginning of the RFT

a = potential of pH decline, given as relative number between 0 and 1

b = time point at which half of the potential pH decline is reached

c = ‘b + c’ means the time point at which a quarter of the potential pH decline is reached

Similarly, the following applies to the **Gompertz function**:

b = time point at which one third of the potential pH decline is reached

c = ‚b + c‘ (where ‘c’ is a negative number)

= time point at which 70% of the potential pH decline is reached

Further items apart of the starting pH were the following:

1) the pH at the end of the test
2) the time point at which the pH first significantly fell below the starting level
3) the time point at which the pH was for the last time significantly different from either the starting or the final level for the first or the last time, respectively
4) the maximum expression (potency) of pH decline
5) the time point at which the pH decline reached its maximum expression.

Table 1 gives an example for curve variables following fitting of RFT readings to a logistic curve with ryegrass incubated alone (CON) or with sucrose (SUC) and a biological additive (LAC), respectively.

**Table 1.** Curve variables[1] for Rostock Fermentation Test readings in a trial with ryegrass incubated alone (CON) or with sucrose (SUC) and a biological additive (LAC), respectively

| Treatment | Convergence[2] | $a_0$ | a | b | b + c |
|---|---|---|---|---|---|
| CON | yes | 6.22 | 0.38 | 14.3 h | 11.5 h |
| SUC | yes | 6.27 | 0.44 | 14.8 h | 10.9 h |
| LAC | yes | 6.20 | 0.43 | 13.7 h | 13.6 h |
| SUC + LAC | yes | 6.22 | 0.49 | 11.6 h | 9.17 h |

RFT, Rostock Fermentation Test; [1]logistic equation; [2]answer the question of the quality of curve fitting; $a_0$, pH value at the beginning of the RFT; a, potential of pH decline, given as relative number between 0 and 1; b, time point at which half of the potential pH decline is reached; c = 'b + c' (with 'c' being a negative number), time point at which a quarter of the potential pH decline is reached

Based on these variables, it was possible to distinguish between treatments. The quality of reading adaptation to the curve can be characterised ('convergence') and thus irregular trends identified indicating failed fermentation. The overall application of the functions to the experimentally derived data showed that only treatments within a given trial should be used for direct comparison. Even apparently identical conditions during different trials lead to gradual deviations in the curve.

**Conclusion** Mathematical modelling of the pH decline during the RFT is possible and allows a more sophisticated interpretation and prediction of the biological process of ensiling. Curve variables can be used for the distinction of treatments with respect to the process step in which the treatments differ.

**References**

Acosta Aragón Y. (2004) Silierung von Mais- und Sorghumkörnern bei unterschiedlichen Tanningehalten und deren Auswirkung auf den Futterwert. Universität Rostock: Dissertation.

Briest G. (1994) Untersuchungen zur Siliereignung von ausgewählten Wildpflanzen eines extensiven Grünlandstandortes. Diploma thesis. University of Rostock, Germany.

Gefrom A. (2012) Die Silierung von Körnern der großsamigen Leguminosen als Methode der Konservierung und der Verbesserung ihres ernährungsphysiologischen Wertes für Monogastrier. Doctoral thesis. University of Rostock, Germany.

Hoedtke S. & Zeyner A. (2011) Comparative evaluation of laboratory-scale silages using standard glass jar or vacuum-packed model silages. J. Sci. Food Agric. 91, 841-849.

Pieper B, Hoedtke S., Wensch-Dorendorf M., Korn U., Wolf P. & Zeyner A. (2017) Validation of the Rostock fermentation test as an *in vitro* method to estimate ensilability of forages using glass jar model silages as a basis for comparison. Grass and Forage Science 72, 568-580.

Pieper B., Kleemann J., Poppe S., Allert H., Losch K., Wittchen H., Milietz E. & Schulz A. (1989) Method for the determination of ensilability in forages. Patent Specification DD 281 255 A5, Patent Office of the German Democratic Republic, Berlin, Germany.

Richter W., Spiekers H., Schuster M. & Baranowski A. (2010) Rostock fermentation test, re-evaluation of biological silage additives with DLG label. Schriftenreihe des Bayerischen Landesamtes für Landwirtschaft, Vol. 11.

Schmidt A. (2007) Einfluss des Vegetationsstadiums auf die Vergärbarkeitseigenschaften von Triticaleganzpflanzen. Master thesis, University of Rostock, Germany.

Zierenberg B. (2000) *In vitro* method for evaluation of the fermentation performance of lactic acid bacteria and its influence on the metabolic activity of other microorganism relevant for ensiling under different ensiling conditions. Doctoral thesis. University of Rostock, Germany.

# First estimation and validation of a new model to predict dry matter loss based on temperature changes – I. A meta-analysis study

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**Keywords:** aerobic stability, dry matter loss estimation, regression, temperature increase

**Introduction** Temperature increase during aerobic exposure of silage – typically during feed out – is a well-established method of describing increased activity of yeast and mould (McDonald et al. 1991). While real-time temperature tracking is relatively easy, the corresponding real-time dry matter (DM) loss during continued aerobic exposure is difficult to establish. Models to predict DM loss as a function of temperature do exist. These are, however, associated with shortcomings, eg. lack of threshold temperatures or using a stepwise logic, that is neither sensitive nor robust, with a corresponding risk of overestimating DM loss. The objective of this meta-analysis study was to establish the regression correlation between DM loss and increased temperature after 24 hours of aerobic exposure.

**Material and Methods** A literature review (n=9) on the DM loss after 24 hours aerobic challenge was used as the basis for this meta-analysis study. Silages (corn, alfalfa, and grass at DM ranging from 15-35%), were all sampled from field stacks; temperature and DM loss were subsequently measured on samples stored for 24 hours in plastic bags, left exposed to air. DM loss (%) and temperature (°C) end points after 24 hours were plotted and submitted to a regression analysis (SAS institute). As a reference method, using a stepwise assessment of dry matter loss as a function of the difference in the temperature vs ambient was compared with the new model.

**Results** Based on the meta-analysis study DM loss significantly increases 0.49% per 1 °C increase in temperature. A linear regression was established with the following equation: DMloss = 0.49T-9.73,

where DMloss (%), and T = temperature peak (°C). The equation establishes DM loss in % (+/- 0.16; *P*=0.02) with intercept -9.73 (+/- 4.12; *P*=0.05); $R^2$ = 0.56. The so far used method describes the following levels of DM loss as a function of temperature (ΔT of recorded temperature during aerobic exposure and ambient temperature): ΔT< 1 °C = no DM loss; ΔT > 1 °C < 2 °C = 3% DM loss; ΔT > 2 °C < 5 °C = 10% DM loss; ΔT > 6 °C = 15% DM loss. Data is shown in Table 1.

**Table 1**. Dry matter loss after 24 hours aerobic exposure (meta-analysis) vs. three different models for predicting DM loss as a function of temperature

| | Ambient temperature during aerobic challenge | Max temperature recorded after 24 h of aerobic exposure | DM loss (%) recorded after 24 h of aerobic exposure | DM loss (%) from McDonald et al. 1991 (ΔT to ambient step logic) | DM loss from Henderson et al. 1979 | DM loss from Woolford et al. 1984 | DM loss (%) using new linear regression model |
|---|---|---|---|---|---|---|---|
| Obs. 1 | 20 | 27 | 4.0 | 15 | 3.11 | 1.99 | 3.50 |
| Obs. 2 | 20 | 22 | 0.0 | 10 | 2.26 | 1.29 | 1.05 |
| Obs. 3 | 21 | 25 | 1.2 | 10 | 2.77 | 1.71 | 2.52 |
| Obs. 4 | 21 | 25 | 1.7 | 10 | 2.77 | 1.71 | 2.52 |
| Obs. 5 | 21 | 25 | 1.5 | 10 | 2.77 | 1.71 | 2.52 |
| Obs. 6 | 21 | 25 | 4.5 | 10 | 2.77 | 1.71 | 2.52 |
| Obs. 7 | 21 | 25 | 2.0 | 10 | 2.77 | 1.71 | 2.52 |
| Obs. 8 | 21 | 30 | 5.0 | 15 | 3.62 | 2.40 | 4.97 |
| Obs. 9 | 20 | 21 | 1.7 | 3 | 2.09 | 1.15 | 0.56 |
| Average deviation from recorded DM loss (%) | | | | 7.93 | -0.37 | 0.69 | -0.12 |

Obs.= Observation, Obs. 1 from Wilkinson and Hall (1965); Obs. 2 from Henderson et al. (1979); Obs. 3 and 4 from Schukkling and Overvest (1980); Obs. 5 and 6 from Honig and Woolford (1980); Obs. 7 and 8 from Honig (1980); Obs. 9 from Rees (1982)

**Discussion** Henderson et al. (1979) developed a model to predict DM loss as a function of temperature increase with the following equation: DMloss = 0.17T-1.480 (*P*=0.01); $R^2$ = 0.9646, based on temperature increase after 24 hours of aerobic challenge. Similarly, Woolford (1984) developed a model to predict DM loss as a function of temperature increase with the following equation: DMloss = 0.1396T-1.7794 $R^2$ = 0.1949, based on temperature increase after 24 hours of aerobic challenge. Both existing models are superior to the step logic. When submitting the data from the present meta study, Henderson's model underestimates DM loss by 0.37% points on average, Woolford's model overestimates DM loss by 0.69% points on average, whereas the new model developed on the basis of the present meta study, underestimates DM loss by 0.12% points on average.

**Conclusion** Based on this meta-analysis study we found a better fit in the prediction of DM loss as a function of temperature. According to the model, no significant DM loss occurs at temperatures below 20 °C. This correlates well with the growth pattern of spoilage organisms - typically yeast - associated with temperature increases in silages upon aerobic exposure. The method needs to be validated on different crops, storage structures, and time points.

**References**

Henderson, A.R., Matthew Ewart, J. & Robertson, G.M. (1979) Studies on the aerobic stability of commercial silages. Journal of the Science of Food and Agriculture, 30 (3), 223-228.

Honig, H. (1980) Mechanical and respiration losses during pre-wilting of grass. Forage Conservation in the 80s, in: Thomas, C. (ed) Occasional Symposium – British Grassland Society. 11:201-204.

Honig, H. & Woolford, M.K. (1980) Changes in silage on exposure to air. Forage Conservation in the 80s, in: Thomas, C. (ed) Occasional Symposium – British Grassland Society. 11:76-87.

McDonald, P., Henderson, A. R. & Heron, S. J. E. eds. (1991) The biochemistry of silage, 2nd edition, Chalcombe Publications, 340 p.

Rees, D.V.H. (1982) The aerobic deterioration of grass silage and its effect on the water-soluble carbohydrate and the associated heat production. Journal of the Science of Food and Agriculture, 33 (6), 499-508.

Schukking, S., & Overvest, J. (1980) Direct and indirect losses caused by wilting. Forage Conservation in the 80s, in: Thomas, C. (ed) Occasional Symposium – British Grassland Society. 11:210-213.

Wilkinson, R. & Hall, C.W. (1965) Respiration rate of harvested forage. Quarterly Bulletin, Michigan State University, Agricultural Experiment Station, 47:518-526.

Woolford, M.K. (1984) The Silage Fermentation. Microbiology Series vol. 14. New York: Marcel Dekker, Inc. 350 p.

# How do time of fermentation and lactic acid bacteria inoculation influence microbial succession during ensiling?

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**Keywords:** alfalfa, corn, inoculation, metasequencing, microbial succession

**Introduction** Silage fermentation is a dynamic microbial process involving the acidification of the forage by lactic acid bacteria activity in order to stabilize the biochemical and microbial constituents. Synthesis of organic acids, mainly lactic acid in the initial phase follows the reactivation and growth of members of the Lactobacillales. A succession of microbial genera occurs as the pH drops. To ensure optimal fermentation and to promote other characteristics, such as improvement of aerobic stability, silage can be inoculated with live microbial additives. The impact of bacterial inoculation on silage quality has been demonstrated in numerous research (Oliveira et al. 2017) but its influence on microbial diversity throughout the fermentation process deserves more attention (Muck 2013). The potential of microbial inoculants to modify the microbial community was observed using traditional microbiology techniques, and also recently using molecular techniques (Eikmeyer et al. 2013). A microbial diversity study using amplicon sequencing has been performed on corn and alfalfa silages to evaluate how a lactic acid bacteria inoculant influenced succession of the microbial communities.

**Material and Methods** Two separated ensiling experiments were performed using similar analytical process, one with corn (352 g dry matter (DM) $kg^{-1}$) and the second one with alfalfa/grass (86/14% botanical composition at 395 g DM $kg^{-1}$). Inoculation was performed using a combination of *Lactobacillus buchneri* NCIMB 40788 and *Lactobacillus hilgardii* CNCM-I-4785 and was compared to the forage without inoculation. The forages were ensiled under vacuum bag mini-silos using 300 g of material. Five repetitions were made for each treatment. Vacuum was applied with a commercial vacuum sealer (Weston 2300). Silage was incubated for 0, 1, (2 for corn), 4, 8, 16, 32 and 64 days. DNA was extracted using MoBio's Power Lyser Power Soil DNA isolation kit prior to amplicon sequencing of the 16S rDNA V4 region (bacteria) and ITS1 region (fungi) on an Illumina MiSeq sequencer. Diversity data were analyzed using edgeR package. Analysis of fermentation parameters was performed at Cumberland Valley Analytical Service (Maryland, USA). One-way ANOVA was used for comparison between treatments. All statistical analyses were performed on R (version 3.3.3).

**Results and Discussion** For corn and alfalfa silage, the fermentation profile followed the expected drop in the pH of the silage, starting within the first 24 hours of fermentation. In that initial 24 hours, the pH reached nearly 5.0 for corn and alfalfa samples. For all treatments, the lowest pH values were of 3.68 (16 days - control) for corn and 4.57 for alfalfa/grass (32 days - control). The concentration of organic acids followed the trends observed by the pH, with higher concentration as the pH drops. As for the bacteria diversity, the fresh forage Enterobacteriales were identifed as the most abundant group in the case of corn and Pseudomonadales for alfalfa/grass (Figure 1). After 24 hours of ensiling, Lactobacillales superseded over the initial population. At that point in the incubation period, corn and alfalfa/grass followed different succession pathways. In corn, *Lactobacillaceae* family was the main population. For alfalfa/grass, *Leuconostoccaceae* dominated the silage for most of the incubation period, with *Weissella* representing the dominant genus. Adding lactic acid bacteria inoculants increased the speed of the microbial succession toward a heterofermentative community. At the Order taxonomy level, the observed changes induced by inoculation were more important for the corn than for alfalfa/grass silage. Inoculated corn silage had a significantly ($P < 0.01$) smaller population of Enterobacteriales compared to the control after 64 days of fermentation. For alfalfa/grass, no difference was visible between the two treatments at the Order community profile. Differences were observed at the Family and Genus level following inoculation with *L. buchneri* 40788 + *L. hilgardii*

4785. The genus *Lactobacillus* was basically absent from non-inoculated samples but represented more than 50% of the bacteria community for the inoculated samples ($P < 0.01$), a trend that started at day 4. Inoculation in alfalfa/grass did contribute to lowering the proportion of other Orders present in the community by more than 12% of the relative abundance for alfalfa/grass ($P < 0.01$). Fungal population also changed throughout the fermentation periods, with yeast (Ascomycota) succeeding over Basiodiomycota in corn silage. Interestingly the combined inoculant contributed to the change in the fungal population by selecting for specific genera of yeast, while overall fungal related reads numbers were reduced. As an example, the yeast *Malassezia* was typical of non-inoculated alfalfa/grass. The results observed here also confirmed the observation of a similar study performed by Gharechahi et al. (2017).

**Conclusion** As previously observed by classic microbiology, microbial population changes are dynamic along the ensiling period. This amplicon based metagenomic diversity study with corn and alfalfa/grass allowed for detailed observation of the succession in bacterial and fungal population, and the impact of inoculation. Inoculation with a mix of two heterofermentative strains, *L. buchneri* 40788 and *L. hilgardii* 4785, positively modified composition of the Eubacteria community for both types of silages. Evolution of the microbial community is a dynamic process that changes along the incubation period used in these two fermentation trials.

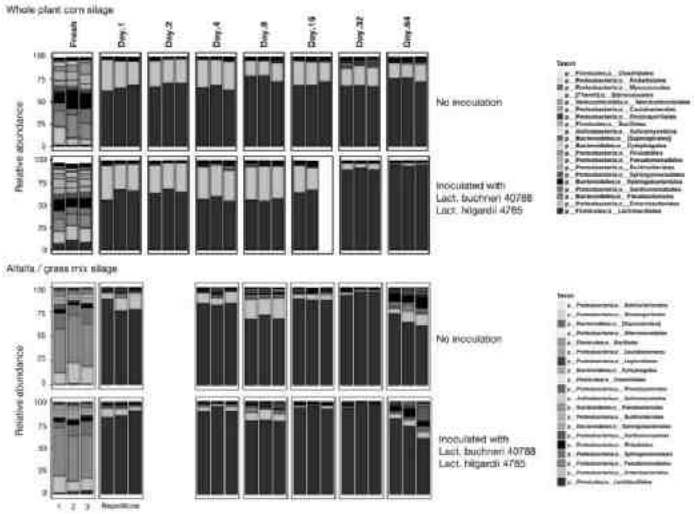


**Figure 1**. Community composition at the Order level following 16S (V4-region) operon metasequencing of whole plant corn silage and alfalfa/grass (86/14% composition) silage fermented for 1, 2 (only for corn), 4, 8, 16, 32, and 64 days. Inoculation with *L. buchneri* 40788 + *L. hilgardii* 4785 was compared to samples not inoculated.

## References

Eikmeyer F., Köfinger, P., Poschenel, A., Jünemann, S., Zakrzewski, M., Heinl, S., Mayrhuber, E., Grabherr, R., Pühler, A., Schwab, H. & Schlüter, A. (2013) Metagenome analyses reveal the influence of the inoculant *Lactobacillus buchneri* CD034 on the microbial community involved in grass ensiling. Journal of Biotechnology, 167, 334-343.

Gharechahi J., Kharazian, Z.A., Sarikhan, S., Jouzani, G.S., Aghdasi, M. & Salekdeh, G.H. (2017) The dynamics of the bacterial communities developed in maize silage. Microbial Biotechnology, 10, 1663-1676.

Muck R.E. (2013) Recent advances in silage microbiology. Agricultural and Food Science, 22, 3-15.

Oliveira A.S., Weinberg, Z.G., Ogunade, I.M., Cervantes, A.A.P., Arriola, K.G., Jiang, Y., Kim, D., Li, X., Gonçalves, M.C.M, Vyas, D. & Adesogan, A.T. (2017) Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. Journal of Dairy Science, 100, 4587-4603.

# Impact of application of foliar fungicide on ensiling properties, feed value and microbiome of barley silage

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**Keywords:** Barley silage, fungicide, microbiome

**Introduction** Scald, net blotch and spot blotch are the most common foliar diseases of barley in western Canada (Turkington et al. 2011). These foliar diseases result in destruction of photosynthetic leaf area, thereby reducing barley yield and quality. Application of foliar fungicide has been reported to improve the yield and quality of barley. However, the effect of foliar fungicide on the quality and microbiome of barley silage has not been evaluated. It is hypothesized that foliar fungicide application would alter nutrient and microbial compositions during ensiling and aerobic exposure of silage.

**Materials and Methods** The study was conducted at the Lacombe (Laco) and Lethbridge (Leth) Agriculture and Agri-Food Canada Research Centres in 2016. Six-row barley was seeded in replicate plots and sprayed with Twinline® fungicide (metconazole and pyraclostrobin) at the flag leaf emergence stage or not sprayed at both locations. Barley was harvested at mid-dough, chopped to a theoretical chop length of 9.5 mm and triplicate mini silos per treatment and day were prepared at each location and opened after 3, 7, 14, 21 and 60 d of ensiling for nutrient, microbial and molecular analyses. Triplicate silage samples from mini silos opened on d 60 of ensiling were used for evaluation of aerobic stability of the silage using Thermochron iButtons. Samples were collected on d 3, 7, 14 and 21 of aerobic exposure for microbial and molecular analyses. Microbial populations were estimated as colony forming units (cfu) and were log transformed prior to statistical analysis. Extracted DNA samples were sequenced and operational taxonomic units (OTUs) were analysed for taxonomic characterization of bacterial and fungal microbiomes. Alpha diversity index (Chao1, Shannon-Weiner index and rarefaction curve) and taxonomic classification were then computed using QIIME software (Duniere et al. 2017). The nutrient composition, ensiling parameters, aerobic stability and microbial data of barley silage were analysed using the mixed model procedure of SAS (version 9.4) for repeated measures with the effect of location × day and treatment × day included in the model. Differences were declared at $P < 0.05$.

**Results and Discussion** Silage pH averaged 4.2 ± 0.08 (Mean ± SD) after 60 d of ensiling across treatments and locations. The pH of fresh forage was greater (7.01 vs 6.53; $P = 0.005$) while that of silage on d 3 of ensiling was lower (4.58 vs 5.06; $P = 0.005$) at Laco relative to Leth. Moreover, pH of fungicide sprayed forage (6.56 vs 6.98; $P = 0.024$) and silage on d 3 (4.53 vs 5.10; $P < 0.001$) was lower relative to untreated barley. Silage pH decreased ($P < 0.001$) with the duration of ensiling while both pH ($P \leq 0.01$) and temperature increased after d 7 of aerobic exposure. Lactic acid concentration of fungicide sprayed silage on d 7 (50.8 vs 34.2 mg/g DM; $P = 0.017$) and d 14 (55.9 vs 39.5 mg/g DM; $P = 0.020$) of ensiling was greater than that of untreated silage. The decrease in silage pH during ensiling correlated to the concurrent increase ($P < 0.001$) in concentration of lactic acid. Moreover, concentration of lactate, acetate, total VFA, Lactate:Acetate and ammonia increased ($P \leq 0.022$) with days of ensiling. The concentration of lactic acid decreased ($P < 0.001$) over the period of aerobic exposure.

The DM content of silage at Laco was higher (39.0 vs 36.5%; $P = 0.002$) than that at Leth while silage produced from fungicide sprayed barley forage was lower (36.7 vs 38.7%; $P = 0.010$) than untreated forage. The CP content of silage averaged 14.0 ± 1.27% (Mean ± SD) during ensiling. Silage produced from fungicide sprayed barley forage had lower NDF content (36.8 vs 41.3%; % DM basis; $P = 0.014$), while both ADF ($P = 0.046$) and NDF ($P < 0.01$) decreased during ensiling. Conversely, the

starch content of barley silage increased (P < 0.001) with days of ensiling. Water soluble carbohydrate (WSC) content of treatments increased (P < 0.001) with days of ensiling while it decreased after d 7 of aerobic exposure. Moreover, WSC content was lower (4.0 vs 24.4 mg/g DM; P = 0.003) at Laco relative to Leth on d 3 of ensiling. The aerobic stability averaged 252 ± 92 h (Mean ± SD) across treatments. The number ($log_{10}$ cfu/g DM) of lactic acid bacteria increased (P < 0.001) with duration of ensiling peaking 3 d post-ensiling and declining thereafter. Moreover LAB count was greater (6.05 vs 4.92 $log_{10}$ cfu/g DM; P < 0.001) in fresh barley forage at Laco relative to Leth. Number of yeasts and moulds decreased (P < 0.001) with days of ensiling. There was greater yeast concentration (6.59 vs 3.49 $log_{10}$ cfu/g DM; P < 0.001) on d 3 and lower yeast concentration (0.37 vs 2.43 cfu/g DM; P = 0.019) on d 7 of ensiling in Leth relative to Laco. Number of moulds were lower (P = 0.01) for fungicide sprayed relative to untreated silage on d 7 of ensiling (Figure 1a). Total number of LAB, yeasts and moulds increased (P ≤ 0.03) upon aerobic exposure (Figure 1b). Bacterial diversity (Chao1, OTUs and Shannon's diversity index) increased (P ≤ 0.001), while fungal diversity decreased (P < 0.001) during ensiling. The bacterial and fungal communities differed between locations and during the ensiling and aerobic exposure periods as determined by Principal Coordinate Analysis. During ensiling, fungicide sprayed barley contained greater abundance of *Xanthomonas, Bacillus* and fungi of the genus *Wickerhamomyces* and lower numbers of unclassified *Leuconostocaceae* relative to untreated barley (Figure 2a, 2b).

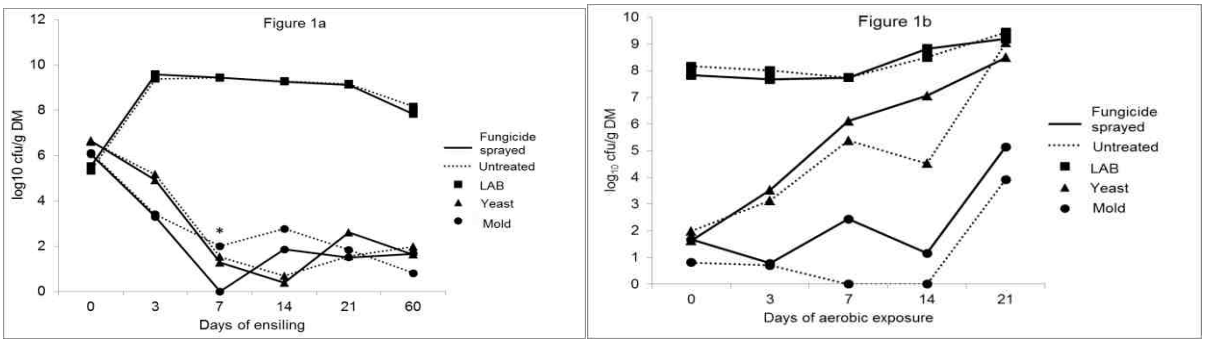


**Figure 1.** Effect of duration of ensiling (a) and aerobic exposure (b) on lactic acid bacteria (LAB), yeast and mould counts. * indicate mould count of fungicide treated silage lower than untreated (P = 0.01)

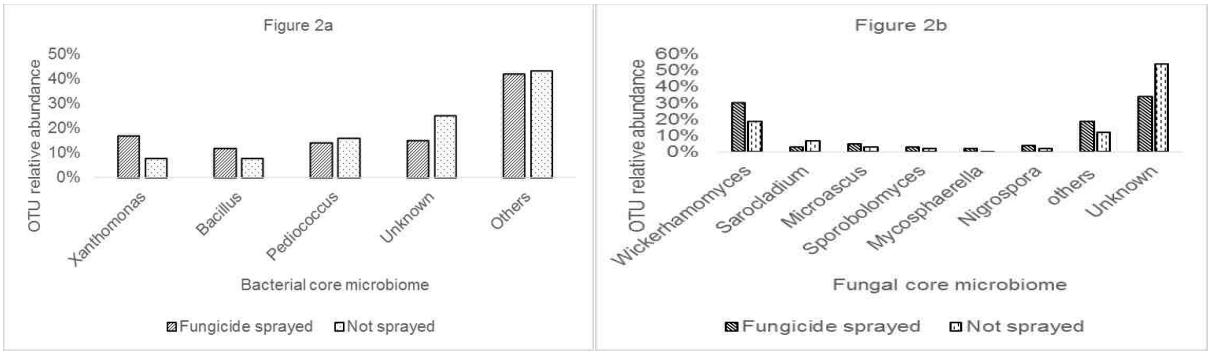


**Figure 2.** Effect of fungicide application on relative abundance of bacterial (a) and fungal core microbiome during ensiling. OTUs were assigned at genus level.

**Conclusion** Results suggest that the location and fungicide application affected LAB, yeast and mould counts during ensiling. Fungicide application can effectively reduce the number of moulds during ensiling but did not influence aerobic stability of barley silage.

**References**

Duniere, L., Xu, S., Long, J., Elekwachi, C., Wang, Y., Turkington, K., Forster, R. & McAllister, T.A. (2017) Bacterial and fungal core microbiomes associated with small grain silages during ensiling and aerobic spoilage. BMC Microbiology, 17, 50, 1-16.

Turkington, T.K., Tekauz, A., Xi, K. & Kutcher, H.R. (2011) Foliar diseases of barley: Don't rely on a single strategy from the disease management toolbox. Prairie Soils and Crops Journal, 4, 142-150.

# Microbial ecology, fermentation, and aerobic stability of conventional and BMR corn hybrids ensiled at high moisture with or without a homo and hetero-fermentative inoculant

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**Keywords:** high-moisture silage, inoculant, next generation sequencing

**Introduction** Ensiling at dry matter (DM) concentrations lower than recommended for corn (< 32% DM) can result in seepage of nutrients and undesirable clostridial fermentation. We evaluated the effects of adding a combination inoculant to four corn (*Zea mays* L.) hybrids harvested at high moisture on the nutritive value, fermentation profile, aerobic stability, bacterial and fungal populations, and community structure.

**Materials and Methods** The treatment design was a factorial combination of four corn hybrids (HYB) ensiled with (INO) and without (CON) inoculant. HYB were TMF2R737 (MCN), F2F817 (MBR), P2089YHR (PCN), and PI144XR (PBR), ensiled at 30.5, 26.3, 31.1, and 31.5% DM, respectively. MBR and PBR were brown midrib mutants. The four corn hybrids were planted in a complete randomized design with plots replicated six times. Two replicated piles were obtained from each corn plot (total of 48 piles). The inoculant contained *Lactobacillus buchneri* and *Pediococcus pentosaceus* ($4 \times 10^5$ and $1 \times 10^5$ cfu/g of fresh corn). Corn was chopped, treated or not with INO, packed into 7.6 L bucket silos, and stored at 23°C (± 1 °C) for 100 d. Sonication was used for microbial extraction (Gutierrez-Rodriguez et al. 2012) and the 16S rRNA V4 (bacteria) and ITS-1 (fungi) regions were amplified. The libraries were sequenced on a Illumina MiSeq platform and analysed with QIIME 1.9.1 using the GreenGenes 13.8 and UNITE fungal ITS reference database. We used the GLM procedure of SAS v.9.4 (SAS Institute 2003) for data analysis. When a two-factor interaction effect was present, we used the SLICE option to analyse the simple effects. Mean separation was based on the PDIFF procedure of LSMEANS.

**Results and Discussion** At d 0 there were differences due to hybrids solely ($P \leq 0.05$). The lactic acid bacteria count (LAB; log cfu/g of fresh corn) was similar for PCN and PBR (8.4) and both were greater than MCN and MBR (7.7±0.23); yeast count was greatest in PCN (6.8 vs. 6.0±0.14), and mold count was lowest in PBR vs. the others (4.6 vs. 5.2±0.15). Bacterial relative abundance (RA, %) of *Enterobacteriaceae* was lower in PBR vs. the other hybrids (~51.3 vs 58.4, respectively; $P < 0.04$). For fungi, MCN and PCN had a higher RA vs. MBR and PBR for *Tremellales* (~25.8 vs. ~13.9 ± 3.91) and lower RA for *Mucoraceae* (~3.64 vs. ~7.51 ± 1.18; $P < 0.04$). PBR had a higher bacterial and fungal operational taxonomic unit (OTU) richness vs. other HYB (640 vs. ~554 ± 21.3 and 82 vs. ~73 ± 1.3, respectively; $P < 0.05$). At opening (d 100), INO had higher ($P \leq 0.05$) LAB (9.3 vs. 7.1 ± 0.29 log cfu/g of fresh corn) and acetic acid (3.44 vs. 1.32 ± 0.35% of DM) and lower yeast (3.1 vs. 4.6 ± 0.45) and molds (1.5 vs. 3.0±0.61 log cfu/g of fresh corn), and also extended the aerobic stability (582 vs. 111±128 h) vs. CON. However, INO had lower ($P \leq 0.05$) DM recovery (95.6 vs. 97.4±1.05%) and lactic acid for all HYB (4.2 vs. 7.6) except MCN (4.9±0.59% of DM; INO × HYB, $P < 0.01$), vs. CON. The increased aerobic stability and decreased DM recovery in INO can be explained by the extensive

acetic acid production that decreased the counts of both yeast and molds (Kleinschmit and Kung, 2006). The INO reduced bacterial (66 vs. 226 ± 10.7) and increased fungal (46 vs. 20 ± 2.95) observed OTUs vs. CON ($P < 0.01$). These results suggest that INO reduces the bacterial and increases fungal diversity consistently across all ensiled hybrids tested in this study. INO had higher RA vs. CON for *Lactobacillaceae* (99.2 vs. 75.7 ± 3.08%) and lower RA for *Enterobacteriaceae* (0.28 vs. 9.93 ± 1.32). INO had a lower RA of *Leuconostocaceae* vs. CON only for PBR (0.21 vs. 14.5) but not for other HYB (~0.21 vs. ~2.70±1.87; HYB × INO, $P < 0.05$). Collectively for the major bacterial families at d 100, RA were 86.6 and 0.4% of genus *Lactobacillus* and *Pediococcus* for *Lactobacillaceae*; 1.7 and 1.1% of genus unidentified and *Leuconostoc* for *Leuconostocaceae*; and 3.7 and 0.4% of genus unidentified and *Cronobacter* for *Enterobacteriaceae*. The overwhelming dominance of *Lactobacillaceae* and the reduction of *Enterobacteriaceae* observed in this study shows that INO has the potential to keep silos hygienic besides the other effects reported on forage quality and conservation. For fungi, INO did not affect *Saccharomycetaceae* (~40.8%) but a lower RA vs. CON for *Monascaceae* (12.6 vs. 44.7 ± 7.30) and increased incertae sedis *Tremellales* (8.0 vs. 1.2 ± 2.31) and incertae sedis *Saccharomycetales* (6.4 vs. 0.3 ± 1.7). Collectively for the major fungal families at d 100, RA were 42.4% of genus *Kazachstania* for *Saccharomycetaceae*; 30.8% of genus *Monascus* for *Monascaceae*; 6.6% of genus Hannaella for incertae sedis *Tremellales*; and 5.9% of genus *Candida* for incertae sedis *Saccharomycetales*. At d 0 vs. 100, we observed a clear separation and difference in the distribution and structure of the bacterial and fungal community using the weighted UniFrac and Bray-Curtis principal coordinates analysis plot, respectively. However, between CON and INO differences were only observed for bacteria at d 100.

**Conclusions** INO used consistently improved aerobic stability across HYB by increasing acetic acid and reducing fungal counts but had lower DM recovery of the corn ensiled at high moisture. This can be partially explained by the *Lactobacillaceae* dominance in INO silages, compared to a more diverse CON, and reduced *Monascaceae*, which increased fungal diversity relative to CON.

**References**

Gutierrez-Rodriguez, E., Gundersen, A., Sbodio, A.O. & Suslow, T.V. (2012) Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of *Escherichia coli* on spinach. Journal of Applied Microbiology, 112, 109-118.

Kleinschmit, D.H. & Kung Jr., L. (2006) A Meta-Analysis of the Effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science, 89, 4005-4013.

# Prediction curve for production of silage effluent based on raw materials dry matter content

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**Keywords:** dry matter, effluent, prediction

**Introduction** Over the last decades, political and social pressure to reduce the pollution arising from agro-industrial activities has led to the food processing industry to modify their production processes, so that their residues can be reused. Several parts of plants that are not exploited during the process of industrial transformation, such as vegetable and fruit residues from commercial processing, have turned these wastes into by-products and become available for animal feeding in areas such as south-eastern Spain with low forage supply (Márquez et al. 2010). However, vegetables and fruits usually have a high amount of water, which causes them to have a rapid degradation and difficult conservation. The ensilage can be an adequate method but the environmental pollution capacity of the effluent and its environmental impact must be considered to prevent the release of effluents, with very high: chemical oxygen demand (COD), biochemical oxygen demand ($BOD_5$), pH, conductivity (C) and total suspended solids (TSS). Several authors have used different models to find the best prediction equation, for crops with low or medium content of dry matter (DM) but no much information has been found about how is produced the flow of the effluents in high moisture content silages. The objective of this study is to know the amount of effluents released in the silage of different vegetable by-products with low dry matter content.

**Material and Methods** Seven common vegetable by-products in the industries producing vegetables (aubergine, lettuce, tomato, zucchini, persimmon, broccoli, and artichoke) were used to find the best equation to predict the effluent production after the silage process. Three replicates of each one was chopped and packed into plastic bags, adjusting the weight to 150 or 250 g, using a vacuum heat-sealer. The by-products were stored for 28 days at 9 °C temperature. The effluent production was measured and the DM content determined. To find the best prediction equation for effluent production based on the DM content of the by-products, the Curve Expert 1.4 program, with 90 fit models incorporated, was used (Hyams 2005).

**Results and discussion** The DM content and the effluent produced of the by-products are shown in Table 1.

**Table 1.** DM content and effluent production of different vegetable by-product silages

| By- product | Dry matter (DM) g/kg | Effluent production mL/kg DM |
|---|---|---|
| Artichoke | 297 | 0 |
| Persimmon | 183.63 | 35.04 |
| Broccoli | 103.8 | 44.8 |
| Aubergine | 76.11 | 75.3 |
| Zucchini | 69.50 | 95.2 |
| Lettuce | 61.83 | 106.09 |
| Tomato | 25.58 | 146.71 |

The date ranged from 24.6 to 297 g/kg of tomato and artichoke by-products, respectively. The best equation found was the quadratic model $Y= 2.05 -1.23x + 1.82 x^2$, with $r^2 = 0.973$ and SE = 16.9

(Figure 1) where can be seen that small increases on moisture content can produce very important elevations on effluent production. The second-best fit found was for the reciprocal equation, Harris model: $Y = 1 /3.082 + 1.314 X^{1,005}$, with $r^2 = 0.953$ and SE = 18.4. For Megías et al. (2014) this model was the best found with less types of by-products, but the $r^2$ was higher.

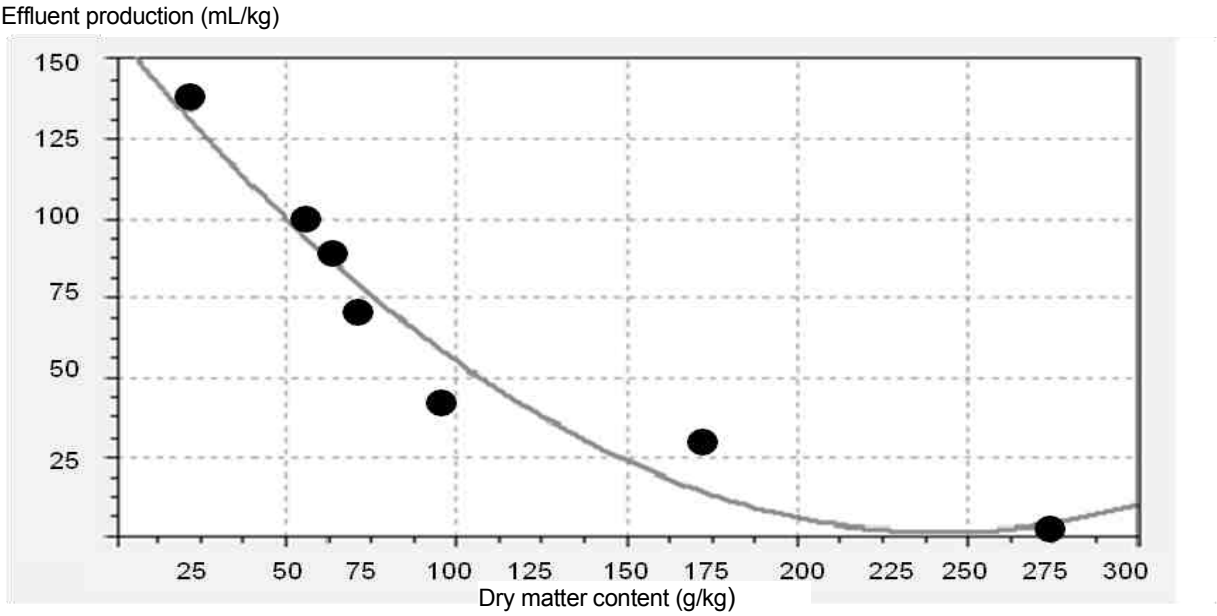


**Figure 1.** Prediction curve of production of silage effluent based on DM content of different vegetable by-products.

**Conclusion** This study showed that changed in the moisture content of seven by-products can produce very important variations of the effluent production. More studies are necessary to know what other parameters, in addition to DM, affect to the production of effluents during silage to decrease the release of them.

**References**

Hyans, D.G. (2005) *Curve Expert version 1.4, for Windows*. Copyright 2005 all right reserved. http://www.curveexpert.net

Márquez, M.A., Diánez F. & Camacho, F. (2010) The use of vegetable subproducts from greenhouses (VSG) for animal feed in the Poniente region of Almería. Renew. Agric. Food Syst. 26, 4-12.

Megías, M.D., Meneses, M., Madrid, J., Hernández, F., Martínez-Teruel A. & Cano, J.A. (2014) Nutritive, fermentative and environmental characteristics of silage of two industrial broccoli (*Brassica oleracea italica*) by-products for ruminant feed. International Journal of Agriculture and Biolology 6, 307-31.

# Relations between silage composition, its metabolome and preference shown by goats

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**Keywords:** dry matter intake, metabolome, preference, small ruminant

**Introduction** The traditional way to analyse silage composition includes the identification and classification of its components in categories such as protein, fat, fibre fractions, vitamins and minerals (VDLUFA 2012). However, first attempts have also been made to establish metabolomics in feed analysis (Johnson et al. 2004). Metabolomics deals with the characterisation of all molecules and chemical components present in biological matrices based on a high methodological turnover (Wishart 2008). By means of implementing this approach into serial analyses allows deeper insights in the complex network and interactions of bioactive molecules in silage. Because the relationships between feed preferences of ruminants and compounds that may affect silage dry matter (DM) intake (DMI) are not yet fully revealed, metabolomics may provide new findings when combined with conventional chemical analyses and preference trials.

**Materials and Methods** Six silage treatments each of first-cut lucerne (*Medicago sativa* L.) and red clover (*Trifolium pratense*) at different DM concentrations (230-384 g/kg), tailored silage additives and intended addition of soil were produced to obtain a range of fermentation qualities. After 120 days of ensiling, silages were sampled for chemical analyses (proximate constituents, fermentation products and other volatile compounds, crude protein fractions), vacuum-packed and refrigerated for a subsequent preference trial with goats. For each plant species a separate run was conducted. During the experimental phase, each possible 2-way combination of the six silages and a standard lucerne hay (n = 21 combinations) was offered for 3-h *ad libitum* intake to Saanen-type wethers (n = 8; mean (SD) body weight 104 kg (± 3.2) and 105 kg (± 2.2), respectively). All data were analysed using SAS 9.4 (SAS®, 2010). Each trial was tested by analysis of variance after averaging DMI of each forage (averaged across each combination, n = 6). The analysis of variance included terms for animal and forage. Within the forage treatments means were separated using the minimum significant difference (MSD) from the Waller-Duncan *k*-ratio *t*-test (*k* = 100) (Burns et al. 2001). The most and least preferred silages from both plant materials were used for metabolome analyses. Non-targeted metabolite profiling comprised analyses by GC-MS (gas chromatography-mass spectrometry) and LC-QTOF/MS (liquid chromatography-quadrupole-time of flight). Using these methodologies metabolites can be analysed in the range of 50-1,700 Da with an accuracy up to 1-2 ppm and a resolution of mass/Δmass = 40,000. The identified metabolites were classified into the chemical taxonomy including super class, class, subclass and direct parent of each metabolite. The classification was conducted with the Human Metabolome Database (HMDB) (Version 3.6).

**Results** Although treatments only produced few differences in fermentation acids and crude protein fractions, preference behaviour of goats was strongly divergent. The most preferred and avoided treatments of lucerne and red clover silages amounted to 860 and 858 g DMI/3 h versus 226 and 283 g DMI/3 h, respectively. Fermentation acid analysis showed the greatest differences in acetic acid (9-62 g/kg DM). Protein fractions showed only small differences within one plant species, but the degree of proteolysis was overall lower in the red clover silages. Metabolome analyses revealed more than 6,400 metabolites in those silages being most different in preference; 2,010 of those were annotated applying the HMDB. The comparison of the most and least preferred silages showed great differences in the silage metabolome. For an initial overview of the analysed samples a principal component (PC) analysis (PCA) was conducted: PC1 separated the different plant species, explaining 40.7% of the overall variance in the data set; PC2 separated the silage treatments explaining 22.9% of the overall variance. Silages with the greatest differences in DMI differed in 29% (lucerne) and 15% (red clover) of their metabolites. Between preferred and avoided silages 1,860 metabolites differed in the lucerne and 934 metabolites in the red clover silages; 475 of those were altered in both plant species; 67 out of the 475 metabolites were more concentrated in the avoided compared with the preferred silages and 95 were more concentrated in the preferred silages. The 67 metabolites were composed of amino acids (10%), oligopeptides (50%), lipids (fatty acyls, glycerolipids and phosphoglycerolipids; 19%), indolacetaldehyde, glucosinolate, styrene, methylfurane,

xanthine, diadenosine tetraphosphate, dialkyldisulfides, phenylpropanoic acid, L-carnitine, hydrocinnamic acid, ethyl 1-(ethylthio)ethyl disulphide, sphingosine, D-threitol and erythritol.

**Discussion** Despite using the same initial plant materials within the runs of the preference trial there were significant differences in preference, suggesting a major influence of the ensiling conditions on silage composition and preference by ruminants. However, no explanation for the differences in preference could be carved out based on proximate constituents and fermentation acid analysis. Since the DMI rankings of the lucerne and red clover silages and thus, the DMI of the most extreme silages (i.e., lowest and highest DMI) were very similar, this offered the possibility to directly compare the metabolites of those silages. The 67 metabolites being more concentrated in the avoided compared to the preferred silages may have had a negative impact on feed intake behaviour. Carnitine as a quarternary ammonium salt for example acts as a taste modifying molecule accounting for a bitter taste (Behrens & Meyerhof 2015). Hydrocinnamic acid has a sweet, floral scent at room temperature (HMDB 2018) and might have contributed to the flavour of the avoided silages in an olfactory way. Precursors such as oligopeptides and amino acids contribute to the flavour of foods by chemical reactions that occur during food processing (Regulation (EC) No 1334/2008 of the European Parliament and the Council 2008). Similar processes may occur during ensiling. A difference of 29% and 15%, respectively, in the entirety of detected metabolites in the lucerne and red clover silages shows clearly that conventional analyses are not sufficient to characterise silages profoundly and to establish reasons for differences in preference behaviour. A certain share of the metabolites may have contributed to the postingestive feedback and to the sensory characteristics of the silages. Volatile compounds could have had the greatest impact on preference and avoidance of silage since they are the most flavour-relevant components in plants and thus in silage. Amongst others, they are composed of monoterpenes, sesquiterpenes, alcohols (mono- and sesquiterpene alcohols), ketones, phenols, aldehydes, coumarins, esters and oxides (Parker 2015), which were all verified in the samples.

**Conclusions** It was shown that conventional analyses are not always sufficient to provide profound explanations for differences in preference between silage treatments, since preference was scarcely related to proximate constituents and fermentation characteristics. Metabolome analyses proofed to be a good foundation for the approach of examining which variables might play a role in preferring and avoiding ensiled forages. Using metabolomics data as a basis to deduce intake related compounds provides a promising track which may lead to progress in silage research.

**References**

Behrens, M., & Meyerhof, W. (2015) Flavour development, analysis and perception in food and beverages. 1st ed. Cambridge: Woodhead Publishing. pp. 297-329.

Burns, J.C., Fisher, D.S., & Mayland, H.F. (2001) Preference by sheep and goats among hay of eight tall fescue cultivars. Journal of Animal Science, 79, 213-224.

HMDB (2018) The human metabolome database. http://www.hmdb.ca/metabolites/HMDB0000764 (accessed 12 March 2018).

Johnson, H.E., Broadhurst, D., Kell, D.B., Theodorou, M.K., Merry, R.J., & Griffith, G.W. (2004) High-throughput metabolic fingerprinting of legume silage fermentations via Fourier transform infrared spectroscopy and chemometrics. Applied Environmental Microbiology, 70, 15831592.

Parker, J.K. (2015) Flavour development, analysis and perception in food and beverages. 1st ed. Cambridge: Woodhead Publishing. pp. 3-30.

Regulation (EC) No 1334/2008 of the European parliament and the Council (2008). http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:354:0034:0050:de:PDF (accessed 12 March 2018).

VDLUFA (2012) VDLUFA-Methodenbuch, Bd. III. Die Chemische Untersuchung von Futtermitteln. 8. Erg. 2012. VDLUFA-Verlag, Darmstadt, Germany (in German).

Wishart, D. (2008) Metabolomics: applications to food science and nutrition research. Trends in Food Science and Technology, 19, 482-493.

# The effect of bacterial inoculant and packing density on corn silage quality and safety

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**Keywords:** Aerobic stability, Lactobacillus buchneri, mycotoxin, silage inoculant, spoilage

**Introduction** The use of inoculants containing homo- or hetero-fermentative lactic acid bacteria (LAB) has been studied largely to improve fermentation and reduce nutrient losses in corn silage (Ranjit and Kung 2000). Yet, farmers very often question the cost effectiveness of using silage inoculants, since corn is considered to be an 'easy-to-ensile' crop. Variation on the effectiveness of silage inoculants can be caused by poor silage management, especially when proper density is not achieved. Gas-filled porosity within the silage mass can facilitate the growth of spoilage microorganisms causing alteration of quality and hygiene due to undesirable fermentation or mycotoxin production. The objective of this research was to assess the effect of the combination of *Lactobacillus buchneri* LB1819 (DSM 22501/1k20738) and *Lactococcus lactis* O-224 (DSM11037/1k2081) on aerobic stability, chemical composition and mycotoxin level of corn silage fermented for 32 days.

**Material and Methods** Triplicate laboratory silos (5 litres) were filled with chopped corn forage (36.6 % DM) simulating 2 densities: low (132 ± 6 kg DM $m^3$) or high (186 ± 6 kg DM $m^3$). Forage was treated with water (Control - CRT), or a combination of *Lactobacillus buchneri* LB 1819 and *Lactococcus lactis* LL O224 (SiloSolve® FC - SS-FC, Chr. Hansen, Denmark). The inoculant was applied at a rate of 250,000 cfu/g of forage. After 32 days of fermentation mini-silos were opened and samples collected to evaluate fermentation parameters, DM losses due to ensiling, biogenic amine and aerobic stability. Mycotoxins produced by *Fusarium spp*., *Penicillum roqueforti* and *Aspergillus fumigatus* were extracted and determined as described previously by Gallo et al. (2016). Data were analysed as a completely randomized design with a 2x2 factorial arrangement of treatments using the GLM procedure of SAS.

**Results** Density did not have an effect on nutritive value or presence of mycotoxins (P>0.05), except mycophenolic acid which tended to be slightly higher in low than high density mini-silos (13 vs. 0.01 µg/kg DM, respectively; P<0.06. Both control and inoculated silages attained pH below 4.0, which is considered to be a threshold for good fermentation (3.44 vs. 3.47; P=0.04). Chemical composition did not differ among treatments, and average values were 32.3% DM for starch, 6.9% DM for CP, 1.3% DM for EE, 42.6% DM for NDF, 20.2% DM for ADF and 4.1% ash. Aerobic stability was improved by the use of SS-FC and this effect was more evident in high than low density mini-silos (TRT x Den interaction, P=0.05). In freshly chopped whole-plants mycotoxins were detected: aflatoxin B1 (0.6 µg/kg DM), fumonisins B1 (4845 µg/kg DM), and B2 (2613 µg/kg DM). On average, the levels of these mycotoxins were numerically reduced during ensiling without differences among treatments. Other mycotoxins produced by *Penicillium* spp. were not detected in fresh material, whereas they were detected in the silage after fermentation with a higher level of roquefortine C in CTR than SS-FC silos (20 vs. 0.1 µg/kg DM; P<0.05).

**Table 1**. Effects of treatment (CTR vs. SS-FC) or density (Low vs. High) on fermentative characteristics of corn silages after 32 days of ensiling

| Items | CTR | | SS-FC | | s.e.m. | P-values | | |
|---|---|---|---|---|---|---|---|---|
| | Low | High | Low | High | | TRT | Den | TRT x Den |
| DM (% as fed) | 34.6 | 34.9 | 34.5 | 34.7 | 0.22 | 0.537 | 0.386 | 0.958 |
| DM loss (% DM) | 7.1 | 4.5 | 7.4 | 6.7 | 0.85 | 0.189 | 0.089 | 0.287 |
| Aerobic stability (AS, hours) | 334 | 557 | 331 | 665 | 24.0 | 0.065 | <0.01 | 0.050 |
| DM loss after AS (% DM) | 17.8 | 22.3 | 23.4 | 21.2 | 1.13 | 0.078 | 0.340 | 0.018 |
| pH | 3.46 | 3.43 | 3.50 | 3.45 | 0.012 | 0.041 | 0.012 | 0.442 |
| Lactate (% DM) | 4.72 | 5.10 | 6.22 | 6.25 | 0.691 | 0.090 | 0.793 | 0.805 |
| Acetate (% DM) | 1.34 | 1.01 | 1.46 | 1.19 | 0.043 | <0.01 | <0.01 | 0.486 |
| Ethanol (% DM) | 0.80 | 0.58 | 0.47 | 0.44 | 0.086 | 0.028 | 0.194 | 0.295 |
| Ammonia nitrogen (% DM) | 3.60 | 3.60 | 3.35 | 3.97 | 0.135 | 0.656 | 0.049 | 0.051 |
| Biogenic amine (mg/kg as fed) | 216 | 194 | 241 | 216 | 4.4 | <0.01 | <0.01 | 0.773 |
| Yeast ($log_{10}$ cfu/g) | 5.3 | 4.7 | 5.7 | 4.2 | 0.38 | 0.981 | 0.019 | 0.301 |
| Mould ($log_{10}$ cfu/g) | 1.0 | 1.0 | 2.2 | 1.8 | 0.65 | 0.161 | 0.728 | 0.728 |
| Aflatoxin B1 (µg/kg DM) | 0.5 | 0.6 | 0.3 | 0.2 | 0.25 | 0.346 | 0.970 | 0.635 |
| Fumonisin B1 (µg/kg DM) | 4107 | 3882 | 3678 | 3249 | 442.3 | 0.264 | 0.480 | 0.824 |
| Fumonisin B2 (µg/kg DM) | 466 | 285 | 311 | 216 | 65.1 | 0.123 | 0.067 | 0.529 |
| Mycophenolic acid (µg/kg DM) | 22 | 0.1* | 4 | 0.1* | 5.8 | 0.167 | 0.057 | 0.167 |
| Roquefortine C (µg/kg DM) | 20 | 20 | 0.1* | 0.1* | 5.3 | <0.01 | 0.988 | 0.988 |

*These values are considered equal to limit of detection of HPLC–MS/MS method.

**Discussion** Despite some differences measured among treatments, the fermentation parameters reflected well fermented silage with or without the use of inoculants. However, higher levels of both lactate (6.22 vs. 4.90% DM, P=0.09) and acetate (1.33 vs. 1.17% DM, P<0.05) as well as lower level of ethanol (0.45 vs. 0.69% DM, P<0.05) were measured in SS-FC with respect to CTR. The presence of *L. buchneri* LB 1819 in the SS-FC resulted in an increased concentration of acetate which explains the tendency for higher aerobic stability in the inoculated silages (Kleinschmit and Kung 2006), at a higher density (TRT x Den interaction, P=0.050). Despite the similar number of yeast and mould between treatments, the concentrations of mycotoxins produced by *Penicillium* spp. were lower for SS-FC when compared to CTR. The concern about the presence of mould in silage is due to the potential risk of mycotoxin production by some of those fungi (e.g. *Aspergillus*, *Fusarium*, and *Penicillium* spp.; Gallo et al. 2015). Recently, Ma et al. (2017) showed how some of the most common silage inoculant bacteria are able to bind aflatoxin B1, with some degree of activity. Additionally, the inoculated silage showed lower incidences of other mycotoxins related to the presence of *Penicillium* spp. thus suggesting that the use of commercial inoculant may impact the growth of different strains of mould depending on the bacteria used as inoculant.

**Conclusion** This study shows the potential of SiloSolve® FC to improve aerobic stability and to reduce the presence of different mycotoxins detected on fresh material (*Aspergillus*-related or *Fusarium*-related mycotoxins) or produced during ensiling (*Penicillium*-related mycotoxins). More studies are necessary to understand the mode of action on how the inoculants influence silage mycotoxin contamination during ensiling.

**References**

Gallo, A., Giuberti, G., Frisvad, J. Bertuzzi, T. & Nielsen, K (2015) Review on Mycotoxin Issues in Ruminants: Occurrence in Forages, Effects of Mycotoxin Ingestion on Health Status and Animal Performance and Practical Strategies to Counteract Their Negative Effects. Toxins (Basel). 7, 3057-3111.

Gallo, A., Bertuzzi, T., Giuberti, G., Moschini, M., Bruschi, S., Cerioli, C. & Maseoro, F. (2016) New assessment based on the use of principal factor analysis to investigate corn silage quality from nutritional traits, fermentation end products and mycotoxins. Journal of the Science of Food and Agriculture 96(2), 437-448.

Kleinschmit, D. H. & Kung, L. (2006) A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of dairy Science, 89(10), 4005-4013.

Ma, Z.X., Amaro, F.X., Romero, J.J., Pereira, O.G., Jeong, K.C. & Adesogan, A.T. (2017) The capacity of silage inoculant bacteria to bind aflatoxin B1 in vitro and in artificially contaminated corn silage. Journal of dairy science, 100(9), 7198-7210.

Ranjit, N.K. & Kung, L. (2000) The Effect of Lactobacillus buchneri, Lactobacillus plantarum, or a Chemical Preservative on the Fermentation and Aerobic Stability of Corn Silage. Journal of Dairy Science, 83(3), 526-535.

# Different models of laboratory mini-silos for the study of the fermentation of Lucerne silage

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**Keywords:** fermentation, laboratory mini-silos, *Lucerne*, silage, vacuum pack

**Introduction** It is difficult to control variables in farm-sized silos. In order to study and understand silage fermentation, there is a need for research using laboratory mini-silos that allow for controlling variables and the assessment of different experimental treatments through replication. Alternatives that are cost effective, require a small sample size and reduce labor are desirable. Most laboratory silos require manual packing. However, it may be possible to use vacuum sealed glass jars as mini-silos as experimental mini-silos. Vacuum packing may allow higher throughput during mini-silo packing and may improve the consistency of packing density. The objective of the study was to evaluate the nutritive, fermentation and aerobic stability (AS) characteristics of *Lucerne* (*Medicago sativa*) silage inoculated or not when ensiled in three different types of laboratory mini-silos for 91 d.

**Materials and Methods** In order to ensile *Lucerne*, three types of laboratory mini-silos were used: A) 3L PVC, B) 1L PVC and C) 1 L glass jars (1L GJ). The PVC mini-silos had one-way mechanics to vent gas while the glass jars were vacuumed sealed. Eight mini-silos of each type (for a total of 24 mini-silos) were filled with wilted *Lucerne* that was inoculated with a homolactic bacterial inoculant (*L. plantarum, E. faecium, L. lactis, P. acidilactici, P.pentosaceus*; HBI, 4 mini-silos) or not (4 mini-silos). The HBI (MikropHerm, Madison, WI) was applied at a rate of $1x10^5$ colony-forming units/g of forage. Pre-ensiled forage and silage samples were analyzed by wet chemistry at Dairyland Laboratories, Inc. (Arcadia, WI). Additionally, fermentation characteristics were analyzed by wet chemistry at the commercial laboratory cited. The silage DM recovered (DMR) at silo opening divided by the DM (dry matter) mass ensiled, multiplied by 100 was used to calculate % DMR. The AS was determined at 6 h intervals over a 174 h period. Nutrient content of pre-ensiled wilted forage and resulting 6 silages were analyzed using the GLM procedure of SAS (SAS Institute, 2004) in a completely randomized design (CRD) with four replicates per analyzed material. Fermentation data from the 6 silages were analyzed using the GLM procedure of SAS (SAS Institute, 2004) in a CRD with four replicates. Data for AS were analyzed using the GLM of SAS (SAS Institute, 2004) as a split plot design using mini-silo as the repetitive measure replicated four times with a factorial arrangement of treatments: 4 treatments x 30 time points when temperature was recorded. Tukey's Test was used for mean separation.

**Results and discussion** Ensiling did not change the DM content varying from 504 g/kg as is for the wilted forage to an average of 487 g/kg as is for the ensiled *Lucerne* (Table 1). Ensiling *Lucerne* increased acid detergent fibre (ADF) from 352 g/kg DM for the wilted forage to an average of 373 g/kg DM for the silages, with the exception for ADF content of the silage from the 3L PVC NI treatment, which did not differ ($P>0.05$) to that of the wilted forage. The highest ADF content was for the silage ensiled in the 1 L GJ NI (385 g/kg DM) which was significantly higher ($P<0.05$) compared with the wilted forage (351 g/kg DM) or the silage from the 3 L PVC regardless of inoculation or not (367 and 363 g/kg/DM) or the 1 L PVC with HBI (368 g/kg DM). This suggests that fermentation could have been influenced by the size or the packing achieved in the 1 L vs the 3 L mini-silos, as there were no statistical differences between the non-inoculated 1 L mini-silos. Ensiling decreased ($P<0.05$) WSC from 131 to an average of 39.4 g/kg DM and tended to decrease ($P<0.10$) NFC from 333 to an average of 317 g/kg DM compared with the wilted forage. There were no other treatment differences in nutrient content due to inoculation. Inoculation decreased ($P<0.05$) silage pH in the 1 L mini-silos regardless of the vessel being PVC or glass, but only numerical differences in the 3L mini-silos were detected (Table 2). Lactic acid was numerically higher (53.3 vs. 46.6 g/kg DM) for the inoculated

silages but did not differ within the same type of mini-silo. Acetic acid (18.4 vs. 12.5 g/kg DM), pH (4.64 vs. 4.40) and ammonia (61.1 vs. 46.0 g/kg of CP) were lower (P<0.05) for the inoculated silages in the 1 L mini-silos. The ratio of Lactic:Acetic (2.59 vs. 4.37) and the proportion of lactic acid of the total acids (0.72 vs. 0.81) were increased (P<0.05) by inoculation in the 1L mini-silos. In the 3L mini-silo the contents of acetic acid and ammonia were numerically lower for the inoculated silage, while the contents of lactic acid, total acids, lactic as a proportion of total acids, the ratio of Lactic:Acetic and ethanol were numerically higher, but did not allow for the detection of treatment differences. Data suggest that 1L mini-silos replicated four-times are suitable for the detection of treatment differences while for 3L mini-silos greater replication may be needed in order to determine treatment differences. These could be explained by the packing densities achieved in the 1L (156 kg/m$^3$) vs 3L (194 kg/m$^3$) mini-silos. The DMR was not affected by treatment and averaged 96.2% (data not shown). All silages were aerobically stable during 174 h (data not shown).

**Table 1**. Nutritional characteristics of wilted *Lucerne* and resulting silage ensiled in different types of mini-silos for 91 d[1,2]

| | | 3L PVC | | 1L PVC | | 1 L GJ | | SE | P< |
|---|---|---|---|---|---|---|---|---|---|
| | **Wilted** | **NI** | **HBI** | **NI** | **HBI** | **NI** | **HBI** | | |
| DM[3], | 504 | 487 | 490 | 488 | 484 | 487 | 488 | 6.1 | 0.35 |
| Crude protein | 179 | 191 | 185 | 180 | 187 | 179 | 183 | 3.1 | 0.10 |
| ADF[4] | 352c | 363bc | 367b | 374ab | 368.7b | 385a | 371ab | 3.3 | 0.001 |
| WSC[5] | 132a | 40b | 41b | 37b | 39b | 40b | 38b | 1.4 | 0.001 |
| NFC[6] | 333 | 313 | 325.1 | 316 | 322.9 | 311 | 315 | 5.3 | 0.09 |

[1]abc within the same row differ P<0.05; 2 Treatments were 3L PVC mini-silo, 1 L PVC mini-silo, 1 L glass jar; NI = not inoculated, HBI= Homolactic bacterial inoculant; [3]Dry matter g/kg as is; all others g/kg DM; [4]Acid detergent fibre; [5]Water soluble carbohydrates; [6]Non-fiber carbohydrates

**Table 2**. Fermentation characteristics of *Lucerne* silage ensiled in different types of mini-silos for 91 d[1,2,3]

| | 3L PVC | | 1L PVC | | 1 L GJ | | SE | P< |
|---|---|---|---|---|---|---|---|---|
| | **NI** | **HBI** | **NI** | **HBI** | **NI** | **HBI** | | |
| pH | 4.53ab | 4.40b | 4.7a | 4.4b | 4.68a | 4.4b | 0.04 | 0.001 |
| Lactic acid, g/kg DM | 51.4ab | 58.6a | 43.6b | 45.1ab | 44.7b | 56.3ab | 3.0 | 0.01 |
| Acetic acid, g/kg DM | 18.7ab | 15.6bc | 16.8a | 9.6c | 19.7ab | 12.4c | 0.9 | 0.001 |
| Total acids, g/kg DM | 70.1ab | 74.1a | 60.4bc | 54.7c | 64.4abc | 68.7ab | 2.6 | 0.001 |
| Lactic:Total acids | 0.73bc | 0.79ab | 0.72c | 0.82a | 0.69bc | 0.82a | 0.17 | 0.001 |
| Lactic:Acetic | 2.88bc | 3.88ab | 2.61bc | 4.70a | 2.27c | 4.54a | 0.31 | 0.001 |
| Ammonia-N, g/kg N | 5.74ab | 5.05bc | 6.40a | 4.50bc | 6.18a | 4.25c | 0.31 | 0.001 |
| Ethanol, g/kg DM | 0.34ab | 0.37a | 0.28c | 0.22c | 0.34ab | 0.29a | 0.01 | 0.001 |

[1]Propionic acid, butyric acid, iso-butyric acid, ethanol, methanol, propanol, propanediol, butanol were analyzed, but not detected at levels above 0.1 g/kg DM; [2]abc within the same row differ P<0.05; 3 Treatments were 3L PVC mini-silo, 1 L PVC mini-silo, 1 L glass jar; NI = not inoculated, HBI= Homolactic bacterial inoculant

**Conclusions** Inoculation exerted positive characteristics to the fermentation process and were detected in the 1 L, but not in the 3L mini-silos, possibly due to differences in packing density. All silages were aerobically stable. Mini-silos as small as 1 L replicated four-times are suitable for the study of the fermentation process of *Lucerne* silage, whether they are hand packed or vacuumed sealed as they allow the detection of treatment differences that are statistically significant.

# Grass silage for biorefinery – A meta-analysis of silage factors affecting liquid-solid separation

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**Keywords:** Biomass, dry matter, meta-analysis, processing, separation

**Introduction** Ensiled grass has potential to be biorefined into a variety of innovative products, which improves the opportunity of a more efficient and sustainable use of green biomass. Usually the first step of the biorefinery process is the separation of liquid and solid fractions. The yield and composition of the liquid and solid fractions varies significantly depending on raw material quality and processing technology. The objective of this work was through a meta-analytical approach to evaluate the effect of silage quality on liquid yield, liquid composition and retained compounds in liquid.

**Material and Methods** A data set was collected from 17 studies that comprised 32 mean values of silage that was separated into liquid and solid fractions. The data include laboratory (150-300 g), pilot (20-80 kg) and farm scale (100-2000 kg) experiments. The data set was analysed using a meta-analysis technique to identify how silage characteristics affect liquid yield and chemical composition and compounds retained in the liquid fraction. Silage was classified according to species (grass or legume [pure or mixed with grass]), additive (control, biological or acid based additive), harvest (primary growth or regrowth) and liquid-solid separation method (farm scale twin screw press, FTS, Haarslev Industries A/S, Søndersø, Denmark; farm scale single screw press, FSS, Pellon Group Ltd., Ylihärmä, Finland; laboratory scale twin screw press, LTS, Angel Juicer Ltd., Busan, South Korea; or laboratory scale pneumatic press, LPP, Luke in-house built equipment, Jokioinen, Finland). Several silages were processed using multiple methods. Silage parameters investigated were chemical composition and in vitro organic matter digestibility (IVOMD), while the evaluated response variables were liquid yield, liquid composition and retained compounds in liquid. As a first step, the silage characteristics with highest correlation to liquid yield were evaluated. Based on that, equations were developed using a mixed model regression analysis with individual experiments as a random effect. The adjustment of equations was measured by the joint evaluation of the coefficient of determination ($R^2$), Akaike's information criterion (AIC) and root mean square error (RMSE). The additive, species and harvest effects were analysed using the mixed procedure and considering the individual experiments and silage dry matter (DM) as random effects.

**Results and Discussion** The chemical composition of the silages displayed large variation (Table 1), with DM ranging from 136 to 320 g $kg^{-1}$ with an average of 224 g $kg^{-1}$, which was lower than typical Finnish silages (321 g $kg^{-1}$; Salo et al. 2014).

**Table 1**. Descriptive statistics of the data set and Pearson correlation coefficients between liquid yield and silage quality for different liquid-solid separation methods.

| Variable | n | Mean | SD[1] | Min | Max | Liquid yield correlation coefficients | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | LPP[2] | FSS[3] | FTS[4] | LTS[5] |
| Silage DM, g $kg^{-1}$ | 32 | 232 | 44.6 | 138 | 320 | **-0.58** | **-0.95** | -0.27 | **-0.86** |
| Silage ash, g $kg^{-1}$ DM | 32 | 87 | 22.1 | 52 | 118 | -0.25 | 0.46 | 0.87 | 0.12 |
| Silage CP, g $kg^{-1}$ DM | 32 | 142 | 29.6 | 84 | 211 | 0.38 | -0.03 | 0.59 | 0.54 |
| Silage NDF, g $kg^{-1}$ DM | 25 | 471 | 61.5 | 342 | 586 | -0.49 | 0.61 | -0.86 | - |
| Silage IVOMD, g $kg^{-1}$ OM | 25 | 740 | 38.9 | 646 | 804 | 0.15 | -0.18 | **0.95** | - |
| Liquid yield | 46 | 0.425 | 0.1509 | 0.179 | 0.702 | | | | |
| Liquid DM, g $kg^{-1}$ DM | 46 | 98 | 31.0 | 33 | 149 | | | | |
| Liquid CP, g $kg^{-1}$ DM | 46 | 190 | 62.1 | 84 | 331 | | | | |
| DM retained in liquid | 46 | 0.184 | 0.0886 | 0.011 | 0.379 | | | | |
| CP retained in liquid | 43 | 0.248 | 0.1379 | 0.073 | 0.736 | | | | |

[1]Standard deviation; [2]LPP: laboratory scale pneumatic press; [3]FSS; farm scale single screw press; [4]FTS: farm scale twin screw press; [5]LTS: laboratory scale twin screw press. Correlation coefficients bolded are significant at 5% probability.

Overall, the separation efficiency showed large variation in the liquid yield as a proportion of fresh weight of the original silage, ranging from 0.179 to 0.702 (Table 1). In general, the silage characteristic most highly correlated with liquid yield was DM followed by neutral detergent fibre (NDF) content. This demonstrates that liquid yield prediction using silage composition is possible especially when silage DM is included in the

prediction equation. Regression equations (Table 2) to predict liquid yield showed a negative correlation with silage DM for LPP, FSS and LTS methods (Figure 1).

**Table 2**. Effect of silage quality on prediction of liquid yield, composition and retained compounds in liquid for different separation methods.

| Press | Y = dependent variable | X = independent variables | α | $\beta_1$ | $\beta_2$ | AIC[1] | $(R^2)^2$ | RMSE[3] |
|---|---|---|---|---|---|---|---|---|
| LPP (n = 22) | Liquid yield | $X_1$ = DM | 0.834 | **-0.0022** | | -43.1 | 0.31 | 0.08 |
| | | $X_1$ = DM; $X_2$ = NDF | 1.21 | **-0.0028** | -0.0005 | -3.2 | 0.57 | 0.08 |
| | Liquid DM | $X_1$ = DM | 31.0 | **0.3346** | | 163 | 0.68 | 18.9 |
| | | $X_1$ = DM; $X_2$ = IVOMD | 49.9 | **0.2946** | -0.0045 | 88.2 | 0.42 | 9.43 |
| | DM retained in liquid | $X_1$ = NDF | 0.665 | **-0.0011** | | -13.7 | 0.41 | 0.06 |
| | | $X_1$ = NDF; $X_2$ = IVOMD | -0.0589 | **-0.001** | 0.0009 | -3.6 | 0.53 | 0.05 |
| | Liquid CP | $X_1$ = DM | 148 | 0.268 | | 218 | 0.12 | 65.4 |
| | | $X_1$ = DM; $X_2$ = NDF | 426 | 0.7933 | -0.893 | 117.5 | -0.06 | 60.6 |
| | CP retained in liquid | $X_1$ = NDF | 0.695 | -0.0011 | | -9.2 | 0.19 | 0.07 |
| | | $X_1$ = NDF; $X_2$ = CP | 0.876 | -0.0013 | -0.0005 | 3.9 | 0.10 | 0.08 |
| FSS (n = 10) | Liquid yield | $X_1$ = DM | 0.581 | **-0.0013** | | -10.9 | 0.89 | 0.02 |
| | | $X_1$ = DM; $X_2$ = NDF | 0.478 | **-0.0011** | 0.0001 | 3.1 | 0.87 | 0.02 |
| FTS (n = 4) | Liquid yield | $X_1$ = IVOMD | -4.14 | **0.0059** | | -4.2 | 0.84 | 0.02 |
| | | $X_1$ = IVOMD; $X_2$ = NDF | -4.27 | **0.0061** | -0.0002 | 4.5 | 0.92 | 0.01 |
| LTS (n = 11) | Liquid yield | $X_1$ = DM | 0.789 | **-0.0009** | | -22.5 | 0.71 | 0.03 |
| | | $X_1$ = DM; $X_2$ = CP | 0.865 | -0.0012 | -0.0002 | -8.9 | 0.68 | 0.04 |

[1]AIC: Akaike's information criterion; [2]Coefficient of determination; [3]RMSE: root mean square error. Coefficients bolded are significant at 5% probability.

For FTS, the equation differed from the other methods, but the data set comprised only 4 observations with small range in silage quality. Inserting a second independent variable in the model to predict liquid yield for the FSS and LTS did not increase $R^2$. However for LPP method, when silage NDF was also included in the regression model, liquid yield was predicted with higher accuracy, based on a joint evaluation of AIC and $R^2$. Parameters related to the liquid composition and retention were estimated only for LPP due to the greater amount of data available. Liquid DM concentration was highly correlated with silage DM (r = 0.83; P < 0.01), while crude protein (CP) concentration resulted in a tendency of correlation with silage DM (r = 0.41; P = 0.07) developing prediction equations with $R^2$ 0.68 and 0.12, respectively. The retention of DM and CP in the liquid fraction could be estimated based on silage NDF content. The accuracy of the equation could be improved by silage IVOMD as a second independent variable only for DM retained in the liquid. Liquid CP content and CP retention in the liquid fraction were poorly estimated based on silage quality as indicated by low $R^2$ and high RSME of the equations. The slope between liquid yield and silage DM concentration was steeper for LPP than the other methods (Figure 1) indicating that this method was more sensitive to changes in silage quality.

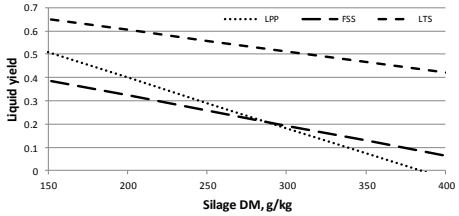


**Figure 1.** Prediction of liquid yield using regression equations based on silage dry matter for different separation methods. LPP: laboratory scale pneumatic press; FSS; farm scale single screw press; LTS: laboratory scale twin screw press.

Since silage DM plays an important influence on liquid yield, this variable was taken as a random effect in the model to identify the differences between additives, species and harvest. There was no effect of these factors on liquid yield for LPP when silage DM content was included in the model.

**Conclusions** The high correlation between silage quality and liquid yield and composition provides potential to predict the biorefinery potential of a particular silage batch based on these equations. This information can also be used to modify the silage production systems so that they best meet the requirements of a green biorefinery process.

**References**

Salo, T., Eurola, M., Rinne, M., Seppälä, A., Kaseva, J. & Kousa, T. (2014) The effect of nitrogen and phosphorus concentrations on nutrient balances of cereals and grass silage. MTT Report 147, Jokioinen, Finland, 37 p. Available at: http://jukuri.luke.fi/bitstream/handle/10024/482918/mttraportti147.pdf.

# Screening of traditional and novel spring maize genotypes for quality silage production

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**Keywords**: Composition, Digestibility, Harvest Maturity, Maize silage

**Introduction** The global demand for dairy products is expected to be 63% higher in 2050, and a large part of this increase will occur in the developing countries such as in Pakistan, because of the rising incomes and rapid population growth (FAO 2011). Much of the recent research shows that the bottle-neck to the increase in milk production under the existing genetic resources and production systems in Pakistan is the low-quality and availability of good quality forages (Nazir et al. 2016).Therefore, a quest for good quality forage resource in terms of high yield, nutritional value and year-round availability is a must to ensure sustainable increase in milk yield, and as such the growth of dairy industry and economic profitability of the dairy farmers. The introduction of high yielding, nutritious fodder varieties can boost up the forage yield and nutrients supply to livestock. Maize is one of the most important forage crops being grown under both irrigated and barani conditions and production of silage from maize can ensure good quality forage availability throughout the year. However, in Pakistan the average forage yield and nutritional quality of maize silages is very low due to low yielding varieties and substandard agronomic practices. Recent research in our laboratory shows that the nutritional value of maize silage produced in Pakistan is highly variable, and most of this variation was caused by differences in genotypes and maize maturity. Genotype of maize has a marked influence on the biomass yield, grain to stover ratio as well as stover/fiber digestibility, and as such silage yield, quality and economic return to farmers (Boon et al. 2005; Khan et al. 2015b). This study is part of systematic research project and aimed to quantify the nutritive value of spring maize hybrids for silage production in terms of: (1) dry biomass and nutrients yield; (2) silage fermentation quality; (3) and fiber digestibility and metabolizable energy (ME) supply to dairy cow.

**Materials and Methods** ***Site selection and maize sowing:*** The study was conducted in the research fields (34°0'N, 71°35'E, 359 m above sea level) of The University of Agriculture Peshawar, Pakistan. Peshawar has a subtropical steppe/low-latitude semi-arid hot climate. Six promising spring maize genotypes, including Pioneer (P1543 and P1429 hybrids), Monsanto (DK 9108 hybrid), new released quality protein maize (QPM-200 and QPM-300) hybrids by CIMMYT and NARC Pakistan, and one local cultivar (Azam) were evaluated. The seeds of each cultivar were sown on March 28, 2016 in 8 replicate plots (5 m × 10 m) that were blocked in two replicate fields (locations). At each field the seeds were sown at a seed of ca. 33000 seeds/acre (plant to plant distance of 20 cm and row to row distance 70 cm) in 4 replicate plots (5 m × 10 m) according to randomized completed block design. The standard agronomic, weed control and irrigation practices were applied uniformly to all plots. ***Monitoring Harvest Maturity and Sampling:*** Maize maturity was strictly monitored by counting the number of leaves from 13 leaves stage onward, appearance of flower (50% flowering date) and silk (50% silking date) on 1 m long randomly selected strip of two randomly selected adjacent rows. Moreover, one week after the silking the DM content of the whole crop was strictly monitored by oven drying randomly collected samples of each plot. Samples from each plot were harvested at a targeted DM content of 25, 30, 35 and 40%. At each harvest time, sample of 1 m2 area of consecutive plants were hand-harvested by cutting 15 cm above the land surface from each harvest area. The harvested crop was weighed, chopped, mixed thoroughly and a sub sample was collected for chemical analysis. ***Laboratory scale Silage production:*** The chopped material of each plot was ensiled in four replicates, 1.5-liter laboratory silos. After three months of silage production duration, the silos were opened and the silages were analyzed for fermentation quality, nutrient composition and fiber digestibility as described earlier (Khan *et al.,* 2014) nutrition. ***Sample processing and chemical***

***analysis:*** The contents of DM (method 930.15), ash (method 942.05), and ether extract (EE, method 920.39), crude protein (CP, method 984.13), acid detergent fiber (ADF, method 973.18) and acid detergent lignin (ADL; ADF method 973.18,) were analyzed per the standard procedures of AOAC (2005). The NDF content was determined as per the procedure of Van Soest *et al.,* (1991). Silage fermentation acids (Lactic acid, Acetic acid, Propionic and Butyric acid were determined at Romer lab Islamabad according to their slandered procedure. ***Statistics:*** The data were analyzed with the PROC MIXED procedure of SAS for the fixed effect of genotype, harvest maturity and their interaction. Replicate plots were considered as a random effect. The following model was used ($Y_{ijk} = \mu + MG_i + HM_j + MG_i \times HM_j + \epsilon_{ijk}$) Post-hoc analyses were carried out using the Tukey-Kramer test to compute pair wise differences in the means. Means with different letters were obtained with "pd mix 800SAS macro".

**Results** Results of the current study are summarized in table 1. It shows that maize hybrid QPM-300 has maximum yield in term of DMY (17.9 t/ha), protein (1.4) and starch yield (5.2 t/ha). Spring maize genotypes affected (P<0.001) the content DM, CP, Starch, ADF, NDF, Acids and *Invitro* dry matter digestibility (IVDMD). The maize genotypes did not differ significantly for DM, EE, and ADL concentration in chemical composition and maize quality of maize silage.

**Table 1**. Chemical composition, ensiling quality of maize silages

| | G1 | G2 | G3 | G4 | G5 | G6 | SEM | P- G |
|---|---|---|---|---|---|---|---|---|
| Yiled (tons/hac) | | | | | | | | |
| DM | $17.9^{A}$ | $16.3^{AB}$ | $15.9^{B}$ | $16.0^{B}$ | $13.1^{C}$ | $14.9^{B}$ | .340 | <.000 |
| CP | $1.41^{A}$ | $1.22^{B}$ | $1.06^{C}$ | $1.07^{C}$ | $0.83^{D}$ | $1.03^{C}$ | .311 | <.000 |
| EE | $0.55^{A}$ | $0.48^{AB}$ | $0.52^{AB}$ | $0.52^{AB}$ | $0.40^{C}$ | $0.47^{AB}$ | .205 | <.000 |
| Starch | $5.26^{A}$ | $4.28^{B}$ | $4.13^{B}$ | $4.26^{B}$ | $3.06^{C}$ | $4.03^{B}$ | .110 | <.000 |
| Composition (%DM) | | | | | | | | |
| CP | $8.05^{A}$ | $7.51^{B}$ | $6.60^{C}$ | $6.66^{C}$ | $6.44^{C}$ | $6.90^{C}$ | 0.11 | <.000 |
| NDF | $41.5^{D}$ | $43.2^{C}$ | $44.9^{B}$ | $45.6^{AB}$ | $47.1^{B}$ | $44.3^{BC}$ | 0.37 | <.000 |
| EE | 3.16 | 2.97 | 3.27 | 3.22 | 3.08 | 3.13 | 0.12 | 0.485 |
| Starch | $28.9^{A}$ | 25.3 | $25.0^{BC}$ | $25.9^{B}$ | $23.2^{C}$ | $26.1^{B}$ | 0.43 | <.000 |
| Silage quality | | | | | | | | |
| pH | 3.7 | 3.7 | 3.8 | 3.8 | 3.9 | 3.8 | 0.11 | NS |
| $NH_3$. N (%) | 0.92 | 0.94 | 0.86 | 0.91 | 0.93 | 0.94 | 0.19 | NS |

Note: Superscript with different alphabets shows significant value
G1: QPM-300, G2: QPM-200, G3: P1543, G4: DK 9108, G5: Azam, G6: P1429

**Discussion** DM yields in current study from whole-crop maize genotypes were within the expected range for maize genotypes (16.6-23.0 t/hac) grown in the tropical area by (Tabacco et al. 2011).The content of CP, starch yield and IVDMD are in line with other studies of (Cone et al*.,* 2008), (Tomoko et al*.* 2007), (Almeida et al*.* 2003).The content of high CP, starch and IVDMD and high lactic acid production of silage at genotype QPM-300 might be due to high number of leaves per plant, maximum number of kernels and it results it reduces the content of NDF and ADF.

**Conclusions** Maize genotypes has markedly differed from each others in term of yield and nutrient composition, and maize genotype QPM-300 was screen out best spring season genotype in term of dry matter yield, starch and protein yield, silage characteristics and fiber digestibility (Invitro).

**References**

Cone J.W., Van Gelder, A.H., Van Schooten H.A. & Groten, J.A.M. (2008) Effects of forage maize type and maturity stage on in vitro rumen fermentation characteristics. Journal of Agriculture Science*.* 55,139-154.

Johnson, L.M., Harrison, J.H., Davidson, D., Robutti, J.L., Swift, M., Mahanna W.C. & Shinners, K.. (2002a) Corn silage management I: Effects of hybrid, maturity, and mechanical processing on chemical and physical characteristics. Journal of Dairy Scince. 85: 833-853.

Khan,N.A., Ali, Y.P., Cone, M., & .Hendriks, J.W.H. (2014) Nutritive value of maize silage in relation to dairy cow performance and milk quality. Journal of Food & Agriculture.95:238-252.

Kononoff, P.J., Heinrichs A.J. & Lehman, H.A. (2003). The effect of corn silage particle size on eating behavior, chewing activities, and rumen fermentation in lactating dairy cows. Journal of Dairy Science .86:3343:3353.

# Oxidative loss of dry matter during storage of grass silage in bunker silos on livestock farms

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**Introduction** Oxidative loss during storage of silage can occur in the peripheral regions of the silo, associated with incomplete protection of silage from oxygen ingress. In addition to financial loss, removal of inedible waste silage is a time-consuming task during feed-out. Inclusion of deteriorated silage in the animals' diet can be associated with reduced feed intake and increased risk of disease. Little is known about the extent of loss due to aerobic deterioration of silage during storage on commercial livestock farms, so a survey was conducted to estimate the incidence and scale of loss.

**Materials and Methods** Bunker silos, on twenty livestock farms in England, were visited in January 2017. The silos contained grass silage that had been stored for more than 200 days and were open for feed-out. Samples of silage were collected from the top surface immediately behind the feed-out face by removing the top covering sheet, and inserting a corer at ca. 1 m intervals across the width of the silo to a depth of 30 cm. The number of sampling points across the top varied depending on the width of the bunker. The samples were bulked, mixed and subsampled for analysis as a single sample from each farm. At the same time 9 samples were obtained from the central core of the silo, as described in Davies et al. (2018), these 9 samples were not bulked, and were analysed as separate samples. Density of silage fresh matter (FM) was assessed in the top 0.5 metre layer (Davies et al. 2018) at 3 points across the width of each silo. Analyses of volatile-corrected dry matter (DM) and ash were undertaken on each sample by wet chemistry. Particle size distribution was assessed with the Penn State Separator (Lammers et al. 1996). Total visible waste DM per metre of silo length (assuming a similar level of loss throughout the length of the silo) was estimated from the depth of visibly discoloured material from the top surface, the DM concentration of the top 30 cm sample, the density of the silage and the dimensions of the silo. Oxidative DM loss was estimated from the concentration of ash in silage in the top 30 cm relative to that in the core of the silo.

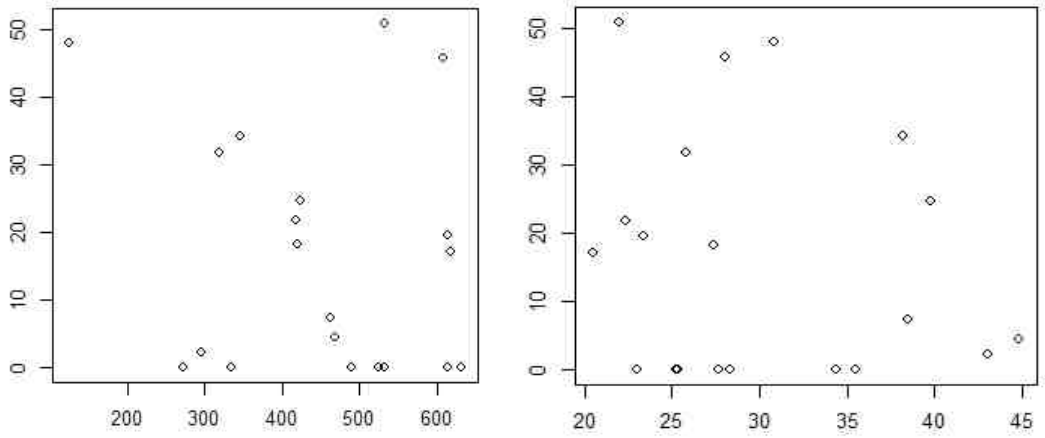
**Results** Table 1 shows the mean, maximum and minimum results of various variables that may influence peripheral oxidative loss in bunker silos together with estimates of oxidative DM loss in the top 30 cm and total visible waste DM in the silos. There was a large range in all variables. Mean silage DM was 30.2%, close to averages of UK grass silage over the past 20 years. However, DM ranged from 20.5 to 44.9%. Mean FM density in the top 1 metre was 452 kg/m$^3$ (range 124 to 632 kg /m$^3$). There was also a large variation in particle size. The mean percentage of total FW retained on the top sieve was 41% of total particles (range 23.2 to 78.3%). The main factor affecting particle size distribution was type of forage harvester, with grass harvested by self-loading wagon having a longer particle size than precision chopped material. Mean oxidative DM loss in the top 30 cm of the silo was 16.3% but ranged from zero to 50.9%. Peripheral oxidative loss was zero on 9 of the 20 farms. Visible waste per metre of silo length ranged from zero to 1.6 t DM. Total visible waste per silo ranged from zero to 68.2 t DM. Statistical analyses indicated that there was no relationship between oxidative DM loss in the top 30 cm layer and size of silo, silage DM content or silage FM density (Figure 1).

**Discussion** The data collected during the assessment of 20 individual farms show a wide variation in the variables assessed as indicated in Table 1. The data also indicates that there are no relationships between estimated peripheral DM loss and commonly quoted variables that affect DM loss during storage such as silage DM content and fresh weight density (McDonald et al. 1991; Ruppel et al. 1995). The zero loss values indicated by the row of data points just above the horizontal

axis in Figure 1 show that it is possible to produce bunker silage with no peripheral loss of DM or visible waste material over a wide range of densities and DM contents. Further study and analysis are therefore required to identify the key multivariate factors that affect peripheral losses in bunker silos.

**Table 1.** Silo dimensions, density of silage in top metre, silage DM, particles in top sieve of Penn State Separator, estimated loss of DM in top 30 cm and estimated total visible waste DM in silo.

| | Width of silo (m) | Height of silo (m) | Total volume of silage in silo ($m^3$) | Density of top metre (kg fresh matter/$m^3$) | Mean silage DM (% of fresh weight) | Particles on top sieve (% of total particles) | DM loss in top 30 cm (%) | Total visible waste in silo (t DM) |
|---|---|---|---|---|---|---|---|---|
| Mean | 12.80 | 2.73 | 935 | 452 | 30.2 | 41.3 | 16.3 | 11.1 |
| Minimum | 8.40 | 1.90 | 336 | 124 | 20.5 | 23.2 | 0 | 0 |
| Maximum | 20.50 | 3.90 | 1872 | 632 | 44.9 | 78.3 | 50.9 | 68.2 |




**DM Loss VS FMDens** **DM Loss VS SDM**

**Figure 1**. Plots of fresh matter density (FMDens, kg/$m^3$, horizontal axis) and silage DM (SDM, %, horizontal axis) against estimated DM loss in top 30 cm (%, vertical axes)

**Conclusions** Oxidative loss was zero on 45% of the farms surveyed, indicating that this source of DM loss is avoidable by adopting silo management practices that prevent oxygen ingress into the silage during storage.

**References**

Davies, D.R., Davies, G.K. & Piotrowski, C. (2018) Development of calibrations for hand-held NIRs instrumentation to measure silage density from the open face of grass silage clamps. This proceedings International Silage Conference.

Lammers, B.P., Buckmaster, D.R. & Heinrichs, A.J. (1996) A simplified method for the analysis of particle sizes of forage and total mixed rations. Journal of Dairy Science 79, 922-928.

Mc Donald P., Henderson A.R. & Heron S.J.E. (1991) The Biochemistry of Silage, 2nd edn. Marlow, UK:Chalcombe Publications.

Ruppel, K.A., Pitt, R.E., Chase, L.E. & Galton, D.M. (1995) Bunker silo management and its relationship to forage preservation on dairy farms. Journal of Dairy Science 78, 141-153.

# Volatile organic compounds and silage: sources, emission, and mitigation

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**Abstract**

Microbial activity during ensiling leads to the production of numerous organic compounds. More than 40 of these compounds are volatile organic compounds (VOC), which readily volatilize when silage is exposed to air. VOC contribute to poor air quality in some areas by participating in atmospheric reactions that produce harmful secondary pollutants, including ozone. Recent measurements and calculations suggest that the silage VOC make a significant contribution to total anthropogenic VOC emission and resulting poor air quality in some areas. Although silage VOC production has been studied for well over a century, only recently have there be efforts to quantify emission. Recent studies have focused on measurement of VOC emission and development of emission models to better understand emission pathways and estimate the magnitude of silage VOC emissions. Studies on silage additives have begun to address the question of mitigation. In this paper we summarize the current understanding of silage VOC production and emission, estimate the contribution of silage to VOC emissions in the US and EU, discuss mitigation methods, and identify research needed to address the problem of silage VOC emissions. Results show that silage VOC emission in Europe and the US is probably equivalent to more than 5% of all other anthropogenic sources, and likely much higher in some locations. However, uncertainty in emission estimates is high, and development and application of more accurate measurement techniques is an important research need.

## Introduction

Silage contains organic acids, alcohols, esters, and other volatile organic compounds (VOC) that volatilize into the atmosphere during silage production, storage, and feeding. Research in the United States over the past decade has shown that these compounds may make a significant contribution to total anthropogenic VOC emission in some areas (Howard et al. 2010; Hu et al. 2012; Hafner et al. 2013). Silage is also an important animal feed and bioenergy feedstock in Europe, but its contribution to VOC emissions has not been assessed. Emission of VOC from silage and other sources contributes to poor air quality by reacting to form tropospheric ozone and other oxidants, resulting in an increase in respiratory disease and premature death (National Research Council 2008; Jerrett et al.

2009). As VOC emission from other sources is reduced, agricultural emission has and will continue to become more important. For example, Anthropogenic VOC emissions in the US have dropped by 55% from 1980 to 2016 (EPA, 2017). The EU has achieved a similar reduction of 57% from 1990 to 2014 (EEA, 2015).

Much is unknown about the problem of silage VOC emission, including the true magnitude of emission and the best approaches for reducing emission. The objectives of this paper are to summarize the current state of knowledge about silage VOC production and emission and to identify research that is needed to better understand and ultimately address the problem of VOC emission from silage.

## Sources of silage VOC

### *Compounds*

There are various definitions, both regulatory and scientific, for VOC, but in general they are organic compounds that readily volatilize (US EPA 2018a). The US Environmental Protection Agency (US EPA) excludes some compounds with low reactivity, including two that are present in silage (acetone and methyl acetate) (US EPA, 2003). And methane is always excluded, leading to the use of the name non-methane VOC (NMVOC) or non-methane hydrocarbons. The name reactive organic gases (ROG) is also used, but in this document we will use the term volatile organic compounds, or VOC. At least 46 different VOC have been identified in silage (Table 1 provides information on some of the most important).

**Table 1**. Major volatile organic compounds (VOC) present in silage, based on data compiled by Hafner et al. (2013) for maize silage. This list includes all compounds with at least one observation with a concentration of 100 mg $kg^{-1}$ (dry matter basis) or an $I_4$ value above 0.1. Compounds are sorted by group (those produced in highest amounts first) and then volatility (lower volatility first).

| Group | Name | CAS number[1] | $Log_{10}$ $H$[2] | MIR[3] | EBIR[4] | Notes |
|---|---|---|---|---|---|---|
| Acid | Acetic acid | 64-19-7 | −5.12 | 0.66 | 0.203 | High production, low volatility, low reactivity. Important for silage preservation. |
| Acid | Propionic acid | 79-09-4 | −5.00 | 1.17 | 0.342 | |
| Alcohol | Methanol | 67-56-1 | −3.74 | 0.65 | 0.197 | Abiotic reaction may be major source. |
| Alcohol | Ethanol | 64-17-5 | −3.67 | 1.45 | 0.571 | Most important compound for air quality |
| Alcohol | 1-Propanol | 71-23-8 | −3.54 | 2.38 | 0.792 | |
| Alcohol | 1-Butanol | 71-36-3 | −3.47 | 2.76 | 0.882 | |
| Aldehyde | Acetaldehyde | 75-07-0 | −2.54 | 6.34 | 1.608 | Product of acetic acid and ethanol oxidation or may be formed directly |
| Aldehyde | Valeraldehyde | 110-62-3 | −2.20 | 4.89 | 1.26 | |
| Aldehyde | Hexanal | 66-25-1 | −2.07 | 4.18 | 1.073 | |
| Aldehyde | 3-Methylbutanal | 590-86-3 | −1.98 | 4.79 | 1.209 | |
| Ester | Methyl acetate | 79-20-9 | −2.33 | 0.067 | 0.043 | Very low reactivity, not considered VOC by US EPA but could affect feed intake |
| Ester | Ethyl acetate | 141-78-6 | −2.20 | 0.59 | 0.238 | |
| Ester | Propyl acetate | 109-60-4 | −2.06 | 0.73 | 0.311 | |

Notes: [1]Chemical Abstract Services registry number (http://support.cas.org/content/chemical-substances/faqs)
[2]$log_{10}$ of unitless Henry's law constant (gas:aqueous) (an indication of volatility).
[3]Maximum incremental reactivity (g $O_3$ per g VOC) (Carter 2009). Generally applicable in urban areas.
[4]Equal benefit incremental reactivity (g $O_3$ per g VOC) (Carter 2009). Generally applicable in rural areas.

Silage VOC are generally small molecules that contain oxygen atoms (they may be referred to as oxygenated VOC). These compounds are all biogenic, meaning they are produced by living organisms. Some of the known silage VOC have been studied for well over a century. For example, acetic acid is important for silage preservation, contributing to both a low pH and inhibition of yeasts (McDonald et al. 1991). Formation of alcohols in silage was studied more than a century ago (Hart and Willaman 1912). The presence of other VOC, including aldehydes and esters, was recognized more than 50 years ago (Morgan and Pereira 1962) but only recently have they been quantified (Nielsen et al. 2007; Chmelova et al. 2009).

***VOC production in silage***

Silage VOC are thought to be produced primarily by microorganisms during silage fermentation and, although some may form during storage. A limited number of studies have shown that concentrations of ethanol and 1-propanol approach increase rapidly during fermentation, approaching final levels within one week of ensiling, while other concentrations of other compounds may continue to increase (Filya and Sucu 2010; Franco 2016). Sources of silage VOC are summarized in this section, and more details can be found elsewhere (McDonald et al. 1991; Hafner et al. 2013).

Acetic acid is produced by numerous silage microorganisms through fermentation, but heterofermentative lactic acid bacteria (LAB), enterobacteria, and clostridia are thought to be the most important. Acetic acid is less effective than lactic acid for lowering pH, but inhibits yeast activity and so can improve aerobic stability (McDonald et al. 1991). Propionic acid is produced by clostridia, and sequentially by *Lactobacillus buchneri* and *L. diolivorans*. Typical concentrations in maize silage after fermentation and storage have been given as between 10 and 30 g $kg^{-1}$ for acetic acid and < 1 g $kg^{-1}$ for propionic acid (Kung and Shaver 2001). A compilation based on literature data showed that acetic acid was generally between 10 and 50 g $kg^{-1}$ and propionic generally between 1 and 10 g $kg^{-1}$ (Hafner et al. 2013).

Ethanol, which is probably the single most important silage VOC from an air quality perspective (Hafner et al. 2013), is thought to be produced primarily by yeasts, and to a lesser degree, obligate heterofermentative LAB by fermentation of forage carbohydrates (McDonald et al. 1991, Driehuis and van Wikselaar 2000). Yeasts use the alcoholic fermentation pathway, resulting in production of 2 moles of ethanol and 2 mole $CO_2$ per mole of glucose substrate, while heterofermentative LAB instead produce one mole each of lactic acid, ethanol, and $CO_2$ (McDonald et al., 1991). Recent results from anti-fungal additive experiments also suggest that yeast dominate ethanol production, at least in maize silage (see Mitigation section below). Ethanol concentrations in maize silage may exceed 10 g $kg^{-1}$ (Hafner et al. 2013).

1-Propanol and propionic acid are produced by *Lactobacillus diolivorans* by degradation of 1,2-propanediol (Krooneman et al., 2002), which is produced by *Lactobacillus buchneri*. Additionally, 1-propanol is a minor product of fermentation by yeasts (McDonald et al. 1991). Production of this alcohol varies widely in silage, with concentrations after storage ranging from below 0.1 to above 10 g $kg^{-1}$ (Hafner et al. 2013).

Methanol has been reported to be the main product from the metabolism of pectin by *Clostridium butyricum* in culture (Schink and Zeikus 1980), and by certain bacteria of the genus *Enterobacter* and *Clostridium* (Lindinger et al. 2006). However, it is likely that methanol formation in silage occurs through the action of pectinesterase and polygalacturonase, which are responsible for de-esterification of pectin in higher plants (Birch et al. 1981; Hou et al. 2008). Also, it is possible that damage to plant cells during harvest and chopping of corn plants for silage triggered the action of pectinesterase and polygalacturonase, which facilitate the degradation of pectins and, thus, the formation of methanol (Pedrolli et al. 2009). Methanol concentrations in silage are generally less than 10% of ethanol (Hafner et al. 2013).

Aldehydes may be produced by some LAB and yeast, using sugar and amino acids as substrates (Liu and Pilone 2001; Østlie et al., 2003), but also through two abiotic pathways, of which alcohol oxidation is probably the most important. Very few measurements of aldehydes in silage exist, but reported concentrations are all below 1 g $kg^{-1}$ and typical concentrations appear to be an order of magnitude lower (Hafner et al. 2013). However, high reactivity increases their contribution to secondary air pollution.

Esters may be formed by LAB and also abiotically through esterification reactions with alcohols (Weiß et al. 2016). More recently, the formation of esters was attributed to the reaction of ethanol and acetyl-CoA, which was found to be mediated by yeast acyltransferases (Piskur and Compagno 2014). Thus, it is likely that production of ethyl acetate and other esters in silage occurs due to yeast activity, and it will probably depend on the concentration of ethanol (or other alcohols) and acetyl-CoA during silage fermentation, as well as pH, temperature, yeast abundance, and strain type (Suomalainen 1981; Peddie 1990). In the case of non-brewing yeasts, ethyl esters can be synthesized by the action of

esterase enzymes (Peddie 1990). Management strategies and practices that promote ethanol formation during ensiling will most likely also stimulate the production of ethyl acetate and other ethyl esters (Weiß et al. 2016). Correlation between ethanol and ethyl ester concentrations in silage provide some evidence of abiotic formation (Weiß and Auerbach 2013). However, limited measurements on the timing of ester production show a high initial rate, before alcohol and acid concentrations have peaked, suggesting the biological routes may dominate (Franco 2016). Most esters are present at concentrations below 1 g $kg^{-1}$ in maize silage after storage (Hafner et al. 2013).

Mitigation is complicated by the redundancy in silage microbial communities, and further characterization of microbial communities responsible for the formation of specific VOC during ensiling is needed. It will be important to investigate both the concentrations of VOC and the changes in microbial communities in the first few days of ensiling as well as after active fermentation in the silo has ceased. A combination of techniques such as selective microbial inhibition, DNA and RNA based methods (*i.e.,* molecular techniques, gene sequencing) for identifying and enumerating both fungi and bacteria, and determination of VOC throughout ensiling would provide valuable data for developing effective mitigation strategies in the control of VOC production and emission from silage (Pang et al. 2011; Gharechahi et al. 2017; Xu et al. 2017; Tennant et al. 2017)

## VOC emission

### *Emission pathways*

Emission of VOC occurs throughout the silage production process, starting during crop growth, generally increasing during harvesting, continuing during fermentation and storage in a silage pile, pit, bag, bunker, or tower silo, and ending only when silage is consumed by animals (the majority of ethanol consumed by cattle, for example, is probably metabolized in the rumen or liver (Kristensen et al. 2007)) or fed to an anaerobic digester for biogas production. The magnitude of VOC emission varies throughout the silage production cycle, and depends on the concentration of VOC present in the material at each stage, and conditions that determine the rate of mass transfer through silage pores and from the exposed surface (Hafner et al. 2012). Or, in the case of emission from growing crops, the production rate may control emission. The maximum concentration of VOC present in silage is a convenient reference point for emission. Assuming little emission occurs before and during storage and VOC production is minor under the aerobic conditions that silage is exposed to once it is removed from a storage structure, maximum VOC concentrations occur after silage fermentation is complete. Expressing the maximum VOC concentration as a fraction of the silage dry matter basis (mass of VOC per kg of silage dry matter) makes it convenient to estimate emission based on silage production or silage feeding estimates, and is the approach used in this section. Production of individual silage VOC and varies with location, crop, and other factors. Based on mean values from a literature review, the sum of all VOC in maize silage is between 30 and 40 g $kg^{-1}$ (silage dry matter basis).

Plants produce volatile compounds as result of abiotic or biotic stresses, and to communicate with insects and other plants (Holopainen, 2004; Holopainen and Gershenzon, 2010). Low molecular weight VOC are also produced as part of secondary metabolism, with methanol, isoprene, and monoterpenes being the most abundant in the atmosphere. Methanol is produced as a byproduct of pectin formation in developing plant cell walls (Galbally and Kirstine 2002; Brunner et al. 2007). Terpenoids, including isoprene and monoterpene, are the largest and most diverse plant secondary metabolites, participating in photosynthesis, respiration, stress adaptation, plant defense mechanisms and attraction of pollinators (Sharkey et al. 2008; Guenther et al. 2012).

Estimates of VOC emission rates from growing crops are scarce. The average flux of volatiles emitted from growing maize crops measured with micrometeorological methods ranges from 230 to 5000 µg $m^{-2}$ $h^{-1}$ and is dominated by methanol, acetic acid, and acetaldehyde (Das et al. 2003; Bachy et al. 2016; 2018). Wiß et al. (2017) and Graus et al. (2013) also measured volatile organic emissions from maize growing crops using controlled flow chamber concentration methods and found VOC flux that was one half the flux measured by the studies that use micrometeorological methods. The range

of volatiles flux measured varied from -300 µg $m^{-2}$ $h^{-1}$ (net uptake) to 3450 µg $m^{-2}$ $h^{-1}$ and was dominated by methanol (55% to 88% of the total VOC emissions), acetic acid (10% to 30%), and acetaldehyde (about 10%). The large variation in flux magnitude was attributed to the interaction between the emission of VOC by maize and the adsorption and desorption from soil, as affected by the physiological stage of the plant, temperature and moisture condition of the soil (Wiß et al., 2017; Bachy et al., 2018; Mozaffar et al., 2018). Graus et al. (2013) also measured VOC emission from switchgrass (*Panicum virgatum* L.) and found the flux to be dominated by methanol, but to be one-half the magnitude of the flux measured on corn fields. Fluxes for white clover and mixed grasses reported in the compilation by Bachy et al. (2016) vary from -200 to 1000 µg $m^{-2}$ $h^{-1}$, and Warenke et al. (2002) report 4000 µg $m^{-2}$ $h^{-1}$ from undisturbed mature alfalfa.

Harvesting the crop triggers stress response mechanisms and enzymatic tissue decomposition that increase emission of VOC. Karl et al. (2001) measurements of VOC emissions from a mixture of grass-legume hay harvesting ranged from 1000 to 8400 µg $m^{-2}$ $h^{-1}$, and Davison et al. (2008) measured fluxes ranged from 500 to 2400 µg $m^{-2}$ $h^{-1}$ after cutting a different grass-legume mixture for hay. Warenke et al. (2002) measure a twofold increase of the VOC flux after alfalfa was cut during harvest. Methanol was the dominant volatile emitted before and during harvest, increasing in proportion immediately after cutting the crop, and diminishing as the crop dries and leaf senescence volatile compounds acetaldehyde, acetic acid and acetone increase in proportion over the following days (Warenke et al. 2002; Bachy el at. 2018; Mozaffar, et al. 2018).

Emissions of volatile organic compounds during crop grow and harvest are unrelated to the post-harvest process and storage, and occur whether the material is processed as silage or not. Cutting the plant material to size for ensiling, transporting it to the silo and packing it to the appropriate density are post-harvest activities directly related to silage production and are expected to enhance VOC emissions through the same mechanisms that occur during harvest. Nonetheless, recommended silage preparation practices limit the time from harvest to covering the silo to a day, and total VOC emissions occurring during silo preparation are expected to be less than those incurred during harvest and drying of the material for hay.

Assuming a silage dry matter yield of 20 t $ha^{-1}$, a growing season of 110 d, dry matter losses during ensiling of 15%, and emission at the maximum observed rate of 5000 µg $m^{-2}$ $h^{-1}$, crop growth could contribute as much as 8 g $kg^{-1}$ (g of VOC per kg silage dry matter). With more typical rates of 200-400 µg $m^{-2}$ $h^{-1}$ (Graus, 2013; Bachy et al., 2016) total emission would be about 0.3-0.6 g $kg^{-1}$. These estimates are < 10% of losses during feeding (discussed below) and < 2% of VOC mass present after silage storage.

During fermentation the most important silage VOC are produced and accumulate. Carbon dioxide ($CO_2$), a byproduct of heterolactic fermentation, mixed acid fermentation, and alcoholic fermentation (McGechan, 1990) causes pressure- and buoyancy-driven advective flow of gas out of the silage storage structure (Williams et al., 1997). This gas flow will carry VOC out of the silage, but the amount transported is limited by the concentration of VOC present at this stage of the fermentation process and by the partitioning of between gas and aqueous phases, which depends on temperature, silage moisture and gas-phase porosity (Hafner et al., 2012). Hafner et al. (2012, supplementary material) presented an approach to predict these losses, assuming that VOC concentrations during the entire fermentation period are similar to the values measured when the silo is open at the end of the storage, which indicates that for the most volatile compounds (esters and aldehydes), predicted losses are < 6% of the total compound mass under typical conditions, but nearly 30% under extreme conditions. For alcohols, which are the most important from an air quality perspective, losses from this route are probably negligible (< 2% even under extreme conditions).

Even in the most carefully sealed silage bags some exchange of mass between the silage and the atmosphere occurs during storage. It is possible to estimate storage losses by using aerobic deterioration losses as an estimate of the air that enters stored silage and assuming that a similar quantity of silage gas has escaped (Hafner et al., 2012, supplementary material). Under typical conditions, predicted ester losses may be 40% to 100% of the total ester mass under extreme

conditions, while alcohol losses would be about 2% under typical conditions. These estimates are probably higher than true values, since the method assumes an even distribution of silage VOC at all times (i.e., insignificant transport resistance within silage). Losses of highly volatile compounds may be significant during storage, therefore concentrations of the most volatile VOC measured after silage storage may significantly underestimate total production.

The term “feedout” is used here to describe the time when silage storage structures are open and silage is removed every day for feeding. Measurements of VOC emission rates have been made on both intact silage at the exposed “face” of a silage pile using low-air flow flux chambers (Alanis et al., 2008; Chung et al., 2010) and on intact samples in the laboratory under a wide range of air velocities (Montes et al., 2010). Results are sensitive to the method used, and in particular, low-air flow flux chamber measurements almost certainly substantially underestimate emission rates (Hafner et al., 2010). Furthermore, emission rates have been found to be sensitive to both temperature and air flow rate in wind tunnel studies (Montes et al., 2010). Despite the uncertainty in emission rates under farm conditions, wind tunnel results show that emission during feedout is almost certainly significant. At 20°C and under moderate air flow of 0.5 m $s^{-1}$ close to the silage surface, ethanol loss from a 15 cm block of silage may exceed 10% of the initial mass over 12 h (Montes et al., 2010). More volatile compounds will be lost at a higher rate (although measurements have not been made under these conditions). With a daily removal of 15 cm during feedout, we might expect around 10% of ethanol to be lost. However, low density, high temperature, high wind speed, and a smaller daily removal thickness could all contribute to greater losses.

During feeding, silage is removed from a storage structure, transported to a mixing wagon, mixed with other feed components, and finally placed in feed lanes, where most of it is consumed by cattle. Wind tunnel measurements show that loss of ethanol is much higher from loose silage than from packed samples (Montes et al. 2010; Hafner et al. 2010). Loose silage at 20°C exposed at wind speeds of 5 m $s^{-1}$ may emit as much as 80% of initial ethanol mass from a 15 cm deep sample over 12 h (Hafner et al. 2010). Limited mass balance measurements under barn conditions with 10 cm deep maize silage and exposed to 0.6 and 0.9 m $s^{-1}$ average wind speed have shown losses of 34-56% of initial ethanol and as much as 92% of initial acetaldehyde present in silage over 6 h (Hafner et al. 2012).

***Measurement of VOC emission***

Quantifying VOC emissions from silage requires accurate measurement of emission rates under normal farm conditions. Although rates can be accurately measured using a variety of approaches, some approaches influence mass transfer and therefore result in inaccurate estimates of emission under farm conditions. The earliest work in California, USA was based on flux chambers that were originally designed for production- or diffusion-limited emission (Alanis et al. 2008; Chung et al. 2010). But silage VOC are relatively soluble in water (Table 1), and emission is therefore unlikely to be production-limited. Also when previously sealed silage is exposed directly to moving air (see Emission pathways section below) VOC loss is unlikely to be diffusion-limited. Both theory and measurements have since shown that emission rates depend on air flow speed at an exposed surface (Hafner et al. 2009; Montes et al. 2010). Comparison of wind tunnel and flux chamber measurements of ethanol emission using two types of silage confirmed that flux chambers are not appropriate for measuring VOC emission rates from silage, and will generally underestimate total emission (Hafner et al. 2010). In selecting a measurement approach, researchers must recognize that air flow affects the rate of VOC mass transfer from silage.

Most emission measurement techniques require determination of gas-phase VOC concentrations. Multiple options exist for making these measurements. A gas chromatograph (GC) with a flame ionization detector (FID) and appropriate column is sufficient for detection and quantification of most silage VOC (Montes et al. 2010; Hafner et al. 2012; Franco 2016). Identification is based on retention time, and for some compounds present at low concentrations, may be inaccurate. An alternative for detection is a mass spectrometer, which enables both quantification and improved identification based on both retention time and mass spectrum (Alanis 2008; Chung et al. 2010). Regardless of the detector, GC methods require that silage VOC are sampled with an acceptable recovery. If direct sampling is not possible, canisters are suitable for the main silage VOC, e.g. ethanol, but polar VOC

(e.g. carboxylic acids), semi-volatile VOC and reactive VOC need special attention in relation to recovery, since poor recovery has been reported (Koziel 2000). Canisters have been used for silage VOC with canisters included in the calibration procedure, which in principle may account for any reduced recoveries (Chung et al. 2010). Sorption-based methods such as adsorption tubes (e.g. Tenax) and solid-phase micro-extraction (SPME) can also be used for sampling VOC, but these methods are problematic for very volatile compounds such as small alcohols and ketones due to the low degree of sorption to the sampling material and challenges in calibration. Alternatively, direct (online) mass spectrometry (proton-transfer-reaction mass spectrometry; PTR-MS) without chromatographic separation has been used for measuring emissions from dairy barns (Shaw 2007; Ngwabie 2008) as well as different sources in cattle operations including feed (Yuan et al. 2017). This method for measuring concentration is applicable to silage emissions in combination with e.g., wind tunnels. Advantages of PTR-MS include high time resolution, high sensitivity, very low risk of sampling bias and relatively easy quantification even of unidentified compounds. Application of a high-resolution time-of-flight (TOF) mass filter enables determination of absolute compound mass. Disadvantages include low selectivity for compounds of identical atomic composition (e.g acetone and propanal) and loss of selectivity due to compound fragmentation (e.g., alcohols with 3 or more carbon atoms (Brown et al., 2010)). For important silage compounds such as ethanol, methanol, acetic acid and acetone, PTR-MS will be a suitable tool for measuring emissions. Photoacoustic gas monitors such as the INNOVA instruments produced by LumaSense (Santa Clara, CA, USA) can provide accurate determination of specific VOC (but not for broad groups, such as VOC or “non-methane hydrocarbons”). The use of photoacoustic monitors with relatively broad optical filters requires that interferences can be excluded. For complex mixtures containing numerous compounds, interferences are likely, and the accuracy of the instrument should be evaluated using a GC or MS (Hafner et al. 2010; Chung et al. 2010).

Emission rates of silage VOC are strongly time-dependent, due to depletion of VOC near an exposed surface and the resulting increase in transport resistance as VOC must travel through silage pores prior to emission. For example, emission rates of ethanol from corn silage measured using a wind tunnel declined by a factor of 70 over 12 h (Hafner et al. 2010). Emission measurements and estimates could be simplified if only a very small fraction of silage VOC mass were volatilized (close to constant emission rates) or if nearly all VOC mass was lost (production limited) (Hafner et al. 2013). Unfortunately, transport properties of silage and the volatility (or solubility) of the most important silage VOC result in a situation that is not close to either of these extremes.

The best methods for measuring emission are those that have no effect on silage properties nor air movement near the silage surface. In some cases, it may be possible to collect silage samples over time while emission occurs. A decline in VOC concentrations within the silage can be used to calculate emission by mass balance (Hafner et al. 2012). It is necessary to confirm that oxidation or other pathways are not responsible for changes, but this can easily be done using closed containers (Hafner et al. 2012). However, it would be difficult to apply this method during consumption of silage.

Dispersion-based methods have been used for many air pollutants from agricultural systems (Leytem et al. 2011; Ogink et al. 2013) and may be useful for silage VOC also. Gas-phase concentrations are generally measured upwind and downwind of a source, and there is no disturbance of the source itself.

Resulting estimates of emission would include all sources within close proximity, so the method could not be used to quantify individual sources within a barn, for example. However, it may be possible to apply it to a silage pile.

Tracer ratio methods have also been used for agricultural air pollutants. They rely on the controlled release (or a known emission rate) of a reference compound, and have the same advantages and disadvantages of dispersion-based methods. Tracer ratio methods have been used extensively for measuring emissions of ammonia from cattle barns (Ogink et al. 2013) either using naturally occurring $CO_2$ produced by the animals as a tracer or using synthetic tracers (e.g. $SF_6$). Using a single tracer, however, only allows for quantification of one source and if silage is stored in connection to a livestock building, this approach may not be possible. Alternatively, a dual tracer method similar to the approach

used by Zeyer et al. (2013) might be used but this requires substantial development and verification. Tracer ratio methods can include both time-averaged and online measurements with the latter (e.g., PTR-MS for VOC) adding the benefit of a high time resolution.

At this time, with only limited measurements of emission rates available (see Emission pathways section, below), those developing inventory estimates should be skeptical of any measurements made under conditions that do not reflect those that silage is exposed to on farms, including silage condition, air speed, temperature, and the duration of exposure. If an appropriate approach from measurement of VOC emission is not available, measurements of VOC within silage are more useful than inaccurate emission measurements that depend on precise conditions that are difficult to replicate. Concentrations within silage can be used to estimate relative differences and total maximum emission (at least for all stages that occur after measurement). Silage VOC concentrations can be measured using water extraction or headspace analysis, combined with GC/FID or GC/MS measurements (Hafner et al. 2014; Weiss et al. 2016).

***Magnitude of silage VOC emission***

Given the paucity of reliable measurements of VOC emission from silage, emission estimates (including those published in national emission inventories) have the potential to be very inaccurate. However, even approximate estimates are useful for assessing the significance of the problem and identifying research needs. In this section we present new estimates of silage VOC emission for the US, EU, and some smaller areas, and compare them to inventory estimates of non-agricultural emissions as a means of evaluating the significance of silage VOC.

We can estimate VOC emission by assuming fixed fractions of the three most important VOC groups are ultimately lost during the stages described above (see Emission pathways section). Mean concentrations from a large compilation of literature data for each of the four most important VOC groups in maize silage were 22.1, 9.7, 1.9, and 0.6 g $kg^{-1}$ for acids, alcohols, esters, and aldehydes (dry matter basis) (Hafner et al., 2013). Our best estimate of typical emission losses from all stages after packing is 40% for alcohols and 70% for esters and aldehydes based on the wind tunnel results for packed silage presented by Montes et al. (2010) and the mass balance results for loose silage reported in Hafner et al. (2012). With a lower volatility, an estimate of 10% for acids may be reasonable. The sum of the product of silage concentrations and these volatilization estimates results in an emission factor of 8 g $kg^{-1}$, which we can take as an estimate of typical VOC emission from maize silage. It is important to remember that this is an approximate estimate at best, and how well it reflects actual emission is unknown. Additionally, the value will vary among locations. Similar approaches for lower and upper limits (assuming only 1% acid emission, 10% for alcohols, and 20% for esters and aldehydes for the lower limit, along with a lower alcohol concentration of 5 g $kg^{-1}$; and 20% loss of acids, 70% for alcohols, and 100% for esters and aldehydes) yield values of 1 and 14 g $kg^{-1}$.

Reflecting on these estimates, and given some confusion in trade publications (e.g., Mitloehner and Cohen 2016), it should be recognized that VOC emission is not the major route of mass loss from silage. Even well-managed silage typically loses > 50 g $kg^{-1}$ (5%) of dry matter between packing and feedout (not including surface waste), and losses may exceed 200 g $kg^{-1}$ (McGechan 1990; Savoie and Jofriet 2003). But this lost mass is primarily as $CO_2$ from both fermentation or aerobic respiration (McGechan 1990). Only under the most extreme conditions (ethanol > 50 g $kg^{-1}$) could VOC emission approach 50 g $kg^{-1}$, and in this case, total mass loss would almost certainly be much higher (assuming ethanol production was from alcoholic fermentation by yeast, fermentation loss by $CO_2$ alone would exceed 50 g $kg^{-1}$ loss as well).

The emission factors calculated above are used here along with estimates of silage production to predict VOC emission. Resulting estimates have been made for the US, the EU, and some smaller areas where silage VOC emission may be more significant: the San Joaquin Valley in California, USA, which has a large fraction of the US dairy cow herd and significant air quality problems (Howard et al. 2010); Germany, which produces more maize silage than any other country in the EU, and France, which is exceeded only by Germany in maize silage production within the EU. In Germany, almost half of the silage maize produced is used for biogas production (S. Kraume, Deutsches Maiskomitee e.V.,

personal communication). Differences in management and handling (including the lack of feed lanes in biogas production) would result in different emission factors than for silage produced for animal feed, and so emission estimates are particularly uncertain for this country.

Resulting estimates (Table 2) clearly show that silage has the potential to be a major source of VOC emissions. But they also show that more precise estimates are needed. For the US, for example, our estimates range from <1% to >9% of non-agricultural emissions. These average values do not show the variability in emission rates. In the San Joaquin Valley, for example, we estimate that silage emissions are at least 7% of non-agricultural sources, but may nearly match the sum from these other sources. Inventory estimates for the SJV range from approximately 13% to 30% of non-agricultural sources (CARB 2017; Howard et al. 2010), which falls in the range of the lower and upper limit of our predictions in Table 2 below.

**Table 2.** Estimates of VOC emissions from national inventories and estimated here, based on silage production and VOC emission factors.

| | Inventory anthropogenic VOC emissions[1] (Gg $yr^{-1}$) | | Total silage production (Tg $yr^{-1}$)[2] | Predicted silage VOC emissions (Gg $yr^{-1}$) | | |
|---|---|---|---|---|---|---|
| Region | Non-agricultural[1] | Agricultural | | Lower limit | Best estimate | Upper limit |
| United States | 15200 | 200 | 125 | 125 | 1000 | 1750 |
| European Union (EU 28) | 5840 | 1170 | 64 | 64 | 510 | 890 |
| San Joaquin Valley, California | 100 | 32[5] | 6.5 | 6.5 | 50 | 90 |
| Germany[6] | 812 | 208 | 26 | 26 | 210 | 360 |
| France | 623 | 1.7 | 15 | 15 | 120 | 210 |

Notes: [1]Emission inventory data for 2014 or 2015. EU data are from EEA (2017), US data are from EPA (2018b), SJV data are from CARB (2017). Agricultural estimates are incomplete in some cases, and known to be underestimates.

[2]Dry matter produced basis. US data are from NASS (2018). The US total includes maize, grass (haylage), and sorghum silage production, and the San Joaquin Valley total is for maize silage only, since no other types are produced in significant amounts. Data for Europe are from EuroStat (2018). All values for Europe are for maize silage only. In all cases, a loss of 15% dry matter from harvest through storage was applied. 1 Tg = 1 million tonnes.

[5]Excludes silage emission, which is reported as 13 Gg $yr^{-1}$.

[6]About 47% of silage maize produced in Germany is for biogas production (S. Kraume, Deutsches Maiskomitee e.V., personal communication), but the same emission factors were used for all locations presented in this table.

**Mitigation**

Silage additives may reduce VOC emission by reducing production of these compounds during ensiling. For given silage handling practices (e.g., sealing, exposure, feed depth) and fixed physical properties (density, particle size), mass transfer theory dictates that total volatilization of a particular compound from silage will be proportional to the mass of that compound produced (Hafner et al. 2012). The use of some antifungal chemical additives have been found to be very effective in reducing production of ethanol and, in some cases, ethyl esters, in corn silage in laboratory trials (Table 3).

Other additives have shown no effect, or, in some cases, increased VOC concentrations (Hafner et al. 2014; Weiß et al. 2016).

**Table 3.** Summary of results from laboratory or full-scale trials on effects on chemical antifungal additives on alcohols and esters in maize silage.

| Study | Additive (dose per forage fresh mass) | Effects | Other details |
|---|---|---|---|
| Kleinschmidt et al. (2005) | 1:1 potassium sorbate:EDTA (1 g $kg^{-1}$) | 80% reduction in ethanol | |
| Teller et al. (2012) | Potassium sorbate (1 g $kg^{-1}$) | 70% or greater reduction in ethanol | |
| Weiß and Auerbach (2013) | 21.9% sodium benzoate, 13.2% potassium sorbate (2 g $kg^{-1}$) | 72% reduction in ethanol, 45% in ethyl acetate and 58% in ethyl lactate | |
| Auerbach and Nadeau (2013) | 250 g/L sodium benzoate, 150 g/L potassium sorbate; 1.0, 1.5, 2.0 L $t^{-1}$ | 73 to 84% reduction in ethanol | Reduction did not depend on dose |
| Hafner et al. (2014) | Potassium sorbate (1 g $kg^{-1}$) | 58% reduction in ethanol, 46% reduction in ethyl acetate | A lower dose of potassium sorbate appeared to increase VOC production |
| Auerbach et al. (2015) | Sodium benzoate, potassium sorbate; 1.0. 2.0 L $t^{-1}$ | 45 to 51% reduction in ethanol | Reduction did not depend on dose |
| Hafner et al. (2015) | Potassium sorbate (1 g $kg^{-1}$) | >70% reduction in ethanol, >65% reduction in ethyl acetate | Also >50% reduction in 1-propanol when *Lactobacillus plantarum* was added along with K sorbate |
| Franco (2016) | Potassium sorbate (1 g $kg^{-1}$) | >80% reduction in ethanol, ethyl acetate, ethyl lactate, >60% reduction in acetaldehyde, >30% reduction in methanol | |
| Weiß et al. (2016) | 21.9% sodium benzoate, 13.2% potassium sorbate (2 g $kg^{-1}$) | 58% reduction in ethanol, 86% in ethyl acetate, 80% in ethyl lactate | Depending on air exposure: 25-40% reduction in ethanol, 63-77% in ethyl acetate and 46-49% in ethyl lactate |
| Brüning et al., In Weiß 2017 | 21.9% sodium benzoate, 13.2% potassium sorbate (2 g $kg^{-1}$) | 62% reduction in ethanol, 88% in ethyl acetate, 50% in ethyl lactate | |

Effects of other management practices are less clear. High compaction of silage (i.e., attaining a high density) has been suggested as an important means for reducing VOC emission (Mitloehner and Cohen 2016). In general sufficient silage density is important for producing high-quality feed. And while lower gas-filled pore space will reduce transport of VOC to the exposed silage face (Hafner et al. 2012), the effect on total silage VOC emission is probably small, since feeding and not storage or feedout is thought to be the major route of VOC loss (see Emission pathways section above). Moreover, production of both ethanol and ethyl esters has been found to be higher in compacted areas within bunker silos in Germany (Weiß et al. 2015) and pilot-scale experiments have shown an increase in response to higher compaction (Brüning et al. 2018). The most recent pilot-scale study with maize found higher acetate, ethanol, ethyl lactate and ethyl acetate in the high density treatments

but similar levels of methanol and 2-butanol between high and low density (Brüning et al. 2018). Overall these results suggest that high density may minimize respiration of sugars, providing more substrate for yeasts to produce ethanol in maize silages, where fermentation by LAB is more likely to be limited by low pH. Whether this holds true in silages where LAB fermentation is more likely to be limited by sugar availability needs to be studied.

Delayed sealing has long been associated with an increase in spoilage microorganisms including yeasts. For a delay in sealing of 16 to 24 h, Weiß et al. (2016) found no consistent effects across three trials in maize silage on ethanol, ethyl acetate and ethyl lactate. Recently, Brüning et al. (2018) studied delays in sealing of 2 and 4 d in maize silage. Both delayed sealing treatments increased ethyl lactate and ethyl acetate and decreased methanol and 2-butanol compared to immediate sealing. Ethanol was unaffected by the sealing treatment. More research in this area is needed and may be useful in uncovering the sources of some VOC. However, from a practical perspective, these results provide an additional reason for farmers to promptly seal silage piles and bunkers.

## Conclusions

Numerous volatile organic compounds (VOC) are produced by yeasts and bacteria during ensiling, and together these compounds make a significant contribution to VOC emission in the US and EU. Silage VOC emission is probably equivalent to > 5% of all other anthropogenic sources in both the US and EU. In some areas with high silage production, its contribution to VOC emissions are undoubtedly larger than national or continental averages, perhaps by an order of magnitude. However, uncertainty in emission estimates is high. Conversely, these is less uncertainty about how VOC emission can be reduced. Chemical additives that inhibit yeasts have the potential to cut silage VOC emission in half. With some exceptions, this topic is not receiving much attention by researchers. Research needs include:

- Development and testing of new, more accurate, methods for measuring VOC emission from silage or whole farms
- Accurate measurements of VOC emission on farms under a range of conditions
- Quantitative assessments of the contribution of silage VOC emission to air pollution for specific locations based on a combination of emission and atmospheric chemistry models
- Identification and quantification of VOC production pathways through application of molecular biology tools
- Evaluation of VOC-reducing silage additives from multiple perspectives (VOC, animal health and milk yield, economy) under diverse conditions
- Assessment of management practices other than use of silage additives on VOC emission

## References

Alanis P., Sorenson M., Beene M., Krauter, C., Shamp, B. & Hasson, A.S. (2008) Measurement of non-enteric emission fluxes of volatile fatty acids from a California dairy by solid phase micro-extraction with gas chromatography/mass spectrometry. Atmospheric Environment 42:6417-6424. doi: 10.1016/j.atmosenv.2008.05.015.

Auerbach H., Nadeau E. (2013) Effects of chemical additives on whole-crop maize silage traits. In: Michalk, D.L., Millar, G.D., Badgery, W.B., Broadfoot, K.M. (eds). Proceedings of the 22nd International Grassland Congress, New South Wales, Australia, 2012, pp. 736-737.

Auerbach, H., Weber, U., Weber, G., Weiss, K. & Theobald, P. (2015) Effects of different chemical additives on the fermentation and aerobic stability of high-moisture corn ensiled in bags. Proceeding of the XVII International Silage Conference, July 2015, Piracicaba, Sao Paulo, Brazil, pp. 542-543.

Bachy A., Aubinet M., Amelynck C., Schoon, N., Bodson, B., Moureaux, C., Delaplace, P., De Ligne, A. & Heinesch, B. (2018) Methanol exchange dynamics between a temperate cropland soil

and the atmosphere. Atmospheric Environment 176:229-239. doi: 10.1016/j.atmosenv.2017.12.016.

Bachy A., Aubinet M., Schoon N., Amelynck, C., Bodson, B., Moureaux, C. & Heinesch, B. (2016) Are BVOC exchanges in agricultural ecosystems overestimated? Insights from fluxes measured in a maize field over a whole growing season. Atmospheric Chemistry and Physics 16:5343-5356. doi: 10.5194/acp-16-5343-2016.

Birch G.G., Blakebrough N. & Parker K.J. (1981) Enzymes and Food Processing. Springer Netherlands.

Brown P., Watts P., Märk T.D. & Mayhew C.A. (2010) Proton transfer reaction mass spectrometry investigations on the effects of reduced electric field and reagent ion internal energy on product ion branching ratios for a series of saturated alcohols. International Journal of Mass Spectrometry 294:103-111. doi: 10.1016/j.ijms.2010.05.028.

Brüning D., Gerlach K., Weiß K. & Südekum K.-H. (2018) Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. Grass and Forage Science 73:53–66. doi: 10.1111/gfs.12288.

Brunner A., Ammann C., Neftel A. & Spirig C. (2007) Methanol exchange between grassland and the atmosphere. Biogeosciences Discussions 4:125-164. doi: 10.5194/bgd-4-125-2007.

CARB (California Air Resources Board). 2017. California Emissions Projection Analysis Model (CEPAM). URL: https://www.arb.ca.gov/app/emsinv/fcemssumcat/fcemssumcat2016.php. Accessed: 14 March 2018.

Carter W.P. (2009) Development of the SAPRC-07 chemical mechanism and updated ozone reactivity scales. Center for Environmental Research and Technology College of Engineering University of California. Final Report to the California Air Resources Board Contract No. 03-318. URL: http://www.engr.ucr.edu/\~carter/SAPRC/.

Chmelova S., Triska J., Ruzickova K. & Kalac P. (2009) Determination of aliphatic aldehydes in maize and grass silages using on-fibre derivatisation with O-(2,3,4,5,6-pentafluoro)benzylhydroxylamine. Animal Feed Science and Technology 152:152-160. doi: 10.1016/j.anifeedsci.2009.03.012.

Chung M.Y., Beene M., Ashkan S., Krauter, C. & Hasson, A.S. (2010) Evaluation of non-enteric sources of non-methane volatile organic compound (NMVOC) emissions from dairies. Atmospheric Environment 44:786-794. doi: 10.1016/j.atmosenv.2009.11.033.

Das M., Kang D., Aneja V.P., Lonneman, W., Cook, D.R. & Weseley, M.L. (2003) Measurements of hydrocarbon air–surface exchange rates over maize. Atmospheric Environment 37:2269-2277. doi: 10.1016/S1352-2310(03)00076-1.

Davison B., Brunner A., Ammann C., Spirig, C., Jocher, M. & Neftel, A. (2008) Cut-induced VOC emissions from agricultural grasslands. Plant Biology 10:76–85. doi: 10.1055/s-2007-965043.

Driehuis F. & van Wikselaar P.G. (2000) The occurrence and prevention of ethanol fermentation in high-dry-matter grass silage. Journal of the Science of Food and Agriculture 80:711-718. doi: 10.1002/(SICI)1097-0010(20000501)80:6<711::AID-JSFA593>3.0.CO;2-6.

EEA 2015. Non-methane volatile organic compounds (NMVOC) emissions. URL: https://www.eea.europa.eu/data-and-maps/indicators/eea-32-non-methane-volatile-1/assessment-4. Accessed: 22 March 2018.

EEA (European Environmental Agency) 2017. Air pollutant emissions data viewer (Gothenburg Protocol, LRTAP Convention). URL: https://www.eea.europa.eu/data-and-maps/dashboards/air-pollutant-emissions-data-viewer. Accessed 15 March 2018.

EPA (United States Environmental Protection Agency) 2003. List of Exempt Compounds. URL: https://www.google.com/url?q=http://www.cdpr.ca.gov/docs/emon/vocs/vocproj/2voc_exempt_list.pdf&sa=D&ust=1521491593714000&usg=AFQjCNFYItW2vZ31A_DMoL_tht_mRND6Xw. Accessed 19 March 2018.

EPA 2017. Air Quality - National Summary. URL: https://www.epa.gov/air-trends/air-quality-national-summary. Accessed 22 March 2018.

EPA (United States Environmental Protection Agency) 2018a. Technical Overview of Volatile Organic Compounds. URL: https://www.epa.gov/indoor-air-quality-iaq/technical-overview-volatile-organic-compounds. Accessed 19 March 2018.

EPA (United States Environmental Protection Agency) 2018b. 2014 National Emissions Inventory (NEI) Data. URL: https://www.epa.gov/air-emissions-inventories/2014-national-emissions-inventory-nei-data. Accessed 14 March 2018.

Eurostat. (2018) Green Maize by Area, Production, and Humidity. URL: http://ec.europa.eu/eurostat/tgm/refreshTableAction.do?tab=table&plugin=1&pcode=tag00101&language=en. Accessed: 16 March 2018.

Filya I. & Sucu E. (2010) The effects of lactic acid bacteria on the fermentation, aerobic stability and nutritive value of maize silage. Grass and Forage Science 65:446-455. doi: 10.1111/j.1365-2494.2010.00763.x.

Franco, R.B. (2016) Measuring Emissions and Developing Strategies to Mitigate Volatile Organic Compounds and Oxides of Nitrogen from Silage. PhD dissertation. University of California at Davis.

Galbally I. & Kirstine W. (2002) The production of methanol by flowering plants and the global cycle of methanol. Journal of Atmospheric Chemistry 43:195-229.

Gharechahi J., Kharazian Z.A., Sarikhan S., Jouzani, G., Aghdasi, M. & Hosseini Salekd, G. (2017) The dynamics of the bacterial communities developed in maize silage. Microb Biotechnol 10:1663-1676. doi: 10.1111/1751-7915.12751.

Graus M., Eller A.S.D., Fall R., Yuan. B., Yaling, Q., Westra, P., de Gouw, J. & Warneke, C. (2013) Biosphere-atmosphere exchange of volatile organic compounds over C4 biofuel crops. Atmospheric Environment 66:161-168. doi: 10.1016/j.atmosenv.2011.12.042.

Guenther A.B., Jiang X., Heald C.L., Sakylyanontvittaya, T., Duhl, T., Emmons, K.L. & Wang, X. (2012) The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions. Copernicus.

Hafner S.D., Franco R.B., Kung L., Rotz, C.A. & Mitloehner, F. (2014) Potassium sorbate reduces production of ethanol and 2 esters in corn silage. J Dairy Sci. doi: 10.3168/jds.2014-8537.

Hafner S.D., Howard C., Muck R.E. et al. (2013) Emission of volatile organic compounds from silage: Compounds, sources, and implications. Atmospheric Environment 77:827-839. doi: 10.1016/j.atmosenv.2013.04.076.

Hafner, S.D., Howard, C., Muck, R.E., Franco, R.B., Montes, F., Green, P.G., Mitloehner, F., Trabue, S.L. & Rotz, C.A. (2013) Emission of volatile organic compounds from silage: Compounds, sources, and implications. Atmospheric Environment 77, 827-839. https://doi.org/10.1016/j.atmosenv.2013.04.076.

Hafner, S.D., Montes, F. & Rotz, C.A. (2009) Modeling emissions of volatile organic compounds from silage, In: Proceedings 2009 ASABE Annual International Meeting, Reno, NV, pp. 1895-1911.

Hafner, S.D., Montes, F. & Rotz, C.A. (2012) A mass transfer model for VOC emission from silage. Atmospheric Environment 54, 134-140. https://doi.org/10.1016/j.atmosenv.2012.03.005.

Hafner, S.D., Montes, F., Rotz, C.A. & Mitloehner, F.M. (2010) Ethanol emission from loose corn silage and exposed silage particles. Atmospheric Environment 44, 4172-4180. https://doi.org/10.1016/j.atmosenv.2010.07.029.

Hafner, S.D., Windle, M., Merrill, C.C., Smith, M.L., Franco, R.B. & Kung Jr., L. (2015) Effects of potassium sorbate and Lactobacillus plantarum MTD1 on production of ethanol and other volatile organic compounds in corn silage. Animal Feed Science and Technology. https://doi.org/10.1016/j.anifeedsci.2015.07.007.

Hart, E. & Willaman, J. (1912) Volatile fatty acids and alcohols in corn silage. Journal of the American Chemical Society 34, 1619-1625.

Holopainen, J.K. (2004). Multiple functions of inducible plant volatiles. Trends Plant Sci. 9, 529-533. https://doi.org/10.1016/j.tplants.2004.09.006.

Holopainen, J.K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. Trends in Plant Science 15, 176-184. https://doi.org/10.1016/j.tplants.2010.01.006.

Hou, C.Y., Lin, Y.S., Wang, Y.T., Jiang, C.M. & Wu, M.C. (2008) Effect of storage conditions on methanol content of fruit and vegetable juices. Journal of food composition and analysis.

Howard, C., Kumar, A., Malkina, I., Mitloehner, F., Green, P., Flocchini, R. & Kleeman, M. (2010) Reactive organic gas emissions from livestock feed contribute significantly to ozone production in central California. Environmental Science & Technology 44, 2309-2314. https://doi.org/10.1021/es902864u.

Hu, J., Howard, C.J., Mitloehner, F., Green, P.G. & Kleeman, M.J. (2012) Mobile source and livestock feed contributions to regional ozone formation in central California. Environ. Sci. Technol. 46, 2781-2789. https://doi.org/10.1021/es203369p.

Jerrett, M., Burnett, R.T., Pope, C.A., Ito, K., Thurston, G., Krewski, D., Shi, Y., Calle, E. & Thun, M. (2009) Long-Term Ozone Exposure and Mortality. New England Journal of Medicine 360, 1085-1095. https://doi.org/10.1056/NEJMoa0803894.

Karl, T., Guenther, A., Lindinger, C., Jordan, A., Fall, R. & Lindinger, W. (2001) Eddy covariance measurements of oxygenated volatile organic compound fluxes from crop harvesting using a redesigned proton-transfer-reaction mass spectrometer. J. Geophys. Res.-Atmos. 106, 24157-24167. https://doi.org/10.1029/2000JD000112.

Kleinschmit, D., Schmidt, R. & Kung, L. (2005) The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. Journal of Dairy Science 88, 2130-2139.

Koziel, J., Jia, M. & Pawliszyn, J. (2000) Air sampling with porous solid-phase microextraction fibers. Analytical Chemistry 72, 5178-5186.

Kristensen, N., Storm, A., Raun, B., Rojen, B. & Harmon, D. (2007) Metabolism of silage alcohols in lactating dairy cows. Journal of Dairy Science 90, 1364-1377.

Krooneman, J., Faber, F., Alderkamp, A., Elferink, S., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J. & Vancanneyt, M. (2002) Lactobacillus diolivorans sp nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology 52, 639-646. https://doi.org/10.1099/ijs.0.01979-0.

Kung, L. & Shaver, R. (2001) Interpretation and use of silage fermentation analysis reports. Focus on Forage 3, 1-5.

Leytem, A.B., Dungan, R.S., Bjorneberg, D.L. & Koehn, A.C. (2011) Emissions of Ammonia, Methane, Carbon Dioxide, and Nitrous Oxide from Dairy Cattle Housing and Manure Management Systems. Journal of Environmental Quality 40, 1383-1394. https://doi.org/10.2134/jeq2009.0515.

Lindinger W., Taucher J., Jordan A., Hansel A. & Vogel W. (2006) Endogenous Production of Methanol after the Consumption of Fruit. Alcoholism: Clinical and Experimental Research 21, 939-943. https://doi.org/10.1111/j.1530-0277.1997.tb03862.x.

Liu Shao-Quan & Pilone Gordon J. (2001) An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. International Journal of Food Science & Technology 35, 49-61. https://doi.org/10.1046/j.1365-2621.2000.00341.x.

McDonald, P., Henderson, N. & Heron, S. (1991) The Biochemistry of Silage. Chalcombe.

McGechan, M.B. (1990) A review of losses arising during conservation of grass forage: Part 2, storage losses. Journal of Agricultural Engineering Research 45, 1-30. https://doi.org/10.1016/S0021-8634(05)80135-0.

Mitloehner, F.& Cohen, M. (2016) Reducing gaseous silage losses protects your bottom line. Progressive Dairyman 05 February 2016. https://www.progressivedairy.com/topics/feed-nutrition/reducing-gaseous-silage-losses-protects-your-bottom-line.

Montes, F., Hafner, S.D., Rotz, C.A. & Mitloehner, F.M. (2010). Temperature and air velocity effects on ethanol emission from corn silage with the characteristics of an exposed silo face. Atmospheric Environment 44, 1987-1995. https://doi.org/10.1016/j.atmosenv.2010.02.037.

Morgan, M.E. & Pereira, R.L. (1962) Volatile Constituents of Grass and Corn Silage. I. Steam Distillates. Journal of Dairy Science 45, 457-466. https://doi.org/10.3168/jds.S0022-0302(62)89427-2.

Mozaffar, A., Schoon, N., Bachy, A., Digrado, A., Heinesch, B., Aubinet, M., Fauconnier, M.-L., Delaplace, P., du Jardin, P. & Amelynck, C. (2018) Biogenic volatile organic compound emissions from senescent maize leaves and a comparison with other leaf developmental stages. Atmospheric Environment 176, 71–81. https://doi.org/10.1016/j.atmosenv.2017.12.020.

National Research Council (2008) Estimating Mortality Risk Reduction and Economic Benefits from Controlling Ozone Air Pollution. National Academies Press, Washington, D.C.

NASS (United States Department of Agriculture National Agricultural Statistics Service). (2018) Data and Statistics. URL: https://www.nass.usda.gov/Statistics_by_Subject/. Accessed: 13 March 2018.

Ngwabie, N., Schade, G., Custer, T., Linke, S. & Hinz, T. (2008) Abundances and flux estimates of volatile organic compounds from a dairy cowshed in Germany. Journal of Environmental Quality 37, 565-573. https://doi.org/10.2134/jeq2006.0417.

Nielsen, T., Kristensen, N. & Weisbjerg, M. (2007) Effect of harvest time on fermentation profiles of maize ensiled in laboratory silos and determination of drying losses at 60 degrees C. Acta Agriculturae Scandinavica Section A-Animal Science 57, 30-37. https://doi.org/10.1080/09064700701440447.

Ogink, N.W.M., Mosquera, J., Calvet, S. & Zhang, G. (2013) Methods for measuring gas emissions from naturally ventilated livestock buildings: Developments over the last decade and perspectives for improvement. Biosystems Engineering, Special Issue: Emissions from Naturally Ventilated Livestock Buildings 116, 297-308. https://doi.org/10.1016/j.biosystemseng.2012.10.005.

Østlie, H.M., Helland, M.H. & Narvhus, J.A. (2003) Growth and metabolism of selected strains of probiotic bacteria in milk. Int. J. Food Microbiol. 87, 17-27.

Pang, H., Qin, G., Tan, Z., Li, Z., Wang, Y. & Cai, Y. (2011) Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and recA gene analysis. Syst. Appl. Microbiol. 34, 235-241. https://doi.org/10.1016/j.syapm.2010.10.003.

Peddie, H.A. (1990) Ester formation in brewery fermentations. Journal of the Institute of Brewing 96, 327-331.

Pedrolli, D.B., Monteiro, A.C., Gomes, E., & Carmona, E.C. (2009) Pectin and pectinases: Production, characterization and industrial application of microbial pectinolytic enzymes. The Open Biotechnology Journal 3: 9-18.

Piškur, J., Compagno, C. (eds.) (2014) Molecular Mechanisms in Yeast Carbon Metabolism. Springer Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-55013-3.

Savoie, P. & Jofriet, J.C. (2003) Silage Storage, in: Buxton, D.R., Muck, R.E., & Harrison, J.H. (eds). Silage Science and Technology. American Society of Agronomy. Crop Science Society of America. Soil Science Society of America, Madison, WI, pp. 405-467.

Schink, B., & Zeikus, J.G. (1980) Microbial methanol formation: A major end product of pectin metabolism. Current Microbiology 4, 387-389. https://doi.org/10.1007/BF02605383.

Sharkey, T.D., Wiberley, A.E., & Donohue, A.R. (2008). Isoprene emission from plants: Why and how. Annals of Botany 101, 5–18. https://doi.org/10.1093/aob/mcm240.

Shaw, S.L., Mitloehner, F.M., Jackson, W., Depeters, E.J., Fadel, J.G., Robinson, P.H., Holzinger, R., & Goldstein, A.H. (2007) Volatile organic compound emissions from dairy cows and their

waste as measured by proton-transfer-reaction mass spectrometry. Environmental Science & Technology 41, 1310-1316.

Suomalainen, H. (1981). Yeast esterases and aroma esters in alcoholic beverages. Journal of the Institute of Brewing 87, 296–300. https://doi.org/10.1002/j.2050-0416.1981.tb04040.x

Teller, R.S., Schmidt, R.J., Whitlow, L.W., & Kung, L. (2012) Effect of physical damage to ears of corn before harvest and treatment with various additives on the concentration of mycotoxins, silage fermentation, and aerobic stability of corn silage. J. Dairy Sci. 95, 1428-1436. https://doi.org/10.3168/jds.2011-4610.

Tennant, R.K., Sambles, C.M., Diffey, G.E., Moore, K.A., & Love, J. (2017) Metagenomic Analysis of Silage. Journal of Visualized Experiments. https://doi.org/10.3791/54936.

Warneke, C., Luxembourg, S., de Gouw, J., Rinne, H., Guenther, A., & Fall, R. (2002). Disjunct eddy covariance measurements of oxygenated volatile organic compounds fluxes from an alfalfa field before and after cutting. Journal of Geophysical Research-Atmospheres 107.

Weiß K., Olbrich, C., & Thaysen, J. (2015) Volatile organic compounds (VOC) in maize silages at German dairy farms. Proceedings XVII International Silage Conference 1.-3.July 2015, Piracicaba, Sao Paulo, Brazil, pp. 98-99.

Weiß, K., & Auerbach, H. (2013) Novel results on the formation of volatile organic compounds in silages, In: Michalk, D.L., Millar, G.D., Badgery, W.B., Broadfoot, K.M. (eds). Proceedings of the 22nd International Grassland Congress, New South Wales, Australia, 2012, pp.721-724.

Weiß, K., Kroschewski, B., & Auerbach, H. (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. J. Dairy Sci. 99, 8053-8069. https://doi.org/10.3168/jds.2015-10323.

Weiß, K. (2017) Volatile organic compounds in silages – Effects of silo management factors on formation. Proceedings of the 5th International Symposium on Forage Quality and Conservation, 16.-18.11.2017, Piracicaba, Brazil, pp.181-210

Williams, A.G., Hoxey, R.P., & Lowe, J.F. (1997) Changes in temperature and silo gas composition during ensiling, storage and feeding-out grass silage. Grass & Forage Science 52, 176-189.

Wiß, F., Ghirardo, A., Schnitzler, J.-P., Nendel, C., Augustin, J., Hoffmann, M., & Grote, R. (2017) Net ecosystem fluxes and composition of biogenic volatile organic compounds over a maize field–interaction of meteorology and phenological stages. GCB Bioenergy 9, 1627-1643. https://doi.org/10.1111/gcbb.12454.

Xu, Z., He, H., Zhang, S., & Kong, J. (2017). Effects of inoculants Lactobacillus brevis and Lactobacillus parafarraginis on the fermentation characteristics and microbial communities of corn stover silage. Sci Rep 7. https://doi.org/10.1038/s41598-017-14052-1.

Yuan, B., Coggon, M.M., Koss, A.R., Warneke, C., Eilerman, S., Peischl, J., Aikin, K.C., Ryerson, T.B., & de Gouw, J.A. (2017) Emissions of volatile organic compounds (VOCs) from concentrated animal feeding operations (CAFOs): chemical compositions and separation of sources. Atmos. Chem. Phys. 17, 4945-4956. https://doi.org/10.5194/acp-17-4945-2017.

Zeyer, K., Schrade, S., Keck, M., & Emmenegger, L. (2013) A dual tracer gas ratio method for the determination of NH3 emissions in naturally ventilated cattle housing, In: Hassouna, M. (ed), Proceedings Emissions of Gas and Dust from Livestock Conference, 2012, Saint-Malo FR, pp. 374-377.

# Effect of wilting and *Lactobacillus buchneri* on the formation of volatile organic compounds in oat silage

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**Introduction** Oat is a winter cereal widely used as whole crop silage for feeding animals. A limiting factor for making high quality silage in pre-flowering stages is the low dry matter (DM) content of oat crop. Thus, wilting is a plausible strategy to decrease moisture content, but the exposure in the field (delayed ensiling) can raise the proliferation of undesirable microorganisms and affect the final quality of the silage, for instance, by decreasing the aerobic stability. Inoculation with heterolactic bacteria (e.g. *Lactobacillus buchneri*) is an alternative to alleviate this problem. However, both DM and inoculation with heterolactic bacteria may alter the profile of fermentation end-products and, in turn, silage conservation and nutritive value. The objective of this study was to evaluate the effects of wilting and a heterofermentative inoculant (*L. buchneri*) on the formation of volatile organic compounds in oat silage.

**Material and Methods** A plot of white oat (*Avena sativa* cv. URS Charrua) at head-emergence stage was split equally. During the morning, half-plot was cut and allowed to wilt for 5 h (Wilted), whereas the other half was directly harvested in the afternoon (Direct-cut, DC). Both forages were mechanically chopped (theoretical length of cut = 8 mm), divided in two piles, treated with *L. buchneri* 40788 [4 × $10^5$ colony-forming units (cfu)/g; LB] or distilled water (5 mL/kg; Ctrl) and packed (0.4 porosity) into 1.96 L jars (4 replicates per treatment). After 112 d of storage, silages were sampled for measuring the pH and contents of DM, $NH_3$-N (colorimetric method), lactic acid (colorimetric method) and volatile organic compounds (GC-MS). Data were analyzed as a 2 × 2 factorial arrangement using the Mixed procedure of SAS. Means were compared by Tukey's test ($\alpha = 0.05$).

**Results and Discussion** Wilting for 5 h was effective to increase DM content from 21% to 30%. In silages without inoculant, higher moisture content extended the fermentation, resulting in higher concentration of lactic acid and lower pH. Butyric acid concentration was low in all silages. In silages inoculated with LB there was a decrease in lactic acid concentration and a greater production of acetic acid, especially in DC silage. The anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *L. buchneri* is well stablished in the literature (Oude Elferink et al. 2001). Interestingly, the content of 1,2-propanediol was high in Wilted-LB silage, but not in DC-LB silage, probably because an indigenous pathway of converting 1,2-propanediol to 1-propanol and propionic acid by *L. diolivorans* (Krooneman et al. 2002). The latter compounds were found in high concentrations in DC-LB silage.

The LB also increased the contents of 2,3-butanediol and $NH_3$-N, perhaps by stimulating the metabolism of enterobacteria (McDonald et al. 1991). Unexpectedly, LB increased the concentrations of alcohols (ethanol, i-propyl alcohol, 2-butanol e methanol), especially in DC silages.

Esters are mainly formed in silages by abiotic esterification (chemical reaction) stimulated by low pH (Weiss 2017). The LB decreased the content of ethyl lactate, due to a reduction in lactic acid concentration, whereas increased the formation of ethyl acetate and propyl acetate, certainly because LB increased acetic acid, ethanol and 1-propanol concentrations.

**Table 1.** Dry matter, pH and fermentation products in oat silages.

| | Direct-cut | | Wilted | | | *P*[1] | | |
|---|---|---|---|---|---|---|---|---|
| Item | Ctrl | LB[2] | Ctrl | LB | SEM[3] | W | I | W × I |
| Dry matter (DM), % fresh matter | 21.6 | 20.5 | 30.8 | 30.1 | 0.31 | <0.01 | 0.02 | 0.61 |
| pH | 3.90$^{c}$ | 4.61$^{a}$ | 4.05$^{b}$ | 4.59$^{a}$ | 0.034 | 0.07 | <0.01 | 0.03 |
| $NH_3$-N, % N | 6.56$^{b}$ | 10.08$^{a}$ | 5.33$^{b}$ | 8.92$^{ab}$ | 0.373 | <0.01 | <0.01 | 0.92 |
| Lactic acid, % DM | 4.05$^{a}$ | 1.85$^{c}$ | 3.05$^{b}$ | 1.74$^{c}$ | 0.130 | <0.01 | <0.01 | <0.01 |
| Acetic acid, % DM | 1.51$^{c}$ | 6.97$^{a}$ | 1.43$^{c}$ | 4.44$^{b}$ | 0.321 | <0.01 | <0.01 | <0.01 |
| Ethanol, % DM | 0.15$^{c}$ | 0.92$^{a}$ | 0.14$^{c}$ | 0.56$^{b}$ | 0.073 | 0.03 | <0.01 | 0.03 |
| 1,2-Propanediol, % DM | 0.14$^{b}$ | 0.75$^{b}$ | 0.25$^{b}$ | 3.37$^{a}$ | 0.227 | <0.01 | <0.01 | <0.01 |
| 2,3-Butanediol, mg/kg DM | 228$^{b}$ | 5671$^{a}$ | 287$^{b}$ | 709$^{b}$ | 650.7 | <0.01 | <0.01 | <0.01 |
| Methanol, mg/kg DM | 152 | 232 | 196 | 208 | 14.3 | 0.58 | 0.03 | 0.09 |
| Propionic acid, mg/kg DM | 96$^{b}$ | 13318$^{a}$ | 87$^{b}$ | 724$^{b}$ | 1518.5 | <0.01 | <0.01 | <0.01 |
| Ethyl lactate, mg/kg DM | 53$^{a}$ | 42$^{b}$ | 49$^{ab}$ | 41$^{b}$ | 4.6 | 0.62 | 0.01 | 0.21 |
| Butyric acid, mg/kg DM | 15$^{b}$ | 19$^{a}$ | 17$^{a}$ | 15$^{b}$ | 0.9 | 0.20 | 0.32 | <0.01 |
| i-Valeric acid, mg/kg DM | 13$^{ab}$ | 13$^{ab}$ | 16$^{a}$ | 10$^{b}$ | 0.9 | 0.97 | <0.01 | 0.02 |
| 1-Propanol, mg/kg DM | 11$^{b}$ | 9068$^{a}$ | 8.8$^{b}$ | 185$^{b}$ | 888.7 | <0.01 | <0.01 | <0.01 |
| i-Butyric acid, mg/kg DM | 3.9$^{b}$ | 4.0$^{b}$ | 3.9$^{b}$ | 19$^{a}$ | 0.9 | <0.01 | <0.01 | <0.01 |
| Ethyl acetate, mg/kg DM | 3.3$^{b}$ | 29$^{a}$ | 2.5$^{b}$ | 25$^{a}$ | 2.9 | 0.34 | <.0.01 | 0.48 |
| Valeric acid, mg/kg DM | 2.6$^{b}$ | 4.3$^{a}$ | 2.9$^{ab}$ | 3.9$^{ab}$ | 0.38 | 0.93 | <0.01 | 0.31 |
| i-Propylic alcohol, mg/kg DM | 1.4$^{b}$ | 12$^{a}$ | 0.9$^{b}$ | 1.6$^{b}$ | 1.09 | <0.01 | <0.01 | <0.01 |
| 2-Butanol,mg/kg DM | 1.1$^{b}$ | 115$^{a}$ | 0.7$^{b}$ | 1.1$^{b}$ | 24.3 | 0.04 | 0.04 | 0.04 |
| Propyl acetate, mg/kg DM | 0.0$^{b}$ | 56$^{a}$ | 0.2$^{b}$ | 3.1$^{b}$ | 7.44 | <0.01 | <0.01 | <0.01 |
| Lactic acid/Acetic acid | 2.80$^{a}$ | 0.27$^{b}$ | 2.23$^{a}$ | 0.41$^{b}$ | 0.228 | 0.36 | <0.01 | 0.14 |
| 1,2-Propanediol + 1-Propanol + Propionic acid, % DM | 0.15$^{b}$ | 3.50$^{a}$ | 0.26$^{b}$ | 3.46$^{a}$ | 0.308 | 0.90 | <0.01 | 0.80 |

[1]W: wilting effect, I: inoculant effect. [2]Ctrl: without inoculant, LB: *L. buchneri* applied at 5 × $10^5$ cfu/g.
[3]Standard error of the mean.
[a,b,c,d] Tukey's test (α = 0.05).

**Conclusion** Both *L. buchneri* and moisture content markedly affected the fermentation profile. Overall, wilting decreased whereas *L. buchneri* increased the formation of volatile organic compounds in oat silage. High moisture content boosted the effect of *L. buchneri* on the formation of volatile organic compounds. Hence, treating oat silage up to 30% of DM with *L. buchneri* might not be recommended.

**References**

Krooneman, J., Faber, F., Alderkamp, A.C., Oude Elferink, S.J.H.W., Driehuis, F., Cleenwerck, L., Swings, J., Gottschal, J.C. & Vancanneyt, M. (2002) *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 52, 639-646.

McDonald, P., Henderson, A.R., & Heron, S.J. E. 1991. The Biochemistry of Silage. 2nd ed. Marlow, UK: Chalcombe Publications. 340 p.

Oude Elferink, S. J., Krooneman, J., Gottschal, J. C., Spoelstra, S. F., Faber, F. & Driehuis, F. (2001) Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Applied and Environmental Microbiology, 67, 125-132.

Weiss, K. (2017) Volatile organic compounds in silages – Effects of management factors on their formation: A review. Slovak Journal of Animal Science, 50, 55-67.

# Formation of volatile organic compounds during the course of maize fermentation depending on sealing time and silage additive use

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**Keywords:** Ethanol, ethyl esters, maize, silage, VOC, yeasts

**Introduction** Ethyl esters, correlated with ethanol, are considered as indicator substances for total volatile organic compounds (VOC) production in silage (Weiss et al. 2016). Delayed sealing and silage additive affect fermentation pattern and VOC production in maize silage (Weiss et al. 2016, Brüning et al. 2017). As no data is available on the accumulation pattern of VOC, our study investigated VOC formation during the course of fermentation.

**Material and Methods** A lab-scale ensiling trial was carried out with forage maize (26.8% DM), ensiled without silage additive (Con) or treated with 2 L $t^{-1}$ of a chemical additive (SA, composed of 257 g $L^{-1}$ sodium benzoate, 134 g $L^{-1}$ potassium sorbate, 57 g $L^{-1}$ ammonium propionate) and either immediately sealed (prompt) or sealed with a delay of 24 h (delay). The four treatments evaluated were Con, Con_Del, SA, SA_Del. Three replicate 1.5-L glass jars per treatment (packing density: 195 kg DM $m^{-3}$) were stored at 22 °C and opened after 3, 7, 16, 34, 62 and 142 days of storage. All samples (n=72) were submitted to analysis for DM losses, fermentation pattern, including ethyl lactate (EL) and ethyl acetate (EA), and yeasts (Weiss et al. 2016). Statistical analysis was performed by procedures MIXED and REG of SAS, 9.4 using fermentation length (F), sealing time (S) and additive (A) as experimental factors. Significance was declared at $P<0.05$.

**Results and Discussion**. With the exception of SA silage, a dramatic yeast development during the early phases of fermentation was observed when compared with fresh maize at ensiling (log 4.3 colony-forming units (cfu) $g^{-1}$, $P<0.001$), but the magnitude was larger in delayed than in promptly sealed silage (day 3: log cfu $^{-1}$ 7.3 *vs* 5.3, $P<0.001$, day 7: log cfu $^{-1}$ 6.8 *vs* 5.2, $P<0.001$). Concurrently, very high concentrations of ethanol had already been produced during the first 16 days of storage in Con, Con_Del and SA_Del silages (20.0 *vs* 8.5 g $kg^{-1}$ DM, SxA interaction, $P<0.01$). As from day 7, promptly sealed silage treated with the additive consistently contained the lowest ethanol concentration, which remained stable until the end of fermentation. The DM losses followed the same pattern (data not shown). Delayed sealing triggered higher losses than found in promptly sealed silage (10.0 *vs* 4.5%, FxSxA interaction, $P<0.01$) but additive use reduced losses (prompt: 3.9 *vs* 5.1%, delay: 8.8 *vs* 11.3%, FxSxA interaction, $P<0.01$). The production pattern over time of lactic acid was similar across treatments during the early stages of fermentation and the peak concentration was detected on day 34 (FxSxA interaction, $P<0.001$). However, with the exception of SA silage, which attained its maximum acetic acid content already after three days of storage, a continuous increase of this fermentation product (FxSxA interaction, $P<0.01$) was observed. The formation of EL persisted throughout the fermentation process, and on day 142 Con_Del silage contained the highest level (508 *vs* 194 …334 mg $kg^{-1}$ DM, $P<0.001$), which confirms data by Weiss et al. (2016). Largely restricted EL accumulation was observed by additive use (prompt: 194 *vs* 334 mg $kg^{-1}$ DM, delay: 264 *vs* 508 mg $kg^{-1}$ DM, $P<0.001$). On the contrary, EA was very rapidly and intensively produced reaching concentrations of about 3,000 mg $kg^{-1}$ DM in Con_Del silage already after three days of storage. On day 34, the highest level was measured in untreated silage sealed with delay (4,556 mg $kg^{-1}$ DM) compared with the other treatments (1,486 mg $kg^{-1}$ DM, SxA interaction, $P<0.001$). Similar accumulation pattern was observed over time in all other treatments but the analysed concentrations differed depending on storage length (FxSxA interaction, $P<0.05$). On this account, the relationship between the concentrations of ethanol and EA ($R^2$=0.35, Root MSE=985.1, $P<0.001$) was not as close as shown by Weiss et al. (2016), or that between ethanol and EL ($R^2$=0.65, Root MSE=74.8, $P<0.001$) in this study. It

can only be speculated about the reasons for the decline in EA concentrations as opposed to the continuous increase in EL content. The pH of silage which could have affected the reaction equilibrium can be ruled out because it was always below 4, with only marginal differences between treatments (data not given). More so, the differences in accumulation pattern between EA and EL may be attributed to different vapour pressures of EA (98 mbar) and EL (2 mbar). Thus, more than the produced EA may have escaped the porous silage and collected in the headspace of the jars to be released along with other fermentation gas whenever a lid-lifting overpressure had built-up. The different concentration levels and the rates of formation of EL and EA, especially during the early fermentation phases, may lead to the assumption that also their synthesis, chemical or biochemical, is different. As certain yeast species can produce EA (Fredlund et al. 2004) and we observed a dramatic increase in yeast count and a significant EA accumulation within 3 days of storage, we believe that the major proportion of EA was produced directly by biochemical pathways, whereas EL was primarily formed by chemical reaction between ethanol and lactic acid as previously suggested by Weiss et al. (2016).

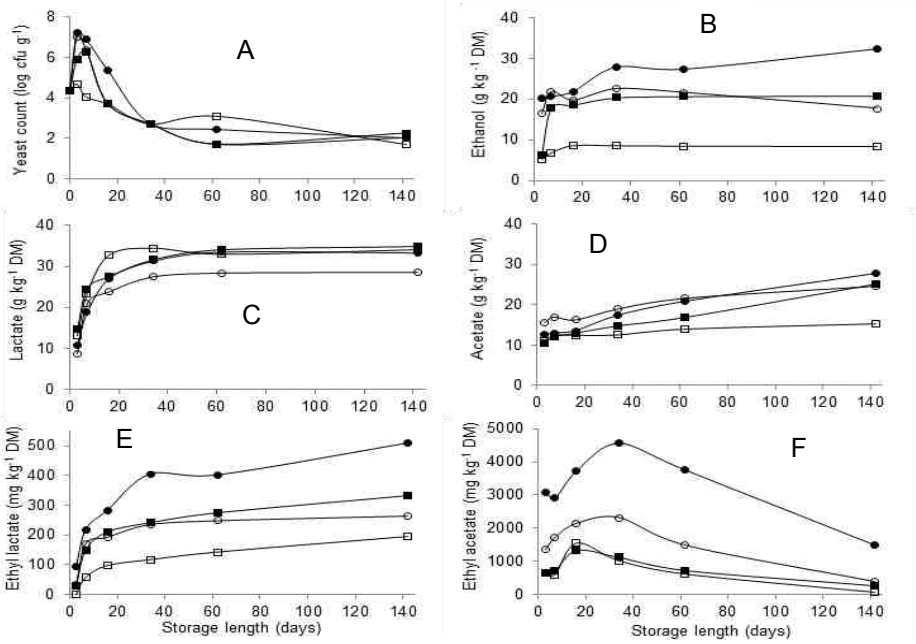


**Figure 1.** Development of yeasts (A) and the concentrations of ethanol (B), lactate (C), acetate (D), ethyl lactate (E), ethyl acetate (F) during the course of fermentation of maize, ■Con, □SA, ●Con_Del, ○SA_Del.

**Conclusions** Delayed sealing stimulates yeast activity resulting in excessive ethanol production, formation of ethyl acetate and high DM losses. The correlation between the concentrations of ethanol and ethyl esters vary depending on the type of ester. Additive use can partially alleviate the detrimental effects of delayed sealing regarding yeast development, DM losses and VOC formation.

**References**

Brüning D., Gerlach K., Weiß K. & Südekum K.-H. (2017) Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. Grass and Forage Science, 73, 53-66.

Fredlund, E., Druvefors, U. Ä., Olstorpe, M. N., Passoth, V. & Schnürer, J. (2004) Influence of ethyl acetate production and ploidy on the anti-mould activity of *Pichia anomala*. FEMS Microbiology Letters, 238, 133-137.

Weiss, K., Kroschewski, B. & Auerbach, H. (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. Journal of Dairy Science, 99, 1-17.

# Formation of climate relevant gases during the ensiling process

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**Keywords:** Greenhouse gases, Methane, Nitrous oxide, Silage

**Introduction** Climate related trace gases are a well discussed topic in agricultural animal husbandry and slurry application to the soil. Formation of emissions associated to the upstream and downstream sectors are mostly neglected. Measurements of climate relevant gas formation by animal or silage are rare. Therefore, this study dealt with the course of gas formation during the process of ensiling under anaerobic conditions with focus on formation of methane ($CH_4$) and nitrous oxide ($N_2O$) immediately after exclusion and depletion of oxygen. Furthermore, the influence of forage type and dry matter (DM) concentration were part of the investigation.

**Material and Methods** Grass (GS) and lucerne (LS) were ensiled at two different levels of DM. Both were produced from a second cut and were cut on an evening in May 2017 and wilted overnight. Additionally, the forages were dried in the next morning on a prepared ground for different lengths in the sun. The more wilted material was four hours longer dried (LD) than the only wilted (W) material. Forages were stored anaerobically in 120-L plastic barrels (n = 3 per treatment). During filling the barrels, density was considered *via* pressing force of a hydraulic press. Furthermore, filling of fresh matter was regulated and controlled by the filled forage mass and the known volume of the barrels. Subsequently, the barrels were closed with a lid and a rubber septum for gas sampling with a double needle and evacuated glass vials. Ensiling time was seven weeks (49 d). There was a constant temperature of 23.7 ± 1.4°C during storage of the silage barrels in an experimental room. The measuring interval was 30 min in the first 12 h and decreased to 2 h afterwards in the first 2 days, up to each 2 days over the period of 49 days. The concentrations of carbon dioxide ($CO_2$), $CH_4$ and $N_2O$ in the glass vials were analysed by gas chromatography (GC-ECD/FID) (Schmithausen et al. 2018). Table 1 shows the DM concentration and the labelling of the used silages.

**Table 1**. The dry matter (DM) content of the forages ensiled in 120 l barrels (n = 3 per treatment)

| Variant | Material per barrel | DM content (%) | Density per m³ (kg $m^{-3}$) |
|---|---|---|---|
| GS W | Grass silage (wilted) | 24.0 ± 2.2 | 170.0 |
| GS LD | Grass silage (longer dried) | 30.6 ± 1.5 | 185.8 |
| LS W | Lucerne silage (wilted) | 23.2 ± 0.3 | 156.7 |
| LS LD | Lucerne silage (longer dried) | 25.5 ± 0.5 | 134.2 |

DM: dry matter

**Results and Discussion** The concentration of $CO_2$ increased within the first 12 h after silo closure up to 50%. After 24 h until 30 h the $CO_2$ concentration increased to 80% to 90% and even up to 100% after 48 h. Therefore, after the first hours $CO_2$ concentration was outside the measuring range of the GC and had to be deluted for analysis. Figure 1 shows the course of $CO_2$ concentration during approximately one day. There are no differences between GS and LS. However, the substrate with higher DM (dashed line) appears with lower $CO_2$ concentration.

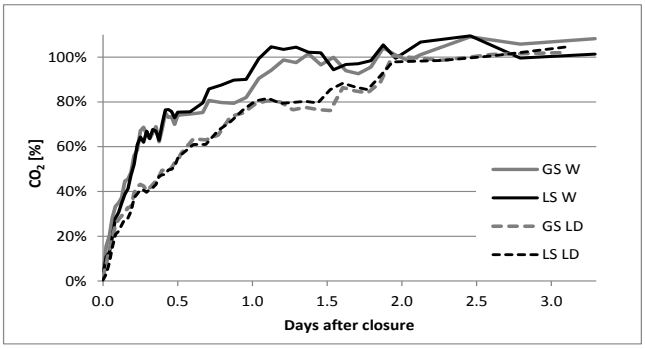


**Figure 1**. Average $CO_2$ concentration in 120 l barrels with lucerne (LS) and grass (GS) over the time.

The $CH_4$ concentration showed a small increase within the first 2 d up to 5-6 ppm following by a decrease to 1 ppm. Later on there was a strong increase in $CH_4$ concentration up to 100 ppm for LS beginning on d 10 (Figure 2, left, dashed line). The GS showed no increase in $CH_4$ concentration. Between day 30 and 40 a low $CH_4$ concentration of 2 ppm was measurable for GS.

A remarkable increase in $N_2O$ concentration within the first 5 d up to 1,200 ppm was measured (Figure 2, right). After that a decrease in the $N_2O$ concentration until d 30 with a constant value between 50 and 300 ppm appeared. Spoelstra (1985) also described formation of $N_2O$ during ensiling by anaerobic activity of *Enterobacteriaceae* species at the beginning of ensilage. A higher DM content results in increasing $N_2O$ concentrations. A reason could be less $CO_2$ formation at the beginning and consequently less suppression of $N_2O$ by $CO_2$. The GS shows higher $N_2O$ concentration than LS and the level remains higher for GS up to the end.

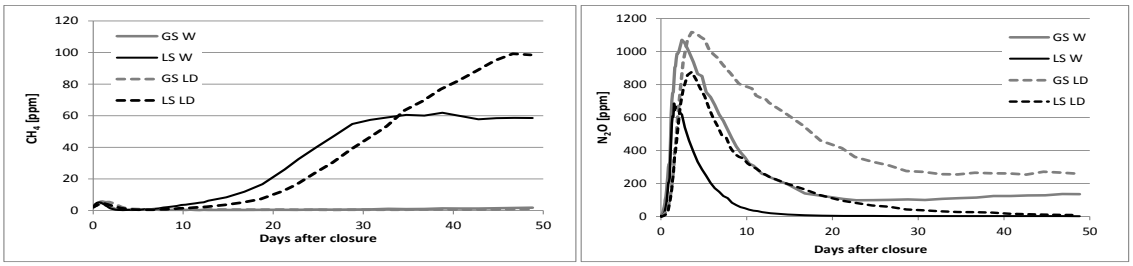


**Figure 2**. Average $CH_4$ (left) and $N_2O$ (right) concentration in 120 l barrels with lucerne (LS) and grass (GS) over the time.

The gas measurements showed remarkable concentrations of $N_2O$. Such a formation of $N_2O$ during the process of ensiling can lead to further outgassing after opening and feeding of the silage. Subsequent $N_2O$ emissions determined from a ruminant animal can originate from the forages eaten by the animal. Possibly the formation of $CO_2$ provides a dilution of the $N_2O$ concentration during the first 10 days. Simultaneously $N_2O$ decreases and remains constant while $CO_2$ production is decreased by the ongoing ensiling process.

**Conclusion** Formation of $N_2O$ takes place at the beginning of the ensiling process and $N_2O$ remains a long time in the silage and is latest released during opening of the silage. The level of $N_2O$ concentration is comparable to other investigations. The courses of $CO_2$, $CH_4$ and $N_2O$ concentration show no similarities, such that the formation of these gases must be completely separate processes. Further research on the amount of gas formation would be instructive.

**References**

Schmithausen, A. J., Trimborn, M., & Büscher, W. (2018) Sources of nitrous oxide and other climate relevant gases on surface area in a dairy free stall barn with solid floor and outside slurry storage. Atmospheric Environment, 178, 41-48.

Spoelstra, S. F. (1985) Nitrate in silage. Grass and Forage Science, 40, 1-11.

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# Nitrate degradation and gas formation in silages

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**Keywords:** Additive, fermentation, nitrite, nitric oxide

**Introduction** The formation of a whole spectrum of gases occurs during the ensiling process. The formation of gases is undesirable, because it is often a sign of undesirable processes in silages, and causes concern about the impact on the global environment. The objective of the study was to investigate nitrate degradation and gas formation from silages treated with a silage additive based on Na-benzoate, K-sorbate and Na-nitrite and compare with untreated and lactic acid bacteria inoculated silages with particular focus on NO formation.

**Materials and Methods** A clover-grass ley (52% grasses) was harvested with lye and shortly wilted on Juli 10$^{th}$ 2016, nearby Uppsala. The forage was chopped, mixed with a suspension of *Clostridium tyrobutyricum* viable spores at the rate of 1 x $10^5$ per g fresh forage (FF) **to create difficult ensilable crop** and divided in three fractions. One was left untreated and served as control, while the rest was treated with either of two silage additives; one with a bacterial inoculant (*Lactococcus lactis, Lactobacillus buchneri*) at the rate of 250000 colony-forming units/g FF, and the second one with Safesil Challenge (Na-benzoate, K-sorbate, Na-nitrite) at the rate of 3 L/ton FF. Forages were ensiled in triplicates 1.7 L laboratory glass silos for 98 days and in steel laboratory silos (25 L) for 7 days. Glass silos were aerated weekly for 2 hours. Two forms of gas collection were applied. The Automatic Methane Potential Test System II (Bioprocess Control AB, Sweden) was used to measure total gas production from glass silos. Steel silos were used to collect escaping gases into Tedlers bags (Supelco) for gas determination. Measurements of gas composition was provided by mobile gas analyzer Optima 7 (MRU GmbH in Neckarsulm, Germany). The analyzer determined the concentrations of $O_2$, CO, NO, $NO_x$, and **gas** temperature. Gas measurement was performed during the first 7 days of storage. After the storage, the same analyses were performed to determine silage quality as described by Knicky and Spörndly (2009).

**Results** The wilting resulted in forage dry matter (DM) content 34.2%, and crude protein and WSC concentrations of 13.3% and 9.9% of DM respectively. The content of nitrate in forage was 3 mg/kg DM. All additive treated silages were found to have a lower silage pH and ammonia-N but higher concentration of lactic acid (Table 1).

**Table1.** Chemical composition of silages after 98 days of storage (n=3).

| Treatment | DM | pH | $NH_4$-N* | $NO_3$-N | $NO_2$-N | Lactic acid | Acetic acid | Butyric acid |
|---|---|---|---|---|---|---|---|---|
| | % | | % of TN | mg/kg DM | | % of DM | | |
| Control | 30.8 | 5.4 | 14.5 | 10.5 | 0.0 | 0.4 | 1.9 | 2.2 |
| S.Challenge | 33.5 | 4.7 | 8.3 | 15.1 | 1.4 | 4.8 | 1.3 | 0.0 |
| Bacteria | 32.4 | 4.7 | 9.1 | 2.0 | 0.1 | 2.3 | 4.2 | 1.7 |
| **$LSD_{0.05}$** | | **0.04** | **0.49** | **3.07** | **0.58** | **0.32** | **0.35** | **0.28** |
| P-value | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

Bacterially inoculated silages had lower formation of butyric acid than control but higher than chemical additive. Concentrations of nitrate and nitrite were remarkably lower in control silages than in Safesil Challenge treated silages during the whole period (Figure 1). The concentration of nitrite-N displayed reduction shortly after ensiling with considerable decrease after 72 hours of storage in Safesil

Challenge treated silages (Figure1). Results of gas measurements revealed the lowest formation of gases in Safesil Challenge treated silages from 4th hour of ensiling to the end of measurement.

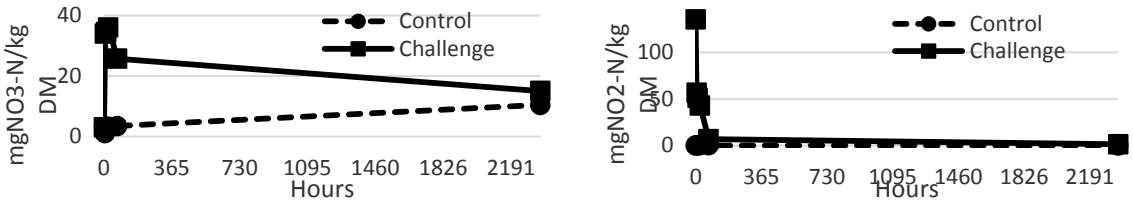


**Figure 1**. Nitrate-N and nitrite-N formations in silages during 98 days of storage (n=3).

The total gas formation in Safesil Challenge treated silages was 4 times and 5 times lower than in bacterially treated and control silages, respectively (Figure 2). Gas composition displayed a significantly higher formation of NO and $NO_x$ gases in Safesil Challenge treated silage (3051ml NO and 2907ml $NO_x$ **per silo**) while formation of these gases in control silage was negligible during the whole measuring time (11ml NO and 10ml $NO_x$ **per silo**).

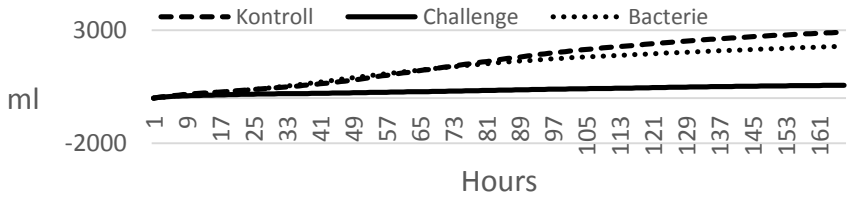


**Figure 2.** Development of total gas formation in silages over the period of 7 days of storage (n=3).

**Discussion** The present study confirmed the importance of nitrate for a successful ensiling process. In agreement with Hein (1970) that ensiling of forages with low nitrate content often results in silages with high butyric acid contents, a low content of natural nitrate in the present crop was probably one of the factor causing butyric acid formation in control and bacterially treated silages. On the contrary, silages treated with Safesil Challenge containing Na-nitrite were free of butyric acid. The rate of nitrite disappearance in Safesil Challenge silages is in agreement with a previous study (Knicky and Spörndly 2009). It is also assumed that the higher nitrate content in Safesil Challenge silage at the end of storage than at the beginning of fermentation was caused by chemical conversion of added Na-nitrite to nitrate (McDonald et al. 1991). Differences in gas formation could be explained by differences in fermentation intensity and variation in bacterial composition among the silage treatments. A high gas formation in bacterially treated silages is assumed to be a consequence of addition of bacterial microflora which intensified the fermentation process. On the other hand, Safesil Challenge possesses a rather selective inhibitory property which restricts particularly undesirable fermentation processes. As the fresh forage was practically free of natural nitrate, the formation of NO and $NO_x$ gases in Safesil Challenge treated silages was the consequence of Na-nitrite degradation present in Safesil Challenge.

**Conclusions** Ensiling of low nitrate content forage resulted in butyric acid formation in silages while the addition of a chemical additive based on Na-benzoate, K-sorbate and Na-nitrite prevented that. Total gas formation was significantly reduced in Safesil Challenge silages and it was associated with increased NO and $NO_x$ presence in the gas composition.

**References**

Hein, E. (1970) Die Beeinflussung des Gärungsverlaufes bei der Grobfuttersilierung durch den Nitratgehalt des Ausgangsmaterials. (The impact of the nitrate content of green fodder on the silage fermentation.), Dissertation, Deutsche Akademie Landwirtschaftswissenschaften, Berlin, Germany.

Knicky, M. & Spörndly, R. (2009) Sodium benzoate, potassium sorbate and sodium nitrite as silage additives. Journal of the Science of Food and Agriculture, 89, 2659-2667.

McDonald, P., Henderson, A.R. & Heron, S.J E. (1991) The Biochemistry of Silage. Chalcombe Publications, Marlow, Bucks, UK, 108 p.

# Sugar beets with varying ensiling partners: losses and volatile organic compounds

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**Keywords:** losses, sugar beets mixtures, volatile organic compounds

**Introduction** The end of the sugar quota system in the EU by 2017 may go along with increasing availability and decreasing market prices of sugar beets. Thus, an alternative use as farm grown feed was tested. Sugar beets can be stored in clamps during the winter time. However, when it is getting warmer in spring, sugar beets tend to spoil. Thus it should be evaluated in terms of losses and silage quality which partners would be apt to ensile broken sugar beets with to absorb the effluent and to serve as appropriate ruminant feed. It was hypothesized that there exist mixing partners which are especially suitable to reduce the loss of mass during ensiling.

**Materials and Methods** Two laboratory scale ensiling experiments were performed in March and in April 2017 with beets stored in a clamp. The mixing partners were selected based on their availability at that time of the year and their contribution to either fibre or protein complementation (Table 1). The ratio was calculated derived from the water holding capacity of the mixing partners determined before, adapted from Barsnick (2003) and the amount of effluent to be expected from the sugar beets. Two elevated percentages of grass silage (30 %) and grass hay (10%) were included as considered for on-farm solutions. Pieces of unwashed pure sugar beets and eight mixtures (Table 1, treatment abbreviations used from here on) were ensiled in quadruplicates in modified Rostock Model Silos (Hoedtke and Zeyner, 2011), i.e. a double layer of vacuum bags, where effluent could drain off in a closed system. Silos were opened after 2 months of anaerobic storage and silages evaluated for sensory and chemical quality parameters, including volatile organic compounds (Weiß et al., 2016) for the ensiling in March. Aerobic stability was assessed according to Honig (1990). Descriptive statistics and univariate analysis with post hoc Tukey HSD test were performed using SPSS (Version 19).

**Table 1.** Ratio of sugar beet (SB) to mixing partner (in % fresh matter, FM) and proximate constituents in silages (g/kg DM)

| Treatment | SB | Partner | Description | Crude ash | Crude protein | Crude fibre |
|---|---|---|---|---|---|---|
| **SB** | 100.0 | 0.0 | Pure sugar beet | 240.9 | 57.6 | 53.5 |
| **Hay10** | 90.0 | 10.0 | Grass hay (85 % DM) | 160.0 | 63.6 | 135.3 |
| **Hay4** | 95.6 | 4.4 | Grass hay (85 % DM) | 171.5 | 61.2 | 93.5 |
| **Sil30** | 70.0 | 30.0 | Grass silage (53 % DM) | 208.4 | 87.5 | 143.5 |
| **Sil7** | 93.0 | 7.0 | Grass silage (53 % DM) | 222.4 | 66.6 | 77.9 |
| **BP** | 88.8 | 11.2 | Pressed sugar beet pulp | 222.5 | 60.1 | 70.8 |
| **FP** | 91.4 | 8.6 | Field pea. coarsely ground | 163.9 | 95.3 | 61.9 |
| **RM** | 93.8 | 6.2 | Rapeseed meal | 185.1 | 121.6 | 71.8 |
| **STR** | 96.7 | 3.3 | Straw. finely shredded | 231.3 | 53.8 | 90.9 |
| | | | *SEM* | *9.61* | *0.98* | *1.57* |

**Results** During the initial phase of the ensiling in March an extreme gas production was observed in general. Resuming both trials, the fresh matter (FM) losses ranged between 4.6-9.4%, dry matter (DM) losses averaged 15%, SB being at the upper end and combinations of Sil30 or of Hay10 or Hay4 resp. at the lower end (Table 2). This corresponded also mostly to the effluent losses where additionally RM at 6% or STR at 3 % of FM proved as efficient absorbents. The fermentation quality of

all treatments was high: no butyric acid was detected despite a mean in crude ash of 201 g/kg DM (Table 1), acetic acid was below 30 g/kg DM, pH corresponded to DM content (Table 2). Only ethanol concentrations were high, at the lower end in Sil30 and Hay10 and in the upper end in BP (Table 2). This went along with very high concentrations of further volatile organic compounds in the March trial (Table 3) compared to the values reported for whole-crop maize silages by Weiß et al. (2016). The ethanol content was linearly correlated to the sum of ethyl lactate+acetate (R=0.62, P<0.001). The aerobic stability as defined by ≥3°C above ambient was very variable and not consistent across the two trials ranging from 2-7 d.

**Table 2.** Fermentation quality of silages (acids, ethanol in g/kg DM) from both trials (n=8 /treatment)

| Treatment | $DM_{cor}$ | FM losses (%) | Effluent* | pH | Lactic acid | Acetic acid | Ethanol |
|---|---|---|---|---|---|---|---|
| **SB** | 236 $^{d}$ | 9.37 $^{a}$ | 3.23 $^{a}$ | 3.63 $^{e}$ | 66.6 | 23.5 | 206.3 $^{ab}$ |
| **Hay10** | 297 $^{a}$ | 4.83 $^{d}$ | 0.05 $^{d}$ | 3.77 $^{bc}$ | 54.3 | 21.5 | 142.8 $^{bcd}$ |
| **Hay4** | 245 $^{d}$ | 5.56 $^{cd}$ | 1.11 $^{cd}$ | 3.69 $^{de}$ | 56.7 | 20.5 | 171.1 $^{abcd}$ |
| **Sil30** | 276 $^{abc}$ | 4.59 $^{d}$ | 0.46 $^{d}$ | 3.81 $^{a}$ | 67.9 | 27.9 | 108.7 $^{d}$ |
| **Sil7** | 239 $^{d}$ | 8.87 $^{ab}$ | 3.13 $^{a}$ | 3.68 $^{de}$ | 63.7 | 17.3 | 164.0 $^{abcd}$ |
| **BP** | 235 $^{d}$ | 9.37 $^{a}$ | 3.25 $^{a}$ | 3.64 $^{e}$ | 58.1 | 19.0 | 224.3 $^{a}$ |
| **FP** | 284 $^{ab}$ | 7.68 $^{abc}$ | 2.92 $^{ab}$ | 3.71 $^{cd}$ | 62.3 | 20.3 | 124.7 $^{cd}$ |
| **RM** | 268 $^{bc}$ | 6.45 $^{bcd}$ | 1.31 $^{abc}$ | 3.80 $^{ab}$ | 70.4 | 24.7 | 157.6 $^{abcd}$ |
| **STR** | 256 $^{cd}$ | 5.92 $^{cd}$ | 0.18 $^{d}$ | 3.74 $^{bcd}$ | 58.8 | 21.3 | 198.5 $^{abc}$ |
| **SEM** | 1.7 | 0.188 | | 0.005 | 2.98 | 0.99 | 5.71 |

$DM_{cor}$: DM corrected for VFA, lactic acid & alcohols (Weissbach and Strubelt, 2008); *% of original FM weight at ensiling

**Table 3.** Volatile organic compounds in the March trial (in mg/kg DM) and water soluble carbohydrates (in g/kg DM) (n=4 per treatment)

| Treatment | Ethyl lactate | Ethyl acetate | Methanol | Propanol* | WSC |
|---|---|---|---|---|---|
| **SB** | 3037 $^{ab}$ | 579 | 1116 $^{bc}$ | 115 $^{cd}$ | 98.2 $^{ab}$ |
| **Hay10** | 1812 $^{c}$ | 1970 | 711 $^{c}$ | 22 $^{cd}$ | 62.2 $^{abcd}$ |
| **Hay4** | 2286 $^{bc}$ | 525 | 1013 $^{bc}$ | 0 $^{d}$ | 76.8 $^{abc}$ |
| **Sil30** | 1698 $^{c}$ | 540 | 760 $^{bc}$ | 1531 $^{a}$ | 14.6 $^{e}$ |
| **Sil7** | 2292 $^{bc}$ | 616 | 816 $^{bc}$ | 205 $^{bcd}$ | 60.5 $^{bcd}$ |
| **BP** | 3461 $^{a}$ | 1033 | 2241 $^{a}$ | 396 $^{bcd}$ | 38.4 $^{cde}$ |
| **FP** | 1867 $^{c}$ | 481 | 952 $^{bc}$ | 139 $^{cd}$ | 92.2 $^{ab}$ |
| **RM** | 1824 $^{c}$ | 647 | 1183 $^{bc}$ | 634 $^{b}$ | 99.7 $^{a}$ |
| **STR** | 2135 $^{bc}$ | 2137 | 1242 $^{b}$ | 452 $^{bc}$ | 29.6 $^{de}$ |
| ***SEM*** | *73.8* | *144.5* | *35.3* | *30.5* | 2.74 |
| ***P*** | *<0.001* | *0.056* | *<0.001* | *<0.001* | *<0.001* |

*propyl acetate was not detected

**Conclusions** For unwashed broken sugar beets, in terms of losses hay, straw, rapeseed meal or a high proportion of high DM grass silage seem to be advantageous ensiling partners, the latter also limiting the amount of ethanol formed and increasing the crude protein content significantly. However, their use in ruminant feeding is limited by their crude ash and volatile compounds concentrations.

**References**

Barsnick R. (2003) Investigations on the acceptance and digestibility of dried sugar beet pulp differently processed in horses [in German]. Hannover, Germany.

Hoedtke S. & Zeyner A. (2011) Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. Journal of the Science of Food and Agriculture, 91, 841-849.

Honig H. (1990) Evaluation of aerobic stability. Proceedings of the EUROBAC Conference, 12-16 August 1986, Uppsala. pp. 76-82.

Weiß K., Kroschewski B & Auerbach H (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. Journal of Dairy Science, 99,1-17; http://dx.doi.org/10.3168/jds.2015-10323.

# Volatile organic compounds in silages – possible effects on intake and metabolism by ruminants and quality of ruminant products: a review

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**Keywords:** alcohol, aldehyde, carboxylic acid, ester, ketone

**Introduction** Silages contain numerous volatile organic compounds (VOC) which are often formed during fermentation or anaerobic storage (via biochemical or microbial processes) but can also be present in the plant already before ensiling or be built later on during aerobic exposure. In literature, the meaning of 'VOC' is diverse and not clearly defined. The European Union defines a VOC as 'any organic compound having an initial boiling point less than or equal to 250 °C (482 °F) measured at a standard atmospheric pressure of 101.3 kPa'. Due to their chemical structure and sensory characteristics several VOC may influence feed intake and metabolism of ruminants and may also be transferred into their products (e.g., meat or milk). The aim of this study is to summarize the literature on VOC in silages and to draw conclusions regarding their impact on ruminants.

**Material and Methods** A literature review was conducted with focus on VOC in silages and possible effects on ruminants during and after ingestion. The VOC were grouped according to their chemical class and the following five classes were studied (number of different compounds used for this study in parentheses): Alcohols (34), aldehydes (14), carboxylic acids (16), esters (37) and ketones (17). For each chemical compound, its reported concentration in silages and, after ingestion by ruminants, in rumen fluid and in milk or cheese was considered. The effects of VOC from silages on rumen metabolism and productivity of ruminants were summarized. Furthermore, studies were evaluated with regard to a possible transfer of VOC from forages into milk or cheese produced by ruminants.

**Results and Discussion** Metabolism: The impact of the VOC belonging to different chemical classes on the metabolism of ruminants turned out to be very diverse. Alcohols seem to impact the rumen metabolism. Here, most research has been conducted with ethanol and propanol. However, a general conclusion cannot be drawn as some alcohols inhibit certain rumen microbes while others seem to stimulate their growth (Kristensen et al. 2007). Alcohols which are not already metabolised in the rumen are absorbed through the rumen epithelium and transported to the liver (Randby et al. 1999). They are not found in faeces such that a complete metabolization seems plausible (Fischer et al. 2015). Their effect on feed intake is variable and due to their high energetic value sometimes increases in productivity are reported. Different alcohols have to be studied separately in terms of their effects on the metabolism of ruminants. In contrast to this, aldehydes are typically completely metabolized in the rumen without impacting the metabolism of ruminants. In different studies, aldehydes were neither detected in rumen fluid nor in faeces of ruminants. The exact way of their degradation or further metabolism is still unclear and studies are rare (Chmelová et al. 2009). Different carboxylic acids are formed during the ensiling process with lactic acid being the most dominant fermentation product. Lactic acid did not affect feed intake or digestion of ruminants (Daniel et al. 2013). Acetic and propionic acids have shown to reduce feed intake several times but without affecting digestive processes (Brown and Radcliff 1972). High concentration of carboxylic acids in silages reduced feed intake and performance (Huhtanen et al. 2002) but low to moderate concentrations had no effects or even positive effects, e.g., on treatment of ketosis (Hueter et al. 1956) such that a kind of threshold level appears to exist for some of them with neutral/positive (below) or negative (above threshold) effects. For most of the other carboxylic acids there is a lack of studies such that general conclusions cannot be drawn. For silage esters and ketones, effects on the ruminal metabolism are not extensively described in literature up to now. Many esters are odorous and highly volatile such that they contribute to the sensory characteristics of forages which might affect feed intake (Gerlach et al. 2013). Besides formation of esters during ensiling esters can also be synthesized in the rumen where an inhibition of some microbes is discussed (Kristensen et al. 2007). However, there is a lack of literature on the effects of esters on rumen microbes or the metabolism of ruminants. Milk: Also the effects of VOC on the composition of milk are diverse and the five classes have to be considered separately. The aldehydes, carboxylic acids and esters found in milk seem to be independent of their occurrence in the ingested forage. For example, in several studies no relationship was observed between ester composition and concentration in feedstuffs and ester

concentration in milk. Esters occurring in products of dairy cows seem to originate from synthesis in the udder or synthesis during the ripening process of cheese. Alcohols, on the other hand, can have effects on milk composition (Raun and Kristensen 2012) and milk flavour (Randby et al. 1999) already in low concentrations, as shown for ethanol and propanol. For ketones, a direct transfer from silage to milk seems probable as increased ketone concentrations in silages resulted in higher concentrations in milk (Villeneuve et al. 2013). Some ketones have also shown to influence milk flavour. Cheese: The results of VOC effects on cheese composition are equivocal and difficult to interpret. Many VOC occurring in cheese are formed during ripening such that a transfer from forage to product is difficult to track, as described for aldehydes, esters and carboxylic acids. Volatile fatty acids in cheese, for example can be a product of lipolysis during ripening or of carbohydrate fermentation by lactic acid bacteria (Verdier-Metz et al. 1998). For alcohols and ketones there is a lack of literature with regard to cheese quality. Ketones are important with regard to the flavour of cheese and can be found in different kinds of cheese in a broad variety; however, in most cases their origin is still unclear (transfer from forage, synthesis by rumen microbes or ruminant, synthesis during milk production or cheese ripening).

**Conclusion** The VOC occurring in silages are very diverse regarding their chemical structure and also regarding their impact on the metabolism of ruminants and ruminant product quality such they have to be considered separately. Major VOC in silages have been studied in literature but for most of the numerous other products the knowledge on their formation and mode of action in ruminants is limited.

**References**

Brown, D.C. & Radcliff, J.C. (1972) Relationship between intake of silage and its chemical composition and in vitro digestibility. Australian Journal of Agricultural Research, 23, 25-33.

Chmelová, S., Tríska, J., Ruzicková, K. & Kalac, P. (2009) Determination of aliphatic aldehydes in maize and grass silages using on-fibre derivatisation with O-(2,3,4,5,6-pentafluoro)benzylhydroxylamine. Animal Feed Science and Technology, 152, 152-160.

Daniel, J.L., Amaral, R.C., Goulart, R.S., Zopollatto, M., Santos, V.P., Toledo Filho, S.G., Cabezas-Garcia, E.H., Lima J.R., Santos M.C. & Nussio, L.G. (2013) Short-term effects of silage volatile compounds on feed intake and digestion in beef cattle. Journal of Animal Science, 91, 2321-2331.

Fischer, S., Trefz, P., Bergmann, A., Steffens, M., Ziller, M., Miekisch, W., Schubert, J. S., Köhler H. & Reinhold, P. (2015) Physiological variability in volatile organic compounds (VOCs) in exhaled breath and released from faeces due to nutrition and somatic growth in a standardized caprine animal model. Journal of Breath Research, 9, 027108.

Gerlach, K., Roß, F., Weiß, K., Büscher, W. & Südekum, K.-H. (2013) Changes in maize silage fermentation products during aerobic deterioration and effects on dry matter intake by goats. Agricultural and Food Science, 22, 168-181.

Hueter, F.G., Shaw, J.C. & Doetsch, R.N. (1956) Absorption and dissimilation of lactates added to the bovine rumen and the resulting effects on blood glucose. Journal of Dairy Science, 39, 1430-1437.

Huhtanen, P., Khalili, H., Nousiainen, J.I., Rinne, M., Jaakkola, S., Heikkila, T. & Nousiainen, J. (2002) Prediction of the relative intake potential of grass silage by dairy cows. Livestock Production Science, 73, 111-130.

Kristensen, N.B., Storm, A., Raun, B.M.L., Røjen, B.A. & Harmon, D.L. (2007) Metabolism of silage alcohols in lactating dairy cows. Journal of Dairy Science, 90, 1364-1377.

Randby, Å.T., Selmer-Olsen, I. & Baevre, L. (1999) Effect of ethanol in feed on milk flavor and chemical composition. Journal of Dairy Science, 82, 420-428.

Raun, B.M.L. & Kristensen, N.B. (2012) Metabolic effects of feeding high doses of propanol and propylacetate to lactating Holstein cows. Livest. Sci. 144, 37-47.

Verdier-Metz, I., Coulon, J.-B., Pradel, P., Viallon, C. & Berdagué, J.-L., (1998) Effect of forage conservation (hay or silage) and cow breed on the coagulation properties of milks and on the characteristics of ripened cheeses. Journal of Dairy Research, 65, 9-21.

Villeneuve, M.P., Lebeuf, Y., Gervais, R., Tremblay, G.F., Vuillemard, J.C., Fortin, J. & Chouinard, P.Y. (2013) Milk volatile organic compounds and fatty acid profile in cows fed timothy as hay, pasture, or silage. Journal of Dairy Science, 96, 7181-7194.

# Fermented feed for pigs and poultry

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**Keywords:** feed fermentation, dietary fibre, lactic acid bacteria, fungi

## Abstract

Due to an increasing competition between humans and livestock animals for food sources, an inefficient utilization of grains as food or feed can be considered as a rising problem worldwide. By-products of the food industry generally comprise a high amount of dietary fibre. This, however, reduces the nutrient digestibility and hence impairs nutrient efficiency in monogastric animals. Currently, different methods of feed pre-treatments are known to improve feed efficiency and warrant sustainability in pig and poultry production systems. To compensate for poor feed efficiency, pretreatment technologies such as fermentation can be applied. Fermentation with bacteria or fungi can degrade dietary fibre, improve the bioavailability of minerals and reduce the pH value in feed and the digesta in the gastro intestinal tract. Furthermore, fermented feedstuffs are known for their prebiotic, antimicrobial and antioxidant properties. As fermentation lowers the pH of the feedstuff the intestinal microbial and physiological balance can be improved, and the gut immune system can be stimulated by an improved barrier function of the gut. Additionally, the application of fermented feed is positively correlated with the main zootechnical performance parameters, resulting from improved nutrient digestibility and reduction of anti-nutritional factors. Thus, fermentation of feedstuffs and and in particular fibre rich industrial by-products for pigs and poultry is of rising interest, as it enables innovative end-use strategies as an animal feed compound.

## Introduction

The use of side-streams accumulating in the food industry as feedstuffs comprises a future-oriented and sustainable approach for an economically and ecologically practicable handling of resources. Hence, owing to volatile prices for feedstuffs, the use of by-products as feedstuffs but also conservation and valorization strategies have become of major interest for animal production systems. The biodegradation of anti-nutritional factors in cereals or by-products via fermentation further enables innovative end-use strategies as an animal feed compound.

Fermentation can be considered as a metabolization of organic material by bacteria, fungi or a combination of both (Madigan et al. 2003). Generally, fermentation is a dynamic process aiming to degrade starch and sugar to lactic acid, alcohol, methane, carbon dioxide and short chain fatty acids (SCFA). However, depending on the water content included in the fermentation process, a more or less pronounced energy loss of the substrate can be expected (Pieper et al. 2011, Humer et al. 2013, 2014). From an agrarian point of view, fermentation can be commonly used to improve storage quality or nutrient utilization of feed (Humer and Schedle 2016). Through fermentation with bacteria and fungi dietary fibre can be disintegrated and bioavailability of minerals can be improved by degrading anti-nutritional factors such as phytate or specific proteins (Feng et al. 2007, Canibe and Jensen 2012, Humer and Schedle 2016). Moreover, fermented feed can improve the intestinal microbial and physiological balance, stimulates the gut immune system and improves the barrier function of the gut by lowering the gut pH (Niba et al. 2009). In agriculture, a popular and widely used kind of feed conversation is ensiling. The advantages of this process are beside an improvement in nutrient

digestibility, a reduction of pathogen bacteria, and an extension of preservation time in animal feed (Canibe and Jensen 2012). However, also substrates fermented with mushrooms are known for prebiotic, antimicrobial and antioxidant properties (Fard et al. 2014). It has already been reported that fungi such as Phlebia floridensis are capable of producing lignocellulolytic enzymes to degrade lignin and can therefore be recommended for improving the digestibility of fibre rich agricultural residues (Sharma and Arora 2010).

Generally, a selective fermentation under determined conditions (temperature, moisture and fermentation time) using lactic acid bacteria should be preferred over an uncontrolled fermentation process (Song et al. 2008). Beside the well-known silage process for ruminants, fermentation of total feed mixtures but also of selected feed compounds became evident in pig production in the past few years (Canibe and Jensen 2003, Jørgensen et al. 2010). However, there is some evidence that a fermentation of single feed compounds is more positively correlated with the main zootechnical performance parameters resulting from improved nutrient digestibility and reduction of anti-nutritional compounds. Therefore, compared to a fermentation of total feed mixtures a fermentation of single feed compounds should be preferred (Canibe et al. 2007). This is evident for the following reasons: Fermentation of whole feed mixtures results in amino acid degradation of the supplemented crystalline amino acids and subsequently in a suboptimal supply of lysine for the organism. Hence, an adequate supply with amino acids for the animal can be harmed by fermentation of selected feed compounds such as cereals or dietary fibre rich by-products like wheat bran or rape-seed meal. Moreover, the palatability of entire fermented feed mixtures may be decreased by the production of biogenic amines such as cadaverin and result in a decreased daily feed intake (Canibe and Jensen 2003, 2010). In the light of the considerations above, fermentation of animal feed can be a future oriented pre-treatment to enhance the digestibility of animal feed and can have positive effects on feed conservation and animal health. The present article will focus on feed/feed stuff fermentation for pig and poultry with lactic acid bacteria or fungi.

**Feed fermentation with lactic acid bacteria**

Lactic acid bacteria, such as Lactobacillus plantarum and Lactobacillus paracasei can be used as starter cultures for compound feed or single feed stuff fermentation (Kraler et al. 2014). In the course of the fermentation lactic acid bacteria convert water-soluble carbohydrates into organic acids, reduce low molecular weight sugars, decrease the pH value and the bacterial count of lactic acid bacteria accumulates (Humer et al. 2013, Chen et al. 2013, Jakobsen et al. 2015). Furthermore, the amount of sucrose, fructan, starch and soluble non-starch polysaccharides can be reduced through fermentation with lactic acid bacteria (Jørgensen et al. 2010). Even if no reduction of non-starch polysaccharides is observed, it is assumed that non-starch polysaccharides are degraded by breaking down parts of the cellulose/β-glucan and pectin, without complete degradation to mono- or disaccharides. This thesis is supported by an increased non-starch polysaccharides digestibility of fermented feed in pigs, resulting from degradation to smaller poly- and oligomers (Jakobsen et al. 2015). Moreover, fermentation can hydrolyze cell wall complexes and release encapsulated nutrients such as fat or improve the availability of macro and trace elements by its phytate degrading properties (Kraler et al. 2014, Humer and Schedle 2016). However, the degradation and quality of fermented feed depends on the material fermented, the starter cultures and fermentation conditions or their content of secondary plant metabolites such as condensed tannins (Humer et al. 2013, Iank Bueno et al. 2018).

**Feed fermentation with fungi**

Solid-state fermentation with fungi is mainly used for fibre rich animal feed compounds, due to the non-starch polysaccharide and lignin degrading properties of enzymes produced by these microorganisms (Lai et al. 2015, Elisashvili et al. 2008). Furthermore, fermentation with fungi increases the phenolic content of feed. Additionally, a viscosity and phytate decreasing effect has been observed (Wanzenböck et al. 2017). Many mushroom species such as Lentinula edodes, Agaricus bisporus, Agaricus blazei, Hericium caput-medusae, Pleurotus ostreatus, Pleurotus eryngii,

Fomitella fraxinea, Flammulina velutipes, Ganoderma lucidum, Cordyceps inensis and Cordyceps militaris can be source of bioactive substances, modulating animal health status (Bederska-Lojewska et al. 2017). Moreover, oyster mushroom waste or by products fermented with mushrooms possess antioxidant and prebiotic properties (Bederska-Lojewska et al. 2017, Xu et al. 2018). White-rot fungi, such as Pleurotus sp, are capable of degrading agro-industrial by-products comprising lignocellulose via their enzymes such as laccases, lignin- and manganese peroxidase, mannase and xylanase (Castoldi et al. 2014, Elisashvili et al. 2008). Previously, it has been reported that solid-state fermentation of wheat bran using Pleurotus eryngii or Pleurotus ostreatus can decrease NDF content, viscosity and phytate content and can therefore be recommended as an appropriate valorization strategy for fibre rich by-products (Wanzenböck et al. 2017). In a further study, a solid-state fermentation of wheat bran for 12 days using white rot fungi resulted in increased laccase and manganese peroxidase activity and higher concentrations of quercitin, phenolics and crude polysaccharides, which is associated with lignin degradation (Wang et al. 2017). In this respect, a fermentation of soybean meal with Aspergillus oryzae resulted in higher crude protein, ash and crude fat contents (Fard et al. 2007). However, to date, the number of studies examining the effect of solid-state fermentation using fungi is still very limited and future studies will be necessary to evaluate the effect of feed fermentation with various fungi species.

**Fermented feed in diets for pigs**

In pig production, feeding fermented liquid feed is a technique mostly used in several northern parts of Europe (e.g. Denmark, north Germany). Thus, feed is produced by a fermentation of solid substrates mixed with water at certain temperatures for a certain time. The process is performed with or without adding starter cultures and mostly lactic acid bacteria (Lactobacillus paracasei, Lactobacillus plantarum and others), yeasts, such as Candida milleri, Kazachstania exigua, Candida pararugosa and Kazachstania buderi and Enterobacteriaceae are involved (Gori et al. 2011, Kraler et al. 2014). In other parts of Europe (south Germany, Austria), ensiling of whole maize or cereal grains is a common used technique for storage of wet maize or cereals. Studies hitherto undertaken showed that such fermented feed stuffs like maize or wheat bran in diets for pigs improved nutrient digestibility and hence performance (Humer et al. 2013, Humer et al. 2014, Humer and Schedle 2016, Gori et al. 2011, Kraler et al. 2014). Generally, fermentation of animal feed can improve the apparent ileal digestibility (AID) of dry matter, organic matter, crude fibre, ash, phosphorus, calcium and energy in pigs (Jørgensen et al. 2010, Kraler et al. 2014). As a result, a modification of the intestinal physiology (microbiome, microbial metabolites, intestinal morphology) was observed (Humer et al. 2014, Kraler et al. 2015). Jakobsen et al. (2015) reported that a fermentation of dried distiller grains with solubles (DDGS) comprising of 80% wheat and 20% barley can improve AID and apparent total tract digestibility (ATTD) of nutrients when administered to pigs. A diet containing 60% fermented DDGS can increase the AID of non-starch polysaccharides from 7.7% to 15.6% and the ATTD from 58.0% to 60.2%. Furthermore, dry matter, crude protein and phosphorus digestibility was enhanced by the supplementation of fermented DDGS. In the aforementioned study using fermented DDGS in pigs´ diets, no effects on Enterobacteriaceae counts in ileum and distal colon/faeces were observed. However, higher lactic acid bacteria counts, presumably originating from the fermented feed in the small intestine and lower counts in the colon/faeces were observed (Jakobsen et al. 2015). In accordance with those findings Canibe et al. (2007) also observed reduced lactic acid bacteria counts in the colon and no effect on Enterobacteriaceae in the gut, resulting from fermented liquid feed or fermented grain. This effect may be due to an absence of low molecular weight carbohydrates in the gut and is further supported by lower concentrations of SCFA in the middle colon of piglets fed with fermented feed. The same study revealed that the Lactobacillus delbrueckii ssp bulgaricus count was reduced in the stomach due to fermented feed supplementation. Furthermore, Clostridium perfringens and Clostridium lituseburense counts increased in the middle colon due to supplementation with fermented feed. However, the pathogenicity of clostridia varies with the strain and its effect on animal health is not predictable (Canibe et al. 2007). Moreover, the supplementation of pigs´ diets with 15% fermented wheat bran seems to promote the growth of beneficial bacteria like lactobacilli in the gut and fermentation of wheat bran may possess the ability to modulate the gastrointestinal microbiota

(Kraler et al. 2016). To sum it up, the fermentation of single feedstuffs applying different fermentation techniques for pigs can enhance nutrient digestibility, as well as the digestibility of macro and trace elements and hence improve performance. Additionally, a modulation of the intestinal microbiota and as result the intestinal physiology has been observed (Canibe et al. 2007, Kraler et al. 2015).

**Fermented feed in diets for poultry**

To investigate the feasibility of feed fermentation with bacteria and yeast several in vitro as well as in vivo trials are available. Studies considered in the following section applied several lactic acid bacterial strains, as well as Bacillus sp., however, yeast and a combination of yeast and bacteria was also often used for feed fermentation. Most feed fermentations were performed with an inoculation of selected microbes, but also natural, spontaneous fermentation processes are evident. The positive effects of fermentation mentioned above (e.g. improved digestibility, performance, feed hygiene, reduced pH, fibre degradation) would render fiber rich feedstuff also more attractive for poultry. Feed fermentation may be a practical and cost-efficient approach to reduce pathogens present in feedstuffs, challenging the poultry industry (Niba et al. 2009). As already known, dietary fibre is poorly digested/fermented by poultry (Lai et al. 2015). Fermentation as a pretreatment may improve the digestibility of by-products by degrading dietary fibre. However, there are a few studies hitherto undertaken that report a successful improvement of by-products via solid-state fermentation using fungi. Similar to pigs, it is recommended to ferment only single feed ingredients such as wheat bran, containing high amounts of dietary fibre (Teng et al. 2017). Feeding broilers with diets supplemented with 1% of mushroom waste can enhance some immune parameters without impairing the performance. However, 2% of mushroom waste in the diet declined feed conversion ratio and weight gain. Mushroom waste in general, increased villus height und crypt depth of the jejunum but showed no influence on relative organ weights. Furthermore, a higher level of antibody titers against influenza virus and a protective effect on the heterophil:lymphocyte ratio were reported by Fard et al. (2014). In a further study, a solid state fermentation of wheat bran for 12 days using white-rot fungi modulated the expression of antioxidant molecular targets in broiler chicken (Wang et al. 2017). Although no effect on performance was found due to the fermentation of wheat bran, a high antioxidant status is aimed to provide cell function and preserve animal health. Thus, feeding fermented wheat bran offers an innovative approach towards decreasing the lipid peroxidation level without impairing the performance (Wang et al. 2017). Nevertheless, literature reports of improved performance parameters due to a feed supplementation with 5% pre-fermented (Cunninghemella elegans) cereal-based product enriched with γ-linolenic acid. In detail, final body weight of the broiler fed with fermented feed increased in parallel with decreased feed consumption and improved feed conversion ratio. Eviscerated carcass yield was improved from 72.4% to 74.2% due to feeding 5% fermented product (Kovalik et al. 2017). A study by Teng et al. (2017) reported decreased NDF and ADF levels resulting from a fermentation of wheat bran using Bacillus amyloliquefaciens. This result can be attributed to the fact that microorganisms like Bacillus amyloliquefaciens can produce great amounts of protease, xylanase and cellulase after only three days of fermentation. Feeding broilers with 10% of the aforementioned fermented wheat bran resulted in higher amounts of lactic acid bacteria in the ileum but did not alter the production of acetic acid, propionic acid or butyric acid in the gut. Lactic acid bacteria prefer low-molecular weight carbohydrates to produce lactic acid. Thus, Bacillus amyloliquefaciens may have degraded complex carbohydrates and therefore promoted lactic acid bacterial growth in the gut (Teng et al. 2017). High proportions of lactic acid bacteria are desirable due to their potential of inhibiting pathogenic bacteria by producing lactic acid and SCFA. Also an increased ileal mucosa sIgA, known for protecting the intestinal epithelium from toxins and pathogens was reported. These effects increased the nutrient absorption and may result in an improved average daily gain and feed conversion ratio of broilers (Peng et al. 2016). Chen et al. (2009) found an increase in growth performance due to a fermentation of feed with Bacillus subtilis var. natto and Saccharomyces cerevisiae using a two-stage fermentation process. Results also showed an increased weight of the proventriculus and liver. These effects may be due to an increased feed uptake up to 8.3%. However, fermentation with Saccharomyces cerevisiae without Bacillus subtilis var. natto showed no effect on performance. It is assumed that Bacillus subtilis produces protease, amylase and lipase, and thus is

inevitable for a successful improvement of digestibility. Lai et al. (2015) fermented Pleurotus eryngii stalk residues and soybean hulls with Aureobasidium pullulans and found a partial elimination of anti-nutritional factors in the substrate and an increase of antioxidant components. Authors observed an improved oxidative status, improved villus height/crypt depth ratio in jejunum and ileum and higher catalase activity of broiler chicken feed with 5% to 10% fermented stalk residues and soybean hulls. To enhance the quality of liquid feed for Landes geese Chen et al. (2013) used Bacillus subtilis var. natto and Saccharomyces cerevisiae for feed fermentation. In case of animal performance, the increased nutrient digestibility resulted in higher feed intake and body weight gain. Authors also observed a significant impact on the microbiota population in the gut. Vast amounts of lactic acid, low pH and large numbers of lactic acid bacteria in the feed resulted in increased Lactobacillus and decreased E. coli counts in the small and large intestine. Furthermore, a reduction of total counts of aerobic bacteria in the large intestine caused by a reduced nutrient availability due to the metabolized low-molecular weight carbohydrates during fermentation was observed. Another study using fermented feed for laying hens obtained similar results Engberg (2009). Although laying hens receiving the fermented diet showed a lower feed intake, animals had higher body weights and the egg shell quality was improved. These results suggest a higher nutrient digestibility and results are also supported by a higher pH value in the ileum and caecum caused by lower microbial activity. A lack of fermentable substrates and an improved digestibility may result in a reduction of bacteria in the gut (Engberg et al. 2009). It is essential to know that secondary metabolites from fungi are capable of raising the anti-oxidative status of laying hens and thus laying performance, egg quality and cholesterol level can be positively influenced (Bederska-Lojewska et al. 2017). Studies with laying hens hitherto undertaken revealed an improved egg quality and egg mass, as well as lower cholesterol levels in egg yolks from laying hens fed with fermented feed using fungi (Lee et al., 2014, Wang et al. 2015). Lee et al. (2014) fed laying hens with 1% to 5% of Flammulina velutipes mycelium fermented with Bacillus subtilis and Klebsiella sp. and observed improved albumen height, Haugh unit, egg shell weight and shell thickness. The diets supplemented with fermented mycel were also capable of suppressing pathogenic bacterial proliferation in the gut and decreased NH3 gas emission. Additionally, Wang et al. (2015) found an improved egg mass and lower cholesterol, when laying hens were fed with 2% rice fermented with Cordyceps militaris. Fermented wheat bran with Pleurotus eryngii improved digestibility of fat and retention of phosphor in laying hens compared to native wheat bran. Furthermore, the fermentation reduced villi length, villi:crypth ratio and the thickness of the mucus layer in jejunum (Steinbauer et al. unpublished data). Based on those studies, it can be concluded that fermentation of feedstuffs can improve the digestibility/retention of nutrients in diets for poultry by degrading dietary fibre. Additionally, there is some evidence for enhanced immune parameters, some health preserving effects, increased nutrient absorption and increased performance of broilers receiving fermented feed.

## Conclusion

In conclusion, feed fermentation of single feedstuffs aims to enhance the digestibility, reduce anti nutritional factors and as a result improve performance. Fermentation with lactic acid bacteria lowers the pH by high lactic acid concentration and improves the hygienic status of animal feed. A promoted growth of beneficial bacteria and a modulation of the intestinal microbiota have been observed in pigs receiving fermented feed. Similar results are relevant for poultry. Fermented feed for broiler can improve immune parameters, but also improved egg quality has been observed for laying hens. As pigs and poultry are in direct competition with humans concerning foodstuffs, fermentation of dietary fibre rich by-products for pigs and poultry is of rising interest, as it enables innovative end-use strategies toward animal feeding.

## References

Bederska-Łojewska, D., Świątkiewicz, S., & Muszyńska B. (2017) The use of basidiomycota mushrooms in poultry nutrition—a review. Animal Feed Science and Technology, 230, 59-69.

Canibe, N., & Jensen, B. B. (2003) Fermented and nonfermented liquid feed to growing pigs: Effect on aspects of gastrointestinal ecology and growth performance. Journal of Animal Science, 81, 2019-2031.

Canibe, N., & Jensen, B. B. (2007) Fermented liquid feed and fermented grain to piglets- effect on gastrointestinal ecology and growth performance. Livestock Science, 108, 198-201.

Canibe, N., & Jensen, B. B. (2010) Fermented liquid feed – Feed processing has a big impact on microbial degradation of free lysine during fermentation. Livestock Science 133, 120-123.

Canibe, N., & Jensen, B. B. (2012) Fermented liquid feed-microbial and nutritional aspects and impact on enteric diseases in pigs. Animal Feed Science and Technology, 173, 17-40.

Canibe, N., Virtanen, E., & Jensen, B. B. (2007) Microbial and nutritional characteristics of pig liquid feed during fermentation. Animal Feed Science and Technology. 134, 108-123.

Castoldi, R., Bracht, A., de Morais, G. R., Baesso, M. L., Correa, R. C. G., Peralta, R. A., Moreira, R. dF. P. M., Polizeli, M. dL. T. dM., de Souza, C. G. M., & Peralta, R. M. (2014) Biological pretreatment of eucalyptus grandis sawdust with white-rot fungi: Study of degradation patterns and saccharification kinetics. Chemical Engineering Journal, 258, 240-246.

Chen, K.L., Kho, W.L., You, S.H., Yeh, R.H., Tang, S. W., & Hsieh, C. W. (2009) Effects of bacillus subtilis var. Natto and saccharomyces cerevisiae mixed fermented feed on the enhanced growth performance of broilers. Poultry Science, 88, 309-315.

Chen, W. Zhu, X.Z., Wang, J.P., Wang, Z.X., & Huang, Y.Q. (2013) Effects of bacillus subtilis var. Natto and saccharomyces cerevisiae fermented liquid feed on growth performance, relative organ weight, intestinal microflora, and organ antioxidant status in landes geese. Journal of Animal Science, 91, 978-985.

Elisashvili, V., Penninckx, M., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Kharziani, T., & Kvesitadze, G. (2008) Lentinus edodes and pleurotus species lignocellulolytic enzymes activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Bioresource Technology, 99, 457-462.

Engberg, R.M., Hammershoj, M., Johansen, N.F., Abousekken, M.S., Steenfeldt, S., & Jensen, B.B. (2009) Fermented feed for laying hens: Effects on egg production, egg quality, plumage condition and composition and activity of the intestinal microflora. British Poultry Science, 50, 228-239.

Fard, S.H., Toghyani, M., & Tabeidian, S.A. (2014) Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. International Journal of Recycling of Organic Waste in Agriculture, 3, 141-146.

Feng, J., Liu, X., Xu, Z.R., Liu, Y.Y., & Lu Y.P. (2007) Effects of Aspergillus oryzae 3.042 fermented soybean meal on growth performance and plasma biochemical parameters in broilers. Animal Feed Science and Technology, 134, 235-242.

Gori, K., Bjorklund, M.K., Canibe, N., Pedersen, A.O., & Jespersen, L. (2011) Occurrence and identification of yeast species in fermented liquid feed for piglets. Microbiology Ecology, 61, 146-153.

Humer, E., & Schedle, K. (2016) Fermentation of food and feed: A technology for efficient utilization of macro and trace elements in monogastrics. Journal of Trace Elements in Medicine and Biology, 37, 69-77.

Humer. E, Wetscherek, W., Schwarz, C., & Schedle, K. (2013) Effect of maize conservation technique and phytase supplementation on total tract apparent digestibility of phosphorus, calcium, ash, dry matter, organic matter and crude protein in growing pigs. Animal Feed Science and Technology, 185, 70-77.

Humer, E., Wetscherek, W., Schwarz, C., & Schedle, K. (2014) Effects of maize conservation techniques on the apparent total tract nutrient and mineral digestibility and microbial

metabolites in the faeces of growing pigs. Animal Feed Science and Technology, 197, 176-184.

Iank Bueno, A. (2018) The use of condensed tannins as silage additive for rehydrated corn grain: effects on silage fermentation pattern losses. Proceedings of the Society of Nutrition Physiology, 27, 133.Jakobsen, G.V., Jensen, B.B., Bach Knudsen, K.E, & Canibe, N. (2015) Impact of fermentation and addition of non-starch polysaccharide-degrading enzymes on microbial population and on digestibility of dried distillers grains with solubles in pigs. Livestock Science, 178, 216-227.

Jørgensen, H., Sholly, D., Pedersen, A.Ø., Canibe, N., & Knudsen, K.E.B. (2010) Fermentation of cereals — influence on digestibility of nutrients in growing pigs. Livestock Science, 134, 56-58.

Kovalík, P., Mačanga, J., Klempová, T., Popelka, P., Marcinčáková, D., Mellen, M., Bartkovský, M., Maskaľová, I., Čertík, M., & Marcinčák, S. (2018) Effect of feeding of 5% prefermented cereal-based bioproduct enriched with γ-linolenic acid on production indicators, chemical composition, fatty acid profile and lipid oxidation of broiler meat. Italian Journal of Animal Science, 17, 408-417.

Kraler, M., Schedle, K., Domig, K.J., Heine, D., Michlmayr, H., & Kneifel, W. (2014) Effects of fermented and extruded wheat bran on total tract apparent digestibility of nutrients, minerals and energy in growing pigs. Animal Feed Science and Technology. 197, 121-129.

Kraler, M., Schedle, K., Schwarz, C., Domig, K.J., Pichler, M., Oppeneder, A., Wetscherek, W., Prückler, M., Pignitter, M., Pirker, K.F., Somoza, v., Heine, D., & Kneifel, W. (2015) Fermented and extruded wheat bran in piglet diets: Impact on performance, intestinal morphology, microbial metabolites in chyme and blood lipid radicals. Achieve of Animal Nutrition, 69, 378-398.

Kraler, M., Ghanbari, M., Domig, K.J., Schedle, K., & Kneifel, W. (2016) The intestinal microbiota of piglets fed with wheat bran variants as characterised by 16S rRNA next-generation amplicon sequencing. Archives of Animal Nutrition, 70, 173-189.

Lai, L.P., Lee, M.T., Chen, C.S., Yu, B., & Lee, T.T. (2015) Effects of co-fermented pleurotus eryngii stalk residues and soybean hulls by aureobasidium pullulans on performance and intestinal morphology in broiler chickens. Poultry Science 94, 2959-2969.

Lee, S.B., Im, J., Kim, S.K., Kim, Y.C., Kim, M.J., Lee, J.S., and Lee, H.G. (2014) Effects of dietary fermented flammulina velutipes mycelium on performance and egg quality in laying hens. International Journal of Poultry Science, 13, 637-644.

Lee, S.Y., Kim, J.S., Kim, J.M., An, B.K., & Kang, C.W. (2010) Effects of multiple enzyme (rovabio® max) containing carbohydrolases and phytase on growth performance and intestinal viscosity in broiler chicks fed corn-wheat-soybean meal based diets. Asian Australasian Journal of Animal Science, 23, 1198-1204.

Madigan, M.T., Marinko, J. M., & Parker, J. (2003) Brock Mikrobiologie. Berlin: Spektrum, Akademischer Verlag. 116 p.

Niba, A.T., Beal, J.D., Kudi, A.C., & Brooks, P.H. (2009) Bacterial fermentation in the gastrointestinal tract of non-ruminants: Influence of fermented feeds and fermentable carbohydrates. Tropical Animal Health and Production, 41, 1393-1407.

Peng, Q., Zeng, X.F., Zhu, J.L., Wang, S., Liu, X.T., Hou, C.L., Thacker, P.A., & Qiao, S.Y. (2016) Effects of dietary lactobacillus plantarum b1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. Poultry Science, 95, 893-900.

Pieper, R., Hackl, W., Korn, U., Zeyner, A., Souffrant W.B., Pieper, B. (2011) Effect of ensiling triticale, barley and wheat grains at different moisture content and addition of Lactobacillus plantarum (DSMZ 8866 and 8862) on fermentation characteristics and nutrient digestibility in pigs. Animal Feed Science and Technology, 164, 96-105.

Schedle, K. (2016) Sustainable pig and poultry nutrition by improvement of nutrient utilisation – a review. Die Bodenkultur: Journal of Land Management, Food and Environment, 67, 45-60.

Sharma, R.K., & Arora, D.S. (2010) Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by phlebia floridensis. Bioresource Technology, 101, 9248-9253.

Teng, P.Y., Chang, C.L., Huang, C.M., Chang, S.C., & Lee, T.T. (2017) Effects of solid-state fermented wheat bran by bacillus amyloliquefaciens and saccharomyces cerevisiae on growth performance and intestinal microbiota in broiler chickens. Italian Journal of Animal Science, 16, 552-562.

Wang, C.C., Lin, L.J., Chao, Y.P., Chiang, C.J., Lee, M.T., Chang, S.C., Yu, .B, & Lee, .TT. (2017) Antioxidant molecular targets of wheat bran fermented by white rot fungi and its potential modulation of antioxidative status in broiler chickens. British Poultry Science, 58, 262-271.

Wang, C.L., Chiang, C.J., Chao, Y.P., Yu, B., & Lee, T.T. (2015) Effect of cordyceps militaris waster medium on production performance, egg traits and egg yolk cholesterol of laying hens. Journal of Poultry Science, 52, 188-196.

Wanzenböck, E., Apprich, S., Tirpanalan, Ö., Zitz, U., Kracher, D., Schedle, K., & Kneifel, W. (2017) Wheat bran biodegradation by edible pleurotus fungi – a sustainable perspective for food and feed. LWT - Food Science and Technology, 86, 123-131.

# Effect of lactic acid bacteria on the reduction of phytate-phosphorus in fermented liquid feed – a contribution to ecology?

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**Keywords:** ecology, fermentation, liquid feed, phytate-phosphorus

**Introduction** Animal production is linked with an undesired entry of nitrogen (N) and phosphorus (P) into soil and groundwater in many areas around the world especially in Western Europe. In this regard farmers are strongly interested to reduce N and P output from animals and thus, they are looking for methods to increase digestibility of P and N from feed. Fermentation of liquid feeds, a young method with increasing significance in pig production, is being discussed to improve the digestibility of P and N (Heinze 2014). With this method cereals and/or by-products are fermented at a dry matter range between 20 to 30% for at least 12 to 24 hours under storage temperatures of >30 °C. The addition of adequate lactic acid bacteria (LAB) is favourable due to a faster reduction of pH and more secure control of undesired microorganism (Lau et al. 2016). Main storage form of P in cereals is inositol-hexaphosphate (Phytate-P). Due to missing endogenous phytase activity pigs are not able to utilize P effectively. Thus, Phytate-P from grain-based diets is largely lost via faeces, if pig feed is not supplemented with phytase. Activity of native phytases on the plants is largely dependent on pH, with an optimal range between pH 4 and 5. Therefore it is not surprising that fermentation of liquid feed can lead to an improved availability of P (Humer et al. 2013) and reduce P losses via faeces. In order to clarify this, the aim of the present study is to assess the effect of a LAB mixture on availability of P in liquid feed.

**Material and Methods** A constant quantity of substrate (25% wheat, 25% barley and 50% solvent extracted canola meal- chemical composition in Table 1) was mixed with water (adjusted to 25% dry matter) as a) untreated control and b) inoculated with a mixture of three homofermentative LAB (*L.plantarum, P.pentosaceus, Lc.lactis*). The fermentation was conducted under laboratory conditions for 24 hours at a temperature of 37 °C (n=8/treatment). PH, amount of lactic acid, acetic, ethanol and concentrations of P and Phytate-P (according to McKie and Mc Cleary, 2016) were recorded after a fermentation time of 0 and 24 hours. Sugar was analyzed according to VDLUFA method III.7.1.1 and starch according to VDLUFA method III.7.2.1. For statistical evaluation, means and standard deviations were calculated for each parameter. The data were examined by SAS evaluation (SAS 9.4.) using the procedure MIXED including the fixed effect of treatment ($P<0.05$).

**Table 1** Chemical composition of the basic material before fermentation

| Item | Basic material |
|---|---|
| Dry matter, g/kg | 896 |
| Crude Protein, g/kg DM | 243 |
| NDF, g/kg DM | 247 |
| ADF, g/kg DM | 153 |
| Crude Fat, g/kg DM | 28.0 |
| Starch, g/kg DM | 480 |
| Sugar, g/kg DM | 53.0 |
| Total phosphorus, mg/kg DM | 4310 |
| Phytate-P, mg/kg DM | 3977 |

**Results and discussion** The addition of LAB mixture yielded a faster pH reduction, a higher amount of lactic acid and also lower amount of acetic acid and ethanol (Table 2). This was also observed by

Heinze (2014) and Lau et al. (2016). Acetic acid and ethanol are mainly products of undesired microorganisms (enterobacteria, yeasts) during fermentation. It is obvious that LAB mixture is able to inhibit these undesired germs and thus reduces their fermentation end products.

The present study also demonstrates a positive effect of LAB addition on P availability. The Phytate-P content of the unfermented raw material was 3977 mg/kg DM (Table 1). After fermentation for 24 hours, the concentration of Phytate-P in the control and treated variant decreased to 3066 mg/kg DM and 2661 mg/kg DM, thus LAB mixture led to significantly lower values ($p < 0.002$). The greatest reduction in Phytate-P can be expected at ≤ pH 4 (Brejnholt et al. 2011), which corresponds to the pH-optimum for most native phytases. The prolonged time at lower pH-values in the LAB treatment might have led to more appropriate conditions for reduction of the inositol-hexaphosphate- complex.

**Table 2** Fermentation end products, ph and Phytate-P (least square means) of liquid feed for control and treatment after 24h of incubation

| Item | Control | Treatment | SEM[2] | P-value |
|---|---|---|---|---|
| Acetic acid, g/kg DM | 13.0 | 3.48 | 0.172 | < 0.001 |
| Ethanol, g/kg DM | 5.78 | 0.58 | 0.167 | < 0.001 |
| Lactic acid, g/kg DM | 33.1 | 59.5 | 0.426 | < 0.001 |
| pH | 4.35 | 3.80 | 0.04 | < 0.001 |
| Phytate-P, mg/kg DM | 3066 | 2661 | 136.5 | 0.002 |

[2]Standard error of means

**Conclusion** The results of this study indicate the potential of fermented liquid feed in pig production for the reduction of P losses via animal faeces. In addition, a corresponding minor risk for an undesired entry of P into soil and groundwater, especially by use of adequate LAB mixtures, is assumed. Thus fermentation of liquid feed seems to be able to contribute to a more ecological pig production.

**References**

Brejnholt, S.M., Dionisio, G., Glitsoe, V., Skov, L.K. & Brich-Pedersen, H. (2011) The degradation of phytate by microbial and wheat phytases is dependent on the phytate matrix and the phytase origin. J. of Food and Agric. 91, pp 1398-1405.

Heinze, A. (2014) Wirtschaftlichere Mastschweinefütterung. Arbeitspaket III: Schweine-futterfermentation. Projektnr. 95.10. Abschlussbericht. pp 4.12.

Humer, E., Wetscherek W., Mair C. & Schedle, K. (2013) Effect of maize conservation technique and phytase supplementation on total tract apparent phosphorus, calcium and nutrient digestibility in growing pigs. Anim. Feed Sci. Technol.185, pp 70-77.

Lau, N., Hummel, J. & Kramer, E. (2016) Effect of lactic acid bacteria on degradation of amino acids to biogenic amines in fermented liquid feeds. Proceedings of the Society of Nutrition Physiology 25, 2016, pp. 40.

McKie, V.A. & McCleary, B.V. (2016) A novel and rapid colorimetric method for measuring total phosphorus and phytic acid in foods and animal feeds. J. AOAC Int. 99, pp 738-743.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) (2012) Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt (Germany).

# Ensiling features of thistle (*Cynara cardunculus* L.) to be used for biogas production

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**Keywords:** aerobic stability, biogas, fermentation quality, thistle silage, yeast.

**Introduction** In recent years, there has been an increasing demand throughout the world for alternative energy crops for biogas. Over the last few years, a growing interest in thistle (*Cynara cardunculus* L.) has been observed, since it produces a large amount of biomass, even when the plants are provided with minimal inputs, which allows the crop to be grown on lands not usually used for cropping (Pari et al. 2017; Pesce et al. 2017). One critical point of alternative sources of biomass for biogas production, such as thistle, concerns their ability to well ferment and to be conserved as silage. The aim of this work was to evaluate the microbial and fermentation quality and the aerobic stability of thistle silage harvested at two different stages of maturity, and treated or not with a lactic acid bacteria (LAB) inoculum.

**Material and Methods** Two trials were conducted on thistle, harvested as a whole plant, at two different growing stages. The fresh forage was untreated (C) or treated with a mixture of *L. buchneri*, *L. plantarum*, and *L. casei* (L) [(theoretical application rate of 300,000 colony-forming units (cfu)/g fresh matter (FM)]. The forages were ensiled in 20-L plastic silos at a density of 716±24 and 378±20 kg/m$^3$ FM for stage I and II, respectively, and opened after 160 and 320 d of ensiling. At opening, the silages were analysed for dry matter (DM) content, pH, fermentative profile and microbial counts [lactic acid bacteria (LAB), yeast and mould]. The DM content was determined at 60°C for 72 h. The fermentative products were determined in the acid extract by HPLC. The microbial counts were determined using the pour plate technique on MRS and YGC agar, for LAB and for the yeast and mould, respectively. The weight losses due to fermentation were calculated as the difference between the weight of the forage placed in each plastic silo at ensiling and at the end of conservation, and were expressed on a DM basis. After each opening, silages were subjected to an aerobic stability test by continuously measuring the temperature during exposure to air. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. Data were analyzed for their statistical significance, via analysis of variance, using the GLM of SPSS (v. 24 for Windows, SPSS Inc., Chicago, IL). The data were analysed utilizing the treatments (T) and harvesting stage (S) as the fixed factor, with three replicates.

**Results and Discussion** The chemical and microbial characteristics of the thistle herbages, prior to ensiling, are reported in Table 1. The DM content at harvest was 24% (wet) and 45% (dry) for harvesting stages I and II, respectively. The buffering capacity was higher for the wet forages than for the dry ones, and no differences were found for the microbial counts. Fermentative profile, microbial counts, aerobic stability and weight losses of silages, after 160 and 320 d, are summarized in Table 2. The use of LAB inoculum did not influence many of the parameters at 160 or at 320 d of ensiling. The harvesting stage affected the fermentative profile to a great extent. Lactic acid was 7 and 10 times higher in wet silages than in dry ones for 160 and 320 d, respectively. Acetic acid was around 20 g/kg DM and 32 g/kg DM for wet and dry silages, respectively. This resulted in a higher lactic-to-acetic ratio than 3 in wet silages and lower than 1 in dry ones. Pari et al. (2017) found that the lactic acid ranged from 9 to 14 g/kg DM and acetic acid ranged from 10 to 19 g/kg DM in 35% DM thistle silages. The different lactic-to-acetic ratio determined the different yeast counts, which reached lower values in dry silages. A larger amount of ethanol (>43 g/kg DM) was found in wet silages than in the dry ones (<2 g/kg DM), with a significant interaction with the treatment, thus confirming a possible yeast activity during early phase of fermentation. The weight losses due to fermentation were influenced by the treatment and harvesting stage, and reached around 6% and 2% for wet and dry silages, respectively. During ensiling, the LAB count remained higher than 7 $\log_{10}$ cfu/g in all dry silage treatments, whereas

it decreased as the ensiling duration increased in the wet silages. Despite the lower amount of LAB in wet silages, the fermentative products indicated a dominant lactic fermentation, probably because of the LAB fermented sugars in early stages of fermentation. The yeast count decreased during conservation, and this reduction improved aerobic stability in both wet and dry silages. Aerobic stability was 4 times higher in the dry than in the wet silages after 320 d. The higher the aerobic stability, the lower the yeast count, as previously reported for other forage crops (Kleinschmit and Kung, 2006).

**Conclusion** Thistle silage seems to represent a good opportunity for use as an energy crop for biogas production. The main factor that affects the fermentation quality appears to be the harvesting stage. Low DM content silages produced large amounts of fermentative products, but were characterized by high weight losses during fermentation and low aerobic stability. Dry thistle silages with low lactic-to-acetic ratios showed reduced yeast counts and improved aerobic stability. Further investigations are needed to evaluate the biogas production of these silages.

**Table 1.** The chemical and microbial characteristics of the thistle herbages prior to ensiling.

| Parameters* | Stage I | Stage II |
|---|---|---|
| DM (%) | 24.3 ± 0.15 | 45.2 ± 3.46 |
| pH | 6.06 ± 0.16 | 6.83 ± .012 |
| Water activity ($a_w$) | 0.996 ± 0.002 | 0.981 ± 0.003 |
| Nitrate (mg/kg) | 491 ± 177 | 578 ± 310 |
| Buffering capacity (mEq/kg DM) | 129 ± 8 | 71 ± 9 |
| Yeast ($log_{10}$ cfu/g) | 6.43 ± 2.06 | 6.14 ± 0.13 |
| LAB ($log_{10}$ cfu/g) | 7.84 ± 0.70 | 7.98 ± 0.47 |

***** The values represent the mean of 6 replications ± SD. DM = dry matter, LAB = lactic acid bacteria.

**Table 2.** Fermentative profile, microbial count and aerobic stability of thistle silages.

| Parameters* | | Stage I | | Stage II | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | d | C | L | C | L | *Treat* | *Stage* | *T*S* | SEM |
| DM (%) | 160 | 20.6 | 22.1 | 44.7 | 43.1 | NS | *** | NS | 3.44 |
| | 320 | 22.1 | 22.4 | 44.1 | 42.2 | NS | *** | NS | 3.00 |
| pH | 160 | 4.13 | 4.11 | 4.90 | 4.78 | ** | *** | NS | 0.110 |
| | 320 | 4.14 | 4.07 | 4.74 | 4.72 | NS | *** | NS | 0.091 |
| Lactic acid (g/kg DM) | 160 | 77.3 | 68.5 | 8.6 | 11.1 | NS | *** | NS | 9.76 |
| | 320 | 55.4 | 61.3 | 6.6 | 6.2 | NS | *** | NS | 7.59 |
| Acetic acid (g/kg DM) | 160 | 21.5 | 18.4 | 31.6 | 32.9 | NS | *** | NS | 2.076 |
| | 320 | 18.4 | 19.5 | 31.7 | 35.2 | NS | *** | NS | 2.26 |
| Lactic-to-acetic ratio | 160 | 3.7 | 3.7 | 0.3 | 0.4 | NS | *** | NS | 0.521 |
| | 320 | 3.0 | 3.1 | 0.2 | 0.2 | NS | *** | NS | 0.420 |
| Ethanol (g/kg DM) | 160 | 49.5 | 43.7 | 0.7 | 1.4 | NS | *** | NS | 6.85 |
| | 320 | 58.5 | 55.4 | 0.6 | 1.7 | * | *** | * | 7.94 |
| LAB ($log_{10}$ cfu/g) | 160 | 5.58 | 5.34 | 7.21 | 7.61 | NS | *** | NS | 0.305 |
| | 320 | 3.91 | 3.30 | 7.06 | 8.04 | NS | *** | * | 0.841 |
| Yeast ($log_{10}$ cfu/g) | 160 | 5.53 | 5.32 | 1.06 | 1.35 | NS | *** | NS | 0.679 |
| | 320 | 3.03 | 1.50 | 1.73 | 1.46 | NS | NS | NS | 0.301 |
| Aerobic stability (h) | 160 | 64 | 69 | 54 | 125 | NS | NS | NS | 14 |
| | 320 | 113 | 134 | 391 | >478 | NS | *** | NS | 48 |
| Weight losses (% DM) | 160 | 6.05 | 5.87 | 1.97 | 1.95 | NS | *** | NS | 0.604 |
| | 320 | 6.65 | 5.96 | 2.23 | 2.21 | *** | *** | *** | 0.593 |

***** d =day of ensiling; DM = dry matter; L = lactic acid bacteria inoculum; LAB = lactic acid bacteria.

### References

Kleinschmit, D.H. & Kung L. (2006) A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of maize and grass and small-grain silages. Journal of Dairy Science, 89, 4005-4013.

Pari, L., Alfano, V., Mattei, P. & Santangelo, E. (2017) Pappi of cardoon (*Cynara cardunculus* L.): The use of wetting during the harvesting aimed at recovering for the biorefinery. Industrial Crops & Products, 108, 722-728.

Pesce, G.R., Negri, M., Bacenetti, J. & Mauromicale, G. (2017). The biomethane, silage and biomass yield obtainable from three accessions of *Cynara cardunculus*. Industrial Crops & Products, 103, 233-239.

# Impact of cultivar, maturity stage and storage period on fermentation quality of wet sorghum seeds

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**Keywords:** hermetic silo, sorghum silage, volatile organic compounds, wet sorghum seeds

**Introduction** Austrian pig fattening farmers are looking for alternative crops instead of maize, like sorghum (*sorghum bicolor*), because increasing problems arise with western corn root worm (*Diabrotica virgifera Le Conte*) and, subsequently, with governmental regulation of crop rotation. Sorghum is an appropriate crop for pig feeding (Puntigam und Wetscherek 2013), but there is little experience in conservation of wet sorghum seeds to silage via hermetic silo (Resch 2016). Analysis of relevant sorghum cultivars at different maturity stages was determined to collect data of nutrients and fermentation quality during the storage period of eleven months, because farmers and consultants of Austrian agricultural institutions need valid results for recommendations to optimal practice.

**Materials and Methods** Wet sorghum seeds of two different sorghum cultivars (Targga, Baggio) and three maturity stages (early [24$^{th}$ Sept. 2014], moderate [3$^{rd}$ Okt. 2014] and late [10$^{th}$ Okt. 2014] degree of seed ripeness) were harvested at the Styrian research station Hatzendorf (46°58'52.2"N and 16°00'13.1"E) and filled in hermetic lockable 60 litre laboratory barrels. All variants were replicated three times. To ensure equal storage conditions the barrels were transported to AREC Raumberg-Gumpenstein and kept there at an average room temperature of 24 °C. Sorghum silages were sampled (mixture of different depths) and analysed by wet chemistry standard methods (VDLUFA 1976) at three different dates during length of storage (4$^{th}$ Feb. 2015, 4$^{th}$ May 2015, 7$^{th}$ Sept. 2015). Dry matter (DM) of silages was corrected by method of Weißbach und Strubelt (2008). Data analysis was executed by software SPSS (version 22). Factor effects were tested via Anova and Tukey-HSD test. Interactions between factors were analysed by GLM (General Linear Model).

**Results** We observed significant differences in DM-content and nutrients (protein, starch, sugar) between maturity stages as well as between cultivars. Silage of wet sorghum seeds did not obtain stable pH-level beneath 5.0. Regarding high ethanol contents, a dominant alcoholic fermentation (55 to 85% of metabolised products) was detected (Table 1).

Consequently, the fermentation process continued during storage period. The average losses of DM were 0.2 to 5.2% depending on DM-content and date of sampling; starch losses went up to maximal 8.7%. Depending on increasing moisture of the wet sorghum seeds fermentation products and fermentation loss increased, as well. Reduction of protein, depending on the production of ammonia ($NH_3$), reached a maximum of 4.9% of total N. However, after eleven months of storage under hermetic-sealed conditions, silage of wet sorghum seeds showed persistent changes of fermentation parameters, indicating suboptimal silage stability. Results of interactions showed a significant impact of DM-content to some fermentation parameters. We partly found strong correlations between fermentation parameters and DM-content resp. storage length (Resch et al. 2017).

**Table 1**. Dry matter content/losses, nutrients and relevant fermentation parameters of ensiled sorghum-seeds in dependence of different cultivars, harvest dates and storage periods

| factor | cultivar (c) | | date of harvest (h) | | | date of sampling (s) | | | interactions (P-value) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| parameter | Targga | Baggio | 24th Sept. | 3rd Okt. | 10th Okt. | 4th Feb. | 4th Mai | 7th Sept. | h x c | h x s | c x s |
| dry matter (DM) g $kg^{-1}$ FM | 692.7$^{b}$ | 682.3$^{a}$ | 648.9$^{a}$ | 676.2$^{b}$ | 737.5$^{c}$ | 700.1$^{c}$ | 677.9$^{a}$ | 684.5$^{b}$ | 0.000 | 0.124 | 0.261 |
| DM-loss % | 2.8$^{b}$ | 3.4$^{a}$ | 4.0$^{a}$ | 2.5$^{b}$ | 2.8$^{b}$ | 1.2$^{c}$ | 4.4$^{a}$ | 3.7$^{b}$ | 0.000 | 0.031 | 0.400 |
| crude protein (XP) g $kg^{-1}$ DM | 86.5$^{a}$ | 90.6$^{b}$ | 90.1$^{b}$ | 89.2$^{b}$ | 86.5$^{a}$ | 89.0$^{a}$ | 88.5$^{a}$ | 88.2$^{a}$ | 0.062 | 0.634 | 0.002 |
| starch (XS) g $kg^{-1}$ DM | 735.2$^{a}$ | 743.4$^{b}$ | 723.7$^{a}$ | 729.5$^{a}$ | 764.8$^{b}$ | 736.6$^{a}$ | - | 742.0$^{a}$ | 0.202 | 0.016 | 0.010 |
| sugar (XZ) g $kg^{-1}$ DM | 19.6$^{a}$ | 27.3$^{b}$ | 34.3$^{c}$ | 26.9$^{b}$ | 9.1$^{a}$ | 19.9$^{a}$ | - | 27.0$^{b}$ | 0.001 | 0.147 | 0.336 |
| pH-value | 5.4$^{b}$ | 5.3$^{a}$ | 5.2$^{a}$ | 5.4$^{b}$ | 5.6$^{c}$ | 5.6$^{b}$ | 5.3$^{a}$ | 5.3$^{a}$ | 0.000 | 0.062 | 0.788 |
| lactic acid g $kg^{-1}$ DM | 1.2$^{a}$ | 1.3$^{a}$ | 1.7$^{b}$ | 1.6$^{b}$ | 0.5$^{a}$ | 0.9$^{a}$ | 1.1$^{a}$ | 1.8$^{b}$ | 0.029 | 0.075 | 0.488 |
| acetic acid g $kg^{-1}$ DM | 1.8$^{a}$ | 2.0$^{a}$ | 2.6$^{c}$ | 2.0$^{b}$ | 1.2$^{a}$ | 1.7$^{a}$ | 1.8$^{a}$ | 2.2$^{b}$ | 0.594 | 0.561 | 0.357 |
| butyric acid g $kg^{-1}$ DM | 0.3$^{a}$ | 0.3$^{a}$ | 0.5$^{c}$ | 0.3$^{b}$ | 0.0$^{a}$ | 0.3$^{b}$ | 0.2$^{a}$ | 0.4$^{c}$ | 0.000 | 0.000 | 0.038 |
| ethanol g $kg^{-1}$ DM | 9.0$^{a}$ | 9.8$^{b}$ | 12.7$^{c}$ | 9.1$^{b}$ | 6.4$^{a}$ | 8.7$^{a}$ | 8.8$^{a}$ | 10.7$^{b}$ | 0.487 | 0.001 | 0.586 |
| ammonia ($NH_3$) % of $N_{total}$ | 2.4$^{a}$ | 2.7$^{b}$ | 3.5$^{c}$ | 2.8$^{b}$ | 1.3$^{a}$ | 1.6$^{a}$ | 2.6$^{b}$ | 3.3$^{c}$ | 0.008 | 0.000 | 0.046 |
| carbon dioxide ($CO_2$) vol% | 49.7$^{a}$ | 60.6$^{b}$ | 55.3$^{a}$ | 58.7$^{a}$ | 51.4$^{a}$ | 63.8$^{b}$ | 59.6$^{b}$ | 41.9$^{a}$ | 0.597 | 0.000 | 0.818 |

Different letters indicate significant differences (P<0.05).

**Conclusions** Optimal conservation of wet sorghum seeds depends on the DM-content (stage of maturity) resp. cultivar to provide acceptable nutrient-contents and fermentation quality determined by sufficient content of acids and $CO_2$ and minimal fermentation losses during the storage period.

**References**

Puntigam, R. & Wetscherek, W. (2013) Einfluss von Körnerhirse auf die Mast-und Schlachtleistung sowie die Fettbeschaffenheit beim Mastschwein. ALVA-Jahrestagung 2013 zum Thema "Pflanzenschutz als Beitrag zur Ernährungssicherung", LFZ für Wein- und Obstbau, Klosterneuburg, Arbeitsgemeinschaft für Lebensmittel-, Veterinär- und Agrarwesen (ALVA), 23. und 24. Mai 2013, 183-185.

Resch, R., Schweiger, M., Bachler, C. & Zentner, E. (2017) Gärqualität von Hirse-GKS in Abhängigkeit von Sorte, Reifestadium und Lagerungsdauer. ALVA-Jahrestagung 2017 zum Thema "Zukunft Obstbau", Waldkirchen am Wesen, Seminarhotel Wesenufer, Arbeitsgemeinschaft für Lebensmittel-, Veterinär- und Agrarwesen (ALVA), 22. - 23. Mai 2017, 379-381.

Resch, R. (2016) Einfluss von Sorte, Reifestadium und Lagerungsdauer auf Gärgaszusammensetzung und Gärungsverluste von Hirse-Ganzkornsilage, Abschlussbericht des Forschungsprojektes "Hirse-GKS", Nr. 3633 (DaFNE 101043), HBLFA Raumberg-Gumpenstein, 31 p.

VDLUFA (1976) Methodenbuch Band III - Die chemische Untersuchung von Futtermitteln, inkl. Ergänzungsblätter 1983, 1988, 1993, 1997, VDLUFA-Verlag, Darmstadt.

Weißbach, F. & Strubelt, C. (2008) Die Korrektur des Trockensubstanzgehaltes von Maissilagen als Substrat für Biogasanlagen. LANDTECHNIK–Agricultural Engineering 63, 82-83.

# Roughage based liquid diets for pregnant sows – Harvest, post-shredding and feeding management of whole-plant silages (wheat and maize)

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**Keywords:** animal welfare, crude fibre, liquid feeding system, pump technology

**Introduction** Restrictive feeding is common practice in large sized units of pregnant sows. Although optimally supplied in terms of nutritional value, the sows are often not sated. Consequently, ranking fights happen regularly. Furthermore, hunger and frustration increase the aggression level during feeding in group housing systems (Meunier-Salaün et al. 2001). As a result, stereotypies often emerge, which may be an indication of reduced animal welfare (Brouns et al. 1994). In order to improve animal welfare and minimise fights, sows can be fed roughage based diets which reduce these problems by decreasing hunger and prolong the time of feed intake (Brouns et al. 1994; Bergeron et al. 2000). Despite these advantages, modern feeding technology in sow husbandry is often not designed for high crude fibre contents in feed rations and even more in premixes without concentrates. Therefore, the objective of the present study was to investigate consistence and material characteristics of whole-plant silages after processing to make them pumpable in a technologically advanced liquid feeding system.

**Material and Methods** In July 2016, wheat with a dry matter (DM) content of 48.3% was harvested as whole plant with a forage harvester. Maize was harvested in September 2016 (34.3% DM, oven-dried for 24 h at 105°C). Both substrates were further shredded using a Corn-Cob-Mix (CCM) mill. Dry sieve analyses of unmilled and milled samples were implemented. Whole-plant wheat silage (WPWS) was ensiled with a chemical silage additive (Kofasil ultra, to ensure the fermentation quality and aerobic stability, containing sodium nitrite, hexamethylene tetramine, sodium benzoate and sodium propionate, 4 litre $t^{-1}$) via baler-wrapper combination in round bales. Whole-plant maize silage (WPMS) was ensiled via a silo press with rotor into a plastic tube without silage additive. Silages were opened after two months. In order to test the pumpability for use in a liquid feeding system, mixtures with WPMS and WPWS of different DM contents were prepared in a specially designed container (12.5$m^3$) equipped with a stirrer. Subsequently, attempts were made to pump the mixtures either with an open hopper pump (OHP) or a double piston pump (DPP) with increasing DM content.

**Results and Discussion** The pH values for WPWS and WPMS were 4.2 and 3.9, respectively. Due to the low pH values, it can be concluded that the forage were well fermented.

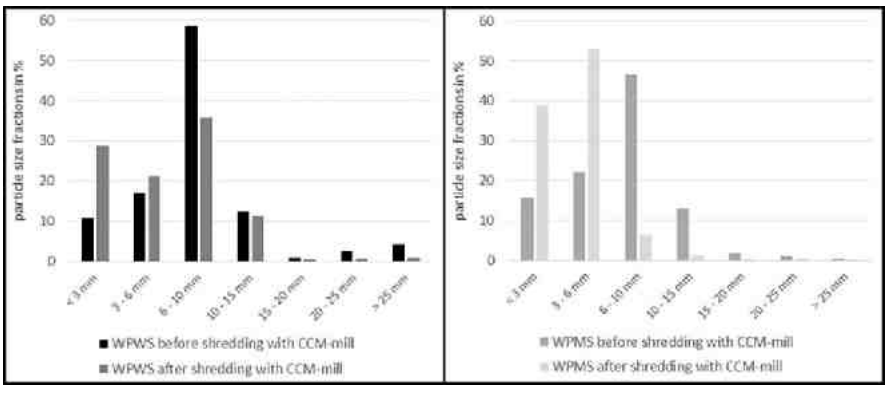


**Figure 1:** Dry sieve analyses of samples from WPWS and WPMS before and after shredding

Dry sieve analyses revealed considerable differences in the shredding process of the CCM-mill (Figure 1). While WPMS was notable shredded with the principle of a hammer mill, considerable particle size fractions of more than 15 mm length occasionally appeared in the WPWS after milling.

Table 1 summarises the experimental setup for testing the pumpability of used silages and the main results. Four stirrer types and two pumps were examined at different DM contents of feed mixtures. The last column shows the maximum pumpable DM-contents of used mixtures (silage + water as premix).

**Table 1**. Type of used stirrer and pump in combination with forage type and DM content

| Trial number | Type of stirrer | Used pump | Silage (+ water) | Max. pumpable actual DM content in % |
|---|---|---|---|---|
| 1 | Cross-arm stirrer | OHP | WPMS | 14.2 ± 0.16 (n=4) |
| 2 | Cross-arm stirrer, lengthened blades | OHP | WPMS | 15.5 ± 0.61 (n=4) |
| 3 | Anchor stirrer, 2 short + 2 long blades | OHP | WPMS | 12.8 ± 1.06 (n=4) |
| | | DPP | WPMS | 10.9 ± 0.92 (n=4) |
| 4 | Anchor stirrer, 1 short + 1 long blade | OHP | WPWS | 6.4 ± 0.80 (n=3) |
| | | DPP | WPWS | Not possible |

OHP = open hopper pump, DPP = double piston pump
WPMS = whole plant maize silage, WPWS = whole plant wheat silage

The mixture of WPMS + water was comparatively easy to homogenise and the highest tested DM content of 15.5% could be mixed with the cross-arm stirrer with lengthened blades and pumped with the OHP. The lengthened blades mixed best but had the highest counter-pressure on the applied gearmotor. In order to reduce the counter-pressure, which was still higher in case of WPWS due to the poor homogenisation, an anchor stirrer was constructed. The anchor stirrer in trial 4 was preferred in case of minimised counter-pressure, but WPWS + water could only be pumped at a very low DM content < 6.5% with the OHP. In another trial in practice with a comparable pump type (OHP) the pumpability of WPWS + water + concentrates was able with 9 - 11% DM content. The present mixture of WPWS + water was not pumpable with the DPP. The bottlenecks inside the pump were blocked by the longer particle size fractions of fibre components.

**Conclusion** The CCM hammer mill was satisfactory able to shred WPMS. However, the mill performance is not sufficient for WPWS. Probably a force-cutting mill would be better for this fibrous material. The pumping of WPMS with the OHP was possible up to a DM content of 15.5%, whereas the DPP showed deficiencies. The WPWS was not pumpable with the DPP, the OHP could pump the WPWS + water but only with a low DM content. This confirms the effect of longer particle fractions after milling, which was already observed in the results of the dry sieve analyses. The high DM content based on the time of harvest is a possible reason for this problem. Therefore, the pumpability of diets and premixes with WPWS or comparable crude fibre sources and higher DM-contents remains a challenge in liquid feeding systems. Finally, further research is needed to improve the animal welfare also in large sized units of pregnant sows by feeding high contents of roughage with concurrent lower energy densities. The pumping of roughage based diets has to run automatically and with a high process reliability of used technology.

**References**

Bergeron, R., Bolduc, J., Ramonet, Y., Meunier-Salaün, M.C. & Robert, S. (2000) Feeding motivation and stereotypies in pregnant sows fed increasing levels of fibre and/or food. Applied Animal Behaviour Science, 70, 27-40.

Brouns, F., Edwards, S.A. & English, P.R. (1994) Effect of dietary fibre and feeding system on activity and oral behaviour of group housed gilts. Applied Animal Behaviour Science, 39, 215-223.

Meunier-Salaün, M.C., Edwards, S.A. & Robert, S. (2001) Effect of dietary fibre on the behaviour and health of the restricted fed sow. Animal Feed Science and Technology, 90, 5369.

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# Roughage based liquid diets for pregnant sows? Apparent digestibility and nutritive value of whole plant silages (wheat and maize)

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**Keywords:** apparent digestibility, crude fibre, liquid feeding system, roughage based diet for sows

**Introduction** Nowadays in modern large sized units of pregnant sows the relevance of "combined feeding" with concentrates and minerals enriched roughages has lost their relevance common in earlier times in smaller farms. Recently, technologically advanced liquid feeding systems facilitate to feed finely cutted whole plant silage (WPS) as basis for pregnant sows - with the objective to feed a ration with high content of roughage with low energy density ad libitum. Thus, the question of this study was at first whether the digestibility rate of the silages can be predicted by using the old equation based on crude fibre content, to design rations for ad libitum liquid feeding systems.

**Material and Methods** Four non-pregnant sows (body weight: 179-277 kg, lactation-No: 2-5) were kept in individual pens which allowed free movement and direct animal contact. All sows were fed diets on maintenance level, calculated on the metabolic body weight (GfE 2006). A ration of concentrates supplemented with minerals was fed as basis. In two consecutive digestive trials a share of concentrates and minerals was substituted by whole plant maize silage (WPMS) resp. whole plant wheat silage (WPWS) (Schiemann 1981; Table 1). Energy and nutrient content was assumed as follow: concentrates 888 g DM and 14.4 MJ ME/ kg DM; WPMS 396 g DM and 10.7 MJ ME/ kg DM (Hohmeier 2015); WPWS 501 g DM and 7.2 MJ ME/ kg DM (Sievers 2017).

**Table 1.** Calculation of isocaloric diets on basis of metabolic body weight

| Sow No | Body weight (kg) | Energy requirement MJ ME (GfE, 2006) | Basis trial | WPMS trial | | WPWS trial | |
|---|---|---|---|---|---|---|---|
| | | | as fed (g) | | | | |
| | | | concentrates | concentrates | WPMS | concentrates | WPWS |
| 1 | 275 | 29.7 | 2311 | 1191 | 3712 | 1719 | 2138 |
| 2 | 184 | 22.0 | 1708 | 880 | 2743 | 1270 | 1580 |
| 3 | 208 | 24.1 | 1875 | 966 | 3012 | 1395 | 1734 |
| 4 | 200 | 23.4 | 1820 | 938 | 2925 | 1355 | 1684 |

whole plant maize silage (WPMS), whole plant wheat silage (WPWS)

After fourteen days of adaptation to the silage based rations seven days of collecting all faeces and refusals of the offered ration followed. In the collection phase, the sows were weighed at the beginning and at the end of the experiment in the morning before feeding. The daily ration was split and fed, twice a day with 7 hours in between. Scattered feed were offered again hourly and possible feed remains weighed the following morning. The faeces were also collected every hour, weighed and frozen. A pooled sample of the respective feed components and a homogenized pooled sample of faeces of each sow from the 7 day trial period were analysed. The apparent digestibility (aD) of WPS was calculated using the difference method (Schiemann 1981). The statistical analysis of the data was done using the software SAS Enterprise Guide 7.1 (SAS Institute Inc., Cary, NC, USA) as descriptive statistic as well as the ANOVA test for mixed models. Due to the small number of animals (n = 4) a normal distribution of the data was assumed.

**Results and Discussion** The calculated values of aD of the organic matter confirmed the expected values predicted with the regression formula. This estimation formula for the aD of the organic matter was developed by Axelsson (1941), cited by Nehring (1972) and established a linear relationship

between the aD of the organic matter and the crude fibre content. The other determined nutrients were digestible as follows (Table2). The energy was calculated by the equation 1 of GfE (2008), the WPMS contained 11.0 MJ ME and the WPWS 8.28 MJ ME per kg DM.

**Table 2**. Apparent digestibility of whole plant silages in non-pregnant sows (n=4)

| | WPMS | WPWS |
|---|---|---|
| aD OM expected, % | 64.6 | 52.7 |
| aD OM, %*** | 64.5 ± 1.26[a] | 52.7 ± 2.93[b] |
| aD XP, % | 58.5 ± 3.66[a] | 51.0 ± 5.38[a] |
| aD XL, %** | 81.0 ± 2,21[a] | 64.8 ± 6.82 [b] |
| aD XF, % | 24.9 ± 3.37[a] | 27.7 ± 2.57[a] |
| aD NFE, %*** | 73.9 ± 0.89[a] | 62.4 ± 2.54[b] |
| Energy, MJ ME/kg DM, (GfE, 2008) | 11.0 | 8.28 |

whole plant maize silage (WPMS), whole plant wheat silage (WPWS), apparent digestibility (aD), organic matter (OM), crude protein (XP), crude fat (XL), crude fibre (XF), nitrogen-free extract (NFE)

**Conclusion** During the trial the offered diets were devoured as calculated. The rations of WPS contained 57 % WPMS resp. 40 % WPWS of total dry matter intake. Thus silages can reduce the need for concentrates in feeding of pregnant sows. Besides the desired expected effects of roughages in feeding pregnant sows there are "side effects" due to the lower digestibility (in comparison to concentrates) especially on the amounts of faeces (slurry, Massé et al. 2003). Considering that the feed intake of WPWS had to be higher for equal energy intake, diets with high crude fibre contents prolong the feed intake, gut fill and feeling of satiety (Jeroch et al. 2008; Ramonet et al. 1999) and therefore should improve the well-being and reduce behavioural disorders (Lawrence and Terlouw, 1993).

**References**

Axelsson, J. (1941) Das Futterverdauungsvermögen der Schweine und seine Konsequenzen für die Bewertung des Schweinefutters. Tierernährung 13, 413-431.

GfE (2006) Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen. DLG-Verlag.

GfE (2008) Prediction of Metabolisable Energy of compound feeds for pigs. Proceedings of the Society of Nutrition Physiology 17, 199-204.

Hohmeier, S. (2015) Einsatz und Futterwert von Mais-Ganzpflanzensilage als Mischfutterbestandteil sowie als Einzelkomponente bei Mastschweinen, DVG.

Jeroch, H., Drochner, W. & Simon, O. (2008) Ernährung landwirtschaftlicher Nutztiere: Ernährungsphysiologie, Futtermittelkunde, Fütterung, UTB.

Lawrence, A. B. & Terlouw, E. (1993) A review of behavioral factors involved in the development and continued performance of stereotypic behaviors in pigs. Journal of animal science 71, 2815-2825.

Massé, D., Croteau, F., Masse, L., Bergeron, R., Bolduc, J., Ramonet, Y., Meunier-Salaun, M. & Robert, S. (2003) Effect of dietary fiber incorporation on the characteristics of pregnant sows slurry. Canadian Biosystems Engineering 45, 6.7-6.7.

Nehring, K. (1972) Lehrbuch der Tierernahrung und Futtermittelkunde.

Ramonet, Y., Meunier-Salaün, M.-C., and Dourmad, J.-Y. (1999) High-fiber diets in pregnant sows: digestive utilization and effects on the behavior of the animals. Journal of animal science 77, 591-599.

Schiemann, R. (1981) Methodische Richtlinien zur Durchführung von Verdauungsversuchen für die Futterwertschätzung. Archives of Animal Nutrition 31, 1-19.

Sievers, S. L. (2017) Untersuchungen zum Einsatz und Futterwert von Ganzpflanzensilagen (Weizen, Roggen, Mais) als Hauptkomponenten in der Fütterung tragender Sauen, DVG, Deutsche Veterinärmedizinische Gesellschaft.

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# Global fermented foods: Ethno-microbiology to next generation sequencing

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**Abstract** Dietary culture is a part of ethnicity, which symbolises the heritage, and socio-cultural aspects of a community. Ancient people have invented the concept of ethno-microbiology involving spontaneous or natural or by adding mixed starter culutre fermentation of edible bio-resources for preservation and production of fermented foods and alcoholic drinks. Fermented foods are the hubs of consortia of both culturable and unculturable microorganisms. There may be more than 5000 varieties of common and uncommon fermented foods and alcoholic beverages being consumed in the world today by billions of people, as staple and other food components.The cultivability of microbiota by culture-dependent techniques including phenotypic and 16S rRNA is still a limiting factor in understanding the natural food fermentation. Application of culture-independent method with next generation sequencing (NGS) technologies has enabled researchers to increase accuracy and relatively in short period of time to profile the entire microbial community both cultivable and non-cultivable in naturally fermented foods.

## Introduction

Millions of diverse ethnic groups of the people in all five continents of the world consume about >5000 types of major/minor and common/uncommon fermented foods and alcoholic beverages representing about 5-40% of daily meals as staple as well as other dietary components (Tamang 2010a). Fermented foods are the eco-system of consortia of microorganisms present as natural microbiota in uncooked plant or animal substrates, containers, earthen pots, and the environment or in traditionally prepared starter culture(s) that modify the substrates biochemically, and organoleptically into edible products and which are culturally and socially acceptable by the ancient people of the world. (Tamang et al. 2016a). Ancient people has invented spontaneous or natural or by adding starter culture fermentation of edible bio-resources using ethno-microbiology method for preservation and production of fermented foods and alcoholic drinks. The concept of ethno-microbiology is very important component in the modern food microbiology since this traditional technology involves the process of conservation and crude sub-culturing of essential and functional microbiota or microbiome using back-sloping method by the ethnic people for centuries. Practicing of ethno-microbiological methods by ancient people has evolved the distinct dietary couture of billions of people representing the diverse communities and ethnicity across the world. The majority of the ethnic fermented foods can be considered essential for food and nutritional security of the region (Tamang et al. 2010b). Ethno-microbiological knowledge of the ancient people for production and management of the available food bio-resources is the prime step of modern food technology, thereby supplementing the food ecosystem and enhancing the regional economy. Based on sensory and physico-chemical properties, fermented foods are are of three types representing 3A connotation (Tamang 2010a): (1) Acidic fermented foods- acidic in taste (<7 pH) mostly fermented by lactic acid bacteria such as *gundruk, kimchi*, *sunki*, yoghurt, *dahi*); (2) Alkaline fermented foods with light ammonical flavour (>7 pH) such as *kinema, dawadawa, pidan*; and (3) Alcoholic beverages with low or high alcohol and esters content such as beer, wine, *saké, pulque, jaanr*. In lactic fermentation, the substrates are kept in air-tight container (less or no oxygen or anaerobic condition) to allow lactic acid bacteria to grow on starchy materials to get the acidic product. In alkaline fermentation, semi-anaerobic or aerobic condition should be maintained to facilitate the growth of aerobic bacilli (mostly *Bacillus subtilis*) as in *kinema, natto*. Sachharification (starch to glucose) and glycolysis (glucose to alcohol and $CO_2$) production is obtained during production of alcoholic beverages. Traditionally the ethnic people know how to get the desirable products using their indigenous knowledge for production of foods for consumption. They did

not know what was the scientific explanation of fermentation mechanisms and identity of functional microorganisms.

**Taxonomy of indigenous microbiome**

Understanding the ethno-microbiology in terms of culture-dependent and independent methods to document a complete profile of microorganisms, and also to study both inter- and intra-species diversity within a particular genus or among genera (Yan et al. 2013). Molecular identification is emerging as an accurate and reliable identification tool, for identification of both culture-dependent and culture-independent microorganisms from fermented foods (Dolci et al. 2015). Due to limitation of only isolation of culturable microorganisms, the culture-dependent methods may not detect the whole microbial community in foods. However, the culture-independent methods by extracting whole genomic DNA directly from small amount (<1 g) samples of fermented food may detect the whole microbial communities in food samples (Puerari et al. 2015). Culture-independent methods including pyrosequencing, PCR-denaturing gradient gel electrophoresis (DGGE) analysis, and recently next generation sequencing such as high throughput metagenomic amplicon sequencing may serve to give more insight into microbial ecology of natural food fermentation with increased accuracy, and relatively short period of time (Ercolini 2004, Alegría et al. 2011, Sha et al. 2017, Shangpliang et al. 2018).

**Microbiome in fermented foods**

Diversity of microbiome consisting of both culturable and unculturable community of microbiota ranges from Gram-positive and Gram-negative bacteria, archae filamentous fungi to enzyme- and alcohol-producing yeasts, are reported from global fermented foods and alcoholic beverages, which has actuay being mooted by the basic principal of ethno-microbiology. Among bacteria, lactic acid bacteria (LAB) mostly species of genera *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Weissella,* etc. are widely present in many fermented foods and beverages (Axelsson et al. 2012, Holzapfel and Wood 2014). Species of *Bacillus* are also present in legume-based fermented foods (Urushibata et al. 2002, Kubo et al. 2011). Few species of *Kocuria, Micrococcus* (members of the *Actinobacteria*) and *Staphylococcus* (belonging to the *Firmicutes*) has been reported for fermented milk products, fermented sausages, meat and fish products (Coton et al., 2010, Shangpliang et al. 2017, 2018). Species of *Bifidobacterium, Brachybacterium, Brevibacterium,* and *Propionibacterium* are isolated from cheese, and species of *Arthrobacter* and *Hafnia* from fermented meat products (Bourdichon et al., 2012). Several genera of yeasts have been isolated from fermented foods, alcoholic beverages and non-food mixed amylolytic starters which mostly include *Candida, Debaryomyces, Geotrichum, Hansenula, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, Saccharomycopsis, Schizosaccharomyces, Torulopsis, Wickerhamomyces,* and *Zygosaccharomyces* (Tamang and Fleet 2009, Lv et al. 2013, Sha et al. 2017). Species of *Actinomucor, Amylomyces, Aspergillus, Monascus, Mucor, Neurospora, Penicillium, Rhizopus* and *Ustilago* are reported for many fermented foods, Asian non-food amylolytic starters and alcoholic beverages (Chen et al. 2014).

**Functionality and Health benefits**

Functional microorganisms transform the chemical constituents of raw materials of plant/animal sources during food fermentation thereby enhancing the bio-availability of nutrients, enriching sensory quality of the food, imparting bio-preservative effects and improvement of food safety, degrading toxic components and anti-nutritive factors, producing antioxidant and antimicrobial compounds, stimulating the probiotic functions, and fortifying with some health-promoting bioactive compounds (Farad et al. 2010, Tamang et al. 2016b). Microorganisms depend on relevant substrates for survival and produce bioactive compounds that enrich the human diet, thereby promoting health benefits to consumers (Bernardeau et al. 2006). Some health-promoting benefits of fermented foods and beverages are: prevention of cardiovascular disease, cancer, hepatic disease, gastrointestinal disorders and inflammatory bowel disease, as well as protection from hypertension, thrombosis, osteoporosis,

allergic reactions, diabetes, spoilage and toxic pathogens, reduction of obesity, increase immunity, alleviation of lactose intolerance, and anti-aging effects. Increased understanding of the viability of probiotic bacteria, interactions between gut microbiota, diet and the host has opened up new possibilities of producing new ingredients for nutritionally optimized foods, which can promote consumers' health through microbial activities in the gut (Tamang 2015).

**Classification of global fermented foods**

Global fermented foods are classified into 9 major groups on the basis of substrates (raw materials) used from plant/animal sources (Tamang 2016, Tamang et al. 2016a,c):

(1) Fermented cereal products (LAB and yeasts): *sourdough, idli, dosa, mawe,* etc.

(2) Fermented dairy products (LAB): yogurt, *dahi*, cheese, *kefir, koumiss, chhurpi,* etc

(3) Fermented legumes (Molds, *Bacillus* spp.): *shoyu, miso, tempe, natto, kinema, chungkokjang*, *thua nao, dawadawa, iru,* etc.

(4) Fermented roots/tubers (LAB + Yeasts): *gari*; *fufu, agbelima*, *tapé,* etc.

(5) Fermented vegetable products (LAB): *sauerkraut, kimchi, gundruk, sunki, saun-cai,* etc.

(6) Fermented and preserved meat products (Micrococcii + LAB): Sausages, *salami*, *nham, nem chua*, *kargyong*, etc.

(7) Fermented, dried and smoked fish products (Micrococcii + LAB): *plara, ngari, tungta*p, etc.

(8) Mixed mylolytic starters of Asia (Molds + Yeasts+ Bacteria): *marcha, nuruk, ragi, bubod, koji,* etc.

(9) Alcoholic beverages (Molds, Yeasts):

- Non-distilled and unfiltered alcoholic beverages produced by amylolytic starters eg. *kodo ko jaanr*, *makgeolli*.
- Non-distilled and filtered alcoholic beverages produced by amylolytic starters eg, *saké*.
- Distilled alcoholic beverages produced by amylolytic starter g., *shochu, soju*, *raksi*.
- Alcoholic beverages produced by involvement of amylase in human saliva eg. *chicha*.
- Alcoholic beverages produced by mono- (single-strain) fermentation eg. beer.
- Alcoholic beverages produced from honey eg. *tej*.
- Alcoholic beverages produced from plant parts eg. *pulque toddy*
- Alcoholic beverages produced by malting (germination) eg. *Bantu* beer, *tchoukoutou*.
- Alcoholic beverages prepared from fruits without distillation eg. wine cider.
- Distilled alcoholic beverages prepared from fruits and cereals eg. whisky and brandy.

(10) Miscellaneous fermented products (Bacteria, Yeast, Molds): vinegar, *nata, kombucha, puer tea, pida*n, etc.

Besides, above mentioned categories of fermented foods and beverages, unique fermented fodder through silage fermentation are fed to animals. Silage fermentation are mostly fermented by LAB (Woolford 1985, Muck 2010).

**References**

Alegría, A., González, R., Díaz, M. & Mayo, B. (2011) Assessment of microbial populations dynamics in a blue cheese by culturing and denaturing gradient gel electrophoresis. *Current Microbiol.* 62, 888-893.

Axelsson, L., Rud, I., Naterstad K, Blom H, Renckens B, Boekhorst J, Kleerebezem M, van Hijum, S., & Siezen, R.J. (2012) Genome sequence of the naturally plasmid-free *Lactobacillus plantarum* strain NC8 (CCUG 61730). *J. Bacteriol.* 194, 2391-2392.

Bernardeau, M., Guguen, M. & Vernoux, J.P. (2006) Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiol. Rev.* 30, 487-513.

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Schure, E.T., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S. & Hansen, E.B. (2012) Food fermentations: microorganisms with technological beneficial use. *Int. J. Food Microbiol.* 154, 87-97.

Chen, B., Wu, Q., & Xu, Y. (2014) Filamentous fungal diversity and community structure associated with the solid state fermentation of Chinese *Maotai*-flavor liquor. *Int. J. Food Microbiol.* 179, 80-84.

Coton, E., Desmonts, M.H., Leroy, S., Coton, M., Jamet, E., Christieans, S., Donnio, P.Y., Lebert, I., & Talon, R. (2010) Biodiversity of coagulase-negative staphylococci in French cheeses, dry fermented sausages, processing environments and clinical samples. *Int. J. Food Microbiol.* 137, 221-229.

Dolci, P., Alessandria, V., Rantsiou, K. & Cocolin, L. (2015) Advanced methods for the identification, enumeration, and characterization of microorganisms in fermented foods. In: Advances in Fermented Foods and Beverages (Ed.) Holzapfel, W.H., Elsevier, London, 157-176.

Ercolini, D. (2004) PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *J. Microbiol. Methods* 56, 297-314.

Farhad, M., Kailasapathy, K. & Tamang, J.P. (2010) Health aspects of fermented foods, In:Tamang J.P., Kailasapathy K. (Eds.) Fermented Foods and Beverages of the World, CRC Press, Taylor & Francis Group, New York, 391-414.

Holzapfel, W.H. & Wood, B.J.B. (2014) Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley-Blackwell, p 632.

Kubo, Y., Rooney, A.P., Tsukakoshi, Y., Nakagawa, R., Hasegawa, H. & Kimura, K. (2011) Phylogenetic analysis of *Bacillus subtilis* Strains applicable to natto (fermented soybean) production. *Appl. Environ. Microbiol.* 77, 6463-6469.

Lv, X-C., Huang, X-L., Zhang, W., Rao, P-F. & Ni, L. (2013) Yeast diversity of traditional alcohol fermentation starters for Hong Qu glutinous rice wine brewing, revealed by culture-dependent and culture-independent methods. *Food Control* 34, 183-190.

Muck, R.E. (2010) Silage microbiology and its control through additives.*Revista Brasileira de Zootecnia* 39: 183-191.

Puerari, C., Magalhâes-Guedes, T.M. & Schwan, R.F. (2015) Physicochemical and microbiological characterization of chicha, a rice-based fermented beverage produced by Umutina Brazilian Amerindians. *Food Microbiol*. 46, 210-217.

Shah, S.P., Jani, K., Sharma, A., Anupma, A., Pradhan, P., Shouche, Y. & Tamang, J.P. (2017) Analysis of bacterial and fungal communities in *Marcha* and *Thiat*, traditionally prepared amylolytic starters of India. *Scientific Reports* 7: 10967 DOI: 10.1038/s41598-017-11609-y.

Shangpliang, H.N.J., Sharma, S., Rai, R. & Tamang, J.P. (2017) Some technological properties of lactic acid bacteria isolated from *Dahi* and *Datshi*, naturally fermented milk products of Bhutan. *Frontiers in Microbiology* 8: doi: 10.3389/fmicb.2017.00116.

Shangpliang, H.N.K., Rai, R., Keisam, S., Jeyaram, K. and Tamang, J.P. (2018) Bacterial community in naturally fermented milk products of Arunachal Pradesh and Sikkim of India analysed by

high-throughput amplicon sequencing. *Scientific Reports* 8: 1532 DOI.10.1038/s41598-018-19524-6.

Tamang, J.P. (2010a) Diversity of fermented foods, In: Tamang JP, Kailasapathy K. (Eds.) Fermented Foods and Beverages of the World, CRC Press, Taylor & Francis Group, New York, 41-84.

Tamang, J.P. (2010b) Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values, CRC Press, Taylor & Francis Group, New York.

Tamang, J.P. (2010c) Diversity of fermented beverages, In: Tamang J.P., Kailasapathy, K. (Eds.), Fermented Foods and Beverages of the World, CRC Press, Taylor & Francis Group, New York, 85–125.

Tamang, J.P. (2015) Health Benefits of Fermented Foods and Beverages. CRC Press, Taylor & Francis Group, New York, pages 636.

Tamang, J.P. (2016) *Ethnic Fermented Foods and Alcoholic Beverages of Asia*. Springer, New Delhi, pages 409. ISBN: 978-81-322-2798-4

Tamang, J.P. and Fleet. G.H. (2009) Yeasts Diversity in Fermented Foods and Beverages. In: *Yeasts Biotechnology: Diversity and Applications* (Eds: Satyanarayana, T. and Kunze, G.). Springer, New York, pp. 169- 198. ISBN: 9781402082917.

Tamang, J.P., Holzapfel, W.H. & Watanabe, K. (2016a) Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology* 7:377. doi: 10.3389/fmicb.2016.00377.

Tamang, J.P., Shin, D.H., Jung, S.J. & Chae, S.W. (2016b) Functional properties of microorganisms in fermented foods. *Frontiers in Microbiology* 7:578. doi: 10.3389/fmicb.2016.00578.

Urushibata, Y., Tokuyama, S., and Tahara, Y. (2002) Characterization of the *Bacillus subtilisyws C* gene, involved in **γ**–polyglutamic acid production. *J. Bacteriol*. 184, 337-343.

Woolford, M.K. (1985) The Silage Fermentation. In: Wood, B.J.B.(Editor), *Microbiologyg of Fermented Foods*, volume 2, pp. 85-112. Elsevier Applied Science Publishers, London.

Yan, Y., Qian, Y., Ji, F., Chen, J. & Han, B. (2013) Microbial composition during Chinese soy sauce *koji*-making based on culture dependent and independent methods**.** *Food Microbiol*. 34, 189195.

# Molecular techniques to develop additives and characterize the microbial ecology of silages

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**Abstract**

Advancements in molecular biology are providing new insight into the complexity and diversity of microbial populations during ensiling. Understanding the epiphytic microbiome on harvested forage could lead to more predictable outcomes and identify those conditions in which additives are likely to be most efficacious for forage conservation. The ability to track specific microbial strains during ensiling could increase our understanding of their contribution to ensiling and the potential for them to elicit probiotic responses in ruminants upon feeding. Comparative genomics could aid in identifying those isolates with the greatest potential as forage inoculants.

## Introduction

Until the development of DNA based community fingerprinting techniques and the metagenomic evaluation of the silage microbiome, characterization of the microbiology of ensiling was limited to isolation and identification of silage microbes grown on selective media. However, many silage microorganisms including lactic acid bacteria (LAB), enterobacteria, yeast and molds can enter a viable but unculturable state, precluding their characterization through conventional plating methods (McAllister et al. 2018). Culture independent techniques enable the identification of microbial communities and the description of complex silage microbial population dynamics during ensiling and aerobic exposure. These molecular techniques also allow for profiling the microbes in silage inoculants and help understand how these microbes survive and alter the ensiling process. Muck (2013) reported that many silage inoculants produce bacteriocins that inhibit the growth of both bacteria and fungi, greatly increasing the survivability of these inoculants in silage. Undesirable silage bacteria such as *Clostridium* spp. and *Bacillus* spp. are of significance especially with respect to dairy cattle as the bacterial spores can enter the food chain through milk, leading to food spoilage and possibly animal and/or human fatalities (Tennant et al. 2017). *Listeria monocytogenes* and VTEC *Escherichia coli,* which can be found in silage have zoonotic significance. Similarly, mycotoxin producing moulds in silage such as *Aspergillus fumigatus* and *Penicillium roqueforti* pose serious health concerns as these toxins can produce detrimental health effects in dairy cattle while causing food safety concerns as a result of secretion in milk (Driehuis et al. 2008). Metagenomic studies can effectively be used to detect these undesirable microorganisms in silage thereby preventing their entrance into the food chain and enhancing food safety.

**Overview of culture independent techniques for silage microbiology**

Most of the culture independent DNA based community fingerprinting techniques rely on polymerase chain reaction (PCR) amplification of targeted sequences (Muck 2013; Nishino 2015). Some of the common techniques employed for the community analysis of the silage microbiome include denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), automated ribosomal intergenic spacer analysis (ARISA) and length heterogeneity PCR (LH-PCR) (Muck 2013, McAllister et al. 2018). The PCR based methods have been widely used to amplify DNA sequences of interest using forward and reverse primers for species identification and to distinguish between multiple microbial species (McAllister et al. 2018). Quantitative or real time PCR (qPCR) has been used for quantifying a particular microbial species present in the sample and for profiling microbial communities (Muck 2013; Nishino 2015; McAllister et al. 2018).

**Sampling for molecular studies**

Proper forage sampling techniques, as for nutrient analysis, need to be followed for molecular evaluation to ensure that the sample is representative of the entire amount of conserved forage. In order to ensure proper sampling prior to ensiling, 3-5 samples of fresh forage need to be collected after each truck load (McAllister et al. 2018). Subsamples are mixed and stored for molecular analysis. While sampling silage, care should be taken to avoid spoiled silage from the silo face by collecting subsamples at least 7-15 cm below the surface of exposed silage. Subsamples are composited and stored in air tight bags for analysis and should be frozen at -20°C for immediate processing and preferably at -80°C if DNA extraction is delayed.

**Sample processing for DNA extraction**

Success of PCR based metagenomic studies depends greatly on the quality and yield of DNA extracted for sequencing. Efficient extraction of microbial DNA is critical for unbiased results from downstream applications like qPCR and metagenomic sequencing (Bahl et al. 2012). McAllister et al. (2018) reported that storing samples intended for DNA extraction at lower (-80°C) temperature ceased the microbial activity and maintained cellular integrity. Preserving samples at ultra-low temperatures also ensures that the microbial diversity profile is maintained. Duration of storage has been reported to affect the diversity of microbial community in biological samples (Lauber et al. 2010, Bahl et al. 2012). These authors reported that even though the DNA yield was not affected by the duration of storage (Bahl et al. 2012), dynamics of microbial community was affected by long (> 50 d), but not short term (< 14 d) storage of samples. Standards for storage and DNA extraction can be used to try and ensure the consistency of findings across studies. The quality of qPCR depends on the quality of DNA extracted, which in turn depends on the method employed for sample processing and DNA isolation.

The yield and quality of nucleic acid extracted depends on the type of silage and the microbial profile during ensiling, and storage. Microbial populations in silage are more concentrated in areas of high soluble carbohydrate and protein content and exist primarily in the form of biofilms. Lysis of biofilms during nucleic acid extraction often results in rapid degradation of RNA (Wang et al. 2011). Samples for RNA extraction are preferably frozen immediately in liquid nitrogen or dry ice and stored at -80°C. Even at this temperature, the integrity of RNA can be lost with prolonged storage. Quaternary ammonium compounds have been reported to be effective in preserving RNA in some studies (van Eijsden et al. 2013). Frozen samples used for DNA extraction are ideally lyophilized as oven drying can affect microbial dynamics and negatively affect the stability of DNA. Moreover, fibre digesting bacteria preferentially colonize the interior of plant cells (Engels 1989) and thus simple washing of the silage with buffers will often fail to extract the DNA associated with these microorganisms.

Samples for DNA extraction are typically ball ground. Frictional impact while ball mill grinding results in effective microbial cell disruption. However, it should be noted that this procedure also increases the amount of plant DNA that is co-extracted along with microbial DNA. This can reduce the concentration

of microbial DNA that is available for sequencing and possibly the sensitivity of downstream analysis. For RNA, grinding of the samples in liquid nitrogen is one way of increasing yield (Wang et al. 2011).

**DNA extraction**

Isolation of DNA from plant tissues can be challenging as plants contain a wide variety of metabolites and biomolecules like polysaccharides, proteins, tannins, alkaloids and polyphenols that can interfere with DNA extraction and purification (Abdel-Latif and Osman 2017). Polysaccharides that co-precipitate with DNA can make DNA pellets slimy and difficult to handle (Healey et al. 2014, Abdel-Latif and Osman 2017), while polyphenols bind to nucleic acids to form phenolic-nucleic acid complexes. Most forage DNA extraction procedures are modifications of the CTAB (cetyl trimethyl ammonium bromide) method with purification of DNA with phenol-chloroform-isoamyl alcohol (Minas et al. 2011). This method can be modified to include polyvinylpyrrolidone (PVP) and high salt solutions to effectively separate polysaccharides and bind polyphenols during DNA extraction. Moreover, the CTAB method is well suited for forage and silage samples processed with liquid nitrogen as it removes plant components. Kits are commercially available and widely used for extraction of DNA/RNA from silage samples.

**DNA extraction kits**

Various commercial DNA extraction kits (QiAamp DNA stool mini kit; Qiagen Sciences, Germantown, MD; FastDNA™ spin kit for soil; MP Biomedicals, Santa Ana, CA; Ultraclean soil DNA isolation kit, MoBio Laboratories Inc., Carlsbad, CA; Quick-DNA™ Plus kit; Zymo Reaserch Corporation, Irvine, CA) can be used for extraction and purification of DNA/RNA from silage (Duniere et al. 2017) and other biological samples (Lebuhn et al. 2016). As silage samples can contain the various PCR inhibitors, DNA extraction kits like the QiAamp DNA stool kit has steps to remove PCR inhibitors, making them more effective for extracting high quality unbiased DNA for downstream analysis. One of the major concerns in any DNA extraction procedure is the ability of the extraction procedure to lyse and recover the DNA from all members of the microbiome (Hazen et al. 2013). It has been reported that of the steps in microbial profiling i.e., DNA extraction, PCR amplification and sequencing, the greatest bias arises from DNA extraction (Vishnivetskaya et al. 2014).

To the authors' knowledge, there has not been any comparison among extraction methods on the yield and purity of DNA/RNA obtained from silage samples. It is logical to assume that the nature of the extraction kit can affect the ability of PCR and downstream analysis to describe the structure of the silage microbial community. In an evaluation of the effect of DNA extraction methods on the quality of DNA extracted from corn, Abdel-Latif and Osman (2017) reported that a modified Mericon extraction method resulted in greater DNA yield and quality at a reduced cost and with less time than either a DNeasy Qiagen Plant mini Kit or CTAB methods.

Most extraction methods result in poor recovery of DNA from Gram-positive bacteria as these microorganisms have thick and resistant cell walls (Fujimoto et al. 2004). In order to extract DNA from Gram-positive bacteria, the cell wall has to be breached to release DNA within the cytoplasm. In a recent evaluation of microbial community composition of permafrost samples, Vishnivetskaya et al. (2014) reported that the Meta-G-Nome™ DNA isolation kit worked for the physical separation of microbes from soil followed by cell lysis and DNA recovery, but had the poorest yield of genomic DNA and did not adequately represent the community genome relative to kits that used bead-beating during DNA extraction (FastDNA® spin kit for soil, PowerSoil® Kit and PowerLyzer® Kit). Similar responses could be anticipated with regard to the isolation of DNA from Gram-positive bacteria in silage.

Spores of anaerobic (*Clostridium* species) and aerobic (*Bacillus* species) frequently found in silages are generally resistant to lysis and DNA yield is limited due to presence of the resistant spore coat. However, in an evaluation of commercial DNA extraction kits for the isolation of bacterial spore DNA from soil samples, Dineen et al. (2010) isolated DNA from spores of *Bacillus* species for qPCR analysis. These authors reported that FastDNA® spin kit that utilized bead-beating for DNA extraction

resulted in highest DNA yield while the E.Z.N.A® Soil DNA kit and PowerSoil® DNA isolation kits effectively removed PCR inhibitors during DNA extraction procedures.

However, bead-beating has to be optimized as it may compromise DNA extraction from thin cell walled microbes due to shearing of the DNA (Miller et al. 1999). The greater abundance of easily lysed β- and α-*Proteobacteria* using the Meta-G-Nome™ DNA isolation kit as compared to bead-beating extraction procedures is likely a reflection of shearing of DNA as a result of this more aggressive isolation technique (Vishnivetskaya et al. 2014). Similarly, Pakpour et al. (2013) reported a greater degree of DNA degradation for DNA extracted via bead-beating relative to kits that did not. These authors reported that kits that do not employ bead-beating can still achieve acceptable yields of DNA. Extraction procedures that employ bead-beating generally results in increased DNA yield at the expense of greater fragmentation of DNA. Considering that silage is typically rich in Gram-positive *Lactobacilli*, that may reside within plant cells, it is logical to assume that bead-beating procedures will result in higher DNA yields relative to kits that do not employ bead-beating. Addition of cell wall degrading enzymes, like lysozyme, during the initial sample preparation steps would reduce integrity of the Gram-positive bacterial cell wall and allow for a shorter time of bead-beating.

**PCR for silage microbiology**

Polymerase chain reaction enables the generation of multiple copies of specific regions of DNA. Similar to conventional PCR, qPCR also follows similar amplification cycles where template DNA is denatured, annealed with specific oligonucleotide primers and extended to generate complementary strands using thermostable DNA polymerases (Postollec et al. 2011). However, in qPCR, the exponential increase in amplicons can be monitored at every cycle in real time. Moreover, qPCR is faster than conventional PCR as it eliminates the post-amplification steps needed for conventional PCR to visualize amplified products and is more sensitive and reproducible (McAllister et al. 2018). Real time PCR has sensitivity and specificity that is equivalent to or even superior to conventional PCR (Espy et al. 2006). Moreover, chances of environmental contamination by amplified DNA are greatly eliminated in qPCR analysis as amplification and detection steps are performed in the same closed vessel.

**Molecular targets for PCR**

Selection of accurate DNA targets for downstream molecular techniques are of prime importance in obtaining non biased microbial community assessment. Target DNA selection depends on the technique used, specificity of primers for the target, the microbiome evaluated and the taxonomic depth required (Bokulich and Mills 2012).

Ribosomal RNA (rRNA) genes are highly conserved regions among all living organisms due to interactions of the products to proteins in the ribosome. Prokaryotic rRNA consists of three transcripts, the 16S (small), the 23S (large) subunits, and the 5S rRNA sequence (McAllister et al. 2018). Sequencing of the 16S rRNA gene is routinely used for the identification of bacterial species and in taxonomic studies. The 16S RNA gene contains 9 hypervariable regions (V1 – V9) (McAlister et al. 2018). The hypervariable regions are interspersed by conserved segments that enable PCR amplification of target sequences using universal primers (McCabe et al. 1999). The hypervariable regions exhibit a considerable degree of diversity among bacterial species, but no single hypervariable region is able to distinguish among all bacterial genera (Chakravorty et al. 2007). However, combining the hypervariable regions can generate adequate sequence diversity to enable the identification of many bacteria to the genus level. Accumulation of taxonomic identification for 16S rRNA gene sequences stored in public databases allows for a more detailed source of comparison for this gene versus the 23S rRNA gene.

The 16S and 23S rRNA genes are separated by a spacer region, known as the internal transcribed spacer (ITS) region that is transcribed along with the ribosomal genes (Osorio et al. 2005). Considerable length and sequence variation exist in this ITS region even among strains of the same

species, making the ITS region useful for identifying closely related strains (Osorio et al. 2005; McAllister et al. 2018). For example, ITS has been used to differentiate between different strains of closely related lactic acid bacteria (Yavuz et al. 2004). Similarly, Rachman et al. (2003) used ITS to distinguish *Lactobacillus farciminis* from *Lactobacillus alimentarius,* even when these species shared 96% similarity in their 16S rDNA. Other divergent loci such as genes encoding the 60 kDa chaperonin protein (CPN-60; Links et al. 2012) and recombinase enzyme (*rec*A; Hofstatter et al. 2016) may also be used to describe bacterial diversity in silage.

Eukaryotic rRNA operons include 18S, 5.8S and 25S/28S rRNA, separated by internal transcribed spacers 1 (ITS1) and 2 (ITS2) that flank the conserved 5.8S region (McAllister et al. 2018). Similar to bacteria, rRNA genes are widely used for the molecular characterization of fungi as multiple copies are present in each genome and ITS regions can be amplified using broad range primers (Khot et al. 2009). Li and Nishino (2011a, b) amplified fungal 18S rRNA to characterize fungal and mold species in grass and whole-crop cereal silages. Even though 18S rRNA has been commonly used for the phylogenetic classification of fungi, compared to 16S rRNA for bacteria, it has fewer hypervariable domains (Schoch et al. 2011). The 28S rRNA, hypervariable regions in the 28S rRNA (D1/D2 region) and ITS are also used for the identification of fungi. The ITS primers target the conserved region of the 18S, 5.8S and 28S RNA genes to amplify these noncoding regions (White et al. 1990). Kurtzman and Robnett (1998) reported that the variable D1/D2 domain of 28S rDNA sufficiently differentiated more than 500 species of *Ascomycetous* yeasts. Fell et al. (2000) and Scorzetti et al. (2002) reported that ITS region was more effective at differentiating among *Basidiomycetous* yeasts as compared to the D1/D2 domain. However, the choice of ITS primers has been shown to result in amplification biases with the preferential amplification of some fungal groups (Bellemain et al. 2010).

**PCR trouble shooting**

Real time PCR allows for generation of millions of amplicons of a specific DNA segment in a short period of time. However, factors such as sample dilution and waiting times in laboratory work flow have been reported to result in amplification of nonspecific DNA products during PCR (Villalba et al. 2017). These nonspecific DNA products appear as a ladder or smear of bands on agarose gels (Lorenz 2012). These authors also reported that unintentional mutations introduced into the amplicons can generate a heterogeneous population of PCR products. If PCR conditions are not optimized, primers preferentially self-anneal or anneal to other primers in the reaction to form primer dimers. PCR bands with varying lengths are formed by nonspecific reaction if PCR stringency is too low. These pitfalls in the PCR procedure could adversely affect the PCR efficiency and downstream genomic analysis. Optimization of PCR with negative and positive control samples is performed to avoid nonspecific DNA products and artefacts (Villalba et al. 2017).

**PCR inhibitors**

PCR inhibitors are a heterogeneous group of chemicals or substances that originate from sample or are introduced during sample processing (Schrader et al. 2012). PCR inhibitors act either through direct interaction with DNA or interference with DNA polymerases (Bessetti 2007). Direct binding of inhibitors to DNA impede amplification while inhibitors of DNA polymerase block enzyme activity. PCR inhibitors relevant to forage and silages include polysaccharides, proteases, phenolics and tannins (McAllister et al. 2018). In general, PCR inhibitors can be removed by dilution, clean-up procedures as recommended within commercial DNA extraction kits, by the use of specific PCR additives, or by the use of column chromatography (Reuter et al. 2009; Schrader et al. 2012).

**Molecular techniques for defining silage microbiology**

Real time PCR has been shown to be a valuable tool in assessing microbial population dynamics during ensiling. This molecular technique raises the possibility of determining the effectiveness of specific bacterial species in silage inoculants as most species of *Lactobacillus* are not easily

differentiated using classical microbiology techniques such as plating. Klocke et al. (2006) were the first to use qPCR in evaluating silage microbiology during ensiling and reported that the copy numbers of genes encoding for 16S rRNA (16S rDNA) of *Lactobacillus plantarum* in inoculated grass silage increased during the first 2 weeks of ensiling and decreased thereafter. Stevenson et al. (2006) using species specific primers to the *rec*A gene reported that the population of *L. plantarum*, *Enterococcus faecium*, *Pediococcus pentosaceus*, *Lactobacillus buchneri* and *Lactobacillus lactis* in alfalfa silage inoculated with various commercial silage inoculants decreased while population of *Lactobacillus brevis* increased after 96 h of ensiling. These authors reported that all the commercial silage inoculants resulted in higher quality silage than uninoculated silage as indicted by the lower pH and higher lactic acid content. It was concluded that the LAB population in commercial silage inoculants influenced the earlier stages of silage fermentation, making their persistency later in ensiling inconsequential (Stevenson et al. 2006). Conversely, in corn silage inoculated with a mixture of *L. buchneri*, *L. plantarum*, *E. faecium*, *Pediococcus acidilactici* and *P.pentosaceus*, Schmidt et al. (2008) reported that qPCR-estimated numbers of *L. buchneri* were 1000 to 10 000 fold greater than in uninoculated silage after 210 d of ensiling. However, Lynch et al. (2012) reported no difference in numbers of *L. buchneri* in inoculated vs uninoculated corn ear silage after 35 or 130 d of ensiling. It was concluded that epiphytic LAB population plays a pivotal role in determining the efficacy of silage inoculants. Recently, Duniere et al. (2015) used qPCR to evaluate the effect of inoculation of corn silage with strains of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* on fermentation and aerobic stability. These authors reported a less pronounced decline in the fungal population during ensiling using qPCR as compared to plate count estimates. It was concluded that fungi contain multiple copies of 18S rRNA and as a result, enumeration by qPCR was not directly proportional to plate counts. Moreover, the primers used to estimate total fungi also targeted yeasts which were excluded from plate counts. These authors reported an increase in population of *S. cerevisiae* and *S. paradoxus* during aerobic exposure, illustrating that at least some of the inoculated yeast remained viable after the ensiling process.

One of the major drawbacks of qPCR based molecular techniques for defining the silage microbiome is the inability to distinguish between viable and non-viable microorganisms as amplified DNA may contain nucleic acids from both live and dead microbial cells. As RNA is more unstable than DNA after cell lysis, reverse transcriptase PCR (RT-PCR) of RNA transcripts serve as an indicator of viability of microbial cells (Bokulich and Mills 2012). Similarly, the DNA-binding dyes, propidium monoazide (PMA) or ethidium monoazide (EMA) can be used to selectively bind the DNA of nonviable cells and prevent it from being amplified during RT-PCR (Bokulich and Mills 2012).

**Tools for silage microbial community analysis**

Advances in the field of silage microbiology through the use of PCR based methods have enabled the profiling of genetic diversity within the silage microbiome (Muck 2013). Some of the tools employed in the past for the community analysis of silage microbiomes have largely been replaced by next generation sequencing (NGS) technologies.

**Next generation sequencing (NGS) in silage microbiology**

Next generation sequencing refers to several technologies that achieve deep, high-throughput DNA sequencing enabling massively parallel sequencing of heterogeneous DNA fragments from multiple samples at substantially lower costs (Kulski 2016).

**NGS platforms**

Most common NGS platforms used for silage microbiology include 454 pyrosequencing, Illumina, Single-molecule-Real-Time (SMRT) sequencing and Nanopore sequencing. Several open source programs exist for processing short amplicon NGS data like QIIME and Mothur which translate the raw reads into microbial community data (Bokulich and Mills 2012).

**Roche 454 pyrosequencing**

Pyrosequencing was the first commercially available next generation sequencing technique (Bokulich and Mills 2012). This system employs detection of light, generated by an ATP sulfurylase, luciferase, DNA polymerase enzymatic system, produced in proportion to the amount of pyrophosphate that is released during repeated nucleotide incorporation into the newly synthesized DNA chain (Kulski 2016). The system produces reads of 600-800 bp which provide greater taxonomic differentiation. However, high cost of reagents and discontinuation of supplies and services for this sequencing technology (Kulski 2016) has resulted in its gradual replacement by other NGS technologies.

**Illumina (Solexa) Hiseq and Miseq sequencing**

Illumina (Solexa) sequencer utilizes sequencing by synthesis technology using removable fluorescently labelled chain-terminating nucleotides that are able to produce larger outputs at lower costs (Kulski 2016). Illumina technology immobilizes random DNA fragments on a surface and then performs solid surface PCR amplification, resulting in clusters of identical DNA fragments (Thomas et al. 2012). The read lengths are shorter (150 bp, initially), output of sequencing data per run is greater and cost is cheaper than pyrosequencing (Kulski 2016).

**Ion Torrent sequencing (Life Technologies)**

Ion Torrent sequencing technology detects nucleotide sequences electronically as the addition of each nucleotide to a DNA polymer releases an $H^+$ ion that change the pH of the surrounding solution in manner which is proportional to the number of incorporated nucleotides (Kulski 2016). Two sequencers that use Ion Torrent technology are high-throughput Ion Proton sequencer and a benchtop Ion Personal Genome Machine (PGM). These sequencers produce relatively long read lengths (200 bp) with reduced turnaround time and lower costs (Kulski 2016).

**Single-Molecule-Real-Time (SMRT) sequencing (Pacific Biosciences)**

The library preparation for SMRT consists of ligating hairpin adapters onto DNA molecules so that after ligation, the double stranded DNA fragments become circular. The method utilizes 150 000 nanoscale observation chambers called zero mode wave guides (ZMWs), where single DNA polymerase enzyme is fixed to the bottom of ZMWs and incorporate fluorescently labelled nucleotides with the single DNA molecule in the library as a template. The SMRT technology is sensitive enough to detect incorporation of one fluorescently labelled nucleotide (Buermans & Dunnen 2014). The fluorescence emitted by nucleotides is recorded in real time and converted into long sequences called continuous long reads (Arudi et al. 2018). Since there is no need for DNA amplification during library preparation or during sequencing, biases such as GC-skewing are avoided (Ardui et al. 2018).

**Nanopore Sequencing (Oxford Nanopore Technologies)**

Nanopore sequencing is characterized by single molecule sensing, label-free ultra long reads with high throughput and low material requirement (Feng et al. 2015). Nanopore sequencing utilizes pores from proteins such as haemolysin for tunneling DNA molecules between two compartments. Conductivity of ion currents change when strands of nucleic acids pass through the pore (Kulski 2016). The target molecules are characterized based on the amplitude and duration of ionic current signals during the translocation events (Feng et al. 2015). There is minimal sample preparation involved with no amplification steps required before sequencing with output of long read lengths, eliminating PCR biases. The simplest units can also be operated through a USB port in a personal lap top computer.

**Metagenomics**

Metagenomics refers to a suite of genomic technologies and bioinformatic tools for the direct genetic analysis of genomes within environmental samples to enable the taxonomic identification of microbial communities (Thomas et al. 2012, Tenant et al. 2017). Next generation sequencing has accelerated the development of sequence based metagenomics (Thomas et al. 2012).

Initially the term metagenomics was applied to functional and sequence based analysis of community microbial genomes (whole shotgun metagenomics), but it is also applied for PCR amplification based studies of genes of interest (Marker gene amplification metagenomics or meta-genetics; Oulas et al. 2015). Shotgun metagenomics has the advantage of avoiding primer biases as the entire microbiome in the community can be sequenced and identified (McAllister et al. 2018).

There is recent interest in describing the microbial ecology of silage using NGS. In an evaluation of dynamics of bacterial community during ensiling of grass, Eikmeyer et al. (2013) used 454 pyrosequencing to report that the inoculant containing *L. buchneri* CD034 not only decreased the abundance of other members of the genus *Lactobacillus* (*L. plantarum* and *L. brevis*), but also decreased the abundance of *Lactococcus* (*Lactococcus. lactis*) and *Leuconostoc* (*Leuconostoc. citreum*) after 58 d of ensiling. These authors also reported a decrease in microbial diversity after 14 and 58 d of ensiling in inoculated as compared to control silage. In an evaluation of microbial communities in commercial silages from bunker silos, Kraut-Cohen et al. (2016) used Ion Torrent sequencing technology in a metagenomic evaluation of the microbiomes associated with preserved vs spoiled silages. These authors reported that well preserved silage had lower taxonomic diversity with greater abundance of LAB, while spoiled silage had a more diverse microbiome with a lower abundance of LAB and higher levels of *Enterobacteriaceae*.

Ni et al. (2017) utilized Illumina MiSeq sequencing to evaluate microbial dynamics during ensiling of Italian rye grass, whole-crop corn and wilted alfalfa. These authors reported that after 60 d of ensiling, *Leuconostoc spp*. and *Pediococcus spp*. predominated in Italian rye grass, *Lactobacillus spp.* in whole crop corn and *Enterococcus spp.* in wilted alfalfa silage. Duniere et al. (2017) reported that the relative abundance of *Actinomycetales* decreased by 11.2 - 32% while that of *Lactobacillales* increased during ensiling of small grain cereal silages. These authors also reported that bacterial diversity decreased during ensiling, and aerobic exposure of small grain cereal silages resulted in an increase in *Bacillales* and decrease in the relative abundance of *Lactobacillales* in aerobically exposed oat, triticale and intercropped silages.

The majority of metagenomic studies have focused on the bacterial ecology of silage as ensiling is largely bacterial driven (McAllister et al. 2018). In a recent evaluation of fungal microbiome dynamics during ensiling and aerobic exposure of small grain cereal silages, Duniere et al. (2017) reported that the diversity of fungal core microbiome of small grain cereal silages decreased during ensiling and aerobic exposure. These authors reported that *Saccharomycetales* represented nearly 70% of the fungal core microbiome during ensiling and aerobic exposure. More recently, Romero et al. (2018) reported that inoculation of corn silage with a mixed bacterial inoculant resulted in an increase in relative abundance in *Debaryomycetaceae* and decrease in *Pichiaceae* relative to untreated silage after 100 d of ensiling.

**Functional sequencing**

Whole shotgun metagenomics allows for sequencing of genes involved in both phylogeny and function in silage microbiome, enabling the characterization of specific metabolic pathways associated with the production or degradation of specific metabolites during ensiling (McAllister et al. 2018). For example, Guo et al. (2014) evaluated the ability of the rat microbiome to detoxify ochratoxin A, which is a common mycotoxin found in corn silages. However, further research in the area of functional genomics during ensiling is needed to know to what extent populations can be manipulated in a manner to produce favourable outcomes in the feed quality of silage.

**Scope of metagenomics in ensiling**

Use of metagenomics and transcriptomic studies are expected to become a standard tool in the field of silage microbiology, owing to the capacity of these techniques for sequencing the available genome in a given community, amount of phylogenetic depth obtained and for the potential for linkages between phylogeny and the function of silage microbial communities. Metagenomics could potentially be used for identifying novel heterofermentative LAB inoculants that can impart enhanced aerobic stability and improved animal performance (Muck et al. 2018). Moreover, these molecular techniques could lead to the development of novel fungal inoculants and shed light on approaches to mitigate the ability of the fungal microbiome to produce mycotoxins in silages. Studies using strains of *Pichia anomala* J121 (Schnürer and Jonsson 2011) show promise as these strains were found to have high activity against fungi and some Gram-negative bacteria. Additionally, inoculation of silage with *Saccharomyces* strains (Duniere et al. 2015) had no effect on LAB or the aerobic stability of silage, although numbers did increase during aerobic exposure, a potential benefit if the strain exhibits beneficial direct-fed microbial (DFM) properties (Duniere et al. 2015). Functional genomics is expected to play a pivotal role in the development of future silage additives that have the capacity to inhibit *Clostridia* and other spoilage and/or pathogenic microorganisms during ensiling. Finally, metagenomics and metatranscriptomic studies could also assist in the development of novel silage additives with unique traits such as the capacity to enhance plant cell wall digestion.

**Conclusion**

Although molecular techniques have their limitations, exponential advancement in this area is poised to rapidly accelerate the depth and breadth of our understanding of the role of the forage microbiome in the preservation of both silage and high-moisture forages and to aid in defining the next generation of forage additives.

**References**

Abdel-Latif, A. & Osman, G. (2017) Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Methods, 13:1, 1-9.

Ardui, S., Ameur, A., Vermeesch, J.R. & Hestand, M.S. (2018) Single molecule real-time (SMRT) sequencing comes of age: applications and utilities for medical diagnosis. Nucleic Acids Research, 46, 2159-2168.

Bahl, M.I., Bergström, A. & Licht. T.R. (2012) Freezing fecal samples prior to DNA extraction affects the Firmicutes to Bacteriodes ratio determined by downstream quantitative PCR analysis. FEMS Microbiology Letters, 329, 193-197.

Bellemain, E., Carlsen, T., Brochmann, C., Coissac, E., Taberlet, P. & Kauserud, H. (2010) ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiology, 10:189, 1-9.

Besetti, J. (2007) An introduction to PCR inhibitors. Available online [https://www.promega.ca/]

Bokulich, N.A. & Mills, D.A. (2012) Next-generation approaches to the microbial ecology of food fermentations. BMB Reports, 45(7), 377-389.

Buermans, H.P.J., & den Dunnen, J.T. (2014) Next generation sequencing technology: Advances and applications. Biochimica et Biophysica Acta, 1842, 1932-1941.

Chakravorty, S., Helb, D., Burday, M., Connell, N. & Alland, D. (2007) A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. Journal of Microbiological Methods, 69(2), 330-339.

Dineen, S.M., Aranda IV, R., Anders, D.L. & Robertson, J.M. (2010) An evaluation of commercial DNA extraction kits for the isolation of bacterial spore DNA from soil. Journal of Applied Microbiology, 109, 1886-1896.

Driehuis, F., Spanjer, M. C., Scholten, J. M., & Giffel, M. C. (2008) Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in Netherlands. Food Additives and Contaminants, 1:1, 41-50.

Dunière, L., Jin, L., Smiley, B., Qi, M., Rutherford, W., Wang, Y. & McAllister, T.A. (2015) Impact of adding *Saccharomyces* strains on fermentation, aerobic stability, nutritive value, and select Lactobacilli populations in corn silage. Journal of Animal Science, 93, 2322-2335.

Dunière, L., Xu, S., Jin, L., Elekwachi, C., Wang, Y., Turkington, K., Forster, R., & McAllister, T.A. (2017) Bacterial and fungal core microbiome associated with small grain silages during ensiling and aerobic spoilage. BMC Microbiology, 17:50, 1-16.

Eikmeyer, F.G., Köfinger, P., Poschenel, A., Jünemann, S., Zakrzewski, M., Heinl, S., Mayrhuber, E., Grabherr, R., Pühler, A., Schwab, H. & Schlüter, A. (2013) Metagenome analysis reveal the influence of the inoculant *Lactobacillus buchneri* CD034 on the microbial community involved in grass ensiling. Journal of Biotechnology, 167, 334-343.

Engels, F.M. (1989) Some properties of cell wall layers determining ruminant digestion. In: Chesson, A. & Orskov, E.R. (ed.). Physico-chemical characterization of plant residues for industrial and feed use. London. Applied Sciences Publishers, p. 80-87.

Espy, M.J., Uhl, J.R., Sloan, L.M., Buckwalter, S.P., Jones, M.F., Vetter, E.A., Yao, J.D.C., Wengenack, N.L., Rosenblatt, J.E., Cockerill III, F.R. & Smith, T.F. (2006) Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clinical Microbiology Reviews, 19, 165-256.

Fell, J.W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman. (2000) Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. International Journal of Systematic and Evolutionary Microbiology. 50, 1351-1371.

Feng, Y., Zhang, Y., Ying, C., Wang, D. & Du, C. (2015) Nanopore-based fourth-generation DNA sequencing technology. Genomics Proteomics Bioinformatics, 13, 4-16.

Fujimoto, S., Nakagami, Y. & Kojima, F. (2004) Optimal bacterial DNA isolation method using bead-beating technique. Memoirs Kyushu University Department of Health Sciences of Medical School, 3: 33-38.

Guo, M., Huang, K., Chen, S., Qi, X., He, X., Cheng, W-H., Luo, Y., Xia, K. & Xu, W. (2014) Combination of metagenomics and culture-based methods to study the interaction between ochratoxin A and gut microbiota. Toxicological Sciences, 141(1), 314-323.

Hazen, T.C., Rocha, A.M., Tachtmann, S.T. (2013). Advances in monitoring environmental microbes. Current Opinion in Biotechnology, 24, 526-533.

Healey, A., Furtado, A., Cooper, T. & Henry, R.J. (2014). Protocol: A simple method for extracting next generation sequencing quality genomic DNA from recalcitrant plant species. Plant Methods, 10:21, 1-8.

Hofstatter, P.G., Tice, A.K., Kang, S., Brown, M.W. & Lahr, D.J.G. (2016) Evolution of bacterial recombinase A (recA) in eukaryotes explained by addition of genomic data of key microbial lineages. Proceedings of Royal Society, 283, 20161453, 1-8.

Khot, P.D., Ko, D.L. & Fredricks, D.N. (2009) Sequencing and analysis of fungal rRNA operons for development of broad-range fungal PCR assays. Applied and Environmental Microbiology, 75, 1559-1565.

Klocke, M., Mundt, K., Idler, C., McEniry, J., O'Kiely, P., & Barth, S. (2006) Monitoring *Lactobacillus plantarum* in grass silages with the aid of 16S rDNA-based quantitative real-time PCR assays. Systematic and Applied Microbiology, 29, 49-58.

Kraut-Cohen, J., Tripathi, V., Chen, Y., Gatica, J., Volchinski, V., Sela, S., Weinberg, Z., & Cytryn, E. (2016) Temporal and spatial assessment of microbial communities in commercial silages from bunker silos. Environmental Biotechnology, 100, 6827-6835.

Kulski, J.K. (2016) Next-generation sequencing – An overview of the history, tools, and "omic" applications. Available Online [https://www.intechopen.com/].

Kurtzman, C.P. & Robnett, C.J. (1998) Identification and phylogeny of *ascomycetous* yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek, 73, 331-371.

Lauber, C.L., Zhou, N., Gordon, J.I., Knight, R. & Fierer, N. (2010) Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples, FEMS Microbiology Letters, 307, 80-86.

Lebuhn, M., Derenkó, J., Rademacher, A., Helbig, S., Munk, B., Pechtl, A., Stolze, Y., Prowe, S., Schwarz, H., Schlüter, A., Liebl, W. & Klocke, M. (2016) DNA and RNA extraction and quantitative real-time PCR-based assays for biogas biocenoses in an interlaboratory comparison. Bioengineering, 3:7, 1-24.

Li, Y., & Nishino, N. (2011a) Bacterial and fungal communities of wilted Italian ryegrass silage inoculated with and without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. Letters in Applied Microbiology, 52, 314-321.

Li, Y. & Nishino, N. (2011b) Effects of inoculation of *Lactobacillus rhamnosus* and *Lactobacillus buchneri* on fermentation, aerobic stability and microbial communities in whole crop corn silage. Grassland Science, 57, 184-191.

Links, M.G., Dumonceaux, T.J., Hemmingsen, S.M. & Hill, J.E. (2012) The chaperonin-60 universal target is a barcode for bacteria that enables de novo assembly of metagenomic sequence data. PLos ONE, 7, e49755, 1-10.

Lorenz, T.C. (2012) Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. Journal of Visualized Experiments. 63, e3998, 1-15.

Lynch, J.P., O'Kiely, P.O., Waters, S.M. & Doyle, E.M. (2012) Conservation characteristics of corn ears and stover ensiled with the addition of *Lactobacillus plantarum* MTD-1, *Lactobacillus plantarum* 30114, or *Lactobacillus buchneri* 11A44. Journal of Dairy Science, 95, 2070-2080.

McAllister, T.A., Dunière, L., Drouin, P., Xu, S., Wang, Y., Munns, K. & Zaheer, R. (2018) Silage review: Using molecular approaches to define the microbial ecology of silage. Journal of Dairy Science, 101, 4060-4074.

McCabe, K.M., Zhang, Y-H., Huang, B-L., Wager, E.A. & McCabe, E.R.B. (1999) Bacterial species identification after DNA amplification with a universal primer pair. Molecular genetics and metabolism, 66, 205-211.

Miller, D.N., Bryant, J.E., Madsen, E.L. & Ghiorse, W.C. (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Applied and Environmental Microbiology, 65, 4715-4724.

Minas, K., McEwan, N.R., Newbold, C.J., & Scott, K.P. (2011) Optimization of a high throughput CTAB-based protocol for the extraction of qPCR grade DNA from rumen fluid, plant and bacterial pure cultures. FEMS Microbiology Letters, 325, 162-169.

Muck, R.E. (2013) Recent advances in silage microbiology. Agriculture and Food Science, 22, 3-15.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C. & Kung Jr.II, J. (2018) Silage review: Recent advances and future uses of silage additives. Journal of Dairy Science, 101, 3980-4000.

Ni, K., Minh, T.T., Tu, T.T.M., Tsuruta, T., Pang, H. & Nishino, N. (2017) Comparative microbiota assessment of wilted Italian ryegrass, whole crop corn, and wilted alfalfa silage using

denaturing gradient gel electrophoresis and next-generation sequencing. Biotechnological Products and Process Engineering, 101, 1385-1394.

Nishino, N. (2015) New trends in silage microbiology, In: Daniel JLP, Morais G., Junges, D., Nussio LG (eds). Proceedings of the 17th international silage conference. Dados Internacionais de Catalogação na Publicação, Piracicaba, Brazil, pp. 52-65.

Osorio, C.R., Collins, M.D., Romalde, J.L. & Toranzo, A.E. (2005) Variation in 16S-23S rRNA intergenic spacer regions in *Photobacterium damselae*: a mosaic-like structure. Applied and Environmental Microbiology, 71, 636-645.

Oulas, A., Pavloudi, C., Polymenakou, P., Pavlopoulos, G. A., Polymenakou, N., Kotoulas, G., Arvanitidis, C. & Iliopoulos, I. (2015) Metagenomics: Tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. Bioinformatics and Biology Insights, 9, 75-88.

Pakpour, S., Olishevska, S.V., Prasher, S.O., Milani, A.S. & Chénier, M.R. (2013) DNA extraction method selection for agricultural soil using TOPSIS multiple criteria decision-making model. American Journal of Molecular Biology, 3, 215-228.

Postollec, F., Falentin, H., Pavan, S., Combrisson, J, & Sohier, D. (2011) Recent advances in quantitative PCR (qPCR) applications in food microbiology. Food Microbiology, 28, 848-861.

Rachman, C.N., Kabadjova, P., Prévost, H. & Dousset, X. (2003) Identification of *Lactobacillus alimentarius* and *Lactobacillus farciminis* with 16S-23S rDNA intergenic spacer region polymorphism and PCR amplification using species-specific oligonucleotide. Journal of Applied Microbiology, 95, 1207-1216.

Reuter, T., Xu, W., Alexander, T.W., Stanford, K., Xu, Y. & McAllister, T.A. (2009) Purification of polymerase chain reaction (PCR)-amplifiable DNA from compost piles containing bovine mortalities. Bioresources Technology. 100, 3343-3349.

Romero, J.J., Joo, Y., Park, J., Tiezzi, F., Gutierrez-Rodriguez, E., & Castillo, M.S. (2018) Bacterial and fungal communities, fermentation, and aerobic stability of conventional hybrids and brown midrib hybrids ensiled at low moisture with or without a homo- and heterofermentative inoculant. Journal of Dairy Science, 101, 3057-3076.

Schmidt, R.J., Emara, M.G. & Kung Jr., L. (2008) The use of quantitative real-time polymerase chain reaction assay for identification and enumeration of *Lactobacillus buchneri* in silage. Journal of Applied Microbiology, 105, 920-929.

Schnurer, J. & Jonsson, A. (2011) *Pichia anomala* J 121: a 30-year overnight near success biopreservation story. Antonie van Leeuwenhoek, 99, 5-12.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. & Fungal Barcoding Consortium (2011) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proceedings of the National Academy of Sciences of the United States of America. 109, 6241-6246.

Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012) PCR inhibitors – occurrence, properties an dremoval. Journal of Applied Microbiology. 113, 1014-1026.

Scorzetti, G., Fell, G., Fonseca, A. & Statzell-Tallman, A. (2002) Systematics of *basidiomycetous* yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. FEMS Yeast Research, 2, 495-517.

Stevenson, D.M., Muck, R.E., Shinners, K.J. & Weimer, P.J. (2006) Use of real time PCR to determine population profiles of individual species of lactic acid bacteria in alfalfa silage and stored corn stover. Applied Microbial and Cell Physiology, 71, 329-338.

Tennant, R.K., Sambles, C.M., Diffey, G.E., Moore, K.A. & Love, J. (2017) Metagenomic analysis of silage. Journal of Visualized Experiments. 119, e54936, 1-11.

Thomas, T., Gilbert, J. & Meyer, F. (2012) Metagenomics – a guide from sampling to data analysis. Microbial Informatics and Experimentation, 2:3, 1-12.

van Eijsden, R.G.E., Stassen, C., Daenen, L., van Mulders, S.E., Bapat, P.M., Siewers, V., Goossens, V.Y., Nielsen, J., Delvaux, F.R., van Hummelen, P., Devreese, B. & Willaert, R.G. (2013) A universal fixation method based on quaternary ammonium salts (RNAlater) for omics-technologies: *Saccharomyces cerevisiae* as a case study. Biotechnology Letters, 35, 891-900.

Villalba, A.R., Verkuil, E, van P., Gunst, Q.D., Ruijter, J.M. & van den Hoff, M. J.B. (2017) Amplification of nonspecific products in quantitative polymerase chain reactions (qPCR). Biomolecular Detection and Quantification. 14, 7-18.

Vishnivetskaya, T., Layton, A.C., Lau, M.C.Y., Chauhan, A., Cheng, K.R., Meyers, A.J., Murphy, J.R., Rogers, A.W., Saarunya, G.S., Williams, D.E., Pfiffner, S.M., Biggerstaff, J.P., Stackhouse, B.T., Phelps, T.J., Shyte, L., Sayler, G.S. & Onstott, T.C. (2014) Commercial DNA extraction kits impact observed microbial community composition in permafrost samples. FEMS Microbiology Letters, 87, 217-230.

Wang, P., Qi, M., Barboza, P., Leigh, M.B., Ungerfeld, E., Selinger, L.B., McAllister, T.A. & Forster, R.J. (2011) Isolation of high-quality total RNA from rumen anaerobic bacteria and fungi and subsequent detection of glycoside hydrolases, Canadian Journal of microbiology, 57, 590-598.

White, T.L., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosome RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., & White, T. J. (ed.). PCR Protocols: A guide to methods and applications. NewYork, NY, Academic Press Inc. p. 315-322.

Yavuz, E., Gunes, H., Bulut, C., Harsa, S. & Yenidunya, A.F. (2004) RFLP of 16S-ITS rDNA region to differentiate *Lactobacilli* at species level. World Journal of Microbiology & Biotechnology, 20, 535-537.

# A survey of silage hygiene on Wisconsin dairy farms

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**Keywords:** Biogenic amines, Clostridia, Enterobacteria, Silage hygiene

**Introduction** Common qualitative measures of silage quality include a fermentation profile and nutrient analysis. However, these measures may not fully define silage quality. Commonly results of silage fermentation and nutrient analysis fall within the “normal” range, when concomitantly producers are reporting depressed animal intake, production, components, reproduction and health. Potential pathogens in silage such as *Clostridia, Enterobacteria*, or biogenic amines are seldom measured, but their implications to animal health and production can be detrimental. Only recently have commercial laboratories offered analytical procedures for silage pathogens and or biogenic amines but to date results are without context. Therefore, the objective of this study was to provide context regarding the measure of unusual indices of silage quality, specifically, biogenic amines, *Clostridia* and *Enterococcus*, and to explore whether any correlations exist with more common fermentation profiles.

**Material and Methods** Corn (n=40) and alfalfa (n=40) silage samples were collected from commercial Wisconsin dairy farms (n=40) between June of 2016 and August of 2016. The ambient temperature ranged from10°C to 35°C during this time. Samples were collected from freshly shaved silo faces (bunkers, bags and piles), and were transported on ice to commercial laboratories using routine wet chemistry procedures for pH, VFA, lactic acid, ethanol (HPLC), biogenic amines (HPLC) and ammonia-N . *Clostridia* (TSC agar, incubated anaerobically), *Enterobacteria* (3M Petrifilm), and yeast, and mold (3M Petrifilm) were enumerated and log-transformed prior to statistical analysis. Relationships between measured silage parameters were made using the “Fit X by Y” function of JMP. Relationships were considered significant, and will only be discussed, when $P \leq 0.05$. “Biologically normal” was defined as the middle two quartiles of the population ($25^{th}$ quartile to $75^{th}$ quartile), and was found using the “distribution” function of JMP.

**Results** Population metrics for both corn and alfalfa silage are shown in Table 1.

**Table 1.** Summary statistics of fermentation parameters, microbiology and biogenic amines in survey alfalfa and corn silages.

| | Alfalfa Silages | | | Corn Silages | | |
|---|---|---|---|---|---|---|
| | Mean | $25^{th}$ Quartile | $75^{th}$ Quartile | Mean | $25^{th}$ Quartile | $75^{th}$ Quartile |
| Subjective feed score | 7.55 | 6.88 | 8.50 | 8.34 | 8.00 | 9.00 |
| Dry Matter, % | 40.69 | 34.09 | 48.56 | 34.11 | 32.11 | 35.94 |
| pH | 4.8 | 4.5 | 5.1 | 3.9 | 3.7 | 3.9 |
| Lactic acid, % | 4.23 | 2.55 | 5.49 | 4.53 | 3.89 | 5.30 |
| Acetic acid, % | 1.52 | 0.78 | 2.14 | 2.44 | 1.09 | 3.88 |
| Lactic:Acetic | 3.52 | 2.05 | 4.46 | 2.70 | 1.36 | 4.22 |
| Propionic acid, % | 0.06 | 0.00 | 0.05 | 0.29 | 0.00 | 0.44 |
| Butyric acid, % | 0.19 | 0.00 | 0.07 | 0.06 | 0.00 | 0.02 |
| Ethanol, % | 0.29 | 0.17 | 0.38 | 0.48 | 0.17 | 0.66 |
| 1,2-Propanediol, % | 0.08 | 0.00 | 0.07 | 0.31 | 0.00 | 0.35 |
| Total Acids, % | 6.00 | 3.82 | 8.38 | 7.31 | 5.57 | 9.31 |
| Ammonia, % CP | 9.03 | 5.85 | 10.77 | 9.11 | 7.84 | 11.03 |
| Enterobacteria, log (cfu/g) | 1.70 | 0.00 | 3.73 | 0.68 | 0.00 | 0.75 |
| Yeast, log (cfu/g) | 3.41 | 0.5 | 5.16 | 4.40 | 2.22 | 6.61 |
| Mold, log (cfu/g) | 1.76 | 0 | 2.87 | 1.15 | 0.00 | 1.98 |
| Sulfite reducing *Clostridia*, log (cfu/g) | 1.55 | 1.00 | 2.10 | 1.06 | 0.00 | 1.83 |
| Cadaverine, ppm | 422 | 44 | 374 | 334 | 149 | 535 |
| Histamine, ppm | 151 | 20 | 150 | 207 | 39 | 395 |
| Phenethylamine, ppm | 66 | 14 | 60 | 34 | 2 | 38 |
| Putrescine, ppm | 227 | 27 | 189 | 289 | 128 | 462 |
| Spermadine, ppm | 10 | 0 | 17 | 2 | 0 | 0 |
| Spermine, ppm | 8 | 0 | 11 | 2 | 0 | 0 |
| Tryptamine, ppm | 48 | 0 | 38 | 13 | 0 | 15 |
| Tyramine, ppm | 348 | 14 | 452 | 315 | 0 | 535 |
| Total Biogenic amines | 1280 | 282 | 1340 | 1196 | 559 | 1599 |

Dry matter and pH were not related in either corn or alfalfa silage. In corn and alfalfa silage there were less total VFA in drier silage ($R^2$ = 0.16 and 0.47)). Likewise, drier alfalfa silage had less lactic acid ($R^2$ = 0.32). There was also a first order relationship between DM and acetic acid in both corn and alfalfa silages ($R^2$ = 0.25 and 0.47).

In corn and alfalfa silage, there was a relationship between pH and butyric acid ($R^2$ = 0.17 and 0.11). As pH increased, the level of *Clostridia* increased in both corn and alfalfa silages ($R^2$ = 0.10 and 0.42). In corn and alfalfa silage, wetter silages had more ammonia (R2 = 0.08 and 0.31) and biogenic amines ($R^2$ = 0.28 and 0.29). In contrast, silage DM was not related to concentrations of *Clostridia* or *Enterobacteria*. In corn and alfalfa silage, as pH increased, so did numbers of *Clostridia* ($R^2$=0.12 and 0.22) and *Enterobacteria* ($R^2$=0.54 and 0.12). There were no relationships between biogenic amines and pH in either silage type. Indicative of proteolysis, biogenic amine concentrations were related to ammonia in both corn and alfalfa silages ($R^2$=0.32 and 0.46). Concentrations of biogenic amines and ammonia were not related to concentrations of with *Clostridia* or *Enterobacteria*.

**Discussion** In general, DM, fermentation profiles, and microbial counts were all within normal known ranges for corn and alfalfa silages. As expected, alfalfa silages had higher pH than corn silages, reflective of the higher buffering capacity of alfalfa.

Characteristically, drier silages had less VFA as compared to wetter silage. It is well defined that lower DM silages are typically able to support a more robust fermentation, and they characteristically have more lactic acid, a lower pH and more acetic acid (Ward, 2000). Wetter silages typically also have more butyric acid, but in this survey, this was not observed.

One challenge of laboratory fermentation analysis is the fermentation time course is not evaluated thus rates of pH decline and VFA production cannot be determined. This probably explains the lack of significant relationships between butyric acid and DM, or between enumerated numbers of *Clostridia* and DM in both silages. It is possible that differences in management practices resulted in a variety of pH declines, and therefore in some situations, the *Clostridia* were not able to thrive and produce butyric acid.

It is generally accepted that *Clostridia* are responsible for the majority of butyric acid production in silages. *Clostridia* characteristically grow in wetter silages, but in this survey, there was no relationships between numbers of *Clostridia* and butyric acid, DM, biogenic amines, or ammonia. However, we did observe relationships between DM and indicators of proteolysis (ammonia and biogenic amines). Silage ammonia concentrations were however weakly related to and butyric acid.. This could mean that unmeasured epiphytic bacteria were responsible for proteolysis and butyric acid production. Alternatively, it could mean that a simple bacterial count of *Clostridia* and *Enterobacteria* is not indicative of the rate of proteolysis in the silo.

**Conclusion** This study provided context for unusual indices of silage hygiene. It underscored the importance of ensiling at the proper DM and achieving a low pH. A simple bacterial plate count may not accurately reflect bacterial activity (such as proteolysis) in the silo.

**References**

Ward, R.T. (2000) Fermentation analysis: use and interpretation. Proceedings of the Tri-State Dairy Nutrition Conference, Fort Wayne, IN, 2000, pp. 117-136.

# Aerobic spoilage of grass silage: *Listeria* and forage quality

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**Keywords:** Aerobic, *Listeria*, NIR, Silage, Spoilage

**Introduction** The role of preserved feed as a potential source of infection for livestock and humans has consistently been underestimated. *Listeria monocytogenes* survival and proliferation during aerobic spoilage of preserved feed is critically important across livestock production sectors (Wilkinson & Davies 2013). The conditions of aerobic spoilage of silage drive changes in specific microorganism populations ultimately impacting forage quality, animal and public health. This study aimed to investigate the role of aerobic spoilage in baled grass silage quality and associated *Listeria* spp. concentration.

**Material and Methods** Cut grass, from permanent pasture predominantly perennial ryegrass with conventional NPK fertilizer application, was baled before being wrapped (4 layers of plastic) less than 24 h after wilting in field and maintained in a stack for 8 weeks. During May 2017 12 bales were characterised by established stakeholder metrics of quality assessment to be deemed 'good' or 'poor'. This assessment included judgement on bale shape, density, wrap integrity and odour. Upon opening 3 'good' and 3 'bad' bales were further selected on visible presence of soil, mould, discolouration of silage and odour.

Systematic sampling of the 6 bales was completed by mechanical silage coring to total 7 kg of silage from each bale. Core samples were mixed as representative of each bale and 750 g sub-samples apportioned into 36 sterile aerobic storage vessels (ASVs) with individual temperature data loggers, 6 ASVs for each bale, one for each destructive sampling time points at: 0,1,2,4,8,16 and 32 days after the experiment start. ASVs were maintained in controlled environment conditions throughout the study at 20.25 ± 0.25℃. All samples were processed and analysed immediately at sampling points, with the exception of 300 g for NIR analysis which was vacuum packed and conventionally frozen at -20℃ for whole batch analysis.

Analysis was completed for pH from 10 g sample in 90 ml Milli-Q water by pH metre, dry matter (DM) and ash from 50 g sample by weight loss at 80℃ for 24 hours and 505℃ for 12 hours, and silage quality parameters by NIRS of defrosted sample corresponding to each time point. Culture confirmation of *Listeria* spp. presence or absence was completed by Oxoid *Listeria* Precis (Oxoid 2010a) method, enumeration of *Listeria* spp. concentration by Oxoid rapid *Listeria* enumeration (Oxoid 2010b) method, confirmation of *Listeria* spp. by selective gram staining and confirmation of *L. monocytogenes* by selective API biochemical testing (Setiani et al 2015) of presumptive (opaque white halo) colonies at each time point. All culture based methodologies were completed in replicate and serial dilution with average results converted to $Log_{10}$ scale. Variation of *Listeria* spp. and *L. monocytogenes* concentration was calculated by blocked general ANOVA. Correlation of NIRS forage quality analysis to the calculated metrics of DM, ash and pH was completed by linear mixed modelling. Variation in forage quality parameters was calculated by blocked general ANOVA to microbial results alongside temperature data.

**Results** Table 1. presents the significant increase in silage samples average total *Listeria* spp. (Log Colony Forming Unit (CFU) $g^{-1}$) at 32 days post aerobic exposure in silage bales except for No.

two and four. This correlates to significant increases of average temperature (℃) at 32 days post aerobic exposure in silage bales except for No. two and four, as presented in Table 2.

**Table 1**. Average total *Listeria* spp. concentration ($Log_{10}$ CFU $g^{-1}$) of silage at time points post aerobic exposure

| Average total *Listeria* spp. concentration ($Log_{10}$ CFU $g^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Days post aerobic exposure | | | | | | |
| Bale No. | 0 | 1 | 2 | 4 | 8 | 16 | 32 |
| 1 | 0 | 0 | 0 | 0 | 0.52 | 1 | 4.37 |
| 2 | 0 | 0.82 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0.52 | 0.82 | 0 | 0.52 | 0.82 | 0 | 4.45 |
| 4 | 0.82 | 0.82 | 1 | 0 | 0 | 0 | 0 |
| 5 | 1 | 4.26 | 1.88 | 0 | 2.71 | 1.22 | 4.47 |
| 6 | 0.52 | 0.52 | 0.52 | 0 | 0.82 | 3.22 | 4.52 |

**Table 2**. Average temperature (℃) of silage at time points post aerobic exposure

| Average temperature (℃) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Days post aerobic exposure | | | | | | |
| Bale No. | 0 | 1 | 2 | 4 | 8 | 16 | 32 |
| 1 | 20.50 | 20.60 | 20.08 | 19.61 | 19.57 | 21.07 | 21.58 |
| 2 | 19.88 | 20.60 | 20.16 | 19.13 | 19.59 | 20.66 | 20.11 |
| 3 | 19.67 | 20.66 | 18.49 | 19.63 | 20.55 | 20.11 | 21.63 |
| 4 | 21.04 | 20.63 | 20.58 | 19.65 | 20.58 | 20.65 | 20.63 |
| 5 | 21.60 | 20.63 | 20.70 | 20.15 | 20.65 | 20.57 | 21.60 |
| 6 | 19.95 | 20.08 | 20.62 | 20.11 | 20.63 | 20.60 | 23.09 |

**Discussion** Culture based enumeration of *Listeria* spp. indicated a significant increase in populations during the study. Across all bales at day 0 *Listeria* spp. populations were equal or less than one $Log_{10}$ CFU $g^{-1}$ this increased in four bales to between four and five $Log_{10}$ CFU $g^{-1}$ 32 days after initial aerobic exposure. No listeria CFU were recorded at day 32 in the two remaining bales and these were confirmed by Oxoid Listeria Precis method. Confirmation of *L. monocytogenes* indicated proportional association with total *Listeria* spp. with maximal concentrations of two $Log_{10}$ CFU g-1 32 days after the experiment start. The observed increase in total *Listeria* spp. and *L. monocytogenes* in four bales correlates to temperature increases indicative of aerobic spoilage.

**Conclusion** These results indicate that increases in total *Listeria* spp. and *L. monocytogenes* concentrations occur alongside temperature increases indicative of aerobic spoilage. This enables the application of positive interventions by stakeholders to preserve optimum forage quality whilst protecting animal and public health. This research has significant impact across livestock production sectors where provision of silage may permit aerobic spoilage over the course of the feeding period and the observed increased risk of listeriosis.

**References**

Oxoid. (2010a) Listeria Precis. Folio No. 1252/MS/11/10. Available at: http://www.oxoid.com/pdf/uk/25939_oxoid_listeria_precis.pdf.(accessed on 9/3/2018).

Oxoid. (2010b) Brilliance Listeria. Folio No. 1270/MS/11/10. Available at: http://www.oxoid.com/pdf/27361_Brilliance_Listeria.pdf. (accessed on 9/3/2018).

Setiani, B.E., Elegado, F.B., Perez, M.T.M., Mabesa, R.C., Dizon, E.I. & Sevilla, C.C. (2015) API Listeria Rapid kit for Confirmatory Fenotypic Conventional Biochemical Test of the Prevalence Listeria monocytogenes in selected meat and meat products. Procedia Food Science, 3, 445-452.

Wilkinson, J.M. & Davies, D.R. (2013) The aerobic stability of silage: key findings and recent developments. Grass and Forage Science, 68, 1-19.

# An attempt to study suppression of feruloyl esterase activity in *Lactobacillus ultunensis* by presence of sugars

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**Keywords:** ferulic acid, feruloyl esterase, *Lactobacillus ultunensis,* methyl ferulate, silage

**Introduction** Improving digestibility of silage during the course of ensiling is of great interest. An interesting approach is inoculation of the ensiling plant material with feruloyl esterase (FAE) producing lactic acid bacteria (LAB) (Lynch et al. 2015). In this approach, the linkage between lignin and hemicelluloses is targeted to improve availability of cellulose and hemicelluloses for microbial degradation in the rumen or anaerobic digesters. However, results have been unsatisfactory. Our objective was to investigate the suitability of this approach, i.e. use of FAE producing LAB as a silage inoculant, based on ensiling conditions (e.g. presence of readily available substrates and low pH). In this trial, we tested the ability of *Lactobacillus ultunensis* for the production of FAE in presence of glucose.

**Material and Methods** *L. ultunensis* DSM 16047 was used. Treatments, in duplicate, were: (i) provision of methyl ferulate (0.2%), (ii) provision of methyl ferulate (0.2%):glucose (0.2%), (iii) sterile control for treatment (i) and (iv) sterile control for treatment (ii). MRS broth with glucose being excluded was used as a basal medium. Methyl ferulate was dissolved in dimethylformamide for medium preparation. The final concentration of dimethylformamide in the growth medium was 5%. The strain was cultured overnight in MRS broth anaerobically at 37ºC before centrifugation of 1 ml of bacterial culture at 2,000 × g for 2 min, resuspension of microbial pellet in MRS basal medium and inoculation. Bacterial cultures and the controls were incubated anaerobically at 37ºC and were sampled after 0, 24 and 48 hours. Bacterial growth was estimated by measuring optical density at 600 nm. Furthermore, samples were centrifuged at 11,000 × g for 10 min and supernatant was subjected to sterile filtration (20 µm pore size) before storage at -20 ºC. After thawing at room temperature, samples were analysed for glucose concentration by HPLC (Popp et al. 2015) and methyl ferulate and ferulic acid concentrations by UPLC according to Hofmann and Schlosser (2016) with a modification of using formic acid for acidification of mobile phase. Statistical analysis was performed by General Linear Model and pairwise comparisons were made by the Tukey method in Minitab 18 (Minitab Inc., State College, PA, USA).

**Results** Concentration of ferulic acid was increased in treatments (i) and (ii) during incubation (Table 1). There was a decrease in the concentration of methyl ferulate in treatment (i) and (ii). In treatment (ii), glucose was completely consumed after 24 h incubation, which coincided with a halt in bacterial growth. A bacterial growth was observed during the first 24 h incubation in treatment (i). In addition, the sum of methyl ferulate and ferulic acid concentrations (on molar basis) did not alter over 48 h incubation in both treatments (i) and (ii).

**Discussion** In silage, a substrate deficit is usually not a limiting factor for microbial growth. Therefore, it is reasonable to suspect that FAE producing inoculants may not produce this enzyme during ensiling. To address this question, in our first trial we tested *L. ultunensis* DSM 16047. The strain has a gene (gene ID: HMPREF0548_1224; Ensemble genome browser 91) that encodes a FAE with secretory signal peptide, suggesting an extracellular activity of the enzyme (Petersen et al. 2011). Methyl ferulate is a standard substrate to screen microorganisms for FAE activity (Donaghy et al. 1998), with ferulic acid being the product of the enzymatic activity.

The methyl ferulate was broken down to ferulic acid in both treatments (i) and (ii), suggesting the presence of FAE activity. However, the sum of methyl ferulate and ferulic acid concentrations did not change throughout the incubation, which implies that the bacteria did not utilize the ferulic acid. This is not surprising as LAB are fastidious species, specialized in the utilization of sugars and not aromatic compounds. The observed bacterial growth in treatment (i) was more likely attributed to a utilization of nutrients such as amino acids and peptides originated from meat and yeast extracts ingredients of MRS medium. It is also evident that glucose did not supress production of FAE, which is more likely due to a low concentration of the glucose in this trial.

**Table 1.** Cultivation of *L. ultunensis* with methyl ferulate (i) or methyl ferulate:glucose (ii).

| Treatment | Sampling (hours) | FA (mg/ml) | MF (mg/ml) | FA+MF (µmol/ml) | Glucose (mg/ml) | Optical density |
|---|---|---|---|---|---|---|
| (i) | 0 | $0.01^{e}$ | $1.74^{ab}$ | 8.39 | $0.00^{b}$ | $0.182^{d}$ |
| | 24 | $0.42^{d}$ | $1.62^{ab}$ | 9.93 | $0.00^{b}$ | $0.366^{b}$ |
| | 48 | $0.76^{b}$ | $1.14^{bc}$ | 9.37 | $0.00^{b}$ | $0.343^{bc}$ |
| (ii) | 0 | $0.01^{e}$ | $1.77^{ab}$ | 8.53 | $2.26^{a}$ | $0.191^{cd}$ |
| | 24 | $0.67^{c}$ | $1.22^{abc}$ | 9.30 | $0.24^{b}$ | $0.840^{a}$ |
| | 48 | $1.25^{a}$ | $0.77^{c}$ | 10.11 | $0.00^{b}$ | $0.785^{a}$ |
| Sterile control for (i) | 0 | $0.00^{e}$ | $1.69^{ab}$ | 8.12 | $0.00^{b}$ | $0.041^{d}$ |
| | 24 | $0.00^{e}$ | $1.81^{ab}$ | 8.70 | $0.00^{b}$ | $0.050^{d}$ |
| | 48 | $0.01^{e}$ | $1.62^{ab}$ | 7.82 | $0.00^{b}$ | $0.052^{d}$ |
| Sterile control for (ii) | 0 | $0.00^{e}$ | $1.70^{ab}$ | 8.17 | $2.24^{a}$ | $0.042^{d}$ |
| | 24 | $0.00^{e}$ | $1.72^{ab}$ | 8.27 | $2.50^{a}$ | $0.054^{d}$ |
| | 48 | $0.00^{e}$ | $1.86^{a}$ | 8.94 | $2.19^{a}$ | $0.050^{d}$ |
| P-value | | | | | | |
| Treatment | | <0.001 | 0.001 | 0.119 | <0.001 | <0.001 |
| Sampling | | <0.001 | 0.004 | 0.180 | <0.001 | <0.001 |
| Treatment x sampling | | <0.001 | 0.012 | 0.623 | <0.001 | <0.001 |

FA=ferulic acid, MF=methyl ferulate. Values with different superscript within a column are different at $P < 0.05$

**Conclusion** *L. ultunensis* was able to break down the methyl ferulate, indicating an extracellular production of FAE by the bacteria. The production of the enzyme, however, was not suppressed by the glucose. A higher concentration of glucose is needed before withdrawing a firm conclusion as to an inhibitory effect of glucose on FAE production in *L. ultunensis*.

**References**

Donaghy, J., Kelly, P.F. & McKay, A.M. (1998) Detection of ferulic acid esterase production by Bacillus spp. and lactobacilli. Applied Microbiology and Biotechnology, 50, 257-260.

Hofmann, U. & Schlosser, D. (2016) Biochemical and physicochemical processes contributing to the removal of endocrine-disrupting chemicals and pharmaceuticals by the aquatic ascomycete Phoma sp. UHH 5-1-03. Applied Microbiology and Biotechnology,100, 2381-2399.

Lynch, J.P., Baah, J. & Beauchemin, K.A. (2015) Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. Journal of Dairy Science, 98, 1214-1224.

Petersen, T.N., Brunak, S., Heijne, G.V., & Nielsen, H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nature Methods, 8, 785-786.

Popp, D., Schrader, S., Kleinsteuber, S., Harms, H. & Sträuber H. (2015) Biogas production from coumarin-rich plants–inhibition by coumarin and recovery by adaptation of the bacterial community. FEMS Microbiology Ecology, 91, article number: fiv103.

# Bacterial and fungal population dynamics, fermentation, and aerobic stability of conventional and BMR corn hybrids ensiled at low moisture with or without a homo- and hetero-fermentative inoculant

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**Keywords:** inoculant, next generation sequencing, low-moisture silage

**Introduction** Harvesting below the recommended range of moisture concentration can result in a cascade of negative events that prevent adequate silage preservation. Our objective was to evaluate the effect of a combo inoculant (homo and heterofermentative lactic acid bacteria) applied to several corn hybrids, which were harvested at low moisture, on the nutritive value, fermentation profile, aerobic stability, bacterial and fungal populations, taxonomic profile, diversity, and community structure.

**Material and Methods** The treatment design was the factorial combination of four corn hybrids (HYB) ensiled with (INO) or without (CON) inoculant. The HYB TMF2R737 (MCN), F2F817 (MBR), P2089YHR (PCN), and PI144XR (PBR) were ensiled at 44.0, 38.1, 42.0, and 41.3% DM, respectively. MBR and PBR were brown midrib mutants. The four corn hybrids were planted in a complete randomized design with plots replicated six times. Two replicated piles were obtained from each corn plot (total of 48 piles). The inoculant contained *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* 12455 (application rate: $4\times10^5$ and $1\times10^5$ cfu/g of fresh corn). Corn was chopped, treated or not with INO, packed into 7.6 L bucket silos, and stored at 23°C (± 1°C) for 100 days. Sonication was used for microbial extraction (Gutierrez-Rodriguez et al. 2012) and the 16S rRNA V4 (bacteria) and ITS-1 (fungi) regions were amplified. The libraries were sequenced on a Illumina MiSeq platform and analysed with QIIME 1.9.1 using the GreenGenes 13.8 and UNITE fungal ITS reference database. We used the GLM procedure of SAS v.9.4 (SAS Institute, 2003) for data analysis. When a two-factor interaction effect was present, we used the SLICE option to analyse the simple effects. Mean separation was based on the PDIFF procedure of LSMEANS. Treatments were considered different when $P \leq 0.05$.

**Results and Discussion** At d 0, we found a higher bacterial observed operational taxonomic units (OTUs) in the brown midrib mutants (MBR and PBR) relative to MCN and PCN (654 and 534 vs. 434 and 444 ± 15.5, respectively; $P < 0.05$). The bacterial and fungal families with the highest relative abundance (RA) were *Enterobacteriaceae* (61.4%) and incertae sedis *Tremellales* (12.5%). At silo opening, we observed no effects of INO on DM recovery (~94.3 ± 1.07%; ~ = average), most likely due to the presence of *P. pentosaceus* in the inoculant which reduces the negative effects of heterolactic fermentation on DM recovery during fermentation (Driehuis et al. 2001). Aerobic stability was extended by all INO-treated HYB (~217 vs. ~34.7 h), except for MBR (~49 ± 38 h), due to a decreased yeast population (3.78 vs. 5.13 ± 0.44 log cfu/g) and increased acetic acid concentration (1.69 vs. 0.51 ± 0.13% of DM) vs. CON ($P < 0.05$). A plausible explanation for the failure of INO to extend the aerobic stability for MBR is the numerically higher concentration of WSC at silo opening

observed in this hybrid compared to the others (2.09 vs. ~1.27 ± 0.17% of DM). Higher residual WSC carry a higher risk of yeast spoilage during silo opening if not enough acetate or propionate is present (McDonald et al. 1991). Furthermore, INO reduced bacterial (61.2 vs. 276 ± 8.70) and increased fungal (59.8 vs. 43.6 ± 2.95) observed OTUs when compared with CON ($P < 0.05$). These results suggest that INO reduces the bacterial and increases fungal diversity consistently across all ensiled hybrids tested in this study. We observed that INO increased the relative abundance (RA) of *Lactobacillaceae* across all HYB (~99.1 vs. ~58.9), and to larger extent for MBR (98.3 vs. 34.3 ± 5.29), and decreased *Enterobacteriaceae* (0.614 vs. 23.5 ± 2.825%) among 4 other bacterial families relative to CON ($P < 0.05$). Collectively for the major bacterial families at d 100, RA were 73.4, 1.38, and 1.32% of genus *Lactobacillus*, unidentified, and *Pediococcus* for *Lactobacillaceae*; 8.82, 0.95, 0.65, 0.62, 0.50, 0.41% of genus unidentified, *Erwinia*, *Serratia*, *Enterobacter*, *Cronobacter*, and *Sodalis* for *Enterobacteriaceae*. Addition of the *L. buchneri* containing inoculant seem to result in silages dominated by the *Lactobacillus* genus with much less diversity than untreated silages, which seem to sustain more *Enterobacteriaceae* and other genera that can potentially include pathogenic species of bacteria. Thus, adding this type of inoculants may have the added benefit of contributing to silage hygiene. For fungi, INO increased the RA of *Debaryomycetaceae* (63.1 vs. 17.3 ± 8.55) and five other fungal families and decreased the RA of *Pichiaceae* (6.47 vs. 47.3 ± 10.95) and incertae sedis *Saccharomycetales* (8.47 vs. 25.9 ± 5.748) vs. CON ($P < 0.05$). Collectively for the major fungal families on day 100, RA were 40.2% of genus *Meyerozyma* for *Debaryomycetaceae*, 25.8 and 1.08% of genus *Issatchenkia* and *Pichia* for *Pichiacea*; 16.8% of genus *Candida* for incertae sedis *Saccharomycetales*. On day 0 vs. day 100 and within day 100 (CON vs. INO), we observed a clear separation and difference in the distribution and structure of the bacterial ($P = 0.001$; $R \geq 0.93$) and fungal ($P = 0.001$; $R \geq 0.79$) community using the weighted UniFrac and Bray-Curtis principal coordinates analysis plot, respectively.

**Conclusion** At the low moisture concentrations evaluated in this study, the INO tested improved whole-crop corn silage quality because of a shift in the bacterial and fungal community composition during ensiling that favoured aerobic stability for all HYB except MBR and a similar DM recovery relative to CON.

**References**

Driehuis, F., Oude Elferink, S.J.W.H. & Van Wikselaar, P.G. (2001) Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass Forage Science, 56, 330-343.

Gutierrez-Rodriguez, E., Gundersen, A., Sbodio, A.O. & Suslow, T.V. (2012) Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of *Escherichia coli* on spinach. Journal of Applied Microbiology 112:109-118.

McDonald, P., Henderson, A.R. & Heron, S.J.E. (1991) The biochemistry of silage. Chalcombe Pub., Marlow, UK.

# Can *Fasciola hepatica* metacercariae survive ensiling and retain their viability?

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**Keywords:** *Fasciola hepatica,* metacercariae, parasitology, viability

**Introduction** *Fasciola hepatica*, the common parasitic liver fluke, is an important cause of morbidity and mortality in ruminant livestock worldwide. Effects of sub-clinical infection on growth rate and milk yield is estimated to cost the UK cattle industry up to £40.4 million annually (Bennett and Ijpelaar 2005). The liver fluke life cycle is complex, due to free-living parasite stages and its dependency on the presence of an intermediate host, a mud snail, *Galba truncatula.* It is understood that up to 50% of metacercariae can overwinter on pasture and infect grazing livestock the following spring (Ollerenshaw 1971). However, the risk of infection posed by feeding silage to livestock is not well understood. The majority of studies investigating metacercarial viability following ensiling are outdated and do not implement molecular techniques to detect the persistence of *F. hepatica* DNA within silage (Wikerhauser and Brglez 1961, Boray and Enigk 1964). Moreover, the impacts of aerobic spoilage on metacercariae survival and the potential development of ensiled metacercariae into infective newly excysted juveniles (NEJs) must be better understood in order to determine the importance of silage in disease transmission.

**Material and Methods** Optimisation of molecular methods to detect *F. hepatica* DNA originating from silage include: i) developing a washing and filtration protocol to remove potential PCR inhibitors from silage samples, ii) ascertaining ITS-2 and COX1 PCR sensitivities (Cucher et al. 2006, Novobilský et al. 2013) by analysing silage effluent samples inoculated with serial dilutions of *F. hepatica* DNA (1 ng/µl-0.1 pg/µl); both PCR assays were conducted as cited in the literature but with the following modifications: (a) using Biomix Red (Bioline) as the reaction mix, (b) the addition of 1 µl Bovine Serum Albumin (BSA) and (c) using 1 µl template DNA originating from silage effluent, iii) optimisation of protocols for e-DNA extraction from nylon filters inoculated with *F. hepatica* DNA (10 ng/µl-300 ng/µl) and iv) shedding of *F. hepatica* metacercariae from laboratory maintained *G. truncatula* specimens onto silage, washing and filtration to isolate metacercariae followed by e-DNA extraction and PCR. Laboratory-based ensiling experiments involved inoculating *F. hepatica* metacercariae onto grass samples of 20% and 30% dry matter (DM) content and ensiling for 2, 6 and 10 week periods. Ensiling vessels were sealed either with an anaerobic fermentation lock or perforated plastic wrap in order to understand the impact of early onset aerobic spoilage on metacercariae survival. Following ensiling, *F. hepatica* DNA persistence within silage was assessed using these optimised molecular methods. Viability of ensiled metacercariae was evaluated by microscopic observations to identify flame cell organelles and via a series of *in vitro* excystment assays.

**Results and Discussion** Optimisation of molecular methods to detect *F. hepatica* DNA originating from inoculated silage effluent was successful, with a PCR sensitivity of 1 pg/µl achieved. The addition of 1 µl BSA to the reaction mix was found to facilitate amplification, potentially through the inactivation of PCR inhibitors within silage (Hedman and Rådström 2013). Successful detection of *F. hepatica* DNA originating from silage contaminated with as few as 6 metacercariae indicated that the washing, filtration and molecular techniques developed were applicable to future post-ensiling forage analysis. Following laboratory-based ensiling experiments, it is evident that *F. hepatica* DNA originating from anaerobically ensiled metacercariae can persist for at least 10 weeks within grass of both 20% and 30% DM content. Despite this, all metacercariae lacked flame cells on observation and failed to develop into NEJs following only two weeks anaerobic storage. This highlights the importance of forage sealing to encourage rapid fermentation onset and demonstrates that successfully detecting *F.*

*hepatica* DNA within silage does not always indicate the presence of viable metacercariae. Following two weeks aerobic storage in grass of 20% and 30% DM content, metacercariae excystment success rates were 95% and 51% respectively. This indicates that conditions within poorly stored forages (particularly those of a lower DM content) are conducive for the survival of viable metacercariae. Interestingly, following 10 weeks aerobic storage within grass of 20% DM content, metacercariae developed into unhatched juvenile parasites without requiring an excystment stimuli. Despite no hatching occurring *in vitro*, this phenomenon suggests that viable metacercariae can persist for lengthy periods within spoiled forages originating from poorly wilted grass and that they could potentially develop when exposed to optimal excystment conditions within the small intestine of the definitive host.

**Conclusion** It has been demonstrated that *F. hepatica* metacercariae quickly lose viability within sealed silages, however they can persist within spoiled silage for a short period of time and retain viability. Development of unhatched juvenile parasites can occur following 10 weeks stored within spoiled silage originating from grass of a low DM content, which highlights the importance of effective grass wilting and anaerobic fermentation to reduce the potential infection risk to livestock. With regards to future developments, additional ensiling experiments using a broader range of grass DM contents to reflect baled silages and studies investigating the impact of additive use on metacercariae survival may be conducted. Developing our understanding of the mechanisms involved in fasciolosis transmission will lead to informed husbandry advice for farmers on how to effectively reduce disease prevalence through improved silage management, particularly at sealing and during the feed out period.

**References**

Bennett, R. & Ijpelaar, J. (2005) Updated estimates of the costs associated with thirty four endemic livestock diseases in Great Britain: A note. Journal of Agricultural Economics, 56(1), pp. 135-144. doi: 10.1111/j.1477-9552.2005.tb00126.x.

Boray, J. & Enigk, K. (1964) Laboratory studies on the survival and infectivity of Fasciola hepatica-and F. gigantica-metzcei-cariae. Zeitschrift fur Tropenmedizin und Parasitologie, 15(3), pp. 324-331.

Cucher, M., Carnevale, S., Prepelitchi, L. & Labbe, J. (2006) PCR diagnosis of Fasciola hepatica in field-collected Lymnaea columella and Lymnaea viatrix snails. Veterinary Parasitology, 137, pp. 74-82. doi: 10.1016/j.vetpar.2005.12.013.

Hedman, J. & Rådström, P. (2013) PCR Detection of Microbial Pathogens. Methods in Molecular Biology, pp. 17-48.

Novobilský, A., Ka, M., Beran, L., Rondelaud, D. & Höglund, J. (2013) Lymnaea palustris and Lymnaea fuscus are potential but uncommon intermediate hosts of Fasciola hepatica in Sweden. Parasites & Vectors, 6(251), pp. 1-10.

Ollerenshaw, C. (1971) Some observations on the epidemiology and control of fascioliasis in Wales. In: International Liverfluke Colloquium (2nd) Wageningen, Central Veterinary Laboratory, Weybridge, Surrey, UK, pp. 103-125.

Wikerhauser, T. & Brglez, J. (1961) O vitalnosti metacerkarija F. hepatica iz salaze. Veterinarski Arhiv, 31, pp. 315-318.

# Characterisation of different yeast species from corn silage and their ability to degrade lactate

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**Keywords:** aerobic stability, corn silage, lactate degradation, osmolality, yeast identification

**Introduction** Acid-tolerant, lactate-assimilating yeasts play a major role in the aerobic deterioration of silage. In general, yeast concentrations of more than $1 \cdot 10^5$ cfu/g silage are regarded as critical in relation to aerobic stability. However, some silages with higher yeast counts are stable upon exposure to air (McDonald et al. 1991). Therefore, it is necessary to look more closely at the composition of the yeast population and to know the favorable growth conditions and inhibitory ranges of these species to keep the silage in a microbiological safe condition and to prevent high nutrient losses during feed-out period. The objective of this study was to identify yeast species from corn silage and to determine their effect on aerobic deterioration.

**Materials and methods** In this study four corn silages from bunker silos of different farms in Brandenburg, Germany were analysed. All silages were prepared by using a biological silage additive (*L. plantarum* DSM 8862 und *L. plantarum* DSM 8866, inoculation $3 \cdot 10^5$ cfu/g fresh matter). The storage period of the silages was 9-12 months at the time of sampling. The samples were treated with demineralized water and macerated in a laboratory homogenizer. The yeast cultivation was carried out by plating out the suspension on Sabouraud 4% dextrose agar (SDA) to obtain all yeast species. To cultivate only lactate-assimilating yeasts, yeast nitrogen base agar with lactate as the carbon source was used. To isolate different yeast species the agar plates were examined using a stereo microscope. The colonies were divided in groups with similar colony morphology and size. One or two colonies of each group were picked and isolated to get pure cultures.

To determine the species, a 579 bp fragment of the variable domains D1 and D2 from nuclear large subunit ribosomal RNA was amplified by PCR (Kurtzman and Robnett 2003) followed by bidirectional Sanger sequencing of the purified PCR product. Identification of the species was carried out by gene sequence comparison with the database of the National Center for Biotechnology Information (NCBI) using the search tool BlastN.

The pure yeast cultures were cultivated in 250 mL bioreactors (*in vitro* model) with air supply. The culture medium contained, in addition to yeast extract and peptone, lactate as the carbon source. To estimate the spoilage capability of the different species two main factors were used: the adaptation period and the degradation rate for lactate during exponential and linear growth phase. In the first trial the parameters lactate level (1.3% (w/v), 1.8% (w/v) and 2.3% (w/v), adjusted with 0.5 M NaOH) and inoculation density ($1 \cdot 10^4$ cfu/ml and $1 \cdot 10^6$ cfu/ml) were varied at constant pH value (pH 3.5) and constant osmolality (1.0 osmol/kg) and in the second trial the parameters osmolality (1.0 osmol/kg, 2.5 osmol/kg and 3.9 osmol/kg) and pH value (pH 3.5 and pH 4.0) were varied at constant inoculation density ($1 \cdot 10^4$ cfu/ml) and constant lactate level (1.8% (w/v)).

**Results and discussion** A total of 18 strains were isolated from the four different corn silages. The strains could be assigned to the seven species *Pichia kudriavzevii*, *Pichia fermentans*, *Candida ethanolica*, *Candida humilis*, *Kazachstania exigua*, *Kazachstania barnettii* and *Saccharomyces cerevisiae* (table 1). Phylogenetic analysis demonstrated that *P. kudriavzevii*, *P. fermentans*, *C. ethanolica* are closely related members of the family *Pichiacae* as well as *C. humilis, K. exigua, K. barnettii* and *S. cerevisiae* closely related belong to the family *Saccharomycetaceae* (figure 1). The latter four species are Crabtree-positive with a high fermentation potential. Under anaerobic condition these yeasts ferment sugars to ethanol and CO2 and thus decrease the amount of sugar available for lactic acid fermentation (Oude Elferink et al. 2002). *P. kudriavzevii*, *P. fermentans*, *C. ethanolica* are

belonging to the Crabtree-negative yeasts. In the presence of oxygen the Crabtree-negative yeasts have a competitive advantage over Crabtree-positive yeasts due to their higher affinity for lactate and glucose (Pahlow et al. 2003).

The developed in vitro model was proven to be suitable to simulate the conditions during the aerobic feed-out period. Under similar initial conditions (osmolality, pH value, lactate level and inoculation density) the values for lactate degradation and growth of the yeasts in vitro were comparable with the determined yeasts populations in corn silage in vivo during aerobic deterioration. Cultivation experiments showed that *P. kudriavzevii*, *P. fermentans*, *C. ethanolica*, *C. humilis* and *K. exigua* poses a high risk to the aerobic stability of silage due to their fast adaption to environmental conditions and their high degradation rate for lactate. It could be observed that osmolality affects yeast growth. The osmotolerance varied between the genera: species belonging to the *Kazachstania*-Clade were totally inhibited while the members of the *Pichia*-Clade could grow in the presence of 2.5 osmol/kg. At this osmotic concentration growth was observed for *S. cerevisiae* at pH 4.0 but not at pH 3.5. While all yeasts could grow in the presence of 1.0 osmol/kg, an osmolality of 3.9 osmol/kg had a toxic effect to all determined species at pH 3.5 and pH 4.0.

**Table 1.** Identification of yeast species isolated from corn silages of four different farms in Brandenburg, Germany

| Name of Species | Classification of the pure yeast cultures by species and number | | | |
|---|---|---|---|---|
| | Farm | | | |
| | 1 | 2 | 3 | 4 |
| *S. cerevisiae* | - | 1 | 1 | - |
| *K. exigua* | - | - | - | 2 |
| *K. barnettii* | - | 1 | - | - |
| *C. humilis* | 1 | 2 | - | - |
| *P. kudriavzevii* | 1 | 1 | - | 1 |
| *P. fermentans* | - | 1 | 3 | 1 |
| *C. ethanolica* | 2 | - | - | - |
| Total number of isolates | 4 | 6 | 4 | 4 |

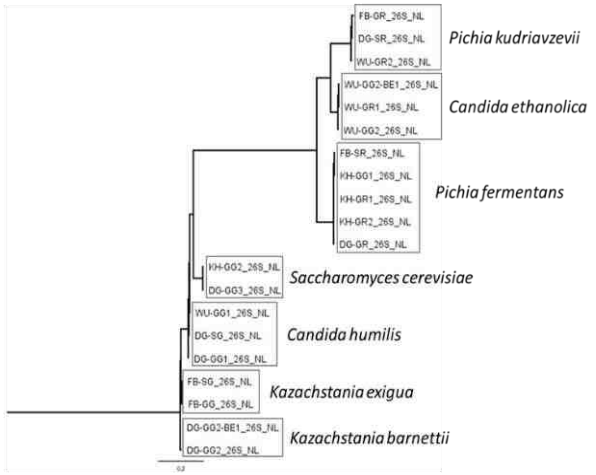


**Figure 1.** Phylogenetic relationships among 18 yeast cultures isolated from four different corn silages and determined from neighbour joining analysis of D1/D2 large subunit ribosomal RNA gene sequences.

**Conclusions** Regarding the examined parameters inoculation density and lactate concentration had no significant effect on yeast growth but lactate concentration influences the increase of pH-value caused by lactic acid degradation. In general, it could be observed that osmolality affects yeast growth and a high osmolality increases the pH-dependent inhibition on yeast growth. However, the high variation in the latter observation warrants further studies.

**References**

Kurtzman C.P., & Robnett C.J. (2003) Phylogenetic relationships among yeasts of the ‚Saccharomyces complex' determined from multigene sequence analyses. FEMS Yeast Research, 3,. 417-432.

McDonald P., Henderson A.R., & Heron S.J.E. (1991) The Biochemistry of Silage. Second edition. Marlow: Chalcombe Publications.

Oude Elferink J.W.H., Driehuis F., Gottschal J.C., & Spoelstra F. (2002) Manipulating silage fermentation. FEED MIX, 10, 20-23.

Pahlow G., Muck R.E., Driehuis F., Oude Elferink S.J.W.H., & Spoelstra S.F. (2003) Microbiology of Ensiling. In: Silage Science and Technology; Agronomy No. 42; D.R. Buxton, R.E. Muck, J.H. Harrison (eds). Madison, Wisconsin: American Society of Agronomy, Inc.; Crop Science Society of America, Inc.; Soil Science Society of America, Inc.; p. 31-93.

# Characterization of the microbial community in lucerne silages differing in fermentation quality

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K**eywords:** fermentation, lucerne silage, microbial colonization, qPCR

**Introduction** The successful production of lucerne (*Medicago sativa L.*) silage depends, amongst others, on an adequate lactic acid fermentation and thus on the sufficient presence and activity of lactic acid-producing bacteria (LAB). However, besides beneficial LAB, various microbial species having a negative impact on silage quality and animal health are present in silages. Information about the microbial community in differently produced lucerne silages (LS) may help to identify effective strategies for promoting the activity of LAB and thus for producing high quality LS. Moreover, those findings may provide indications at which silage qualities potentially pathogenic microorganisms could be expected in higher abundance.

**Material and Methods** In 2015, a pure lucerne ley was harvested in Vinxel, North Rhine-Westphalia, Germany, to produce six LS of varying quality. Lucerne was wilted to 25% and 35% dry matter (DM), respectively and 125 g sugar/kg DM was added to the plant material to compensate the naturally low content of water-soluble carbohydrates. Subsequently, 25% DM lucerne was ensiled 1) without treatment (25CON), 2) with an artificially induced soil contamination (25S) or 3) after treatment with formic acid (Amasil NA, BASF, Germany) (25FA). 35% DM lucerne was ensiled 4) without treatment (35CON), 5) with sodium nitrite and hexamine (Kofasil liquid, Addcon Europe, Germany) (35KOFA), or 6) with a silage additive containing *Lactobacillus plantarum* (BIO-SIL, Dr. Pieper, Germany) (35BIO). Each variant was ensiled in triplicate and stored for 30 days. Afterwards, 10g of each LS were grinded in liquid nitrogen using pestle and mortar. An aliquot was then used for microbial DNA extraction using a modified protocol of the First-DNA all-tissue Kit (Gen-IAL GmbH, Germany), which included a beat-beating step and RNase A treatment. Quantitative real-time polymerase chain reaction (qPCR) analysis was applied to quantify 16S rRNA gene copies of total bacteria, *Lactobacillus spp.*, *L. plantarum*, *L. buchneri*., *Clostridium* spp. and *Enterobacteriaceae*. All primer pairs were selected from literature and optimized (Table 1). For each target, 20µl qPCR reactions were carried out in duplicate using a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, USA) containing 10 µl of 2x primaQUANT-CYBR-Green Mastermix (Steinbrenner Laborsysteme GmbH, Germany), 1 µl of template DNA, primer concentration as described in table 1 and was filled up with PCR grade water (VWR International GmbH, Germany). Thermal cycling conditions were as follows: 10 min at 95 °C, 40 cycles of 5 s at 95 °C, 20 s at the appropriate annealing temperature (Table 1), 1 s at 72 °C. Finally, the melt curve was generated by stepwise (0.3 °C) temperature increase from 60 °C to 95 °C. Reaction specificity and PCR products were checked by 2% agarose gel electrophoresis and melt curve analysis.

**Table 1**. Overview of selected primer pairs used for qPCR analysis

| Target species/group | Primer concentration (nM) | Annealing temperature (°C) | Product size (bp) | Reference |
|---|---|---|---|---|
| Total bacteria | 600 | 52 | 147 | Fuller et al. (2007) |
| *Lactobacillus* spp. | 300 | 59 | 341 | Rinttilä et al. (2004) |
| *L. plantarum* | 200 | 59 | 75 | Klocke et al. (2006) |
| *L. buchneri* | 200 | 59 | 130 | Schmidt et al. (2008) |
| *Enterobacteriaceae* | 500 | 63 | 195 | Bartosch et al. (2004) |
| *Clostridium* spp. | 500 | 62 | 270 | Zheng et al. (2017) |

**Results and Discussion** Significantly different abundances of the genus *Lactobacillus* were obtained between the LS variants with 25CON and 25S having more lactobacilli compared to 35KOFA (Table 2). Besides, 25% DM LS showed higher gene copies of *Lactobacillus* spp. (P=0.039, data not shown) and *L. buchneri* (P<0.001) than LS with 35% DM, which might be due to a higher water availability in the silo that could have promoted their growth. The inoculation with viable *L. plantarum*

culture resulted in the highest abundance of this species in 35BIO (Table 2). In contrast, inoculation with *L. plantarum* seemed to diminish the colonization by *L. buchneri*, thus indicating a strong competitive behaviour of *L. plantarum* against other LAB. Besides, also DM content strongly affected the abundance of *L. buchneri* in LS ($P<0.001$) with 42.6% more gene copies in 25% DM LS compared to 35%DM LS.

While aiming to reduce potentially harmful bacteria, treatments with formic acid (25FA) or sodium nitrite and hexamine (35KOFA) had no effect on abundances of *Enterobacteriaceae* and *Clostridium* spp. However, abundances of these two groups were on the same high level across all LS (Zheng et al. 2017), although higher gene copy numbers might have been expected only in the soil contaminated variant (clostridia endospores $2.4x10^3$ MPN/g soil). *Listeria* spp. could not be detected in the LS using genus-specific primers from Somer und Kashi (2003). The successful application of these primers by others indicates a lack of *Listeria* in the present LS to be more likely and thus beneficial for LS quality; however, insufficiencies in primer design or during qPCR analysis cannot be excluded.

**Table 2.** Means (with standard deviation in parentheses) of respective gene copy numbers ($log_{10}$ 16S rRNA gene copies/g silage) for the different LS variants.

| Target species/group | 25CON | 25S | 25FA | 35CON | 35KOFA | 35BIO | P-value |
|---|---|---|---|---|---|---|---|
| Total bacteria | 9.61 (.13) | 9.64 (.22) | 9.43 (.13) | 9.35 (>.01) | 9.53 (.52) | 9.70 (.22) | <0.689 |
| *Lactobacillus* spp. | $9.33^{a}$(.15) | $9.45^{a}$(.10) | $9.18^{ab}$(.02) | $9.16^{ab}$(.16) | $8.48^{b}$(.65) | $9.20^{ab}$(.13) | <0.028 |
| *L. plantarum* | $8.19^{ab}$(.13) | $8.49^{a}$(.15) | $8.41^{a}$(.19) | $8.12^{ab}$(.18) | $7.33^{b}$(.87) | $9.01^{a}$(.08) | <0.004 |
| *L. buchneri* | $6.83^{a}$(.38) | $7.26^{a}$(.29) | $6.09^{ab}$(.05) | $4.65^{ab}$(.83) | $5.55^{abc}$(n/a) | $4.06^{c}$ (n/a) | <0.001 |
| *Enterobacteriaceae* | 5.82 (.22) | 5.87 (.12) | 4.84 (.82) | 5.09 (.14) | 5.20 (n/a) | 5.05 (.39) | <0.143 |
| *Clostridium* spp. | 6.09 (.44) | 6.02 (.24) | 5.93 (.04) | 6.14 (.21) | 6.01 (.05) | 6.22 (.63) | <0.915 |

Means with different superscripts with in a row indicate significant difference($P<0.05$). n/a, not available

**Conclusion** The study showed that certain silage additives increase the abundance of beneficial LAB in LS. Additionally, the DM content was found to have a strong impact on the microbial community in LS. No treatment affected the presence of potentially harmful bacteria in LS, which, however, were on a high level for all LS variants. Further investigation should be conducted on the composition, but also on the metabolic activity of the microbial community in LS.

**References**

Bartosch, S., Fite, A., Macfarlane, G.T. & McMurdo, M.E.T. (2004) Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. Applied and Environmental Microbiology 70 (6), p. 3575-3581.

Fuller, Z., Louis, P., Mihajlovski, A., Rungapamestry, V., Ratcliffe, B. & Duncan, A.J. (2007) Influence of cabbage processing methods and prebiotic manipulation of colonic microflora on glucosinolate breakdown in man. The British Journal of Nutrition 98 (2), p. 364-372.

Klocke, M., Mundt, K., Idler, C., McEniry, J., O'Kiely, P. & Barth, S. (2006) Monitoring Lactobacillus plantarum in grass silages with the aid of 16S rDNA-based quantitative real-time PCR assays. Systematic and Applied Microbiology 29 (1), p. 49-58.

Rinttilä, T., Kassinen, A., Malinen, E., Krogius, L. & Palva, A. (2004) Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. Journal of Applied Microbiology 97 (6), p. 1166-1177.

Schmidt, R.J., Emara, M.G. & Kung, L. (2008) The use of a quantitative real-time polymerase chain reaction assay for identification and enumeration of Lactobacillus buchneri in silage. Journal of Applied Microbiology 105 (3), p. 920-929.

Somer, L. & Kashi, Y. (2003) A PCR Method Based on 16S rRNA Sequence for Simultaneous Detection of the Genus Listeria and the Species Listeria monocytogenes in Food Products. Journal of Food Protection 66 (9), p. 1658-1665.

Zheng, M.L., Niu, D.Z., Jiang, D., Zuo, S.S. & Xu, C.C. (2017) Dynamics of microbial community during ensiling direct-cut alfalfa with and without LAB inoculant and sugar. Journal of Applied Microbiology 122 (6), p. 1456-1470.

# Comparison between a *Clostridium tyrobutyricum*-specific quantitative polymerase chain reaction (qPCR) method and a traditional method for determining total spore-forming bacteria in clover-grass silage

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**Keywords:** *Clostridium tyrobutyricum*, qPCR, silage

**Introduction** *Clostridium (C.) tyrobutyricum* is the most common type of bacteria to contaminate milk and cause late blown cheese due to high spore counts in silage (Pahlow et al. 2003). A traditional and commonly used method to estimate total spore counts is the most probable number (MPN) method (Jonsson 1990). As this method is rather time consuming and not specific for *C. tyrobutyricum*, there is an interest in finding a more rapid and specific method. The quantitative polymerase chain reaction (qPCR) method can be used to quickly quantify and identify microorganisms by their DNA sequences. This method has previously been used to identify species of clostridia of which *C. tyrobutyricum* was one of them (Cremonesi et al. 2012). The purpose of this study was to test a qPCR method to quantify *C. tyrobutyricum* in silage, by investigating potential differences and relationships in the clostridia estimate of silage between the qPCR method and the MPN method. Furthermore, relationships were evaluated between silage fermentation characteristics and *C. tyrobutyricum* copies or total spore-forming bacteria count.

**Material and Methods** A sward (75% red clover/25% grass) was harvested September 4, 2011 in the third cut at Skara, southwest Sweden, and ensiled in 14 1.7-L silos with or without inoculants, containing homo- and/or heterofermentative lactic acid bacteria, for 142 days and analysed for fermentation parameters. Also, 0.5-L silos were filled and opened after 3 days to analyse pH as a measure of acidification rate. For the qPCR method, which is specific for *C. tyrobutyricum*, a kit for DNA extraction from soil was used with a lysozyme to disrupt the cell walls. Sequences of primers and probes were the same as described by Bassi et al. (2013). The number of gene copies per gram of silage was calculated based on the cycle threshold values for the samples and the standard. The Bryant Burkey broth, containing calcium lactate, meat extract, sodium acetate, peptone from casein, cysteine HCl, resazurin and yeast extract, was used as a substrate and the total spore content was estimated by using an MPN index table with 95% confidence intervals for the MPN method (FDA, 1978). Relationships between total spore-forming bacteria count by the MPN method and the *C. tyrobutyricum* numbers by the qPCR method were investigated by linear regression analysis using PROC REG of SAS. Least-square (LS) means of counts from the methods were compared in PROC GLM of SAS. Stepwise regressions on the relationships between silage fermentation characteristics and *C. tyrobutyricum* by the qPCR method and total spore count by the MPN method were performed in PROC REG of SAS using $P<0.15$ as the significance level for inclusion of a variable in the model. For other statistical analyses, differences were considered significant when $P<0.05$.

**Results** Comparisons between the LS means of the gene copies of *C. tyrobutyricum* by the qPCR and total spore-forming bacteria by the MPN method did not reveal significant differences (qPCR: 4.5 log copies $g^{-1}$ *vs* MPN: 3.7 log MPN $g^{-1}$, SEM=0.51, $P=0.301$). Furthermore, the MPN method showed a strong positive relationship with the qPCR method (Figure 1). Stepwise regression analysis indicated that gene copies of *C. tyrobutyricum* by the qPCR method had strong relationships ($R^2=0.98$ Root MSE=0.239) with silage pH after 3 days of fermentation ($P<0.001$) and contents of lactic acid ($P<0.001$), acetic acid ($P<0.05$) and 1,2-propanediol ($P=0.107$). Plotting *C. tyrobutyricum* gene copies from the qPCR method as a function of silage pH after 3 days of fermentation and as a function of acetic acid content showed positive relationships, whereas the relationships with the contents of lactic acid and 1,2-propanediol were negative. Total spore-forming bacteria count analysed by the MPN

method showed a very strong positive relationship ($R^2$=0.90, Root MSE=0.719) with butyric acid (*P*<0.001) and butanol (*P*=0.113).

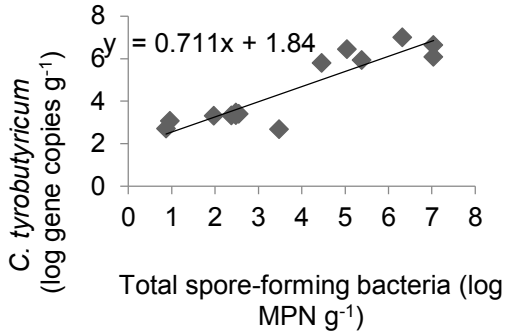


**Figure 1**. Relationship between the counts of total spore-forming bacteria (MPN method) and *C. tyrobutyricum* (qPCR method) in red clover-grass silage, n=14.

**Discussion** Results from this study showed that the total population of spore-forming bacteria (MPN method) was vastly composed of *C. tyrobutyricum* in the clover-grass silage as the variation in the MPN method could explain 83% of the total variation in *C. tyrobutyricum* content of clover-grass silage analyzed by the qPCR method. However, other bacteria such as facultative anaerobic *Bacillus* species can ferment sugars and give false positive results in the MPN method. In this study, presence of *Bacillus* in the silage was confirmed by growth of *Bacillus* on plates containing a medium of yeast and beef extracts, peptone and agar (Jonsson 1990), which might have affected the results from the MPN method. The negative relationship between *C. tyrobutyricum* by the qPCR method and the lactic acid content and the positive relationships with acetic acid and pH reflect fermentation of lactic acid to butyric acid and acetic acid by *C. tyrobutyricum*, resulting in an increasing pH-value (Pahlow et al. 2003). Furthermore, the negative relationship with 1,2-propanediol is most likely related to conversion of 1,2-propandiol to propionic acid and propanol by *Lactobacillus diolivorans* (Krooneman et al. 2002). The positive relationships between total spore count by the MPN method and contents of butyric acid and butanol show degradation of hexoses and lactic acid to these fermentation products by saccharolytic clostridia and most likely by facultative anaerobic *Bacillus* species (Jonsson, 1990).

**Conclusions** The qPCR method for specific detection of *C. tyrobutyricum* in clover-grass silage seems promising but needs to be investigated further in comparison to the MPN method. Using a larger data set, more robust relationships with the fermentation products can be developed.

**References**

Bassi, D., Fontana, C., Zucchelli, S., Gazzola, S. & Cocconcelli, P.S. (2013) TaqMan real time quantitative PCR targeting the phosphotransacetylase gene for *Clostridium tyrobutyricum* quantification in animal feed, faeces, milk and cheese. International Dairy Journal, 33, 75-82.

Cremonesi, P., Vanoni, L., Silvetti, T., Morandi, S. & Brasca, M. (2012) Identification of *Clostridium beijerinckii, Cl. butyricum, Cl. sporogenes, Cl. tyrobutyricum* isolated from silage, raw milk and hard cheese by a multiplex PCR assay. Journal of Dairy Research, 79, 318-323.

FDA Bacteriological Manual. (1978) Bacteriological Analytical Manual, 5th ed. Food & Drug Administration, Washington, D. C. p. 5-6.

Jonsson, A. (1990) Enumeration and confirmation of *Clostridium tyrobutyricum* in silages using neutral red, D-cycloserine, and lactate dehydrogenase activity. Journal of Dairy Science, 73, 719-725.

Krooneman, J., Faber, F., Alderkamp, A.C., Oude Elferink, S.J.H.W., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C. & Vancanneyt, M. (2002) *Lactobacilllus diolivorans* sp. Nov., a 1,2 propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 52, 639-646.

Pahlow, G., Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H., & Spoelstra, S.F. (2003) Microbiology of ensiling. In: Buxton, D.R., Muck, R.E., & Harrison, R.E. (ed.). Silage Science and Technology. ASA, CSSA, SSSA, Madison, WI. p. 31-93.

# Effect of sealing strategies and sampling site on microbial communities of corn silage

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**Keywords:** chemical additives, ensiling, fermentation

**Introduction** Different sealing strategies are used to preserve nutrients and facilitate adequate fermentation. Chemical additives such as sodium benzoate have antimyotic activity thereby promoting aerobic stability of silage by controlling yeasts and fungi. The aim of this study was to evaluate the effect of sealing strategies and sampling site on microbial communities in corn silage.

**Material and Methods** The corn hybrid used for silage preparation was AG 1051. Whole corn plants were harvested at 35% of dry matter (DM) and ensiled in bunker silos (45 t). Two sealing strategies were adopted: (1) polyethylene film black-on-white 200 µm covered with sugarcane bagasse (10 cm thick layer) (BG) or (2) application of sodium benzoate on the top surface of ensiled mass (150 g/m$^2$, dilution of 1:4 = 0.6 L/m$^2$) and sealing it immediately with polyethylene film black-on-white 200 µm (BZ). After 343 days of storage, the silos were opened and samples of silage were collected from top and middle site of the silo. The determination of lactic acid bacteria, yeast, filamentous fungi, clostridium and bacillus was done by plating techniques. Data were analysed using the MIXED procedure of SAS.

**Results** The application of sodium benzoate on the top surface was not efficient (P< 0.01) in controlling yeasts in the top layer of the silo (3.98 log cfu g$^{-1}$) compared to the middle location (2.25 log cfu g$^{-1}$) and the silage with sugarcane bagasse coverage (2.82 and 2.99 log cfu g$^{-1}$), respectively. There was no sealing strategy effect on the other microorganisms evaluated (*P*≥0.1). Sampling site affected Clostridium (*P*=0.06) and Bacillus (*P*=0.02), where both populations were higher in the top compared to the middle of the silo. Lactic acid bacteria community was greater (*P*=0.04) in the middle of the silo than in the top. There was no effect of sealing strategy and sampling site on fungi (P≥0.1).

**Table 1.** Microbiological characteristics of the middle and top layers of corn silage with sugarcane bagasse coverage or sodium benzoate application on the top surface.

| | BG[2] | | BZ[2] | | | *P* | | |
|---|---|---|---|---|---|---|---|---|
| **Item**[1] | Middle | Top | Middle | Top | EPM | S | L | S × L |
| pH | 3.72 | 3.87 | 3.85 | 3.96 | 0.06 | 0.12 | 0.02 | 0.59 |
| LAB, log cfu /g | 4.36 | 4.08 | 4.69 | 4.12 | 0.19 | 0.27 | 0.04 | 0.34 |
| Yeasts, log cfu /g | 2.99b | 2.82b | 2.25b | 3.98a | 0.48 | 0.74 | 0.02 | 0.01 |
| Fungi, log cfu /g | 2.48 | 3.12 | 2.83 | 3.51 | 0.68 | 0.39 | 0.15 | 0.96 |
| Clostridium, log cfu /g | 2.30 | 3.35 | 2.31 | 3.52 | 0.39 | 0.83 | 0.06 | 0.85 |
| Bacillus, log cfu /g | 3.34 | 4.30 | 3.00 | 4.30 | 0.30 | 0.62 | 0.02 | 0.60 |

[1]LAB: Latic acid bacteria; [2]BG: Corn silage sealed polyethylene film black-on-white 200 µm covered with sugarcane bagasse (10 cm thick layer); [2]BZ: application of sodium benzoate on the top surface of ensiled mass (150 g/m$^2$, dilution of 1:4 = 0.6 L/m$^2$) and sealing it immediately with polyethylene film black-on-white 200 µm S= effect of treatment; L = effect of sampling site; S x L = effect of interaction treatment by sampling site.

**Discussion** Anaerobic environment is essential for the development of lactic acid bacteria that ferment soluble carbohydrates producing acids that reduce the pH of the silage (Muck 1991). Due to the lower possibility of oxygen entry, the middle site of the silages is more favourable for lactic acid bacteria (LAB) to grow compared to the top site. Bacillus is anaerobic or facultative aerobic microorganism that may be involved in the initial deterioration of silages (Te Giffel et al. 2002). Oxygen penetration allows deteriorating microorganisms to grow. Likely because of that, greater spore counts of undesirable microorganisms and lower LAB have been found in the top site of silos.

The silages were storage for 343 days before opening and to minimize the effects of the solar radiation on the silage plastic cover, BG was used as physical barrier and BZ as chemical agent to control the undesirable microorganisms that may develop in the top layer of the ensiled material. However, BZ treatment was not efficient in controlling the growth of deteriorating microorganisms, as the yeast population was greater in the top site of the silages treated with sodium benzoate. The low efficiency of sodium benzoate in control yeast in the top layer might be associated with the method used to apply the additive. Sodium benzoate was not mixed into the top layer, was spread.

The solar radiation throughout the time likely improved the oxygen penetration by drying the polyethylene, increasing oxygen permeability. Although, when BG was used that growth of yeast was controlled, probably due the physical barrier that avoided dryness of the polyethylene cover.

**Conclusions** Sugarcane bagasse is more effective to protect silage polyethylene cover exposed for long time than sodium benzoate as chemical barrier.

**References**

Te Giffel, M.T., Wagendorp, A., Herrewegh, A. & Driehuis, F. (2002) Bacterial spores in silage and raw milk. *Antonie van Leeuwenhoek*, *81*(1-4), 625-630.

Muck, R.E. (1991) Silage fermentation. In: Zeikus, G.; Johnson, E.A. (eds.). Mixed cultures in biotechnology, New York, USA, 1991, pp. 171-204.

# Effects of dry matter, silage additive and bagging technology on fungal counts and aerobic stability of pressed sugar beet pulp silage

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**Keywords:** aerobic stability, fungi, plastic bags, sugar beet pulp

**Introduction** Silage from pressed sugar beet pulp (PSBP) represents a valuable dietary component in animal nutrition but its poor aerobic stability (ASTA) restricts its use (Weber et al. 2006). To counteract the detrimental effects of air on stability during feed-out, chemical additives have been successfully used (Potthast et al. 2014). However, new developments regarding higher dry matter (DM) content and bagging technology (direct bagging from truck) required additional studies on fungal contamination and ASTA of PSBP silage.

**Material and Methods** In 2015 and 2016, fresh PSBP of different DM contents (28 and 45%) were obtained from sugar factories of Südzucker AG, Mannheim, Germany and pressed into plastic bags by either using a rotor bagger (RT 8000, Budissa Bag GmbH, Germany), or by direct transfer from the truck (only 45% DM), which was equipped with horizontal bottom augers. The liquid chemical additive contained potassium sorbate (154 g $L^{-1}$), sodium benzoate (257 g $L^{-1}$) and ammonium propionate (57 g $L^{-1}$) and was applied on the bagging machine at 1.5 L $t^{-1}$, or 2.0 L $t^{-1}$ (2016, rotor bagger, 45% DM). One bag per treatment was kept outside for several months until opening in late June/early July of the following year. Sampling was performed upon bag opening (fresh) and after one week of air exposure (old), according to Potthast et al. (2014). Fresh cutting faces were repeatedly produced by removal of one meter of PSBP silage which were, again exposed to air for one week so that, in total, fresh and old surfaces were sampled three times. Packing density was measured only on fresh PSBP with 45% DM. Fungal counts were determined by spread-plating of serial dilutions. When the silage temperature increased by 3 °C over ambient, silage was considered aerobically unstable. Data were subjected to statistical analysis by SAS 9.4. The effects of year (Y), DM, air exposure (A), additive (AD) and bagging technology (BT, only in 45% DM material), respectively, were tested by the non-parameteric ANOVAF-option, and the procedure NLIN ("broken line"-model) was employed to describe the relationship between yeast count and ASTA. Significance was declared at $P<0.05$.

**Results and Discussion** The use of the rotor bagger resulted in higher packing density (299 kg DM $m^{-3}$) than measured in the truck-bagged material (245 kg DM $m^{-3}$, $P<0.001$). There were no differences detected in fungal counts between years, and the effects on ASTA was only marginal (table 1), which is in line with data by Weber et al. (2006). Fungal counts were highest and ASTA lowest in old, untreated PSBP. Additive use consistently improved ASTA, but the magnitude of the effect differed between old and fresh PSBP silage (A x AD interaction, $P<0.01$). Although DM affected yeast count ($P<0.01$), ASTA was not influenced. This was likely caused by only small differences between DM levels and the fact that, in general, yeast count was well below the threshold value of $10^5$ colony-forming units (cfu) $g^{-1}$ (Jonsson and Pahlow 1984). Regardless of DM, ASTA was enhanced by additive treatment although its effect was stronger in 28% DM PSBP (DM x AD interaction, $P<0.001$). There was no clear effect of bagging technology observed on fungal counts and aerobic stability due to many interactions with other factors (data not shown). This can be attributed to insufficient additive dosage and homogeneity of application on the truck in year 2015. The analysis of samples for sodium benzoate content and subsequent calculation of the additive application rate substantiated this assumption (mean: 0.8 L $t^{-1}$; SD: 0.4 L $t^{-1}$). Fresh, rotor-bagged PSBP had lower yeast count than truck-bagged material (log cfu $g^{-1}$ 2.9 *vs* 4.5), whereas no difference was observed in old PSBP silage (log cfu $g^{-1}$ 5.8 *vs* 6.0, A x BT interaction, $P=0.003$). The ASTA of PSBP silage was highly variable and

ranged between 0.8 and 6.8 days. In 2015, silage additive use did not lead to lower yeast count in truck-bagged PSBP silage whereas a consistent decrease was observed in rotor-bagged material. Considering rotor-bagged material only, untreated air-exposed material had lower ASTA than treated PSBP (1.1 days *vs* 4.2 days). Additive use could vastly counterbalance the detrimental effects of air on ASTA regardless of year (fresh, untreated: 5.3 days *vs* 4.2 days, old, treated). In 2016, there were no technology-related differences observed regarding ASTA. Silage additive treatment improved ASTA in old PSBP silage by 4.1 day in rotor-bagged material and by 3.5 days when truck-bagging was employed.

**Table 1.** Effects of year (Y), dry matter (DM), air exposure (A) and additive (AD) on fungal counts and ASTA of PSBP silage packed by rotor bagger (data given as means and SEM per treatment, log cfu $g^{-1}$ unless stated otherwise, n=9).

| Year | A | DM | AD | Yeast count | | Mould count | | ASTA (days) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean | SEM | Mean | SEM | Mean | SEM |
| 2015 | | | | | | | | $3.8^{A}$ | 0.3 |
| 2016 | | | | | | | | $4.1^{B}$ | 0.3 |
| | fresh | | 0 | $2.9^{b}$ | 0.19 | $2.2^{a}$ | 0.08 | $4.8^{b}$ | 0.2 |
| | | | 1.5 l $t^{-1}$ | $2.3^{a}$ | 0.12 | $2.2^{a}$ | 0.04 | $6.2^{c}$ | 0.1 |
| | old | | 0 | $6.8^{d}$ | 0.22 | $4.0^{c}$ | 0.31 | $0.8^{a}$ | 0.2 |
| | | | 1.5 l $t^{-1}$ | $4.3^{b}$ | 0.30 | $2.7^{b}$ | 0.17 | $4.5^{b}$ | 0.4 |
| | | 28 | | $3.8^{A}$ | 0.26 | | | | |
| | | 45 | | $4.4^{B}$ | 0.25 | | | | |
| | | 28 | 0 | | | | | $2.3^{a}$ | 0.4 |
| | | | 1.5 l $t^{-1}$ | | | | | $5.5^{c}$ | 0.3 |
| | | 45 | 0 | | | | | $2.9^{b}$ | 0.4 |
| | | | 1.5 l $t^{-1}$ | | | | | $5.0^{c}$ | 0.4 |
| Level of significance (ANOVAF-option) | | | | | | | | | |
| Y | | | | ns | | ns | | *** | |
| A | | | | *** | | *** | | *** | |
| DM | | | | ** | | ns | | ns | |
| AD | | | | *** | | * | | *** | |
| A x AD | | | | ** | | ** | | *** | |
| DM x AD | | | | ns | | ns | | * | |

$^{A,B}$marginal means for each factor within columns bearing unlike superscripts differ, $^{a,b}$cell means within columns with unlike superscripts differ, other possible interactions not presented because of no significance.

Regardless of air exposure, increasing the additive application rate from 1.5 to 2.0 L $t^{-1}$ decreased the yeast count ($P$<0.001), in a dose-dependent manner (log cfu $g^{-1}$ 3.9 *vs* 2.6) when compared with untreated PSBP silage (log 5.5 cfu $g^{-1}$). However, application rate had a stronger effect on ASTA in old PSBP silage as reflected by an improvement ($P$<0.001) from 1.2 days in untreated material to 5.3 and 6.9 days, respectively (A x AD interaction, $P$<0.01). The relationship ($R^2$=0.66, $P$<0.0001, RMSE=1.47) between yeast count (x) and ASTA (y), was best characterized by the “broken-line”-model by the equations: y=5.77 (if x ≤ 3.89, break point) and y=11.1-1.38x (if x > 3.89).

**Conclusion** The study showed that rotor-bagging was superior to truck-bagging regarding packing density of PSBP silage. Rapid aerobic deterioration caused by fungi can be prevented by the use of the chemical additive, which must be applied homogeneously and at a sufficient rate. If this is guaranteed, also high DM PSBP can be directly packed into plastic bags attached to a truck.

**References**

Jonsson, A. & Pahlow, G. (1984) Systematic classification and biochemical characterization of yeasts growing in grass silage inoculated with *Lactobacillus* cultures. Animal Research and Development, 20, 7-22.

Potthast, C., Brinker, S. & Maier, K. (2014) Assessment of the effects of chemical silage additives in pressed pulp silage. In: IIRB (ed). Proceedings of the 74th IIRB Congress, Dresden, Germany, pp.116-121.

Weber, U., Kaiser, E. & Steinhöfel, O. (2006) Studies on ensiling pressed sugar beet pulp in plastic tubes. Part 2: Effect of storage length, addition of ensiling aids and extraction end closure on the aerobic stability of pressed pulp silage. Sugar Industry, 131, 857-862.

# Estimating fungal biomass during aerobic spoilage of silage

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**Keywords:** aerobic spoilage, enzyme-linked immunosorbent assay (ELISA), fungal biomass, silage

**Introduction** Fungi have a critical role in the aerobic spoilage of silage which results in considerable economic losses, estimated to be £110 million in the UK alone (Williams 1995). This is due to rejection of feed, reduction in nutritional value, and wastage due to the potential risk of wild yeast and mycotoxin contamination. Methods to monitor silage quality are shifting from reliance on in-lab analysis towards on-farm tests where results are immediately available and potentially more accurate because changes in the sample may occur between collection and laboratory analysis. Currently, most on-farm silage assessments are based on temperature, smell and visual inspection of silage before feeding. More recently, some farms have begun to employ high tech methods such as near-infrared spectroscopy (NIRS) to predict silage quality on-farm. Although there are a range of methods available for studying microbial populations in silage, including culture, qPCR and DNA sequencing, these all require specialist scientific knowledge and equipment (Muck, 2013). Here, we assessed the competence of an enzyme-linked immunosorbent assay (ELISA) based method to estimate fungal biomass in silage during a controlled aerobic spoilage incubation experiment. We hypothesized that we would be able to detect an increase in fungal biomass over time during aerobic exposure and to distinguish between bales of contrasting quality, as assessed by conventional methods.

**Material and Methods** Cores of grass silage making up a composite sample of 7 kg taken from round bales made on the Rothamsted Research North Wyke farm platform were used in a controlled laboratory incubation study. Aerobic stability vessels (ASV) containing 750 g silage were incubated in the dark at 20 ˚C and were destructively sampled at 0, 1, 2, 4, 8, 16 and 32 days after the onset of the aerobic incubation study. Samples were processed immediately or frozen at -20 °C in vacuum bags. The internal temperature of each vessel was individually recorded. Active fungal hyphal biomass was quantified using an antibody-based ELISA method which has been previously developed as a method of fungal detection in soil (Thornton 2008). ELISA absorbance values gained from an ascomycete specific monoclonal antibody were converted to biomass using a calibration curve made with known quantities of a *Trichoderma* isolate. As a secondary indicator of fungal proliferation, ergosterol was extracted from each sample and the quantity was measured using high-performance liquid chromatography (HPLC). The area under the peak in each sample was integrated and compared with a standard calibration curve constructed from known quantities of ergosterol. In addition, silage quality indicators were assessed by NIRS and wet chemistry analysis. Statistical analysis was carried out in GenStat (18$^{th}$ Edition). Values were transformed through natural Log transformation before analysis.

**Results** The results obtained by both the ELISA based method and the ergosterol method showed an increase in fungal biomass over the period of aerobic exposure (Table 1). Statistical analysis through Analysis of Variance showed that there was a significant increase in fungal biomass during the aerobic exposure time course ($F_{6,35}$=4.68, P=0.001) in the ELISA method. This result was supported by the results observed in the corresponding ergosterol measurements ($F_{6,32}$ =3.41, P=0.01). This indicated that the method has the capability to detect temporal changes in fungal biomass within silage.

**Table 1.** Mean values of ergosterol and biomass in silage during an aerobic spoilage time course.

| | Timepoint (days) | | | | | | | F Stat. | P value |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | 8 | 16 | 32 | | |
| Mean Biomass mg $g^{-1}$ DM | 0.007 | 0.005 | 0.003 | 0.006 | 0.005 | 0.168 | 0.376 | (6,35) 4.68‡ | 0.001‡ |
| Mean Ergosterol mg $g^{-1}$ DM | 0.3† | 0.02 | 0.04 | 0.03 | 0.02 | 0.04 | 0.14 | (6,32) 3.41‡ | 0.01‡ |

† n=3 due to 3 samples being below the level of detection. ‡ reported based on Natural log of values.

**Discussion** Monitoring silage quality can be unreliable due to the heterogeneous nature of the material, which increases the difficulty in obtaining a representative sample. In addition, the ability to independently assess samples from different sections of the feed-out face of a silo, will enable the farmer to identify weaknesses in their silage management and rectify them at future harvests. Here, we showed that an antibody-based ELISA method has the potential to be developed into a rapid on-farm analysis tool. Although the method showed that it was concurrent with the results gained from ergosterol measurements there are still some potential questions that require further assessment, such as the specificity of the antigens used in the method and the variable levels of ergosterol within different species of fungi at different growth stages (Seitz et al. 1979). Plate count methods were not used as they do not provide an assessment of fungal biomass. The increase in ergosterol over time enables an assessment of both residual fungal biomass at silo opening (active or otherwise) and by subtraction, the proliferation of fungal biomass during aerobic exposure. This study used a slow rate of aerobic spoilage which resulted in an incomplete increase in temperature to replicate feed-out face oxygen penetration. This is reflected in the results which indicate that true aerobic spoilage was not seen in all bales, however it did provide a suitable platform for testing the method. New techniques need to be developed that can detect small changes in fungal populations brought about by the slow ingress of oxygen. There is considerable scope to develop and refine this method further for use on farm.

**Conclusion** We conclude that application of this method both in research and on-farm has potential to improve our understanding of fungal growth population dynamics occurring due to aerobic exposure during both the storage and feed-out phases of ensilage. In addition, it has the potential to be applied as an on-farm test to determine the level of fungal contamination and therefore the suitability of silage for feeding. This work demonstrates that the novel application of this method is suitable for quantifying biomass of fungi in silage which have an important role in silage preservation, feeding, animal health and farm profitability.

**References**

Muck, R.E. (2013) Recent advances in silage microbiology, Agricultural and Food Science. 22, 3-15.

Seitz, L.M., Saner, D.B, Burroughs, R, Mohr, H.E & Hubbard J.D. (1979) Ergosterol as a measure of fungal growth. Phytopathology. 69, 1202-1203.

Thornton, C.R. (2008) Tracking fungi in soil with monoclonal antibodies. European Journal of Plant Pathology. 121, 347-53.

Williams, A., Critten, D.L., Reynolds, A.M. (1995). A mathematical model of the aerobic deterioration of silage. Grass and Forage Science 50(2), 132-146.

# Identification of lactic acid bacteria isolated from *Stipa grandis* silage and native grass silage in Eurasian steppe

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**Keywords:** Eurasian steppe, lactic acid bacteria species, native grass silage *Stipa grandis* silage

**Introduction** *Stipa grandis* P. Smirn is a unique and dominant species in the eastern Eurasian steppe, although its silage has been used as roughage of ruminant for long time, the species of lactic acid bacteria (LAB) in the silage is rarely studied. The objective of this study was to identify LAB isolated from *S. grandis* silages and native grass silages from different sites in the Eurasian steppe of northern China.

**Material and Methods** *Stipa grandis* and native grass were harvested in 9 sites on Eurasian steppe, chopped to 1cm to 2cm, and ensiled in plastic film bags (28 × 35 cm) with 300 g and then stored for 60 days (d). After 60 d, the 33 strains and 29 strains of LAB were isolated from *S. grandis* silages and native grass silages respectively and identified using 16S rRNA sequencing method.

**Results** All isolates were identified as gram-positive and catalase-negative. There were 10 strains isolated from *S. grandis* silages identified as *Enterococcus mundtii*, 7 strains as *Enterococcus faecalis* and 11 strains as *Leuconostoc pseudomesenteroides* with minimum 99% similarity in 16S rDNA gene sequences. There were *Lactobacillus brevis*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Weissella halotolerans* and *Weissella cibaria* identified with minimum 99% similarity in 16S rDNA gene sequences, each of which had only one strain isolated from *S. grandis* silages (Table 1).

There were 6, 10, 3, 4 and 3 strains isolated from native grass silages which were most closely related to *E. mundtii*, *E. faecals*, *L. plantarum*, *P. pentosaceus* and *L. pseudomesenteroides*, respective, with minimum 99% similarity in 16S rDNA gene sequences. There were only one strain of *L. graminis*, *P. acidilactici* and *W. viridescens* identified respectively (Table 1).

The percentages of *E. mundtii*, *E. faecalis* and *L. pseudomesenteroides* isolated from *S. grandis* silages were 30.3%, 21.2% and 33.3% respective; *L. brevis*, *P. acidilactici*, *P. pentosaceus*, *W. halotolerans* and *W. cibaria* were 3.03% respective. The percentages of *E. mundtii*, *E. faecalis*, *L. plantarum*, *P. pentosaceus* and *L. pseudomesenteroides* isolated from native grass silage were 20.7%, 34.5%, 10.3%, 13.8% and 10.3% respective; *L. graminis*, *P. acidilactici* and *W. viridescens* were 3.45% respective (Table 1). The percentages of *Enterococcus* isolated from *S. grandis* silages and native grass silages were 51.5% and 55.2% respectively. There were 3.03% of *Lactobacillus*, 6.06% of *Pediococcus*, 6.06% of *Weissella* and 33.3% of *Leuconostoc* in LAB isolated from *S. grandis* silages, and 13.8% of *Lactobacillus*, 17.2% of *Pediococcus*, 3.45% of *Weissella* and 10.3% of *Leuconostoc* in LAB isolated from native grass silages (Table 1).

**Table 1. S**pecies and gunera of lactic acid bacteria isolated from *Stipa grandis* silages and native grass silages

| Items | | *Stipa Grandis* Silages | | Native Grass Silages | |
|---|---|---|---|---|---|
| | | Counts | Percentage | Counts | Percentage |
| species | *E. mundtii* | 10 | 30.3 | 6 | 20.7 |
| | *E. faecalis* | 7 | 21.2 | 10 | 34.5 |
| | *L. brevis* | 1 | 3.03 | - | - |
| | *L. graminis* | - | - | 1 | 3.45 |
| | *L. plantarum* | - | - | 3 | 10.3 |
| | *P. acidilactici* | 1 | 3.03 | 1 | 3.45 |
| | *P. pentosaceus* | 1 | 3.03 | 4 | 13.8 |
| | *W. halotolerans* | 1 | 3.03 | - | - |
| | *W. cibaria* | 1 | 3.03 | - | - |
| | *W. viridescens* | - | - | 1 | 3.45 |
| | *L. pseudomesenteroides* | 11 | 33.3 | 3 | 10.3 |
| | Total | 33 | 100 | 29 | 100 |
| Genera | *Enterococcus* | 17 | 51.5 | 16 | 55.2 |
| | *Lactobacillus* | 1 | 3.03 | 4 | 13.8 |
| | *Pediococcus* | 2 | 6.06 | 5 | 17.2 |
| | *Weissella* | 2 | 6.06 | 1 | 3.45 |
| | *Leuconostoc* | 11 | 33.3 | 3 | 10.3 |
| | Total | 33 | 100 | 29 | 100 |

**Discussion** In general, the main genus of LAB in silage are *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactoccus*, *Streptococcus*, *Weissella* (Cai *et al*. 1998). In this study, the strains isolated from *S. grandis* silages and native grass silages were identified as 5 genera, which were *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Weissella*, and *Leuconostoc*. The percentage of *Enterococcus* isolated from two silages was more than 50% in this study, whereas Zhang *et al*. (2015) reported that 96.6% of LAB isolated from *S. grandis* silage was identified as *Enterococcus*. Some *Enterococcus* from silages has been identified as *E. faecium*, *E. faecalis*, *E. mundtii*, *E. casseliflavus* and *E. hirae* (Tohno *et al.* 2012). In the study, the *Enterococcus* was identified as *E. faecalis* (21.2% vs 34.5%) and *E. mundtii* (30.3% vs 20.7%) isolated from *S. grandis* silages and native grass silages. Several previous reports have observed the presence of *L. pseudomesenteroides* in silage (Cai *et al.* 1998), in this study, there were also *L. pseudomesenteroides* (33.3% vs 10.3%) in *S. grandis* silages and native grass silages.

**Conclusions** *Enterococcus* was the dominant genus in *S. grandis* silages and native grass silages, *E. mundtii*, *E. faecalis* and *L. pseudomesenteroides* were most frequently isolated from *S. grandis* silages, and *E. mundtii*, and *E. faecalis* were most frequently isolated from native grass silages.

**References**

Cai, Y., Benno, Y., Ogawa, M., Ohamomo, S., Kumai, S. & Nakase, T. (1998) Influence of Lactobacillus spp. from an inoculant and of *Weissella* and *Leuconostoc* spp. from forage crops on silage fermentation. Applied and Environmental Microbiology, 64, 2982-2987.

Tohno, M., Kobayashi, H., Nomura, M., Uegaki, R. & Cai, Y. (2012) Identification and characterization of lactic acid bacteria isolated from mixed pasture of timothy and orchard grass, and its badly preserved silage. Animal Science Journal, 83, 318-330.

Zhang, Q., Yu, Z. & Wang, X.G. (2015) Isolating and evaluating lactic acid bacteria strains with or without sucrose for effectiveness of silage fermentation. Grassland Science, 61, 167-176.

# Impact of inoculation with *Lactobacillus buchneri* and *Lactobacillus hilgardii* on bacterial and fungal communities during feed-out period of corn silage

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**Keywords:** aerobic stability, microbial population, metasequencing, bacteria, fungi

**Introduction** The last phase of the ensiling process corresponds to the feed-out period. The protection against ingress of oxygen is then removed, increasing the risk for aerobic deterioration of the fermented forage. Presence of oxygen allows yeast and bacteria to consume the organic acids produced during fermentation. Physico-chemical conditions are then more favourable and other microorganisms resume their growth, a concept known as aerobic stability (AS). Silages prepared from different forages show different levels of response to aerobic stability. AS is usually measured by an increase in temperature within the silage due to microbial metabolism. Corn silages are generally rather unstable due to high residual sugar concentration. The presence of the acetate producing bacteria genus *Acetobacter* has been reported during the early feed-out (Spoelstra et al. 1988) along with lactate degrading yeasts (Pahlow et al. 2003). Inoculation by heterofermentative lactic acid bacteria can delay deterioration due to synthesis of acetic acid and propionic acid during the fermentation phase, which improves yeast inhibition. In this study, we studied the effect on AS and on microbial diversity during a 10-day aerobic exposition of corn silage inoculated or without a combination of *Lactobacillus buchneri* and *Lactobacillus hilgardii*.

**Material and Methods** Corn (370 g dry matter (DM) $kg^{-1}$ forage) was ensiled in bag silos after addition of water (control) or an inoculant based on a combination of *Lactobacillus buchneri* NCIMB 40788 and *Lactobacillus hilgardii* CNCM-I-4785 at 1.5 x $10^5$ colony-forming units /CFU)/g each. The silos were incubated outdoor for 150 days prior to opening. From each treatment, 6 buckets (repetitions) were filled with 2 kg of silage collected at silo opening from the middle of the bag, fitted with temperature probes, and incubated for 10 days. Additionally, 30 more buckets (diversity trial) filled with 2 kg of silage were prepared for each treatment. All buckets were incubated in a controlled temperature room set at 20°C. For the diversity trial, every 24 h of incubation, three buckets per treatment were divided into subsamples for chemical analysis, DNA extraction, and mycotoxin quantification. DNA was extracted using MoBio's Power Lyser Power Soil DNA isolation kit prior to amplicon sequencing of the 16S rDNA V4 region (bacteria) and ITS1 region (fungus) on an Illumina MiSeq sequencer. Results were analyzed using a temporal pseudo-replication model using package nmle. Diversity data were analyzed using edgeR package. One-way ANOVA was used for comparison between treatments for the samples prior to air exposition. All statistical analyses were performed on R (version 3.3.3).

**Results and Discussion** At the opening, the pH of the inoculated silo (3.97) was slightly higher than the control (3.87). The lactate:acetate ratio (1.65 and 3.74 for treated and control, respectively) showed that inoculation orientated fermentations toward an heterofermentative profile . The untreated silage heated more rapidly than the treated one which indicated a poor AS in the control (for the control: 129 h to reach the 2°C over ambient level (145 h for +3°C), for the treated silage: 206 hours (224 h for +3°C)). The initial bacterial population of the inoculated silage had more Proteobacteria (~5%) than the control (~2%), but Firmicutes (among which lactic acid bacteria) dominated in all samples, representing 94% of the overall diversity (Figure 1A). In the control, the diversity profile of the bacterial community was stable for 120 h, and afterwards the lactic acid bacteria were rapidly replaced by *Acetobacteriaceae*. This change in bacteria succession corresponds to the temperature increase over threshold values. For the inoculated samples, after 10 days of incubation, *Lactobacilliaceae* still constituted more than 50% of the bacterial diversity (not shown in the abstract). Fungal diversity also presented important differences between treatments (Figure 1B). The main

observation for fungal diversity was that in inoculated samples, *Saccharomycetaceae* became strongly dominant from 72 h of air exposure and represented ~90% of the total reads in the inoculated samples after 10 days, which was not observed in control samples where Zygomycota were also abundant, together with other families of Ascomycota, such as *Pichiaceae* or *Trichocomaceae* (*Penicillium* genus). The only mycotoxin detected was roquefortin C, which was only presents in the untreated samples following 7 days of incubation. It is currently not possible to conclude if inoculation with the lactic acid bacteria directly contributed to this specific succession. Deeper identification of the *Lactobacillaceae* members at the species level would be necessary to know the specific contribution of the inoculated strains to bacterial diversity.

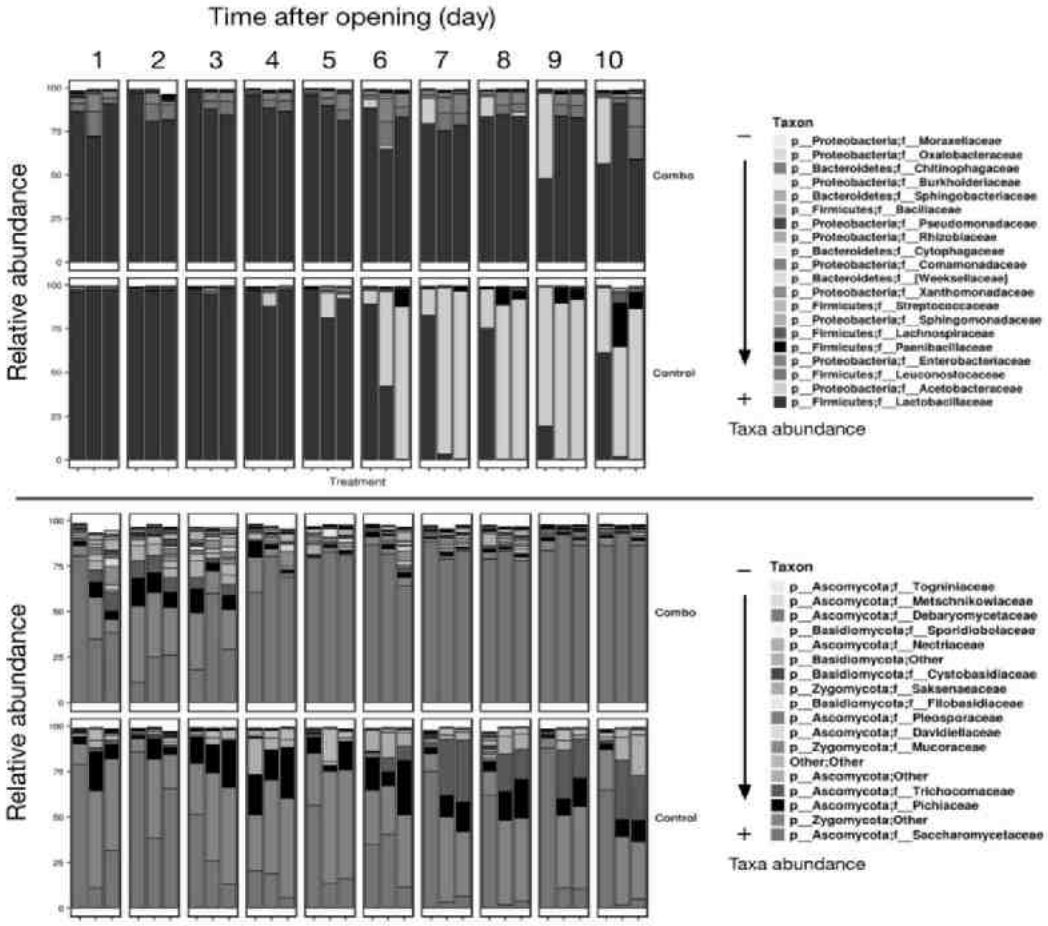


**Figure 1.** Eubacteria (A) and Fungi (B) diversity in whole plant corn silages exposed to air during a 10-day aerobic stability assay. The silages were either untreated (control) or inoculated with *Lact. buchneri* 40788 and *Lact. hilgardii* 4785 and stored for 150 days in a bag silo before opening.

**Conclusion** Inoculation with a combination of heterofermentative microbial additives (*L. buchneri* 40788 and *L hilgardii* 4785) led to improving aerobic stability of corn silage. As *Lactobacillaceae* and *Saccharomycetaceae* families were dominant in the inoculated silage, we suspect that their development could limit the growth of spoilage microorganisms such as members of the *Acetobacteriaceae* and of the *Trichocomaceae*.

**References**

Pahlow G., Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H., Spoelstra, S.F. (2003) Microbiology of ensiling. Pages 31-93 in Silage science and technology. D.R. Buxton, R.E. Muck, J.H. Harrison, ed. American Society of Agronomy. Madison, Wisconsin, USA.

Spoelstra S.F., Courtin, M.G. & van Beers, J.A.C. (1988) Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. Journal of Agricultural Science, Cambridge 111, 127-132.

# Impact of plant biostimulants on maize forage and subsequent silage quality: A field experiment using mini-silos

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**Keywords:** plant biostimulants, maize, silage, aerobic stability

**Introduction** Maize forage crops with high numbers of yeast and mould are susceptible to diseases that can result in aerobic spoilage and mycotoxin production post-ensiling when the silo is opened. Plant biostimulants (PBs) are becoming more popular in crop production. In the present study, a commercial PB named Imprograin®, available from Alltech Inc. (Dunboyne, Ireland) and marketed as a proprietary liquid product containing a mixture of spent lactobacillus culture media and an aqueous yucca extract surfactant specifically formulated to optimise crop performance and yield, was applied as diluted foliar spray. The aim of the present study was to determine the impact of Imprograin® when applied to maize on: the forage and subsequent silage nutritional quality; the aerobic stability of the silage; and the key natural microbial communities that are involved in the ensiling process [Enterobacteria (ENB), lactic acid bacteria (LAB) and Yeasts]. Imprograin® is a complex product, thus the primary focus of this study was on assessing whether it had an effect on reducing the aerobic spoilage of the silage.

**Material and Methods** Imprograin® was tested on maize cultivar Rodriguez (KWS, Hertfordshire, UK). Maize (12.500 seeds/ha) were planted in a 4-ha field, on a commercial farm (Cornwall, UK) on April 24th 2017. Imprograin® (1 L in 200 L of deionised water per ha) was sprayed on the crops at the same time as herbicide on June 13th 2017. Within the 4-ha field, experimental treatments consisted of either maize treated with Imprograin® (⅔ of the field) or maize with no treatment (Control, ⅓ of the field). The field was harvested on October 11th 2017 by a contractor using a 950 CLAAS harvester with a corn cracker. The field was harvested in 8 straight lines (4 m width) at 15 cm stubble height and was chopped to an average length of 12 mm. The treated crop (PB) was harvested first, followed by the control. Harvested maize samples were collected from the maize pile left at the end of each line and were placed in polythene bags (n=8 for control and n=8 for treatment, *ca.* 5 kg/bag). Maize in the bags was mixed well and then packed into 3 L Kilner glass jars (mini-silos) fitted with a clip top, to ensure an airtight seal was maintained (Kilner, Liverpool, UK). Each jar contained *ca.* 1.6 kg of maize at a density of 168 kg DM $m^{-3}$. The mini-silos were incubated in two plant growth cabinets (Panasonic, Leicestershire, UK) at 22 ± 0.5 °C at Rothamsted Research (North Wyke, Devon, UK) for 3 months in a randomised blocked design based on incubator/shelf blocks. Culturable bacterial and fungal populations were enumerated and biochemical and nutritional analysis was performed for both pre- and post-ensiled maize according to official methods of analysis of AOAC (Horwitz 2000). Following the ensiling process, subsample (1 kg) from each replicate underwent a 20-day aerobic stability test (3 °C temperature increase above the ambient temperature). For the statistical analysis of the harvested forage data from the field, a t-test was performed. General analysis of variance (ANOVA) was used for the statistical analysis of the silage data based on chemical and microbiological parameters using the GenStat Software (version 18.1)(Lawes Agricultural Trust). Significance level was set at 95-% ($P \leq 0.05$).

**Results** The biochemical and microbiological characteristics of pre-ensiled maize are shown in Table 1. Imprograin® treated maize had a lower pH value than the control. Dry matter (DM) and starch values were higher in treated maize than in the control. Differences were also found in the fibre content; with treated maize, having less acid detergent fibre (ADF) and neutral detergent fibre (NDF)

than the control. LAB and yeast counts differed between treatments with treated maize forage containing higher LAB and lower yeast counts. The post-ensiling parameters, along with aerobic stability values, are presented in Table 2. Treated maize silage had higher LAB and lower yeast counts in comparison to the control. There were no mould counts on the silage samples. Dry matter and starch values were higher in treated silage than in the control while pH values were lower. ADF and NDF fibre values were lower in treated silage than in the control. It took significantly more time (*ca*. 124 h) for the treated silage to heat upon exposure to air than the control. There were no significant differences between treatments for fermentation by-products (VFAs, lactic, NH3-N; results not presented).

**Table 1**. Chemical and microbiological parameters of pre-ensiled maize forage.

| Parameters | Units | Control | Treatment | SED | P value |
|---|---|---|---|---|---|
| pH | | 6.14 | 5.72 | 0.140 | 0.015 |
| DM | g $kg^{-1}$ FM | 307 | 323 | 4.3 | 0.004 |
| ADF | g $kg^{-1}$ DM | 311 | 288 | 6.4 | 0.006 |
| NDF | g $kg^{-1}$ DM | 502 | 473 | 4.6 | <0.001 |
| Starch | g $kg^{-1}$ DM | 182 | 230 | 14.2 | 0.007 |
| Enterobacteria | $Log_{10}$ CFU $g^{-1}$ | 6.17 | 5.81 | 0.193 | 0.089 |
| Lactic acid bacteria | $Log_{10}$ CFU $g^{-1}$ | 6.08 | 6.18 | 0.012 | <0.001 |
| Yeast | $Log_{10}$ CFU $g^{-1}$ | 7.20 | 7.01 | 0.032 | <0.001 |

kg, kilogram; DM, dry matter; FM, fresh matter; CFU, colony-forming units; SED, standard error of the difference

**Table 2**. Chemical and microbiological parameters; and aerobic stability of post-ensiled maize.

| Parameters | Units | Control | Treatment | SED | P value |
|---|---|---|---|---|---|
| pH | | 3.84 | 3.68 | 0.033 | <0.001 |
| DM | g $kg^{-1}$ FM | 293 | 310 | 5.2 | 0.007 |
| ADF | g $kg^{-1}$ DM | 281 | 260 | 3.0 | <0.001 |
| NDF | g $kg^{-1}$ DM | 471 | 457 | 5.2 | 0.026 |
| Starch | g $kg^{-1}$ DM | 268 | 300 | 10.6 | 0.012 |
| Aerobic stability | hours | 288 | 413 | 56.6 | 0.050 |
| Enterobacteria | $Log_{10}$ CFU $g^{-1}$ | ND | ND | ND | ND |
| Lactic acid bacteria | $Log_{10}$ CFU $g^{-1}$ | 7.81 | 7.99 | 0.061 | 0.018 |
| Yeast | $Log_{10}$ CFU $g^{-1}$ | 3.86 | 3.62 | 0.012 | <0.001 |

kg, kilogram; DM, dry matter; FM, Fresh matter; CFU, colony-forming units; SED, standard error of the difference; ND, not detected. Aerobic stability was determined as the number of hours needed for the silage temperature to increase 3 °C above ambient temperature.

**Discussion** The observed differences in biochemical and microbiological parameters between control and Imprograin® treated maize pre-ensiling, were maintained through the ensiling process with corresponding differences seen at the end-point. These differences resulted in higher aerobic stability of the treated silage. Silage aerobic stability is affected by DM, acetic acid and yeast concentrations (Wilkinson & Davies 2013). In our study, there were no differences in acetic acid, lactic acid or ethanol (data not shown). The DM content was higher in the more stable silage contrasting with relevant published work, which suggests that higher DM silages are generally more aerobically unstable. However, as the yeast population was lower in the treated silage, it indicates that lower yeast populations are involved with improved aerobic stability (Wilkinson & Davies 2013). Moreover, the increased DM and starch content along with the lower ADF and NDF of the treated silage, implies that treatment decreased the time required for the maize crop to mature.

**Conclusion** Imprograin® treatment influenced both forage quality and microbial numbers. These parameters benefited the ensiling process and following pre-ensiling trends resulted in a better-quality silage.

**References**

Horwitz, W. 2000. Official methods of analysis of AOAC International.

Wilkinson, J.M., & Davies, D.R. (2013) The aerobic stability of silage: key findings and recent developments. Grass and Forage Science, 68, 1-19.

# *In vitro* fermentation profiles of *Clostridia* at different ensiling pH

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**Keywords:** Clostridia, fermenter, organic acid synthesis, pH

**Introduction** Clostridia are often present in low quality silage and origin either by soil particles or following contamination of the silage by animal carcasses or manure. One group of clostridia, the butyric spore formers, is sometimes present in silage. The group includes *Clostridium tyrobutyricum*, *Clostridium beijerinckii*, *Clostridium butyricum*, and *Clostridium sporogenes*. These bacteria are generally recognized as unable to grow under the low pH conditions observed in corn silage. A pH lower than 4.2 is considered deleterious for their vegetative cell. However, clostridia spores could survive the ensiling process. Inhibition of *Clostridium* has been associated with rapid silage fermentation and high lactate concentration (Thylin et al. 1995). In a study performed with *Cl. tyrobutyricum* (type strain ATCC 25755), a species frequently observed in contaminated silage, it was observed that changes to the pH of cells grown in fermenters led to shifts in the organic acid production profile (Zhu et al. 2004). At pH below 5.5, a shift from butyrate to acetate and lactate production were observed. To test if strains isolated from silage are responsive to pH in terms of fermentation metabolic capacities, a steady-state growth assay was performed in fermenters.

**Material and Methods** Three strains of *Cl. beijerinckii* isolated and purified from different grass silages (E401 - *Festuca arundinacea*; E502 - *Poa pratensis*; E602 - *Phleum pratense*) were grown in fermenters set for continuous growth using a defined culture media containing one of three main sources of carbon (lactic acid, glucose, xylose - 7 g/L). The culture media also includes malt extract, yeast extract, peptone and sodium acetate (2.5 g/L, each) as a source of amino acids and essential cofactors. Comparison with strain *Cl. tyrobutyricum* ATCC 25755 were performed, as this strain of butyric spore forming bacteria is well studied. The fermenters were flushed with nitrogen and they are set to change the pH from the initial starting value of 6.5 down to 4.0, in 0.5 increment steps, then to 3.8. Incubation temperature was of 28°C and the pH was adjusted using sulfuric acid (50% w/v). Adjustment of the pH was performed once stable $OD_{600}$ was reached. Before each pH adjustment, a sample was collected for quantification of lactic acid, acetic acid, butyric acid, and propionic acid by HPLC. Fermentation runs (means of 16 days) were repeated three times. One-way ANOVA were performed with pH as the independent variable.

**Results and Discussion** The response to change in pH was dependent on the main C source. With glucose, it was possible to maintain growth for strains ATCC 25755 and E401 from a pH of 6.0 down to 3.8 (Table 1). The other two strains had a low growth at pH below 4.5. With lactate as the main C source, the growth rate significantly dropped starting at pH 4.0 for all four strains. As of the metabolic results with glucose as the main C source, ATCC 25755 production of n-butyrate was similar at all pH level (Table 1), while iso-butyric acid peaked at the pH of 5.0, with lowest production at pH 6.0. Production of propionate increased at the lower pH for ATCC 25755. The main difference for n-butyric and iso-butyric acid, was that the relation between the pH and butyrate production was strain-dependent. With glucose, synthesis of both butyrate isoforms by E401 and E502 (not shown) strains started at pH 5.0 and increased as the pH decreased. For those two strains, the production of butyrate under glucose as main C source was lower than for ATCC 25755. For strain E602 (not shown), synthesis of both butyrate isoforms was similar to the level observed by *Cl. tyrobucyricum* ATCC 25755.

With lactate as the main C source, the tolerance to pH varied between strains (Table 2). The ATCC 25755 and E401 growth was lower at pH below 4.5. For E502 and E602, that drop-in growth was observed at pH below 5.0 (not shown). Lactate was readily consumed by the clostridia at the higher

pH, as no residual lactate was detected in the medium. As growth rate decreased as pH was lowered, catabolism of lactate was reduced, and iso-butyric acid production dropped. Similar to the results with glucose, peak production of butyrate was observed at pH around 5.0. A link with cell growth rate is possible, and since acetate (from the culture media) was also highest at pH of 3.8, the cells were possibly not active. For lactate as main C source, butyrate production was similar between *Clostridium* species. The results from the fermenters using xylose as main C source were similar to glucose.

**Table 1**. Glucose fermentation parameters of *Clostridium tyrobutyricum* ATCC 25755 and *Clostridium beijerenckii* E401.

| | pH | | | | | | *P* |
|---|---|---|---|---|---|---|---|
| Organic acids (mg/g) | 6.0 | 5.5 | 5.0 | 4.5 | 4.0 | 3.8 | |
| ATCC 25755 | | | | | | | |
| Acetic acid | 7.68 | 6.75 | 5.46 | 5.59 | 6.02 | 6.17 | 0.150 |
| Propionic acid | 7.34 $^{b}$ | 7.53 $^{b}$ | 7.89 $^{b}$ | 7.78 $^{b}$ | 8.12 $^{a}$ | 8.60 $^{a}$ | 0.025 * |
| n-butyric acid | 1.15 | 0.62 | 0.51 | 0.58 | 0.65 | 0.66 | 0.245 |
| iso-butyric acid | 2.25 $^{c}$ | 5.37 $^{b}$ | 9.37 $^{a}$ | 8.07 $^{a}$ | 5.75 $^{b}$ | 4.65 $^{b}$ | 0.054 |
| E401 | | | | | | | |
| Acetic acid | 7.86 | 8.01 | 7.38 | 9.13 | 9.06 | 8.85 | 0.091 |
| Propionic acid | 11.79 | 10.21 | 7.59 | 8.03 | 8.88 | 8.84 | 0.144 |
| n-butyric acid | 0.00 $^{d}$ | 0.00 $^{d}$ | 0.64 $^{c}$ | 0.87 $^{b}$ | 0.74 $^{b}$ | 1.73 $^{a}$ | 0.018 * |
| iso-butyric acid | 0.00 $^{d}$ | 0.00 $^{d}$ | 0.64 $^{c}$ | 0.98 $^{b}$ | 1.08 $^{b}$ | 1.27 $^{a}$ | 0.001 ** |

**Table 2**. Lactate fermentation parameters of *Clostridium tyrobutyricum* ATCC 25755 and *Clostridium beijerenckii* E401.

| | pH | | | | | | *P* |
|---|---|---|---|---|---|---|---|
| Organic acids (mg/g) | 6.0 | 5.5 | 5.0 | 4.5 | 4.0 | 3.8 | |
| ATCC 25755 | | | | | | | |
| Lactic acid | 0.00 $^{c}$ | 0.00 $^{c}$ | 0.00 $^{c}$ | 0.00 $^{c}$ | 2.25 $^{b}$ | 4.42 $^{a}$ | 0.037 * |
| Acetic acid | 2.42 | 2.34 | 1.76 | 1.77 | 2.67 | 3.29 | 0.223 |
| iso-butyric acid | 4.36 $^{a}$ | 4.25 $^{a}$ | 4.77 $^{a}$ | 4.97 $^{a}$ | 3.29 $^{b}$ | 1.71 $^{c}$ | 0.029 * |
| E401 | | | | | | | |
| Lactic acid | 0.86 | 2.66 | 0.00 | 0.00 | 2.25 | 4.61 | 0.133 |
| Acetic acid | 3.00 | 3.53 | 2.49 | 2.12 | 2.67 | 3.37 | 0.970 |
| iso-butyric acid | 4.16 | 3.81 | 5.33 | 4.79 | 3.29 | 1.64 | 0.096 |

**Conclusion** Quantification of metabolic efficiency from microorganisms is difficult to measure in situ. A chemostat trial was set up to test growth and fermentation profile of different clostridial strains isolated from silage. We observed that the tested strains were able to grow under the selected conditions down to pH of 3.8 and that n-butyric and iso-butyric acids production was pH-dependent, with peak production around pH of 5.0. Synthesis level was also dependent on the strain and important variations were observed. Thus, butyric acid could not always be used as a criterion for presence of butyric spores in silage, but these observations were linked to the specie and the substrate more than on pH level. Different between strains were observed previously (Ruusunen et al. 2012), and this could play an important part of our results.

**References**

Ruusunen, M., Surakka, A., Korkeala, H., & Lindström, M. (2012) *Clostridium tyrobutyricum* strains show wide variation in growth at different NaCl, pH, and temperature conditions. Journal of Food Protection, 75, 1791-1795.

Thylin I., Schulsky, P., Lindgren, S., & Gottschal, J. C (1995) Influence of pH and lactic acid concentration on *Clostridium tyrobutyricum* during continuous growth in a pH-auxostat. Journal of Applied Bacteriology, 79, 663-670.

Zhu Y., & Yang, S.-T. (2004) Effect of pH on metabolic pathway shift in fermentation of xylose by Clostridium tyrobutyricum. Journal of Biotechnology, 110, 143-157.

# *In vitro* screening of technical lignins for their antifungal activity against three molds and one yeast isolated from spoiled forage

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**Keywords:** mold, yeast, spoilage, technical lignins.

**Introduction** Several technical lignin types have well-recognized antimicrobial properties against bacteria and fungi (Baurhoo et al. 2008). The objective of this study was to evaluate a set of technical lignins for their antifungal activity *in vitro*, against fungi isolated from spoiled hay and identify potential candidate lignins that could be further tested as hay preservatives. We hypothesize that technical lignins will have different antifungal activities, depending on their chemical properties, against fungi causing forage spoilage.

**Material and Methods** For experiment 1, treatments (TRT) were 1) LignoBoost Kraft lignin (UW), 2) TRT 1 acetone insoluble fraction (AI), 3) TRT 1 acetone soluble fraction 1 (Hexane insoluble; HEX), 4) TRT 1 acetone soluble fraction 2 (Hexane soluble; PI), 5) alkali Kraft lignin (AKL), 6) sodium lignosulfonate (NaL), 7) magnesium lignosulfonate (MgL), 8) ammonium lignosulfonate (AMOL), 9) propionic acid (positive control; PRP), and 10) Control (untreated). The antifungal activity of treatments (40 mg/ml) was determined using the poisoned food technique (Balouiri, et al. 2016). *Mucor circinelloides*, *Penicillium solitum*, *Aspergillus amoenus*, and *Debaryomyces hansenii* were isolated from spoiled forage and identified. For experiment 2, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of selected technical lignins from experiment 1 and PRP were determined by the broth macrodilution assay (NCCLS, 2002a, b) against the above mentioned fungi at pH 4 and 6.

**Table 1.** Minimal inhibitory concentration (MIC, mg/ml) and minimal fungicidal concentration (MFC, mg/ml) of treatments against fungi isolated from spoiled hay as a function of media pH.[1]

| | | *A. amoenus* | | *P. solitum* | | *M. circinelloides* | | *D. hansenii* | |
|---|---|---|---|---|---|---|---|---|---|
| TRT | pH | MIC | MFC | MIC | MFC | MIC | MFC | MIC | MFC |
| NaL | 4 | 20.0 ± 0[2] | 40.0 ± 0 | 33.3 ± 5.77 | 60.0 ± 0 | 25.0 ± 0 | > 60 | 40.0 ± 0 | 40.0 ± 0 |
| | 6 | >60 | n.c.[3] | >60 | n.c. | > 60 | n.c. | > 60 | n.c. |
| MgL | 4 | 33.3 ± 5.77 | 40.0 ± 0 | 46.7 ± 5.77 | > 60 | 36.7 ± 5.77 | >60 | 26.7 ± 2.89 | 30.0 ± 0 |
| | 6 | > 60 | n.c. | > 60 | n.c. | > 60 | n.c. | > 60 | n.c. |
| AKL | 4 | > 60 | n.c. | > 60 | n.c. | > 60 | n.c. | > 60 | n.c. |
| | 6 | > 60 | n.c. | > 60 | n.c. | > 60 | n.c. | > 60 | n.c. |
| PRP | 4 | 1.25 ± 0 | 5.0 ± 0 | 1.25 ± 0 | 10.0 ± 0 | 3.33 ± 1.44 | 20.0 ± 0 | 1.25 ± 0 | 5.0 ± 0 |
| | 6 | 5.0 ± 0 | 10.0 ± 0 | 5.0 ± 0 | 16.7 ± 5.77 | 10.0 | 40.0 ± 0 | 4.17 ± 1.44 | 15.0 ± 0 |

[1] *Aspergillus amoenus*, *Penicillium solitum*, *Mucor circinelloides* (molds), and *Debaryomyces hansenii* (yeast). NaL= Sodium lignosulphonate, MgL= Magnesium lignosulphonate, AKL= Alkali Kraft lignin, PRP= Propionic Acid (positive control).
[2] Mean ± standard deviation.
[3] Not calculated.

The MIC was defined as the lowest concentration of TRT that prevents visible growth and the MFC was defined as the lowest concentration of TRT that decreases 99.9% of the initial fungal concentration.

Experiment 1 had a complete randomized block design (CRBD, 4 runs) and a factorial arrangement of 3 molds (MLD) × 10 TRT. A CRBD also was used to test for the yeast (*D. hansenii*). Data were analyzed with PROC GLM (SAS 9.4), when an interaction was present the SLICE option was used. TRT were considered different when $P \leq 0.05$. For experiment 2, MIC and MFC assays were carried out independently three times in duplicate and values are reported as mean concentrations (mg/ml ± standard deviation; SD).

**Results** In experiment 1, we found an interaction effect of MLD × TRT on antifungal activity ($P < 0.001$). For *A. amoenus* we observed 100 ± 2.77% antifungal activity for PRP and NaL, followed by MgL (40.9%) and to a lesser extent AKL (12.1%, $P \leq 0.05$). Inhibition by other TRT was < 5%. For *M. circinelloides*, the most effective TRT were PRP and NaL (100 ± 2.77%), followed by MgL (73%) and to a lesser extent AKL (49.7%; $P \leq 0.05$). Inhibition by other TRT was < 10%. For *P. solitum*, the most effective TRT were PRP and NaL (100 ± 2.77%), followed by MgL, (28.1%), and PI (8.1%; $P \leq 0.05$). Inhibition by other TRT was < 1%. For NaL, PRP, and HEX there were not significant differences across MLD. However, for MgL, different antifungal activities were observed across *M. circinelloides, A. amoenus,* and *P. solitum* (72.9, 40.9, and 28.1 ± 2.77%, respectively; $P \leq 0.05$) and a similar trend was observed for AKL (49.7, 12.1, and -8.0 ± 2.77 %, respectively; $P \leq 0.05$). Overall, *M. circinelloides* was the most sensitive mold, followed by *A. amoenus,* and *P. solitum*. For *D. hansenii*, we identified PRP, NaL, and MgL as the most effective TRT with 100 ± 3% antifungal activity, followed by AKL, AMOL, PI, and UW (9.8, 9.9, 10.6, and 8.4% respectively; $P \leq 0.05$). Inhibition by other TRT was < 4%. In experiment 2, we observed that among technical lignins at pH 4, NaL had the lowest MIC across molds, with values ranging from 20 for *A. amoenus* to 33.3 mg/ml for *P. Solitum* (Table 1). For *D. hansenii*, we identified MgL as the TRT with smallest MIC (26.7 mg/ml). Under the conditions tested in this study, we did not observe inhibition activity for AKL nor for any of the lignosulfonates at a pH of 6. At pH 4, NaL and MgL had similar fungicidal activity against *A. amoenus* (40 mg/ml, MFC). However, NaL had a higher fungicidal activity against *P. solitum* and lower for *D. hansenii* when compared to MgL. Overall, technical lignins had lower antifungal activity compared to PRP. A limited number of studies have evaluated the antifungal activity of technical lignins. For sodium lignosulfonate, MIC values ranging from 50 to 128 µg/ml against *Candida* spp. have been reported (Jha and Kumar 2018). Inhibition of *D. hansenii* by sodium and calcium-magnesium lignosulfonate was shown by Núñez-Flores et al. (2012), but no inhibition was observed for *A. niger*, and *P. expansum*. For Alkali kraft lignin, Dong et al. (2011) reported a MIC of 10 mg/mL for *Candida lipolytica*. Differences in responses and their extent can be partially explained by differences in methodologies and organisms used across studies. Among these factors, we believe that pH plays a major role in the extent of antifungal activity (Dong et al. 2011). Unfortunately, several of these studies did not report media pH values after the addition of technical lignins.

**Conclusion** NaL and MgL were found to be the technical lignins with the most antifungal activity when evaluated against fungi isolated from spoiled hay, but their activity was limited when compared to PRP. These treatments will be further evaluated using hay as a substrate.

**References**

Balouiri, M., Sadiki, M. & Ibnsouda, S.K. (2016) Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6, 71-79.

Dong, X., Dong, M., Lu, Y., Turley, A., Jin, T. & Wu, C. (2011) Antimicrobial and antioxidant activities of lignin from residue of corn stover to ethanol production. Industrial Crops and Products, 34, 1629-1634.

Jha, A. & Kumar, A. (2018) Deciphering the role of Sodium Lignosulfonate against Candida spp. As persuasive anticandidal agent. International Journal of Biological Macromolecules, 107, 1212-1219.

NCCLS, M27-A2. (2002a) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Second Edition. (Electronic Document), https://clsi.org/standards/products/microbiology/m27/. (Accessed 29/01/2018).

NCCLS, M38-A. (2002b) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard. (Electronic Document), https://clsi.org/standards/products/microbiology/documents/m38/. (Accessed 29/01/2018).

Núñez-Flores, R.,B. Giménez, F. Fernández-Martín, M. López-Caballero, M. Montero & Gómez-Guillé, M. (2012) Role of lignosulphonate in properties of fish gelatin films. Food hydrocolloids, 27, 60-71.

# Influence of storage period on the quality of a maize silage

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**Keywords:** feed-out, maize silage, microbiological quality, silage additives, storage period

**Introduction** On farms, sometimes maize is ensiled after harvest, and the feed-out of a maize silage begins immediately. Wichert et al. (1998) showed that silages with poor hygienic quality cause decreases in feed intake by about 10-20%. An experiment was conducted to investigate the influence of the storage period of a maize silage on fermentation quality, microbiological quality and aerobic stability.

**Material and Methods** Six 700-L containers were filled with maize at an average dry matter (DM) content of 37%. The density of the forage amounted to 171 +/- 10 kg DM/$m^3$. With three of the containers, the feed-out period started the day after ensiling; three other containers were sealed, and the feed-out phase started after a two-month storage period. The following three treatments were applied both to the silage removed immediately and to the silage fed out after the two-month storage period: (1) 5-cm layer fed out daily; (2) 10-cm layer fed out daily; and (3) 5-cm layer fed out daily, with the maize being treated with a silage additive (chemical product, active ingredient ammonium propionate, dosage 6 kg/t) at the time of ensiling. The maize silage removed daily was weighed, and on days 0, 1, 4, 7, 10, 14 and 18, samples were taken to analyse pH and microbiological parameters (yeasts and moulds) and aerobic stability. For the statistical analysis all microbial counts were log10-tranformed. Data were analysed using analysis of variance (Systat 13).

**Results and Discussion** In the silages whose feed-out period started the day after ensiling, the fermentation process began modestly, and the pH decreased slowly to 4.7 on average (Figure 1). On the other hand, after the two-month storage period, the lactic fermentation process in the silages was completed, and an average pH of 4.1 was reached. Between the feed-out periods the pH values were significant (P<0.01). Between the treatments the pH values showed a tendency (p=0.051).

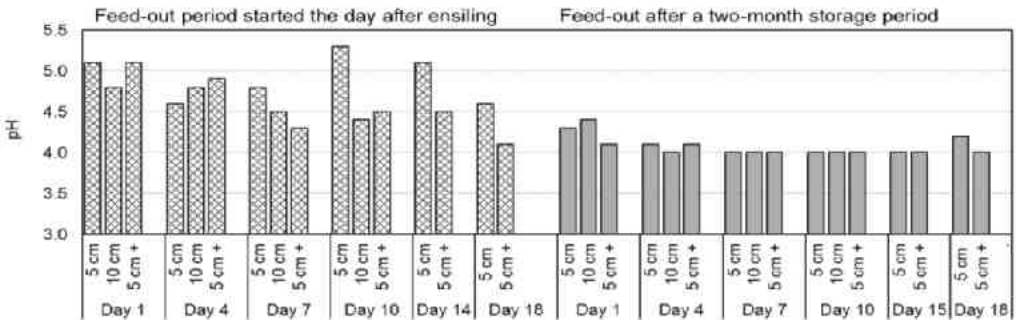


**Figure 1.** pH values in the maize silages.

In the silages whose feed-out period started immediately, the silages were characterised by high numbers of yeasts (Figure 2) and moulds (Figure 3). According to the VDLUFA quality assessment (VDLUFA 2012), many samples were classified as category 4, meaning they were spoiled. For the yeasts the feed-out periods (p<0.01) and the treatments (p<0.01) were significant. For the moulds only the feed-out periods were significant (p<0.001). In nearly all 700 L containers, where the feed-out period started the day after ensiling, the silages were already warm. The test of aerobic stability showed they were aerobically instable (Figure 5). This was also the case when the silage was treated with the additive. After the two-month storage period, the numbers of various microorganisms were in most cases within the normal range, and the silages had a better aerobic stability. On average the aerobic stability amounted 77 hours instead of 6 hours for the immediately feed-out (p<0.001). Both

the removal of a larger layer and treatment with a silage additive further improved the aerobic stability of the silages ($p<0.5$).

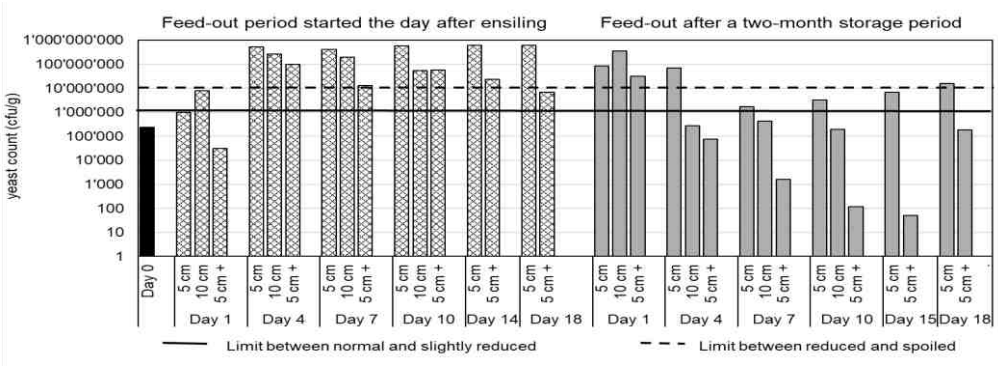


**Figure 2.** Yeast count in the maize silages (cfu: colony forming units).

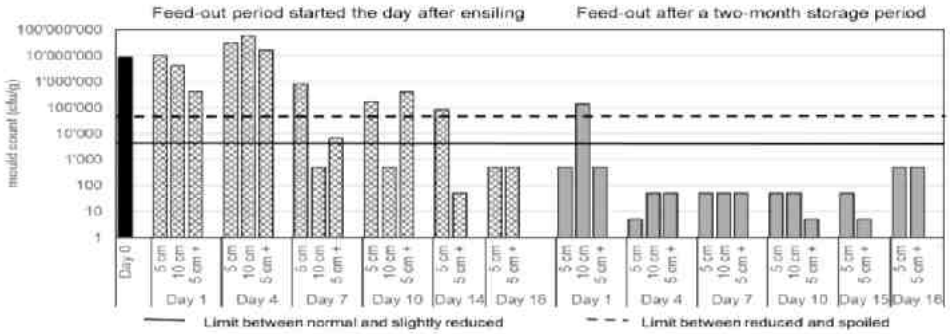


**Figure 3.** Moulds in the maize silages (cfu: colony forming units).

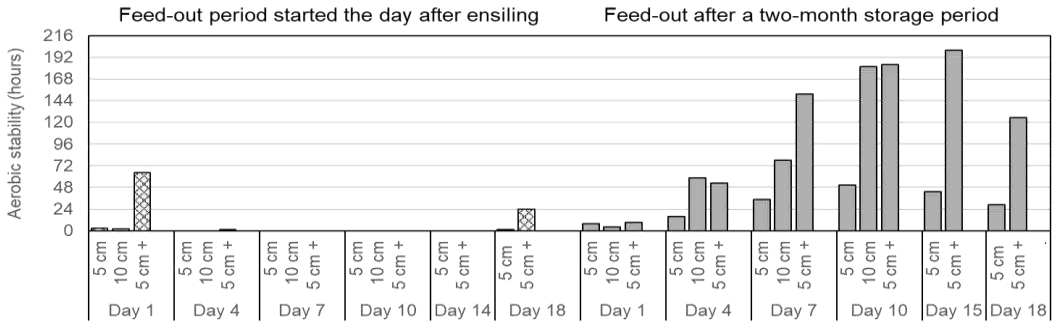


**Figure 4.** Aerobic stability of the maize silages.

**Conclusion** Due to the high numbers of yeasts and moulds, feeding out the maize silage immediately after ensiling cannot be recommended. After the two-month storage period, various microorganisms were in most cases within the normal range, and the silages had a better aerobic stability.

**References**

VDLUFA (2012) Keimgehalte an Bakterien, Hefen, Schimmel- und Schwärzepilzen. Methodenbuch III, Die chemische Untersuchung von Futtermitteln, 8. Ergänzungslieferung 2012.

Wichert, B., Kienzle, E. & Bauer, J. (1998) Palatability and intake of silage in dairy cows, in relation to hygienic quality. Journal of Animal Physiology and Animal Nutrition, 80, 253–259.

# Interaction between lactic acid bacteria and two species of the *Penicillium roqueforti* group: an *in vitro* and *in vivo* approach

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**Keywords:** heterofermentative homofermentative lactic acid bacteria, mycotoxins, *Penicillium roqueforti*, silage

**Introduction** *Penicillium roqueforti sensu stricto* (*s.s.*) and *P. paneum*, designated together as *P. roqueforti*, are the main toxigenic moulds contaminating silages in temperate climate regions (O'Brien et al. 2007, Samson & Frisvad 2004, Tangni et al. 2017). Spores of these moulds are present on forage crops prior to ensiling, as well as homo- and heterofermentative lactic acid bacteria (resp. HoLAB and HeLAB). The effect of LAB metabolites on *P. roqueforti* growth and roquefortine C (ROC) production was studied *in vitro* with corn infusion agar, and *in vivo* by means of a microsilo experiment.

**Material and Methods** For the *in vitro* experiment, corn infusion agar (CIA) was prepared from fresh whole-crop corn (Niderkorn 2007) and poured into 90-mm diameter Petri dishes. The inoculant 1188 (HoLAB) or 11A44 (HeLAB) was incorporated into the medium at $1x10^6$ cfu $ml^{-1}$ (Pioneer Hi-Bred, 1188 contains *L. plantarum* DSM 4784 ATCC 53187, 318 DSM 4785, 319 DSM 4786 and 346 DSM 4787 ATCC 55943, and *E. faecium* 301 DSM 4789ATCC 55593 and 202 DSM 4788 ATCC 53519; 11A44 contains *L. buchneri* LN4637). The LAB were allowed to proliferate anaerobically during seven days at 20 °C. Centrally in the medium, a five-mm plug was removed and 20 µl of spore solution of *P. roqueforti s.s.* MUCL 46746 (PR) or *P. paneum* CBS 112295 (PP) at $0.5x10^6$ spores $ml^{-1}$ was inserted, prior to the anaerobic incubation period or after (n=4). Mould growth was facilitated by aerobic incubation at 20 °C, and after seven days colony diameter was registered. For ROC determination, three nine-mm agar plugs were pooled, extracted according to Delmulle (2009) and quantified by LC-MS/MS (Monbaliu et al. 2010). From the remaining agar medium, a 1:10 (w/w) aqueous extract was made to determine pH, lactic acid and acetic acid (Ohmomo et al. 1993). To determine the acid production by the LAB inoculants, plates not inoculated with *P. roqueforti* were additionally analysed (n=4).

For the *in vivo* experiment, microsilos (2.75 L) equipped with a Bunsen valve were used (Wambacq et al. 2013). A mixture of perennial ryegrass and white clover (second cut) was mown, prewilted and chopped to 10-12 cm particles. At a dry matter (DM) content of 420 g $kg^{-1}$ fresh matter (FM), the mixture was ensiled with the following treatments (n=4): 1) negative control, 2-4) infected with PR: no additive – HoLAB – HeLAB, 5-7) PP with the same additives. At silo opening after 56 days of storage, *P. roqueforti* numbers (by dilution plating on Potato Dextrose Agar supplemented with 5 ml acetic acid $l^{-1}$ according to O'Brien et al. 2008), pH (on a 1:10 (w/w) aqueous extract), DM content (by air drying) and aerobic stability (Honig 1991) were determined. Statistical analysis was performed with the SPSS Statistics 24 program. Significance was declared at 95%. Normality and homoscedasticity were checked resp. by Shapiro-Wilk's test (with Bonferroni correction) and Levene's test. A multiple Anova was performed. In case of significant interaction between factors, an Anova analysis was performed for each level of one factor to assess the effect of the other factors. Normally distributed variables were subjected to an Anova with Tukey as *post hoc* test in case of homoscedasticity; otherwise a Welch Anova with Dunnett T3 *post hoc* was executed. Not normally distributed variables were subjected to a non-parametric Kruskal-Wallis test. In absence of significant interactions, the main effect of each factor was determined likewise over all levels of the other factors.

**Results and Discussion** The *in vitro* experiment revealed significantly stronger growth of PR compared to PP, while inoculant and incubation regime did not have a significant effect on fungal growth. Incubation regime did not significantly influence ROC production, while PR produced more ROC than PP, and HeLAB inoculation resulted in significantly higher ROC levels compared to HoLAB inoculation. The non-infected agar medium containing HoLAB contained not only lactic acid, but also some acetic acid after seven days of anaerobic incubation: *L. plantarum* can occasionally display heterofermentative metabolism, converting

certain pentose sugars into lactic acid, acetic acid and ethanol (Oude Elferink et al. 2000). Compared to non-infected agar, mould growth triggered a reduction of lactic acid as well as acetic acid in the medium, demonstrating metabolism by *P. roqueforti* of these acids.

From the *in vivo* experiment with microsilos can be concluded that HoLAB inoculation significantly lowered *P. roqueforti* numbers compared to non-inoculated silage, while HeLAB inoculation resulted in intermediate *P. roqueforti* counts. No clear explanation for this finding could be found in literature. Both inoculants significantly decreased the pH of PR-inoculated silage compared to no additive, with HoLAB inoculation resulting in a significantly lower pH than HeLAB inoculation. HoLAB inoculation significantly lowered the pH of PP-inoculated silage compared to no additive and HeLAB inoculation. The DM content at silo opening was significantly higher in case of inoculant application, while aerobic stability was not significantly influenced. Lactic acid and acetic acid contents, which might shed more light on these observations, were not determined.

**Conclusion** *P. roqueforti s.s.* and *P. paneum* are very well adapted to silage conditions (Samson and Frisvad 2004, Taniwaki et al. 2009). This can partly be attributed to their ability to metabolize lactic acid and acetic acid, as demonstrated in the *in vitro* experiment. The precise effect of lactic acid and acetic acid on ROC production by *P. roqueforti* cannot be unravelled from the current *in vitro* experiment, but acetic acid seemed to trigger ROC production. Still, it remains elusive whether or not HeLAB inoculants trigger ROC production by *P. roqueforti* in silages. The *in vivo* experiment with microsilos did not comprise ROC determination. Inoculation with HoLAB resulted in significantly lower *P. roqueforti* counts compared to no additive application, while HoLAB inoculants usually reduce aerobic stability due to increased yeast and mould growth in general (Oude Elferink et al. 2000).

**References**

Delmulle, B. (2009) Investigation of mycotoxin production in water-damaged mouldy interiors in connection with the sick building syndrome. PhD thesis, Ghent University, Belgium.

Honig, H. (1991) Reducing losses during storage and unloading of silage, in: Forage Conservation towards 2000. Volkenrode: Sonderheft 123. pp. 116-123.

Monbaliu, S., Van Poucke, C., Detavernier, C.I., Dumoulin, F., Van De Velde, M., Schoeters, E., Van Dyck, S., Averkieva, O., Van Peteghem, C. & De Saeger, S. (2010) Occurrence of mycotoxins in feed as analyzed by a multi-mycotoxin LC-MS/MS method. Journal of Agricultural and Food Chemistry, 58, 66-71.

Niderkorn, V. (2007) Activités de biotransformation et de séquestration des fusariotoxines chez les bactéries fermentaires pour la détoxification des ensilages de maïs. PhD thesis, Université Blaise-Pascal, France.

O'Brien, M., Egan, D., O'Kiely, P., Forristal, P.D. & Fuller, H.T. (2008) Morphological and molecular characterization of *Penicillium roqueforti* and *P. paneum* isolated from baled grass silage. Mycological Research, 112, 921-932.

O'Brien, M., O'Kiely, P., Forristal, P.D. & Fuller, H.T. (2007) Quantification and identification of fungal propagules in well-managed baled grass silage and in normal on-farm produced bales. Animal Feed Science and Technology, 132, 283-297.

Ohmomo, S., Tanaka, O. & Kitamoto, H. (1993) Analysis of organic acids in silage by high-performance liquid chromatography. Bulletin of the National Grassland Research Institute, 48, 51-56.

Oude Elferink, S.J., Driehuis, F. & Gottschal, J.C. (2000) Silage fermentation processes and their manipulation. FAO Plant Production and Protection Papers, 17-30.

Samson, R. & Frisvad, J. (2004) Studies in Mycology 49. Utrecht: Centraalbureau voor Schimmelcultures. 251 p.

Tangni, E., Wambacq, E., Bastiaanse, H., Haesaert, G., Pussemier, L., De Poorter, J., Foucart, G. & Van Hove, F. (2017) Survey of fungal diversity in silage supplied to cattle in Belgium over a two-year period. Journal of Animal Science Advances, 3, 598-612.

Taniwaki, M., Hocking, A, Pitt, J. & Fleet, G. (2009) Growth and mycotoxin production by food spoilage fungi under high carbon dioxide and low oxygen atmospheres. International Journal of Food Microbiology, 132, 100-108.

Wambacq, E., Latré, J.P. & Haesaert, G. (2013) The effect of *Lactobacillus buchneri* inoculation on the aerobic stability and fermentation characteristics of alfalfa-ryegrass, red clover and maize silage. Agricultural and Food Science, 22, 127-136.

# Isolation and identification of lactic acid bacteria from fermented juice of tropical crops in Thailand

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**Keywords:** fermented juice, lactic acid bacteria, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*

**Introduction** Silage is the most common preserved ruminant feed. Epiphytic Lactic Acid Bacteria (LAB), naturally presents on forage crops, is responsible for silage fermentation and also influence silage quality (Cai 1999). Species of the genus *Lactobacillus* are major components of the microbial flora in various types of forage crops and silages and they usually play an important role in lactic acid production and pH reduction during silage fermentation (Cai et al. 1998, Pang et al. 2011). An inoculation of LAB in the ensiling process has been recommended in order to make good quality of silage. Fermented juice of epiphytic lactic acid bacteria (FJLB) have been used as silage additive for improving the manipulating numbers of the epiphytic LAB (Bureenok et al. 2005). Moreover, the specificity between the forage species and its epiphytic microflora has been studied by isolation and identification of the main microorganism groups present in the forage used for silage. Therefore, the present study aimed to isolate and identify LAB from fermented juice of different kinds of tropical crops in Thailand by using physiological and biochemical tests.

**Material and Methods** The FJLB was individually prepared from napier grass, ruzi grass, guinea grass, stylo grass and leucaena before harvesting, and 25 g of grass was macerated with 50 ml of distilled water using a blender. This was filtered through sterilized double cheese cloths and the filtrate was added with 1% glucose (Bureenok et al. 2005). The FJLB was kept in an incubator at 30°C for 3 days. After that, LAB were isolated from each FJLB by spread on lactobacilli de Man, Rogosa, Sharpe (MRS) agar incubated at 35°C for 48 h under anaerobic conditions. LAB colonies were isolated and purified twice by streaking on MRS agar plates. Fifteen strains were selected due to high acidification activity (pH is lower than 4). Gram stain, colony morphology and catalase activity were determined according to the methods for LAB identification described by Kozaki et al. (1992). Morphology, physiology and biochemical tests of LAB morphology and Gram-staining response were examined after 24 h of incubation on MRS agar. The biochemical characterization and sugar fermentation pattern of the strains was tested using an API 50 CHL test kit (BioMérieux, Marcy PEtoile, France).

**Results and Discussion** A total of 15 strains were obtained from FJLB of napier grass, ruzi grass, guinea grass, stylo grass and leucaena. All isolates were Gram-positive and catalase-negative bacteria and able to have high acidification activity. All 15 strains could ferment D-glucose, D-galactose, L-Arabinose, Ribose, D-fructose, D-Trehalose and D-maltose (Table 1). Moreover, this analysis resulted in the delineation of three groups of isolates. Group I was classified as *Pediococcus pentosaceus*, representing strains R1, R4, R5, R8, R11 (ruzi grass) and L1 isolated from leucaena. LAB strains within group I were able to ferment xylose but not D-Lactose, D-Melibiose D-Saccharose. This charateristic could discriminate Group I from Group II and Group III. Group II was classified as *Lactobacillus plantarum* composed of strain N3 (napier grass), G4 (guinea grass), St1, St 2 and St3 (stylo grass). Group II LAB strains fermented $\alpha$-Methyl-D-mannopyranoside, D-Lactose separating this group from others. Group III was classified as *Lactobacillus fermentum* and comprised strain N4 (napier grass) and strains G3, G6 and G7 (guinea grass). LAB within group III fermented D-Raffinose but not N-acetyl glucosamine which differentiated this group from others. The Group I and Group II showed 99.9% of similarity to *Pediococcus pentosaceus* and *Lactobacillus plantarum* in API 50CH database, respectively. In case of Group III, strain N4 and G3 showed 99.9% of similarity and G6, G7 showed 96.3% of similarity to *Lactobacillus fermentum* in API 50CH database (Table 2). However,

high level of phenotypic variability was observed among LAB species. This variability limits the usefulness of the phenotypic identification method API 50CH test. Therefore, further studies are required using genomic methods such as 16S rRNA sequencing in combination with phenotypic test for LAB identification.

**Table 1.** Results of API 50 CH tests of LAB strains isolated from FJLB of napier grass, ruzi grass, guinea grass, stylo grass and leucaena.

| Items | Group I | | | | | | Group II | | | | | Group III | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R1 | R4 | R5 | R8 | R11 | L1 | N3 | G4 | St1 | St2 | St3 | N4 | G3 | G6 | G7 |
| D-Xylose | + | + | + | + | + | + | - | - | - | - | - | - | - | - | - |
| D-Mannose | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - |
| D-Mannitol | - | - | - | - | - | - | + | + | + | + | + | - | + | - | - |
| D-Sorbitol | - | - | - | - | - | - | - | - | + | + | + | - | + | - | - |
| α-Methyl-D-mannopyranoside | - | - | - | - | - | - | + | + | + | + | + | - | + | - | - |
| *N*-acetyl glucosamine | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - |
| Amygdaline | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - |
| Arbutine | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - |
| Salicine | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - |
| D-Cellobiose | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - |
| D-Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D-Lactose | - | - | - | - | - | - | + | + | + | + | + | - | + | - | - |
| D-Melibiose | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + |
| D-Saccharose | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + |
| D-Trehalose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D-Melezitose | - | - | - | - | - | - | + | + | + | + | + | - | + | - | - |
| D-Raffinose | - | - | - | - | - | - | - | - | + | - | - | + | + | + | + |

-,Negative reaction; +, Positive reaction.

**Table 2.** Percentage of identity of LAB strains isolated from FJLB of napier grass, ruzi grass, guinea grass, stylo grass and leucaena, in API 50CH database

| Group | Strain | %identity | Closely related strain |
|---|---|---|---|
| I | R1, R4, R5, R8, R11 and L1 | 99.9% | *Pediococcus pentosaceus* |
| II | N3, G4, St1, St2 and St3 | 99.9% | *Lactobacillus plantarum* |
| III | N4 and G3 | 99.9% | *Lactobacillus fermentum* |
| | G6 and G7 | 96.3% | |

**Conclusion** Fifteen strains isolated from FJLB of napier grass, ruzi grass, guinea grass, stylo grass and leucaena were divided in three groups and identified as *Pediococcus pentosaceus, Lactobacillus plantarum* and *Lactobacillus fermentum* based on the results of API 50CH. There were differences between the LAB dominant species among FJLB from each grasses.

**References**

Bureenok, S., Namihira, T., Tamaki, M., Mizumachi, S., Kawamoto, Y. & Nakada, T. (2005) Fermentation Quality of Guineagrass Silage by Using Fermented Juice of the Epiphytic Lactic Acid Bacteria (FJLB) as a Silage Additive. Asian-Australasian Journal of Animal Science, 18 (6), 807-811.

Cai, Y. (1999) Identification and characterization of *Enterococcus* species isolated from forage crops and their influence on silage fermentation. Journal of Dairy Science, 82, 2466-2471.

Cai, Y., Benno, Y., Ogawa, M., Ohmomo, S., Kumai, S. & Nakase, T. (1998) Influence of *Lactobacillus* spp. from an inoculant and of *Weissella* and *Leuconostoc* spp. from forage crops on silage fermentation. Applied and Environmental Microbiology, 64, 2982-2987.

Kozaki, M., Uchimura, T. & Okada, S. (1992) Experimental Manual of Lactic Acid Bacteria. Asakurashoten, Tokyo, Japan.

Pang, H., Qin, F., Tan, Z., Li, Z., Wang, Y. & Cai, Y. (2011) Natural populations of lactic acid bacteria associated with silage fermentation as determined by phenotype, 16S ribosomal RNA and *recA* gene analysis. Systematic and Applied Microbiology, 34, 235-241.

# Isolation and identification of lactic acid bacteria in sorghum silage

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**Keywords:** fermentation, PCR, *Sorghum bicolor*, succession

**Introduction** Sorghum (*Sorghum bicolor* (L) Moench) is considered the fifth most important grain crop in the world. Sorghum silage is widely used as food source for ruminant livestock in many countries around the world. Sorghum silage, however, is susceptible to a high degree of alcohol fermentation by yeasts, leading to high rates of DM loss (Pinho et al., 2015). It is knows that the use of specific strains isolated from the same forage can improve the fermentation pattern and to obtain better quality silage (Muck, 2008). The microbial inoculants sold in Brazil comprise strains isolated from temperate climate crops, indicating that is necessary to know the silage microbiota under tropical conditions. The objective of this study was to isolate and identify lactic acid bacteria (LAB) in sorghum silage.

**Materials and Methods** Lactic acid bacteria (LAB) were isolated from samples of sorghum in different fermentation periods (0, 1, 3, 7, 14, 28, 56, and 90 days). The DNA isolated from plants and silages was extracted by using the Wizard® Genomic DNA Purification kit (Promega), with modifications. The 16S rRNA sequences were amplified by PCR using the primers p027F (GAGAGTTTGATCCTGGCTCAG) and 1492R (TACGG(C/T)TACCTTGTTACGACTT).

The sequences of each isolate were compared to those available in the GenBank database and were aligned by using the algorithm BLAST (Basic Local Alignment Search Tool) (http://www.ncbi.nlm.nih.gov/BLAST) for nucleotides. The 16S rRNA sequences that showed similarity equal or greater than 97% were considered as belong to the same Operational Taxonomic Unit.

**Results and Discussion** One hundred and ninety-four strains of LAB were identified based on sequence analyses of their 16S rDNA. There was a high diversity of species during the fermentation process (Figure 1). The most prevalent LAB species in the epiphytic microbiota of fresh forage sorghum were *Weissella cibaria and Enterococcus sulfureus*. *Lactobacillus plantarum* predominated during almost all fermentation periods, except for day 1.

The disappearence of genus *Weissela* from third day of fermentation unlike occured due the predominance of more acidic tolerant species such as *Lactobacillus spp* (Fusco et al., 2015; Fessard and Remize, 2017). The predominance of the main LAB species of sorghum silages, considering isolates in MRS agar for all fermentation period were *Lactobacillus plantarum* 53%, *Weissella cibaria* 15%, *Lactobacillus pentosus* 9%, *Lactobacillus farciminis* 3%, *Enterococcus sulfureus* 2%, *Lactobacillus casei* 2%, *and Lactobacillus pentosaceus* 2%.

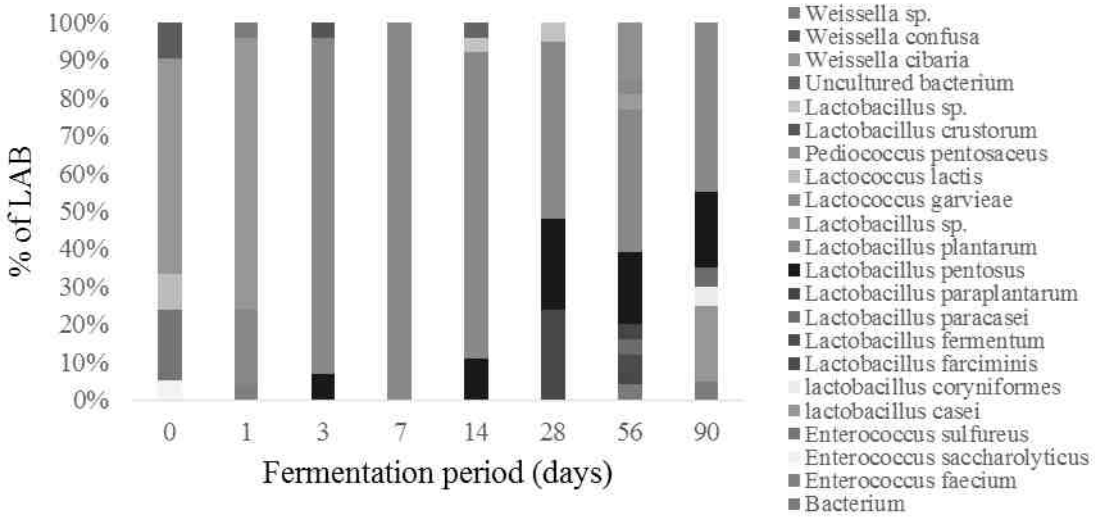


**Figure 1** Succession of lactic acid bacteria in plants and sorghum silage.

**Conclusions** The *Lactobacillus plantarum* was the predominant specie of LAB in sorghum silages produced in tropical conditions.

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**References**

Fessard, A. & Remize, F. (2017) Why Are *Weissella* spp. Not Used as Commercial Starter Cultures for Food Fermentation? Fermentation, 3, 38, doi:10.3390/fermentation3030038.

Fusco, V., Quero, G.M., Cho, G.S., Kabish, J., Meske, D., Neve, H., Bockelmann, W. & Franz, C.M.A.P. (2015) The genus Weissella: taxonomy, ecology and biotechnological potential. Frontiers in Microbiology, 6, 1-22.

Muck R.E. (2008) Advances in inoculants for silage. In: Pereira O.G., Obeid J.A., Fonseca D.M. and Nascimento Júnior D. (eds) Proceedings of 4th Symposium on Strategic Management of Pasture and 2nd International Symposium on Animal Production Under Grazing, Viçosa, MG, Brazil, 13-15 November 2008, pp. 221-232. Viçosa, Brazil: UFV Press.

Pinho, R.M.A., Santos, E.M., Oliveira, J.S., Freitas, P.M.D. & Perazzo, A.F. (2015) Sorghum cultivars of different purposes silage. Ciência Rural 45, 298-303.

# Modulation of bacterial community and metabolome in whole crop corn silage by inoculating homofermentative *Lactobacillus plantarum* and heterofermentative *Lactobacillus buchneri*

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**Keywords:** bacterial community, corn silage, metabolome, SMRT

**Introduction** Silage fermentation quality mainly depends on the microbial communities and their succession as well as fermentative metabolites during ensiling. Although metagenomic analysis by 454 high-throughput sequencing provided more comprehensive insight into the composition of the whole microbial community involved in ensiling, this technique is restricted to genus-level identification because only a partial sequence of the 16S rRNA gene is evaluated. A recent metagenomic approach, the PacBio single molecule, in conjunction with real-time sequencing technology (SMRT) can be used to reveal the bacterial profile of target samples at the species level because it can generate long sequence reads. LAB can produce a large number of metabolites during fermentation, such as amino acids, fatty acids, oligosaccharides, vitamins, and aromatic compounds. However many of the metabolites in silages have not been identified. Therefore, modulation of bacterial community and metabolome dynamics in corn silage by inoculating *Lactobacillus plantarum* and *Lactobacillus buchneri* were profiled in the present study.

**Material and Methods** Vacuumed 45 silos of corn (vacuum-sealing polyethylene plastic bags packed with approximately 300 g of fresh forage) were individually prepared for each of the following treatments: (a) untreated (control), (b) *L. plantarum*, and (c) *L. buchneri*. The silos were stored at ambient temperature (22–25°C) and sampled on days 3,7,14, 30, and 45. Fresh and ensiled corn silages were sampled for total bacteria DNA extraction. PCR amplification of the full-length 16S rRNA gene for SMRT sequencing was carried out using the forward primer 27F and reverse primer 1492R. Sequencing of the amplicons was performed on a PacBio Sequel instrument (Pacific Biosciences, Menlo Park, CA, USA). The metabolites were analyzed by chromatograph system coupled with a Pegasus 4D time-of-flight mass spectrometer (GC-TOF-MS).

**Results and discussion** Based on SMRT sequencing of the full-length 16S rRNA gene of silage bacteria, about 14 species of lactic acid bacteria were identified from the 45 samples. Compared with the control, samples treated with inoculations increased the abundances of *L. buchneri, L. paralimentarius* and *L. paraplantarum* but decreased the abundances of *L. farciminis, L. brevis* and unclassified *Rahnella* (Figure 1). Furthermore, inoculation of *L. plantarum* promoted growth of *L. hammesii* and unclassified *Lactobacillus* but inhibited growth of unclassified *Pediococcus*. However, the contrast results occurred in samples inoculated with *L. buchneri.* It is interesting that in samples inoculated with *L. buchneri* and *L. plantarum*, the two species were not dominant strains during ensiling, which might be due to the abundant epiphytic microbes in fresh corn as well as the two inoculated strains being less competitive during ensiling (Guo et al. 2018). Based on the GC-TOF-MS of 45 silage samples, a total of 608 substances were detected, and 259 different metabolites were identifed with their relative concentrations. According to principal component analysis (data not shown), clear differences with the fermentation process were observed between samples within treatments. Higher relative contents of ornithine, oxoproline, proline, methionine and phenylalanine were detected in the silage samples inoculated with *L. plantarum* compared to the control silages. The *L. buchneri* inoculated samples showed higher relative contents of serine, proline, methionine, isoleucine, phenylalanine and 4-aminobutyric acid but lower relative content of oxoproline than the control group (Table 1). In addition, some antibacterial substances were observed to be higher in *L. buchneri*

inoculated silages, such as homogentisic acid, 4-hydroxycinnamic acid, chlorogenic acid, neohesperidin and hesperitin, and higher relative contents of glutaraldehyde and 3-phenyllactic acid were detected in samples treated with *L. plantarum*. The results suggested that there are many other substances that have antibacterial function in silages besides acetic acid and 1,2-propanediol.

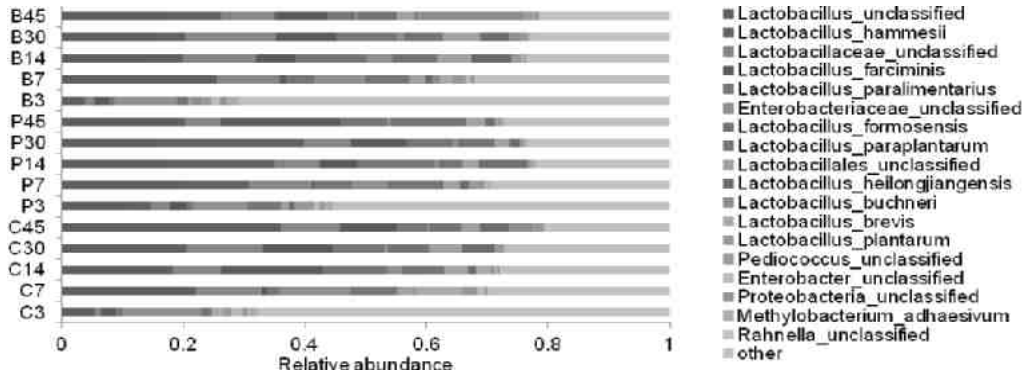


**Figure 1**. Relative abundances of the main silage bacterial species after fermentation (C, control; B, samples treated with *L. buchneri*; P, samples treated with *L. plantarum*; Silos were opened at 3, /, 14, 30 and 45 days after ensiling)

**Table 1**. Relative concentration[1] in partial of major metabolites in corn silages with inoculation of *L. plantarum* and *L. buchneri* after ensiling

| Metabolite name | Treatments and sampling days[2] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C3 | C7 | C14 | C30 | C45 | B3 | B7 | B14 | B30 | B45 | P3 | P7 | P14 | P30 | P45 |
| Ornithine | 0 | 0.310 | 0.266 | 0.816 | 1.436 | 0.061 | 0.054 | 0.689 | 1.015 | 1.134 | 0.304 | 0.546 | 1.476 | 1.522 | 0.907 |
| Oxoproline | 9.600 | 11.095 | 12.449 | 5.582 | 14.77 | 4.833 | 3.850 | 5.480 | 7.102 | 7.787 | 8.729 | 11.21 | 13.10 | 16.03 | 10.71 |
| Proline | 2.581 | 4.613 | 4.730 | 5.841 | 7.455 | 3.057 | 4.882 | 6.663 | 6.492 | 9.374 | 3.353 | 5.910 | 6.693 | 7.329 | 6.060 |
| Methionine | 0.234 | 0.644 | 0.762 | 1.084 | 1.445 | 0.242 | 0.524 | 1.048 | 1.107 | 1.575 | 0.304 | 0.795 | 1.041 | 1.203 | 1.139 |
| Isoleucine | 3.237 | 4.046 | 3.123 | 3.761 | 6.134 | 2.795 | 4.063 | 5.539 | 6.316 | 6.960 | 2.366 | 4.140 | 5.580 | 6.641 | 4.111 |
| Phenylalanine | 1.177 | 1.870 | 2.188 | 2.133 | 2.545 | 0.740 | 1.790 | 2.933 | 2.329 | 2.688 | 0.869 | 1.863 | 1.960 | 2.549 | 3.755 |
| 4-Aminobutyric acid | 0.507 | 0.446 | 0.468 | 0.721 | 0.739 | 0.803 | 1.030 | 1.295 | 1.370 | 1.329 | 0.412 | 0.465 | 0.512 | 0.584 | 0.590 |
| 4-Hydroxycinnamic acid | 0.077 | 0.171 | 0.074 | 0 | 0.105 | 0.317 | 0.285 | 0.349 | 0.318 | 0.410 | 0 | 0 | 0 | 0 | 0 |
| Chlorogenic acid | 0.003 | 0.003 | 0.004 | 0.005 | 0.006 | 0.004 | 0.004 | 0.006 | 0.004 | 0.001 | 0.006 | 0.006 | 0.008 | 0.005 | 0.003 |
| Neohesperidin | 0.006 | 0.004 | 0.002 | 0.001 | 0.001 | 0.007 | 0.004 | 0.004 | 0.003 | 0.002 | 0.002 | 0 | 0.001 | 0 | 0 |
| Hesperitin | 0 | 0 | 0.001 | 0.002 | 0.003 | 0.001 | 0.001 | 0.002 | 0.002 | 0.003 | 0 | 0.001 | 0.003 | 0.002 | 0.003 |
| Glutaraldehyde | 0.060 | 0.097 | 0.097 | 0.206 | 0.222 | 0.041 | 0.101 | 0.159 | 0.230 | 0.194 | 0.057 | 0.075 | 0.167 | 0.272 | 0.257 |
| 3-Phenyllactic acid | 0.615 | 0.759 | 0.740 | 0.858 | 0.901 | 0.591 | 0.737 | 0.889 | 0.989 | 0.990 | 0.769 | 0.891 | 1.164 | 1.144 | 0.928 |

[1]The relative concentration of each metabolite is an average of data from three biological replicates using GC-TOF-MS; [2]C, control; B, samples treated with *L. buchneri*; P, samples treated with *L. plantarum*; Silos were opened at 3, 7, 14, 30 and 45 days after ensiling.

**Conclusion** Inoculation of *L. plantarum* and *L. buchneri* altered the microbial composition in ensiled corn silage in very different ways, but the two strains did not occupy the bactierial community as dominant species during silage fermentation process. Inoculation of *L. plantarum* and *L. buchneri* also changed the fermentative metabolite dynamics of the ensiled corn in different ways, and some new metabolites with biofunctions were discovered in corn silage inoculated with *L. plantarum* or *L. buchneri*.

**References**

Guo, X.S., Ke, W.C., Ding, W.R., Ding, L.M., Xu, D.M., Wang, W.W., & Yang, F.Y. (2018). Profiling of metabolome and bacterial community dynamics in ensiled *Medicago sativa* inoculated without or with *Lactobacillus plantarum* or *Lactobacillus buchneri*. Scientific reports, 8, 357.

# Recovery and PCR-based characterization of *Listeria* strains from total mixed ration and maize silages with different silo management practices

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**Keywords:** aerobic deterioration, *Listeria spp*., maize silage, silo management

**Introduction** The penetration of air into a silage stimulates aerobic bacteria, yeasts and moulds, and causes aerobic deterioration. This aerobic activity results in dry matter (DM) and nutrient losses, the accumulation of pathogens and mycotoxins, and reduced DM intake (Borreani et al. 2018). Among pathogens, *Listeria monocytogenes* is a potentially dangerous foodborne pathogen, which represents a primary concern for the production of Gorgonzola, an Italian Protected Designation of Origin (PDO) blue-veined cheese produced only in the Piedmont and Lombardy regions. The aim of this research was to investigate the occurrence of *Listeria spp*. strains in maize silage fed to cows that produce milk destined for Gorgonzola production, and to characterize the isolated strains by PCR-based method.

**Material and Methods** A survey was carried out over 4 years in the western Po plain (Novara, Italy) on 18 dairy farms (Italian Friesian cows) that supply milk to a Gorgonzola producing plant. Each farm was visited seven times, and one maize silage (bunker or pile open for feed-out) was examined in detail on each farm (for a total of 120 maize silage silos). Total mixed ration (TMR) samples were collected at the same time as the visit (n = 117). A questionnaire was completed, on each studied farm, with information on the silage-making process, silo covering, silo management and dairy ration composition. Each silo face was examined in detail and silage samples were collected in central (CORE, n = 120) and peripheral (PERIPHERAL, n = 151) zones of the silo, as reported by Borreani and Tabacco (2010). The ISO 11290-1:1996/Amd 1:2004 (2004) method was applied to all collected samples for the isolation of *Listeria spp*. and PCR was adopted to identify *L. monocytogenes*. The other *Listeria* species were identified after 16S rRNA gene sequencing. All silage samples were analysed for DM content, pH, fermentative profile and microbial counts.

**Results and Discussion** Almost all maize silos had at least one visible spoiled area. The 16S rRNA sequencing resulted in all *Listeria spp*. being classified as *L. innocua*. On 10 farms out of 18, at least one strain of *L. monocytogenes* was isolated from maize silage or TMR, whereas at least one strain of *L. innocua* was isolated from all the surveyed farms. *L. innocua* was detected in 82 and *L. monocytogenes* in 8 out of 271 maize silage samples, respectively, and 91% of the positive samples were collected in peripheral zones or in a part of the silo where spoiling silage was visible (Figure 1). The silage sample collected in the peripheral areas of the silo that were positive to *Listeria spp*. had lower DM, and higher pH, yeast and mould counts than non-contaminated peripheral samples (Table 1). Six maize silage samples from the visible spoiled area were positive to both *L. monocytogenes* and *L. innocua*. More than half of the TMR samples (52%) were contaminated by *L. innocua*, whereas *L. monocytogenes* was detected in 11 out of 117 TMR samples. The contaminated TMR samples had a higher mould count than non-contaminated samples (Table 1). A total of 286 and 46 strains of *L. innocua* and *L. monocytogenes* were PCR-typed, respectively. Non-pathogenic *L. innocua* isolates clustered all at 35% similarity level, thus showing a great diversity. However, 94% of the isolates (n=268) clustered at a 70.9% similarity level, thus indicating a degree of relatedness which may be explained by considering an adaptation to the environmental conditions of the farm. This large cluster gathered strains belonging to TMR and maize silage collected over four different years. In 7 farms out of 18, there were isolates from TMR clustering with those isolated from maize silage at the same sampling date, with values of similarity ranging from 81% up to 94.4%, thus probably indicating a contamination pathway. *Listeria monocytogenes* strains were all clustered at 39.7%, with a less extent of variability than the other species, however the populations were considered quite heterogeneous, considering that all isolates belonged to the same species. However, 87% of the isolates (n=40) grouped at 69.7%, thus also indicating a degree of selection based on the farm environment. On 4

farms, the similarity of TMR and maize silage isolates ranged from 75.9% to 87.6%, probably indicating a route of transmission (Figure 2). The higher proportion of TMR positive to *L. monocytogenes* than silage from the peripheral area of the silo could indicate that some other sources of contamination were present on the farms, and these could have been found in not well preserved baled silage, as reported by Nucera et al. (2016).

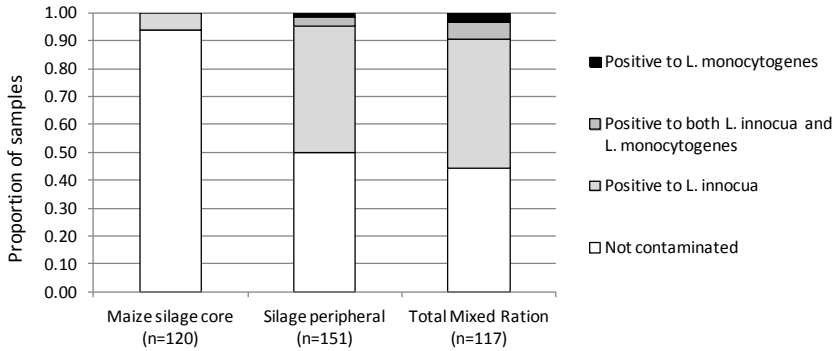


**Figure 1.** Proportion of samples not contaminated, or positive to *L. innocua*, to *L. monocytogenes* or both, in different zones of maize silage and in total mixed ration, on 18 dairy farms in Novara, Italy.

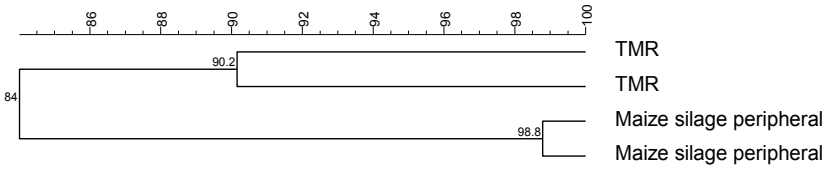


**Figure 2.** Example of cluster analysis of *L. monocytogene* strains isolated from samples collected in the peripheral area of the silages and TMR on one farm at the same sampling date.

**Table 1.** Characteristics of maize silage samples collected in the peripheral areas and TMR that were contaminated or not by *Listeria spp*. in the 4-year survey in Novara, Italy.

| Variables | Maize silage peripheral (n=151) | | *P value* | TMR (n=117) | | *P value* |
|---|---|---|---|---|---|---|
| | Not contaminated | Contaminated | | Not contaminated | Contaminated | |
| DM content (g kg$^{-1}$) | 31.9 | 28.0 | *** | 52.9 | 51.2 | NS |
| pH | 4.44 | 5.84 | *** | 4.90 | 5.03 | NS |
| Yeast (log cfu g$^{-1}$) | 4.48 | 5.96 | *** | 6.04 | 5.98 | NS |
| Moulds (log cfu g$^{-1}$) | 3.79 | 6.22 | *** | 3.87 | 4.47 | * |

**Conclusion** Results of this survey show that spoiled maize silage from the peripheral area of a silo could be one of the most relevant sources of the direct contamination of *L. innocua* and *L. monocytogenes* for TMR fed to dairy cows, even though some other farm sources could also be present.

**References**

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes B.J. & Muck R.E. (2018) Factors affecting dry matter and quality losses in silages. Journal of Dairy Science, 101, in press.

Borreani, G. & Tabacco, E. (2010). The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science, 93, 2620-2629.

Nucera D.M., Grassi A., Morra P., Piano S., Tabacco E. & Borreani G. (2016). Detection, identification and typing of *Listeria* species from baled silages fed to dairy cows. Journal of Dairy Science, 99, 6121-6133.

# Selection of heterofermentative lactic acid bacteria in sugarcane silages

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**Keywords:** acetic acid, ethanol, lactic acid, 16S rRNA

**Introduction** Sugarcane is widely used as supplemental winter forage in Brazil and other tropical countries. However, this culture has limitations in silage-making due to its high water soluble carbohydrate content and high population of epiphytic yeasts that favor alcoholic fermentation, which can cause aerobic deterioration of the silage, thereby reducing its nutritional value after silo opening. Calcium oxide (CaO) has been used as a silage additive resulting in a reduction of yeasts and ethanol production and improved DM recovery. It can be inferred that CaO selects more competitive strains of lactic acid bacteria (LAB) that can dominate the fermentation process. The use of specific LAB strains isolated from the same forage is a strategy that has been adopted by researchers with a view to obtaining better quality silage. The objective of this study was to isolate and identify strains of heterofermentative LAB with potential for use as microbial inoculant in sugarcane silage.

**Material and Methods** Five hundred grams of chopped sugarcane untreated (Control) or treated with 0.5 and 1% CaO were ensiled in bags followed by evacuation of air and heat-sealing. The bags were stored at room temperature and triplicate bags were opened after 1, 3, 7, 14, 28, 56 and 100 d of fermentation. Samples of silages (25 g) were mixed with 225 mL Ringer Solution and serial dilutions were prepared and plated on MRS agar for LAB count after 48h at 37ºC. After incubation, colonies were selected and inoculated on MRS agar containing 5 g/L $CaCO_3$ and 0.04 g/L bromocresol purple to evaluate their acid production. Isolates that were characterized as acid-positive and catalase-negative were inoculated on MRS broth and maintained at -80 °C for further analysis. Organic acid (lactic, acetic) and ethanol contents were evaluated by HPLC using the aqueous sugar cane extract obtained after cultivation of the LAB isolates at 37 °C for 48 h. The aqueous extract of sugarcane was prepared by blending fresh sugarcane and distilled water at the ratio of 5:1 for five minutes. The extract was filtered through layers of cheesecloth and sterilized at 121 $^{o}$C for 15 min. The LAB isolates that produced higher concentrations of acetic acid (AA) than lactic acid (LA) and did not produce ethanol (class 3) or produced it at concentrations < 6.0 mmol/L were selected and further identified by 16S rRNA gene sequencing. The DNA was extracted by using the Wizard® Genomic DNA Purification kit (Promega), with modifications. The product of the PCR, one fragment of approximately 1500 pb, was sent to Macrogen, Korea, for purification and sequencing. Sequences were compared with the GenBank database using the BLAST algorithm (National Center for Biotechnology Information, Maryland, USA).

**Results** A total of 648 strains were isolated, consisting of 207 from control silage and 223 and 218 from the silages treated with 0.5% and 1% CaO, respectively. Based on the analysis of organic acids in aqueous-extract of sugarcane, the LAB isolates were classified into three distinct groups of interest: A) those which produced an AA concentration higher than that of LA and did not produce ethanol; B) those which produced higher concentrations of AA than LA and the ethanol <6.0 mmol/L; and C) isolates that produced close value concentrations of AA, LA and ethanol (standard deviation of 0.9). A higher number of isolates (n = 34) with a desirable acid production profile was observed on day 7 of fermentation. No isolate was selected from silage stored for 100 days (Table 1). Of the 83 isolates selected, the sequencing of the 16S rRNA gene enabled the identification of 79 LAB with similarity equal to or greater than 97% of the available sequences on GenBank. The main LAB species found were *Leuconostoc mesenteroides* (68.68%), *Lactobacillus plantarum* (20.48%), *Weissella confusa* (2.42%), *Weissella cibaria* (1.20%), *Leuconostoc citreum* (1.20%), *Lactobacillus* sp. (1.20%) and unidentified bacteria (4.82%). The species *L. mesenteroides* (89.47%) prevailed in silage treated with

CaO. *Leuconostoc mesenteroides* was found on all days of fermentation, except on day 14, when *L. plantarum* was predominant (Figure 1).

**Table 1**. Number of heterofermentative LAB isolates grown in aqueous extract of sugarcane for the different organic acid production profile groups.

| Day of fermentation | Group | | |
|---|---|---|---|
| | A | B | C |
| 0 | 1 | 1 | 1 |
| 1 | 7 | 15 | 2 |
| 3 | 0 | 9 | 1 |
| 7 | 17 | 14 | 3 |
| 14 | 5 | 4 | 0 |
| 28 | 2 | 0 | 0 |
| 56 | 1 | 0 | 0 |
| Total | 33 | 43 | 7 |

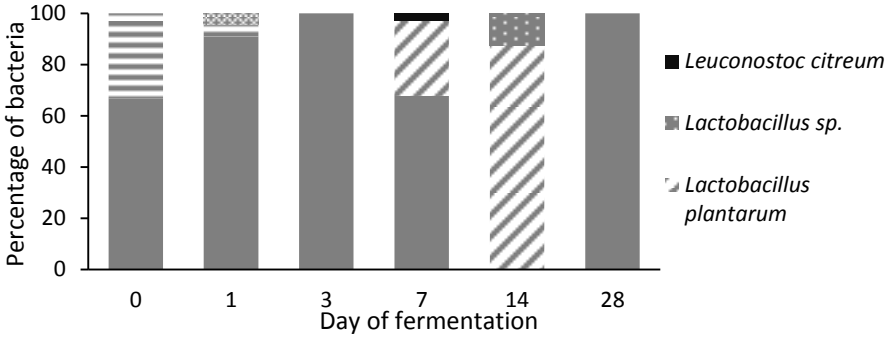


**Figure 1**. Succession of heterofermentative LAB in sugarcane silages as a function of fermentation period

**Discussion** Organic acid (lactic, acetic) production screening via HPLC is a technique largely used today for selecting LAB with potential traits for use as microbial inoculant in silages (Ávila et al. 2014).

Inoculants containing heterofermentative LAB that produce high concentrations of acetic inhibits yeast and mold growth, increasing aerobic stability of silages (Kung Jr. et al. 2003). The genus *Leuconostoc* belongs to the group of heterofermentative LAB and is usually found in plant material and dairy products. Some studies reported this genus as the dominant microbial population in forage crops and silage (Cai et al. 1994). *Lactobacillus plantarum* was identified as a predominant species for most crops, in addition to being one of the main LAB used in inoculants for silages. To continue the study, two LAB - *L. mesenteroides* 7.10T1 (control silage) and 7.19T3 (silage with 1% CaO) were selected to be tested as biological additives in future experiments with sugarcane silage to evaluate the fermentation profile and aerobic stability, because of their high production of acetic acid.

**Conclusion** *Leuconostoc mesenteroides* was the predominant species of heterofermentative LAB in sugarcane silages produced in tropical conditions, based on the production of organic acids.

**Acknowledgments**: This research was supported by the FAPEMIG, CNPq, and CAPES foundations, and INCT-CA.

**References**

Ávila, C.L.S., Carvalho, B.F., Pinto, J.C., Duarte, W.F. & Schwan, R.F. (2014) The use of *Lactobacillus* species as starter cultures for enhancing the quality of sugar cane silage. Journal Dairy Science. 97, 940-951.

Cai, Y., Ohmomo, S. & Kumai, S. (1994) Distribution and lactate fermentation characteristics of lactic acid bacteria on forage crops and grasses**.** Japanese Journal of Grassland Science. 39, 420-428.

Kung Jr., L., Stokes, M. R., Lin, C. J. 2003. Silage additives. In: Buxton, D. R.; Muck, R. E.; Harrison, J. H. (eds.) Silage science and technology. Wisconsin: ASA/CSSA/ SSSA, p. 305-360.

# Silage additives suppress fungal growth and mycotoxin formation in whole-crop rye silage exposed to air

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**Keywords:** fungi, mycotoxin, roquefortine C, silage, whole-crop rye

**Introduction** Whole-crop rye silage (WCR) plays an important role in ruminant nutrition due to its high dry matter (DM) yield and the opportunity to grow corn after its harvest, thereby enabling farmers to produce a high yearly DM output per hectare. As published data on fungal contamination, aerobic stability (ASTA) of and mycotoxin formation in WCR silage is scarce, this study aimed at filling this gap by testing the effects of different additive types on selected silage quality traits.

**Material and Methods** The crop was harvested before ear emergence on a German dairy farm, wilted overnight to 25% DM and chopped to about 30 mm particle size. After treatment with either tap water (CON), a dual-purpose inoculant (LAB), containing *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673, or with 2 L $t^{-1}$ of a chemical additive (CHEM), which contained sodium nitrite (195 g $L^{-1}$), hexamethylene tetramine (71 g $L^{-1}$) and potassium sorbate (106 g $L^{-1}$), the forage was transferred into 1.5-L glass jars (packing density: 87 kg DM $m^{-3}$). Jar body and lid had a 6-mm hole closed by rubber stoppers, which were removed on storage day 28 and 57 to allow air ingress for 24 hours. Silages (n=3 per treatment) were stored for 64 days at 22 °C. Fungal counts were analysed by ISO 21527, and ASTA was denoted by the hours that elapsed before a 2 °C increase in silage temperature above ambient. A multi-species method was employed for mycotoxin detection (Lopez et al. 2016). Data were analysed using the MIXED (non-parametric ANOVAF-option) and REG procedures of SAS, 9.4. Fungal counts were $\log_{10}$-transformed and values below the detection limit were set at log 1.7 colony-forming units (cfu) $g^{-1}$. Significance was declared at $P<0.05$.

**Results and Discussion** Untreated WRC silage showed visible signs of moulding already before silo opening, which was likely caused by induced air ingress one week before then end of storage. This visual observation was confirmed by higher counts of yeasts, moulds and total fungi than any treated silages (table 1), in which all fungal counts were below the limit of detection regardless of the applied additive type. As a consequence, untreated silages had very poor ASTA of less than three days, whereas additive treatment maintained ASTA over the entire period of aeration of 10 days. Auerbach et al. (2013) determined higher ASTA in untreated WCR silage of almost 5 days, but this treatment contained antimycotic butyric acid (0.15% of DM). The counts of yeasts and moulds were highly correlated ($y=-0.091+1.085x$, $R^2=0.89$, $P<0.0001$). Strong negative relationships existed between the counts of yeasts ($y=570.3x^{-1.666}$, $R^2=0.92$, $P<0.0001$), moulds ($y=530.0x^{-1.515}$, $R^2=0.97$, $P<0.0001$) and total fungi ($y=512.4x^{-1.436}$, $R^2=0.99$, $P<0.0001$), and ASTA, respectively. These observations support results by Auerbach et al. (2013) who found a highly significant, negative linear relationship between the count of lactate-assimilating yeasts and ASTA. Neither of the 46 detectable mycotoxins, including those produced by *Fusarium* species, were found in fresh WCR. This may be explained by the early forage harvest date before ear emergence. Roquefortine C, which can be produced by all three representatives of the *Penicillium roqueforti*-group and other species, e. g. *Penicillium expansum* (Boysen et al. 2000), was the only mycotoxin detected in WRC silage. This mycotoxin was found already at silo opening in untreated silage only, and its concentration increased to 85.2 mg $kg^{-1}$ DM during aeration, which was much higher than reported for maize and grass silage by Auerbach et al. (1998). Regardless of additive type, treated silage did not contain roquefortine C before air exposure, and its accumulation was largely restricted during aeration as reflected by concentrations never exceeding 0.05 mg $kg^{-1}$ DM (figure 1). The reasons why only roquefortine C was detected in our study although other mycotoxins of the *Penicillium roqueforti*-group have been found in silage (Schneweis et

al. 2000) and can be detected by the employed method, remain unclear. According to Müller & Amend (1997), some of those mycotoxins can react with silage constituents, such as ammonia and free amino acids, rendering the parent compounds undetectable. In addition, not all species of the *Penicillium roqueforti*-group have the capacity to produce all mycotoxins (Boysen et al. 2000).

**Table 1.** Effects of additives on fungal counts and aerobic stability of whole-crop rye silage (n=3)

| Parameter | CON[1] | | LAB[2] | | CHEM[3] | | *P* |
|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | level |
| Yeast count (log cfu $g^{-1}$) | $3.6^{b}$ | 0.39 | $<2.0^{a}$ | 0 | $<2.0^{a}$ | 0 | ** |
| Mould count (log cfu $g^{-1}$) | $4.0^{b}$ | 0.36 | $<2.0^{a}$ | 0 | $<2.0^{a}$ | 0 | ** |
| Total fungal count (log cfu $g^{-1}$) | $4.2^{b}$ | 0.29 | $<2.0^{a}$ | 0 | $<2.0^{a}$ | 0 | ** |
| Aerobic stability (hours) | $65^{a}$ | 3.7 | $240^{b}$ | 0 | $240^{b}$ | 0 | ** |

[1]untreated, [2]combination of *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673, [3]liquid blend of sodium nitrite, hexamethylene tetramine potassium sorbate.

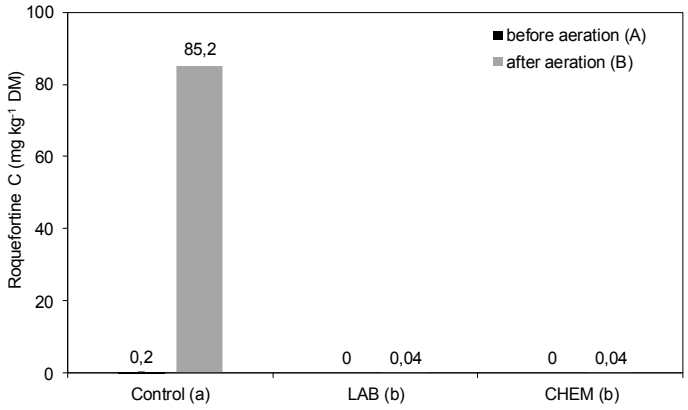


**Figure 1.** Effects of aeration and silage additive on roquefortine C concentrations in whole-crop rye silage. $^{(A,B)}$effect of aeration ($P$<0.001), $^{(a,b)}$effect of additive ($P$<0.001), interaction not significant, n=3.

**Conclusion** This study showed that untreated WCR silage rapidly deteriorated upon exposure to air, and large quantities of roquefortine C were formed. The use of silage additives is highly recommended to control fungal development and mycotoxin formation during feed-out.

**References**

Auerbach, H., Oldenburg, E., & Weissbach, F. (1998) Incidence of *Penicillium roqueforti* and roquefortine C in silages. Journal of the Science of Food and Agriculture, 76, 565-572.

Auerbach, H., Weiss, K., Theobald, P., & Nadeau, E. (2013) Effects of inoculant type on dry matter losses, fermentation pattern, yeast count and aerobic stability of green rye silages. In: Mair, C., Wetscherek., W. & Schedle, K (eds). 12. BOKU-Symposium, Vienna, Austria, pp. 179-185.

Boysen, M. E., Jacobsson, K.-G., & Schnürer, J. (2000) Molecular identification of species from the *Penicillium roqueforti* group associated with spoiled animal feed. Applied and Environmental Microbiology, 66, 1523-1526.

López, P., de Rijk, T., Sprong, R. C., Mengelers, M. J. B., Castenmiller, J. J. M., & Alewijn, M. (2016) A mycotoxin-dedicated total diet study in the Netherlands in 2013: Part II – occurrence. World Mycotoxin Journal, 9, 89-108.

Müller, H.-M., & Amend, R. (1997) Formation and disappearance of mycophenolic acid, patulin, penicillic acid and PR Toxin in maize silage inoculated with *Penicillium roqueforti.* Archives of Animal Nutrition, 50, 213-225.

Schneweis, I., Meyer, K., Hörmansdorfer, S., & Bauer, J. (2000) Mycophenolic acid in silage. Applied and Environmental Microbiology, 66, 3639-3641.

# The use of *Lactobacillus diolivorans* as silage inoculant

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**Keywords:** *Lactobacillus diolivorans*, 1,2-propanediol production, 1-propanol, propionic acid

**Introduction** Heterofermentative lactic acid bacteria (LAB), e.g. *Lactobacillus buchneri* and some close relatives are capable of converting lactic acid into equimolar amounts of 1,2-propanediol and acetic acid and small amounts of ethanol under anaerobic conditions (Oude Elferink et al. 2001). Furthermore *L. diolivorans* has been shown to be unique among lactic acid bacteria in its ability to degrade 1,2-propanediol under anaerobic conditions to 1-propanol and propionic acid (Kronemann et al. 2002). Recent research has shown the ability of *L.* diolivorans producing 1,2-propanediol, acetic acid and ethanol such as *L. buchneri.* The aim of this study was to investigate the heterofermentative metabolism of *L. diolivorans* in a chemically defined medium under anaerobic conditions.

**Material and Methods** *L. diolivorans* was grown anaerobically at 30 °C in a chemically defined basal medium with 55 mM glucose as only carbon source in the first experiment (Mc Feeters at al. 1986). In the second one 55 mM mannitol and 30 mM 1,2-propanediol as electron acceptor was used. A 24-h culture was centrifuged, washed once with sterile saline, and the cell pellet was suspended in sterile saline. For all experiments, each tube was inoculated with washed cells to give at least $10^7$ colony-forming units (cfu) $ml^{-1}$ and incubated to the extent of 52 days in an anerobic jar. Duplicate tubes were cultivated and analyzed separately in all experiments. Quantitative HPLC analysis of sugars, organic acids and alcohols, the pH-value and the optical density at 600 nm were determined at different times of anaerobic cultivation at 30 °C.

**Results** *L. diolivorans* was grown in anaerobic batch cultures with 55 mM glucose as a substrate. During glucose degradation significant growth, measured as an increase in cell density, was observed. The disappearance of glucose, product formation and the optical density of the cultures are depicted in Figure 1. During the degradation of glucose (from 55.2 to 3.1 mM), lactic acid increased from 0 to 36.8 mM, acetic acid increased from 0 to 12.0 mM, ethanol increased from 0 to 35.0 mM and 1-propanol increased from 0 to 1.2 mM under acidic and anaerobic conditions after 52 days. In contrast to silages the amount of alcohols in a chemically defined basal medium is higher, because no alternative electron acceptors are available. Furthermore 1,2-propanediol could be measured after 14 days and increased from 1.3 to 4.5 mM by the end of this experiment. For the first time it was found that *L. diolivorans* is able to degrade glucose to lactic acid, acetic acid, 1,2-propanediol, ethanol as well as small amounts of 1-propanol.

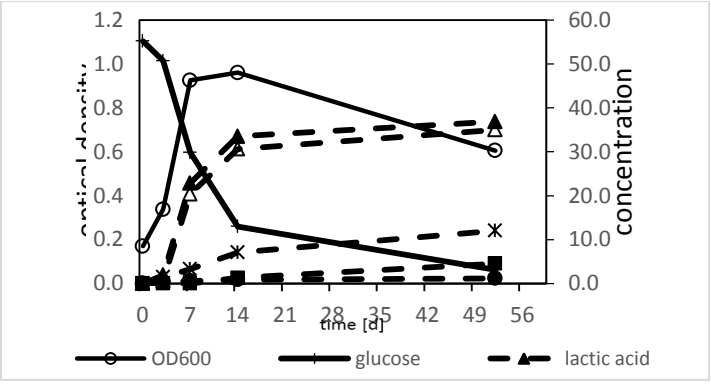


**Figure 1.** Time course of the batch cultivation of *L. diolivorans* in a chemically defined basal medium with 55 mM glucose under anaerobic conditions at 30 °C.

A second batch experiment was carried out to further investigate the degradation of 1,2-propanediol. *L. diolivorans* was grown in anaerobic batch cultures with 55 mM mannitol and 30 mM 1,2-propanediol

as substrates. During the first 2 weeks of incubation the cell density increased, after that it slightly decreased until day 32. Mannitol was degraded into lactic acid, acetic acid and ethanol. 1,2-propanediol was converted into 1-propanol and propionic acid by *L. diolivorans* (Fig. 2). During 1,2-propanediol degradation (from 32.4 to 0 mM), 1-propanol increased from 0 to 25.4 mM and propionic acid increased from 0 to 3.0 mM. The extent of mannitol degradation (from 51.2 to 36.7 mM) was directly related to the concentration of 1,2-propanediol (from 32.4 to 0 mM) as electron acceptor.

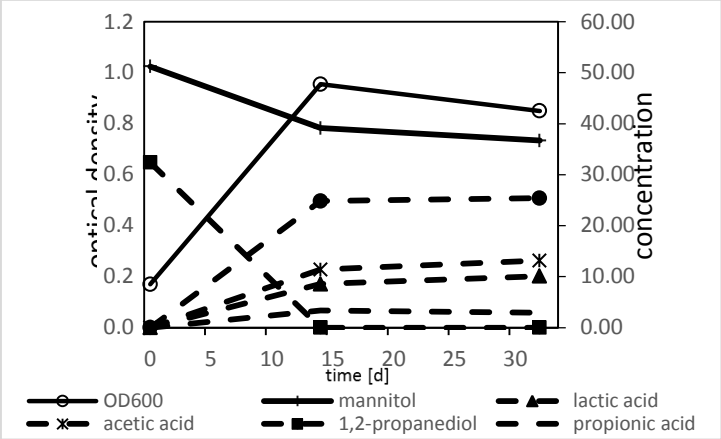


**Figure 2:** Time course of the batch cultivation of *L. diolivorans* in a chemically defined basal medium with 55 mM mannitol and 30 mM 1,2-propanediol under anaerobic conditions at 30 °C.

**Discussion** Production of 1,2-propanediol has only been found for *L. buchneri* and some close relatives but not for *L. diolivorans.* Based on the results obtained in our experiments, we propose that *L. diolivorans* is able to conduct a similar metabolism as *L. buchneri*, that leads to the production of 1,2-propanediol as metabolite. Furthermore we can confirm the anaerobic degradation of 1,2-propanediol in 1-propanol and propionic acid based on the knowledge of literature. This could be the reason why accumulated 1,2-propanediol was only detected under specific conditions as in our experiments.

**Conclusion** *L. diolivorans* is very interesting as silage inoculant because of the manifold metabolism. The initial fermentation of water-soluble carbohydrates into lactic acid leads to the fast acidification of the environment. The production of acetic acid and also propionic acid improves the aerobic stability of silages, because these two acids have a stronger effect in inhibiting yeast growth than lactic acid.

**References**

Krooneman, J., Faber, F., Alderkamp, A.C., Oude Elferink, S.J.H.W., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C., & Vancanneyt, M. (2002) *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 52, 639-646.

McFeeters, R.F., & Chen, K.-H. (1986) Utilization of electron acceptors for anaerobic mannitol metabolism by *Lactobacillus plantarum*. Compounds which serve as electron acceptors. Food Microbiology, 3, 73-81.

Oude Elferink, S.J.H.W., Krooneman, J., Gottschal, J. C., Spoelstra, S.F., Faber, F., & Driehuis, F. (2001) Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri.* Applied and Environmental Microbiology, 67, 125-132.

# Uncorrected silo management increases the risk of contamination of the milk production chain with *Clostridium* spp. and *Paenibacillus* spp.

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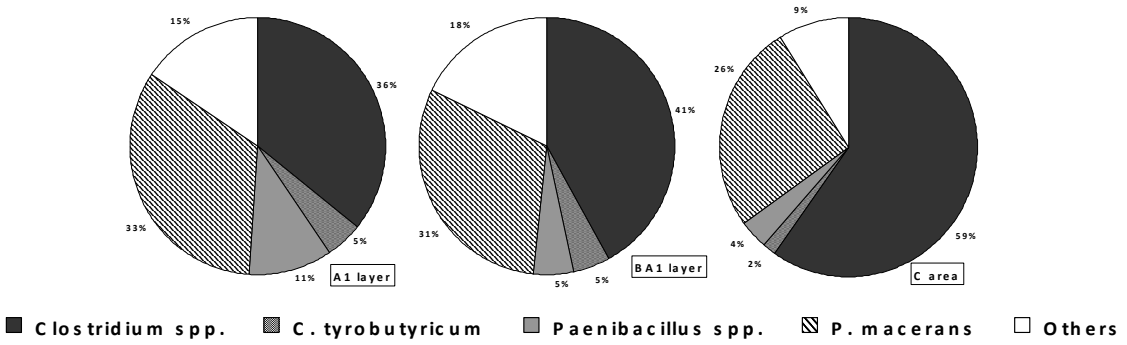
**Keywords:** anaerobic sporeformer, aerobic deterioration, maize silage, milk, TMR

**Introduction** The occurrence of *Paenibacillus* and *Clostridium* spores in silage is of great concern for dairy producers, because their spores can survive milk and cheese processing, and some strains are capable of subsequently germinating and causing damage to conserved milk and dairy products (te Giffel et al. 2002). Anaerobic spore-formers can multiply in silage (Borreani et al. 2013), and, at critical levels of contamination, their control becomes very difficult during the process of making hard cheeses (Vissers et al. 2007). The objectives of this study were to verify the role of the aerobic deterioration of maize silage on the proliferation of *Paenibacillus* and *Clostridium* spp. spores, and to evaluate the efficacy of different management strategies on reducing aerobic deterioration and on mitigating these undesirable bacterial outbreaks.

**Material and Methods** A survey was carried out, over a 2 year period, on 15 dairy farms in northern Italy, which produce milk destined for the production of Grana Padano cheese. A questionnaire, containing information on the silo management practices, the amount of silage consumed daily, the filling and packing methods, the silo cover, the typology of weigh down the silo cover and the feeding rates, was filled in on each farm. Samples of maize silage, total mixed ration (TMR), faeces (mixed sample of 5 milking cows), bedding, water and bulk tank milk were collected on each farm. Three silage sampling areas were chosen in each silo: centre (C), and two peripheral areas, close to the sealant film (A1, first 15 cm– visibly spoiled) and below A1 (BA1, 15 to 30 cm immediately below the A1 layer). Each silage and TMR sample was analysed to establish their chemical, microbial and fermentative characteristics. Faeces, milk and water were also collected and analysed for anaerobic spore-former enumeration. Anaerobic spores were counted after pasteurisation of the serial dilutions at 80°C for 10', and this was followed by the streak plate technique on RCM agar, 0.005% neutral red, and 200 ppm of d-cycloserine, incubated anaerobically for 7 d at 35°C. Five to ten bacterial colonies per sample, representative of distinct morphologies, were chosen for isolation and identification. Bacterial identification was performed by sequencing the V1-V3 region of the 16S rRNA gene, according to Borreani et al. (2013).

**Results and Discussion** Spoiled maize silage was confirmed to be a source of anaerobic spores, with *Paenibacillus* and *Clostridium* being present at higher levels than 5 log cfu/g of silage in more than 80% of the samples (Figure 1). *Paenibacillus* (4 species) and *Clostridium* (10 species) represented 29% and 61% of all the maize silage spores, respectively. The frequently dominant clostridia species were: *C. tyrobutyricum*, *C. aerotolerans*, *C. aminovalericum*, *C. amygdalium*, *C. celerecrescens*, *C. jejuense*, *C. sporogenes*, *C. xylanoliticum*; while the *Paenibacillus* species were *P. macerans*, *P. thermophylus*, and *P. cookii*. The zones immediately below the visible spoiled silage (BA1), even though no evident spoiling was visible, also showed spore content levels of up to 7.49 log colony-forming units (cfu)/g. This aspect determines the potential capability of this masked spoiled area to become the main source of spores for dairy cow TMR. The TMR spore counts were as follows: 30% of samples below 3 log cfu/g (47% *Clostridium* spp., 11% *C. tyrobutyricum*, 13% *P. thermophylus*, 3% *P. macerans*); 50% from 3.00 to 4.99 log cfu/g (51% *Clostridium* spp., 9% *C. tyrobutyricum*, 9% *P. thermophylus*, 4% *P. macerans*); and 20% greater than 5 log cfu/g (46% *Clostridium* spp., 3% *C. tyrobutyricum*, 13% *P. thermophylus*, 3% *P. macerans*). The dominant anaerobic sporeformers of the bulk milk are reported in Figure 2. Among the spore-forming species, clostridia represented 36, 52 and 0% in the milk samples with <200, from 200 to 1000 and >1000 spores/L, respectively; with 5 species frequently identified: *C. beijerinckii*, *C. bifermentans*, *C. butyricum*, *C. sporogenes*, *C. tyrobutyricum*, and *C. aminovalerianicum*. The *Paenibacillus* species

represented 59, 36 and 84% in the milk with <200, from 200 to 1000 and >1000 spores/L, respectively; *P. macerans*, *P. cooki, P. thermophylus*, and *P. polymixa* were the most frequently identified species.




| | A1 layer (average_min_max) | BA1 layer | Centre |
|---|---|---|---|
| Anaerobic spore (log CFU/g) | 6.03 (3.46-7.76) | 4.99 (2.48-7.49) | 2.66 (1.88-3.83) |
| PH | 6.39 (3.83-7.74) | 4.62 (3.68-7.54) | 3.77 (3.52-4.04) |
| Yeast (log CFU/g) | 5.98 (4.45-8.02) | 5.10 (<1.00-7.30) | 1.40 (<1.00-3.29) |
| Sample temperature (°C) | 38.3 (26.8-59.8) | 37.5 (20.1-49.4) | 23.4 (19.1-30.2) |

**Figure 1.** Distribution of the anaerobic spore-forming bacteria isolates in different areas of the maize silage, mean spore and yeast counts, pH and temperature of the sampling area of 49 commercial farms in northern Italy.

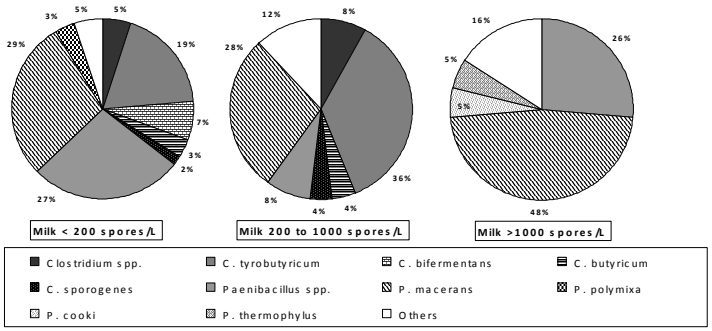



**Figure 2.** Distribution of the anaerobic spore-forming bacteria in the bulk milk of 49 commercial farms in northern Italy.

**Conclusion** The adoption of good silage management practices that are able to reduce spoiled zones in a silo could greatly decrease the risk of spore contamination with *Clostridium* spp. and *Paenibacillus* spp. in the successive phases of the milk production chain.

**References**

Borreani, G., Dolci, P., Tabacco, E., & Cocolin, L. (2013). Aerobic deterioration stimulates outgrowth of spore-forming *Paenibacillus* in corn silage stored under oxygen-barrier or polyethylene films. Journal of Dairy Science, 96, 5206-5216.

te Giffel, M.C., Wagendorp, A., Herrewegh, A. & Driehuis, F. (2002). Bacterial spores in silage and raw milk. Antonie van Leeuwenhoek, 81, 625-630.

Vissers, M.M.M., Driehuis, F., Te Giffel, M.C., De Jong, P., & Lankveld, J.M.G. (2007). Quantification of the transmission of microorganisms to milk via dirt attached to the exterior of teats. Journal of Dairy Science, 90, 3579-3582.

# Using molecular microbial ecology to define differential responses to the inoculation of barley silage

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**Keywords:** Barley silage, high throughput sequencing, microbial communities, third-generation inoculant

**Introduction** Ensiling of forages is recognized as a microbial process that relies on the generation of lactic acid by lactic acid bacteria (LAB) under anaerobic conditions. To improve the ensiling process, microbial inoculants are frequently applied to forages to more rapidly decrease pH and extend the aerobic stability of silage (Romero et al. 2017, Addah et al. 2014). It is well known that responses to silage inoculants can vary depending on the nature of the epiphytic microbial population, the characteristics of the forage and the ensiling practices employed (Addah et al. 2011, Ni et al. 2015, Romero et al. 2017). We investigated the impact of a mixed *Lactobacillus buchneri*, *L. plantarum*, *L. casei* inoculant possessing ferulic acid esterase (FAE) activity on fermentation, aerobic stability and nutritive value of barley silage over two years (Addah et al. 2012a, 2012b, 2014). In the first year, a classical response to inoculation was obtained with a more rapid decline in pH and an increase in acetic acid concentration during the ensiling process in both laboratory and Ag-Bag® silos. In the second year, this classical response was not observed in laboratory silos, but was observed in Ag-Bag® silos. The objective of this study was to determine if molecular microbial ecology could provide insight as to why differential responses to the inoculant were observed in barley silage between the two years.

**Materials and Methods** Whole-crop barley (*Hordeum vulgare* L.) was ensiled with or without a FAE inoculant containing *L. buchneri*, *L. plantarum* and *L. casei* in mini or Ag-Bag® silos at a rate of $1 \times 10^5$ colony-forming units (CFU) of LAB per g of fresh forage in 2009 and 2010 as described in Addah et al. (2012a, 2012b; 2014). DNA was extracted from samples collected before and after ensiling from both silo types over both years according to the procedures of Yu and Morrison (2004). Extracted DNA samples were pair-end sequenced using Illumina MiSeq at Genome Quebec (McGill University, Montreal, QC) with amplification of 16S (V3-V4) and 18S rDNA (V4-V5) for bacteria and fungi, respectively. Sequence data used to define bacterial and fungal communites were analyzed in a bioinformatic pipeline that involved applications of UCHIME, Greengenes, QIIME and MOTHUR in a similar manner as described by Duniere et al. (2017). An analysis of variance for the effect of silo type, sampling time and year on the abundance of predominant OTUs was performed using SAS Mixed procedures to determine differences at $P < 0.05$.

**Results** Sequencing results from both years showed similar temporal shifts in bacterial communities in both mini and Ag-Bag® silos, with the phyla *Proteobacteria* and *Actinobacteria* being predominant pre-ensiling and *Firmicutes* post-ensiling. Post-ensiling, *Lactobacillus* was the most predominant genera observed in all silages. In 2009, inoculation increased ($P < 0.05$) the abundance of *Lactobacillus* in barley silage in both silo types. In 2010, this observation was confirmed in Ag-Bag® silos, but not in mini silos. Changes in silage chemistry corresponded with differences in microbial ecology. Differences in the composition of epiphytic bacterial communities was found in fresh control forages from both years, with *Lactobacillaceae* and *Leuconostocaceae* being more ($P < 0.05$) predominant in 2010 than 2009. In addition, inoculation did not alter the core fungal community in either silo type in either year, but temporal shifts in the community were noted. Members of *Cladosporium*, *Leptosphaeria* and *Cryptococcus* were abundant in fresh forage, but were superseded ($P < 0.05$) by *Pichia* and *Kazachstania* after ensiling in both silo types.

**Discussion** Different characteristics of epiphytic communities associated with fresh forage could impact silage fermentation and terminal silage quality (Ni et al. 2015). Understanding the epiphytic microbiome on fresh forage might lead to more predictable ensiling outcomes and identify conditions where silage inoculants deliver the greatest benefit (McAllister et al. 2017). In this study, members of *Lactobacillaceae* and *Leuconostocaceae* were more predominant in fresh forage from 2010 than 2009, probably resulting in different responses in the amount of acetic acid produced in barley silage between these two years. Inoculation may have less impact when high concentrations of lactic acid bacteria are associated with fresh forage. However, differential responses observed between mini and Ag-Bag® silos suggested that ensiling conditions in mini silos may not adequately represent the more variable ensiling conditions in farm-scale Ag-Bag® silos. Currently, there are only a few studies that have used molecular ecology to characterize epiphytic fungal communities associated with forage. Our study demonstrated that epiphytic members of *Cladosporium*, *Leptosphaeria* and *Cryptococcus* decreased with ensiling, reflecting the acidification and transition from an aerobic to an anaerobic environment (Duniere et al. 2017). As in our study, others also confirmed that members of *Sacchromycetaceae* including *Pichia* and *Kazachstania* are the predominant fungi in terminal silage (May et al. 2001), suggesting that these fungal members are capable of fementative growth and can survive the ensiling process.

**Conclusion** Advancements in next generation sequencing are providing new insight into the complexity of microbial communities in silages. Our results suggest that differences in the composition of epiphytic bacteria likely are responsible for the differential responses observed in inoculated barley silage.

**References**

Addah, W., Baah, J., Groenewegen, P., Okine, E.K. & McAllister, T.A. (2011) Comparison of the fermentation characteristics, aerobic stability and nutritive value of barley and corn silages ensiled with or without a mixed bacterial inoculant. Canadian Journal of Animal Science, 91, 133-146.

Addah, W., Baah, J., Okine, E.K. & McAllister, T.A. (2012a) A third-generation esterase inoculant alters fermentation pattern and improves aerobic stability of barley silage and the efficiency of body weight gain of growing feedlot cattle. Journal of Animal Science, 90, 1541-1552.

Addah, W., Baah, J., Okine, E.K. & McAllister, T.A. (2012b) Use of thermal imaging and the in situ technique to assess the impact of an inoculant with feruloyl esterase activity on the aerobic stability and digestibility of barley silage. Canadian Journal of Animal Science, 92, 381-394.

Addah, W., Baah, J., Okine, E.K., Owens, F.N. & McAllister, T.A. (2014) Effects of chop-length and a ferulic acid esterase-producing inoculant on fermentation and aerobic stability of barley silage, and growth performance of finishing feedlot steers. Animal Feed and Technology, 197, 34-46.

Duniere, L., Xu, S., Jin, L., Elekwachi, C., Wang, Y., Turkington, K., Forster, R., & McAllister, T.A. (2017) Bacterial and fungal core microbiomes associated with small grain silages during ensiling and aerobic spoilage. BMC Microbiology, 17, 50.

May, L. A., Smiley, B., & Schmidt, M. G. (2001) Comparative denaturing gradient gel electrophoresis analysis of fungal communities associated with whole plant corn silage. Canadian Journal of Microbiology, 47, 829-841.

McAllister, T.A., Duniere, L., Drouin, P., Xu, S., Wang, Y., Muuns, K. & Zaheer, R. (2017) Using molecular approaches to define the microbial ecology of silage. Journal of Dairy Science, accepted.

Ni, K., Wang, Y., Cai, Y., & Pang, H. (2015) Natural lactic acid bacteria population and silage fermentation of whole-crop wheat. Asian-Australasian Journal of Animal Sciences, 28, 1123-1132.

Romero, J.J., Zhao, Balseca-Paredes, Y.M.A., Tiezzi, F., Gutierrez-Rodriguez, E. & Castillo, M.S. (2017) Laboratory silo type and inoculation effects on nutritional composition, fermentation, and bacterial and fungal communities of oat silage. Journal of Dairy Science, 100, 1812-1828.

Yu, Z. & Morrison, M. (2004) Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques, 36, 808-812.

# Changes in pH and chemical composition of fungi-treated wheat straw, stored anaerobically, with or without additives

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**Keywords:** Anaerobic storage, fungi, wheat straw

**Introduction** Lignocellulosic biomass, derived from agricultural processes, such as wheat straw (WS), has a great potential to be exploited as a ruminant feed. However, the presence of recalcitrant lignin hampers the degradation of the polysaccharides cellulose and hemicellulose in the rumen. Compared with other pre-treatment methods, white rot fungi offer a cheap and sustainable alternative since some of these are selective, i.e. they degrade lignin and hardly any cellulose. Many studies have already shown that these fungi can increase the digestibility of lignocellulose by ruminants (Tuyen et al. 2012, 2013, Van Kuijk et al. 2015). After a successful aerobic incubation with the fungus, termination is necessary for maintaining the nutritive value, enabling the fungi-treated substrate to be fed to animal over a prolonged period of time. Ensiling is a storage method based on the fermentation of water soluble carbohydrates by lactic acid producing bacteria (LAB), resulting in a rapid reduction in pH. Under such acidified environment forages are successfully preserved. In the current experiment, we treated WS with the fungus *Ceriporiopsis subvermispora* for 39 days, and the untreated WS and fungal treated WS were subsequently stored anaerobically as such, with the addition of LAB, or the addition of a combination of LAB and molasses. The aim of this study was to investigate which method is the best to store fungal treated wheat straw for longer periods.

**Materials and Methods** ***Fungus and spawn preparation*** The white rot fungus strain used was *C. subvermispora* (strain code: CBS 347.63; Origin: USA). The fungus was grown on an agar plate, then two agar pieces (~1.5 $cm^2$) were transferred to plastic boxes with sterilized sorghum grain and incubated in a climate controlled room at 24°C. The fully colonized grains were stored at 4 °C before use. ***Fungal solid state fermentation and storage study*** The WS was chopped to a theoretical length of 0.5 cm. The WS was soaked in tap water for 3 days, and thereafter drained for a period of 5h. Fungal treatment of the WS was carried out in plastic containers (3000 ml) with a lid with filter, allowing gas exchange and avoiding contamination. Before inoculation with the fungus, the wet WS was mixed, distributed into the containers and autoclaved at 121 °C for 1 h. After cooling overnight to room temperature, 12-13 g (10% of dry WS) of spawn was added to each box and mixed gently by hand under aseptic conditions. Autoclaved wheat straw (AWS, 121 °C for 1 h) was incubated with *C. subvermispora* for 39 days. Untreated AWS and *C. subvermispora* treated AWS were then packed into 500 ml airtight glass jars "as is", with $1\times10^6$ cfu *Lactobacillus plantarum* $g^{-1}$ wet substrate or with a combination of LAB and molasses (3% wet weight). The jars were filled by pressing the substrate into the jars, leaving as little air as possible before being closed and stored at 20°C in a climate-controlled chamber. The substrates density in the glass jars was 699.4-753.5 $kg/m^3$ for AWS and 794.2-812.0 $kg/m^3$ for *C. subvermispora* treated AWS, on a fresh basis. Three jars of each treatment were collected at 0, 2, 4, 8, 16, 32 and 64 days of storage for analysis of pH, and chemical composition (only 0 and 64 days).

**Results and Discussion** A drop in pH during ensiling of AWS is only achieved when Lactic acid bacteria and molasses is added (Figure 1A). The fungus decreases the pH of AWS from 5.14 to 3.41 and addition of lactic acid bacteria with or without molasses did not had a large effect on the pH after 64 days of anaerobic storage (Figure 1B). The chemical analysis showed that the fungal treated wheat straw is very stable, also without any additions (Table 1).

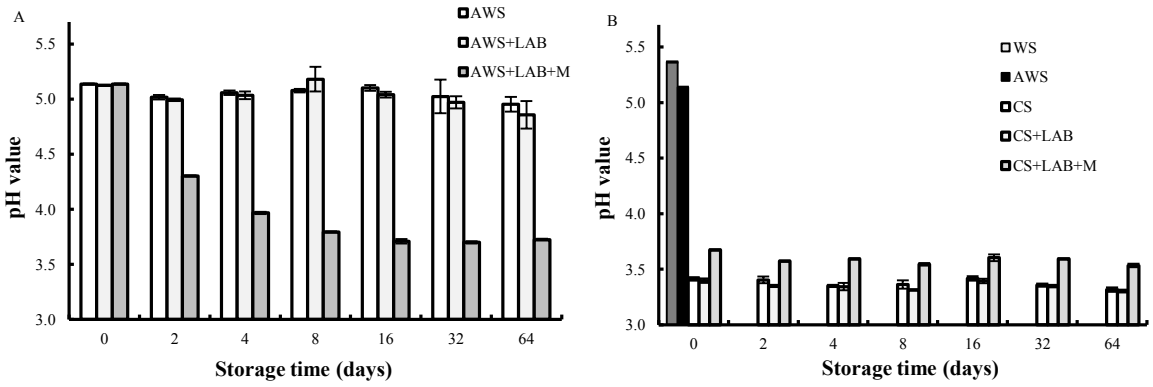


**Figure 1**. pH value of (A) autoclaved wheat straw (AWS) and (B) AWS treated with *C. subvermispora* (CS) after 0, 2, 4, 8, 16, 32 and 64 days of storage. Substrates were stored as is, with addition of lactic acid bacteria (LAB) or with addition of a combination of LAB and molasses (LAB+M).

**Table 1.** Chemical composition and *in vitro* gas production (IVGP) of *Ceriporiopsis subvermispora* treated autoclaved wheat straw (CS, solid state fermentation for 39 days) stored anaerobically as is, with addition of lactic acid bacteria (LAB) or with addition of a combination of LAB and molasses (LAB+M) for 0 (T0) and 64 (T64) days.

| Treatment | CS as is | | CS+LAB | | CS+LAB+M | |
|---|---|---|---|---|---|---|
| | CS-T0 | CS-T64 | CSL-T0 | CSL-T64 | CSLM-T0 | CSLM-T64 |
| Ash (g $kg^{-1}$ DM) | 24.3 | 25.2* | 24.9 | 24.9 | 35.8 | 36.2 |
| Crude protein (g $kg^{-1}$ OM) | 36.3 | 37.2 | 35.3 | 37.8 | 39.9 | 41.4 |
| Cellulose (g $kg^{-1}$ OM) | 475.2 | 473.8 | 475.9 | 472.4 | 435.2 | 427.3 |
| Hemicellulose (g $kg^{-1}$ OM) | 165.7 | 173.4 | 184.2 | 179.7 | 161.3 | 168.3 |
| Lignin (g $kg^{-1}$ OM) | 40.9 | 35.9* | 29.5 | 34.8 | 28.6 | 30.1 |
| IVGP (ml $g^{-1}$ OM) | 269.6 | 268.5 | 264.5 | 253.9 | 263.1 | 273.5 |

DM, dry matter; OM, organic matter;
* Significantly different ($P<0.05$) from 64 days to the corresponding value at day 0

**Conclusions** It is possible to conserve *C. subvermispora* treated straw under anaerobic conditions, without additives, at least up to 64 days. This finding is important for practical applications to supply fungi treated feed to ruminant animals for a prolonged period.

**References**

Tuyen, D.V., Cone, J.W, Baars, J.J.P., Sonnenberg, A.S.M. & Hendriks, W.H. (2012) Fungal strain and incubation period affect chemical composition and nutrient availability of wheat straw for rumen fermentation. Bioresource Technology 111, 336-342.

Tuyen, D.V., Phuong, H.N., Cone, J.W., Baars, J.J.P., Sonnenberg, A.S.M. & Hendriks, W.H. (2013) Effect of fungal treatments of fibrous agricultural by-products on chemical composition and in vitro rumen fermentation and methane production. Bioresource Technology 129, 256-263.

Van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H. & Cone, J.W. (2015) Fungal treatment of lignocellulosic biomass: Importance of fungal species, colonization and time on chemical composition and in vitro rumen degradability. Animal Feed Science and Technololy 209, 40-50.

# Chemical composition of silages produced in Mecklenburg-Western Pomerania

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**Keywords:** Dietary cation-anion difference, energy content, major elements, protein value

**Introduction** For nutritional-physiological and economic reasons forages make up the main part of the ruminant's diet, such that the knowledge of the exact feeding value of silages is of utmost importance. Most variables of this complex characteristic can be deduced from feeding tables, hence facilitating ration planning and calculation. However, the chemical composition of forages might be subject to wide variation because of, *e.g.*, distinct harvesting dates, soil composition or the fertilization management. The objective of this study was to assess variations of selected variables associated with the feeding value of silages produced in Mecklenburg-Western Pomerania in northern Germany.

**Material and Methods** Silage samples produced in 2017 of grass (n = 444 for 1$^{st}$, n = 304 for 2$^{nd}$ and n = 169 for 3$^{rd}$ cut, respectively, unless otherwise stated), maize (n = 2486, unless otherwise stated), lucerne (n = 48, unless otherwise stated) and whole-plant cereal crops other than maize (n = 280, unless otherwise stated) submitted by small and large-scale farms from Mecklenburg-Western Pomerania were analysed for selected variables of the feeding value.

Proximate composition, fibre fractions ADFom (acid detergent fibre exclusive residual ash) as well as aNDFom (amylase-treated neutral detergent fibre exclusive residual ash) and *in vitro* digestibility variables (24-h gas production [GP] and enzyme soluble organic matter [ESOM]) were determined by near infrared reflectance spectroscopy according to VDLUFA methods (VDLUFA 2012). The contents of utilizable crude protein at the duodenum (uCP) and net energy for lactation (NEL) were subsequently calculated according to the recommendations of the Society of Nutrition Physiology (GfE 2001, 2008). An X-ray fluorescence spectrometer was used to determine selected major elements. The dietary cation-anion difference (DCAD) was calculated in terms of meq/kg dry matter (DM) using the concentrations of sodium, potassium, chlorine and sulphur (Oetzel 2002). Nutrient contents and selected variables of the feeding value are presented as medians and ranges (minimum – maximum).

**Results and Discussion** Although mean values of DM contents in all silage types meet the demands of the good agricultural practice in view of successful ensilage (Table 1), extreme low or high contents imply the danger of secondary fermentations respectively aerobic instability. The elevated crude ash contents of grass silages indicate that half of the analyzed samples were presumably contaminated with soil, indicating that butyric acid fermentation might have occurred (not analyzed). In average, ADFom and aNDFom contents were slightly elevated in all cuts of grass silages with negative effects on the digestibility. As a consequence of the mentioned wide variations of crude nutrient contents in all kind of silages, the energy content calculated as net energy for lactation (NEL) varied substantially (*e.g.* 2$^{nd}$ cut grass silage 3.7-7.4 MJ NEL/kg DM and lucerne 4.6-7.2 MJ NEL/kg DM) and did in average not meet the target values for grass and lucerne. Among the major elements Ca (*e.g.* 2$^{nd}$ cut grass silage 1.7-23.3 g/kg DM and lucerne silages 3.6-19.3 g/kg DM), K (*e.g.* 2$^{nd}$ cut grass silage 6.3-41.2 g/kg DM and lucerne silages 16.6-39.1 g/kg DM) and Cl (*e.g.* 1$^{st}$ cut grass silage 2.1-20.9 g/kg DM and lucerne silages 1.0-11.4 g/kg DM) were found to vary most. Since K and Cl have a considerable influence on the DCAD value, this variable therefore largely varied mainly in grass (*e.g.* 1$^{st}$ cut silage -56-690 meq/kg DM) and lucerne (103-664 meq/kg DM) silages.

**Conclusion** In order to prevent the occurrence of feed-induced diseases, exact data about the diet's chemical composition is crucial to meet the animal's requirements and to prevent under- or oversupply of certain nutrients. Apart of the crude nutrients, contents of minerals may vary widely and might be not in accordance with tabulated values, implying the necessity for chemical analyses if, *e.g.*, the

concept of DCAD is applied when formulating rations for dry as well as lactating cows, where requirements differ substantially.

**Table 1**. Chemical composition of silages produced in Mecklenburg-Western Pomerania.

| | Grass (1st cut) | Grass (2nd cut) | Grass (3rd cut) | Maize | Lucerne | WPC |
|---|---|---|---|---|---|---|
| Dry matter (g/kg) | 347 (*187 - 780*) | 375 (*217 - 681*) | 369 (*179 - 730*) | 354 (*186 - 539*) | 346 (*130 - 576*) | 354 (*179 - 517*) |
| Crude ash (g/kg DM) | 96 (*64 - 200*) | 103 (*34 - 227*) | 106 (*50 - 340*) | 33 (*17 - 109*) | 105 (*72 - 162*) | 56 (*27 - 121*) |
| Crude protein (g/kg DM) | 159 (*82 - 248*) | 159 (*56 - 243*) | 152 (*76 - 238*) | 67 (*29 - 197*) | 180 (*109 - 308*) | 90 (*41 - 215*) |
| Crude fat (g/kg DM) | 31 (*11 - 47*) | 30 (*17 - 43*) | 30 (*13 - 40*) | 25 (*12 - 39*) | 28 (*9 - 34*) | 23 (*14 - 37*) |
| aNDFom (g/kg DM) | 528 (*362 - 648*) | 533 (*435 - 675*) | 539 (*406 - 681*) | 402 (*296 - 589*) | 478 (*386 - 586*) | 438 (*292 - 666*) |
| ADFom (g/kg DM) | 294 (*176 - 385*) | 292 (*213 - 402*) | 286 (*179 - 402*) | 234 (*163 - 370*) | 343 (*213 - 457*) | 320 (*219 - 470*) |
| GP (ml/200 mg DM) | 45.9 (*23.8 - 66.0*) | 43.3 (*18.8 - 58.4*) | 43.7 (*18.4 - 55.0*) | nd | nd | nd |
| ESOM (g/kg DM) | nd | nd | nd | 718 (*389 - 830*) | nd | nd |
| uCP (g/kg DM) | 132 (*101 - 159*) | 130 (*91 - 162*) | 128 (*101 - 151*) | 130 (*110 - 145*) | 135 (*107 - 170*) | 111 (*91 - 147*) |
| NEL (MJ/kg DM) | 5.9 (*4.5 - 7.4*) | 5.8 (*3.7 - 6.9*) | 5.7 (*4.5 - 6.7*) | 6.7 (*5.1 - 7.6*) | 5.4 (*4.6 - 7.2*) | 5.1 (*4.0 - 6.1*) |
| Ca (g/kg DM) | 5.2[1] (*1.9 - 20.2*) | 6.3[3] (*1.7 - 23.3*) | 6.4[5] (*3.7 - 14.2*) | 1.6[7] (*0.9 - 3.2*) | 12.2[9] (*3.6 - 19.3*) | 3.4[11] (*1.6 - 11.9*) |
| P (g/kg DM) | 2.8[1] (*1.4 - 4.3*) | 2.7[3] (*1.3 - 4.4*) | 2.8[5] (*1.7 - 4.5*) | 2.1[7] (*0.5 - 3.2*) | 3.0[9] (*2.2 - 4.0*) | 2.5[11] (*1.7 - 3.6*) |
| Na (g/kg DM) | 1.3[1] (*0.1 - 9.6*) | 1.6[3] (*0.1 - 8.6*) | 1.7[5] (*0.1 - 7.1*) | 0.1[7] (*0.1 - 0.8*) | 0.5[9] (*0.1 - 2.3*) | 0.1[11] (*0.1 - 1.2*) |
| Mg (g/kg DM) | 1.6[1] (*0.8 - 3.1*) | 1.9[3] (*0.7 - 4.4*) | 2.0[5] (*0.7 - 3.4*) | 1.1[7] (*0.4 - 2.1*) | 2.0[9] (*1.5 - 3.8*) | 1.1[11] (*0.7 - 2.8*) |
| K (g/kg DM) | 22.4[1] (*4.3 - 37.7*) | 19.9[3] (*6.3 - 41.2*) | 19.8[5] (*8.2 - 38.4*) | 9.4[7] (*1.5 - 18.5*) | 27.7[9] (*16.6 - 39.1*) | 15.3[11] (*9.4 - 29.3*) |
| Cl (g/kg DM) | 7.9[2] (*2.1 - 20.9*) | 8.9[4] (*2.5 - 21.6*) | 9.6[6] (*0.3 - 19.1*) | 1.3[8] (*0.4 - 3.0*) | 3.0[10] (*1.0 - 11.4*) | 2.7[12] (*1.5 - 3.9*) |
| S (g/kg DM) | 2.2[2] (*1.0 - 3.3*) | 2.4[4] (*1.3 - 3.5*) | 2.5[6] (*1.1 - 3.6*) | 0.8[8] (*0.6 - 1.3*) | 2.1[10] (*1.3 - 2.7*) | 1.5[12] (*1.1 - 2.1*) |
| DCAD (meq/kg DM) | 235[2] (*-56 - 690*) | 198[4] (*-99 - 501*) | 199[6] (*-22 - 378*) | 152[8] (*21 - 222*) | 404[10] (*103 - 664*) | 189[12] (*163 - 320*) |

DM, dry matter; GP, gas production; ESOM, enzyme soluble organic matter; nd, not determined; WPC, whole-plant cereals
[1] n = 373, [2] n = 53, [3] n = 229, [4] n = 43, [5] n = 126, [6] n = 34, [7] n = 544, [8] n = 112, [9] n = 30, [10] n = 13, [11] n = 30, [12] n = 5

**References**

GfE [Society of Nutrition Physiology] (2001) Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt a. M.

GfE [Society of Nutrition Physiology] (2008) Communications of the committee for requirement standards of the society of nutrition physiology: New equations for predicting metabolisable energy of grass and maize products for ruminants. Proceedings of the Society of Nutrition Physiology, 17, 191-198.

Oetzel, G.R. (2002) The dietary cation-anion difference concept in dairy cattle nutrition: possibilities and pitfalls. Proceedings of the XXII World Buiatrics Congress, Germany, pp. 198-208.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten [VDLUFA, ed.] (2012) Die chemische Untersuchung von Futtermitteln, In: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Vol. III. VDLUFA-Verlag, Darmstadt.

# Determination of the fermentation characteristics and nutritive value of mixed alfalfa and sweet corn stalk silages ensiled at six ratios

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**Keywords:** Alfalfa, fermentation profile, mixed silage, nutritive value, sweet corn stalk

**Introduction** Alfalfa (*Medicago sativa* L.) is a legume forage difficult to ensile successfully, mainly due to low contents of dry matter and water soluble carbohydrates and high buffering capacity and pH (Wen et al. 2017). Compared with alfalfa, the ensilability of sweet corn (*Zea mays* L.) stalk is higher as a result of a lower buffering capacity and a higher level of water soluble carbohydrates. Alfalfa and sweet corn stalk are complementary to each other in terms of chemical properties given above. Ensiling alfalfa as mixture with sweet corn stalk has the potential to improve the fermentation characteristics of alfalfa silage. Therefore, the objective of this work was to evaluate the efficacy of mixture ratio on the conservation profile and nutritive value of mixed alfalfa and sweet corn stalk silages.

**Material and Methods** A second regrowth of alfalfa at the early bud stage was cut at 187 g $kg^{-1}$ dry matter and chopped to 1 cm theoretically by a forage chopper on 25th August 2017. Simultaneously, sweet corn stalk was cut at 222 g $kg^{-1}$ dry matter and chopped to 1 cm in theory by another forage chopper. The chopped forages were made into six mixed alfalfa-sweet corn stalk proportions (containing 0, 200, 400, 600, 800 and 1000 g $kg^{-1}$ sweet corn stalk based on fresh weight basis). The mixed forages (300 g each) were packed into polythene bags, followed by air removal using a vacuum sealer. Bags of each ratio were prepared in triplicate and stored for 65 days at ambient conditions (temperature, 25-26°C; relative humidity, 34%).

At bag opening, silage mass was mixed thoroughly. A subsample of 20 g was weighed into a blender jar, diluted with 180 ml of distilled water, homogenized for 2 min, and analyzed for pH value by an electrode. Lactic, acetic, propionic and butyric acids were determined by high performance liquid chromatography. Ammonia-N was determined according to Broderick and Kang (1980). Another subsample of 200 g from each forage or silage was put in an oven at 65°C for 48 hours to determine dry matter. Buffering capacity was measured by suspending 1 g of sample in 100 ml distilled water at 150 rpm for 30 min, followed by titration from pH 6.0 to pH 4.0 with lactic acid. Neutral and acid detergent fibers were measured according to Van Soest et al. (1991). Relative feed value was calculated referring to Linn and Martin (1989). Crude protein was analyzed by Kjeldahl method. Water soluble carbohydrates were determined using the anthrone method (Murphy 1958).

Simple correlation analysis (SAS 9.1) was used to examine the linear effect of mixture ratio on the fe rmentation characteristics and nutritive value of silage.

**Results** ***Chemical composition of ensiling materials*** According to Table 1, the fermentation coefficient of alfalfa was lower, reflected by lower concentrations of dry matter and water soluble carbohydrates and a greater buffering capacity compared with sweet corn stalk. ***Fermentation characteristics of silages*** The pH and $NH_3$-N levels reduced and AA content increased significantly with an increase of sweet corn stalk rato in mixed forages ($P<0.05$), reflected from Table 2. ***Nutritive value of silages*** DM and NDF contents significantly increased, whereas RFV and CP values remarkably decreased as sweet corn stalk ratio in mixed forages elevated ($P<0.05$) (Table 3).

**Table 1**. Chemical composition of alfalfa and sweet corn stalk prior to ensiling.

| Item | alfalfa | sweet corn stalk |
|---|---|---|
| DM, g $kg^{-1}$ | 187.66 ± 0.97 | 222.77 ± 4.99 |
| Buffering capacity, lactic acid, g $kg^{-1}$ DM | 55.55 ± 0.68 | 32.77 ± 0.37 |
| Water soluble carbohydrates, g $kg^{-1}$ DM | 40.20 ± 1.97 | 144.25 ± 7.27 |
| Fermentation coefficient[1] | 24.56 ± 0.27 | 57.50 ± 2.21 |
| pH | 6.51 ± 0.02 | 5.56 ± 0.17 |
| Crude protein, g $kg^{-1}$ DM | 281.62 ± 3.52 | 112.01 ± 2.74 |
| Neutral detergent fiber, g $kg^{-1}$ DM | 416.71 ± 3.83 | 572.74 ± 7.58 |
| Acid detergent fiber, g $kg^{-1}$ DM | 307.43 ± 0.35 | 295.50 ± 5.94 |

**Note:** DM, dry matter; [1]Fermentation coefficient = dry matter% + 8 water soluble carbohydrates/buffering capacity.

**Table 2**. Fermentation characteristics of mixed alfalfa and sweet corn stalk silages.

| Item | 0%SS | 20%SS | 40%SS | 60%SS | 80%SS | 100%SS | SEM | Linear |
|---|---|---|---|---|---|---|---|---|
| pH | $5.47^{f}$ | $4.84^{c}$ | $4.23^{d}$ | $4.13^{c}$ | $3.79^{b}$ | $3.61^{a}$ | 0.15 | <0.0001 |
| LA | $5.71^{c}$ | $10.71^{b}$ | $14.40^{a}$ | $14.17^{a}$ | $13.96^{a}$ | $13.97^{a}$ | 0.78 | 0.0002 |
| AA | $7.13^{a}$ | $5.68^{b}$ | $4.40^{c}$ | $5.27^{b}$ | $4.17^{c}$ | $3.50^{d}$ | 0.29 | <0.0001 |
| PA | $1.35^{bc}$ | $1.17^{c}$ | $1.19^{c}$ | $2.07^{a}$ | $1.75^{ab}$ | $1.65^{ab}$ | 0.10 | 0.0333 |
| BA | 1.08 | 0.49 | ND | ND | ND | ND | - | - |
| $NH_3$-N | $316.39^{a}$ | $176.04^{b}$ | $113.22^{c}$ | $83.18^{cd}$ | $52.15^{de}$ | $39.95^{e}$ | 23.22 | <0.0001 |

**Note:** SS, sweet corn stalk; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; these fermentation products were shown on dry matter% basis; SEM, standard error of mean; ND, not detected. Ammonia-($NH_3$)-N was expressed as g $kg^{-1}$ total N.

**Table 3**. Nutritive value of mixed alfalfa and sweet corn stalk silages.

| Item | 0%SS | 20%SS | 40%SS | 60%SS | 80%SS | 100%SS | SEM | Linear |
|---|---|---|---|---|---|---|---|---|
| DM | $181.64^{e}$ | $195.65^{d}$ | $205.11^{c}$ | $207.30^{c}$ | $21.80^{b}$ | $225.83^{a}$ | 3.71 | <0.0001 |
| NDF | $395.23^{f}$ | $434.61^{e}$ | $467.38^{d}$ | $514.64^{c}$ | $530.49^{b}$ | $550.23^{a}$ | 13.39 | <0.0001 |
| ADF | 309.42 | 305.51 | 306.13 | 316.48 | 310.32 | 316.62 | 1.82 | 0.0992 |
| RFV | $153^{e}$ | $139^{de}$ | $130^{d}$ | $116^{c}$ | $114^{b}$ | $109^{a}$ | 3.83 | <0.0001 |
| WSC | $7.19^{c}$ | $7.21^{c}$ | $7.32^{c}$ | $9.90^{b}$ | $10.04^{b}$ | $14.79^{a}$ | 0.66 | <0.0001 |
| CP | $207.01^{a}$ | $201.89^{a}$ | $179.32^{b}$ | $161.95^{c}$ | $136.89^{d}$ | $105.57^{e}$ | 8.69 | <0.0001 |

**Note:** SS, sweet corn stalk; DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; RFV, relative feed value; WSC, water soluble carbohydrates; CP, crude protein; SEM, standard error of mean. All indexes except RFV were shown as g $kg^{-1}$ dry matter.

**Discussion** Ensiling alfalfa alone was poorly conserved, shown by high pH and this was mainly due to low levels of water soluble carbohydrates and dry matter and a high buffering capacity. The pH and ammonia-N levels of silages decreased with elevated ratio of sweet corn stalk in mixed forages possibly because of sweet corn stalk having a higher content of water soluble carbohydrates and less protein degradation. In contrast to the fermentation characteristics, the relative feed value of silages decreased as the proportion of sweet corn stalk in mixed forages increased.

**Conclusion** Ensiling alfalfa as mixture with sweet corn stalk enhanced significantly the fermentation characteristics and decreased the relative feed value of mixed alfalfa and sweet corn stalk silages.

**References**

Broderick, G.A. & Kang, J.H. (1980) Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. Journal of Dairy Science, 63, 64-75.

Linn, J.G. & Martin, N.P. (1989) Forage quality tests and interpretation. Veterinary Clinics of North America: Food Animal Practice, 7, 509-523.

Murphy, R.P. (1958) A method for the extraction of plant samples and the determination of total soluble carbohydrates. Journal of the Science of Food and Agriculture, 9, 714-717.

Van Soest, P.J., Robertson, P.J. & Lewis, B.A. (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

Wen, A.Y., Yuan, X.J., Wang, J., Desta, S.T. & Shao, T. (2017) Effects of four short-chain fatty acids or salts on dynamics of fermentation and microbial characteristics of alfalfa silage. Animal Feed Science and Technology, 223, 141-148.

# Dry matter loss, fermentation profile and aerobic stability of wet brewers grains ensiled with or without increasing concentrations of dry ground corn

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**Keywords:** Aerobic stability, dry matter loss, fermentation profile, wet brewers grain

**Introduction** Wet brewers grain (WBG) is a brewing by-product with high concentrations of protein and digestible fiber. However, it often has inadequate amounts of water-soluble carbohydrates (WSC) for silage fermentation due to the removal of sugars from the grain throughout the malting process. Thus, WBG is an unstable feed with high potential for deterioration due to its low concentrations of DM and WSC (Orosz and Davies 2015). The addition of soy hulls to enhance dry matter (DM) and WSC concentrations of the mixture resulted in greater DM and nutrient losses compared with pure WBG in a previous study from our group (Moriel et al. 2015). Recently, however, WBG was suscesfully used as a moisture source to rehydrate and ensile dry ground corn (DGC; Ferraretto et al. 2018). Perhaps DGC would provide adequate substrate concentration for silage fermentation and improve storage and aerobic stability of WBG. Therefore, the objective of this study was to evaluate the fermentation profile, dry matter loss, and aerobic stability of WBG ensiled pure or with increasing concentrations of DGC. Our hypothesis was that both WBG/DGC mixtures would have enough WSC to allow desirable anaerobic fermentation during ensiling, and thereby, reduce DM loss and increase aerobic stability in comparison with pure WBG.

**Material and Methods** Samples of DGC and WBG were obtained at the University of Florida Dairy Unit (Gainesville, FL, USA), weighed separately and mixed following these treatments: pure WBG (**WBG**; 23% of DM concentration), mixture of DGC and WBG targeting for 35% (**M35**) or 50% (**M50**) of DM. Samples were ensiled in laboratory silos (3.78 l buckets) and allowed to ferment for 0, 14 and 28 days. The experiment consisted of 9 treatments (3 mixtures of WBG and DGC × 3 ensiling time points) ensiled in triplicate. Mini-silos were weighed at ensiling and at opening to measure DM loss. Subsequently, a subsample of each mini-silo was collected for pH, fermentation profile, DM and nutrient concentrations and WSC analysis. After subsample collection, temperature sensors were placed in the center of remaining silo material (approximately 2 kg) and set to record the temperature every 30 min for 7 days. Aerobic stability was denoted by hours that elapsed before sample temperature increased by at least 2°C above room temperature (3 sensors monitored room temperature continuously). Data were analyzed as a completely randomized design in a 3 × 3 factorial arrangement of treatments using Proc Glimmix of SAS with treatment, time and their interaction as fixed effects. Laboratory silo was used as the experimental unit. Statistical significance and trends were declared at $P \leq 0.05$ and $P \geq 0.05$ to $P \leq 0.10$, respectively.

**Results** A treatment × time interaction was observed ($P = 0.001$) for DM concentration; DM content of WBG remained constant throughout the ensiling period, but was decreased for M35 and M50 after 28 d of ensiling. As expected, concentrations (DM basis) of CP, ether extract, NDF and ash were greater ($P = 0.001$) for WBG (30.0, 6.9, 49.3 and 4.1%, respectively), intermediate for M35 (18.4, 5.5, 24.1 and 2.6%, respectively) and lower for M50 (13.7, 4.5, 16.4 and 2.1%, respectively). In contrast, starch concentration (DM basis) was greater ($P = 0.001$) for M50 (63.8%), intermediate for M35 (47.6%) and lower for WBG (5.6%). Unexpectedly, WSC concentration was greater for WBG than other treatments ($P = 0.001$; Table 1). Concentration of WSC was greater on 0 compared with 14 and 28 days of ensiling ($P = 0.001$; 11.4 vs. 1.8%, on average). A treatment × time interaction was observed ($P = 0.001$) for pH; pH of WBG was lower, similar and greater than M35 at 0, 14 and 28 days of ensiling, respectively, but lower at 0 and 14 days and similar at 28 days compared with M50. Lactic acid concentration was greatest for WBG and lowest for M50 ($P = 0.001$; Table 1). However, acetic

acid concentration was greater ($P$ = 0.001; Table 1) for M35 compared with other treatments. A time effect was observed ($P$ = 0.001) for both, lactate and acetate concentrations; greater concentration of lactate was observed after 14 and 28 days (5.13% of DM, on average) compared with 0 days (1.70% of DM), whereas acetate concentration was greater for 28 days than 0 days (0.65% vs. 0.22% of DM, respectively). Propionate and butyrate concentrations were not detected. A treatment × time interaction was observed ($P$ = 0.001) for ammonia-N; WBG had greater concentration at 0 days, but lower at 14 and 28 days compared with other treatments. Aerobic stability tended ($P$ = 0.08; Table 1) to be 12 h greater for M50 than M35. In addition, aerobic stability was greater for 28 than 14 days ($P$ = 0.001; 53.7 vs. 44.2 h, respectively). Loss of DM was lower ($P$ = 0.001; Table 1) for WBG than other treatments. Furthermore, DM loss increased with ensiling time ($P$ = 0.001; 2.7 vs. 10.6% for 14 and 28 days, respectively).

**Table 1**. Effect of treatment of fermentation profile, dry matter loss and aerobic stability of wet brewers grains fermented with or without dry ground corn[1]

| Item | WBG | M35 | M50 | SEM | *P*-value |
|---|---|---|---|---|---|
| Lactate, % of DM | 5.15[a] | 4.14[b] | 2.68[c] | 0.51 | 0.001 |
| Acetate, % of DM | 0.26[b] | 0.65[a] | 0.39[b] | 0.08 | 0.001 |
| WSC, % of DM | 6.3[a] | 4.5[b] | 4.1[b] | 0.5 | 0.001 |
| DM loss, % | 1.5[b] | 10.2[a] | 8.2[a] | 1.2 | 0.001 |
| Aerobic stability, h | 51.7 | 41.3 | 54.0 | 5.3 | 0.08 |

[1]Treatments were pure WBG (WBG), mixture of DGC and WBG targeting for 35% (M35) or 50% (M50) of DM.

**Discussion** Greater WSC for WBG than other treatments was unexpected and in disgreement with our previous experiment (Ferraretto et al. 2018). These data highlight that the malting process within and across breweries vary and thus, concentrations of DM and WSC are often unpredictable. Despite no benefits on aerobic stability and greater DM loss, adequate fermentation was achieved for DGC/WBG mixture treatments based on the pH drop observed. Furthermore, lactate and acetate concentrations resemble those of high-moisture corn or rehydrated and ensiled corn. However, further research is warranted to elucidate strategies to reduce DM loss and improve aerobic stability.

**Conclusion** Under the conditions of the present study, mixing WBG with DGC did not improve ensiling of WBG. However, fermentation profile was adequate for WBG/DGC mixtures.

**References**

Ferraretto, L.F., Silva Filho, W.I., Fernandes, T., Kim, D.H. & Sultana, H. (2018) Effect of ensiling time on fermentation profile and ruminal in vitro starch digestibility in rehydrated corn with or without varied concentrations of wet brewers grains. Journal of Dairy Science, 101, 4643-4649.

Moriel, P., Artioli, L.F.A., Poore, M.H. & Ferraretto, L.F. (2015) Dry matter loss and nutritional composition of wet brewers grains ensiled with or without covering and with or without soybean hulls and propionic acid. The Professional Animal Scientist, 31, pp. 559-567.

Orosz, S. & Davies, D.R. (2015) Short and long term storage of wet by-products fed by ruminants, In: Daniel, J. L. P., Morais, G., Junges, D., & Nussio, L. G. (eds.). Proceedings of the XVII International Silage Conference, Piracicaba, SP, Brazil, 2015, pp. 202-242.

# Fermentative profile, microbial and chemical characteristics and aerobic stability of whole crop soybean silage affected by the stage of growth and inoculation with lactic acid bacteria

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**Keywords:** crude protein, fermentation products, fibre digestibility, soybean silage

**Introduction** Finding new sources of home-grown protein is crucial for the future profitability and environmental sustainability of the dairy sector in Italy (Borreani et al. 2013). Soybean meal is the most frequently used source of protein in dairy cow diets, but its overseas origin poses many threats to the economic and environmental impact of milk production. In Italy, soybean is often double cropped with winter cereal, but an autumn harvest is, in most cases very difficult, due to difficulties in reaching the right maturity stages for grain harvesting (Undersander et al. 2007). Directly harvesting whole crop soybean and conserving it through ensiling could partially overcome these adverse effects and offer opportunities to increase the amount of on-farm produced protein (Mustafa and Seguin 2003). The aim of the study was to investigate the effects of stage of maturity and lactic acid bacteria (LAB) inoculant application on the fermentation quality, microbial and chemical characteristics of soybean grain harvested and ensiled as a direct-cut whole crop in the Po plain, northern Italy.

**Material and Methods** The research was conducted at the Research Centre of the University of Turin on loamy-sand soil. Two soybean varieties (VR) with low trypsin inhibitor activity (Ascasubi, VR1, medium-tall plant size, and Aires, VR2, low size plant, SIS, San Lazzaro di Savena, Bologna, Italy) were sown at the end of May (after wheat was harvested as whole-crop for ensilage) and then harvested at two stages of growth (R4-5, complete pod development and R7, early plant maturity). The whole crop was chopped to a theoretical length of 10 mm, using a self-propelled forage harvester (Claas Jaguar 960, equipped with a direct disc whole-crop header). The chopped materials were sampled for analyses and then ensiled as untreated (C) or treated (T) with a mixture of LAB (*Lactobacillus plantarum*, *Pediococcus acidilactici*, and *L. casei*, at an inoculation rate of $3x10^5$ cfu/g of wet weight), in 20-l laboratory silos, which were weighed and kept indoors for about 200 days. After 200 days, the silos were weighed, opened and silage aerobic stability (AS) was determined by monitoring the temperature increases and defined as the number of hours (h) the silage remained stable before rising more than 2°C above room temperature. The DM yield and grain-to-whole-plant ratio was determined at harvesting. The whole-crop was analysed prior to ensiling to establish the water activity, pH, buffering capacity (BC), water-soluble carbohydrates (WSC), starch, microbial counts (yeast, mould and LAB), ash, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, crude protein (CP, Dumas nitrogen 6.25), and in vitro NDF digestibility after 48 h (NDF-D). The silages were analysed, by means of HPLC, to establish the fermentative profiles, pH, ammonia, microbial counts (yeast, mould and LAB), ash, EE, NDF, ADF, lignin, CP and NDF-D. The significance of variety (VR), stage of growth (S) and LAB inoculation (T) effects were analysed via analysis of variance.

**Results and Discussion** The pre-ensiling characteristics of the two VR are reported in Table 1. The VR1 had lower WSC, starch, CP, nitrate and NDF-D and higher ash, NDF, ADF and water activity than VR2. The stage of growth affected almost all the measured characteristics, with the advanced stage of maturity showing a greater DM yield, grain to plant ratio, CP, NDF, ADF, lignin and lower BC, water activity, WSC, and starch and ash contents. All the silages, except the C silage of VR1 at the R4-5 stage, were well preserved. A huge fermentation activity, which led to high lactic and acetic acid contents, especially at the early stage of growth, was observed. The use of LAB inoculum reduced the risk of butyric fermentation, but failed to prevent it in VR1 at a lower DM content (Table 2).

**Conclusion** Directly ensiling whole crop soybean has been shown to be a feasible conservation option. The LAB inoculum reduced, but did not totally prevent butyric fermentation at lower DM contents. Harvesting at the R7 stage of growth increases the DM yields. Moreover, fermentation was found to be good, even without LAB inoculation.

**Table 1.** Pre-ensiling characteristics of two soybean crops at the R4-5 and R7 stages of growth.

| Item | VR1 | | VR2 | | SE | *VR* | *S* | *VR·S* |
|---|---|---|---|---|---|---|---|---|
| | R4-5 | R7 | R4-5 | R7 | | | | |
| DM yield (t $ha^{-1}$) | 5.28 | 5.61 | 4.86 | 5.33 | 0.066 | ** | * | NS |
| Grain/plant ratio | 0.21 | 0.42 | 0.20 | 0.49 | 0.001 | NS | *** | NS |
| DM content (%) | 26.7 | 37.4 | 22.0 | 30.9 | 0.002 | *** | *** | NS |
| pH | 6.34 | 6.05 | 6.13 | 6.14 | 0.029 | NS | *** | *** |
| BC (meq $kg^{-1}$ DM) | 310 | 215 | 313 | 286 | 12.4 | NS | * | NS |
| Water activity ($a_w$) | 0.991 | 0.989 | 0.995 | 0.992 | 0.001 | ** | * | NS |
| Nitrate (mg/kg DM) | <100 | <100 | 666 | 802 | - | - | - | - |
| WSC (% DM) | 3.7 | 3.6 | 7.6 | 5.4 | 0.160 | *** | ** | * |
| Starch (% DM) | 5.0 | 1.4 | 8.1 | 1.2 | 0.061 | *** | *** | *** |
| Crude protein (% DM) | 16.7 | 18.4 | 20.8 | 25.0 | 0.607 | ** | * | NS |
| Ash (% DM) | 11.6 | 9.2 | 8.0 | 7.4 | 0.087 | *** | *** | ** |
| Ether extract (% DM) | 4.7 | 5.9 | 3.1 | 6.8 | 0.057 | * | *** | *** |
| NDF (% DM) | 43.3 | 47.0 | 35.4 | 39.3 | 0.544 | *** | ** | NS |
| ADF (%DM) | 35.6 | 38.3 | 26.7 | 30.6 | 0.483 | *** | ** | NS |
| Lignin (% DM) | 7.6 | 8.9 | 5.7 | 9.1 | 0.218 | NS | *** | NS |
| NDF-D (% NDF) | 47.2 | 46.1 | 52.6 | 53.5 | 0.285 | *** | NS | NS |
| Yeast (cfu/g silage) | 6.51 | 7.31 | 6.46 | 6.73 | 0.085 | NS | * | NS |
| LAB (cfu/g silage) | 7.92 | 9.16 | 8.31 | 8.64 | 0.095 | NS | ** | * |

VR = variety effect; S = stage of growth effect.

**Table 2.** Fermentative, chemical and microbial characteristics of two untreated and LAB inoculated soybean silages harvested at the R4-5 and R7 stages of growth.

| | VR1 | | | | VR2 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R4-5 | | R7 | | R4-5 | | R7 | | | | | | |
| Item | C | T | C | T | C | T | C | T | *SE* | *VR* | *S* | *T* | *VR·S* |
| DM content (%) | 22.9 | 23.9 | 34.1 | 36.2 | 20.5 | 20.9 | 27.2 | 28.1 | 0.154 | *** | *** | * | *** |
| pH | 5.04 | 4.69 | 4.68 | 4.52 | 4.23 | 4.19 | 5.15 | 5.07 | 0.021 | NS | *** | ** | *** |
| Crude protein (% DM) | 18.9 | 18.7 | 20.0 | 20.3 | 21.3 | 21.1 | 24.4 | 24.3 | 0.176 | *** | *** | NS | *** |
| $NH_3$ (% total N) | 12.6 | 10.9 | 5.5 | 5.6 | 12.5 | 12.5 | 16.2 | 14.8 | 0.271 | *** | ** | NS | *** |
| Ash (% DM) | 13.4 | 13.2 | 9.5 | 9.6 | 8.5 | 9.0 | 8.0 | 8.3 | 0.094 | *** | ** | NS | *** |
| NDF (% DM) | 46.7 | 45.8 | 50.6 | 47.0 | 36.8 | 37.4 | 39.3 | 38.7 | 0.270 | *** | ** | * | NS |
| ADF (% DM) | 40.5 | 39.6 | 41.9 | 38.9 | 31.3 | 30.8 | 33.6 | 33.0 | 0.168 | *** | ** | ** | * |
| Lignin (% DM) | 8.1 | 8.4 | 9.0 | 9.2 | 6.3 | 6.0 | 6.4 | 6.7 | 0.093 | *** | ** | NS | NS |
| NDF-D (% NDF) | 45.7 | 46.8 | 46.2 | 47.0 | 52.9 | 52.1 | 51.5 | 49.7 | 0.466 | *** | NS | NS | NS |
| Lactic ac. (g/kg DM) | 14.7 | 43.4 | 37.7 | 54.4 | 116.3 | 122.1 | 17.7 | 25.2 | 2.808 | *** | *** | NS | *** |
| Acetic ac. (g/kg DM) | 54.2 | 63.1 | 46.3 | 37.7 | 70.3 | 67.6 | 70.9 | 69.6 | 1.230 | *** | ** | NS | ** |
| Propionic ac (g/kg DM) | 11.3 | 8.7 | 3.8 | 3.2 | 10.4 | 10.1 | 4.9 | 5.4 | 0.211 | * | *** | NS | NS |
| Butyric ac. (g/kg DM) | 38.7 | 11.3 | 0.0 | 0.0 | 0.0 | 0.0 | 1.9 | 0.1 | 1.564 | ** | ** | * | ** |
| Ethanol (g/kg DM) | 22.0 | 21.7 | 10.0 | 9.4 | 22.3 | 21.9 | 35.3 | 36.6 | 0.363 | *** | NS | NS | *** |
| Weight loss (% DM) | 12.2 | 8.3 | 4.5 | 4.6 | 7.7 | 7.4 | 8.5 | 8.5 | 0.371 | NS | ** | NS | *** |
| Yeast ($log_{10}$ cfu/g) | 2.28 | 2.42 | 1.73 | 1.40 | 1.71 | 1.61 | 1.91 | 1.73 | 0.086 | NS | NS | NS | NS |
| LAB ($log_{10}$ cfu/g) | 7.43 | 7.29 | 7.36 | 6.82 | 6.84 | 6.52 | 7.30 | 6.89 | 0.295 | NS | NS | NS | NS |
| Aerobic stability (h) | >336 | 238 | >336 | >336 | 306 | 284 | 234 | 300 | - | - | - | - | - |

S = stage of growth effect; T = LAB inoculum effect; the VR·T, S·T, and VR·S·T interactions were not significant.

## References

Borreani, G., Coppa, M., Revello-Chion, A., Comino, L., Giaccone, D., Ferlay, A. & Tabacco, E. (2013) Effect of different feeding strategies in intensive dairy farming systems on milk fatty acid profiles, and implications on feeding costs in Italy. J. Dairy Sci., 96, 6840-6855.

Mustafa, A.F. & Seguin, P. (2003) Characteristics and in situ degradability of whole crop faba bean, pea, and soybean silages. Can. J. Anim. Sci. 83: 793-799.

Undersander, D., Jarek, K., Anderson, T., Schneider, N. & Milligan, L. (2007) A guide to making soybean silage. Online. Forage and Grazinglands doi:10.1094/FG-2007-0119-01-MG.

# Grass silage for biorefinery – Effects of type of additive and separation method

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**Keywords:** Enzyme, formic acid, green biorefinery, liquid-solid separation, round bale

**Introduction** There is increasing interest of using grass silage as a feedstock for biorefineries for multiple end-products. The vast information about management factors affecting silage quality originating from the traditional use as feed can be transferred to this new area. Further, the ensiling process could in some cases be used as a pre-treatment for the biorefining process. Rinne et al. (2017, 2018) recently demonstrated that using fibrolytic enzymes improved silage fermentation quality and increased the yield of silage juice in a mechanical liquid-solid separation process when used in combination with formic acid based additive. The objective of the present experiment was to evaluate the effects of different types of additives on the quality of grass silage as a feedstock for a biorefinery process using four different separation techniques to produce liquid and solid fractions from silage, including two methods suitable for farm scale silage fractionating.

**Material and Methods** Grass silage was harvested into ca. 700 kg round bales from 3$^{rd}$ cut in Jokioinen, Finland on 19 September 2016. Silages were prepared without any additive (Control), using a fibrolytic enzyme (E; liquid Flashzyme Plus with cellulase and hemicellulase activities, Roal Ltd., Rajamäki, Finland) at a rate of 1.1 ml kg$^{-1}$ dry matter (DM) or a formic acid based additive (FA; AIV2 Plus, Eastman Chemical Company, Oulu, Finland) at a rate of 6.8 l t$^{-1}$. The bales were stored outdoors with the average daily temperature being -0.5°C ranging from -18.5 to 12.7°C during the ensiling period of 161 days. Three bales were analysed for each additive treatment. After opening, the bales were mixed in a feed mixer wagon for 10 min and representative samples were taken for routine laboratory analyses for chemical composition and fermentation quality. Further, liquid-solid separation which would typically be the first step of a biorefinery process was conducted using four different techniques: Farm scale twin screw press using 100 kg batch from each bale (FTS; Haarslev Industries A/S, Søndersø, Denmark); Farm scale single screw press using 20 kg batch for each bale (FSS; Pellon Group Ltd., Ylihärmä, Finland); Laboratory scale twin screw press (300 g batch, LTS; Angel Juicer Ltd., Busan, South Korea); and Laboratory scale pneumatic press (450 g batch; LPP, Luke in-house built equipment, Jokioinen, Finland).

**Results and Discussion** The DM content of the grass before ensiling was 253 g kg$^{-1}$, the ash, crude protein (CP) and neutral detergent fibre contents were 105, 152 and 446 g kg$^{-1}$ DM, respectively. There were clear differences in the efficiency of the liquid-solid separation between the different methods. The twin screws resulted in higher yield of liquid on fresh matter basis (0.561 for FTS and 0.579 for LTS) than the FSS (0.266) and LPP (0.288). All silages were well preserved but FA clearly restricted the fermentation compared to C and E treatments and FA silage was also the driest and had the lowest CP concentration (Table 1). In previous studies (Rinne et al. 2017, 2018), enzyme application improved silage fermentation quality and liquid yield, but these effects could not be repeated in the current experiment, where the silage was stored in autumn/winter conditions for ca. 5 months. The liquid yield for C, E and FA over all methods was 0.439, 0.431 and 0.401 while 0.202, 0.204 and 0.183 of the original DM and 0.343, 0.345 and 0.240 of the original CP was retained in the liquid, respectively. The lower yields of FA treated silages reflect at least partly the higher DM and lower CP concentrations in that silage. The FA treatment may result in higher effluent losses which could be linked with these changes, although the DM of the silage raw material was above 250 gkg$^{-1}$ which is generally considered the limit when effluent production ceases. Further, the potentially lower losses during storage and higher nutritional quality (e.g. lower $NH_3$-N proportion) of the FA treated silage fractions need to be considered in choosing silage additives for a biorefinery process similarly as when producing feeds for livestock.

**Table 1**. Composition of the silages ensiled in round bales without additive (Control), with fibrolytic enzyme application (Enzyme) or with a formic acid based additive (FA) and the results of mechanical liquid-solid separation of them.

| | | Control | Enzyme | FA | SEM[1] | Mean±SEM[2] |
|---|---|---|---|---|---|---|
| Silage | Dry matter, g kg$^{-1}$ | 235$^{a}$ | 243$^{b}$ | 265$^{c}$ | 1.7 | |
| | pH | 4.76$^{b}$ | 4.82$^{b}$ | 4.51$^{a}$ | 0.057 | |
| | $NH_3$-N, g kg$^{-1}$ N | 84$^{b}$ | 82$^{b}$ | 50$^{a}$ | 4.1 | |
| | Ash, g kg$^{-1}$ DM | 109 | 112 | 113 | 2.6 | |
| | Crude protein (CP), g kg$^{-1}$ DM | 168$^{b}$ | 161$^{b}$ | 142$^{a}$ | 3.6 | |
| | Fermentation acids, g kg$^{-1}$ DM | 81$^{b}$ | 74$^{b}$ | 14$^{a}$ | 2.8 | |
| | Neutral detergent fibre, g kg$^{-1}$ DM | 445 | 448 | 460 | 5.6 | |
| | In vitro OMD, g kg$^{-1}$ OM | 742 | 738 | 746 | 5.5 | |
| Farm scale twin screw press | Liquid yield | 0.564 | 0.539 | 0.580 | 0.0235 | 0.561$^{B}$ |
| | Liquid DM, g kg$^{-1}$ | 113 | 116 | 115 | 1.3 | 115$^{B}$ |
| | Liquid CP, g kg$^{-1}$ DM | 270$^{b}$ | 259$^{b}$ | 188$^{a}$ | 3.5 | 239 |
| | DM retained in liquid | 0.268 | 0.256 | 0.274 | 0.0164 | 0.266$^{B}$ |
| | CP retained in liquid | 0.408 | 0.411 | 0.342 | 0.0203 | 0.387$^{B}$ |
| Farm scale single screw press | Liquid yield | 0.288$^{b}$ | 0.282$^{b}$ | 0.229$^{a}$ | 0.0089 | 0.266$^{A}$ |
| | Liquid DM, g kg$^{-1}$ | 115 | 118 | 118 | 1.1 | 117$^{B}$ |
| | Liquid CP, g kg$^{-1}$ DM | 270$^{b}$ | 262$^{b}$ | 190$^{a}$ | 3.9 | 241 |
| | DM retained in liquid | 0.138$^{b}$ | 0.136$^{b}$ | 0.102$^{a}$ | 0.0043 | 0.125$^{A}$ |
| | CP retained in liquid | 0.218$^{b}$ | 0.218$^{b}$ | 0.133$^{a}$ | 0.0087 | 0.190$^{A}$ |
| Lab scale twin screw press | Liquid yield | 0.595$^{b}$ | 0.596$^{b}$ | 0.546$^{a}$ | 0.0082 | 0.579$^{B}$ |
| | Liquid DM, g kg$^{-1}$ | 129$^{a}$ | 134$^{ab}$ | 139$^{b}$ | 1.8 | 134$^{C}$ |
| | Liquid CP, g kg$^{-1}$ DM | 265$^{c}$ | 250$^{b}$ | 187$^{a}$ | 2.6 | 234 |
| | DM retained in liquid | 0.274$^{b}$ | 0.288$^{b}$ | 0.251$^{a}$ | 0.0045 | 0.271$^{B}$ |
| | CP retained in liquid | 0.534$^{b}$ | 0.537$^{b}$ | 0.375$^{a}$ | 0.0136 | 0.482$^{C}$ |
| Lab scale pneumatic press | Liquid yield | 0.308$^{b}$ | 0.308$^{b}$ | 0.248$^{a}$ | 0.0058 | 0.288±0.0078$^{A}$ |
| | Liquid DM, g kg$^{-1}$ | 105$^{a}$ | 108$^{b}$ | 108$^{ab}$ | 0.8 | 107±0.7$^{A}$ |
| | Liquid CP, g kg$^{-1}$ DM | 271$^{c}$ | 256$^{b}$ | 185$^{a}$ | 3.1 | 237±1.9 |
| | DM retained in liquid | 0.126$^{ab}$ | 0.134$^{b}$ | 0.103$^{a}$ | 0.0059 | 0.121±0.0066$^{A}$ |
| | CP retained in liquid | 0.212$^{b}$ | 0.215$^{b}$ | 0.111$^{a}$ | 0.0107 | 0.179±0.0207$^{A}$ |

[1]SEM = Standard error of the mean. Means within the same row without same superscript differ significantly (P<0.05, Tukey test).
[2]For each individual variable, means in the same column without same capital superscript letter differ significantly (P<0.05, Tukey test).

**Conclusion** In this experiment, we could not confirm the positive effect of fibrolytic enzyme addition on fermentation quality or the liquid-solid separation of grass silage. The reasons for this could likely be due to the low temperature during storage, being considerably below the optimum temperature of the used enzymes (45-50 °C), or potentially also due to differences in silage raw material. The results demonstrate that the method used for liquid-solid separation greatly influences the separation of the liquid fraction. Almost 40% of the CP in the original silage could be retained in the liquid fraction with farm scale twin screw press.

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**References**

Rinne, M., Winquist, E., Pihlajaniemi, V., Niemi, P., Seppälä, A. & Siika-aho, M. (2017) Fibrolytic enzyme treatment prior to ensiling increases press-juice yield from grass silage. Nordic Feed Science Conference 13-14 June 2017. Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management. Report 296. pp. 71-76. http://www.slu.se/globalassets/ew/org/inst/huv/nfsc/nfsc-2017-proceedings.pdf

Rinne, M., Jalava, T., Stefanski, T., Kuoppala, K., Timonen, P., Winquist, E. & Siika-aho, M. (2018) Optimizing grass silage quality for green biorefineries. 27th European Grassland Federation General Meeting, Cork, Ireland 17-21 June 2018. www.europeangrassland.org.

# Grass silage for biorefinery – Palatability of silage juice for growing pigs and lactating cows

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**Keywords:** Green biorefinery, liquid feed, milk, pork, potassium

**Introduction** In a green biorefinery approach, liquid feed can be produced from grass silage by simple liquid-solid separation. The silage juice contains soluble components from the grass silage the main components being minerals (ash), soluble crude protein, fermentation end products (lactic acid, volatile fatty acids, ethanol) and possibly water soluble carbohydrates depending on the concentration in the original silage, and it has a pH typically in the range of 4-4.5. Silage juice can be used for pig feeding where it provides nutrients, stabilizes pH of the liquid feed and may help maintaining intestinal health of pigs. Inclusion of grass derived feeds in pig diets provides opportunity to include grass into crop rotation in pig farms and brings new source of non-human edible feed into pig diets. In ruminant feeding, silage juice can be used e.g. to fortify the total mixed ration (TMR) of dairy cows thus increasing the forage based feeds in their diets without compromising feed intake and milk production. Potentially the amino acids included in the liquid fraction may escape rumen degradation and could increase the intestinally absorbable amino acids derived from the forage. The aim of this study was to demonstrate the suitability of silage juice in pig and dairy cow feeding and to evaluate the palatability of it.

**Material and Methods** The grass silage used was made from a mixed timothy-meadow fescue sward which was precision chopped and ensiled into a clamp using a formic acid based additive (AIV 2 Plus, Eastman Chemical Company, Oulu, Finland) in Jokioinen, Finland. The silage was fractionated into liquid and solid fractions at farm scale using a twin screw press (Haarslev Industries A/S, Søndersø, Denmark). The composition of the liquid used in the palatability trials as well as the solid fraction and the original silage are shown in Table 1. The methods used for laboratory analyses have been described by Seppälä et al. (2016). The pig palatability trial was conducted using eight fattening pigs (live weight 52 kg, SD 3.8) grouped in a pen. They received increasing amounts of silage juice containing 8 MJ net energy (NE) $kg^{-1}$ dry matter (DM), and 186 g $kg^{-1}$ DM crude protein for 5 days in addition to a complete feed diet containing 11.1 MJ NE $kg^{-1}$ DM, and 176 g $kg^{-1}$ DM crude protein so that the amount of liquid feed reached 4 l $pig^{-1}$ $day^{-1}$ in the end of the trial period which gave the pigs 11.6% of the feed energy from silage juice. In the palatability trial on dairy cows, five cows (live weight 692 kg (s.d. 80.0), energy corrected milk production 28.1 (SD 3.67) kg per day and 408 days in milk (SD 61.6)) in individual pens were used. The cows received 20 kg of silage juice per day in 2 portions for 4 days on top of their normal diet (*ad libitum* intake of grass silage with average consumption of 10.9 (SD 2.13) kg DM $day^{-1}$, 8.2 (SD 0.48) kg concentrate DM $day^{-1}$ and free access to water).

**Results and Discussion** The yield of liquid in the separation of the liquid and solid fractions was 0.488 on fresh matter basis from the amount of original silage. From DM, ash and CP present in the original silage, 0.182, 0.774 and 0.575, respectively, were captured in the juice. When silage juice was mixed with the complete feed, pigs consumed max. 400 g juice DM $day^{-1}$. The daily growth rate was not compromised. After second day in the trial, the faeces of the pigs started to become loose but it became normal two days after the trial period ended. The loose faeces was probably due to too high potassium (K) intake as the concentration of K in the liquid feed was 7 g $kg^{-1}$. Also the crude protein content in the mixture of grower feed and juice was relatively high (183 g $kg^{-1}$ DM) which may have affected the consistency of faeces. The silage juice contained 6.6 g $kg^{-1}$ formic acid, which originated from the silage as a formic acid based additive had been used at the time of ensiling. At the end of pig palatability trial the formic acid content in the mixture of complete feed and silage juice was 1 g $kg^{-1}$. The recommended maximum level of formic acid as feed preservative for pigs is set to 12,000 mg $kg^{-1}$ (feed moisture 120 g $kg^{-1}$) and that in drinking water

4,000 mg $l^{-1}$ in the EU (EFSA 2014). The formic acid may aid in stabilizing the juice and even the other feeds it is mixed with. Formic acid may also commercially be used in pig farms to stabilize liquid feed and to improve the intestinal health of pigs. It has been shown to reduce the *Enterobacteriaceae* counts in the liquid feed (Canibe et al. 2007). As the formic acid does not affect yeasts in liquid feed (Canibe et al. 2001), commercially liquid feed may be treated with mixture of organic acids reducing the yeast and mould growth. The commercially used amounts of organic acids usually are 1-1.5 g $kg^{-1}$ liquid feed. If the concentration of formic acid is above 2 $gkg^{-1}$, it may limit feed intake of pigs. The average juice consumption of the dairy cows was 14.7 kg $day^{-1}$. One cow consumed all juice every day and for other cows, no clear pattern in consumption could be detected. The intake of other feeds or milk production were not affected by the trial period when compared with the time before and after the juice inclusion. In this case, the juice was offered from separate containers. Another option could be to mix the juice in TMR. Water is often added to TMR to decrease the separation of concentrate and forage particles so that could be a practical approach on dairy farms, and it could potentially also increase the stability of TMR.

**Table 1**. Composition of the silage juice, solid fraction and original silage.

| | Original silage | Solid fraction | Liquid fraction |
|---|---|---|---|
| Dry matter, g $kg^{-1}$ | 264 | 428 | 100 |
| In dry matter, g $kg^{-1}$ | | | |
| Ash | 102 | 71 | 255 |
| Crude protein | 126 | 117 | 166 |
| NDF | 547 | 645 | Nd* |
| Water soluble carbohydrates | 37 | 18 | 120 |
| Ethanol | 6 | 2 | 21 |
| Lactic acid | 55 | 25 | 183 |
| Acetic acid | 21 | 9 | 65 |
| Propionic acid | 0.5 | 0.3 | 0 |
| Butyric acid | 0.4 | 0.2 | 0.4 |
| In total N, g $kg^{-1}$ N | | | |
| Soluble N | 347 | 232 | Nd* |
| Ammonium-N | 47 | 23 | 102 |
| *In vitro* cellulase solubility | 775 | 739 | Nd* |

*Not determined. By definition, silage juice is totally soluble

**Conclusion** Silage juice could successfully be included in the diets of fattening pigs and lactating dairy cows in a short term palatability trial. The high K content may be a limiting factor determining the daily portion of silage juice suitable for pigs. To determine the production responses, longer term feeding experiments should be conducted.

**References**

Canibe, N., Miquel, N., Miettinen, H. & Jensen, B.B. (2001) Addition of formic acid or starter cultures to liquid feed. Effect on pH, microflora, composition, organic acid concentration and ammonia concentration. Medical Faculty Landbouw, University of Gent, 66/3b, 431-434.

Canibe, N., Virtanen, E., & Jensen, B. B. (2007). Effect of acid addition to pig liquid feed on its microbial and nutritional characteristics. Livestock Science, 108, 202-205.

EFSA FEEDAP Panel (2014) Scientific Opinion on the safety and efficacy of formic acid when used as a technological additive for all animal species. EFSA Journal, 12, 3827-3851.

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M. (2016) Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass and Forage Science, 71, 458-471.

# Influence of ensiling and thermal treatment of peas on their feed value

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**Keywords:** Ensiling, heat treatment, legume grain

**Introduction** The cultivation of grain legumes has increased in recent years in Germany. In addition to the cultivation benefits, state subsidy programs have contributed to this trend. To expand the use of locally produced grain legumes for the feeding of farm animals, processing may enhance the nutritional value. High starch contents and high protein solubility (PS) limit the possible use in ruminant diets. The combination of ensiling and toasting might be a promising method for the on-farm processing of grain legumes. The objective of our study was to examine the effects of ensiling and heat treatment on the characteristics of starch and protein especially with respect to their ruminal degradation. We hypothesized that both treatments enhance the starch and protein stability in the rumen and thus improve the feed value of grain legumes.

**Material and Methods** ***Study A*** Grains of two pea varieties (flower colour: white vs. coloured) were re-moistened to 70% of dry matter (DM) and ground. They were ensiled in the following treatments: control, lactic acid bacteria (LAB) 1 (mixture of *Lactobacillus plantarum* & *P. acidilactici* strains), LAB 2 (*L. plantarum* LMG 18053). They were fermented as Rostock Model Silages (Hoedtke and Zeyner 2011) for 2 months. The resulting silages were then either not further treated or toasted at laboratory scale at different temperatures and for different time periods. The effect of the treatments was evaluated by chemical analysis (proximate composition and crude protein fractions at Licitra et al. 1996). Additional chemical analysis (dry matter [DM], crude protein [CP], true protein [TP], protein solubility [PS]). Starch and sugar were carried carried out polarimetrically according to VDLUFA (2012). The statistical analysis was carried out using SAS 9.4 (MIXED), considering fixed effects of varieties, treatment and inoculants (subordinated to treatment), interaction effects were considered at the $P<0.05$ significance level. ***Study B*** Mini model silages of the green pea variety Astronaute (30% DM) applying the above mentioned treatments (native, ensiled LAB1 and LAB2, ensiled and toasted and native toasted) were prepared and samples scanned by electron microscopy.

**Results and Discussion** ***Study A*** During ensiling, the pH was lowered to < 4.8 only when LAB were inoculated. The LAB treatments had higher lactic acid content (7.2 g/kg DM vs. 18.2 g/kg DM; $P<0.05$) and lower ethanol contents ($P<0.05$) than the control. No butyric acid was formed and ammonia production was negligible (< 1.4 g/kg CP). The LAB-treated silages were stable under aerobic conditions for at least 7 days. Only a treatment of the ensiled material at >120 °C for 30 min reduced the PS ($P<0.05$) (native: 87% of CP; ensiled + toasted at 120 °C for 30 min: 67% of CP; ensiled + toasted at 200 °C for 30 min: 39% of CP) and increased the proportion of the CP fraction B2. Table 1 shows results of selected features of the variety Alvesta as related to treatment. There were no differences in these characteristics with respect to the applied LAB. The data of both treatments were therefore combined. The silages were toasted at 160 °C for 30 min. The CP and sugar contents remained unchanged (Table 1). The differences in starch content probably due to the lack of regression equations for grain legumes. The silages showed a reduction in PS after ensiling. The additional heat treatment increased the effect on the PS significantly ($P<0.05$). Heat treatment without ensiling showed no effect on the PS (Table 1). Consequently, the treatment must have increased the proportion of slowly soluble CP fractions (B2, B3) and reduced the ruminal CP degradation. This relationship cannot be clearly recognized by the contents of the TP of the combined treatments.

**Table 1.** Selected characteristics of different treatments of the field pea Alvesta.

| | native | ensiled *(LAB)* | ensiled *(LAB)* + toasted | toasted | | |
|---|---|---|---|---|---|---|
| | n=18 | n=31 | n=13 | n=3 | SEM | P-value |
| DM [g/kg] | 805.0[b] | 658.1[c] | 774.3[b] | 909.6[a] | *11.15* | <0.001 |
| CP [g/kg DM] | 205 | 209 | 206 | 213 | *2.74* | 0.066 |
| TP [g/kg DM] | 176[ab] | 169[b] | 173[b] | 188[a] | *10.4* | 0.138 |
| PS [%] | 71.5[a] | 65.9[b] | 58.9[c] | 75.0[a] | *1.17* | <0.001 |
| Starch [g/kg DM] | 512[c] | 543[b] | 562[a] | 542[ab] | *30.4* | <0.001 |
| Sugar [g/kg DM] | 44.7 | 44.6 | 43.7 | 44.6 | *2.11* | <0.001 |

[a, b] different letters indicate significant difference with P<0.05; LAB, lactic acid bacteria; TP, true protein; PS, protein solubility

The scanning electron microscopy pictures of ***Study B*** showed no difference between the control (not shown) and the starting material (Fig. 1 a). The addition of lactic acid bacteria apparently reduces the protein-lipid-complex (Fig. 1 b). The starch granules remained unchanged regardless of the amylolytic activity. Heat treatment leads to a modification in the starch granules (Fig. 1 c).

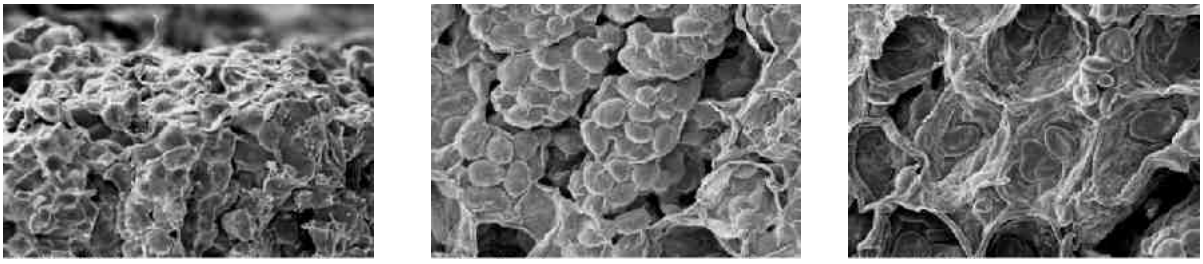

Figure 1 (a, b, c): scanning electron microscopy pictures of a green pea Astronaute (30 % DM) in the treatments native (a), ensiled with lactic acid bacteria (LAB) (b) and ensiled (LAB) + toasted (160°C, 30 min) (c)

The morphological changes were strongly dependent from the treatment intensity (ensiling duration, seed quantity to be toasted, temperature and duration).

**Conclusions** Morphological alterations induced by treatment were observed. However, these are heavily dependent on treatment intensity. The results indicate an increase in rumen undegradable CP for ruminant diets for peas through ensiling plus thermal treatment.

**References**

Hoedtke, S. & Zeyner, A. (2011) Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. Journal of the Science of Food and Agriculture, 91, 841-849.

Licitra, G., Hernandez, T.M. & Van Soest, P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science Technology, 57, 347-358.

VDLUFA 2012. Die chemische Untersuchung von Futtermitteln. Methodenbuch. Vol. 3. Darmstadt. VDLUFA-Verlag.

# Laboratory silo type and inoculation effects on nutritional composition, fermentation, and bacterial and fungal communities of oat silage

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**Keywords:** inoculant, mini-silo type, next generation sequencing

**Introduction** Vacuum bags and fixed volume vessels are common methods for screening additives in silage. In contrast to other silage crops, little is known about silo type effects on of small grain cereals like oats (*Avena sativa* L.). Furthermore, there are few reports of the use of next generation sequencing in characterizing silage ecology. The objectives were to evaluate: 1) the use of 2 types of experimental silos (S) to characterize whole-crop oats silage with or without addition of an inoculant (I), and 2) the effect of inoculation on the microbial community structure of oats ensiled using only plastic bucket silos (BKT).

**Materials and Methods** From each of 6 sections in a field, oats were mowed at the heading stage, wilted for 21 hours (h) to 45% DM, and chopped to a 1.3 cm theoretical length of cut. Material collected from each section was divided into 4 piles and each forage pile was randomly assigned to a 2 (mini-silo types) × 2 (inoculation) factorial arrangement of treatments in a complete randomized design replicated 6 times. Mini-silo types were embossed vacuum bags (BAG) and 19-l buckets (BKT) and inoculation was done with either an inoculant that contained *Lactobacillus buchneri* and *Pediococcus pentosaceus* ($4 \times 10^5$ and $1 \times 10^5$ colony forming units [cfu]/g of fresh oats, respectively; INO) or sterile double distilled water (CON). Sonication was used for microbial extraction (Gutierrez-Rodriguez et al., 2012) and the 16S rRNA V4 (bacteria) and ITS-1 (fungi) regions were amplified. The libraries were sequenced on a Illumina MiSeq platform and analysed with QIIME 1.9.1 using the GreenGenes 13.8 and UNITE fungal ITS reference database. We used the GLM procedure of SAS v.9.4 for data analysis. When a two-factor interaction effect was present, we used the SLICE option to analyse the simple effects. Mean separation was based on the PDIFF procedure of LSMEANS.

**Results and Discussion** At d 0 there were no differences between BAG and BKT, and between INO and CON on DM (44.0 ± 1.0%), pH (6.11 ± 0.07), and counts (cfu/g of fresh oats) of lactic acid bacteria (9.28 ± 0.13), yeasts (4.88 ±0 .09), and molds (3.97 ± 0.08). *Leuconostocaceae* (82.9 ± 4.27%) and *Enterobacteriaceae* (15.2 ± 3.52) were the predominant bacterial families and unidentified sequences were predominant for fungi. A higher relative abundance (RA) of the *Davidiellaceae* fungal family (34.3 vs. 19.6 ± 4.47%) was observed in INO vs. CON. Some differences existed between BAG vs. BKT at silo opening (217 days), including lower CP (7.73 vs. 7.04 ± 0.247% of DM) and ethanol (1.93 vs. 1.55 ± 0.155) and greater lactic acid (3.65 vs. 4.28 ± 0.241), respectively ($P < 0.05$). Also, WSC and mold counts were lower in BAG vs. BKT for CON (1.78 vs. 2.70 ± 0.162% of DM and 0.8 vs. 2.82± 0.409 log cfu/fresh g) but not for INO (~1.53 and 1.55), respectively ($P < 0.05$). Application of INO increased DM recovery (96.1 vs. 92.9 ± 0.63%), aerobic stability (565 vs. 133 ± 29.2 h), acetic acid (2.38 vs. 1.22 ± 0.116% of DM), and reduced NDF (65.0 vs. 67.0 ± 0.57), ADF (36.7 vs. 38.1 ± 0.60), ethanol (0.63 vs. 2.85 ± 0.155) and yeast counts (1.10 vs. 4.13 ± 0.484) in INO vs. CON, respectively ($P < 0.05$). Greater DM recovery in INO

compared to CON could be partially explained by a lower ethanol production indicating less inefficient secondary fermentation by yeasts and heterofermentative bacteria and a similar production of lactic acid that assured rapid acidification. At 217 days, INO had a lower RA of *Leuconostocaceae* (42.3 vs. 95.8 ± 4.64) and higher of *Lactobacillaceae* (57.4 vs. 3.9 ± 4.65) vs. CON. In CON, *Weissella* and *Leuconostoc* were the prevailing identifiable genera for *Leuconostocaceae* (6.83 and 4.54%, respectively) and for *Lactobacillaceae* was *Pediococcus* (2.43%). In the case of INO, the predominant genus for *Lactobacillaceae* was *Lactobacillus* (56.9%) and *Weissella* and *Leuconostoc* were the prevailing identifiable genera for *Leuconostocaceae* (4.11 and 2.82%). A tendency for INO to increase the RA of an unidentified sequence of the *Ascomycota* phyla was observed, when compared to CON (11.3 vs. 0.5 ± 4.00%, respectively). No effect of INO ($P > 0.21$) was observed for *Pichiaceae* (~61.9 ± 20.68%), *Trichocomaceae* (~7.9 ± 6.71%), an unidentified sequence from the *Saccharomycetales* order (~13.9 ± 11.39%, respectively), and unidentified fungi (~1.5 ± 0.96%), when compared to CON. At day 0 vs. 217, we observed a clear separation and difference in the distribution and structure of the bacterial and fungal community using the weighted UniFrac and Bray-Curtis principal coordinates analysis plot, respectively. However, between CON and INO differences were only observed for bacteria at day 217.

**Conclusions** Despite some differences were found between BKT and BAG, both techniques can be comparable for characterizing effects of INO on the most basic measures used in silage evaluation. The use of inoculant improved oats silage quality partially by a shift in the bacterial community composition during ensiling which mainly consisted of an increased RA of *Lactobacillaceae* and reduction of *Leuconostocaceae* relative to CON.

**References**

Gutierrez-Rodriguez, E., Gundersen, A., Sbodio, A.O. & Suslow, T.V. (2012) Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of *Escherichia coli* on spinach. Journal of Applied Microbiology, 112, 109-118.

# Nutritive value and fermentation characteristics of sweet sorghum silage

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**Keywords:** Nutritive value, silage, sweet sorghum

**Introduction** Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a potential bioenergy crop with high biomass and sugar yields. The high concentration of soluble sugars in the stem can be converted to ethanol and the remaining bagasse could also be used for advanced biofuel production. However, due to the favorable agronomic characteristics, there is interest to test the potential of using sweet sorghum as forage for livestock. It is hypothesized that the increased concentration of soluble, highly fermentable sugars in the sweet sorghum stem would result in silage with superior fermentation characteristics. In addition, even if the juice from the stem is extracted to biofuel production, the bagasse may still have sufficient soluble sugar concentration to produce silage with desirable nutritive value and fermentation characteristics. The objective of this study was to test the nutritive value and fermentation characteristics of sweet sorghum silage with different degrees of processing.

**Material and Methods** The experimental area was located at the Range Cattle Research and Education Center, Ona, FL, USA. The sweet sorghum cultivar 'EJ 7281' (Blade Energy Crops, Thousand Oaks, CA, USA) was seeded on May 8 2013 in 5 · 4 m plots to achieve plant population of 88,000 plants/ha. Plots were fertilized with 135 kg N (divided in three applications), 61 kg $P_2O_5$, and 120 kg $K_2O$ $ha^{-1}$ (divided in two applications). Irrigation was applied as needed when rainfall was < 25 mm per week. The harvest occurred at early hard dough stage on October 7 2013. Treatments were: Whole plant (WP), stem (ST), or bagasse (BAG, stem was squeezed and juice removed) in a randomized complete block design with 4 replicates. The mini-silos (PVC pipes with rubber caps with capacity of 2 kg of green forage) were filled immediately after harvest and chopping the forage at approximately 5-cm particle size. The silos were opened on January 4 2014. Forage samples were ground in an Udy mill (Udy Corporation, Fort Collins, CO, USA) to pass a 1-mm screen. Samples of the ensiled forage were analyzed for dry matter (**DM**), crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), in vitro true digestibility (**IVTD**), neutral detergent fiber digestibility (**NDFD**), $NH_3$-N, pH, and volatile fatty acids (**VFA**) concentrations. Silage samples destined for microbial analysis will be placed in bags, heat sealed, and analyzed for yeast and mold counts (AOAC, 2005) at the Dairyland Laboratory Inc. (Sauk Rapids, MN, USA).

**Results** The BAG treatment had the greatest ($P$<0.05) DM concentration, followed by WP and ST had the least DM concentration. The WP had the greatest CP concentration ($P$=0.003) but there was no difference ($P$=0.12) between ST and BAG. The BAG had the greatest levels of ADF and NDF while there was no difference between ST and WP ($P$<0.35). The WP had the greatest ($P$<0.01) IVTD, followed by ST, and BAG had the least IVTD concentrations. The WP had the greatest ($P$<0.01) levels of lactic acid and the least pH but there was no difference ($P$=0.09) in acetic acid concentration between WP and ST. The BAG had the least levels of lactic and acetic acid and the greatest levels of propionic acid. There was no difference ($P$<0.69) in butyric acid and ammonia (g $kg^{-1}$ N) concentrations among treatments. The BAG had greater ($P$=0.04) yeast count than ST and WP and there was no difference in mold count among treatments ($P$=0.69).

**Table 1.** Nutritive value of sweet sorghum silage with different degrees of processing.

| Item | Treatment | | | *P* value | SE |
|---|---|---|---|---|---|
| | Whole Plant | Stem | Bagasse | | |
| DM, g kg$^{-1}$ | 250b | 210c | 280a | 0.006 | 0.7 |
| NFC, g kg$^{-1}$ | 251a | 242a | 140b | 0.02 | 18.0 |
| CP, g kg$^{-1}$ DM | 55a | 31b | 28b | 0.003 | 1.9 |
| ADF, g kg$^{-1}$ DM | 420b | 456b | 518a | 0.04 | 25.1 |
| NDF, g kg$^{-1}$ DM | 587b | 615b | 737a | 0.01 | 21.5 |
| IVTD, g kg$^{-1}$ DM | 600a | 560b | 510c | 0.01 | 5.0 |
| NDFD, g kg$^{-1}$ DM | 530 | 520 | 520 | 0.64 | 10.0 |

[a,b, c] Means within rows with different superscripts differ (*P*<0.05).

**Table 2.** Fermentation characteristics of sweet sorghum silage with different degrees of processing.

| Item | Treatment | | | *P* value | SE |
|---|---|---|---|---|---|
| | Whole Plant | Stem | Bagasse | | |
| pH | 3.6b | 3.8a | 3.8a | 0.04 | 0.04 |
| Lactic acid, g kg$^{-1}$ DM | 37a | 30b | 20c | 0.01 | 3.4 |
| Acetic acid, g kg$^{-1}$ DM | 23a | 24a | 20b | 0.02 | 2.3 |
| Propionic acid, g kg$^{-1}$ DM | 0.6b | 1.0b | 4.0a | 0.009 | 0.4 |
| Butyric acid, g kg$^{-1}$ DM | 0.2 | 0.2 | 0.2 | 0.69 | 0.01 |
| Ammonia, g kg$^{-1}$ CP | 50 | 50 | 50 | 0.79 | 5.0 |
| Mold, log cfu g$^{-1}$ | 28 | 29 | 27 | 0.59 | 1.2 |
| Yeast, log cfu g$^{-1}$ | 34b | 41b | 63a | 0.04 | 6.6 |

[a,b] Means within rows with different superscripts differ (*P*<0.05).

**Discussion** The presence of leaves and juice in the WP silage increased nutritive value and fermentation characteristics of the silage when compared to the ST and BAG. Amer et al. (2012) compared the feeding value of sweet sorghum and alfalfa (*Medicago sativa* L.) silage and observed that dry matter intake, energy-corrected milk production and milk efficiency did not differ between the treatments, confirming the superior feeding value of sweet sorghum silage. Although the BAG had decreased nutritive value, VFA concentrations, and greater yeast count, the silage was relatively well-preserved and could still be used to feed animals with decreased nutrient requirements or be used as roughage in high concentrate diets.

**Conclusions** The bagasse by-product from sweet sorghum sugar extraction can be conserved as silage and may have merit to be fed to livestock. It is likely that the bagasse silage will be more economically attractive than WP because of the potential income generated from the juice and biofuel production.

**References**

Amer, S., Seguin, P. & Mustafa, A.F. (2012) Effects of feeding sweet sorghum silage on milk production of lactating dairy cows. Journal of Dairy Science, 95, 859-863.

# Reducing hydrocyanic acid in roots and leaves of cassava by ensiling

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**Keywords:** Cassava, ensiling, hydrocyanic acid

**Introduction** Cassava (*Manihot esculenta* Crantz) is a widely grown crop in many countries in tropical regions. It is extremely reliable to grow, especially on sloping rain-fed soils of low fertility, survives drought periods and grows well with limited water (Nhu Phuc et al. 2000). The roots and leaves of cassava are rich in nutrients and are often used as food and feed. However, the contents of hydrocyanic acid (HCN) in fresh roots and leaves of cassava are high, being 15-400 mg $kg^{-1}$ (Rogers 1963) and 100-1100 mg $kg^{-1}$ (Shen 1991), respectively. High content of HCN limits effective cassava use due to its strong toxicity to humans and animals. In order to improve the utilization of cassava, the effects of ensiling time and temperatures on the silage fermentation and HCN contents of cassava roots and leaves were studied.

**Material and Methods** Cassava, sown on an experiment field of South China Agricultural University (Guangzhou, China), was harvested after 8 months growth and roots and leaves were collected for analyses and silage making as described in detail elsewhere (Tian et al. 2017). In experiment 1, the effects of ensiling time on the silage fermentation and HCN content were studied. The silage samples were taken for the measurements after ensilage for 7, 14, 21, 28 and 56 days, respectively. In experiment 2, the effects of ensiling temperatures on silage fermentation and HCN content were studied. The silages kept at 20, 30 or 40°C for 56 days were investigated. All data were analyzed by one-way analysis of variance using the Statistical Packages for the Social Sciences (SPSS 18.0 for Windows). The means were then compared for significance by Duncan's multiple range method.

**Results and Discussion** Ensiling time had significant effects on pH, lactic acid content and HCN content in both roots and leaves of cassava ($P$<0.01). With the increase of ensiling time, pH values and HCN contents decreased, and HCN reduction mainly took place in the first 14 days of ensiling (Figure 1).

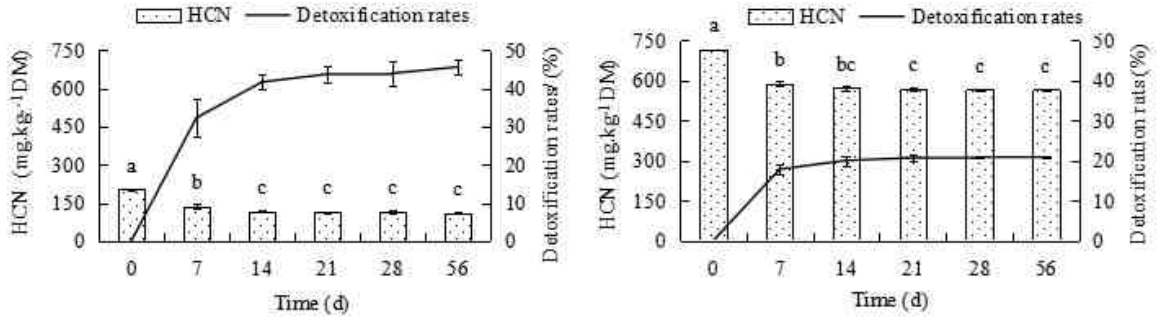


**Figure 1.** HCN contents and detoxification rates of cassava roots (left) and leaves (right) during ensiling.

Linamarase, which hydrolyses cyanogenic glycosides, is very active at a pH range of 5-6, but no hydrolysis of HCN takes place at pH of 2-4 (Oke 1994). That is, the low pH at the later stage of ensiling would not improve the hydrolysis of HCN. Ensiling temperature had significant effects on pH, lactic and acetic acids contents of cassava leaves ($P$<0.01), but in roots these variables were not affected. With the rise in ensiling temperature, the reduction of HCN was significantly enhanced, and it reached 558 g $kg^{-1}$ for roots and 636 g $kg^{-1}$ for leaves at 40°C (Figure 2). The high temperature promoted the reduction of HCN, which was consistent with results reported by Nambisan (1994).

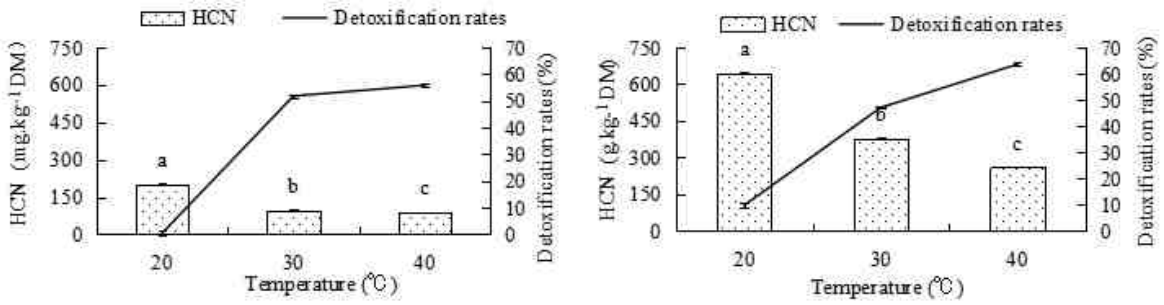


**Figure 2.** HCN contents and detoxification rates of cassava roots (left) and leaves (right) silages at different temperatures.

**Conclusions** Ensiling could reduce HCN content either in roots or leaves of cassava and such reductions mainly took place in the first 14 days of ensiling. In addition, high ensiling temperature intensified HCN reduction.

**References**

Nambisan, B. (1994) Evaluation of the effect of various processing techniques on cyanogen content reduction in cassava, Acta Horticulturae, 375,193-202.

Nhu Phuc, B.H., Ogle, B. & Lindberg, J.E. (2000) Effect of replacing soybean protein with cassava leaf protein in cassava root meal based diets for growing pigs on digestibility and N retention. Animal Feed Science and Technology, 83, 223-235.

Oke, O.L. (1994) Eliminating cyanogens from cassava through processing: technology and tradition. Acta Horticulturae, 375, 163-174.

Rogers, D.J. (1963) Studies of *Manihot esculenta* Crantz (cassava) and related species. Bulletin of the Torrey Botanical Club, 90, 43-54.

Shen, Y.R. (1991) Using cassava leaves as feed. Guangdong Journal of Animal and Veterinary Science, 4, 34-37.

Tian, J., Zhu, L., Dong, Z.X., Wang, X.Y. & Zhang, J.G. (2017) Effects of treatment methods on the hydrocyanic acid content and nutrient composition of cassava roots. Acta Agrestia Sinicca, 25, 875-879.

# True protein conservation in a forage legume comparing drying to ensiling

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**Keywords:** drying, *Medicago sativa*, proteolysis, silage additives, true protein

**Introduction** Crude protein (CP) quality plays an increasing role with rising animal performance. During ensiling plant enzymes and microbes degrade forage protein. This is particularly important for high protein forages such as lucerne. It was hypothesized that there exist conservation alternatives which preserve the true protein fraction of the original forage plant better than any ensiling treatment. The aim of the study was to compare ensiling and drying (field drying and technical drying at different temperatures) of lucerne (*Medicago sativa*) regarding their impacts on protein quality variables.

**Materials and Methods** For drying and ensiling, lucerne (variety Plato) was cut in June 2016 in Northern Saxony, Germany. It was field-wilted for 20 h, then harvested and chopped. Part of the forage was further wilted to achieve ≥ 400 g/kg dry matter (DM) and a third part was wilted until achieving field-dried hay. Lucerne of the two wilting levels 350 and 450 g/kg DM was then further dried in forced air drying ovens at 40, 80 or 180 °C respectively, in triplicates until achieving ≥ 860 g/kg DM. For ensiling, the two wilting levels were treated as follows: control, *Lactobacillus plantarum* (biological, B), buffered formic acid (chemical, C), combination of *L. plantarum* + formic acid (BC), $NaNO_2$ + hexamethylenetetramine (NA), ByProQ (water extracted tannin from quebracho) at 30 g/kg forage DM. The ensiling trial was repeated in August 2016. The forage was ensiled in glass jars in triplicates for 60 d. Dried and ensiled lucerne was analyzed chemically including CP fractionation (Licitra et al., 1996), where A means the easily soluble non-protein nitrogen fraction, B and C comprise the true protein whereby C is indigestible for the animal. For the statistical evaluation, further samples treated with NA from a farm silage bag from 2015 and 2016, and dried samples from a commercial drying plant dried at 180 °C from both years were included when considering the CP fractions. Descriptive statistics and univariate analysis with post hoc Tukey HSD test were performed using SPSS (V. 19).

**Table 1.** Fermentation quality of lucerne ensiled at two DM levels and with different additives (acids in g/kg DM)

| Silage | pH | | Lactic | *SD* | Acetic | *SD* | Butyric | $NH_3$-N | *SD* | *n* |
|---|---|---|---|---|---|---|---|---|---|---|
| **350 g/kg DM** | **4.64** | *0.2* | 69.9 | *14.0* | 13.7 | *6.0* | **0** | 86 | *20.0* | **46** |
| CON | 4.6 | abc | 67.2 | cd | 13.3 | abc | 0 | 88 | ab | 6 |
| B | 4.6 | ab | 72.0 | cd | 16.6 | abc | 0 | 100 | b | 6 |
| BC | 4.6 | ab | 62.5 | abcd | 6.3 | a | 0 | 61 | a | 6 |
| C | 4.7 | abcd | 59.6 | abcd | 10.3 | abc | 0 | 78 | ab | 6 |
| NA | 4.7 | abcd | 80.0 | d | 16.2 | abc | 0 | 96 | b | 16 |
| ByProQ | 4.7 | abcd | 78.0 | cd | 19.5 | c | 0 | 95 | b | 6 |
| **450 g/kg DM** | **4.91** | *0.2* | 54.2 | *14.4* | 12.6 | *7.0* | **4** | 88 | *21.0* | **36** |
| CON | 4.9 | bcd | 63.6 | bcd | 15.4 | abc | 0 | 100 | b | 6 |
| B | 4.8 | abcd | 66.2 | cd | 15.7 | abc | 0 | 99 | b | 6 |
| BC | 4.9 | bcd | 39.9 | a | 6.4 | a | 0 | 66 | a | 6 |
| C | 5.0 | d | 41.5 | ab | 7.1 | ab | 0 | 64 | a | 6 |
| NA | 4.9 | bcd | 58.7 | abcd | 12.9 | abc | 0 | 96 | b | 6 |
| ByProQ | 5.0 | cd | 55.6 | abc | 17.8 | bc | 23 | 102 | b | 6 |
| P (Additive) | 0.438 | | 0.010 | | <0.001 | | 0.3 | <0.001 | | |
| P (DM level) | <0.001 | | <0.001 | | 0.59 | | 0.2 | <0.001 | | |
| P (Additive * DM level) | 0.879 | | 0.19 | | 0.75 | | 0.3 | 0.506 | | |

Different letters within the same column mean significant differences among treatments (P < 0.05)

**Results and Discussion** The fermentation quality of lucerne silages was good with mean values: pH 4.8, lactic acid 62 g/kg DM, acetic acid 13 g/kg DM and almost no butyric acid (Table 1). $NH_3$-N was 87 g/kg of total N on average. The A-fraction amounted in the dried treatments remained stable compared to the fresh forage independent from temperature whereas it varied between 50 and 70% of CP in the silages, ByProQ at 450 g/kg DM showing the lowest values (Table 2). However, in the dried treatments, there was a shift within the true protein fractions from fresh to dried lucerne: $B_2$ decreased from in favour of $B_3$ (Table 2). The C-fraction, which can be an indicator of heat damage, went up slightly (Table 2), which is tolerable. As a contrast, the $B_2$-fraction in the silages decreased by more than half, mainly in favour of the A-fraction (Table 2). The rumen undegradable CP ($RUP_{5h}$) estimated by the equation of Kirchhof (2007) varied between 8.5 and 24 % of CP in the silages and 28 to 36% of CP in the dried treatments. For all CP fractions the conservation effect was significant (P<0.001).

**Table 2.** CP fractions (in % of CP) in silage treatments and in dried lucerne (mean values as there was no treatment effect in the latter)

| | | A | | B1 | | B2 | | B3 | | C | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fresh** | **All wilting levels** | **27.3** | *A* | **4.3** | *B* | **54.0** | *C* | **8.9** | *B* | **5.5** | *B* |
| **Silage** | **Mean** | **63.1** | *B* | **1.1** | *A* | **26.3** | *A* | **4.9** | *A* | **4.6** | *A* |
| | **220 g/kg DM** (NA) | **69.7** | *c* | **1.5** | *c* | **25.1** | *abcd* | **1.4** | *a* | **2.3** | *a* |
| | **350 g/kg DM** | **65.6** | | **1.0** | | **25.5** | | **3.4** | | **4.6** | |
| | CON | 69.9 | *c* | 0.7 | *ab* | 23.5 | *a* | 2.9 | *a* | 3.1 | *ab* |
| | B | 68.5 | *c* | 0.7 | *ab* | 24.5 | *ab* | 2.7 | *a* | 3.6 | *abc* |
| | BC | 63.4 | *bc* | 1.1 | *abc* | 27.9 | *de* | 2.7 | *a* | 4.9 | *cd* |
| | C | 64.6 | *bc* | 0.9 | *abc* | 26.9 | *bcde* | 2.5 | *a* | 5.2 | *cd* |
| | NA | 65.3 | *bc* | 1.4 | *bc* | 24.9 | *abc* | 3.6 | *a* | 4.7 | *bcd* |
| | ByProQ | 62.2 | *bc* | 0.5 | *a* | 26.4 | *abcde* | 5.3 | *a* | 5.5 | *d* |
| | **45 % DM** | **57.6** | | **1.1** | | **27.8** | | **8.0** | | **5.4** | |
| | CON | 62.0 | *bc* | 1.0 | *abc* | 26.5 | *bcde* | 5.7 | *a* | 4.8 | *bcd* |
| | B | 61.8 | *bc* | 1.0 | *abc* | 26.4 | *abcde* | 6.0 | *a* | 4.8 | *bcd* |
| | BC | 57.1 | *ab* | 1.2 | *abc* | 28.6 | *e* | 7.4 | *a* | 5.7 | *d* |
| | C | 56.5 | *ab* | 1.4 | *bc* | 28.9 | *e* | 7.7 | *ab* | 5.4 | *cd* |
| | NA | 58.5 | *ab* | 1.0 | *abc* | 27.5 | *cde* | 7.4 | *a* | 5.6 | *d* |
| | ByProQ | 49.7 | *a* | 1.1 | *abc* | 28.8 | *e* | 14.1 | *b* | 6.4 | *d* |
| **Dried** | **Mean** | **27.1** | *A* | **5.2** | *C* | **41.2** | *B* | **19.6** | *C* | **6.9** | *C* |
| | SEM (conserved) | 0.497 | | 0.157 | | 0.255 | | 0.385 | | 0.113 | |
| P | Conservation type | <0.001 | | <0.001 | | <0.001 | | <0.001 | | <0.001 | |
| | Wilting level | 0.016 | | 0.016 | | 0.020 | | 0.049 | | 0.123 | |
| | Cons.*wilting | <0.001 | | 0.424 | | 0.010 | | 0.001 | | <0.001 | |

Different letters in lower case mean significant differences among ensiling treatments. Different letters in upper case mean significant differences between conservation types (P < 0.05)

**Conclusion** Although proteolysis could be reduced by more intensive wilting and chemical or tannin additives, ensiling cannot compete with any of the drying treatments under ideal conditions concerning the true protein concentration. For practical application farmers should assess whether there exists an economical opportunity to rapidly dry high protein forages for use as valuable ration component.

**References**

Kirchhof, S. (2007) Kinetik des ruminalen in situ-Nährstoffabbaus von Grünlandaufwüchsen des Alpenraumes unterschiedlicher Vegetationsstadien sowie von Maissilagen und Heu – ein Beitrag zur Weiterentwicklung der Rationsgestaltung für Milchkühe. Dissertation, Christian-Albrechts-Universität Kiel.

Licitra G., Hernandez T.M. & Van Soest P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology, 57, 347-358.

# Methodology of ensiling trials and effects of silage additives

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**Keywords:** approval schemes, ensiling trials, methodology, silage additives

## Introduction

Many farmers and agricultural entrepreneurs find it difficult to choose a suitable silage additive among the large variety of additives on the market. Often additive retailers offer farmers an early season discount, which encourages farmers to buy an entire season's supply of additive long before they know which type of challenges they will encounter and hence which type of additive they require. The only way farmers can get unbiased information about an additive is through repeated ensiling tests made by independent test institutes. Without independent testing of silage additives, it is more or less impossible for the farmer or adviser to judge objectively the efficacy of an additive and to make a sensible choice from the vast variety of products. This contribution will focus on different approval schemes for silage additives within Europe, in particular in which way they test the efficacy of silage additives for the benefit of silage-producing farmers and entrepreneurs. The focus will be on today's two active approval systems, the EU authorisation of silage additives (compulsory) and the German DLG approval scheme (voluntary).

## National silage additive schemes in Europe

The first countries in Europe started already in 1979 to test silage additives. At the 11$^{th}$ International Silage Conference in Aberystwyth, Wales in 1996 five approval schemes for silage additives had been presented. In Table 1 characteristics of these silage additive schemes are presented.

**Table 1**. Characteristics of European silage additive approval schemes active in 1996.

| Country | Start | Compulsory | Positive control required | Farm or lab scale silos | Reference |
|---|---|---|---|---|---|
| Finland | 1987 | Yes | Yes | Both | Mannerkorpi et al. 1996 |
| France | 1979 | Yes | Yes | 4m$^3$-silo | Demarquilly and Andrieu 1996 |
| Germany | 1990 | No | No | Lab | Honig and Pahlow 1993<br>Pahlow and Honig 1996<br>Staudacher et al. 1999<br>Honig and Thaysen 2002 |
| Ireland | 1994 | No | No | Both | Fitzgerald et al. 1996 |
| UK | 1995 | No | No | Both | Haigh et al. 1996<br>Weddell et al. 1996<br>Weddell et al 2002 |
| Switzerland | 1979 | Yes | Yes | Lab | Wyss and Vogel 1997<br>Wyss 1997 |

Unfortunately, although this commonality exists, no uniformity in either the methodology used or the interpretation of test results was obtained. This lack of common methodology and interpretation presented a particular problem for the manufacturers and retailers of additives, who demanded a single approval scheme, which was valid in all European countries. One by one national approval

schemes were abolished and by the time EU regulation No. 1831 acquired legal force (2004), only the German DLG approval scheme was still in use and is so still today.

**The EU authorisation of silage additives**

At the 11th International Silage Conference in Aberystwyth, Wales, Haigh et al. (1996) presented a proposal for an EU additive approval scheme. Haigh stated that active ingredient authorisation has to be the responsibility of the EU. The prove of effectiveness of formulations had to be delegated to the respective national authorities of individual member states. It was envisaged that approval at EU level allowed an active ingredient to be used throughout the entire EU. However, individual member states could appeal against the decision, if they could present good reasons against it. In Haigh's proposal only chemical active ingredients were listed. At this time, microorganisms and enzymes had not been incorporated into the scheme.

Since 2004 all silage additives in the European Union (EU) require authorisation according to EC Regulation No. 1831/2003 (Article 10) before they can appear on the market. Silage additives are considered to be 'technological additives' if their primary effect targets the improvement of silage quality (EFSA 2012). Additives that are expected to exert their primary effect on animals, are categorised as 'zootechnical additives' and their authorisation is stipulated by other regulations and guidelines, which usually require animal trials. When active components have passed through the authorisation process, which is administrated by EFSA (European Food Safety Authority), and appear on an official whitelist, they can be marketed within the entire EU. The EU authorisation process focuses on safety (regarding handling and intake) and efficacy (regarding mainly improved fermentation or aerobic stability) of single, active components of an additive. All active components of an additive must be authorised before the additive is allowed to appear on the market. Once an active component is authorised, it can be used by any additive company thereafter. This means that the EU certification has only limited value for farmers since most additives contain more than one active component. But the main objective with the EU approval system is to make sure that only safe products are sold within the EU and not to help farmers to choose a suitable silage additive.

To prove the active component's efficacy at least three successful lab-scale trials, lasting ≥90 days, are required. Depending on the claimed mode of action, treated silages have to show a significant improving effect against an untreated control treatment. Guidelines resemble the German DLG approval system (see below), but are less versatile regarding which problems they might be able to alleviate. Aerobic stability is determined by monitoring silage temperature over time as applied by most research institutes, but unlike the German guidelines, stability should be determined after about 90 days of anaerobic storage and without any air stress treatment (i.e. air infusion in silos during storage). The lack of an appropriate air stress treatment during storage increases the risk that the less well-fermented silages – usually the untreated controls – will demonstrate better aerobic stability than additive-treated silages. Completely anaerobic conditions such as in lab-scale silos do not mimic farm conditions and make it difficult to demonstrate an additive effect with regard to aerobic stability.

For prove of statistical significance between treated and untreated silages, EU guidelines recommend the use of non-parametric statistical tests such as the Wilcoxon-Mann-Whitney test. This type of test has the advantage that the collected data do not have to follow normal distribution like with commonly practiced analysis of variance (ANOVA) tests. ANOVA evaluations make it sometimes difficult to explain significant differences because not normally distributed parameters have to be mathematically transformed to make them normally distributed. This means that non-parametric tests usually produce probabilities, which are often more reliable and easier to interpret for a majority of readers.

**The German DLG additive approval scheme**

The German approval system for silage additives was introduced in 1990 by DLG (German Agricultural Society in Frankfurt). DLG is a non-governmental agricultural organisation that has a long history in quality approval of agricultural commodities such as concentrates, plastic films, disinfectants for stables and milking parlours, teat dips, fuels and lubricants, fertilisers, food and wines and other agricultural goods. Quality approved goods receive a 'DLG Quality Mark', which is usually printed on

the package of the approved product and signals to the user that this product had passed through a series of tests and complies with the minimum quality criteria set up by DLG. These tests must be carried out at independent research institutes and in accordance to detailed DLG guidelines (DLG 2018; Thaysen et al. 2007). The DLG committee for silage additives, consisting of 10 independent and 2 DLG-employed scientists, recommends then, based on the delivered trial dossiers, to approve or not approve the 'DLG Quality Mark' for the tested additive. The certification process is kept confidential and only approved products appear on an open DLG website (www.dlg.org/siliermittel). Several of the listed additives are identical and are sold under different names by different retailers. All identical products that wish to carry the DLG Quality Mark of the original product must apply for it and are checked by DLG if they really are identical. Once each year all products on the DLG list of approved additives are sampled and analysed to check that composition and recommended application rates of each additive comply with values from the time of approval.

If an additive company or retailer considers an application for a DLG Quality Mark, the first step would be to choose, which of the different 'action categories' (AC) would be suitable for the additive. Fermentability coefficients (FC) define how easy or difficult forages are expected to ensile. FC values are calculated from DM, sugar (WSC) and buffering capacity (BC) values of the respective forage (Weissbach, 1975; Weissbach 1996). Table 2 lists the available AC within the DLG approval system.

The DLG committee requires a dossier describing at least 5 successful lab-scale ensiling trials for AC1 (fermentation quality), AC2 (aerobic stability) and AC5a (*Clostridium* reproduction) and at least 3 feeding trials for AC4 (animal performance). For AC 6 (methane yield) at least 3 or 5 lab-scale trials are required depending on if the DLG Quality Mark is intended for a single substrate (3 trials) or for several different substrates (5 trials). In addition, the applicant is encouraged not to withhold unsuccessful trials. Unsuccessful trials often come to the DLG committee's attention anyway because its members are part of an informal silage science network in Northern Europe. Other trial reports not complying with DLG guidelines are appreciated as additional information.

AC1 tests (improved fermentation quality) are carried out with lab-scale silos (approx. 1.5 L volume, at least 3-fold replication) comparing untreated controls with additive-treated silages. Silos are stored anaerobically at 25°C for at least 90 days before silo contents are sampled and analysed for DM (corrected for volatiles lost during drying), pH, ammonia-N, organic acids and alcohols. Weight losses (% of initial DM) of silo contents during storage are determined by frequent weighing of silos.

AC2 tests (improved aerobic stability) require an air infusion 28 and 42 days after sealing. The air infusion is achieved by removing plugs from two holes (∅ 6 mm) on the lid and bottom of each silo for the duration of 24 h. This will stimulate yeast growth and make most control silages aerobically instable – a vital prerequisite to test the claimed effect of the additive. Exactly 7 days after the last air infusion (i.e. on day 49), silos are sampled and analysed. Aerobic stability is determined by transferring silo contents aseptically to insulated vessels (approx. 1-2 litres). Electronic temperature sensors inserted into the centre of each vessel, monitor individual silage temperatures for a period of at least 7 days at 20°C ambient temperature. A temperature increase in a silage sample is interpreted as increased activity of aerobic microorganisms (commonly yeasts or acetic acid bacteria), which consume mainly sugars (WSC) and lactate for their growth. Aerobic instability is defined as the time for the silage sample to reach 3°C above ambient temperature. Other analyses such as pH, weight losses and yeast counts at start and end of the stability test are use as supporting information.

The AC3 (reduced effluent formation) test will not be described here since no DLG Quality Marks were ever awarded in this category.

**Table 2**. Action categories within the DLG approval system. FC values indicating ensilability of herbage: FC = DM, % + (8 x WSC, % DM / BC, g lactate/100 g DM).

| | |
|---|---|
| **Action category 1** | **Improved fermentation processes** |
| Field of application | |
| **a** | Difficult to ensile forages<br>Fermentability coefficient (FC) < 35<br>Roughage forages with an insufficient content of water-soluble carbohydrates and/or dry matter (DM) |
| **b** | Moderately difficult to easy to ensile forages in the lower DM range<br>FC ≥ 35; DM < 35%<br>e.g. grasses, forage legumes, silage maize, whole cereal plants, millet, Sudan grass |
| **c** | Moderately difficult to easy to ensile forages in the upper DM range<br>FC ≥ 35; DM ≥ 35 to ≤ 50%<br>e.g. grasses, forage legumes, silage maize, whole cereal plants, millet, Sudan grass<br>Each with a sufficient content of water-soluble carbohydrates |
| **d** | Grain silage<br>e.g. corn cob mix, earlage, moist cereal grains |
| **e** | Special types of forages<br>Forages requiring ensiling agents to develop specific actions<br>e.g. beets, pulps, pressed pulp, stillage, brewers grains or forages for which an ensiling agent is specifically designed |
| **Action category 2** | **Improved aerobic stability** |
| Forage/substrate type | • Grasses or forage legumes, preferably wilted<br>• Silage maize and maize cob products<br>• Whole cereal plants<br>• Cereal crops (cereals, maize) and forage legumes<br>• Root crops<br>• By-products of the food and fermentation industries<br>Depending on the test reports submitted with the application, the use of the DLG Quality Mark may be limited to specific forages/substrate types |
| **Action category 3** | **Reduced effluent production** |
| Field of application | Forage with low dry matter contents |
| **Action category 4** | **Secondary effect** |
| **a** | Ensiling agents also capable of improving the feed intake value of treated silage |
| **b** | Ensiling agents also capable of improving the digestibility of treated silage |
| **c** $_{\text{Meat}}$ | Ensiling agents also capable of improving the beef production value of treated silage |
| **c** $_{\text{Dairy}}$ | Ensiling agents also capable of improving the milk production value of treated silage |
| **Action category 5** | **Additional effects** |
| **a** | Prevention of *Clostridium* endospore reproduction |
| **b** | Specific effects defined by the applicant |
| **Action category 6** | **Improved methane yield value of silage by:** |
| **a** | Reducing fermentation losses |
| **b** | Preventing secondary heating |
| **c** | Specific effects defined by the applicant |

AC4 tests (improved animal performance value) require feeding trials with growing or lactating cattle depending on if the AC4 application concerns improved DM intake (AC4a), improved forage digestibility (AC4b), improved beef production value (AC4c $_{MEAT}$) or improved milk production value (AC4c $_{MILK}$). Understandably these studies are considerably costlier than lab-scale ensiling trials. This might be the reason why no new applications were handed in during the last decade. Another complication might be a possible conflict with EU regulations, which require that feed additives improving animal performance, are authorised according to guidelines for 'zootechnical additives'. This is why the DLG approval system emphasises that all AC4 claims are secondary effects in contrast to primary effects in categories AC1 (improved fermentation) and AC2 (improved aerobic stability). If companies would be willing to conduct such trials, they have to prioritize compulsory EU legislation over the voluntary DLG approval system.

The AC5a test (reduced clostridial spore reproduction) should be conducted with wet forages analogous to the AC1a test. However, the forage should be inoculated with a sufficiently high amount of clostridial endospores (≥$10^3$ cfu/g silage) and the test requires the quantification of spores at the start and end of the storage period (≥90 days). Increased spore counts, butyrate and ammonia-N levels are taken as an indication of increased clostridial activity in silages. As to the question of suitable spore strains for the inoculation of forages, Pauly et al. (2008) tested 10 different *Clostridium* spore cocktails in 4 different forages with respect to their ability to produce clostridial fermentation in silage. Each cocktail contained between 1 to 3 different strains. This study confirmed that our previously selected *Cl. tyrobutyricum* strain (strain 213) produced reliably clostridial activity compared to other inoculated silages and was found to be a suitable challenge organism for ensiling trials that focus on the inhibition of clostridial activity.

AC6 tests (improved methane yield value) determine the effect of a silage additive on the methane yield from ensiled crops by comparing each substrate to untreated controls in two procedure tests.

These tests are (Fig. 1):

- Procedure test 1: 90 days fermentation, no air stress (analogous to AC1);
- Procedure test 2: 49 days fermentation, with air stress and aerobic stability test (analogous to AC2).

The ensiling tests and associated test methods are analogous to those in AC1 or AC2.

The specific methane yield is determined:

- in the fresh material;
- in procedure test 1 immediately after removal from the silo, i.e. after 90 days fermentation without air stress;
- in procedure test 2 after 49 days fermentation with air stress after aerobic stability has been tested (ASTA test).

Silage must be removed from the aerobic stability test after ten days at the latest, or three days after control silages have heated up. The control silage is classified as heated up, if two of the three sample replicates have heated up (>23°C).

However, the weight losses during the fermentation period plus the losses during the aerobic stability test (both in g DM) must be taken into account in any event when calculating the overall effects.

This test was developed by Nussbaum (Nussbaum and Staudacher 2012) and Thaysen (Thaysen and Ohl 2015). In 2015, the first product received the DLG Quality Mark in this category.

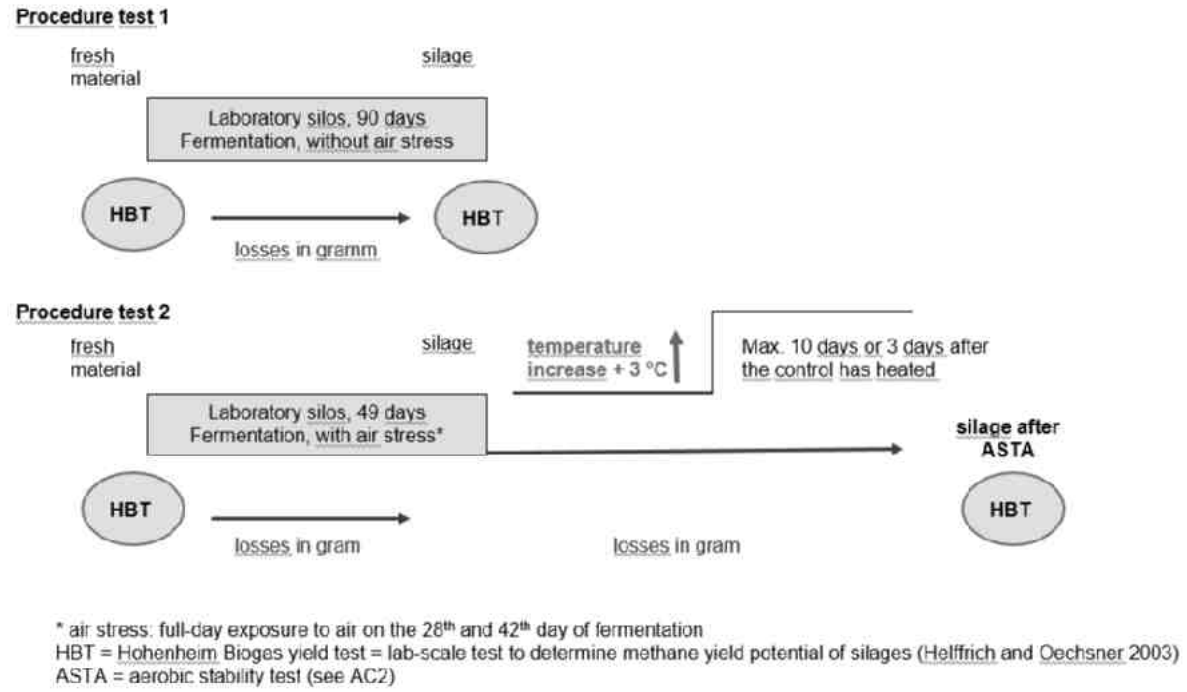


**Figure 1**. Test scheme for changes in methane yield values of silages associated with the use of ensiling agents for DLG Quality Mark purposes.

The number of DLG-approved silage additives and number of brand owners with at least one DLG-approved silage additive in their portfolio is depicted in Fig.2 below. The number of DLG-approved silage additives was rather constant and varied between 60 – 72 per year during the last years.

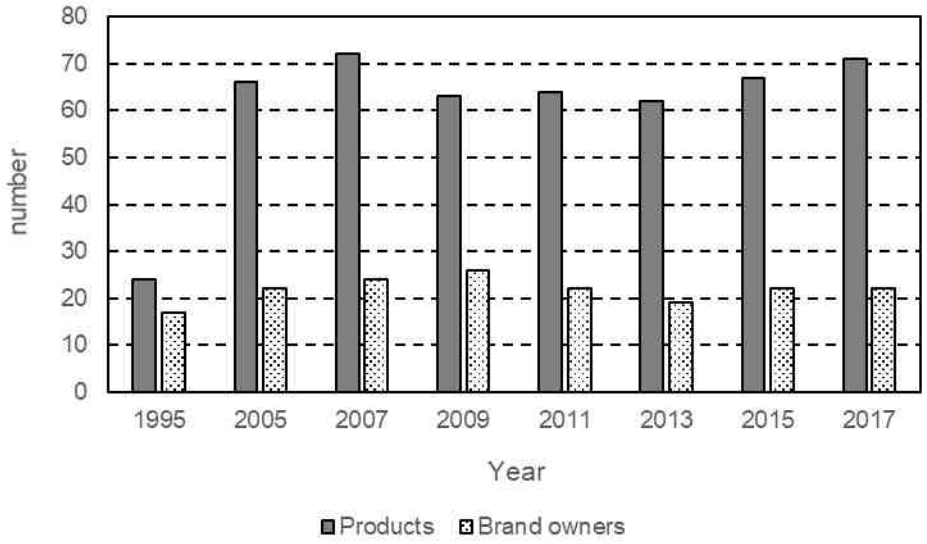


**Figure 2**. Products and brand owners with a DLG Quality Mark.

**Osmotolerance**

All biological ensiling agents are additionally tested for osmotolerance during the annual quality test. Lactic acid bacteria with a low osmotolerance do not perform well in high DM silages such as in AC1c. Where the detected osmotolerance levels in products certified for action category 1c are below 30% of

the declared microbial counts over three consecutive years, the respective manufacturer is advised accordingly.

The osmotolerance-test or “Rostock Fermentation Test” (RFT) is an *in vitro* test using forage juice in test tubes. By adding a certain amount of potassium chloride (KCl) it is possible to increase osmolality and simulate higher dry matter levels in the test tubes. The test analyses the activity of natural occurring and supplemented lactic acid bacteria together with the contents of fermentable carbohydrates in the forage (Richter et al. 2010). The pH decrease after 3 days fermentation in tubes with and without KCl will give a good indication about how osmotolerant the additive is. The basic principle of the test is the adaption of the fermentation media to the conditions of a three days fermented silage using the osmolality, a parameter that includes the total concentration of soluble ingredients with osmotic behaviour.

One application of the RFT is the check-up of different silage additives from one year to the next for the DLG. A great advantage of this test is the good standardisation of the test conditions and the fastness compared to ensiling trials. With the help of a cluster analysis it is possible to identify additives that work not very well (Cluster IV).

**Comparison of the German DLG and the French INRA schemes**

The big difference between the German DLG and the French IRA schemes was the size of the silos and the wilting degree of the forage (Pflaum et al. 1997). In France the test silos had a capacity of 4 $m^3$, which were close to practical conditions and the forage was cut and ensiled without any wilting. In Germany, laboratory silos with a volume of 1.5 litres were used and the forage was wilted to different DM contents. Furthermore, the French approval scheme was compulsory for the authorisation for a product and included an obligatory determination of silage intake and digestibility with sheep for chemical additives. In Germany, the DLG scheme is on a voluntary basis and the applicant chooses among various AC tests according to the additive’s specific mode of action.

In 1994 and 1995 comparative ensiling trials with the same forage and the same wilting degree were carried out in Theix, France, for a direct comparison between the DLG and INRA schemes. In 1995, Switzerland joined in on the comparison. The Swiss approval system is similar to the DLG method.

In Figure 3 fermentation acids of the first trial of 1995 are presented. All silages treated with the inoculant contained in comparison to the untreated silages more lactic acid and less acetic and butyric acid. Also the second trial, where besides a negative control without additive, a chemical additive and an inoculant were tested in 1 L laboratory silos (Germany) and 4 $m^3$ silos (France), showed similar results (Fig. 4). The differences between the fermentation acids between the three countries can partly be explained by the different storage temperatures (inside or outside).

In general, the aim of the silage additive testing system was fulfilled with both methods.

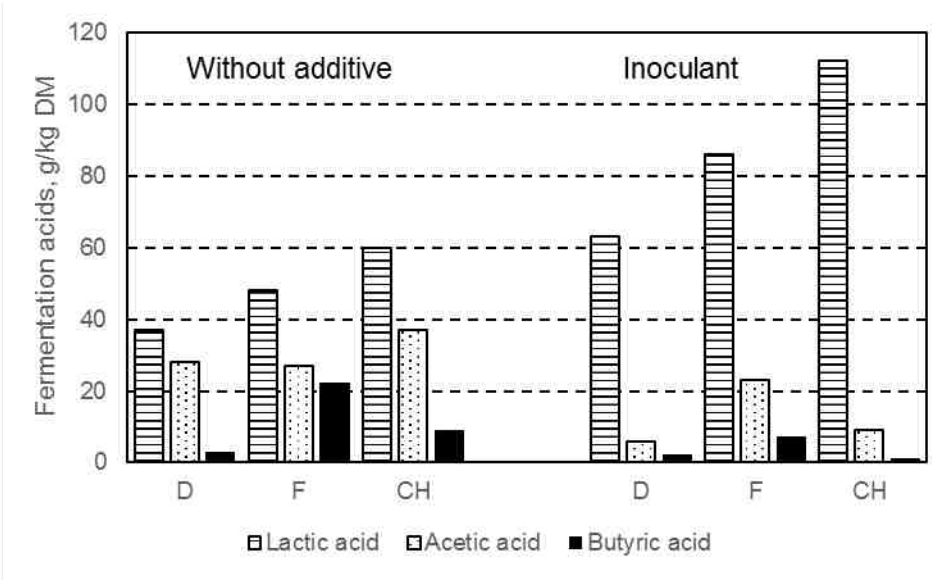


**Figure 3**. Results of trial 1 in 1995 – ryegrass, second cut, 25% DM, 84 g crude protein/kg DM and 120 g WSC/kg DM (D: Germany; F: France; CH: Switzerland).

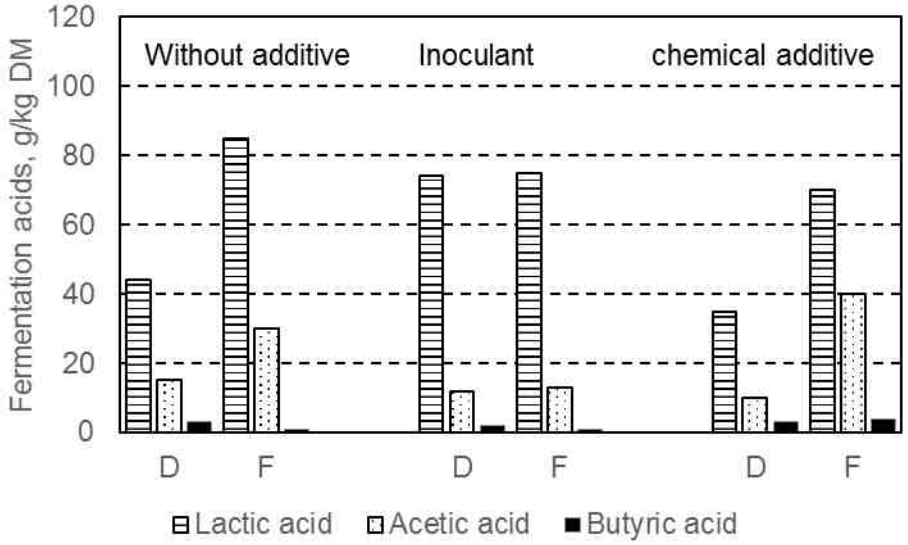


**Figure 4**. Results of trial 2 in 1995 – ryegrass, second cut, 25% DM, 80 g crude protein/kg DM and 122 g WSC/kg DM (D: Germany; F: France).

**Testing silage additives in round bales**

Experience from many round bale experiments indicated that some additives, which have proved their efficacy in bunker silos, were often failing in round bales. Two important differences to bunker silage are a) bale silage is recommended to be wilted to 45 – 55% DM and b) bale silage is usually unchopped. We believe that the key issue is how the additive is distributed within the herbage. During baling the additive is sprayed on top of the windrow just when it is fed into the pick-up unit of the baler. A reasonable assumption is that any blending of additive and forage in a baler is rather inefficient given that the forage usually is unchopped. Efforts to apply an additive to moist hay (Charlick et al. 1980; Holden & Sneath 1980) demonstrate the problem of distributing an additive evenly in unchopped forage.

A test scheme for the approval of silage additives for big bales was already presented at the 15th International Silage Conference in Madison (Pauly and Rubenschuh 2009). In 2010 and 2011 trials were carried out in Germany, Sweden and Switzerland with the main goal to compare laboratory silos (1.5 Liter) with round bales (Wyss et al. 2012). In 2010 round bale trials with identical protocols were conducted in Germany, Sweden and Switzerland to compare the effect of two additives against an untreated control. The inoculant contained the strains *L. plantarum, L. rhamnosus, P. pentosaceus, L. buchneri* and *L. brevis* and was applied at a rate of 1 g per tonne, respectively 100.000 cfu/g FM. The product was diluted with water and the application rate was 4 litres / tonne FM. The chemical product contained hexamine, sodium nitrite, sodium benzoate and sodium propionate and was applied undiluted at a rate of 4 litres / tonne FM. The applied dosage of the inoculant amounted 118, 148 and 108% and for the chemical product 131, 138 and 103% of the targeted doses in Germany, Sweden and Switzerland, respectively. This showed us that the application of silage additives in round bales, especially sticking to the target rate, was not easy and required skill and experience. In 2011, the study was repeated in Germany and Switzerland but with a slightly modified protocol. The DM contents were 37 and 41% in Germany and Switzerland, respectively. This time the applied rate for the inoculant amounted to 67 and 113%, respectively. In addition a part of the laboratory silos and round bales were exposed to an air stress treatment. In laboratory silos two 6 mm holes were opened for 24 h (stress 1) one week before silos were sampled. In bales four holes (diameter 20 mm) were made and closed again (taped) after 24 h (stress 2). For another air stress variant 20 holes were made with a nail (diameter 2 mm) and holes were not sealed until bales were sampled seven days later.

In general, the silages from the laboratory silos and round bales had a good fermentation quality. The fermentation was more intensive and the pH was lower in small scale laboratory silos in comparison to round bale silages. The acid profiles of the silages from Sweden and Switzerland (Fig. 5 and 6) show similar responses to the additive treatments and bales versus lab silos. As expected acid formation was larger in the wetter Swiss than drier German forages. The more intensive fermentation in the laboratory silos can be partly explained by the different length of cut of forages. The results of the aerobic stability tests are presented in fig. 7 and 8. In Germany, the aerobic stability in the treated bales was improved in only 2 of 5 cases. Here the low dose rate (67%) can explain this result. In Switzerland, the inoculant did improve the aerobic stability of all laboratory and round bale silages. The experiments indicated that silage additives can be tested in round bales when treated and untreated forages have the same DM content and when silage additives have been applied evenly and at the targeted dose. Furthermore, it is possible to expose round bales to an air stress treatment and thereby create more suitable conditions (i.e. aerobically instable controls) for the testing of silage additives. Aerobic stability of bales is usually not an issue for most farmers since bales are consumed within a day or two. However, an increasing number of horse owners taking care of only few animals require a long aerobic stability when they buy silage or haylage from farmers.

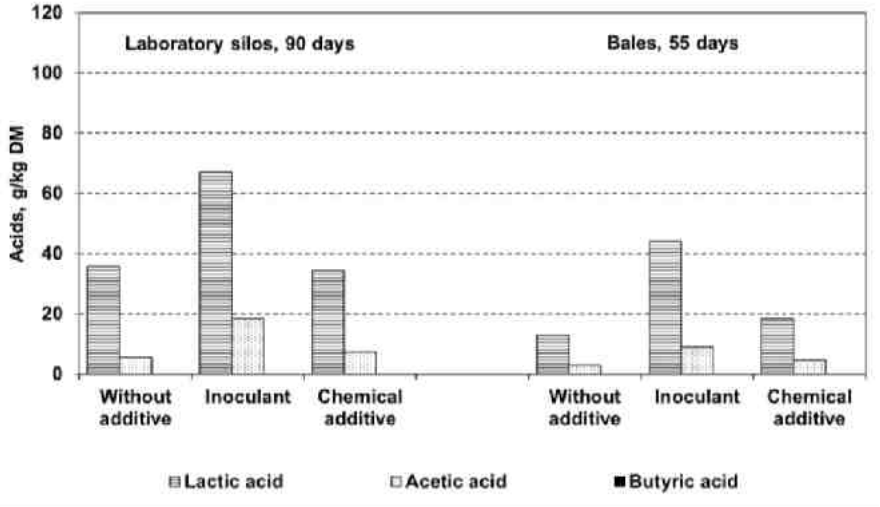


**Figure 5**. Fermentation acids of treated and untreated silages from Sweden (Herbage: DM 50.2%, crude protein 116 g/kg DM, NDF 495 g/kg DM, WSC 153 g/kg DM)

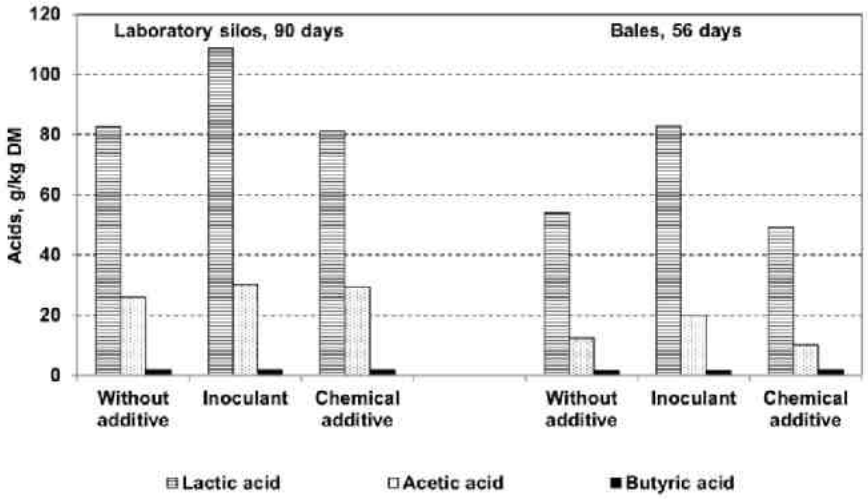


**Figure 6**. Fermentation acids of treated and untreated silages from Switzerland (Herbage: DM 38.0%, crude protein 195 g/kg DM, crude fibre 214 g/kg DM, WSC 145 g/kg DM)

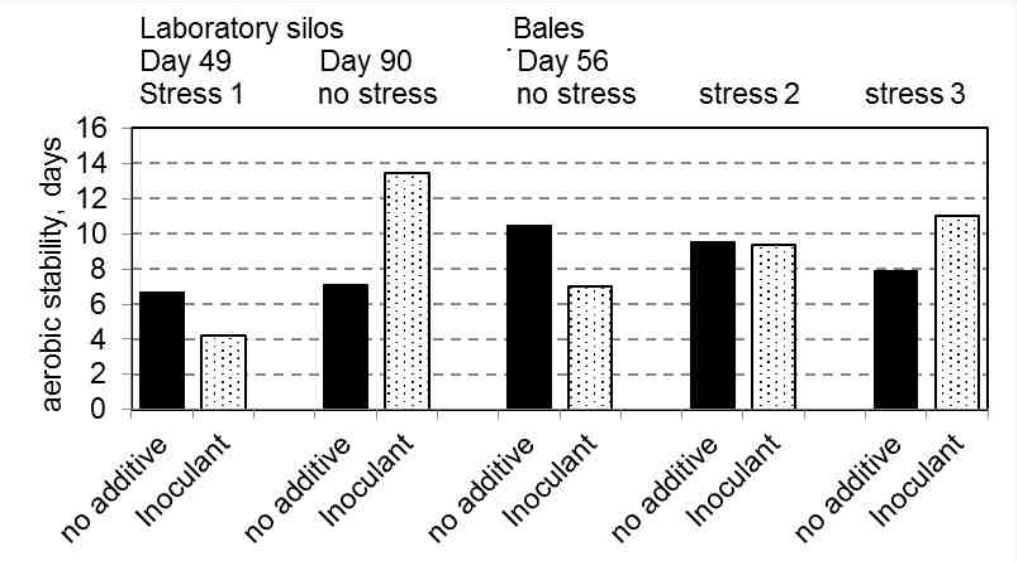


**Figure 7**. Aerobic stability of the silages from Germany made from the same herbage (Herbage: DM 37.1%, crude protein 128 g/kg DM, crude fibre 290 g/kg DM, WSC 98 g/kg DM). 'Stress' indicates different air stress treatments applied during storage.

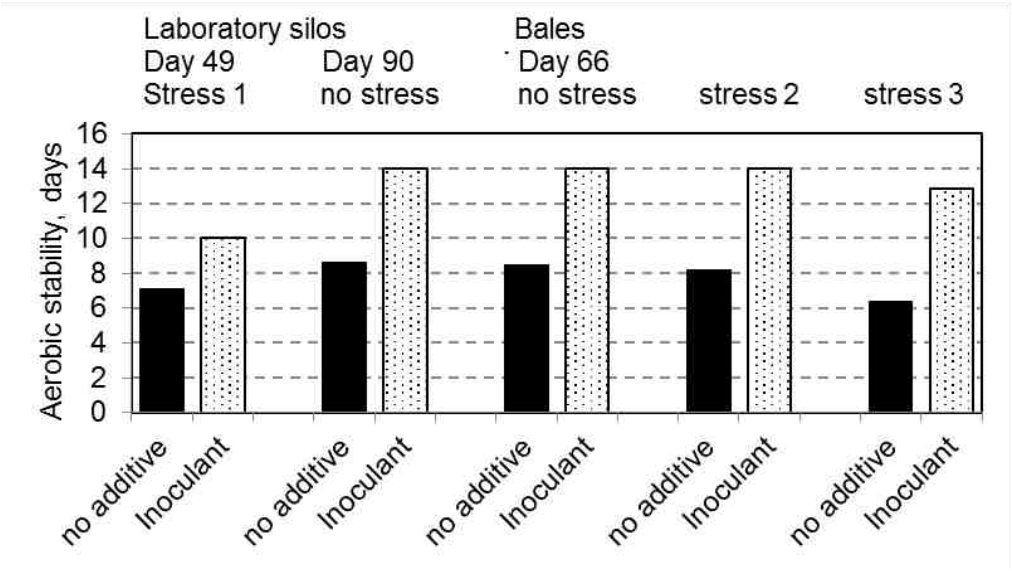


**Figure 8**. Aerobic stability of the silages from Switzerland made from the same herbage (Herbage: DM 40.6%, crude protein 147 g/kg DM, crude fibre 231 g/kg DM, WSC 167 g/kg DM). 'Stress' indicates different air stress treatments applied during storage.

**Future perspectives**

Guidelines for the test of silage additives should not be static but should be updated regularly to meet new arising challenges. The DLG Commission for Silage Additives investigates currently the possibility to introduce new test protocols for: a) silage additives, which show a positive response after a shorter storage time (AC2), b) silage additives that reduce the extent of protein degradation during ensilage or c) TMR additives, which extend the aerobic stability of total mixed rations (TMR).

**Summary**

In the period between 1979 and 1995 five national silage additive approval schemes appeared in Europe (Table 1). Today only two approval schemes are still in use, the EU authorization of additive components (compulsory) and the German DLG approval scheme of complete additives (voluntary). The EU authorization focuses on safety and environmental properties. Efficacy is primarily tested with regard to fermentation quality and aerobic stability (without air stress). Since EU authorization is compulsory for all additives and most additives are composed of more than one single active component, it offers no immediate help to advisors or farmers to help selecting a suitable additive.

The DLG approval scheme has a more consumer-oriented approach and can test complete additives under a rather large variety of conditions, so called 'action categories' or ACs. There are 6 different ACs and most ACs have several subgroups (Table 2). That offers a rather wide variety of conditions under which an additive can be tested. Approved additives get the privilege to carry a 'DLG Quality Mark' with the approved ACs on their package, which signals to the consumers the specific qualities the additive has demonstrated in a series of impartial tests. Additives which fail a test are not made public. After 2005 between 60 and 72 approved additives (Figure 2) are listed each year on an open DLG website (in German: http://www.dlg.org/siliermittel.html). This web list represents the only source of impartial information about silage additives in the German speaking regions of Europe and is used extensively by many advisors and farmers.

Comparative trials from 1995 between the German and the French approval schemes were described as well as trials from 2010 to evaluate a DLG test protocol for testing additives in round bales. Round bales present a challenge because additives are usually sprayed on top of the windrow with very limited blending of additive and forage (which remains unchopped!). Many additives do not perform well when not properly distributed in the forage. Other challenges were controlling the application rate

in each bale and minimizing DM variation between the first and the last bale in the experiment. Even experiments which introduce an air stress in bales to create bales with short aerobic stability were presented. An air stress in bales might be important for horse owners, who require a longer period of aerobic stability. With aerobically instable bales it is possible to test additives, which are able to extend the time from bale opening until bales start to heat and mould.

**References**

Charlick, R.H., Holden, M.R, Klinner, W.E & Shepperson, G (1980) The use of preservatives in haymaking. Journal of Agricultural Engineering & Research 25, 87-97.

Demarquilly, C. & Andrieu J.P. (1996) Legislation for use of silage additives in France. Proceedings of the XI International Silage Conference, Aberystwyth, Wales, 138-139.

DLG (2018) DLG Testing Guidelines for the award und use of the DLG Quality Mark for ensiling agents", prepared under the auspices of the DLG commission for ensiling agents, at http://www.dlg.org/siliermittel.html.

EFSA (2012) Guidance for the preparation of dossiers for technological additives. EFSA Panel on Additives and Products or Substances used in Animal Food. EFSA Journal 2012; 10 (1): 2528, 1-23.

Fitzgerald, L., O'Kiely, P., Fitzgerald, S. & Murphy, J.J. (1996) Silage Additive Scheme – Republic of Ireland (1996) Proceedings of the XI International Silage Conference, Aberystwyth, Wales, 150-151.

Haigh, P.M., O'Kiely, P., Pahlow, G. & Viuf, B.T. (1996) European Union proposed silage additive scheme. Proceedings of the XI International Silage Conference, Aberystwyth, Wales, 140-141.

Haigh, P.M., Weddel, J.R & Agnew, R. (1996) The United Kindom forage additive approval scheme. Proceedings of the XI International Silage Conference, Aberystwyth, Wales, 142-143.

Helffrich, D. & Oechsner H. (2003) Hohenheimer Biogasertragstest. Landtechnik 3, 148-149.

Holden, MR & Sneath, RW (1980) Application of preservative chemicals to hay at baling. EGF Occasional Symposium No.11, Proceedings of a Conference on Forage Conservation in the 80's (ed. C. Thomas), British Grassland Society, Brighton, UK, 248-251.

Honig, H. & Pahlow, G. (1993) The DLG-evaluation scheme for silage additives. Proceedings of the XI International Silage Conference, Aberystwyth, Wales 148-149.

Honig, H. & Thaysen, J. (2002) 10 years testing of silage additives by DLG – a comprehensive data evaluation. Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland, 232-233.

Mannerkorpi, P., Jaakkola, S., Toivonen, V., Heikkila, T., Huhtanen, P. & Rankanen, R. (1996) The Finnish scheme for official approval of silage additives. Proceedings of the XI International Silage Conference, Aberystwyth, Wales 148-149.

Nussbaum, H. & Staudacher, W. (2012) Yield of methane gas – a new DLG-test scheme for silage additives. Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 450-451.

Pahlow, G. & Honig, H. (1996) The German silage additive approval scheme. Proceedings of the XI International Silage Conference, Aberystwyth, Wales, 146-147.

Pauly, T.M. & Rubenschuh, U. (2009) A test scheme for the approval of silage additives for big bales. Proceedings of the XV International Silage Conference, Madison, USA, 291-292.

Pauly, T.M., de Paula Sousa, D., Spörndly, R. & Christiansson, A. 2008. Inoculation of experimental silages with different *Clostridium* spores. In: Proceedings of the 22$^{th}$ General Meeting of the European Grassland Federation (EGF) 'Biodiversity and Animal Feed', Uppsala, Sweden. Grassland Science in Europe, vol. 13, p. 678-680.

Pflaum, J., Gartner, L., Demarquilly, C., Andrieu, J.P., Honig, H., Staudacher, W. & Wyss, U. (1997) Silage Additive Testing. Comparison of the German DLG and the French INRA Schemes. Das wirtschaftseigene Futter, 42 (3), 217-248.

Richter, W., Spieker, H. Schuster, M. & Baranowski, A. (2010) Rostocker Fermentationstest – Nachprüfung biologischer Siliermittel mit DLG-Gütezeichen. Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft 11, 1-65.

Staudacher, W., Pahlow, G. & Honig H. (…) Certification of silage additives in Germany by DLG. Proceedings of the XII International Silage Conference, Uppsala, Sweden, 239-241.

Thaysen, J., Honig, H., Kalzendorf, C., Spiekers, H. & Staudacher, W. (2007) Silage Additives: Aspects of feed legislation, efficacy of DLG-approved products and recommendations for application. Übersichten Tierernährung 35, 55-91.

Thaysen, J. & Ohl, S. (2015) Effects of silage additives in whole-crop maize and grain silages to enhance methane yield. Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 86-87.

Weddell, J.R., Haigh, P.M. & Steen R.W.J. (1996) The UK Forage additives Approval Scheme – Development and Guidelines. Proceedings of the XI International Silage Conference, Aberystwyth, Wales 144-145.

Weddell, J.R., Agnew R. & Cottrill B. (2002) The UK Forage Additives Approval Scheme – Developments and Product Approvals. Proceedings of the XIII$^{th}$ International Silage Conference, Auchincruive, Scotland, 230-231.

Weissbach, F. (1975) Methoden und Tabellen zur Schätzung der Vergärbarkeit. Akademie der Landwirtschaftswissenschaften der DDR. Agra Markkleeberg.

Weissbach, F. (1996) New developments of crop conservation. Proc. of the 11$^{th}$ International Silage Conference (eds. Jones *et al*., IGER), Aberystwyth, Wales. 11-25.

Wyss, U. (1997) Siliermittel zur Verbesserung der aeroben Stabilität. Agrarforschung 4 (7), 273-276.

Wyss, U. & Vogel, R. (1997) Ergebnisse der Siliermittelprüfung 1996. Agrarforschung 4 (3), 121-124.

Wyss, U., Thaysen, J., Pauly, T. & Rubenschuh, U. (2012) Testing inoculant and chemical additives in round bales in comparison to laboratory silos. Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 294-295.

# Action of lactic acid bacteria used as silage inoculants on the digestive tract of ruminants

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**Keywords:** bacteriocin**,** microbial additives, probiotic, rumen

**Introduction** The lactic acid bacteria *Lactobacillus plantarum* and *Lactobacillus buchneri,* used as silage additives, produce several bacteriocins, which can control pathogens such as *Listeria monocytogens* that may occur in silages (Lewus et al., 1991; Olasupo, 1996; Yildrim et al., 2002). The use of inoculants in silages may influence the digestive tract environment, with consequent effects on animal performance. The objective was to verify the probiotic effect of lactic acid bacteria (LAB), used as silage inoculants, and its products on the ruminal ambient and consequences in animal performance, by an *in vitro* assay.

**Material and Methods** There were used two LAB inoculants, *Lactobacillus plantarum (*$5x10^5$ cfu/g fresh forage) and *Lactobacillus buchneri, (*$5x10^5$ cfu/g fresh forage), besides the control treatment, totalizing three treatments, with four replicates each to compose the treated corn silages. Experimental silos were stored for up to 170 days, and after opening samples were collected for the *in vitro* assay. The inoculum from a cannulated cow, fed *ad libitum* with hay for three consecutive days, was used to compose the treatments as described by Craig et al. (1984), in a completely randomized design. Treatments were: 1) Control, with no treatment, to preserve rumen microorganisms, LAB and the possible bacteriocins from silages; 2) Autoclaved inoculum, to eliminate rumen microorganisms (Nowroozi et al., 2004), and keep LAB and bacteriocins from silages; 3) Inoculum plus protease, to inhibit bacteriocin effect; 4) Autoclaved inoculum plus protease, to keep only LAB from silages effect. Sampling was done after 48h incubation. Samples of the *in vitro* assay (n=96) were analysed for pH, LAB population and bacteriocins, by a qualitative method of critical dilution according to Mayr-Harting et al. (1972). *Bacillus cereus* was used as sensitive indicator culture. Commercial nisin (Nisaplin, Danisco) and two bacteriocin-producing lactic cultures were included in the assay as positive control of bacteriocin activity. *In vitro* assay was performed according to Weinberg et al. (2004), using 2.5 g of fresh forage in 25 mL of inoculum, which refers to an animal fed 20 kg of silage, in a rumen with 150 L capacity. Statistical analysis was done using the proc GLM of SAS 9.1.3 version, and means were compared by Student t- test with 5% significance.

**Results and Discussion**

**Table 1.** Characteristics of silages used in the *in vitro* assay

| | Treatment | | | | Effect[2] | | |
|---|---|---|---|---|---|---|---|
| Variable[1] | Control | LB | LP | SEM[3] | C*LB | C*LP | LB*LP |
| pH | 3.78 | 3.82 | 3.81 | 0.008 | 0.0081 | 0.1003 | 0.2792 |
| LAB, log cfu/g | 3.39 | 8.39 | 6.38 | 0.150 | <0.0001 | <0.0001 | <0.0001 |

LAB: lactic acid bacteria;[2]C – control; LB – *L. buchneri*; LP – *L. plantarum;* [3]SEM: standard error mean

In the *in vitro* assay the protease addition does not seem to interfere in silage pH (Table 2). Probably, the low initial LAB population in control treatment (Table 1) was not able to compete with ruminal microbiota and besides that the higher pH in the autoclaved treatments may have inhibited the increase in LAB population. In this assay, the *L. plantarum* seems to have a lower survival force when compared to *L. buchneri* due to its population reduction after autoclaving and protease addition. Weinberg et al (2004), evaluated the survival of LAB coming from silage inoculants in the rumen and observed higher LAB population in autoclaved rumen fluid, and attributed this to the greater competition when preserving rumen microbiota, which occurred in the present study to the *L. buchneri* treatment with no protease. For the silages containing *L. plantarum*, we observed the opposite effect. Although it was believed that there could be a possible probiotic effect of these inoculants inside the

rumen by the action of bacteriocins, it was not detected, where inhibition halo formation was observed only for the positive standard (nisin solution).

**Table 2.** Lactic acid bacteria (LAB) counts and pH after *in vitro* assay (n=96)

| Treatment | pH | LAB, log cfu/g[1] |
|---|---|---|
| *Control* | | |
| Control | 4.05 | 1.65[b] |
| *L. buchneri* | 4.04 | 8.44[a] |
| *L. plantarum* | 3.98 | 8.46[a] |
| SEM[2] | 0.031 | 1.06 |
| *Autoclaved inoculum* | | |
| Control | 4.27[a] | 0.83[b] |
| *L. buchneri* | 4.11[b] | 8.81[a] |
| *L. plantarum* | 4.06[b] | 6.31[a] |
| SEM | 0.031 | 1.06 |
| *Inoculum plus protease* | | |
| Control | 3.91 | 1.08[b] |
| *L. buchneri* | 3.93 | 8.45[a] |
| *L. plantarum* | 3.90 | 8.30[a] |
| SEM | 0.031 | 1.06 |
| *Autoclaved inoculum plus protease* | | |
| Control | 4.24[a] | 0.00[b] |
| *L. buchneri* | 4.05[b] | 8.23[a] |
| *L. plantarum* | 4.01[b] | 4.20[ab] |
| SEM | 0.031 | 1.06 |

[1]LAB: lactic acid bacteria; [2]SEM: standard error mean; [ab]Different letters, in each fluid treatment, differ by Student t test at 5% significance

According to Olasupo (1996), *L. plantarum* produces various bacteriocins, amongst them the plantaricins B, BN, A, C, S, T, F, C19 and SA6. However, in some strains bacteriocin activity is low and in many of them absent, which may be the case of the strains evaluated in this study. Besides that, the antibacterial activity expressed by an inoculant containing LAB strains depends on the water activity, pH and the presence of volatile fatty acids in the silage, which are specific for each bacterial strain (Gollop et al. 2005). It is possible that the environment promoted during the forage fermentation allows a smaller or larger production of bacteriocins than in the pure inoculant. However, even the tested silages did not produce bacteriocins.

**Conclusion** The inoculants evaluated were able to survive in the rumen fluid, but the evaluated strains did not produce bacteriocins.

**References**

Craig, W.M., Hong, B.J., Broderick, G.A. & Bula, R.J. (1984) In vitro inoculum enriched with particle-associated microorganisms for determining rates of fiber digestion and protein degradation. Journal of Dairy Science, 67, 2902-2909.

Gollop, N., Zakin, V. & Weinberg, Z. (2005) Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. Journal of Applied Microbiology**,** 98 (3), 662-666.

Lewus, C.B., Kaiser, A. & Montville, T.J. (1991) Inhibition of food-borne pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl. Environ. Microbiol.*, 57(6): 1683-1688.

Mayr-Harting, A., Hedges, A.J. & Berkeley, R.C.W. (1972). Methods for Studying Bacteriocins. In: Ribbons, J.R.NA.D.W. (Ed.). Methods in Microbiology: Academic Press, v.7, Part 1, 315-422.

Nowroozi, J., Mirzaii, M. & Norouzi, M. (2004) Study of *Lactobacillus* as probiotic bacteria. Iranian Journal of Public Health**,** 33 (2), 1-7.

Olasupo, N. (1996) Bacteriocins of *Lactobacillus plantarum* strains from fermented foods. Folia Microbiologica, 41 (2), p. 136.

Weinberg, Z.G., Chen, Y. & Gamburg, M. (2004) The passage of lactic acid bacteria from silage into rumen fluid, in vitro studies. Journal of Dairy Science**,** 87 (10), 3386-3397.

Yildirim, Z., Avsar, Y.K. & Yildirim, M. (2002) Factors affecting the adsorption of buchnericin LB, a bacteriocin produced by *Lactobacillus buchneri*. Microbiol Res 157:103-107.

# Additive type and composition affect fermentation pattern, yeast count, aerobic stability and formation of volatile organic compounds in whole-crop rye silage

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**Keywords:** aerobic stability, silage, volatile organic compounds, VOC, whole-crop rye, yeast

**Introduction** Whole-crop cereal silage may be prone to clostridial fermentations (Nadeau et al. 2007) or undergo severe fungal spoilage during feed-out (Knicky 2005). Therefore, the objective of this study was to evaluate the effects of different additive types and compositions on relevant quality traits of silage, including volatile organic compounds (VOC), made from whole-crop rye (WCR) under challenging conditions of short fermentation length and repeated air ingress during storage.

**Material and Methods** WCR was harvested at dough stage (DM 43.5 %) and contained 214 g $kg^{-1}$ DM water-soluble carbohydrates (WSC), 33 g $kg^{-1}$ DM crude ash, 44 g $kg^{-1}$ DM crude protein and 362 g $kg^{-1}$ DM crude fibre. The material was chopped to 30 mm theoretical particle size by a commercial chopper (Krone, Big X 650) and thereafter transferred into 1.5-L glass jars, which had 6 mm-holes in the jar body and in the lid. These holes were closed by rubber stoppers, which were removed for 24 hours on day 28 and 46 of storage to enable air ingress. Before packing, fresh WCR were treated (n=3) with commercial silage additives including different types of lactic acid bacteria (LAB): *Lactobacillus plantarum* DSM 16627 and *Lactobacillus paracasei* NCIMB 30151 ($LAB_{ho}$), *Lactobacillus buchneri* CNCM-I 4323 ($LAB_{he}$), *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673 ($LAB_{heho}$), or chemical additives containing sodium nitrite (195 g $L^{-1}$), hexamethylene tetramine (71 g $L^{-1}$), potassium sorbate (106 g $L^{-1}$) (NHS, 2 L $t^{-1}$), or sodium benzoate (257 g $L^{-1}$), potassium sorbate (154 g $L^{-1}$), ammonium propionate (57 g $L^{-1}$) (BSP, 1.5 L $t^{-1}$). After 53 days of storage at 22 °C, silages were subjected to routine analytical procedures for fermentation pattern and fungal counts. The contents of ethyl esters of lactic and acetic acids were determined by gas-chromatography and its sum is referred to as total ethyl esters. Aerobic stability (ASTA) was defined as the number of hours that the silage remained stable before reaching a temperature of 2 °C above the ambient temperature. Statistical evaluation was performed by using the procedures MIXED, REG and CORR of SAS, 9.4. The ANOVAF-option for not normally distributed data or the Tukey´s test were used as global tests for significance, which was declared at $P<0.05$.

**Results and Discussion** Additives improved the efficiency of the fermentation process as reflected by largely reduced DM losses (table 1). The very large DM losses (>10%) in untreated silages and those inoculated with $LAB_{ho}$ were very likely caused by yeast activity to form ethanol, whose concentration was highly correlated with this parameter ($y=8.23x^{0.2853}$, $R^2=0.86$, $P<0.001$). The additives $LAB_{he}$ and $LAB_{heho}$ stimulated acetic acid production ($P<0.001$) and inhibited yeast development ($P<0.001$), thereby improving ASTA ($P<0.001$). Strong relationships were found between acetic acid and yeast count ($y=-1.81x^2+2.21x+6.385$, $R^2=0.86$, $P<0.001$) and ASTA ($y=3.37e^{2.194x}$, $R^2=0.91$, $P<0.001$) respectively, and between yeast count and ASTA ($y=542.6-74.53x$, $R^2=0.92$, $P<0.001$). Only treatments NHS and BSP protected WSC from microbial degradation ($P<0.001$). As high WSC levels may negatively affect ASTA (Auerbach et al. 2013), this can explain why the chemicals only marginally improved ASTA over that of CON and $LAB_{ho}$. In addition, the used application rate of the chemical additive may have been too low to sustainably suppress yeast growth under repeated air stress during storage. However, the additives must have inhibited anaerobic yeast metabolism in earlier stages of fermentation because their use resulted in the lowest ethanol concentrations. Accumulation of ethyl esters of lactic and acetic acids was largely restricted by treatments NHS and BSP ($P<0.001$). As shown for other crops (Weiss 2017), ethanol played the prominent role in ethyl ester formation also in

WCR silage. Ethyl acetate and ethyl lactate levels increased linearly with increasing ethanol concentration (figure 1). The measured total ethyl ester concentrations corresponded very well with the predicted values according to Weiss (2017) ($R^2$=0.93, y=1.40x, *P*<0.001).

**Table 1.** Effects of additives on DM losses, fermentation pattern, yeast count, aerobic stability and VOC in whole-crop rye silage (data presented as LSmeans in g $kg^{-1}$ DM unless stated otherwise, n=3)

| Parameter | CON | $LAB_{ho}$ | $LAB_{he}$ | $LAB_{heho}$ | NHS | BSP | SEM | *P* |
|---|---|---|---|---|---|---|---|---|
| DM loss (%) | $10.9^{d}$ | $12.6^{e}$ | $6.8^{bc}$ | $7.3^{c}$ | $5.0^{a}$ | $5.3^{ab}$ | 0.50 | *** |
| WSC | $62.5^{a}$ | $55.4^{a}$ | $50.7^{a}$ | $40.2^{a}$ | $131.1^{b}$ | $138.2^{b}$ | 6.23 | *** |
| pH | $3.94^{bc}$ | $3.98^{cd}$ | $3.78^{a}$ | $3.81^{a}$ | $4.00^{d}$ | $3.92^{b}$ | 0.010 | *** |
| Lactic acid | $33.8^{b}$ | $33.8^{b}$ | $33.3^{b}$ | $32.6^{b}$ | $27.8^{a}$ | $31.5^{b}$ | 0.70 | *** |
| Acetic acid | $8.9^{a}$ | $5.8^{a}$ | $19.6^{c}$ | $21.0^{c}$ | $13.3^{b}$ | $12.7^{b}$ | 0.73 | *** |
| Ethanol | $27.1^{y}$ | $28.9^{y}$ | $5.8^{x}$ | $7.5^{xy}$ | $1.5^{w}$ | $4.1^{wx}$ | - | *** |
| 1,2-propanediol | $0.4^{x}$ | $0^{w}$ | $1.1^{y}$ | $1.4^{z}$ | $0^{w}$ | $0^{w}$ | - | *** |
| Yeast count[1] | $6.6^{b}$ | $7.1^{b}$ | $2.8^{a}$ | $3.5^{a}$ | $6.7^{b}$ | $6.3^{b}$ | 0.26 | *** |
| ASTA[2] (hours) | $19^{w}$ | $15^{w}$ | $303^{y}$ | $336^{y}$ | $52^{x}$ | $63^{x}$ | - | *** |
| Ethyl lactate[3] | $307^{z}$ | $359^{z}$ | $127^{xy}$ | $127^{y}$ | $11^{w}$ | $62^{x}$ | - | *** |
| Ethyl acetate[3] | $108^{z}$ | $87^{yz}$ | $44^{x}$ | $35^{xy}$ | $0^{w}$ | $0^{w}$ | - | *** |
| Total ethyl esters[3] | $415^{z}$ | $446^{z}$ | $171^{y}$ | $162^{yz}$ | $11^{w}$ | $62^{x}$ | - | *** |

[1]log cfu $g^{-1}$, [2]aerobic stability, [3]mg $kg^{-1}$ DM, $^{w,x,y,z}$values in rows bearing different superscripts differ, non-parametric ANOVAF-option, $^{a,b,c,d}$values in rows bearing different superscripts differ, Tukey`s test.

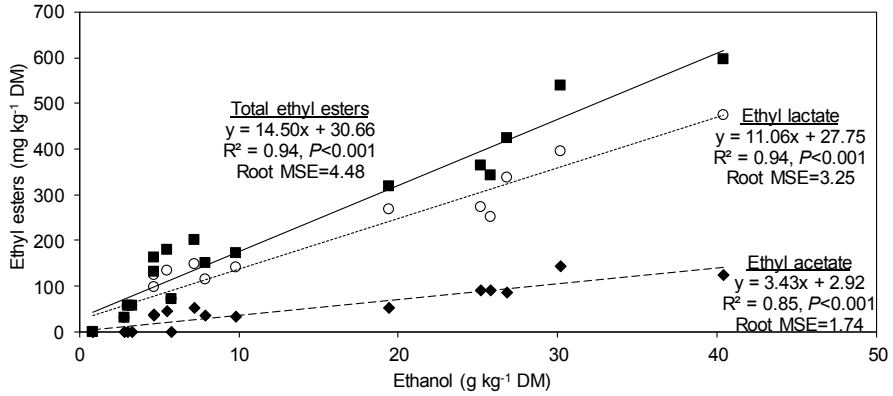


**Figure 1.** Relationship between the concentrations of ethanol and ethyl esters in whole-crop rye silage, ■ total ethyl esters, ○ ethyl lactate, ◆ ethyl acetate, n=18

**Conclusion** This study demonstrated positive effects of the tested additives on important silage quality traits. For the first time, ethyl ester concentrations were reported for WCR silage, which corresponded well with those found in other types of silage. Ethanol played the primary role in ethyl ester formation. Chemical additives were shown, again, to be best suitable to reduce VOC accumulation in silages.

**References**

Auerbach, H., Weiss, K., Theobald, P. & Nadeau, E. (2013) Effects of inoculant type on dry matter losses, fermentation pattern, yeast count and aerobic stability of green rye silages. In: Mair, C., Wetscherek, W. & Schedle, K (eds). 12. BOKU-Symposium, Vienna, Austria, 2013, pp. 179-185.

Nadeau, E. (2007) Effects of plant species, stage of maturity and additive on the feeding value of whole-crop cereal silage. Journal of the Science of Food and Agriculture, 87, 789-801.

Knicky, M. 2005. Possibilities to improve silage conservation. Acta Universitatis Agriculturae Suecia, doctoral thesis no. 62, chapter IV, p. 1-20.

Weiss, K. (2017) Volatile organic compounds (VOC) in silages - Effects of silo management factors on its formation. In: Nussio, L.G., de Sousa, D.O., Gritti, V.C., Salvati, G.G., Santos, W.P. & Salvo, P.A.R. (eds.). V International Symposium on Forage Quality and Conservation, Piracicaba, SP, Brazil, 2015, pp. 181-210.

# Aerobic stability of crimped wheat grain manipulated by additive treatments detected using different methods

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**Keywords:** Cereal, Carbon dioxide, Gas production, Spoilage, *Triticum*

**Introduction** Crimping grains enables farmers to harvest moist cereals and preserve them as animal feed. Crimped grain preservation is based on lactic acid fermentation by anaerobic lactic acid bacteria and the recommended grain moisture content (MC) is 300-400 g/kg. In good weather conditions the grains may be drier and reduced moisture of the grains has also technological advantages in harvesting and logistics. In crimped grain with MC between 200 and 300 g/kg, fermentation is restricted so that efficient protection is needed against aerobic deteriorating organisms. The objective of the current study was to evaluate the fermentation quality, microbial composition and aerobic stability of dryer than optimal crimped wheat grain ensiled with different additives. The traditional method of determining aerobic stability after the preservation is measuring the raise in temperature. This may not always be detected in relatively dry samples so we compared three methods in evaluating the aerobic stability: 1) increase in temperature, 2) measurement of $CO_2$ produced by aerobic bacteria 3) visual appearance of mould.

**Material and Methods** Crimped wheat grain with 270 g/kg MC was used as raw material. Eight treatments were used including a control (C) without additive; $FA_1$ (80% formic acid [FA], 5 l/t); $FA_2$ (58% FA, 20% propionic acid [PA], 2.5% potassium sorbate, 5.2% sodium formate [SF]; AIV Ässä Na, 5 l/t); $FA_3$ (AIV Ässä Na, 7 l/t); $FA_4$ (76% FA, 5.5% SF; AIV 2000 Plus Na, 7 l/t); $FA_5$ (37% FA, 22% SF, 18% PA, 7.3% sodium, 1% sorbic acid; GrasAAT SX, 5 l/t); $PA_1$ (54% PA; Eastman Stabilizer Crimp, 5 l/t); and $PA_2$ (37% PA, 14% sodium benzoate, 10% FA, 11% sodium propionate; Kofa Feed EP, 4 l/t). Additives $FA_2$, $FA_3$, $FA_4$ and $PA_1$ were products of Eastman Chemical Company (Oulu, Finland) and $FA_5$ and $PA_2$ were produced by ADDCON GmbH (Bitterfeld-Wolfen, Germany). Three replicates were made for each treatment. Additives were diluted in water (25 mL for one 4 kg batch) and mixed separately for each replicate and then compacted into glass jars, which were filled as full as possible to minimize the volume of air in the headspace. Jars were opened after 57 days of ensiling and samples were taken to evaluate fermentation quality and microbial quality (Seppälä et al. 2016). Aerobic stability was measured through three different approaches. For the standard method, aerobic stability was evaluated according to Seppälä et al. (2016) by following the temperature rise. For the second method, 50 g of sample was put into 0.5 l glass bottles and closed air tight with lid allowing sampling of the gas from the headspace of the bottle. The headspace gas was sampled once daily and analysed for $CO_2$ using a gas chromatograph (Perkin Elmer Arnel Clarus 500 equipped with a thermal conductivity detector TCD and a Supelco Carboxen™ 1010 PLOT fused silica capillary column (30 m x 0.53 mm)). Third method to evaluate aerobic stability was the visual appearance of mould from the glass bottles once a day using a score scale: 0 = no mould; 1 = slight mouldiness; 2 = moderate mouldiness; and 3 = severe mouldiness. The data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. The sum of squares was further partitioned into contrasts with treatment as a fixed effect and replicates as a random effect.

**Results and Discussion** Wheat raw material MC was 194 g/kg containing 20 g of ash, 139 g crude protein (CP), 670 g starch and 126 g neutral detergent fibre (NDF)/kg DM. The MC of wheat grain after addition of water was 270 g/kg. Total bacteria, yeasts and moulds of the wheat raw material were $1.4 \times 10^7$, $7 \times 10^4$ and $2.3 \times 10^5$ CFU/g, respectively. Overall, MC was similar for all treatments (Table 1), ranging from 269 to 276 g/kg and this resulted in virtually no lactic acid fermentation of the grains but in control, significant ethanol formation occurred. $FA_3$ resulted in the lowest pH value among treatments. Ammonia-N was similar for control and additive treatments but higher for $PA_1$. $PA_1$, $FA_1$ and $FA_4$ presented the highest number of moulds, which were even higher than in the raw material while control and $FA_3$ showed the lowest mould numbers. Aerobic stability of crimped wheat grain preserved with $FA_1$, $FA_5$, $PA_2$ and control treatment was the shortest, followed by $FA_4$. The longest aerobic stability was reached for $FA_3$ treatment, where temperature did not increase 2 °C above the ambient during the whole observation period of 174 h; and $PA_1$ treatment with 168 hours. According to commercial instructions, $PA_2$ should not be mixed with water, which

was done for all additives to promote even distribution in the raw material. This practise may have resulted in crystallization of benzoic acid and subsequently reduced the efficiency of $PA_2$ treatment in the current study.

Cumulative $CO_2$ production of crimped wheat grain preserved with $FA_1$ was the highest and fastest among the treatments (Figure 1A). Control, $FA_4$, $FA_5$ and $PA_2$ presented the same pattern in cumulative $CO_2$ while that of $FA_3$ remained close to zero throughout the evaluation period.

**Table 1.** Aerobic stability, fermentation quality and microbial composition of crimped wheat grain according to additive treatments.

| | C | $FA_1$ | $FA_2$ | $FA_3$ | $FA_4$ | $FA_5$ | $PA_1$ | $PA_2$ | SEM[6] | $(R^2)$[7] |
|---|---|---|---|---|---|---|---|---|---|---|
| Aerobic stability, hours | | | | | | | | | | |
| Increase in °C[1] | 80[a] | 72[a] | 137[b] | 174[b,*] | 108[ab] | 87[ab] | 168[b] | 82[a] | 12.5 | |
| $(CO_2)$[2] | 55[a] | 54[a] | 108[c] | 168[d,*] | 82[b] | 71[ab] | 127[c] | 64[ab] | 6.0 | 0.936 |
| $(CO_2)$[3] | 80[ab] | 64[a] | 126[c] | 168[d,*] | 99[bc] | 95[bc] | 164[d] | 79[ab] | 6.9 | 0.979 |
| Score[4] | 112[bcd] | 48[a] | 114[cd] | 168[f,*] | 68[a] | 86[abc] | 168[f,*] | 132[de] | 9.9 | 0.567 |
| Score[5] | 128[cd] | 62[a] | 127[cd] | 168[e,*] | 92[ab] | 97[bc] | 168[e,*] | 144[d] | 7.1 | 0.528 |
| Moisture content, g/kg | 276 | 271 | 272 | 269 | 271 | 273 | 272 | 273 | 1.1 | |
| pH | 6.16[e] | 4.89[b] | 5.20[c] | 4.57[a] | 5.01[bc] | 5.79[d] | 5.75[d] | 5.87[d] | 0.052 | |
| In DM, g/kg | | | | | | | | | | |
| Water soluble carbohydrate | 19.0[a] | 33.7[bc] | 41.8[c] | 37.6[bc] | 35.2[bc] | 28.3[ab] | 30.6[ab] | 22.0[ab] | 2.59 | |
| Ethanol | 7.80[d] | 0.19[a] | 0.28[a] | 0.26[a] | 0.24[a] | 0.57[a] | 1.33[b] | 3.25[c] | 0.122 | |
| Lactic acid | 0[a] | 0[a] | 0[a] | 0[a] | 0[a] | 0[a] | 0.22[a] | 0.74[b] | 0.048 | |
| Acetic acid | 1.11[b] | 0.58[a] | 0.67[a] | 0.55[a] | 0.58[a] | 0.78[a] | 1.29[b] | 1.35[b] | 0.063 | |
| Propionic acid[8] | 0.13[a] | 0.16[a] | 1.09[bc] | 1.74[c] | 0.75[ab] | 1.01[b] | 4.28[d] | 1.77[c] | 0.148 | |
| Butyric acid | 0.04 | 0.03 | 0.03 | 0.03 | 0.04 | 0.03 | 0.04 | 0.04 | 0.003 | |
| Amm-N, g/kg N[8] | 5.85[a] | 4.65[a] | 4.95[a] | 4.65[a] | 5.40[a] | 4.35[a] | 16.94[b] | 4.35[a] | 0.418 | |
| Yeasts, CFU/g | $2.7\times10^3$ | $<1\times10^1$ | $<1\times10^1$ | $<1\times10^1$ | $<1\times10^1$ | $1.5\times10^3$ | $4.2\times10^3$ | $4.1\times10^4$ | - | |
| Moulds, CFU/g | $9\times10^2$ | $4.3\times10^5$ | $1.2\times10^5$ | $1.7\times10^2$ | $3.7\times10^5$ | $6.1\times10^4$ | $7.2\times10^5$ | $5.5\times10^4$ | - | |

Values with same letter in a row are not significantly different at 5% Tukey test. For additive treatments, see Material and Methods section; [1]Time taken to increase 2 °C above the ambient temperature; [2,3]Time taken to increase the $CO_2$ concentration up to 1 and 2 mg/g DM, respectively; [4,5]Time taken to sample reach cumulative mould score 1 and mould score 2, respectively; [6]Standard error of the mean; [7]Correlation coefficients between increase in temperature[1] and the alternative methods to evaluate aerobic stability; [8]Not corrected for the amount of propionic acid and ammonia from the additive. *Treatment did not reach the threshold during the evaluation period.

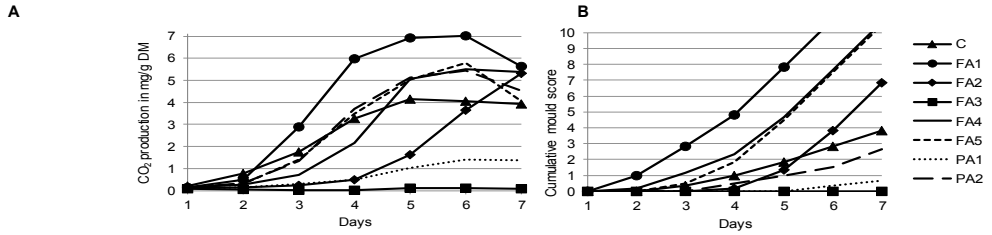


**Figure 1. A**. Cumulative $CO_2$ production (mg/g DM) and **B**. cumulative mould score of ensiled crimped wheat grain according to days after air exposure and additive treatments.

There was a positive correlation between aerobic stability and $CO_2$ production (Table 1), and both methods classified the treatments in the same order, but at different intensities, because there were different concentrations of $CO_2$ at the time the treatment heated 2 °C above ambient temperature. The visual evaluation of mould (Figure 1B) did not classify treatments exactly as the production of heat and $CO_2$, but this method appeared practical, easy, efficient, low cost and yet providing useful results of differences between additives.

**Conclusion** There were clear differences in the efficacy of additives in improving the aerobic stability of relatively low moisture crimped wheat grains, with $FA_3$ (high dose of additive including several ingredients) being the most efficient. All methods used to evaluate aerobic stability provided useful information of the efficacy of the additives. The correlation between temperature and $CO_2$ production was very high indicating that $CO_2$ produced by aerobic bacteria can be used as a method to evaluate aerobic stability, while visual appearance ranked the additives somewhat differently.

**References**

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M. (2016) Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass and Forage Science, 71, 458-471.

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# Additives with Lactobacillus spp. mix and cellulose enzymes affect the chemical quality and in situ ruminal degradability of whole –plant corn silage

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**Keywords:** Cellulose enzymes, corn silage, Lactobacillus spp, ruminal degradability

**Introduction** Silage of whole-plant corn with high dry matter (DM) content could result in lower contents of soluble carbohydrates, lower fiber degradability and could affect the fermentation process (Bal et al. 1997, Saldaña and Cañeque Martinez 1998). Biological additives are useful for make faster the ensiling process, Also, some additives have been added fibrolytic enzymes or are produced by the same Lactobacillus spp.(LAB), which could improve the degradation of the cell wall (Muck et al. 2010). For this study was used Biotrato (Biogrow Argentina Co.), this is an inoculant for high fiber forage, contain six types of LABs (60 x $10^9$ CFU $g^{-1}$) and cellulose enzyme. We hypothesize that corn silage treated with Biotrato (TBIO) have less aNDF, ADF and more degradability in situ than the original harvested plant (OPH) and non-inoculated control silage (CS). The aim of this trial was studied the effect of TBIO in the fiber content and In Situ degradability of corn silage harvesting with ~40% DM.

**Materials and Methods** Trial was carried out at Clemos Laboratories, Villa María, Córdoba, Argentina, since April to August of 2016. We used whole-plant corn chopped and refrigerated at ~3°C in farm, and then moved to the lab. The hybrid was Dow 510 PW, harvested at 112 days from planting, with DM 40.27 ± 1.80 %. Whole-plant corn chopped were ensiled in 10 mini silos of high density polyethylene (n = 5 for TBIO; n = 5 for CS) of 10 litres capacity each one. The mini silos were filling at 600 kg as feed $m^{-3}$ with air press. The addition of Biotrato (16% *Lactobacillus curvatus*: 16% *Lactobacillus plantarum*, 16% *Lactobacillus acidophilus,* 16% *Pediococcus acidilactici*, 16% *Enterococcus faecium*, 12% *Lactobacillus buchneri* and 8 % cellulose enzyme) for TBIO was $10^6$ CFU $g^{-1}$ of fresh forage sprayed with the distilled water. Mini silos were stored at controlled temperature (25.0 ± 1.0 °C) and opened at 63 days. Silage samples were analyzed by properly accredited commercial laboratory by wet chemistry for DM, crude protein (CP), aNDF, ADF, ash and starch (STCH) for OPH. For TBIO and CS also were analyzed pH, NH3 $Nt^{-1}$, acid-detergent insoluble nitrogen (ADIN) and acid detergent lignin (ADL). For In situ ruminal degradability (ISRD, %), one sample by mini silo (n = 5 for TBIO; n = 5 for CS), were incubated in nylon bags into three rumen-cannulated steers in INTA Manfredi Lab. (Córdoba, Argentina). The incubation times were 0, 8, 16, 24 and 48 hours. The data set from wet chemistry was submitted to analysis of variance and test LSD Fisher. For ISRD were analyzed as a randomized complete block using Infostat 2017 (Di Rinzo et al. 2017).

**Results** For TBIO aNDF was lower and significant ($P < 0.05$). CP and STCH were greater and significant ($P < 0.05$) for TBIO compared with OPH and CS.. For ISRD was greater and significant ($P < 0.05$) at 0 hours and 8 hours (Table 2).

**Discussion** This study shown that fiber levels (aNDF and ADIN) of whole-plant corn silage treatment with Biotrato decrease compared with OPH and CS, which can be attributed to the enzymatic effect. Similar results were found by Ruiz et al. (2009) and Spoelstra et al. (1992). ISRD was higher at 0 and 8 hours for inoculated treatment (TBIO), but no effect were found after them. Because after enzymes effect NDF decrease, soluble sugars concentrations increase and the remaining of cell walls is less digestible during ensiling (Nadeau et al. 2000).

**Conclusions** this study shown the positive effect of TBIO on the whole-plant corn silage fiber content compared OPH and CS, suggesting the possible to enhance the digestibility of corn silage.

**Table 1.** Chemical value of original plant and by treatment.

| Variable | OPH | CS | TBIO | SEM | P Value |
|---|---|---|---|---|---|
| DM, % | 40.27[a] | 38.85[ab] | 37.79[b] | 0.55 | 0.024 |
| CP, % | 8.54[a] | 8.73[a] | 9.15[b] | 0.09 | <0.001 |
| aNDF, % | 39.00[a] | 35.00[b] | 33.29[c] | 0.49 | <0.001 |
| ADF, % | 20.24[a] | 18.76[ab] | 17.06[b] | 0.88 | 0.071 |
| Ash, % | 7.48[a] | 6.27[b] | 6.65[b] | 0.15 | <0.001 |
| STCH, % | 22.71[a] | 29.67[b] | 28.71[b] | 1.90 | 0.048 |
| ADIN, % | - | 0.21[a] | 0.16[b] | 0.01 | 0.020 |
| ADL | - | 3.14 | 2.92 | 0.11 | 0.177 |
| NH3 $Nt^{-1}$ | - | 4.09 | 3.72 | 0.15 | 0.128 |
| pH | - | 3.50 | 3.49 | 0.02 | 0.572 |

OPH: original harvested plant; CS: non-inoculated control silage; TBIO: silage treated with Biotrato *Means in the same row with different letters differ significantly (P<0.05).*

**Table 2.** In situ ruminal degradability of corn silage by treatment.

| Hours | CS | TBIO | SEM | P Value |
|---|---|---|---|---|
| 0 | 31.72[a] | 33.27[b] | 0.43 | 0.016 |
| 8 | 53.68[a] | 55.73[b] | 0.69 | 0.044 |
| 16 | 65.61 | 66.32 | 0.64 | 0.439 |
| 24 | 70.56 | 72.04 | 0.85 | 0.086 |
| 48 | 78.35 | 78.83 | 0.45 | 0.454 |

CS: non-inoculated control silage; TBIO: silage treated with Biotrato*. Means in the same row with different letters differ significantly (P<0.05).*

**References**

Bal, M.A., Coors, J.G., & Shaver, R.D. (1997) Impact of the maturity of corn for use as silage in the diets of dairy cows on intake, digestion, and milk production. Journal of Dairy Science, 80(10), 2497-2503.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. InfoStat versión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar.

Muck, R.E. (2010) Silage microbiology and its control through additives. R. Bras. Zootec., Viçosa , v. 39, supl. spe, p. 183-191.

Nadeau, E.M.G., Buxton, D.R., Russell, J.R., Allison, M.J., & Young, J.W. (2000) Enzyme, Bacterial Inoculant, and Formic Acid Effects on Silage Composition of Orchardgrass and Alfalfa1, 2. Journal of Dairy Science, 83(7), 1487-1502.

Ruiz, B.O., Castillo, Y., Anchondo, A., Rodríguez, C., Beltrán, R., La O, O., & Payán, J. (2009) Efectos de enzimas e inoculantes sobre la composición del ensilaje de maíz. Archivos de Zootecnia, 58 (222), 163-172.

Saldaña, S. (1998) Ensilado de forrajes y su empleo en la alimentación de rumiantes. Vicente Cañeque Martinez, José Luis Sancha Saldaña.

Spoelstra, S.F., Van Wikselaar, P.G. & Harder, B. (1992) The effects of ensiling whole crop maize with a multienzyme preparation on the chemical composition of the resulting silages. Journal of the Science of Food and Agriculture, 60(2), 223-228.

# An evaluation of monopropionine as chemical additive to improve aerobic stability of corn silage

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**Keywords:** aerobic stability, chemical additive, corn silage, monopropionine.

**Introduction** One of the main problems on farm throughout the world is the aerobic deterioration of silages due to the action of yeast and others aerobic bacteria during the feed-out phase. High environmental temperatures may increase the growth rates of spoilage microorganisms, thereby intensifying the deterioration process (Borreani et al. 2018). Since yeasts are usually believed to initiate aerobic spoilage, additives containing antifungal components have been used to decrease their numbers and improve the aerobic stability (Da Silva et al. 2015). The aim of this study was to evaluate the effect of a new chemical additive, composed of a mixture of monoglycerides of short chain fatty acids, on the aerobic stability of whole crop corn silage.

**Material and Methods** Two trials were carried out on 40% dry matter (DM) whole crop corn. The fresh forage was untreated **(C)** or treated with a new additive [composed of a mixture of monoglycerides, mainly consisting of monopropionine and monobutyrin (SILO S.p.A., Firenze, IT)] applied at a rate of 0.5% fresh matter (FM) **(0.5%)**, 1.0% FM **(1.0%)** and 1.5% FM **(1.5%)**. The forages were ensiled in 20-L plastic silos with four replications and opened after 100 and 240 d of ensiling for Trial I and Trial II, respectively. At opening, the silages were analyzed for their DM content, pH, fermentative profile and microbial counts [lactic acid bacteria (LAB), yeast and mold. The DM content was determined at 60°C for 72 h, fermentative profiles was characterized in the acid extract by HPLC (Canale et al., 1984). The microbial counts were determined using the pour plate technique on MRS and YGC agar, for LAB, and for yeasts and molds, respectively. The weight losses due to fermentation were calculated as the difference between the weight of the forage placed in each plastic silo at ensiling and the weight at the end of conservation, and were expressed on a DM basis. After each opening, the silages were subjected to an aerobic stability test by continuously measuring the temperature during exposure to air. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. The aerobic stability tests were made at ambient temperature. The obtained data were analyzed for their statistical significance, via analysis of variance using version 24 of SPSS or Windows (SPSS Inc., Chicago, IL). The data were analyzed utilizing treatments as the fixed factor. When the calculated values of F were significant, the REGWF test ($P$<0.05) was used to interpret any significant differences among the mean values.

**Results and Discussion** The DM content, fermentative profile, yeast count and aerobic stability of the silages at opening are reported in Table 1. No practical differences were found in either trial concerning the pH value or the DM content between treatments. The fermentation was typical of corn silage with a 40% DM content, and showed a dominant homofermentative fermentation and a higher lactic-to-acetic-acid ratio than 4 in all the silages. The 1,2-propanediol was absent in all the silages. Butyric and propionic acids were below the detection limit (<0.01 g/kg DM) in the C treatment, whereas the treated silages revealed increasing amounts as the additive dose was increased. This was only due to an additive effect and not to microbial activity, since propionic and butyric acids were also detected immediately before ensiling after the addition of the additive (data not shown). Since the silage was extracted at pH 1.5, the additives were detected by the HPLC as free acids. The ethanol content was significantly reduced as a result of the increasing additive application in both trials. The addition of the SILO product decreased the LAB count at opening of the silage without negatively affecting silage fermentation. It could be hypothesized that the decrease in LAB occurred after they had fermented the main sugar in the plant 30 to 40 d after ensiling. The weight losses were affected by the treatments; the lower the DM losses, the higher the dose of monoglyceride additive applied. The addition of the additive decreased the yeast count below 3 $\log_{10}$ cfu/g in the treated silages,

whereas higher values than 4 $log_{10}$ cfu/g were found in the C silages in both trials. The reduction in the yeast count was reflected in a higher aerobic stability in both trials. The higher the application dose of the additive was, the higher the aerobic stability. In Trial II, the aerobic stability of the silages was lower than in Trial I in all the treatments, when compared at the same number of yeast, and this is mainly attributable to a higher temperature during the aerobic stability test (27 vs. 21°C), as previously observed by Ashbell et al. (2002).

**Conclusion** A positive action of a mixture of monopropionine and monobutyrin as a silage additive to improve the aerobic stability of silages was found in a dose dependent manner. The higher environmental temperature during air exposure reduced aerobic stability. This new additive seems to be very promising to improve aerobic stability of corn silage and further investigation could be conducted to establish the cost effectiveness of the treatment and suitable management practices.

**Table 1.** DM, fermentative profile, yeast count and aerobic stability of corn silages in Trials I and II.

| Parameters* | Trial I | | | | | | Trial II | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | 0.5% | 1.0% | 1.5% | *P* | SE | C | 0.5% | 1.0% | 1.5% | *P* | SE |
| DM (g/kg) | 40.0$^{b}$ | 41.1$^{ab}$ | 42.2$^{a}$ | 41.8$^{a}$ | * | 0.305 | 39.9$^{b}$ | 41.2$^{ab}$ | 42.0$^{a}$ | 41.5$^{a}$ | * | 0.277 |
| pH | 3.74$^{a}$ | 3.72$^{b}$ | 3.71$^{b}$ | 3.71$^{b}$ | ** | 0.004 | 3.73 | 3.69 | 3.70 | 3.71 | NS | 0.0059 |
| Lactic acid (g/kg DM) | 49.2 | 42.1 | 44.2 | 43.9 | NS | 1.23 | 49.9 | 48.6 | 45.1 | 45.7 | NS | 0.946 |
| Acetic acid (g/kg DM) | 11.2$^{a}$ | 7.5$^{b}$ | 7.5$^{b}$ | 7.2$^{b}$ | *** | 0.512 | 10.6$^{a}$ | 9.7$^{ab}$ | 8.8$^{bc}$ | 8.7$^{bc}$ | ** | 0.259 |
| Lactic-to-acetic ratio | 4.4 | 5.6$^{a}$ | 5.9$^{a}$ | 6.1$^{a}$ | *** | 0.212 | 4.7 | 5.0 | 5.1 | 5.3 | NS | 0.884 |
| Propionic acid (g/kg DM) | 0.0$^{d}$ | 1.5$^{c}$ | 3.0$^{b}$ | 4.2$^{a}$ | *** | 0.494 | 0.0$^{d}$ | 1.2$^{c}$ | 3.0$^{b}$ | 4.2$^{a}$ | *** | 0.507 |
| Butyric acid (g/kg DM) | 0.0$^{d}$ | 0.9$^{c}$ | 1.8$^{b}$ | 3.0$^{a}$ | *** | 0.34 | 0.0$^{d}$ | 0.6$^{c}$ | 1.5$^{b}$ | 2.4$^{a}$ | *** | 0.279 |
| Ethanol (g/kg DM) | 16.9$^{a}$ | 9.9$^{ab}$ | 7.4$^{bc}$ | 4.8$^{bc}$ | *** | 1.39 | 26.8$^{a}$ | 18.4$^{b}$ | 8.8$^{c}$ | 5.7$^{c}$ | *** | 2.64 |
| LAB ($log_{10}$ cfu/g) | 6.29$^{a}$ | 5.66$^{ab}$ | 5.03$^{b}$ | 5.16$^{b}$ | * | 0.197 | 5.16$^{a}$ | 4.71$^{a}$ | 3.55$^{b}$ | 3.39$^{b}$ | ** | 2.56 |
| Yeast ($log_{10}$ cfu/g) | 4.29$^{a}$ | 1.83$^{b}$ | 2.81$^{ab}$ | 1.93$^{b}$ | * | 0.362 | 4.72$^{a}$ | 2.77$^{b}$ | 0.50$^{c}$ | 1.05$^{b}$ | *** | 0.523 |
| Aerobic stability (h) | 89$^{b}$ | 141$^{ab}$ | 147$^{ab}$ | 207$^{a}$ | * | 15.6 | 52$^{b}$ | 67$^{ab}$ | 131$^{ab}$ | 173$^{a}$ | * | 18.5 |
| Weight losses (% DM) | 2.25$^{a}$ | 1.77$^{ab}$ | 1.38$^{bc}$ | 0.77$^{c}$ | ** | 0.190 | 3.69$^{a}$ | 2.52$^{ab}$ | 1.53$^{b}$ | 1.16$^{b}$ | * | 0.391 |

*C = Control silage; 0.5, 1.0 and 1.5% = monopropionine and monobutyrin additive rate on fresh forage; DM = dry matter; LAB = lactic acid bacteria; SE = standard error of the mean.

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**References**

Ashbell, G., Weinberg, Z.G., Hen Y. & Filya I. (2002) The effects of temperature on the aerobic stability of wheat and corn silages. Journal of Industrial Microbiology and Biotechnology, 28, 261-263.

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes B.J. & Muck R.E. (2018) Factors affecting dry matter and quality losses in silages. Journal of Dairy Science, 101, 3952-3979.

Canale, A., Valente M.E., & Ciotti A. (1984) Determination of volatile carboxylic acids (C1–C5) and lactic acid in aqueous acid extracts of silage by high performance liquid chromatography. Journal of the Sciences of Food and Agriculture, 35, 1178-1182.

Da Silva, T.C., Smith, M.L., Barnard A.M. & Kung L. (2015) The effect of a chemical additive on the fermentation and aerobic stability of high-moisture corn. Journal Dairy Science, 98, 8904-8912.

# Biological and chemical additives maintain nutritive value of grass silage during air exposure

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**Keywords:** additives, aerobic stability, digestibility, grass silage

**Introduction** Aerobic instability of grass silage negatively affects its hygienic quality due to fungal development and potential mycotoxin formation. Moreover, during exposure to air, digestible nutrient losses may occur, resulting in reduced intake and performance, and lower farm profitability. Silage additives have been successfully used to mitigate aerobic deterioration. However, as their mode of action differs, this study aimed at comparing different additive types on fermentation, aerobic stability and organic matter digestibility.

**Material and Methods** A second cut grass ley was harvested on a dairy farm near Skara, Sweden, wilted overnight to 42% dry matter (DM), and chopped to 20 mm theoretical particle size. Herbage was manually treated by spraying with the following additives, applied in water at a total volume of 10 ml $kg^{-1}$ fresh forage: *Lactobacillus buchneri* CNCM-I 4323 (LB); *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673 (LBPA); or a chemical blend (SNHEPS) composed of sodium nitrite (195 g $L^{-1}$), hexamethylene tetramine (71 g $L^{-1}$) and potassium sorbate (106 g $L^{-1}$), applied at 2.5 L $t^{-1}$. All additives were kindly provided by KONSIL Europe GmbH, Germany. Untreated grass (CON) received tap water at 10 ml $kg^{-1}$. Subsequently, herbage was packed to 172 kg DM $m^{-3}$ in 1.6-L glass jars, which were equipped with a hole (6 mm diameter) in the body and in the lid. These holes were closed by rubber stoppers that were removed for 24 hours on day 28 and 49 of fermentation enabling air ingress to stimulate fungal development. Silages were stored for 56 days at about 22 °C. Standard analytical procedures for silage evaluation were used. *In vitro* organic matter digestibility (IVOMD) was determined and metabolizable energy (ME) content was calculated according to Lindgren (1979, 1983). Aerobic stability (ASTA) was evaluated by the temperature method over 276 hours (11.5 days) of aeration. Silage was considered aerobically unstable once the sample temperature was higher by 2 °C than the ambient temperature. All data were subjected to statistical analysis using the mixed model procedure of SAS 9.4. Significance was declared at $P<0.05$, and the Tukey´s test was employed for pairwise comparisons between least-square means (LSmeans).

**Results and Discussion** Our results substantiate previous observations by Nadeau et al. (2012) that the use of the chemical additive (SNHEPS) resulted in the most efficient fermentation process as reflected by the lowest DM losses, highest water-soluble carbohydrate concentrations and largely restricted formation of ethanol as well as proteolysis as indicated by the lowest ammonia-N content (table 1). Combining the homofermentative *Pediococcus acidilactici* with the heterofermentative *Lactobacillus buchneri* in LBPA counteracted the increase in DM losses and proteolysis and the decrease in WSC that were observed in silages solely treated with LB. Yeast counts were lowest when *Lactobacillus buchneri*-containing additives were used, resulting in aerobically stable silages over the entire period of aeration. However, there was only a trend observed for the additive SNHEPS to improve ASTA ($P=0.073$). This can be explained by the fact that one of the three replicate silages showed low ASTA of 50 hours, which may have been caused by too low of an application rate, or inhomogeneous additive distribution. Both IVOMD and ME content of the silage at silo opening were high, and no differences between treatments were detected (table 2). Aeration of untreated silage largely reduced IVOMD and ME, whereas these parameters remained unaffected when additives were applied. This confirms results on maize silage by Nadeau et al. (2011). Assuming that the nutritive

value declines only when silages heat-up, these changes caused a reduction per day of heating of 0.71 digestibility units and 0.12 MJ ME $kg^{-1}$ DM, respectively.

**Table 1.** Fermentation characteristics, yeast count and aerobic stability of grass silage (data presented as LSmeans in g $kg^{-1}$ DM unless stated otherwise, n=3)

| Parameter | CON[1] | LB[2] | LBPA[3] | SNHEPS[4] | SEM | *P*-value |
|---|---|---|---|---|---|---|
| DM loss (%) | 5.0$^{c}$ | 6.6$^{a}$ | 5.3$^{b}$ | 4.1$^{d}$ | 0.03 | *** |
| WSC[5] | 74.1$^{b}$ | 17.9$^{d}$ | 30.8$^{c}$ | 91.2$^{a}$ | 7.17 | *** |
| $NH_3$-N (% total N) | 7.4$^{b}$ | 8.2$^{a}$ | 6.8$^{c}$ | 5.4$^{d}$ | 0.05 | *** |
| pH | 4.16$^{c}$ | 4.31$^{a}$ | 4.07$^{d}$ | 4.24$^{b}$ | 0.005 | *** |
| Lactic acid | 43.4$^{b}$ | 30.5$^{d}$ | 46.7$^{a}$ | 37.8$^{c}$ | 0.47 | *** |
| Acetic acid | 11.0$^{b}$ | 21.0$^{a}$ | 14.2$^{ab}$ | 11.0$^{b}$ | 1.43 | ** |
| Ethanol | 5.9$^{ab}$ | 8.6$^{a}$ | 2.4$^{b}$ | 1.1$^{b}$ | 1.14 | ** |
| 1,2-propanediol | 4.1$^{b}$ | 14.3$^{a}$ | 9.9$^{a}$ | 3.2$^{b}$ | 1.12 | ** |
| Yeast count (log cfu $g^{-1}$) | 6.4$^{a}$ | 2.2$^{c}$ | 3.3$^{bc}$ | 4.3$^{ab}$ | 0.57 | ** |
| ASTA[6] (hours) | 44$^{b}$ | 276$^{a}$ | 276$^{a}$ | 201$^{ab}$ | 37.8 | * |
| pH after ASTA[6] test | 6.43$^{a}$ | 4.34$^{b}$ | 4.10$^{b}$ | 4.92$^{ab}$ | 0.363 | * |

[1]untreated control, [2]*L. buchneri* CNCM I-4323, [3]*L. buchneri* CNCM I-4323 and *P. acidilactici* DSM 11673, [4]liquid blend of sodium nitrite, hexamethylene tetramine, potassium sorbate, [5]water-soluble carbohydrates, [6]aerobic stability, LSmeans in rows with unlike superscripts differ at *P*<0.05 (Tukey´s test).

**Table 2.** *In vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) of grass silage before and after aeration as affected by additive use (data presented as LSmeans, n=3)

| Parameter | CON[1] | LB[2] | LBPA[3] | SNHEPS[4] | SEM | *P* - value* |
|---|---|---|---|---|---|---|
| IVOMD (%) | | | | | | <0.001 |
| before aeration | 85.0$^{x}$ | 82.6$^{x}$ | 84.3$^{x}$ | 84.3$^{x}$ | 0.81 | |
| after aeration | 78.1$^{y}$ | 85.2$^{x}$ | 84.9$^{x}$ | 85.8$^{x}$ | | |
| ME[5] (MJ $kg^{-1}$ DM) | | | | | | <0.001 |
| before aeration | 10.8$^{x}$ | 10.4$^{x}$ | 10.7$^{x}$ | 10.7$^{x}$ | 0.13 | |
| after aeration | 9.6$^{y}$ | 10.8$^{x}$ | 10.7$^{x}$ | 10.8$^{x}$ | | |

[1]untreated; [2]*L. buchneri* CNCM I-4323; [3]*L. buchneri* CNCM I-4323 and *P. acidilactici* DSM 11673, [4]liquid blend of sodium nitrite, hexamethylene tetramine, potassium sorbate, [5]calculated from IVOMD, [x,y]LSmeans in rows (between additives) and columns (within additive for each parameter) bearing unlike superscripts differ at *P*<0.05, *denotes significance level of the interaction aeration-by-additive treatment.

**Conclusion** Silage additives affected the fermentation process according to their mode of action, and the chemical treatment was most efficient. The detrimental effects of air during feed-out can be avoided by using additives with a proven record to improve aerobic stability, thereby maintaining the nutritive value of the silage until fed to the animal.

**References**

Lindgren, E. (1979) The nutritional value of roughages determined in vivo and by laboratory methods. Report 45. Department of Animal Nutrition & Management, SLU, Uppsala, Sweden, p. 66.

Lindgren, E. (1983) Nykalibrering av VOS-metoden för bestämning av energivärdehos vallfoder. Working Paper. Department of Animal Nutrition and Management, SLU, Uppsala, Sweden, p. 4.

Nadeau, E., Svensson, E., Zaralis, K., Helander, C., Pauly, T. & Arnesson, A. (2011) Effects of additives on aerobic stability and nutritive value of maize silage stored during different time periods when harvested at advancing maturity stages. Proceedings of the 8th International Symposium on the Nutrition of Herbivores, Aberythwyth, Wales, UK, 2011, p. 363.

Nadeau, E., Arnesson, A. & Auerbach, H. (2012) Effects of additives and particle size on fermentation and aerobic stability of grass silage. In: Kuoppala, K., Rinne, M. & Vanhatalo, A. (eds.). Proceedings XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 380-381.

# Carrot by-product fermentation quality and aerobic stability could be modified with silage additives

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**Keywords:** *Daucus carota*, Feed preservation, Feed value, Formic acid, Lactic acid bacteria

**Introduction** Incremental demand in consumer choices and growth of population result in large quantities of biowaste production worldwide (Karak et al. 2012). There is a requirement for a more sustainable production in the food industry, where many by-products become biowaste along the process chain. One of the options would be to use these by-products as animal feeds, but the challenge is how to preserve and store such residues as they are typically wet and prone to fast deterioration. Large amounts of by-products from carrot industry are generated and could potentially be stored as silage for subsequent use in animal diets. Additives can be added to materials to preserve and/or enhance the quality, such as increasing aerobic stability and shelf life. The objective of the current study was to evaluate the carrot by-product ensiling process with or without commercial silage additives (lactic acid bacteria inoculants and formic acid) on chemical composition, fermentation pattern and aerobic stability.

**Material and Methods** Treatments were designed in a factorial 2 × 3, where first factor was two types of raw material, carrot by-product with pieces and without pieces; second factor was three additive treatments, including a control without additive, a heterofermentative lactic acid bacteria (LAB) inoculant (*Lactobacillus paracasei, L. plantarum, Lactococcus lactis* and *L. buchneri*, $1.25\times10^{11}$ cfu/g, Bonsilage Alfa, 2 g/t; Schaumann, Pinneberg, Germany) and a formic acid (FA) based product (AIV2 Plus, 5 l/t; Eastman Chemical Company, Oulu, Finland). The carrot mass was carefully mixed with the additive and packed tightly in plastic bags (10 kg fresh matter), which were placed in buckets. Two replicates per treatment were prepared. The plastic bags were tightly sealed and a gas valve was placed on top of the bag to allow the gas escape. Silos were opened after a three-week preservation period. Chemical and microbiological characteristics and fermentation quality of the samples was determined by standard laboratory methods. Aerobic stability of the carrot samples before and after fermentation was measured by placing a 2 cm layer of the carrot mass in a plastic container which was covered by a perforated plastic film and maintained at +20 °C. Aerobic stability was evaluated once daily by visually observing growth of yeasts and moulds on the surface of the carrot mass. The data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability. The sum of square was further partitioned into control vs. additives, LAB vs. FA and types of raw material.

**Results and Discussion** The dry matter (DM) concentration of carrot by-products prior to ensiling was low (76 and 70 g/kg) and they contained on average 69 and 71 g ash, 65 and 68 g crude protein (CP), 282 and 106 g water soluble carbohydrates (WSC), and 220 and 290 g neutral detergent fibre in kg DM, respectively, for carrot with and without pieces. Yeast and moulds for carrot with and without pieces were $1.1\times10^{5}$ and $1.5\times10^{6}$, $8.2\times10^{3}$ and $1.0\times10^{5}$ cfu/g, respectively. Carrot with and without pieces presented, respectively, $6.2\times10^{4}$ and $7.8\times10^{5}$ cfu/g enterobacteria and $2.1\times10^{6}$ and $1.8\times10^{7}$ cfu/g LAB.

There was a higher concentration ($P<0.05$) of ash, WSC, lactic, acetic and propionic acids for carrot by-product with pieces than in the carrot without pieces (Table 1). All treatments showed extensive fermentation with low pH (on average 3.71) after the three-week fermentation period. Formic acid increased ($P<0.05$) DM and CP content of both carrot by-product types. Use of FA efficiently reduced ($P<0.05$) conversion of WSC into lactic, acetic and propionic acids compared to control and LAB, but resulted in very high ammonia N proportion of total N. Formic acid also decreased ($P<0.05$) butyric acid of the silages. There was no effect ($P>0.05$) of LAB treatment compared to control in the

parameters evaluated, except for losses (P<0.05), which increased with the addition of the inoculant. Some fresh samples (before ensiling), deteriorated in two to three days during aerobic phase (Figure 1). The stability of the FA treated materials was slightly prolonged, but all samples deteriorated quickly compared to previous experiment (Rinne et al. 2017). In the ensiled materials, FA increased the aerobic stability up to 3 days; control and LAB were deteriorated more quickly. FA decreased (P<0.05) total bacteria and LAB for both types of carrot compared to raw material (Table 1). Ensiling had an effect (P<0.05) on microbial quality of the silage since counts of yeasts, moulds and enterobacteria decreased for all additive treatments, including control.

**Table 1**. Type of raw material and additive effects on the composition and quality of ensiled carrot by-products after 21 days at room temperature.

| | Carrot with pieces | | | Carrot without pieces | | | SEM[1] | P-value[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | LAB | FA | Control | LAB | FA | | R | C LAB | C FA | LAB FA |
| Dry matter, g/kg | 60 | 61 | 75 | 60 | 64 | 75 | 0.9 | 0.46 | 0.17 | <0.01 | <0.01 |
| Chemical composition, g/kg DM | | | | | | | | | | | |
| Ash | 88 | 87 | 67 | 79 | 78 | 66 | 0.7 | <0.01 | 0.47 | <0.01 | <0.01 |
| CP | 72 | 73 | 78 | 71 | 71 | 82 | 0.6 | 0.95 | 0.62 | <0.01 | <0.01 |
| WSC | 10 | 11 | 394 | 6 | 6 | 245 | 1.7 | <0.01 | 0.89 | <0.01 | <0.01 |
| pH | 3.60 | 3.58 | 3.80 | 3.73 | 3.77 | 3.78 | 0.012 | <0.01 | 0.73 | <0.01 | <0.01 |
| $NH_3$-N, g/kg N | 29 | 28 | 150[3] | 29 | 28 | 165[3] | 6.1 | 0.51 | 0.82 | <0.01 | <0.01 |
| Fermentation acids, g/kg DM | | | | | | | | | | | |
| Lactic acid | 192 | 190 | 2 | 139 | 141 | 6 | 3.0 | <0.01 | 0.98 | <0.01 | <0.01 |
| Acetic acid | 53.1 | 52.3 | 5.9 | 33.6 | 35.7 | 6.4 | 0.70 | <0.01 | 0.61 | <0.01 | <0.01 |
| Propionic acid | 4.7 | 4.5 | 0.9 | 4.0 | 4.2 | 0.9 | 0.09 | 0.03 | 0.92 | <0.01 | <0.01 |
| Butyric acid | 0.34 | 0.33 | 0.27 | 0.33 | 0.31 | 0.27 | <0.01 | 0.41 | 0.14 | <0.01 | <0.01 |
| Weight loss, % | 0.21 | 0.63 | 0.25 | 0.38 | 0.46 | 0.13 | 0.094 | 0.65 | 0.03 | 0.30 | <0.01 |
| Yeast[4] | $<1.0\times10^3$ | $4.8\times10^3$ | $6.1\times10^3$ | $2.8\times10^3$ | $<1.0\times10^3$ | $<1.0\times10^3$ | | | | | |
| Mould[4] | $<1.0\times10^3$ | $<1.0\times10^3$ | $<1.0\times10^3$ | $<1.0\times10^3$ | $<1.0\times10^3$ | $<1.0\times10^3$ | | | | | |
| Enterobacteria[4] | $<1.0\times10^1$ | $<1.0\times10^1$ | $<1.0\times10^1$ | $<1.0\times10^1$ | $<1.0\times10^1$ | $<1.0\times10^1$ | | | | | |
| LAB[4] | $2.0\times10^8$ | $2.2\times10^8$ | $1.8\times10^4$ | $1.5\times10^8$ | $1.1\times10^8$ | $3.1\times10^5$ | | | | | |

[1]SEM = Standard error of the mean; [2]R: raw material effect, Carrot with pieces vs Carrot without pieces; C LAB: Control vs LAB; C FA: Control vs FA; LAB FA: LAB vs FA; [3]Corrected for the amount of ammonia in the additive; [4]cfu/g.

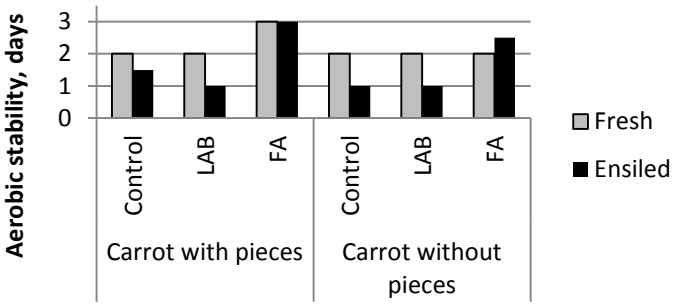


**Figure 1**. Aerobic stability (days) of fresh and ensiled carrot by-products according to raw material types and additive treatments.

**Conclusions** Preserving carrot by-products proved to be challenging. Spontaneous fermentation could efficiently be restricted with the use of FA, and benefits in aerobic stability were achieved.

**References**

Karak, T., Bhagat, R.M. & Bhattacharyya, P. (2012) Municipal solid waste generation, composition, and management: the world scenario. Critical Reviews in Environmental Science and Technology, 42, 1509-1630.

Rinne, M., Jalava, T., Siljander-Rasi, H., Kuoppala, K., Blasco, L., Kahala, M. & Järvenpää, E. (2017) Improving the usability of carrot by-products as animal feeds by ensiling, In: Nordic Feed Science Conference; Uppsala, Sweden, 2017, pp. 163-168. Available at: http://www.slu.se/globalassets/ew/org/inst/huv/nfsc/nfsc-2017-proceedings.pdf

# Changes in the chemical composition of sugarcane silages treated with microbial and chemical additives

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**Keywords:** indigestible neutral detergent fiber, legume silage, ruminal digestibility

**Introduction** The production of sugarcane silage is considered difficult because of its high concentration of water-soluble carbohydrates (WSC) which leads to ethanolic fermentation, resulting in high dry matter (DM) loss. The effectiveness of the additives used to control the yeasts is commonly evaluated by measuring the fermentation end products (e.g., organic acids and ethanol) and the population of yeasts. However, the evaluation of the chemical composition over the period of ensiling is directly related to the fermentation process (consumption of WSC) and consequently to the growth of yeasts, especially in sugarcane. Therefore, the objective of this experiment was to evaluate the changes in the chemical composition of sugarcane silages treated with microbial and chemical additives.

**Materials and Methods** Whole-plant sugarcane (32% average initial DM) was harvested from the dairy farm of Universidade Federal de Viçosa, MG, Brazil, and chopped using a stationary chopper (2-mm theoretical chop length). Chopped forage was further divided into five piles and treated with 1) nothing (Control), 2) Lactobacillus buchneri 40788 (LB; Lallemand, Brazil; theoretical application rate of 1×105 cfu/g of fresh weight); 3) calcium oxide (CaO) - 10 g/kg of fresh weight, 4) 2 L of Safesil (Salinity/Agro, Halmstad, Sweden)/t (S2); 5) 3 L of Safesil/t, (S3); and 6) 5 L of Safesil/t (S5). The active ingredients in the additive Safesil were sodium benzoate (200 g/kg), potassium sorbate (100 g/kg), and sodium nitrite (50 g/kg). Fresh, treated forage from each pile from d 0 was sampled and analyzed for DM, pH, lactic acid bacteria, yeasts, and molds. Forage from each pile was packed into five 10-L laboratory silos at a packing density of approximately 170 kg of DM/$m^3$ and sealed with plastic lids. Silos were opened after 3, 10, 21, and 100 d of storage. Representative samples of the fresh crop and of the silages were collected to evaluate the chemical composition: dry matter (DM), mineral matter (MM), crude protein (CP), neutral detergent fiber (NDF), and water-soluble carbohydrates. Data were analyzed considering the effects of additive (A), day of ensiling (D), and their interaction (A×D) by using the SAS software, version 9.1 ($P < 0.05$).

**Results** There was effect of interaction on the concentrations of DM, CP, NDF and WSC ($P<0.05$). Silages treated with chemical additives showed higher ($P<0.05$) DM concentration and lower ($P<0.05$) NDF concentration compared to Control and LB-treated silages over the fermentation periods. Sugarcane treated with CaO showed the highest concentration of MM ($P<0.05$) independent of the fermentation period. There were no differences among treatments at the day of ensiling for the WSC concentration. After 100 days, sugarcane silage treated with S2 also showed lower concentration of WSC than S3 and S5.

**Discussion** The decrease in DM content in the control and LB-treated silages was due to the DM losses caused by the ethanol fermentation and probably because the DM content was not corrected for the volatile compounds (Silva et al., 2018). The increase in the NDF portion in the control and LB silages is commonly observed because in sugarcane silages and indicates that the fermentation was more intense in those treatments compared to the others, which can be proved by the DM losses from the consumption of WSC in those treatments since the third day of ensiling. However, sugarcane treated with Safesil (at a miminum dose of 3 L/t) showed consistent preservation of the WSC during

the fermentation period. It could be suggested that the differences observed in the WSC concentrations among the treatments are related to the metabolism of yeasts. The dynamics of WSC concentrations demonstrate the effectiveness of the chemical additives on controlling the yeasts growth during the fermentation and it was confirmed by the increase in the DM recovery and aerobic stability observed in this experiment (Da Silva et al., 2015). In conclusion, treating sugarcane with Safesil at 3 L/t preserves the WSC during the fermentation process until 100 days of ensiling.

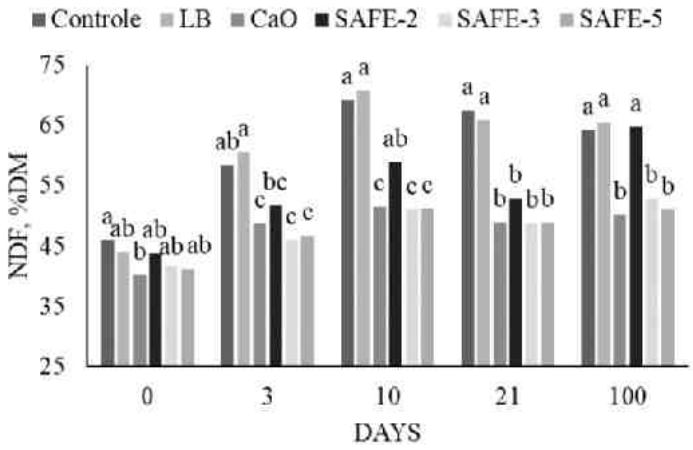


**Figure 1**. Concentrations of neutral detergente fiber (NDF) in the DM basis of sugarcane silages treated with additives* over the fermentation periods (days). *Control = no treatment; LB = *Lactobacillus buchneri* 40788 (application rate of $1\times10^5$ cfu/g); CaO = calcium oxide (10 g/kg); S2 = 2 L Safesil (sodium benzoate (200 g/kg), potassium sorbate (100 g/kg), and sodium nitrite (50 g/kg)) /t fresh weight; S3 = 3 L Safesil/t; S5 = 5 L Safesil/t. [a-b]Means followed by the same letter within the day of fermentation in the bars are not different based on Tukey's test ($P < 0.05$).

**Conclusions** Treating sugarcane with chemical additives before ensiling is effective on preserving the chemical composition of the fresh forage. The chemical additive containing a mixture of sodium nitrite, potassium sorbate, and sodium benzoate does not show increase in the fibre content because of WSC consumption, which could be related to the control of yeasts growth and ethanolic fermentation.

**References**

Da Silva, T.C., Pereira, O.G., Kung Jr., L., Da Silva, L.D., De Paula, R.A., Martins, R.M., Da Silva, V.P., & Ribeiro, K.G. (2015) Effect of a chemical additive containing sodium benzoate, potassium sorbate, and sodium nitrite on the microbial populations and aerobic stability of sugarcane silage. In: Daniel, J.L.P., Morais, G., Junges, D. & Nussio, L.G. XVII International Silage Conference, Piracicaba: ESALQ, 623p.

Silva, L.D., Pereira, O.G., Silva, T.C., Leandro, E.S., Paula, R.A., Santos, S.A., Ribeiro, K.G. & Valadares Filho, S.C. 2018. Effects of Lactobacillus buchneri isolated from tropical maize silage on fermentation and aerobic stability of maize and sugarcane silages. Grass and Forage Science, 1:1-11.

# Combination of chemical additives or microbial inoculants affects aerobic stability of whole corn silage differently

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**Keywords:** aerobic stability, chemical additive, corn silage

**Introduction** Corn silage is the most common fiber source for dairy and beef cattle in Brazil, although due the hot and wet climate, yeast spoilage is a big concern that decreases aerobic stability, reducing quality and quantity of the total mass ensiled. Chemical additives and microbial inoculants have been evaluated in order to inhibit yeast growth, promoting long aerobic stability after silage opening. However, combination of chemical additives or microbial inoculants may enhance the aerobic stability of silages. Our hypothesis is that whole corn silage treated with a combination of chemical additives will present greater aerobic stability compared to the silage with no additive, but similar to treatments inoculated with mid-high doses of a combination of microbial inoculants. The aim of this study was to evaluate the effect of combination of chemical additives or microbial inoculants on aerobic stability of whole corn silage.

**Materials and methods** The corn crop was harvested with 33% of DM and ensiled in experimental plastic containers (20 L) to reach 650 kg FM/m³. Before ensiling, the mass was inoculated with one of the treatments (1) Control (distilled water at 8.3 ml/kg FM); (2) 0.4% Chemical combo (38% propionic acid, 34% formic acid, 8% sodium – Lupro Mix NA – BASF); (3) Microbial combo at $1.4 \times 10^5$ cfu/g FM (*L. curvatus, L. plantarum, L. acidophilus, L. buchneri, L. acidilactici, E. faecium*, and *L. lactis* – LactoSilo® Gold - BASF); (4) Microbial combo at $7 \times 10^5$ cfu/g FM; (5) Microbial combo at $3.5 \times 10^6$ cfu/g FM; (6) Microbial combo at $7 \times 10^6$ cfu/g FM. The additives were diluted in distilled water at 8.3 ml/kg FM. Treatments were evaluated in quadruplicate and silos were kept within controlled temperature (25 °C) room for 120 d. To determine aerobic stability (h before a 2 °C increase in ambient temperature after exposure to air at 25 °C), approximately 5 kg of silage was placed on plastic buckets and the temperature was recorded every 30 minutes, during 10 d, using data loggers placed in the middle of the mass. Data were analyzed using the Mixed procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC).

**Results and discussion** The aerobic stability was affected ($P<0.01$) by treatments, where the greater stability was observed for the silages treated with Chem. This higher effect on the aerobic stability of the ensiled mass is due to the action of formic and propionic acids in controlling fungi and yeasts groth (Selwet, 2009). As described by Selwet (2008), propionic acid has the power to control up to 85% of the fungal action of whole plant corn silages. The action of formic and propionic acids on fungi and yeasts is due to the mechanism of the cells' exudates of these microorganisms, where the protonated acid enters the cells by diffusion and dissociates, the generated hydrogen is withdrawn from the cell by active transport, generating energy expenditure, as described by Lambert and Stratford (1999). There was no difference among silages treated with Micro at any dose and Cont. HTemp was affected ($P=0.03$) by the treatments, where the lowest HTemp was observed in the silage treated with Chem, with no statistical difference among Micro1, 2, and 3. Silages without additive and inoculated with Micro4 showed the highest HTemp, with no difference between them or among Micro1, 2, and 3. The presence of L. plantarum in the microbial combo at high dose may have led to higher production of lactic acid, which does not act as an antifungal (Moon, 1983). HHTemp was affected ($P=0.1$) by treatments, where the greater HHTemp was observed for the silages treated with Chem, with no statistical difference between Chem and Micro4. HHTemp of Micro4 treated silages also did not differ among the other treatments that shoed lower HHTemp, compared to Chem. There was no effect ($P>0.2$) of treatments on Σ5d and Σ10d of the silagens evaluated in this study.

**Table 1.** Aerobic stability (AS), highest temperature (HTemp), hours to the highest temperature (HHTemp), sum of the temperature variation within five days (Σ5d), sum of the temperature variation within ten days (Σ10d) of the silages treated with a combination of chemical additives or microbial inoculants.

| | Cont | Chem | Micro1 | Micro2 | Micro3 | Micro4 | SEM | P-value |
|---|---|---|---|---|---|---|---|---|
| AS | $77.5^{b}$ | $140.3^{a}$ | $74.7^{b}$ | $76.7^{b}$ | $75.8^{b}$ | $97.0^{b}$ | 10.2 | <0.01 |
| HTemp | $38.1^{b}$ | $29.2^{a}$ | $36.0^{ab}$ | $34.3^{ab}$ | $34.3^{ab}$ | $38.5^{b}$ | 1.8 | 0.03 |
| HHTemp | $153.2^{b}$ | $228.5^{a}$ | $136.7^{b}$ | $151.8^{b}$ | $156.2^{b}$ | $174.0^{ab}$ | 15.0 | 0.01 |
| Σ5d | 176.2 | 171.5 | 176.0 | 166.6 | 176.6 | 166.8 | 3.7 | 0.24 |
| Σ10d | 349.0 | 343.8 | 350.7 | 333.1 | 352.3 | 333.8 | 7.6 | 0.35 |

**Conclusions** The combination of chemical additives was more efficient in increase aerobic stability of whole corn silage ensiled for 120 d, when compared to the combination of microbial inoculants containing hetero and homofermentative bacteria or no additive silage. Increasing the dosage of the microbial inoculant seemed to do not affect aerobic stability consistently, although further investigation is need.

**References**

Kleinschmidt, D.H and Kung JR, L. 2006. A meta-analysis of the effects of lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science, Champaign, v. 89, n. 10, p. 4005-4013.

Lambert, R.J and Stratford, M. 1999. Weak acid preservatives: modeling microbial inhibition and response. Journal of Applied Microbiology, Oxford, v. 86, p. 157-164.

Moon, J.N. 1983. Inhibition of the growth of acid-tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. J. Appl. Bacteriol. 55, 453–460.

Selwet M. 2008. Effect of organic acids on numbers of yeasts and mould fungi and aerobic stability in the silage of corn. Polish Journal of Veterinary Sciences, 11, 119–123.

Selwet M. 2009. Effect of propionic and formic acid mixtures on the fermentation, fungi development and aerobic stability of maize silage. Polish Journal of Agronomy, 1, 37-42

# Effect of silage additives on the fermentation and the protein quality of clover-grass mixture

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**Keywords:** clover-grass mixture, silage additives, fermentation, crude protein fractions

**Introduction** Forage legumes and their mixtures with grasses are an important protein source for ruminant nutrition in Slovakia. These forages represent approximately 50 % of all forages grown on the arable land. Protein degradability in forage legumes is of global importance because utilization efficiency of forage has economic and environmental consequences. Rumen protein degradation and the resulting imbalance between carbohydrate and protein supply leads to lower N-use efficiency by ruminants (Broderick 1995, Hakl and Šantrůček 2016). Both chemical and biological additives can reduce effective lucerne protein degradability during ensiling. The effectiveness of inoculants on protein degradability is dependent on the WSC concentration of the forage. In forages with moderate to high WSC concentrations (Nadeau et al. 2016), inoculants will restrict it. The objective of our study was to evaluate the effect of application of different silage additives on the fermentation and protein quality of clover-grass mixture silages from the late autumn harvest.

**Materials and method** The experiment was conducted on a mixture of 40% clover and 60% grass from the late autumn harvest. The harvest clover-grass mixture was wilted for 36 hours, chopped and after homogenization ensilaged in laboratory silos. The fermentation process during ensilaging was observed in one control variant without treatment of the mixture and two experimental variants, in which the ensilaged matter was treated with additives: T1 (biological silage additive, *Lactobacillus plantarum* DSM 3676 and 3677; the application rate was 2 l/t) and T2 (chemical additive, 24.4% of sodium nitrite (E 250) and 16% of hexamine; the application rate was 2.5 l/t). During fermentation, the silages were stored for 180 days in a dark room at temperature 20 – 22$^{o}$ C. Samples of clover-grass matter as well as silage samples were chemically analysed. The parameters of organic analysis were determined according to EC No 152/2009, parameters of fermentation process were determined: pH by electrometric method, acids by gas chromatography and alcohol by Conway. The results were statistically processed by one-way analysis of variance, by the ANOVA multifactorial procedure and by the subsequent POST-HOC Tukey test. The analysis of crude protein fractions was based on the method involving soluble and insoluble nitrogen fractions, which has been developed to meet the need for uniform procedures for partitioning feed nitrogen into the individual fractions A, B, and C (Licitra et al. 1996). Nitrogen fractions are classified according to their solubility into fractions A (non-protein nitrogen), B1 (rapidly degradable protein), B2 (intermediately degradable protein), B3 (slowly degradable protein), and C (unavailable protein).

**Results and Discussion** Harvested mixture (Table 1) has low dry matter content, good crude protein content, with low content of crude fibre but high content of total sugar and ash.

**Table 1.** Nutrition value in wilted clover-grass mixture in g/kg DM.

| Dry matter | Crude protein | Crude fibre | Fat | Ash | Total sugars | NEL MJ/kg DM |
|---|---|---|---|---|---|---|
| 276.20 | 162.09 | 228.95 | 21.39 | 104.67 | 93.83 | 6.1 |

In nutrient content of silages, no significant differences were observed with the exception of the content of dry matter and total sugar (Table 2). The only positive impact of the bacterial inoculant application in comparison to the untreated silage was observed in the ammonium-N concentration. All other parameters of the fermentation process were on the same levels as in the untreated silage. Application of the chemical additive compared to the untreated silage led to decrease of fermentation

losses, lactic acid, butyric acid contents, ammonia-N concentration and to increase of pH, alcohol and acetic acid content. The results of experiments in Table 3 suggest that bacterial and chemical application of silage additives had positive impact on the qualitative parameters of the protein quality. In the control silages was observed higher content of ammonia-N and protein fractions A, B1 too. The fractions B2, B3 were the least from our experimental groups. Application of the bacterial additive showed up on reduced content of ammonia-N, protein fractions A, B1 and higher content of fractions B2, B3 in feed. In silages with the chemical application, we found the lowest content of ammonia-N, protein fractions A, B1 and the highest content of fractions B2, B3.

**Table 2.** Parameters of fermentation process and nutrition value in silages from clover-grass mixture.

| Item | Control | | T1 | | T 2 | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | SD | $\bar{x}$ | SD | $\bar{x}$ | SD |
| Dry matter (g/kg FW) | 261.42 $^{a}$ | 3.30 | 266.82 $^{a}$ | 1.43 | 270.71$^{b}$ | 3.43 |
| Crude protein (g/kg DM) | 179.06 | 3.70 | 184.37 | 1.47 | 184.76 | 2.23 |
| Crude fibre (g/kg DM) | 232.72 | 0.25 | 236.78 | 7.24 | 227.68 | 4.95 |
| Fat (g/kg DM) | 31.98 | 0.68 | 31.15 | 0.69 | 32.12 | 0.97 |
| Ash (g/kg DM) | 112.55 | 0.43 | 110.11 | 0.86 | 111.04 | 1.52 |
| Total sugars (g/kg DM) | 13.99 $^{a}$ | 0.42 | 20.21$^{b}$ | 0.40 | 17.26 $^{ab}$ | 2.79 |
| NEL (MJ/kg DM) | 5.89 | 0.01 | 5.90 | 0.01 | 5.92 | 0.02 |
| Losses of DM (%) | 0.84 $^{a}$ | 0.03 | 0.73 $^{ab}$ | 0.18 | 0.72 $^{b}$ | 0.04 |
| pH | 4.02 $^{a}$ | 0.01 | 3.99 $^{a}$ | 0.07 | 4.26 $^{b}$ | 0.04 |
| Acids (g/kg DM) | | | | | | |
| - lactic | 102.27 $^{a}$ | 4.76 | 101.44 $^{a}$ | 1.13 | 88.81 $^{b}$ | 3.75 |
| - acetic | 18.93 $^{ab}$ | 0.83 | 16.71 $^{a}$ | 2.21 | 21.97 $^{b}$ | 1.99 |
| - butyric | 0.75 $^{a}$ | 0.11 | 0.73 $^{a}$ | 0.21 | 0.32 $^{b}$ | 0.09 |
| Alcohol (g/kg DM) | 1.13 | 0.93 | 1.48 | 0.70 | 1.64 | 0.44 |
| $NH_3$-N of total N (%) | 9.42 $^{a}$ | 0.36 | 7.89 $^{b}$ | 0.30 | 6.91 $^{c}$ | 0.43 |

n = 6, FW – fresh weight , DM – dry matter, NEL - netto energy lactation
Different superscripts within a row mean statistical difference ($P \leq 0.05$); $^{ab}$ vs $^{a,b}$ is not different.

**Table 3.** Crude protein fractions in silages from clover-grass mixture.

| Silage | Crude protein fractions (in % of CP) | | | | |
|---|---|---|---|---|---|
| | A | B1 | B2 | B3 | C |
| Control | 54.53 | 1.47 | 27.89 | 7.31 | 8.80 |
| T1 | 52.87 | 1.40 | 28.09 | 8.35 | 9.29 |
| T2 | 51.35 | 1.37 | 28.90 | 9.06 | 9.32 |

n = 3, Crude protein fractions: A = non-protein nitrogen, B1 = buffer soluble protein, B2 = neutral detergent soluble protein, B3 = acid detergent soluble protein, C = acid detergent insoluble protein.

**Conclusion** The results of the study suggest that the fermentation process in conservation of clover-grass mixture harvested at the end of the vegetation season after wilting can be quite good. Application of bacterial inoculant treatment, compared to untreated matter, reduced the content of ammonia nitrogen, but it was unable to change the level of fermentation process. Application of chemical additive improved fermentation process of the silage matter, decreased the butyric acid content and the concentration of ammonia nitrogen too. The application of bacterial and chemical additives improved the protein quality in the clover-grass silages.

**References**

COMMISSION REGULATION (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official J. of EU, L 54 (52), 2009, 130 p.

Hakl, J., Šantrůček, J. (2016) Utilization of perennial fodder crops for production of high quality silages. In: 17th Int. Conf. Forage Conservation. (Proc.), Horný Smokovec, Slovakia, 2016, pp. 19-26.

Nadeau, E., Richardt, W., Nørgaard, P. (2016) Fibre and protein quality of silages and their effects on ruminant performance. In: 17th International Conference Forage Conservation (Proc.), Horný Smokovec, Slovakia, 2016, pp. 39-53.

Broderick G.A. (1995) Desirable characteristics of forage legumes for improving protein utilization in ruminants.Journal of Animal Science, 73, pp. 2760–2773.

Licitra, G., Hernandez, T.M., Van Soest, P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology, 57, 347-358.

# Effects of additive, herbage dry matter concentration and clostridia inoculation on fermentation quality of a red clover-grass silage

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**Keywords:** clostridia, formic acid, nitrite, silage additive

**Introduction** The preservation of legumes is a challenge. In particular, wet grain-legume silages tend to undergo clostridial fermentation because of their low dry matter (DM) concentration, their high buffering capacity and their relatively low concentrations of water-soluble carbohydrates (WSC) and nitrate (König et al., 2017). This silage study compared formic acid with a solution of sodium nitrite as silage additive. The aim of the study was to find the preservative that effectively prevents butyric acid formation and improves the red clover - grass silage quality. It was hypothesized that 1) additives suppress butyric acid fermentation and clostridial growth compared to the untreated control, 2) sodium nitrite treatment is superior compared to formic acid in preventing clostridial fermentation.

**Material and Methods** Two ensiling trials with two DM concentrations of ensiled herbages were carried out. A red clover-grass herbage was cut on 4 August 2016 and wilted to DM concentrations of 199 (DM1) and 314 (DM2) g/kg (Table 1) and ensiled in 1.5 L glass silos with four replicates per treatment. Half of the herbage was inoculated with *Clostridium tyrobutyricum* $1*10^5$ colony forming units/g fresh matter to increase the challenge for additives. The additive treatments were as follows: Control (no treatment), formic acid (100%) (FA) 4 l/t kg fresh herbage, sodium nitrite (SN) 900 g/1000 kg fresh herbage. Silages were analyzed for pH, fermentation acids, ammonia, ethanol and water-soluble carbohydrates (König et al., 2017). In addition, four clostridia species were examined by qPCR. The results were analyzed by ANOVA using the mixed method of SAS (SAS 9.3, Institute Inc., Cary, NC). Squares sums for treatment effects were further separated using orthogonal contrasts in comparisons with a single degree of freedom. Non-normally distributed data were tested with the non-parametric Kruskall-Wallis test (SPSS, version 21, IBM, Armonk, USA), and if significant, the differences between the treatments were analyzed by pairwise testing (Dunn-Bonferroni).

**Table 1.** Chemical composition and ensilibility characteristics of un-wilted and wilted red clover-grass herbage (g/kg dry matter, unless otherwise stated).

| | Un-wilted | Wilted |
|---|---|---|
| Dry matter (DM), g/kg | 199 | 314 |
| Calculated $DM_{min}$, g/kg [1)] | 260 | 293 |
| Ash | 88.3 | 83.3 |
| Crude protein | 188 | 177 |
| Soluble N, g/kg N | 367 | 318 |
| Neutral detergent fibre | 460 | 467 |
| Water soluble carbohydrates (WSC) | 82.6 | 95.7 |
| WSC, g/kg fresh matter | 16.4 | 30.1 |
| Digestible organic matter in dry matter | 679 | 673 |
| Buffer capacity (BC) as lactic acid g/kg DM | 78.6 | 72.6 |
| BC, mekv/kg DM | 872 | 805 |
| Nitrate, g/kg DM | 4 | 4 |
| Fermentation coefficient [2)] | 28.3 | 42.0 |
| Clostridia, log copies/g fresh matter | 13.3 | 9.9 |

[1)] Minimum DM content of ensiled herbage needed to ensure high fermentation quality of silage, $DM_{min}$=45-8*WSC/BC, [2)] Fermentation coefficient = DM (%) + 8*WSC/BC

**Table 2.** Fermentation quality of silages (g/kg dry matter unless otherwise stated).

| | Not inoculated | | | Inoculated | | | | Contrasts | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C[1)] | FA[2)] | SN[3)] | C[1)] | FA[2)] | SN[3)] | SEM[4)] | 1 | 2 | 3 |
| **Dry matter 1** | | | | | | | | | | |
| pH | 4.08[ab] | 4.15[b] | 4.04[ab] | 4.08[ab] | 4.01[a] | 4.06[ab] | 0.026 | | | |
| Amm-N, g/kg N[5)] | 86.0[b] | 50.8[ab] | 44.6[ab] | 76.0[b] | 41.2[a] | 45.2[ab] | 2.87 | | | |
| WSC[6)] | 3.2[a] | 86.7[bc] | 3.9[ab] | 5.4[abc] | 93.7[c] | 4.7[abc] | 3.10 | | | |
| Lactic acid | 136 | 26 | 139 | 131 | 21 | 133 | 2.6 | 0.03 | <0.001 | <0.001 |
| Acetic acid | 36.3 | 10.6 | 34.8 | 35.8 | 7.4 | 37.9 | 0.77 | 0.74 | <0.001 | <0.001 |
| Butyric acid | 0.0 | 4.7 | 0.0 | 0.0 | 0.7 | 0.0 | 1.75 | | | |
| Ethanol | 3.3[abc] | 5.0[bc] | 1.2[a] | 3.2[abc] | 5.8[c] | 1.5[ab] | 0.53 | | | |
| Clostridia[7)] | 10.4[ab] | 11.2[a] | 10.2[b] | 10.3[ab] | 10.7[ab] | 10.4[ab] | 0.29 | | | |
| **Dry matter 2** | | | | | | | | | | |
| pH | 4.14[abc] | 4.21[c] | 4.11[ab] | 4.11[ab] | 4.18[bc] | 4.09[a] | 0.009 | | | |
| Amm-N, g/kg N[5)] | 72.1 | 47.7 | 44.8 | 71.4 | 50.4 | 47.2 | 0.94 | 0.07 | <0.001 | <0.01 |
| WSC[6)] | 14.1[a] | 84.1[c] | 17.8[abc] | 14.1[ab] | 74.6[bc] | 19.7[abc] | 2.32 | | | |
| Lactic acid | 114[abc] | 35[a] | 109[abc] | 122[c] | 38[ab] | 111[abc] | 2.5 | | | |
| Acetic acid | 27.1 | 8.2 | 23.2 | 26.0 | 9.7 | 25.0 | 0.70 | 0.22 | <0.001 | <0.001 |
| Butyric acid | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.00 | | | |
| Ethanol | 3.2[abc] | 3.1[abc] | 0.9[a] | 4.1[c] | 3.8[bc] | 1.1[ab] | 0.23 | | | |
| Clostridia[7)] | 10.4 | 10.8 | 10.5 | 10.3 | 11.9 | 11.8 | 0.82 | 0.25 | 0.22 | 0.84 |

[1)] Control, [2)] Formic acid, [3)] Sodium nitrite, [4)] Standard error of the means, [5)] Ammonia-N concentration is corrected by deducting all nitrogen applied through SN additive, [6)] Water soluble carbohydrates, [7)] Clostridia expressed as log copies/g fresh matter

Contrasts: 1=Not inoculated vs. Inoculated with clostridia; 2=Additives vs. Control; 3=FA vs.SN

For non-normal distributed data, means within the same row without same superscript differ significantly (P<0.05).

**Results and Discussion** Inoculation with clostridia resulted in minor changes in the silage fermentation profile, since the number of clostridia was initially high (Table 2). The use of FA resulted in lower levels of lactic acid and acetic acid and higher concentrations of water soluble carbohydrates compared to the untreated control and the SN treated silage. In un-wilted FA silage, the ethanol concentration was higher than in SN silages. The nitrate content of the herbage almost reached the minimum concentration of 4.3 g nitrate/kg DM proposed by Kaiser and Weiss (1997) for producing butyric-free silages from forage with a higher fermentation coefficient (FC) than 45. Thus, the nitrate concentration in the herbage can explain why even the untreated silages were free of butyric acid. In contrast to the other treatments, FA did not prevent completely butyric acid fermentation in wet silages. This was possibly due to the low application rate resulting in an insufficient pH effect of FA in relation to DM content. Furthermore, the copy number of clostridia was significantly higher in un-wilted, non-inoculated FA silage than in SN silage.

**Conclusion** The nitrate concentration in the herbage was high enough to completely prevent butyric acid fermentation in wilted silages. In un-wilted silages, formic acid was less effective than sodium nitrite and control treatments to suppress butyric acid fermentation and clostridia.

**References**

Kaiser, E. and Weiss, K. (1997). Zum Gärungsverlauf bei der Silierung von nitratarmem Grünfutter 2. Mitteilung: Gärungsverlauf bei Zusatz von Nitrat, Nitrit, Milchsäurebakterien und Ameisensäure. Archiv Für Tierernährung, 50, 187-200.

König, W., Lamminen, M., Weiss, K., Tuomivirta, T. T., Sanz Muñoz, S., Fritze, H., Elo, K., Puhakka, L., Vanhatalo, A. and Jaakkola, S. (2017). The effect of additives on the quality of white lupin-wheat silages assessed by fermentation pattern and qPCR quantification of clostridia. Grass and Forage Science, 72, 757-771.

# Effect of different additives and their interactions on alfalfa silage quality

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**Keywords:** alfalfa silage, bacillus, cellulase, fermentation quality, lactic acid bacteria.

**Introduction** It is well documented that the addition of LAB can help to improve silage quality. Silage material treated with cellulase has been proposed as an approach for breaking down forage fiber to support WSC for LAB fermentation, and the reduction of fibre content resulted in the rapid increase in degradable dry matter. Previous studies have shown that fibrolytic enzymes could improve the fermentation quality and the *in vitro* digestibility. *Bacillus* species are not common microbiological additives in silages due to their lower efficiency to produce lactic acid compared with LAB. However, many species of the genus *Bacillus* (*B*) have cellulase activity and can produce various antimicrobial substances preventing the deterioration caused by undesirable microorganisms. As the initial chemical ingredients, especially for carbohydrate compositions, vary substantially among forage crops, the mechanisms of actions of cellulases as additives in various silages are not clear, and these positive responses are therefore not always consistent. The main objective of the current study was to evaluate the effects of LAB, cellulase, cellulase-producing *Bacillus pumilus* and their combinations on fermentation, bacterial community, nutritive value and *in vitro* digestibility of alfalfa silage.

**Materials and Methods** The alfalfa used in this study was harvested in Zhuozhou, Hebei Province, China. The treatments included an originally selected LAB (*Lactobacillus plantarum* (a214)) isolated from alfalfa silage, a cellulase-producing *Bacillus pumilus* (CB) from fresh alfalfa, cellulase (C), mixed additives (a214+CB and a214+C), additionally, two commercial additives (GFJ and Chikuso-1) both containing *Lactobacillus plantarum*, were used to compare their effectiveness with that of the other additives. These strains were applied at 6.0 log cfu $g^{-1}$ FM. Cellulase additive used in this experiment were 20 mg $kg^{-1}$ FM. Chopped alfalfa (200g) was packed into plastic bags and ensiled for periods of 30, 60 days and then exposed to air for five days (At the days of 60, samples of approximately 100 g were transferred from each bag to 0.5 L plastic jars, then, the plastic jars capped with sterile gauze for aerobic exposure, and kept at room temperature for five days ). Fermentation characteristics, chemical compositions, bacterial community and *in vitro* digestibility of the silages were determined to assess the effects of different treatments (Zhang et al. 2016). The data were analysed by two-way analysis of variance to evaluate the effects of the storage days (D), additive treatments (T) and their interactions (D×T). All statistical analyses were performed using the general linear model procedure in SAS 9.0 (SAS Institute, Cary, NC, USA, 2002). Significance was declared at $P < 0.05$ unless otherwise noted.

**Results** Compared with the other treatments, a214, a214+C and a214+CB treatments showed lower pH value and higher lactic acid concentration during the ensiling process ($P < 0.05$) (Table 1). No evident aerobic deterioration was observed in the silages after exposed to air, and no significant fiber degradation was found in C and CB treatments (data not shown). The a214 treatment showed the highest *in vitro* digestibility of dry matter (591.3 $\mathrm{g\ kg^{-1}DM}$) after ensiling for 60 days, and the addition of a214 could enhance the *Lactobacillus* abundance greatly (Figure 1). Overall, the a214 treatment exhibited better fermentation quality compared with the other treatments including the two commercial inoculants, which indicated the potential use of this strain as an alfalfa silage inoculant.

**Discussion** The inefficiency of the cellulase additives in silage quality depends on many factors, such as the environmental conditions, type of enzyme preparation, type and properties of the forages to be ensiled, epiphytic microflora, WSC and ensiling technique (Moharrery et al. 2009). Li et al. (2014) reported that the activity of cellulase could be inhibited by microorganisms in king grass silage. Some other researchers reported that the high lignin concentration in lucerne was responsible for the less

effective of enzymatic attack on cellulose during ensiling (Moharrery et al. 2009). In general, the reasons for the above mentioned inefficient results are complex and differed among experiments, which may explain among silages treated in this study, C or CB alone treatment had no significant effect for silage fermentation and IVDMD. In this study, it was expected that addition of LAB to alfalfa silage would help to increase lactic acid production, thereby accelerating fermentation, shortening the time to reach pH stability and inhibiting the activity of undesirable microorganisms. The increased lactic acid concentration, decreased of pH and $NH_3$-N content by addition of LAB strain a214 in our study support this expectation, and favorable fermentation characteristics were also obtained from treatments a214+C and a214+CB.

**Table 1** Fermentation quality of alfalfa silage

| Items | Storage day (D) | Treatment(T) | | | | | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | GFJ | Chikuso-1 | C | CB | a214 | a214+C | a214+CB | | D | T | D×T |
| pH | 30 | $5.41^{abA}$ | $5.21^{bcA}$ | $4.95^{cd}$ | $5.40^{ab}$ | $5.66^{aA}$ | $4.72^{d}$ | $4.69^{dA}$ | $4.78^{d}$ | 0.016 | <0.001 | <0.001 | 0.325 |
| | 60 | $5.34^{aB}$ | $4.81^{bB}$ | $4.76^{bc}$ | $5.34^{a}$ | $5.24^{aB}$ | $4.63^{c}$ | $4.62^{cB}$ | $4.63^{c}$ | | | | |
| | 65 | $5.16^{aC}$ | $4.87^{bAB}$ | $4.74^{bc}$ | $5.24^{a}$ | $5.32^{aB}$ | $4.63^{c}$ | $4.68^{cA}$ | $4.63^{c}$ | | | | |
| LA | 30 | $22.04^{bc}$ | $23.14^{bC}$ | $33.49^{aB}$ | $19.26^{bc}$ | $16.37^{c}$ | $35.10^{aB}$ | $34.79^{aB}$ | $35.40^{aB}$ | 0.333 | <0.001 | <0.001 | <0.001 |
| | 60 | $22.97^{d}$ | $42.88^{bA}$ | $47.80^{aA}$ | $24.07^{c}$ | $24.03^{c}$ | $48.22^{aA}$ | $48.28^{aA}$ | $47.64^{aA}$ | | | | |
| | 65 | $24.05^{b}$ | $33.21^{aB}$ | $38.47^{aB}$ | $22.81^{b}$ | $18.85^{b}$ | $37.05^{aB}$ | $36.24^{aB}$ | $38.30^{aB}$ | | | | |
| AA | 30 | $29.46^{cB}$ | $34.12^{ab}$ | $35.15^{ab}$ | $31.63^{bcB}$ | $28.21^{cA}$ | $37.86^{aA}$ | $34.46^{ab}$ | $37.40^{a}$ | 0.334 | 0.772 | <0.001 | <0.001 |
| | 60 | $27.32^{eB}$ | $37.52^{abc}$ | $35.18^{abcd}$ | $40.72^{aA}$ | $28.38^{deA}$ | $30.60^{deB}$ | $30.98^{de}$ | $33.57^{bcd}$ | | | | |
| | 65 | $33.69^{bA}$ | $38.56^{a}$ | $35.74^{ab}$ | $31.48^{bB}$ | $22.17^{cB}$ | $32.72^{bB}$ | $34.32^{ab}$ | $35.48^{ab}$ | | | | |
| $NH_3$-N | 30 | $5.02^{bcB}$ | $5.86^{a}$ | $5.42^{bB}$ | $5.41^{bB}$ | $5.69^{abB}$ | $3.18^{cB}$ | $3.46^{cB}$ | $2.38^{dB}$ | 0.127 | <0.001 | <0.001 | 0.644 |
| | 60 | $7.33^{aA}$ | $6.55^{ab}$ | $6.56^{abB}$ | $5.75^{bB}$ | $6.91^{aB}$ | $3.53^{cB}$ | $3.57^{cB}$ | $3.75^{cAB}$ | | | | |
| | 65 | $8.83^{aA}$ | $6.88^{ab}$ | $7.12^{abA}$ | $8.00^{aA}$ | $9.06^{aA}$ | $5.06^{cA}$ | $5.04^{cA}$ | $4.55^{cA}$ | | | | |

LA, lactic acid (g $kg^{-1}$ DM); AA, acetic acid (g $kg^{-1}$ DM); $NH_3$-N, $NH_3$-N (% total nitrogen). Means in the same column ($^{A-C}$) or in the row ($^{a-d}$) with different superscript letters differ significantly ($P < 0.05$); SEM, standard error of the mean.

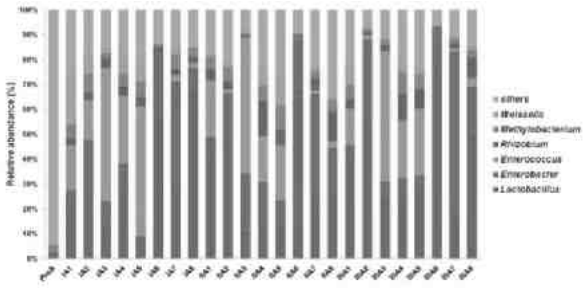

**Figure 1** Genus-level microbiota analysis for pre-ensiled alfalfa and silage. Pre, pre-ensiled; A, alfalfa; I, silages of 30 days; II, silages of 60 days; III, exposed to air for 5 days after 60 days; 1,Control; 2, GFJ; 3, Chikuso-1; 4, C; 5, CB; 6, a214; 7, a214 + C; 8, a214 +CB.

**Conclusions** The effects of adding LAB, C, CB and their combinations on alfalfa silage were investigated in this study. Compared with the other treatments, the addition of LAB strain a214 showed lower pH value, higher lactic acid concentration ($P < 0.05$). It also could enhance the *Lactobacillus* abundance and the *in vitro* digestibility of silage. Since C or CB alone had no significant effect on silage quality, the effects of combination were caused mostly by the strain a214 alone. The results implied that the more resistant fibers of alfalfa probably limited the effect of cellulase to degrade plant cell walls and indicated that the strain a214 isolated from alfalfa silage could thus be considered a promising starter culture or inoculant for alfalfa silage.

**References**

Li, M., Zi, X., Zhou, H., Hou, G., Cai, Y. (2014) Effects of sucrose, glucose, molasses and cellulase on fermentation quality and *in vitro* gas production of king grass silage. Animal Feed Science and Technology, 197, 206212.

Moharrery, A., Hvelplund, T., Weisbjerg, M.R. (2009) Effect of forage type, harvesting time and exogenous enzyme application on degradation characteristics measured using *in vitro* technique. Animal Feed Science and Technology, 153, 178-192.

Zhang, Q., Yu, Z., Yang, H., Na, R.S. (2016) The effects of stage of growth and additives with or without cellulase on fermentation and in vitro degradation characteristics of Leymus chinensis silage. Grass and Forage Science, 71, 595-606.

# Effect of chemical additives on silage composition, aerobic stability and feed intake of maize silage depending on aerobic storage

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**Keywords:** aerobic deterioration, feed intake, silage additives

**Introduction** When silage is exposed to air during feed-out, aerobic deterioration may occur with significant changes in silage composition. However, it is unclear to what extent ruminants respond to these changes by reducing feed intake. One aim of the use of chemical additives is to improve the aerobic stability. Recently, Weiß and Auerbach (2012) observed a higher aerobic stability compared to untreated silage when using chemical additives based on formic acid as well as additives based on sodium benzoate and potassium sorbate. The formic acid-treated silages, however, showed higher ethanol and ethyl ester contents as well as higher dry matter (DM) losses than the silages treated with sodium benzoate and potassium sorbate. The aim of the present study was to determine the effect of two chemical additives based on formic acid as well as on sodium benzoate and potassium sorbate on changes in maize silage composition throughout an aerobic storage phase and to study the effects on short-term feed intake by goats.

**Material and Methods** Silages were prepared from whole-crop maize containing 282 g $kg^{-1}$ DM. Forage was treated as follows: no additive (**CON**, control silage); silage additive 1 (**SBPS**, forage treated with a mixture of sodium benzoate and potassium sorbate (2 L/t)); silage additive 2 (**FASF**, forage treated with a mixture of formic acid, sodium formate, propionic acid and sodium benzoate (3 L/t)). The treated forages were ensiled in 120-L plastic barrels (six replicates each). After opening the barrels, the silages were sampled and exposed to air for 6 days. During aerobic storage, samples were taken from the silages at 2-day intervals to determine silage composition and for usage as feed during subsequent preference trials. A 15-day preference trial was carried out with goats ($n$ = 5) for each treatment. During the experimental phase, each possible two-way combination ($n$ = 10) of the exposed silages and a lucerne hay was offered for 3 hours (h) to each goat and the DM intake (DMI) was determined. Treatment effects on silage composition were statistically analysed by one-factorial ANOVA and effects on DMI by the Waller-Duncan k-ratio $t$-test. The silage preparation, all analyses and the preference trials were conducted as described by Brüning et al. (2018a, b).

**Results and Discussion** At silo opening, all silages had low pH values (Table 1). On day 4 of aerobic storage, the pH was higher in CON than in FASF and SBPS, which indicated that aerobic deterioration had occurred in CON. The aerobic deterioration was initiated by yeasts whose counts were highest (at least numerically) in CON throughout the storage phase. The yeasts metabolised lactic acid and generated heat. As a result, CON showed both the lowest contents of lactic acid on day 4 and 6 and the lowest aerobic stability (Figure 1a, overall $P<0.001$). The degradation of lactic acid and other fermentation acids (not shown) led to corresponding increases in aNDFom and ADFom. Concomitantly with its strong changes during aerobic storage, CON showed lower DMI on day 6 (Figure 1b) than FASF and SBPS (233 vs. 423 and 233 vs. 692 g 3 $h^{-1}$, overall $P<0.001$). SBPS, on the other hand, was most preferred with the highest DMI likely due to the only slightly altered silage composition throughout the aerobic storage phase.

**Conclusions** Deterioration in feed value and microbial status appears to have been responsible for the decline in feed intake in CON after prolonged aerobic storage. Best results in both silage quality and feed intake were achieved with SBPS. Therefore, the use of chemical additives based on sodium benzoate and potassium sorbate is recommended to ensure a high silage quality and the highest possible feed intake even after prolonged exposure to air during feed-out.

**Table 2.** Treatment effects on silage composition during aerobic storage post-opening.

| | Day of aerobic storage | CON[1] | SBPS[2] | FASF[3] | SEM[4] | Treatment effect P-value |
|---|---|---|---|---|---|---|
| pH | 0 (opening) | 3.63[a] | 3.59[a] | 3.73[b] | 0.02 | <0.001 |
| | 2 | 3.69[a] | 3.62[a] | 3.76[b] | 0.02 | <0.001 |
| | 4 | 5.51[c] | 3.65[a] | 3.82[b] | 0.02 | <0.001 |
| | 6 | 6.66[b] | 4.50[a] | 4.35[a] | 0.06 | <0.001 |
| Lactic acid (g kg$^{-1}$ DM) | 0 (opening) | 68.1[b] | 72.4[b] | 43.2[a] | 1.1 | <0.001 |
| | 2 | 66.6[b] | 68.4[b] | 42.6[a] | 1.2 | <0.001 |
| | 4 | 16.5[a] | 71.0[c] | 31.5[b] | 3.5 | <0.001 |
| | 6 | 6.09[a] | 35.0[b] | 21.4[ab] | 7.5 | 0.048 |
| aNDFom (g kg$^{-1}$ DM) | 0 (opening) | 423 | 419 | 424 | 4.6 | 0.683 |
| | 2 | 413[ab] | 405[a] | 434[b] | 7.5 | 0.043 |
| | 4 | 449[b] | 404[a] | 436[b] | 5.0 | <0.001 |
| | 6 | 466[b] | 421[a] | 441[a] | 6.4 | 0.001 |
| ADFom | 0 | 232 | 230 | 235 | 3.4 | 0.663 |
| | 2 | 228 | 232 | 242 | 5.5 | 0.188 |
| | 4 | 241 | 228 | 242 | 6.1 | 0.087 |
| | 6 | 259[b] | 237[a] | 238[a] | 6.1 | 0.003 |
| Yeasts (log cfu g$^{-1}$ FM) | 0 (opening) | 5.20 | 2.70 | 4.15 | - | - |
| | 2 | 6.52 | 3.48 | 3.85 | - | - |
| | 4 | 8.60 | 5.00 | 7.08 | - | - |
| | 6 | 7.70 | 6.30 | 6.70 | - | - |

1 = control silage; 2 = forage treated with sodium benzoate and potassium sorbate; 3 = forage treated with formic acid, sodium formate, propionic acid and sodium benzoate; 4 = Standard error of the mean; aNDFom = neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; ADFom = acid detergent fibre expressed exclusive of residual ash; cfu = colony-forming units; FM = fresh matter; Means within rows with differing superscripts (a-c) differ (based on Tukey's test, P<0.05); each *n* = 6 (except yeasts counts, *n* = 1).

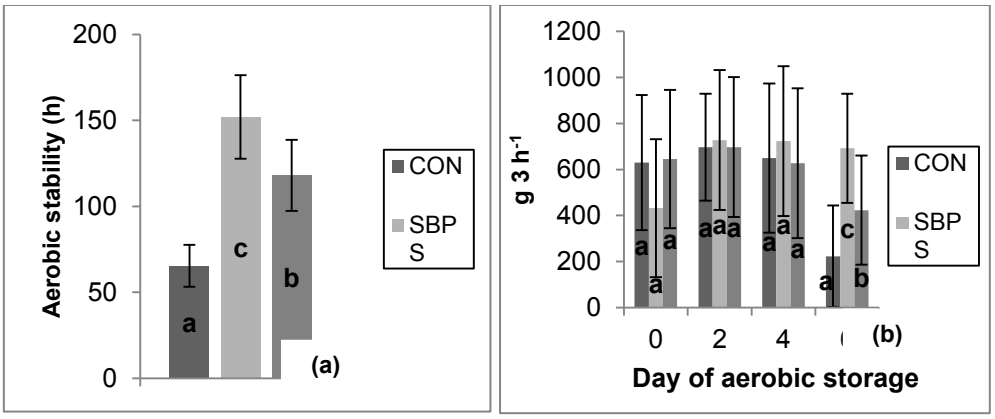


**Figure 1 (a)** Effect of the chemical additives on aerobic stability (n = 6); for treatments abbreviations see Table 1; aerobic stability was defined as the number of hours the silage remained stable before a temperature rise by ≥ 2°C above ambient temperature; Means with different superscripts (a-c) differ (P<0.05). **(b)** Effect of the chemical additives on DMI (g 3 h$^{-1}$, n = 20); Means within days of aerobic storage with different superscripts (a-c) differ (P<0.05); vertical bars indicate the standard deviation.

**References**

Brüning, D., Gerlach, K., Weiß, K. & Südekum, K.-H. (2018a) Effect of compaction, delayed sealing and aerobic exposure on maize silage quality and on formation of volatile organic compounds. Grass and Forage Science, 73, 53-66.

Brüning, D., Gerlach, K., Weiß, K. & Südekum, K.-H. (2018b) Effect of compaction, delayed sealing and aerobic exposure on forage choice and short-term intake of maize silage by goats. Grass and Forage Science, 73, 392-405.

Weiß, K. & Auerbach, H. (2012) Occurrence of volatile organic compounds and ethanol in different types of silages, In: Kuoppala, K., Rinne, M., & Vanhatalo, A. (eds). Proceedings of the XVI. International Silage Conference, Hämeenlinna, Finland, 2012, pp. 128-129.

# Effect of chemical additives, lactic acid bacteria and their combinations on the fermentation of low dry matter crops

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**Keywords:** chemical additives, LAB, low dry matter content, low S/BC

**Introduction** Low dry matter (DM) silages quite often suffer from butyric acid fermentation. Chemical additives like nitrite and hexamethylentetramin (HMTA) can control clostridia and their spores. Nitrite alone or in combination with benzoic acid is also quite effective (Knicky & Spörndly 2009). But in case of the absence of HMTA it may lead to a poorer result. These chemicals mainly control clostridia and enterobacteria. The pH- drop in the silage comes from the spontaneous fermentation. Therefore, an equal proportion of homo- and heterofermentative lactic acid bacteria (LAB) may expected. A not effective pH drop may come from an unsufficient number of homofermentetive LAB. A combination of additives and LAB could therefore improve the fermentation pattern. Thus, the objective of this study was to investigate the effects of different chemical additives and the addition of LAB on the fermentation at unsufficient ensiling conditions.

**Material and Methods** The first cut of *Dactylus glomerata* (Orchard grass) and a vetch rye mixture were ensiled. The crop traits for grass or vetch rye mixture were (g/kg DM): DM 246/162, crude protein (CP) 170/131, crude ash (CA) 96/74, sugar (S) 72/53, nitrate 0.0/1.0, buffer capacity (BC) 3.7/4.3 g lactic acid/100 g DM, S/BC 1.9/1.2 and the fermentation coefficient was 40/26. Thus, the fermentation conditions were poor. Furthermore, 30 g/kg fresh matter (FM) of dirt (dried soil and old silage from a bunker floor) was added to the crop material in order to simulate stress conditions. The number of LAB in fresh crop was high (>800.000 CFU/g). The crop material was ensiled in 1.5 litre jars. Each treatment was prepared from 10 kg fresh crop and treated with: **1)** nitrite, HMTA (KL2, 2 litres/t FM, KOFASIL liquid, ADDCON Europe GmbH, **2)** KL3, 3 litres/t FM, **3)** nitrite, sodium benzoate, potassium sorbate (EP2, 2 litres/t FM), **4)** EP3, 3 litres/t FM, **5)** *Lactobacillus plantarum*, DSM 3676/3677 (LAC, $1x10^5$ CFU/g FM, KOFASIL LAC, ADDCON Europe GmbH), **6)** EP2+LAC and **7)** control, added with the adequate amount of water. The silages were opened after 90 days. Fermentation traits from fresh crop were evaluated based on the VDLUFA protocol and the following parameters of the silage were analysed: pH (VDLUFA 2012), lactic acid (HPLC), acetic acid (GC), $NH_3$ (auto-analyzer, VDLUFA 2012). The fermentation loss was calculated after Weißbach (2005). The setup of the experiment was a random block design 3 times replicated. Statistical analyses were done using the ANOVA procedure, program “R”. When the overall P-value was significant at 5% level, pair wise comparisons between LSMEANS of treatments were done using Tukey´s test.

**Results and Discussion** Caused by the poor fermentation conditions control silages of both crops had pH-values of 4.6. Treated silages showed lower pH-levels. The pH and DM-loss was closely correlated (Fig.1). If all data are considered the correlation was not that strong especially in the vetch mix silages. If the KL treatments were excluded the correlation was improved drastic. This indicates that KL is controlling the DM-loss quite effective also at higher pH and poor fermentation conditions. The KL treatments and the EP2 LAC were significantly different to the control in case of fermentation loss which is given for both crops. In both crops KL could reduce the amount of acetic acid as well (Tab.1). The positive effect of the EP additive on fermentation was not consistent but given in the vetch rye mixture. However, the nitrite/benzoic/sorbic additive alone does not outperform the nitrite/HMTA combination. Under the chosen poor fermentation conditions the $NH_3$-N content of $N_t$ was high (Tab.1). But the KL treatments could decrease the $NH_3$-N significantly. However unexpectedly the butyric acid concentration went to zero in all silages (data not shown). This was not expected for the grass, due to the low nitrate content in the plant material (Kaiser et al. 1997). But the intensive acetic acid formation and the high $NH_3$ content of total N in the control and in some treated silages showed a

not well fermentation, except for the KL treatments in both silages. The combination of EP2/LAB led to an improved fermentation in the grass silage.

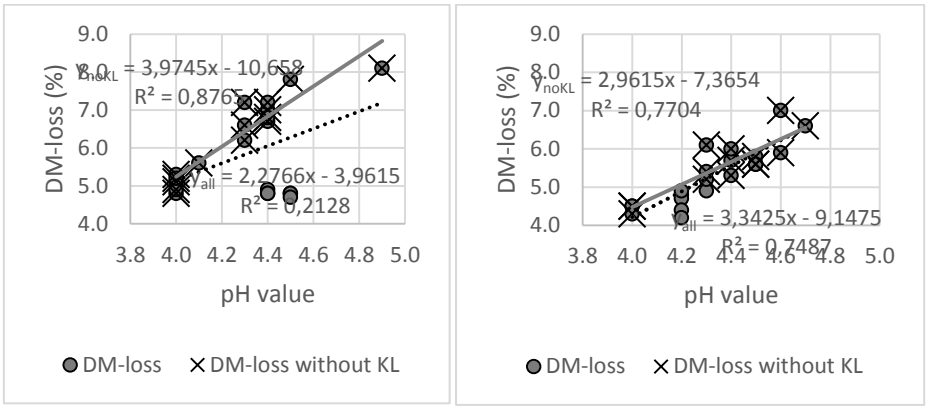


**Figure 1.** Interaction between pH level and DM-loss (%) in *Dactylus glomerata* (left) and vetch rye mixture (right) silages, all date considered ($y_{all}$) or nitrite HMTA (KL) treated silages excluded ($y_{noKL}$)

**Table 1.** Fermentation acids (g/kg DM), content of $NH_3$-N of total N (%) and fermentation loss (% of DM) of silages of *Dactylus glomerata* and the vetch rye mixture.

| | *Dactylis glomerata* | | | | Vetch rye mixture | | | |
|---|---|---|---|---|---|---|---|---|
| | Lactic-acid | Acetic-acid | NH3-N | FL | Lactic-acid | Acetic-acid | NH3-N | FL |
| Control | 26.7$^{a}$ | 27.6$^{a}$ | 12.5$^{a}$ | 6.5$^{a}$ | 57.9$^{ac}$ | 44.9$^{a}$ | 18.6$^{a}$ | 7.7$^{a}$ |
| KL2 | 62.5$^{bd}$ | 22.0$^{b}$ | 10.3$^{b}$ | 4.8$^{b}$ | 58.7$^{ac}$ | 17.3$^{c}$ | 12.7$^{b}$ | 4.8$^{c}$ |
| KL3 | 67.0$^{cd}$ | 15.6$^{c}$ | 10.2$^{b}$ | 4.5$^{c}$ | 60.6$^{a}$ | 18.5$^{bc}$ | 14.3$^{b}$ | 4.8$^{c}$ |
| EP2 | 33.3$^{a}$ | 29.9$^{a}$ | 11.5$^{ac}$ | 5.7$^{a}$ | 89.7$^{b}$ | 26.7$^{d}$ | 16.8$^{ab}$ | 5.2$^{c}$ |
| EP3 | 40.2$^{a}$ | 28.0$^{ab}$ | 11.6$^{ac}$ | 5.7$^{a}$ | 74.1$^{ab}$ | 17.9$^{c}$ | 15.3$^{ab}$ | 5.1$^{c}$ |
| LAC | 49.6$^{b}$ | 19.9$^{bc}$ | 11.0$^{ac}$ | 5.6$^{a}$ | 32.4$^{d}$ | 43.4$^{a}$ | 16.9$^{ab}$ | 7.0$^{a}$ |
| EP2 LAC | 69.8$^{cd}$ | 14.6$^{c}$ | 9.9$^{b}$ | 4.4$^{bc}$ | 40.3$^{cd}$ | 40.2$^{a}$ | 16.5$^{ab}$ | 6.5$^{b}$ |

KL=nitrite, HMTA (KL2, 2 litres/t fresh crop, KL3, 3 litres/t; EP=nitrite, sodium benzoate, potassium sorbate (EP2, 2 litres/t, EP3, 3 litres/t; LAC=*Lactobacillus plantarum*; EP2+LAC=combination of both; FL=fermentation losses; different letters show significant (P=0.05) differences within a column

**Conclusion** In case of poor fermentable crops additives may help to improve fermentation. In this trial the KL treatment was the best additive to control undesirable fermentation in wet crops and low S/BC. The effect of the EP treatments was not consistent but showed in the EP2/LAC combination and at the grass crop a positive effect.

**References**

Kaiser, E., Weiß, K. & Zimmer, J. (1997) Zum Gärungsverlauf bei der Silierung von nitratarmem Grünfutter, 1. Mitteilung: Gärungsverlauf in unbehandeltem Grünfutter. Archiv Animal Nutrition, 50, 87-102.

Knicky, M. & Spörndly, R. (2009) Sodium benzoate, potassium sorbate and sodium nitrite as silage additives. Journal of Science of Food and Agriculture, 89, 2659-2667.

VDLUFA (2012) Versuchs- und Untersuchungsmethodik -Methodenbuch Bd. III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt**.**

Weißbach, F. (2005) A simple method for correction of fermentation losses measured in laboratory silos. In: Proceedings of the XIV International Silage Conference, Belfast, Irland, 2005, pp. 278.

# Effect of different inocula on aerobic stability of corn silage

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**Keywords:** aerobic stability, corn silage, heterolactic inocula, *Lactobacillus hilgardii*.

**Introduction** Aerobic deterioration of silages causes dry matter (DM) and nutritive value losses, and leads to risks for human and animal health (Borreani et al. 2018). In order to improve the aerobic stability of silages, heterolactic bacteria, mainly *Lactobacillus buchneri* inocula have been used because of their ability to produce a greater amount of acetic acid, which inhibits yeasts and increases aerobic stability. New strains of LAB inoculant have been evaluated in recent years (Assis et al., 2014) to meet the needs of dairy farmers to feed silages from early opening silos (after 30 to 40 d of ensiling). The aim of the work was to evaluate the effect of *L. hilgardii* (LH), alone or in combination with *L. buchneri,* on aerobic stability of corn silage after different ensiling durations.

**Material and Methods** Corn was harvested as a whole plant (42% DM), and not treated **(C)** or inoculated with *L. buchneri* NCIMB 40788 **(LB)** [(theoretical application rate of 300,000 cfu/g fresh matter (FM)], *L. hilgardii* CNCM I-4785 **(LH-)** (theoretical application rate of 100,000 cfu/g FM), *L. hilgardii* CNCM I-4785 **(LH+)** (theoretical application rate of 300,000 cfu/g FM) and with a combination of *L. hilgardii* and *L. buchneri* **(LB+LH)** (theoretical application rate of 150,000 cfu/g FM of each one). The fresh forage was ensiled in 20-L plastic silos and opened after 15, 30 and 100 d of ensiling. At opening, the silages were analysed for DM content, pH, fermentative profile and microbial counts. The DM content was determined at 60°C for 72 h and corrected in order to consider the losses of volatile compounds. The fermentative products were determined in the acid extract by HPLC. Yeast and mold counts were determined using the pour plate technique on Yeast Extract Glucose Chloramphenicol agar. The weight losses due to fermentation were calculated as the difference between the weight of the forage placed in each plastic silo at ensiling and the weight at the end of conservation, and were expressed on a DM basis. At each opening, the silages were subjected to an aerobic stability test by continuously measuring the temperature during exposure to air. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. The obtained data were analyzed for their statistical significance, via analysis of variance, using version 24 of SPSS for Windows (SPSS Inc., Chicago, IL). The data were analyzed utilizing the inocula as the fixed factor, with five replicates. When the calculated values of F were significant, the REGWF test ($P < 0.05$) was used to interpret any significant differences among the mean values.

**Results and Discussion** DM content, pH, fermentative profile, yeast count and aerobic stability are reported in Table 1. The pH decreased as the duration of the ensiling increased, with a significant effect among treatments on 30 and 100 d, Lactic acid increased in all the treatments during conservation, with higher values in C and LH- silages after 100 d of ensiling, even though the differences were not of practical relevance. No differences were found in the acetic acid content between treatments after 15 d of ensiling, whereas it was higher in the LH+ and LB+LH than in the C silages at 30 and 100 d. In line with Assis et al. (2014), the fermentative results indicate the presence of 1,2-propanediol in silages treated with *L. hilgardii*. The yeast count decreased as the ensiling duration increased, with the lowest value in LH treated silages. The aerobic stability increased as the ensiling duration increased, mainly due to a reduction in the yeast count (Figure 1), with a *P-value* <0.001 and an adjusted $R^2$ of 0.77. The highest value of aerobic stability was observed in LH+ silages at 100 d. Confirming results reported by several authors, the increase in aerobic stability was related to the large amount of acetic acid and lower yeast count (Kleinschmit & Kung 2006).

**Conclusion** The use of *L. hilgardii* improved the aerobic stability at 15 d of ensiling when used in combination with *L. buchneri*, and at 100 d of ensiling when used alone with comparable effect to *L. buchneri*. The reduction in the yeast count during ensiling have been confirmed to be the most relevant factor influencing the increase of aerobic stability.

**Table 1.** Dry matter (DM) content, fermentative profile, yeast count and aerobic stability of corn silage after 15, 30 and 100 d of ensiling.

| Days | C | LB | LH- | LH+ | LB+LH | *P-value* | SEM |
|---|---|---|---|---|---|---|---|
| | | | **DM (%)** | | | | |
| **15** | 42.1$^{ab}$ | 42.4$^{a}$ | 41.5$^{ab}$ | 41.9$^{ab}$ | 41.2$^{b}$ | 0.023 | 0.142 |
| **30** | 42.7 | 42.2 | 41.5 | 41.9 | 42.3 | 0.270 | 0.175 |
| **100** | 41.6 | 41.5 | 41.8 | 40.9 | 40.7 | 0.353 | 0.200 |
| | | | **pH** | | | | |
| **15** | 3.80 | 3.81 | 3.80 | 3.80 | 3.81 | 0.697 | 0.00321 |
| **30** | 3.76$^{bc}$ | 3.77$^{ab}$ | 3.76$^{c}$ | 3.77$^{ab}$ | 3.77$^{a}$ | 0.002 | 0.00170 |
| **100** | 3.74$^{c}$ | 3.77$^{a}$ | 3.74$^{c}$ | 3.75$^{b}$ | 3.77$^{a}$ | <0.001 | 0.00261 |
| | | | **Lactic acid (g/kg DM)** | | | | |
| **15** | 31.4 | 30.0 | 31.6 | 28.5 | 29.5 | 0.175 | 0.464 |
| **30** | 32.9$^{b}$ | 35.6$^{ab}$ | 37.4$^{a}$ | 34.8$^{ab}$ | 35.6$^{ab}$ | 0.022 | 0.459 |
| **100** | 39.6$^{a}$ | 35.0$^{b}$ | 39.8$^{a}$ | 38.5$^{ab}$ | 36.6$^{ab}$ | 0.021 | 0.572 |
| | | | **Acetic acid (g/kg DM)** | | | | |
| **15** | 6.1 | 6.2 | 6.1 | 5.9 | 6.1 | 0.840 | 0.0911 |
| **30** | 6.3$^{c}$ | 6.7$^{bc}$ | 7.7$^{a}$ | 7.8$^{a}$ | 7.3$^{ab}$ | <0.001 | 0.141 |
| **100** | 8.5$^{b}$ | 10.0$^{b}$ | 9.7$^{b}$ | 12.4$^{a}$ | 12.0$^{a}$ | <0.001 | 0.363 |
| | | | **1,2-propanediol (g/kg DM)** | | | | |
| **15** | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.00$^{b}$ | 0.17$^{a}$ | 0.014 | 0.0203 |
| **30** | 0.00$^{d}$ | 0.34$^{c}$ | 0.57$^{b}$ | 0.88$^{a}$ | 0.41$^{c}$ | <0.001 | 0.0616 |
| **100** | 0.00$^{c}$ | 1.86$^{ab}$ | 1.31$^{b}$ | 3.32$^{a}$ | 2.78$^{ab}$ | <0.001 | 0.280 |
| | | | **Yeast ($log_{10}$ cfu/g)** | | | | |
| **15** | 5.11$^{abc}$ | 5.38$^{ab}$ | 5.38$^{a}$ | 5.00$^{bc}$ | 4.89$^{c}$ | 0.006 | 0.0575 |
| **30** | 4.49$^{ab}$ | 4.71$^{a}$ | 4.40$^{b}$ | 4.23$^{b}$ | 4.39$^{b}$ | 0.003 | 0.0438 |
| **100** | 4.00$^{a}$ | 3.18$^{ab}$ | 2.88$^{b}$ | 2.80$^{b}$ | 2.98$^{b}$ | 0.010 | 0.132 |
| | | | **Aerobic stability (h)** | | | | |
| **15** | 51$^{b}$ | 50$^{b}$ | 52$^{b}$ | 53$^{b}$ | 74$^{a}$ | 0.014 | 2.822 |
| **30** | 58$^{ab}$ | 55$^{b}$ | 57$^{ab}$ | 67$^{ab}$ | 67$^{a}$ | 0.037 | 1.644 |
| **100** | 96$^{c}$ | 160$^{ab}$ | 170$^{ab}$ | 191$^{a}$ | 137$^{b}$ | <0.001 | 7.778 |
| | | | **Weight losses (% DM)** | | | | |
| **15** | 1.81 | 1.66 | 1.73 | 1.70 | 1.82 | 0.556 | 0.0339 |
| **30** | 1.96 | 1.86 | 1.87 | 1.89 | 1.97 | 0.551 | 0.0236 |
| **100** | 2.20 | 2.23 | 2.18 | 2.16 | 2.34 | 0.180 | 0.0261 |

C = control; DM = dry matter; LB = *L. buchneri*; LH = *L. hilgardii*; SEM = standard error of the mean.

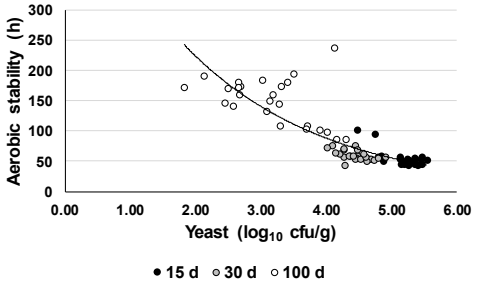


**Figure 3.** Relation of the aerobic stability and yeast count at silo opening, as affected by different ensiling period.

### References

Assis, F.G.D., Avila, C.L.D., Pinto, J.C. & Schwan R.F. (2014). New inoculants on maize silage fermentation. Revista Brasileira de Zootecnia, 43, 395-403.

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J. & Muck, R.E. (2018). Factors affecting dry matter and quality losses in silages. Journal of Dairy Science, 101, 3952-3979.

Kleinschmit, D.H, & Kung L. (2006) A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of maize and grass and small-grain silages. Journal of Dairy Science, 89, 4005-4013.

# Effect of length of ensiling on fermentation characteristics, aerobic stability and structural microexamination of the grain in corn silages treated with bacterial inocula in a tropical climate

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**Keywords:** Corn silage, Additive, Fermentation Length, Micro-examination

**Introduction** Differences in epiphytic microbial populations and inherent plant characteristics (i.e. chemical composition) have been reported in forages harvested in temperate and tropical climates. Ensiling forages in tropical areas results in lower quality silage compared to plant material fermented in temperate environments. Alternatives to improve the fermentation characteristics, aerobic stability and ruminal degradability of starch are a priority for the production and use of corn silage in tropical climates. Adding microbial inocula containing lactic acid-producing bacteria (LAB) and increasing the lenght of fermentation might enhance the fermentation characteristics and aerobic stability of whole plant corn ensiled in Puerto Rico.

**Material and Methods** Whole corn plants (*Zea maiz*) were harvested at 28% DM (dough stage) at a commercial farm located in the southwest area of the island (18.0047° N, 66.3898° W). Sixteen silos, each filled with 5 kg of wholecorn plant chopped into 2 cm pieces including the ears, were assigned to two experimental treatments; no additive or treated with LAB containing *L. plantarum, E. faecium, L. lactis, P. acidilactici,* and *P.pentosaceus* ($10^5$ CFU/g, MikropHerm, Madison, WI). Four silos per treatment were opened after two lenghts of fermentation (LOF, 120 and 180 days) and analyzed to determine chemical composition, pH, fermentation products, and changes in the microstructure of the starch granules. Aerobic stability was determined by changes in temperature observed every 6 hours during 7 days. Data on chemical composition, pH and fermentation products were analyzed according to a completely randomized design with a factorial arrangement 2 x 2 (with or without LAB and 2 (LOF). Aerobic stability data were analyzed as a split-plot design with a factorial arrangement 2 (with or without inoculum) x 2 (LOF) x 28 (periods of aerobic exposure). Tukey‘s test was used for mean separation (SAS, 2004).

**Results** Ensiling corn during periods of either 4 or 6 months with or without bacterial inoculum did not affect the chemical composition of the resulting silage (data not shown). However, addition of the LAB improved the fermentative characteristics of the silage by decreasing ($P<0.05$) the content of acetic acid below the control while increasing ($P<0.05$) the total acid content while it tended ($P<0.10$) to improve lactic:acetic acid ratio (Table 1). Structural changes on the surface, agglutination, and pitting (presumably due to the action of amylase) properties of the starch granules after the fermentation process were observed (Figures 1 and 2). These changes were most visible in the silages treated with the inoculum and fermented for 180 days and are attributed to the effect of the LAB on the starch protein matrix, allowing the agglutination of the starch granules. These structural changes could be beneficial for animal production due to enhanced ruminal degradation of the starch granules resulting from break down of this starch protein matrix, thus making the starch more available for digestion. With both LOF, the silages treated with the bacterial additive were more stable during the period of aerobic exposure than those fermented without the additive. The increase in temperature indicates aerobic deterioration of silages without additive after 66 hours while the inoculated silages lasted 108 hours without heating.

**Table 1.** Effect of the inoculum on the corn silage's fermentation products after 120 and 180 days of fermentation.

| Component | Control | Inoculum | Probability | |
|---|---|---|---|---|
| | | | TRT | TRT x LOF |
| pH | 3.71 | 3.75 | 0.20 | 0.66 |
| Lactic Acid[1] | 98.1 | 109.6 | 0.16 | 0.44 |
| Acetic Acid[1] | 22.4[a] | 18.0[b] | 0.03 | 0.85 |
| Lactic/Acetic Ratio | 4.45 | 6.57 | 0.09 | 0.46 |
| Propionic Acid[1] | 0.20 | 0.20 | 0.64 | 0.92 |
| Butyric Acid[1] | 0.90[b] | 13.0[a] | 0.01 | 1.00 |
| Total Acids | 121.5[b] | 141.1[a] | 0.05 | 0.54 |
| Amoniacal-N/Total-N | 7.63 | 8.38 | 0.15 | 0.15 |
| Volatile Fatty Acids[1] | 94.4 | 96.3 | 0.30 | 0.42 |

[1] g/kg DM

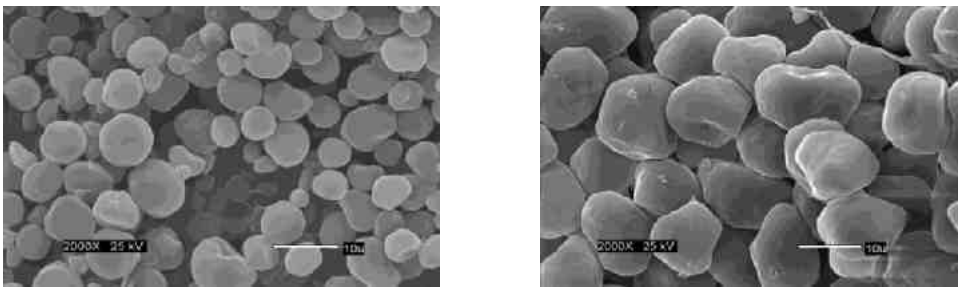

**Figure 1.** Starch granules scanning electron microscopy on the control silage after 120( Left) and 180 (right) days of fermentation. Some pitting is observed in the grain of corn ensiled, presumably due to the action of amylase.

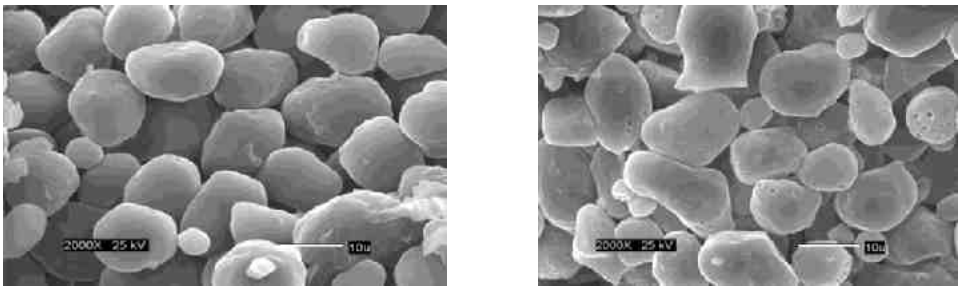

**Figure 2.** Starch granules scanning electron microscopy on the inoculated silage after 120 (Left) and 180 (right) days of fermentation. Intense pitting is observed in the grain of corn ensiled, presumably due to the action of amylase.

**Discussion** Ensiling whole-plant corn for fermentation periods of 6 versus 4 months had no significant effect on the chemical composition of the silage. The use of the bacterial inoculant improved the fermentative characteristics by increasing the percentage of total acids and reducing that of acetic acid resulting in an improved lactic:acetic acid ratio. More marked structural changes were observed in starch granules of the inoculated silage with the longer LOF, thus presumably making the starch granules more available for ruminal degradation. Finally, in both LOF, the silages treated with LAB inoculum were more stable under aerobic conditions than those without the additive.

**Conclusion** Corn ensiled in a tropical climate and treated with a microbial inocula did not improved the fermentation characteristics, but enhanced aerobic stability of the resulting silage. Microexamination showed the the effect of the inocula on the starch protein matrix of corn kernels.

# Effect of microbial inoculant and fermentation period on the fermentation profile of silage of rehydrated corn and sorghum grains

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**Keywords:**acetic acid,lactic acid, microbial population

**Introduction** The use of microbial inoculants may improve the silage fermentation through efficient lactic acid production, which contributes to a fast pH decline, inhibiting other bacteria and preserving the silage quality. Ensiling rehydrated grains improves their digestibility, besides preserving other nutritional characteristics of the fresh material. We hypothesized that the use of a microbial inoculants associated with the fermentation period improves the fermentation pattern of silages of ground and rehydrated grains. In this context, the objectives of this study were to evaluate the fermentation profile and microbial population in silage of ground and rehydrated corn and sorghum grains treated or untreated with microbial inoculant, in different fermentation periods.

**Material and Methods** A 8 x 3 x 2 factorial scheme was used, with eight fermentation periods (P: 1, 3, 7, 21, 56, 90, 180 and 270 days), three inoculants (I: control, Lalsil AS and Lalsil Corn) and two types of grains (G: corn and sorghum), with three replicates in a completely randomized design. Grains were ground through a 3-mm sieve and then rehydrated to 70% dry matter. In the treatments with inoculants, $10^5$cfu $g^{-1}$ of each one were used, in addition to the water used for rehydration. The inoculants used were Lalsil Corn (LC) (Lallemand®, Brazil) (sacarose, *Lactobacillus plantarum*>3,0 x $10^{10}$ UFC/g and *Propionibacterium acidipropionici*>3,0 x $10^{10}$ UFC/g) and Lalsil AS (LAS) (Lallemand®, Brazil) (*Lactobacillus buchneri* CNCM-I 4323 1,0 x $10^{11}$ UFC/g e sacarose). The material was ensiled in vacuum-sealed bags. Dry matter (DM); crude protein (CP); pH; ammonia ($NH_3$-N, % of total N); lactic (LA), acetic (AA), propionic (PA), and butyric (BA) acids, lactic acid bacteria (LAB); fungi (FUN); and enterobacteria (ENT) populations were evaluated. Data were analyzed using the PROC MIXED procedure of SAS software (version 9.4) considering 0.05 as the critical level of significance and also subjected to regression analysis as a function of the fermentation period.

**Results and Discussion** The *P*-value and SEM of fermentation characteristics of silages are shown in Table 1. There was an effect of the I x G x P interaction ($P < 0.05$) on all parameters evaluated, except for the concentrations of dry matter, crude protein and propionic acid ($P > 0.05$). Nonlinear models were fitted for the pH of silage of both grains, whose values stabilized from the seventh day of fermentation. The highest AA content was observed at 56, 90, 180, and 270 days of fermentation in LAS-treated rehydrated corn grain silage and at 90, 180, and 270 days in the LAS-treated rehydrated sorghum grain silage. Rehydrated corn grain silage treated with LAS showed highest contents of $NH_3$-N at 7, 90, 180, and 270 days of fermentation. This may be an effect of butyric acid-producing bacteria, which worked with higher proteolytic activity, resulting in ammonia production (Ferreira et al., 2015). This fact was evidenced by the higher butyric acid level observed in those silages. However, based on the criteria of Mahanna (1994) and Kung et al. (2001), all silages can be considered high-quality, since their $NH_3$-N contents on DM basis were lower than 10%. Silages treated with LC showed the highest LA contents and the lowest pH. The highest LA content in corn silage was observed at 3 and 21 days and in sorghum silage at 3, 7, 56, and 90 days. The lower pH of LC-treated silages is likely due to the higher LA production. It is a known fact that an improvement in LA production may lead to a faster pH decline (McAllister and Hiristov, 2000). Irrespective of the inoculants evaluated, from 21 days of fermentation, all corn silages had pH within the adequate range for the fermentation of moist or rehydrated-grain silage, which stabilizes at a higher pH (Kung & Shaver, 2001). Only after 56 days of fermentation the silages of rehydrated sorghum presented pH lower than 4.5. However, the

use of LC led to the highest pH decline rate in the silages of rehydrated sorghum (1.18 vs. 0.25 units day$^{-1}$ for control). Rehydrated sorghum grain silages treated or untreated with LC showed higher DM content than rehydrated corn grain silages. The LC inoculant provided the lowest and highest DM and CP contents in the corn silage, respectively. The LAS inoculant provided the highest (P<0.05) CP content in sorghum silage. In control silage and in that treated with LAS of both grains, the LAB population decreased linearly (P<0.05) as a function of the fermentation period.

**Table 1.** Statistical analysis for the fermentation profile variables of rehydrated corn and sorghum grain silages (G) treated with microbial inoculants (I) at different fermentation periods (P)

| Item | SEM[1] | P-value | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | I | G | P | IxG | G×P | I×P | I×G×P |
| Dry matter | 0.50 | 0.02 | <0.01 | <0.01 | <0.01 | <0.01 | 0.77 | 0.50 |
| Crude protein | 0.18 | 0.45 | <0.01 | <0.01 | <0.01 | 0.01 | 0.51 | 0.60 |
| pH | 0.09 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Ammonia N | 0.32 | <0.01 | <0.01 | <0.01 | 0.17 | <0.01 | <0.01 | 0.01 |
| Lactic acid | 0.12 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Acetic acid | 0.04 | <0.01 | 0.67 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Propionic acid | <0.01 | 0.03 | 0.10 | <0.01 | 0.48 | <0.01 | <0.01 | 0.63 |
| Butyric acid | 0.02 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| Enterobacteria | 0.71 | <0.01 | 0.71 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 |
| Lactic acid bacteria | 0.42 | <0.01 | <0.01 | <0.01 | 0.23 | <0.01 | <0.01 | <0.01 |
| Fungi | 0.54 | <0.01 | <0.01 | <0.01 | 0.13 | <0.01 | <0.01 | 0.01 |

The maximum LAB population (9.70 cfu g$^{-1}$) was observed at seven days of fermentation for the LAS-treated corn grain silage. The marked increase in LAB counts in the first days of fermentation was possibly benefited by the grinding of the material, which improved the nutrient availability. A quadratic effect (P<0.05) of P was observed on the fungal population in rehydrated corn grain silage. In control and LAS sorghum silage, the population of this microbial group decreased linearly (P<0.05) as a function of P. However, a linear increase was observed in the LC silage. Only the LAS inoculant had a beneficial effect on the fungal population in both silages.

**Conclusion** The inoculant containing homofermentative LAB (LC) improved the fermentation profile of the evaluated silages, by increasing the lactic acid content and lowering the pH. The LC inoculant showed a better response to the fermentation of rehydrated sorghum grain silage.

**Acknowledgments**: This research was supported by the FAPEMIG, CNPq, and CAPES foundations, and INCT-CA.

**References**

Ferreira, D.J., Zanine, A.M., Santos, E.M., Oliveira, J.S, & Pinho, R.M.A. (2015) Lactic Acid Bacteria of Potential as a Means of Inhibiting Undesirable Microorganisms in Warm Season Grass Silages. American Journal of Experimental Agriculture, 8 (1), 1-11.

Kung, L. & Shaver, R. (2001) Interpretation and use of silage fermentation analysis reports. Focus on Forage, v.3, p.1-5.

Mahanna, B. (1994) Proper management assures high-quality silage, grains. *Feedstuffs*, 10, 12-18.

McAllister, T.A. & Hristov, A.N. (2000) The fundamentals of making good quality silage. Proc. 2000 Western Can. Dairy Seminar. University of Alberta, Dept. of Agric., Food and Nutr. Sci., Edmonton, AB.

# Effect of microbial inoculant, particle size, and storage length on chemical composition and starch degradability of rehydrated sorghum grain silage

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**Keywords:** degradability, *Lactobacillus buchneri*, rehydration, sorghum grain silage

**Introduction** Sorghum grain is known to have lower starch degradability when compared to corn, due the complex arrange of proteins that protects starch from hydrolysis. Rehydration of grains for ensiling is a technology that is feasible at farm and may improve starch degradability if properly processed, inoculated, and ensiled. Our hypothesis is that rehydrated sorghum grain treated with microbial inoculant that contains heterofermentative bacteria will increase starch degradability along the storage time, if finely ground. The aim of this study was to evaluate the effects of microbial inoculant (I), particle size (PS), and storage time (T) on dry matter and starch contents and degradability of rehydrated sorghum grain silage.

**Materials and Methods** Sorghum grains were ground into three PS (8, 2, and 1 mm), rehydrated to reach 650 g DM/kg OM and ensiled in mini silos (300 g). Before ensiling, the mass was inoculated with one of the treatments (1) Control (distilled water); (2) LB, *Lactobacillus buchneri* CNCM I-4323 at $1 \times 10^5$ cfu/g OM (Lalsil® AS – Lallemand Brasil Ltda.); (3) PP, *Pediococus acidilactici* CNCM MA 18/5M at $3 \times 10^4$ cfu/g OM and *Lactobacillus plantarum* CNCM MA 18/5U at $1 \times 10^5$ cfu/g OM (Lalsil® CL – Lallemand Brasil Ltda.); and (4) LBpp, LB + PP. Treatments were evaluated in quintuplicate during 0, 15, 30, 120, and 360 days of storage. Silos were kept inside the lab within controlled temperature (25 °C). Samples were dried in forced air ventilation oven for DM determination. Starch content was determined by chemical solubilisation and enzymatic digestion. Dry matter and starch degradability was performed by *in situ* incubation for 7 h, using 4 ruminally cannulated Holstein cows. Data were analyzed by split-split plot using the Mixed procedure of SAS version 9.2 (SAS Inst. Inc., Cary, NC).

**Results** Dry matter content of the silage was affected by PS*T interaction ($P<0.01$), where the DM of all treatments decreased throughout storage time, with greater decrease for the treatments with 1 mm of PS (Figure 1). There was PS*I*T interaction ($P = 0.05$) for starch content, where a wide variation was observed during the time of conservation, however the final concentration did not differ from the initial (Figure 1). Dry matter and starch degradability were affected by PS*I*T interaction ($P<0.01$), presenting the same behavior throughout storage time (Figure 1). Degradability increased linearly with time, with greater enhancement for treatments with 1 and 2 mm of PS, and lower for 8 mm of PS. Besides the effect of PS, inoculant containing only *L. buchneri* increased degradability when rehydrated sorghum grain was ensiled for over 120 days (Figure 1).

**Discussion** The results showed a positive effect of storage time on starch degradability of reconstituted sorghum grain. Although, the PS of the grain affected the enhancement of the degradability, once the treatments with 8 mm of PS presented lower degradability compared to the others PS, likely due the physical barrier that avoids the attack of the microorganisms that contribute to the proteolysis of the protein matrix. It is known that 60% of the proteolysis that occur during the fermentative process is done by bacterial activity (Junges et al. 2017). However, the specie that contributes more to the proteolysis process is unknown. Overall, the inclusion of *L. buchneri* enhanced the increasing in starch and DM degradability of reconstituted sorghum grain storage for longer than 120 d, once there was a greater degradability for the silages treated with *L. buchneri* in each PS and

followed by the treatments that had a mix of inoculants contacting LB in the composition, indicating that the positive effect of LB on starch and consequently, DM degradability.

**Conclusions** Storage length increases starch degradability with greater enhancement when rehydrated sorghum grain is finely ground (1 and 2 mm) and inoculated with *L. buchneri*.

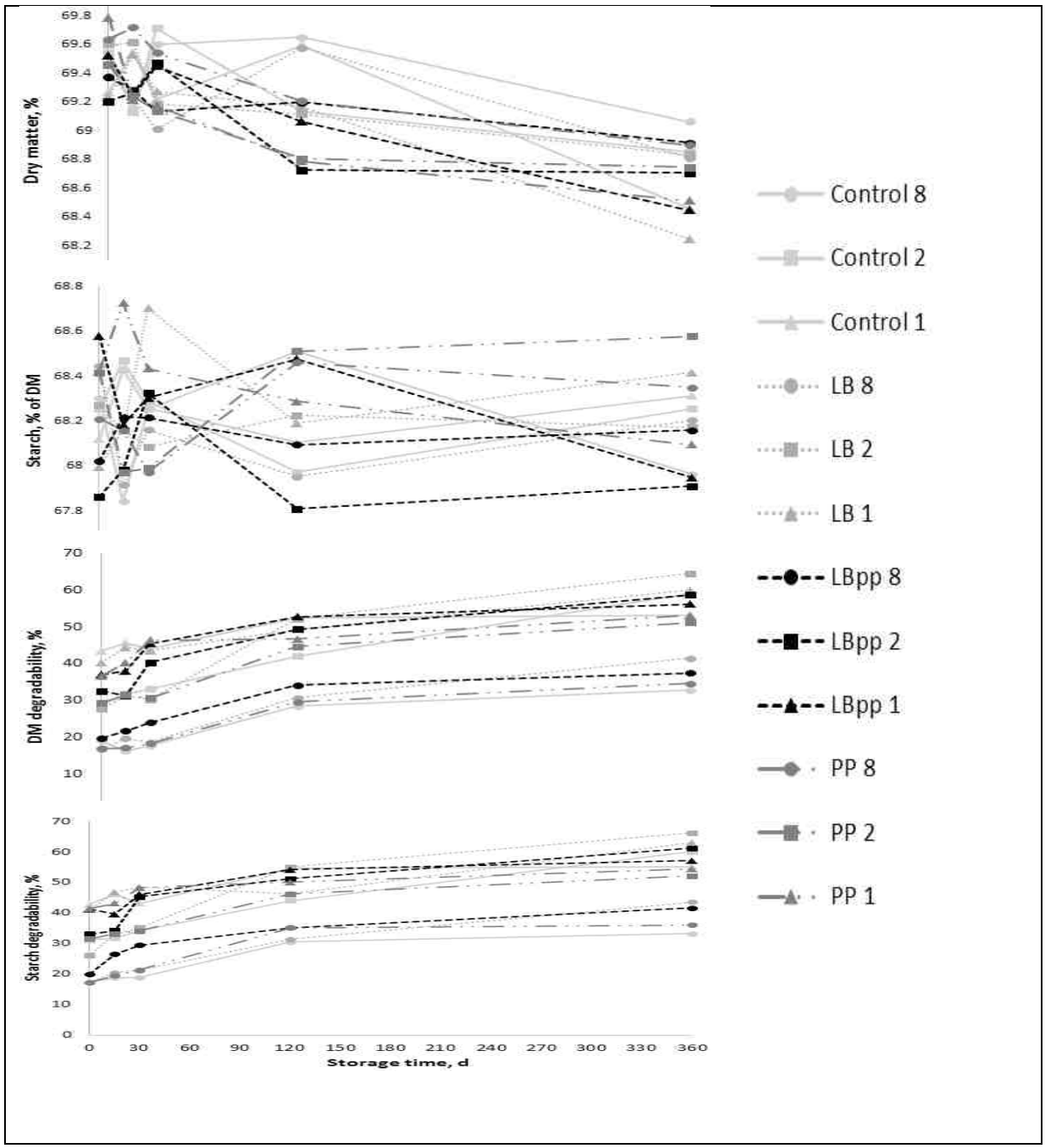


**Figure 1** Microbial inoculant, particle size, and storage time effects on dry matter and starch contents and degradability of rehydrated sorghum grain silage depending on storage.

**Reference**

Junges, D., Morais, G., Spoto, M.H.F., Santos, P.S., Adesogan, A.T., Nussio, L.G. & Daniel, J.L.P. (2017) *Short communication:* Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. Journal of Dairy Science, 100, 9048-9051.

# Effect of sealing strategies and sampling site on fermentation profile of corn silage

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**Keywords:** aerobic deterioration, aerobic stability, chemical additive, sealing

**Introduction** The aerobic deterioration process can be decreased when adopting different sealing strategies to reduce the air influx into the silage, such as the sugarcane bagasse physical barrier under the film plastic, to minimize the effects of the solar radiation. To improve the aerobic stability of silages, the use of the chemical additives as sodium benzoate are recommend, because of the antimicrobial activity (Kleinschmit et al., 2005). The aim of this study was to evaluate the fermentation profile of corn silages from two different sealing strategies.

**Materials and methods** The corn hybrid used for silage was AG 1051. Whole corn plants were harvested at 35% of dry matter (DM) and ensiled in bunker silos (45 t) for a year. Two sealing strategies were adopted: (1) polyethylene film black-on-white 200 µm covered with sugarcane bagasse (10 cm thick layer) (BG) or (2) application of sodium benzoate on the top surface of ensiled mass (150 g/m$^2$, dilution of 1:4 = 0.6 L/m$^2$) and sealing it immediately with polyethylene film black-on-white 200 µm (BZ). After 343 days of storage, the silos were opened and samples of silage were collected from top and middle sites of the silo. Extracts of silages were made for determination of the fermentation profile and analyzed by gas chromatograph with mass detector (GC-MS). Concentration of lactic acid was performed by colorimetrich method. Was made one bunker silo for each treatment. Data were analyzed using the MIXED procedure of SAS.

**Results** There was an effect of sampling location for pH values, where the silages of the middle location showed lower pH when compared to the top location (*P<0.01*). There was an effect of sampling location for the concentration of lactic acid, where the concentration was larger in the middle location (*P=0.02*). The concentration of acetic acid (*P=0.04*), 1,2-propanediol (*P<0.01*) and ethanol (*P=0.03*) was lower in the top location of silos in both treatments. There was no treatment effect on concentration of butyric acid (*P>0.1*).

**Table 1.** Influence of sealing strategies and sampling site in the fermentation profile of corn silages

| | BG[1] | | BZ[2] | | | *P* | | |
|---|---|---|---|---|---|---|---|---|
| Item | Middle | Top | Middle | Top | SEM | S[3] | L[3] | S×L[3] |
| pH | 3.72 | 3.87 | 3.85 | 4.00 | 0.05 | 0.11 | <0.01 | 0.96 |
| Lactic Acid % | 2.71a | 2.30ab | 3.15a | 1.00b | 0.34 | 0.3 | 0.02 | 0.07 |
| Acetic Acid % | 2.46 | 1.96 | 3.2 | 1.76 | 0.39 | 0.58 | 0.04 | 0.23 |
| Butyric Acid % | 0.01 | 0.2 | 0.04 | 0.31 | 0.13 | 0.67 | 0.13 | 0.74 |
| 1,2-propanediol % | 1.01 | 0.35 | 1.52 | 0.52 | 0.35 | 0.51 | <0.01 | 0.46 |
| Ethanol % | 0.34 | 0.18 | 0.3 | 0.09 | 0.07 | 0.42 | 0.03 | 0.72 |

[a-b] Means with different letters in a row differ significantly (Tukey-Kramer, α = 0.10)

[1]BG: Corn silage sealed with polyethylene film black-on-white 200 µm covered with sugarcane bagasse (10 cm thick layer) (BG); [2]BZ: corn silage with application of sodium benzoate on the top surface of ensiled mass (150 g/m$^2$, dilution of 1:4 = 0.6 L/m$^2$) and sealing it immediately with polyethylene film black-on-white 200 µm (BZ)

[3]S= Effect of treatment in ensiling; L = Effect of sample site on silo; S x L = Effect of interaction between treatment and sample site.

**Discussion** An anaerobic environment and soluble carbohydrates are required for lactic acid bacteria, which results in high quality fermentation silages (Muck, 1988). Lactic acid reduces silage pH rapidly, however the top site of the silos are known to present high dry matter losses, due to the oxygen that remains or penetrates trough the polyethylene cover, allowing the development of undesirable microorganisms. The silages were stored for 343 days before opening and to minimize the effects of the solar radiation on the silage plastic cover, BG was used as physical barrier and BZ as chemical controller of the undesirable microorganisms that may grow on the top site of the ensiled material. However, BZ treatment was not efficient in controlling the growth of deteriorating microorganisms, as the lactic acid concentration was lower in the top site, indicating that there was yeast consuming the local lactic acid. The solar radiation throughout the time likely enhanced the dryness of the polyethylene cover even with BG as physical barrier, increasing oxygen penetration that inhibited anaerobic bacteria growth and greater volatilization, resulting in lower acetic acid, 1,2-propanediol, and ethanol.

**Conclusion** The silages sampled in the top site of the silo showed worse fermentation profile than silages sampled in the middle site of the silo.

**References**

Kleinschmit, D. H., Schmidt, R. J., & Kung, L., Jr. (2005). The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. Journal of Dairy Science, 88, 2130-2139.

Muck, R. E. (1988). Factors influencing silage quality and their implications for management. Journal of Dairy Science, 71, 2992-3002.

# Effect of wilting and additives on fatty acid composition of red clover silage

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**Keywords:** formic acid, lactic acid bacteria, red clover, wilting

**Introduction** Forage lipids are typically rich in polyunsaturated fatty acids (PUFA) 18:2n-6 and 18:3n-3, but conservation method greatly affects lipid content and composition. Wilting of grass decreases the proportion of PUFA in forage whereas harvesting at early growth stage and using formic acid to restrict fermentation in silo increases their proportions (Dewhurst et al. 2006). However, the effect of wilting and extent of in silo fermentation on fatty acids in red clover (*Trifolium pratense*) is less studied. It was hypothesized that 1) wilting decreases PUFA content of red clover and 2) restricting microbial fermentation in silo by formic acid preserves fatty acids better than inoculation with lactic acid bacteria.

**Material and Methods** Pure red clover herbage (cultivar Saija, Boreal Plant Breeding Ltd) was grown in Finland (Jokioinen 60˚49'N, 23˚28'E) and cut at early flowering. One part of the cut herbage was ensiled immediately and other part was wilted to two higher dry matter (DM) contents. The DM content of ensiled material was 194, 358 and 522 g/kg for 0, 24 and 48 h of wilting, respectively. Additive treatments were as follows: untreated, formic acid (4 L pure formic acid per tn herbage) or lactic acid bacteria (*Lactobacillus plantarum* $10^6$ colony-forming units (cfu)/g herbage, Lactofast, Kemira Ltd). The amount of added liquid (water plus additive) in herbage was the same (10 ml/kg) for all treatments. The herbage was compacted into minisilos in triplicate (958, 875 and 708 kg/$m^3$ for 0, 24 and 48 h of wilting, respectively). The 120 ml minisilos were of glass and sealed with a rubber stopper and a plastic screw cap. They were opened 191 days after ensiling. Chemical composition of forages was determined as described earlier by Koivunen et al. (2015) with exception of lipid extraction and fatty acid methylation that was according to Halmemies-Beauchet-Filleau et al. (2013). Results were analyzed by ANOVA using the Mixed procedure of SAS (SAS 9.3, Institute Inc., Cary, NC). Sums of squares for treatment effects were further separated into single degree of freedom comparisons using orthogonal contrasts.

**Results and Discussion** Wilting improved the fermentation quality of all silages by decreasing ($P<0.01$) the concentration of acetic and butyric acids (Table 1). Forage ensiled with formic acid was the most restrictively fermented one. The fatty acid content of ensiled material decreased gradually as the wilting time prolonged (Table 2). The proportion of 18:3n-3 decreased markedly during wilting from 50 to 43 g per 100 g fatty acids, whereas that of 18:2n-6 remained constant. The fatty acid content of silages was slightly higher than that of ensiled material (Table 2). This is probably due to fermentative losses of carbohydrates and proteins in silo as well as lipid synthesis by microbes. Changes in silage fatty acid composition due to additive treatment and extent of fermentation in silo were in general marginal. Nevertheless, using formic acid slightly decreased the proportion of 18:3n-3 and increased that of 18:2n-6 relative to lactic acid inoculum ($P<0.04$, interaction) in silages prepared without wilting. In addition, using lactic acid bacteria in silage preparation increased the proportion of 16:0 and 18:0 inherent to bacterial lipids relative to silage prepared with formic acid ($P<0.01$).

**Conclusion** Wilting decreases markedly red clover's fatty acid and 18:3n-3 content, whereas the extent of fermentation in silo has marginal effects on fatty acids.

**Table 1.** Dry matter content (g/kg) and fermentation quality (g/kg dry matter) of red clover silages

| Wilting time, h | Additive | Dry matter | pH | Sugars | Lactic acid | Acetic acid | Butyric acid | Ammonia-N, g/kg N |
|---|---|---|---|---|---|---|---|---|
| 0 | No | 213 | 4.07 | 12 | 114 | 33 | 2.9 | 30.4 |
| | Formic acid | 215 | 4.03 | 19 | 53 | 33 | 5.3 | 16.0 |
| | Lactic acid bacteria | 217 | 4.07 | 28 | 113 | 27 | 3.3 | 21.6 |
| 24 | No | 387 | 4.31 | 41 | 94 | 30 | 1.2 | 33.7 |
| | Formic acid | 391 | 4.26 | 27 | 53 | 32 | 1.6 | 17.4 |
| | Lactic acid bacteria | 386 | 4.21 | 52 | 89 | 15 | 1.7 | 17.9 |
| 48 | No | 534 | 5.11 | 115 | 30 | 17 | 0.8 | 24.4 |
| | Formic acid | 534 | 4.67 | 134 | 16 | 14 | 1.1 | 18.2 |
| | Lactic acid bacteria | 530 | 4.36 | 88 | 56 | 11 | 0.9 | 18.6 |
| SEM | | 3.2 | 0.028 | 4.8 | 1.4 | 2.7 | 0.49 | 1.59 |
| | | | | | | | | |
| Statistical significance | | | | | | | | |
| Wilting, 0 vs 24 and 48 h (1) | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.40 |
| Length of wilting, 24 vs 48 h (2) | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.16 | 0.06 |
| No additive vs. additives (3) | | 0.83 | <0.01 | 0.57 | <0.01 | 0.03 | 0.08 | <0.01 |
| Formic acid vs. lactic acid bacteria (4) | | 0.39 | <0.01 | 0.27 | <0.01 | <0.01 | 0.09 | 0.11 |
| Interaction 1x3 | | 0.53 | <0.01 | 0.07 | <0.01 | 0.46 | 0.17 | 0.81 |
| Interaction 1x4 | | 0.21 | <0.01 | 0.03 | <0.01 | 0.38 | 0.04 | 0.08 |
| Interaction 2x3 | | 0.54 | <0.01 | 0.70 | <0.01 | 0.65 | 0.82 | <0.01 |
| Interaction 2x4 | | 0.93 | <0.01 | <0.01 | 0.09 | 0.02 | 0.80 | 0.97 |

**Table 2.** Total fatty acid (FA) content (g/kg DM) and fatty acid composition (g/100 g FA) of ensiled plant material and silages

| Wilting time, h | Additive | Total FA | 16:0 | 18:0 | 18:1n-9 | 18:2n-6 | 18:3n-3 |
|---|---|---|---|---|---|---|---|
| Ensiled material | | | | | | | |
| 0 | All treatments | 15 | 15 | 2.0 | 1.9 | 25 | 50 |
| 24 | All treatments | 10 | 17 | 2.3 | 1.7 | 25 | 46 |
| 48 | All treatments | 7.9 | 18 | 2.5 | 1.7 | 24 | 43 |
| | | | | | | | |
| Silages | | | | | | | |
| 0 | No | 16 | 18 | 2.4 | 1.7 | 20 | 54 |
| | Formic acid | 17 | 17 | 2.2 | 1.7 | 22 | 53 |
| | Lactic acid bacteria | 16 | 17 | 2.5 | 1.7 | 19 | 55 |
| 24 | No | 12 | 19 | 2.6 | 1.5 | 21 | 51 |
| | Formic acid | 12 | 18 | 2.4 | 1.5 | 20 | 50 |
| | Lactic acid bacteria | 11 | 19 | 2.6 | 1.4 | 19 | 48 |
| 48 | No | 12 | 18 | 2.5 | 1.7 | 20 | 47 |
| | Formic acid | 10 | 19 | 2.6 | 1.6 | 20 | 47 |
| | Lactic acid bacteria | 9.2 | 20 | 2.7 | 1.6 | 20 | 46 |
| SEM | | 0.39 | 0.33 | 0.05 | 0.04 | 0.5 | 0.8 |
| | | | | | | | |
| Statistical significance for silages | | | | | | | |
| Wilting, 0 vs 24 and 48 h (1) | | <0.01 | <0.01 | <0.01 | <0.01 | 0.72 | <0.01 |
| Length of wilting, 24 vs 48 h (2) | | <0.01 | 0.05 | 0.36 | <0.01 | 0.94 | <0.01 |
| No additive vs. additives (3) | | 0.03 | 0.50 | 0.37 | 0.05 | 0.54 | 0.21 |
| Formic acid vs. lactic acid bacteria (4) | | 0.02 | <0.01 | <0.01 | 0.09 | 0.02 | 0.75 |
| Interaction 1x3 | | 0.04 | 0.16 | 0.35 | 0.50 | 0.09 | 0.47 |
| Interaction 1x4 | | 0.52 | 0.11 | 0.16 | 0.86 | 0.04 | 0.04 |
| Interaction 2x3 | | 0.05 | <0.01 | 0.06 | 0.28 | 0.20 | 0.38 |
| Interaction 2x4 | | 0.68 | 0.72 | 0.08 | 0.68 | 0.94 | 0.61 |

## References

Dewhurst, R.J., Shingfield, K.J., Lee, M.A. & Scollan, N.D. (2006) Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Animal Feed Science and Technology, 131, 168-206.

Halmemies-Beauchet-Filleau, A., Kairenius, P., Ahvenjärvi, S., Crosley, L.K., Muetzel, S., Huhtanen, P., Vanhatalo, A., Toivonen, V., Wallace, R.J. & Shingfield, K.J. (2013) Effect of forage conservation method on ruminal lipid metabolism and microbial ecology in lactating cows fed diets containing a 60:40 forage-to-concentrate ratio. Journal of Dairy Science, 96, 2428-2447.

Koivunen, E., Jaakkola, S., Heikkilä, T., Lampi, A.M., Halmemies-Beauchet-Filleau, A., Lee, M.R.F., Winters, A.L., Shingfield, K.J. & Vanhatalo, A. (2015) Effects of plant species, stage of maturity, and level of formic acid addition on lipolysis, lipid content, and fatty acid composition during ensiling. Journal of Animal Science, 93, 4408-4423.

# Effects of storage conditions and additive type on fermentation quality, aerobic stability and nutritional value of grass-clover silage

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**Keywords:** additive, aerobic stability, digestibility, grass silage

**Introduction** Fermentation and aerobic stability are affected by silage additives and storage conditions (Weiss et al. 2016). Short fermentation with air ingress may favour fungal development (Auerbach and Nadeau 2018), whereas long strict anaerobic storage may stimulate clostridia activity (Auerbach et al. 2012). As there is very limited evidence, our trial studied the interactions between silage additive use and storage conditions on grass-clover silage quality.

**Material and Methods** In July 2016, a second-cut grass-clover ley was wilted overnight to 35% DM and chopped to 20 mm theoretical particle size. Herbage was manually treated by spraying with the following additives, applied in water at a total volume of 10 ml $kg^{-1}$ fresh forage: *Lactobacillus buchneri* CNCM-I 4323 ($LAB_{he}$), *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673 ($LAB_{heho}$), or a liquid chemical mixture (NHS, 2.5 L $t^{-1}$), composed of (g $L^{-1}$) sodium nitrite (195), hexamethylene tetramine (71) and potassium sorbate (106). Untreated forage received tap water at 10 ml $kg^{-1}$. Subsequently, herbage was packed in 1.5-L glass jars (n=3 per treatment), which had 6-mm holes in the body and in the lid and stored at 22 °C for 56 days. These holes were closed by rubber stoppers that were removed for 24 hours on day 28 and 49 of fermentation, enabling air penetration to stimulate fungal development (short). A second set of silages was kept strictly anaerobically in 1.5-L glass jars at 22 °C for 105 days (long). Standard procedures for silage quality evaluation were used. The *in vitro* organic matter digestibility (IVOMD) was determined and metabolizable energy (ME) content was calculated according to Lindgren (1979, 1983). Aerobic stability (AS) was tested for 288 hours by temperature measurement. All data were subjected to statistical analysis by the mixed model procedure of SAS 9.4. The Tukey´s test was used to detect differences between LSmeans at $P<0.05$.

**Results and Discussion** Storage (S) conditions and additive (A) use affected many silage traits, and numerous interactions were detected (table 1). Long storage resulted in higher DM loss than short storage (7.7 *vs* 7.0%) but an interaction with additive use was found ($P<0.001$). Within storage condition, NHS silage showed the smallest and $LAB_{he}$ the largest losses. In general, water-soluble carbohydrates were vastly degraded during fermentation, and only short-NHS silage contained relevant concentrations when compared with all other treatments (3.39 *vs* 0.78% of DM). Proteolysis as reflected by ammonia-N concentrations was restricted by long anaerobic storage (11.1 *vs* 12.2% of total N, $P<0.001$) and by additive use (10.2 *vs* 15.9% of total N). The chemical NHS was superior to any other additive. Production of lactic and butyric acids was highest in untreated silage stored anaerobically for a long period, indicating the risk for clostridia development to increase with storage length (Auerbach et al. 2012). Additives containing *L. buchneri* stimulated the production of acetic acid compared with untreated silage regardless of storage conditions (3.35 *vs* 1.74% of DM) but the effect was stronger during long (3.66 *vs* 1.89% of DM) than short storage with repeated air ingress (3.05 *vs* 1.59% of DM) showing that the process of anaerobic degradation of lactate into acetate, and the co-product, 1,2-propanediol, is time-dependent, and continues for longer periods of time. The sole use of *L. buchneri* in $LAB_{he}$ restricted the formation of this co-product compared with $LAB_{heho}$ and stimulated its utilization, probably by *L. diolivorans* to produce higher concentrations of n-propanol and propionic acid although the reasons for this observation remain to be elucidated. Why the chemical additive increased 1,2-propandiol concentrations over those of untreated regardless of storage conditions cannot be explained but an effect on certain representatives of the silage microflora seems likely.

Ethanol accumulation was affected by additive, and the use of the chemical was most efficient to minimize it compared with all other treatments (0.39 *vs* 1.26% of DM). Long anaerobic storage of silage led to yeast counts below the detection limit resulting in AS of 288 hours (SxA interaction, $P<0.001$). Untreated silage stored over short fermentation length with air ingress became aerobically unstable after 37 hours of aeration, followed by NHS (164 hours) and $LAB_{he}$ and $LAB_{heho}$ (288 hours). The lower AS in NHS silage can be attributed to low AS (52 hours) of one replicate, which may have been caused by too low of an application rate, or inhomogenous additive distribution. Air exposure reduced silage IVOMD from 79.1 to 77.2% ($P<0.01$) and ME from 10.0 to 9.6 MJ $kg^{-1}$ DM ($P<0.001$), and only the chemical additive improved IVOMD (79.7 *vs* 76.6, $P<0.01$) and ME (10.0 *vs* 9.5 MJ ME $kg^{-1}$ DM, $P<0.01$) over that of untreated silage. Our data substantiate findings by Auerbach and Nadeau (2018) on the detrimental effects of aeration on the nutritive value of grass silage. In this study, however, all used additive types were capable of preventing the reduction of IVOMD and ME.

**Table 1.** Effects of storage (S) conditions and additive (A) use on fermentation pattern, yeast count and aerobic stability of grass-clover silage (LSmeans in % of DM unless stated otherwise, n=3)

| Treatment | DML[1] | WSC[2] | $NH_3$[3] | LA[4] | AA[5] | PA[6] | BA[7] | EOH[8] | POH[9] | PD[10] | YC[11] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Long anaerobic storage* | | | | | | | | | | | |
| Untreated | 8.2$^{b}$ | 0.78$^{c}$ | 15.0 | 5.64$^{a}$ | 1.89$^{c}$ | 0.16$^{b}$ | 0.37$^{a}$ | 1.32 | 0.22$^{b}$ | 0.35$^{f}$ | 1.7$^{d}$ |
| $LAB_{he}$[12] | 9.1$^{a}$ | 0.51$^{cd}$ | 12.7 | 2.84$^{d}$ | 3.88$^{a}$ | 0.39$^{a}$ | 0.03$^{c}$ | 1.67 | 0.96$^{a}$ | 0.64$^{e}$ | 1.7$^{d}$ |
| $LAB_{heho}$[13] | 7.2$^{c}$ | 0.79$^{c}$ | 9.3 | 3.15$^{cd}$ | 3.43$^{ab}$ | 0.02$^{d}$ | 0.06$^{bc}$ | 0.70 | 0.05$^{cd}$ | 3.02$^{a}$ | 1.7$^{d}$ |
| NHS[14] | 6.4$^{d}$ | 1.22$^{b}$ | 7.4 | 3.30$^{c}$ | 3.29$^{ab}$ | 0.08$^{c}$ | 0.04$^{c}$ | 0.43 | 0.12$^{c}$ | 2.47$^{b}$ | 1.7$^{d}$ |
| *Short storage with air ingress* | | | | | | | | | | | |
| Untreated | 6.8$^{cd}$ | 0.95$^{bc}$ | 16.8 | 4.25$^{b}$ | 1.59$^{c}$ | 0.02$^{d}$ | 0.15$^{b}$ | 1.37 | 0.00$^{d}$ | 0.19$^{f}$ | 6.3$^{a}$ |
| $LAB_{he}$[12] | 8.6$^{ab}$ | 0.41$^{d}$ | 13.1 | 2.82$^{d}$ | 3.20$^{b}$ | 0.19$^{b}$ | 0.02$^{c}$ | 1.84 | 0.29$^{b}$ | 0.74$^{e}$ | 1.7$^{d}$ |
| $LAB_{heho}$[13] | 7.0$^{c}$ | 0.77$^{c}$ | 10.4 | 4.36$^{b}$ | 2.90$^{b}$ | 0.00$^{d}$ | 0.03$^{c}$ | 0.66 | 0.00$^{d}$ | 1.82$^{c}$ | 2.1$^{cd}$ |
| NHS[14] | 5.5$^{e}$ | 3.39$^{a}$ | 8.3 | 4.56$^{b}$ | 1.98$^{c}$ | 0.01$^{d}$ | 0.00$^{c}$ | 0.34 | 0.03$^{d}$ | 1.10$^{d}$ | 3.9$^{b}$ |
| SEM[15] | 0.10 | 0.065 | 0.28 | 0.092 | 0.131 | 0.011 | 0.020 | 0.008 | 0.017 | 0.060 | 0.21 |
| Level of significance of global *F*-test | | | | | | | | | | | |
| S | *** | *** | *** | *** | *** | *** | *** | ns | *** | *** | *** |
| A | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| S x A | *** | *** | 0.098 | *** | ** | *** | *** | ns | *** | *** | *** |

[1]DM loss, %, [2]water-soluble carbohydrates, [3]$NH_3$-N, % of total N, [4]lactic acid, [5]acetic acid,[6]propionic acid, [7]butyric acid, [8]ethanol, [9]n-propanol, [10]1,2-propanediol, [11]yeast count, log cfu $g^{-1}$, [12]*L. buchneri* CNCM I-4323, [13]*L. buchneri* CNCM I-4323 and *P. acidilactici* DSM 11673, [14]liquid blend of sodium nitrite, hexamethylene tetramine, potassium sorbate, LSmeans in columns bearing unlike superscripts differ at $P<0.05$, [15]relates to 2-factor interaction, ns not significant.

**Conclusions** Long strict anaerobic storage stimulates clostridia development whereas short storage with air ingress promotes yeast activity resulting in low aerobic stability. These findings are important to consider in designing silage experiments to study effects of different additive types. The used chemical additive showed the most promising results in terms of efficiency of fermentation and nutritional quality of grass-clover silage.

**References**

Auerbach, H. & Nadeau, E. (2018) Biological and chemical additives maintain nutritive value of grass silage during air exposure. Accepted by 17th International Silage Conference, Bonn, Germany.

Auerbach, H., Weiss, K. & Nadeau, E. (2012) Benefits of using silage additives. In: Auerbach, H., Lückstädt, C. & Weissbach, F. (eds). Proceedings of the 1st International Silage Summit, Worthington, UK: Anytime Publishing Services, p. 75-144.

Lindgren, E. (1979) The nutritional value of roughages determined in vivo and by laboratory methods. Report 45. Department of Animal Nutrition and Management, SLU, Uppsala, Sweden, p. 66.

Lindgren, E. (1983) Nykalibrering av VOS-metoden för bestämning av energivärdehos vallfoder. Working Paper. Department of Animal Nutrition & Management, SLU, Uppsala, Sweden, p. 4.

Weiss, K., Kroschewski, B. & Auerbach, H. (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. Journal of Dairy Science, 99, 1-17.

# Effects of storage time and silage additives on aerobic stability of maize silages

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**Keywords:** aerobic stability, maize silage, silage additive, storage period

**Introduction** Limited maize silage resources on dairy farms often lead to minimized fermentation periods of the freshly ensiled maize silage piles. Due to fermentation periods of sometimes only a few days to a few weeks those silages tend to be more sensitive to aerobic deterioration compared with silages stored with a sufficient storage period of at least seven weeks. In this study we compared maize silages stored for either two or seven weeks and the impact of treatment different silage additives on aerobic stability of the maize silages.

**Material and Methods** Freshly chopped maize (see Table 1) was ensiled as untreated control or treated with two silage additives designed to improve aerobic stability in 1,5- L glass jars in triplicates. The laboratory silos were stored for either two or seven weeks in a temperature controlled room at 25 °C.

**Table 3**. Chemical composition and microbiological counts of the fresh harvested maize crop

| | | |
|---|---|---|
| Dry matter (DM) | g/kg | 294 |
| Crude protein | g/kg DM | 73 |
| Crude ash | g/kg DM | 39 |
| Crude fibre | g/kg DM | 175 |
| Starch | g/kg DM | 296 |
| Water solubole carbohydrates (WSC) | g/kg DM | 107 |
| Buffering capacity (BC) | g lactic acid/100 g DM | 33 |
| Lactic acid bacteria (LAB) | colony-forming units (cfu) log | 6.1 |
| Yeast | cfu log | 6.0 |
| Mould | cfu log | 5.4 |
| Fermentation coefficient (FC)* | | 55 |

*FC = DM(%)+8x(WSC/BC)

One additive contained three different strains of lactic acid bacteria (LAB) (*lactobacillus diolivorans* (DSM 32074)*, lactobacillus buchneri* (DSM 12856) and *lactobacillus rhamnosus* (NCIMB 30121)) dosed with 1g additive diluted in 1.0 L water per tonne fresh material. 400 g potassium sorbate (PSORB) diluted in 1 L water per tonne fresh material were applied to the second treatment. Silage additives were applied prior to filling of the laboratory silos. Silos were sealed directly after filling. After a storage period of either two or seven weeks the laboratory silos got balanced to estimate fermentation losses. Silages were analysed for nutrients, fermentation products, microbiological counts and aerobic stability. Aerobic stability was determined by automatic measuring silage temperatures every 4 hours of silage samples stored under aerobic conditions in a temperature controlled room with an ambient temperature of 20°C. Start of heating is defined as: silage temperature being three Kelvin higher than room temperature. Statistical differences depending on treatment or storage period in this trial were determined with Kruskal-Wallis Test.

**Results and Discussion** Table 2 shows the fermentation products, fermentation losses, microbiological counts and aerobic stability as affected by additive treatment and length of storage. The increased length of storage affected most fermentation parameters, such as increased concentrations of lactic- and acetic acid, $NH_3$-N and the concentration of ethanol resulting in increased

fermentation losses. With increased formation of lactic acid, the pH values decreased. Independent from treatment the increased storage period resulted in reduced yeast counts and thus improved aerobic stability. Despite the fact that heterofermentative lactic acid bacteria are reported to show no effect on improving aerobic stability after only two weeks of storage (Driehuis et al., 1999) the treatment with the LAB additive increased aerobic stability by more than three days after just those two weeks.

**Table 4**. Fermentation products, fermentation losses, microbiological counts and aerobic stabilities of the silages after two or seven weeks of storage.

| | | 2 weeks storage | | | 7 weeks storage | | | SEM |
|---|---|---|---|---|---|---|---|---|
| | | Cont. | LAB | Psorb | Contr. | LAB | Psorb | |
| Lactic acid | g / kg DM | 63Aa | 62Aa | 58Aa | 82Aa | 52Aa | 73Aa | 2.82 |
| Acetic acid | g / kg DM | 16Aa | 17Aa | 15Aa | 22Ba | 32Ba | 19Ba | 1.47 |
| $NH_3$-N | % of total N | 5.8Aa | 6.0Aa | 5.9Aa | 7.8Ba | 7.5Ba | 6.7Ba | 0.20 |
| pH-value | | 3.8Aa | 3.8Aa | 3.8Aa | 3.7Aa | 3.9Aa | 3.7Aa | 0.03 |
| Ethanol | g / kg DM | 20Aa | 18Aa | 9Aa | 21Aa | 18Aa | 10Aa | 1.24 |
| 1-Propanol | g / kg DM | n.d.Aa | 2Aa | n.d.Aa | 5Ba | 12Ba | 5Ba | 1.03 |
| Yeast | cfu log | 4.1Aa | 3.3Aa | 3.1Aa | 3.0Ba | 2.5Ba | n.d.Ba | 3.07 |
| Aerobic stability | days | 7.3Aa | 10.5Ab | 13.2Ab | 10.0Ba | 20.8Bb | 20.8Bb | 1.34 |
| Ferment.losses[#] | % | 4.8Aa | 4.9Aa | 4.2Abc | 5.4Ba | 6.2Ba | 4.7Bbc | 0.15 |

[#] Weissbach, 1998; n.d. = not detected; Cont.= Control; LAB= lactic acid bacteria inoculant; PSORB = potassium sorbate; differing capital letters (A,B) show significantly different values ($p<=0.05$) depending on storage time; differing small letters (a,b,c) those depending on treatment

As *lactobacillus buchneri* is reported to ferment carbohydrates partly to 1,2-propanediol (Oude Elferink et al., 2001) the strain of *lactobacillus diolivorans* in the LAB additive must have converted the 1,2-propanediol directly to 1-propanol (Krooneman et.al., 2002), as no 1,2-propanediol could be detected either after two or seven weeks of storage. As typical for silages treated with heterofermentative LAB the fermentation of lactic acid to acetic acid with increased storage period could as well be detected as well as the increased $NH_3$-N and fermentation losses (Driehuis et al., 1999). The antifungal effect of the treatment with potassium sorbate was detected after two and seven weeks of storage as shown by low counts of yeasts and improved aerobic stability (Kung et al., 2003).

**Conclusion** As expected untreated maize silage with only two weeks of storage showed a reduced aerobic stability compared to silage stored sealed for seven weeks. Different from older research results a newly composed LAB inoculant can improve aerobic stability after only two weeks of storage. This can help to prevent aerobic deterioration on farms with limited maize recourses and early bunker opening. The treatment with potassium sorbate led to even better aerobic stabilities.

**References**

Driehuis, F.; Oude Elferink, S. J. H. W., & Spoelstra, S. F. (1999) Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with Lactobacillus buchneri inhibits yeast growth and improves aerobic stability, Journal of Applied Microbiology, 87, 4, 583–594

Krooneman, J., Faber, F., Alderkamp, A.C., Oude Elferink, S.J.H.W., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J. C. & Vancanneyt, M. (2002) Lactobacillus diolivorans sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 52, 639-646.

Kung, L.; Stokes, M.R. & Lin, C.J. (2003) Silage Additives in: Silage Science and Technology (editors: Buxton, D.R.; Muck, R.E. & Harrison, J.H.); American Society of Agronomy, Inc. (2003), 340.

Oude Elferink, S.J.W.H.; Krooneman, J.; Gottschal, J.C.; Spoelstra, S.F.; Faber, F. & Driehuis, F. (2001) Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. Journal of Applied Microbiology, 67, 125-132.

Weissbach, F. (1998) Zur Methodik der Ermittlung der Gärverluste bei der Silierung; Jahresbericht der FAL, 26.

# Effects of *Lactobacillus buchneri* PJB/1 alone and in combination with *Lactobacillus plantarum* MTD-1 on the bacterial community composition and aerobic stability of high moisture corn stored with or without air stress

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**Keywords:** air stress, high moisture corn, inoculant, microbiome

**Introduction** High moisture corn (**HMC**) can spoil rapidly when exposed to air because of its limited fermentation and high number of yeasts. Yeasts that assimilate lactate usually initiate aerobic spoilage. Inoculants that produce organic acids with antifungal properties can inhibit yeasts and increase aerobic stability of HMC (Kung et al. 2007). The objective of this study was to evaluate the effects of *Lactobacillus buchneri* PJB/1 alone or in combination with *L. plantarum* MTD-1 on the bacterial community composition and aerobic stability of HMC stored with or without air stress.

**Materials and Methods** HMC was harvested at 68% dry matter (DM) and untreated (**CTR**) or treated with *L. buchneri* PJB/1 at 400,000 cfu/g of fresh forage weight (**LB**) or *L. buchneri* PJB/1 and *L. plantarum* MTD-1 at 400,000 and 100,000 cfu/g, respectively (**LB + LP**). Five individual replicates of HMC per treatment (30 silos) were packed in buckets (7.5 L) at a density of 653 kg DM/m$^3$ and opened after 30 or 90 d. Five individual replicates of HMC untreated (**CTR**-**AS**) and treated with LB (**LB-AS**) (10 silos), were prepared to evaluate the effects of air stress during storage and allowed to ensile for 90 d. Buckets used for air stress had 3 holes of 1.60 cm diameter (2 holes were 5 cm above the bottom of the silo, 180° from each other, and 1 was on the lid). All holes were opened for 2 h every 2 weeks until 42 d, and then 2 h per week until 90 d. At 30 d, only CTR, LB, and LB + LP were sampled for analysis. At 90 d, CTR, LB, LB + LP, CTR-AS and LB-AS were sampled at time of opening and after 50 and 140 h of aerobic exposure. Samples were analyzed for pH, numbers of LAB and yeast, and concentration of organic acids and alcohols by high-performance liquid chromatography. Aerobic stability was calculated as the number of hours before the temperature of silage mass rose 2°C above the baseline after exposure to air at 22°C. Bacterial community composition of four replicates from each treatment was analyzed by the amplification of the V4 and V5 regions of 16S rRNA using Illumina two-step process and sequencing on Illumina MiSeq (2 x 250 bp paired-end). QIIME (version 1.9.1) was used to filter paired-end reads for quality, trim primers, pick operational taxonomic units (OTU), and filter singles and doubles reads. OTU picking was done using open reference clustering at 97% similarity against Greengenes (DeSantis et al. 2006). Diversity analysis was performed using Phyloseq package in R (McMurdie and Holmes 2013). Statistical analysis was done using JMP version 12 (SAS Institute, Cary, NC). Data from the silo openings were analyzed separately as a completely randomized design. Data from post aerobic exposure samples were analyzed as a 5 × 2 factorial arrangement of treatments, including the fixed effects of treatment, sampling time, and their interaction. Mean comparisons were performed by Tukey's test at $P \leq 0.05$.

**Results and Discussion** HMC treated with LB and LB + LP had equally higher* abundance of *L. buchneri* than CTR after 30 d (96 *vs.* 3% of the total bacterial community) and 90 d of ensiling (86% vs. 12%). In CTR, *L. buchneri* was 5 and 25% of the total abundance of the order *Lactobacillaes* after 30 and 90 d of ensiling. In treated silages, this strain represented 97% and 91% of the order *Lactobacillaes* after 30 and 90 d. After 90 d, there was no difference in *L. buchneri* abundance in treated silages despite air-stress status (86%), however CTR-AS had higher* abundance of this strain than CTR non-stressed (40 *vs.* 12%). No differences in *L. buchneri* and *L. plantarum* abundances were observed between LB and LB+LP. Treated silage after 90 d had lower* abundance of *Enterobacteriales* and *Clostridiales* than CTR (Figure 1), even when air-stressed. *L. buchneri* dominated the fermentation in treated silages as soon as 30 d after ensiling, even when combined

with *L. plantarum* or when subjected to air stress. In contrast, CTR had high abundance of microorganisms that can cause secondary fermentation and negatively affect nutritional and sanitary properties of silage. At 30 d, there were no differences in the concentration of lactic acid among treatments, but treated silages had higher* concentrations of acetic acid (0.81 *vs.* 0.17% of DM) than CTR. Additionally, treated silage had 1,2-propanediol at 0.54% of DM, whereas this alcohol was not detected in CTR. At 90 d, for both non-stressed and air-stressed silos, LB and LB + LP had lower* concentration of lactic acid (0.91 *vs.* 1.48% of DM) and higher* concentrations of acetic acid (1.28 *vs.* 0.34% of DM) and 1,2-propanediol (0.86 *vs.* 0.12% of DM) compared to CTR. Fermentation end-products analysis showed that *L. buchneri* was active and produced acetic acid and 1,2-propanediol. At both ensiling times and during aerobic exposure, CTR and CTR-AS had higher* numbers of total yeasts than LB, LB + LP and LB-AS. CTR at 30 and 90 d, without or with air stress, had aerobic stabilities of 32, 52, and 25 h, respectively. LB and LB + LP after 30 or 90 d of ensiling, even when subjected to air stress, were more* stable (> 240 h) than CTR.

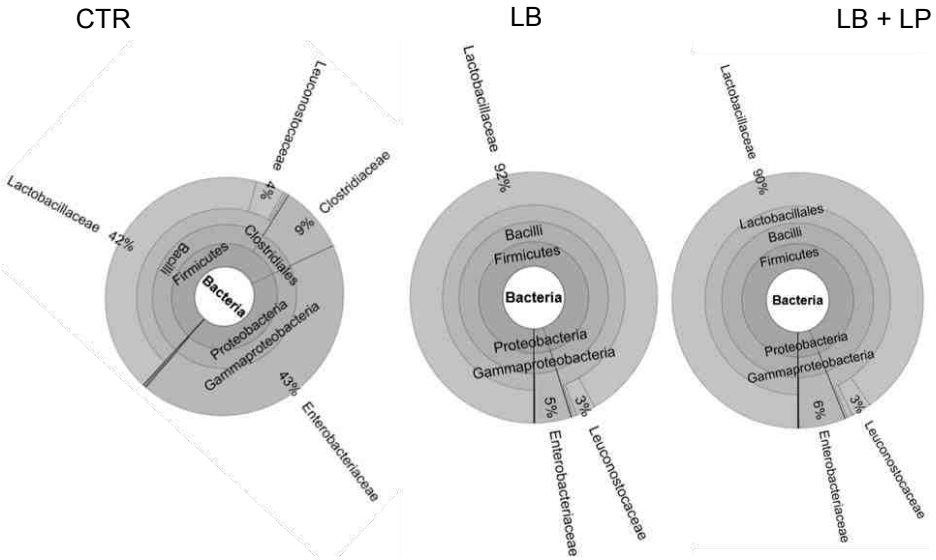


**Figure 1.** Krona plot illustrating the bacterial community composition, based on High-Throughput Paired-end Illumina Sequencing of the V4-V5 region of the 16S rRNA, of silage untreated (CTR), treated with *Lactobacillus buchneri* PJB/1 at 400,000 cfu/g of fresh forage weight (LB) or *L. buchneri* PJB/1 and *L. plantarum* MTD-1 at 400,000 and 100,000 cfu/g respectively (LB + LP) ensiled for 90 d. Rings represent different taxonomic ranks (kingdom, phylum, class, order, and family, respectively) and numbers represent the relative abundance of each family.

**Conclusions** *L. buchneri* dominated the fermentation in LB, LB + LP, and LB-AS HMC. It reduced the relative abundance of *Enterobacteriales* and *Clostridiales and* inhibited the growth of yeasts. Consequently, treatment markedly improved the aerobic stability of HMC as soon as after 30 d of ensiling, and after 90 d it increased stability even when silages were subjected to air-stress at storage.

**References**

DeSantis, T.Z. et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology, 72, 5069-5072.

Kung, L., Schmidt, R.J., Ebling, T.E. & Hu, W. (2007) The effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of ground and whole high-moisture corn. Journal of Dairy Science, 90, 2309-2314.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE, 8, e61217.

# Effects of a chemical additive on the microbial community composition and aerobic stability of short-term ensiled corn silage

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**Keywords:** chemical additive, corn silage, microbiome

**Introduction** Silages must often be fed very soon after ensiling due to feed shortages. Exposure of silage to air at feed-out can cause the growth of yeasts and/or acetic acid bacteria that initiate spoilage. The oxidation of lactic and/or acetic acids by those microorganisms, increases silage pH and allows for the growth of aerobic bacteria and/or molds that cause further spoilage. The objective of this study was to evaluate the effects of a chemical additive on the microbial community composition and dynamics of whole-plant corn silage, and its effectiveness on improving aerobic stability of short-term ensiled silages.

**Materials and Methods** Whole-plant corn was harvested at 39% dry matter (DM), chopped, and ensiled untreated (**CTR**), or treated with Safesil (**SF**, 20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite, Salinity, Göteborg, Sweden) at 2 L/t of fresh forage weight. Four individually replicated silos (7.5 L) were packed (224 kg of DM/$m^3$) and opened after 1, 4, and 46 d of ensiling. CTR and SF silages ensiled for 46 d were exposed to air and sampled after 48, 96, and 240 h of exposure. Samples were analysed for microbial community composition, numbers of yeasts and molds, and aerobic stability. Aerobic stability was calculated as the number of hours before the temperature of the forage mass rose 2°C above baseline after exposure to air at 22°C. Yeasts and molds were plated on malt extract agar acidified with lactic acid. The V4 and V5 regions of the 16S rRNA, for bacteria, and the internal transcribed space 1 (ITS1), for fungi, were amplified using the Illumina two-step process and sequenced on Illumina MiSeq (San Diego, CA, USA) (2 x 250 bp paired-end). QIIME (version 1.9.1) was used to filter paired-end reads for quality, trim primers, pick operational taxonomic units (OTU), and filter singles and doubles reads. OTU picking was done using open reference clustering at 97% similarity against Greengenes (DeSantis et al. 2006) or UNITE (Kõljalg et al. 2005), for bacteria and fungi, respectively. Diversity analysis was performed using Phyloseq package in R (McMurdie and Holmes 2013). Statistical analysis was done using JMP version 12 (SAS Institute, Cary, NC). Data from the silo opening was analyzed as a 2 × 3 factorial arrangement of treatments in a completely randomized design, including the fixed effects of treatment, day, and their interaction. Data from the post aerobic exposure samples were analyzed as a 2 × 3 factorial arrangement of treatments in a completely randomized design, including the fixed effects of treatment, sampling time, and their interactions. Pairwise mean comparisons were performed using Tukey's test at $P \leq 0.05$.

**Results** The composition of the microbial community changed with time of ensiling. Figure 1a shows that after 1 d, *Leuconostocaceae* family represented 72% of the total bacterial abundance, *Lactobacillaceae* 13%, and Enterobacteriaceae 4%. At d 4, *Leuconostocaceae* abundance decreased* to less than 7%, whereas *Lactobacillaceae* increased* to 80% of the total bacterial community. At 46 d, abundance of *Lactobacillaceae* declined* to 64% and *Enterobacteriaceae* increased* to 17%. Numbers of total yeasts were similar between treatments from 1 to 4 d, however at 46 d there were fewer* yeasts in SF silages than CTR (<1.00 *vs.* 3.22 log cfu/g fresh weight). The abundance of the genus *Candida,* in both treatments, was similar from 1 to 46 d at approximately 50% of the total fungal community. *Acremonium* abundance declined*** with time from 15, 3, to 1% from 1, 4 to 46 d. After 46 d of ensiling, silages were exposed to air. Numbers of yeasts were lower*** in SF compared to CTR at all post-aerobic exposure sampling times, and mold counts were lower* after 240 h. Figure 1b shows that the population of *Lactobacillaceae* in SF was more*** stable to air exposure than in CTR (77 vs.

52%). After 48 h of air exposure, *Enterobacteriaceae* abundance in CTR silages was 47% greater* than in SF silages. After 240 h of aerobic exposure, *Acetobacteraceae* was the most abundant bacterial family in CTR silages (68%), whereas its abundance in SF silages was lower* (4%). After 1, 4, and 46 d of ensiling, SF silages (224, >240, and >240 h) were more* stable than CTR (32, 81, and 117 h).

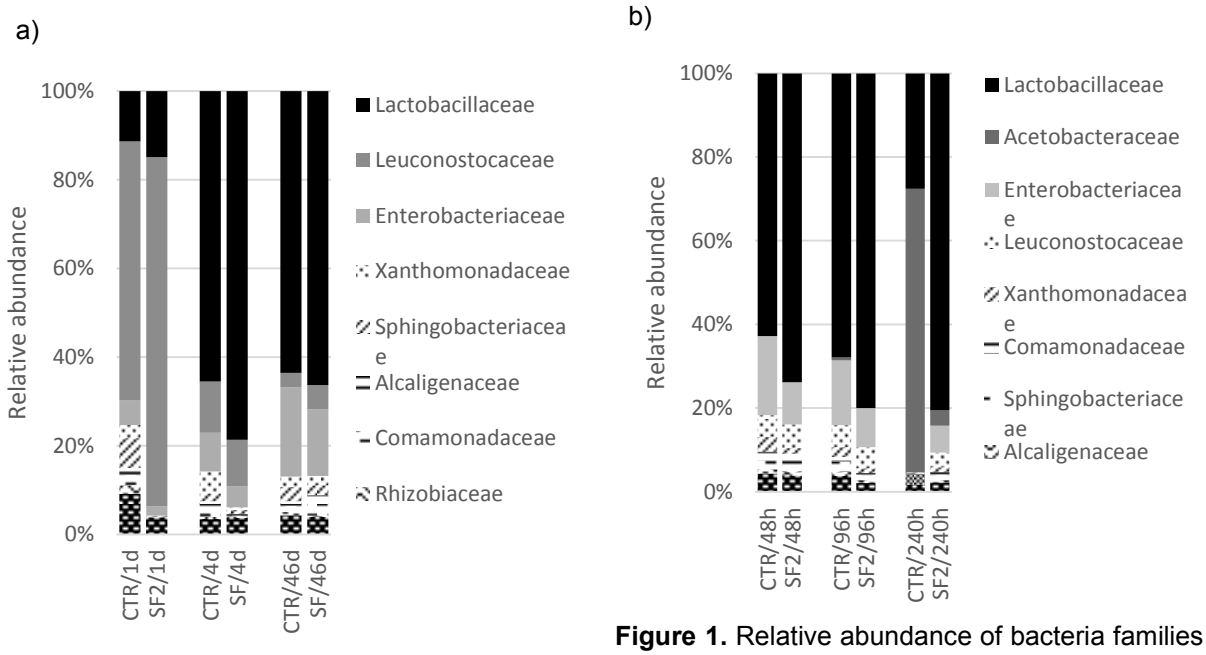


**Figure 1.** Relative abundance of bacteria families as analyzed by the sequencing of the V4-V5 region of the 16S rRNA of four replicates per treatment using Illumina MiSeq in a) silages untreated (CTR) or treated with an additive containing sodium benzoate, potassium sorbate, and sodium nitrite at 2 L/t of fresh forage weight (SF) ensiled for 1, 4, and 46 d, and in b) CTR and SF silages ensiled for 46 d exposed to air for 48, 96, and 240 h.

**Discussion** The increased stability in treated corn silage after short periods of ensiling could not be explained by changes in the bacterial or fungal community composition during ensiling or by a reduction in the total numbers of culturable yeasts. Only after 46 d of ensiling did the additive reduce the numbers of culturable yeasts in the silage. Therefore, after short periods of ensiling (1 and 4 d) the additive might have had a fungistatic effect whereas after a long term exposure the effect appeared to be fungicidal. The additive affected bacterial community composition of silage subjected to air exposure after 46 d of ensiling, especially by reducing the abundance of *Acetobacteraceae.* Strains belonging to the family *Acetobacteraceae* were found to initiate the aerobic deterioration of corn silage, usually simultaneously with lactate-assimilating yeasts (Spoelstra et al.1988).

**Conclusions** The additive containing sodium benzoate, potassium sorbate, and sodium nitrite markedly improved the stability of corn silage after short periods (1 and 4 d) of storage, probably due to fungistatic (rather than fungicidal) effects.

**References**

DeSantis, T.Z. et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology, 72, 5069-5072.

Kõljalg, U. et al. (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist, 166, 1063-1068.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE, 8, e61217.

Spoelstra, S.F., Courtin, M.G. & Van Beers, J. A. C. (1988) Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. Journal of Agricultural Science, 111, 127-132.

# Effects of a chemical additive on the microbial community composition, fermentation, and aerobic stability of corn silage stored with or without air stress

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**Keywords:** air stress, chemical additive, corn silage, microbiome

**Introduction** It is well accepted that during feed out, silage is exposed to air that can stimulate the growth of undesirable microorganisms leading to spoilage. However, silage can also be exposed to air during storage. The objective of this study was to evaluate the effects of a chemical additive on the microbial community composition and fermentation dynamics of whole-plant corn silage, and its effectiveness on improving aerobic stability, and overcoming an air-stress challenge during storage.

**Materials and Methods** Whole-plant corn was harvested at 39% DM, chopped, and ensiled untreated (**CTR**), or treated with Safesil (Salinity, Göteborg, Sweden; active ingredients: 20% sodium benzoate, 10% potassium sorbate, and 5% sodium nitrite) at 2 L/t of fresh forage weight (**SF2**) or at 3 L/t (**SF3**). Ten individually replicated silos for each treatment were packed in 7.5 L buckets (density of 224 kg of DM/$m^3$). Half of the silos were not air-stressed (**NS**), and the other half were air-stressed (**AS**). Buckets used for air stress had 3 holes of 1.60 cm diameter (2 holes were 5 cm above the bottom of the silo, 180° from each other, and 1 was on the lid). All holes were opened for 2 h/week, then resealed. After 63 d of ensiling, all silos were opened and exposed to air. CTR and SF2 were sampled after 115 and 260 h of exposure. Samples were analyzed for pH, DM recovery, numbers of LAB and yeast, and concentrations of organic acids and alcohols. Aerobic stability was calculated as the number of hours before the temperature of the forage mass rose 2°C above baseline after exposure to air at 22°C. Four replicates of each fresh forage, CTR-NS, CTR-AS, SF2-NS, or SF2-AS, and post aerobic exposure samples were analyzed for bacterial and fungal community composition. The V4 and V5 regions of the 16S rRNA, for bacteria, and the internal transcribed space 1 (ITS1), for fungi, were amplified using the Illumina two-step process and sequenced on Illumina MiSeq (San Diego, CA, USA) (2 x 250 bp paired-end). QIIME (version 1.9.1) was used to filter paired-end reads for quality, trim primers, pick operational taxonomic units (OTU), and filter singles and doubles reads. OTU picking was done using open reference clustering at 97% similarity against Greengenes (DeSantis et al. 2006) or UNITE (Kõljalg et al. 2005), for bacteria and fungi, respectively. Diversity analysis was performed using Phyloseq package in R (McMurdie and Holmes 2013). Statistical analysis was done using JMP version 12 (SAS Institute, Cary, NC). Data from the silo opening was analyzed as a 3 × 2 factorial arrangement of treatments in a completely randomized design, including the fixed effects of treatment, air stress, and their interaction. Data from post aerobic exposure samples were analyzed as a 2 × 2 × 2 factorial arrangement of treatments, including the fixed effects of treatment, air stress, sampling time, and their interactions. Pairwise mean comparisons were performed using Tukey's test at $P \leq 0.05$.

**Results and Discussion** After 63 d of ensiling, the pH was higher* in CTR than treated silages. In NS silages, SF2 had similar and SF3 lower* numbers of yeasts compared to CTR. In AS silages, neither additive level affected the numbers of yeasts. Figure 1a shows that the relative abundance of the genus *Candida* was higher in CTR-NS than in SF2-NS silages (83 *vs.* 47% of the total abundance). CTR-AS has lower* abundance of *Candida* than SF2-AS (8 vs. 99%) and was dominated by *Pichia* (87%). Air stress during storage modified the fungal community composition of corn silage, increasing the abundance of the fungi genus *Pichia.* SF2 and SF3 reduced* the production of ethanol compared to CTR, in both NS and AS silos. CTR silages lost approximately 13% of DM during 63 d of ensiling, whereas SF2 and SF3 silages lost less* DM (8% and 5%, respectively). Air stress tended to increase

(*P* = 0.09) *Acetobacteraceae* abundance (Figure 1b). Figure 1c shows the relative abundance of bacteria families after air exposure. In AS silos after 115 h of aerobic exposure, CTR and SF had high abundance of *Acetobacteraceae* (82%), but CTR had higher* pH and numbers of yeasts, and lower* concentrations of lactic and acetic acids than SF2. After 260 h, there were no differences in numbers of yeasts, pH, and concentrations of lactic acid, acetic acid, and ethanol between CTR-AS and SF2-AS. In NS silage after 115 h of air exposure, CTR and SF2 had high abundance of *Lactobacillaceae*, the same pH, and concentrations of lactic and acetic acids. However, SF2 had lower* numbers of yeasts and concentration of ethanol than CTR. In NS silos after 260 h of aerobic exposure, CTR had higher* numbers of yeasts and pH, lower* concentrations of lactic acid, acetic acid, and ethanol, and higher* abundance of *Bacillaceae* than SF2, which remained stable and with high abundance of *Lactobacillaceae* (85%). SF2-AS (98 h) and SF3-AS (239 h) were more* stable than CTR-AS (41 h), and SF2-NS and SF3-NS (> 260 h) were more* stable than CTR-NS (132 h).

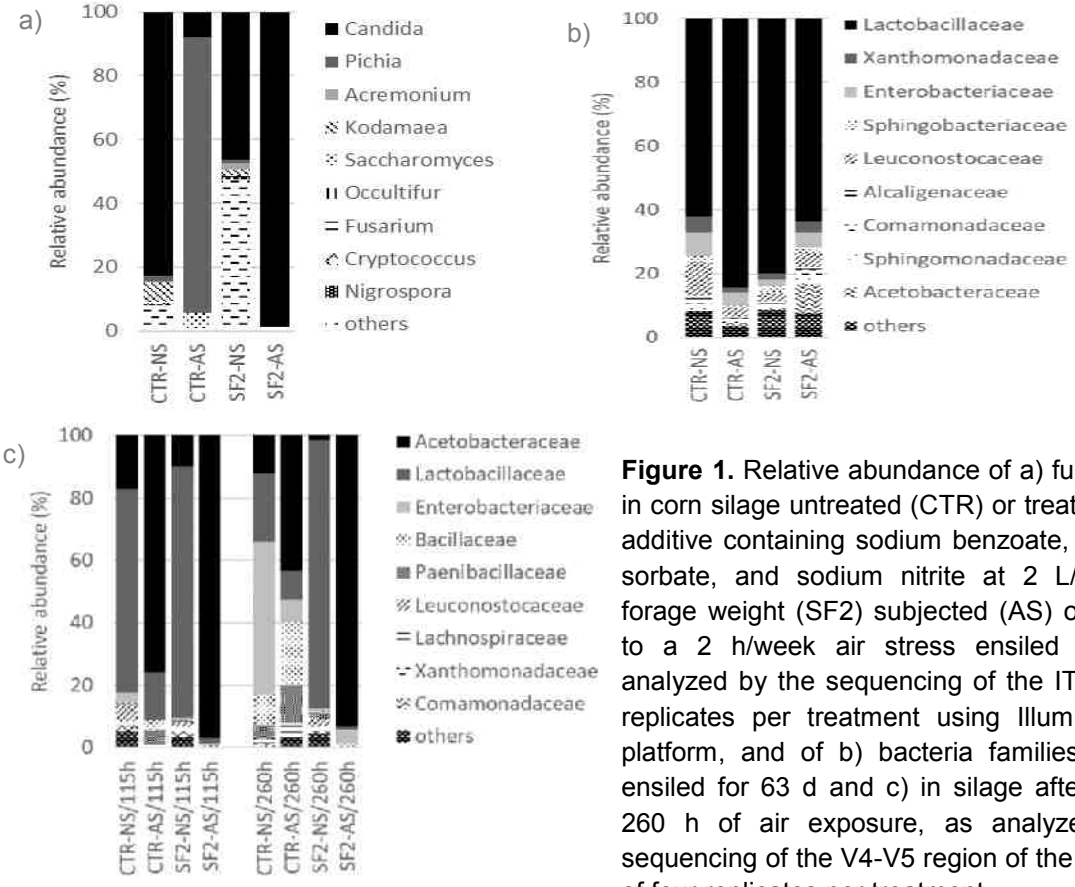


**Figure 1.** Relative abundance of a) fungi genera in corn silage untreated (CTR) or treated with an additive containing sodium benzoate, potassium sorbate, and sodium nitrite at 2 L/t of fresh forage weight (SF2) subjected (AS) or not (NS) to a 2 h/week air stress ensiled for 63 d, analyzed by the sequencing of the ITS1 of four replicates per treatment using Illumina MiSeq platform, and of b) bacteria families in silage ensiled for 63 d and c) in silage after 115 and 260 h of air exposure, as analyzed by the sequencing of the V4-V5 region of the 16S rRNA of four replicates per treatment.

**Conclusions** The low dose of the additive containing sodium benzoate, potassium sorbate, and sodium nitrite did not reduce the numbers of agar-culturable yeasts but still markedly improved aerobic stability of corn silage. In NS silos, the improvement in aerobic stability by the low dose of the additive can be explained by a reduction of the relative abundance of the genus *Candida*. In AS silos, the additive overcame the negative effects of air possibly by inhibiting specific microorganisms that were stimulated by oxygen, such as *Pichia* and *Acetobacteraceae*.

**References**

DeSantis, T.Z. et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology, 72, 5069-5072.

Kõljalg, U. et al. (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist, 166, 1063-1068.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE, 8(4), e61217.

# Effects of a homolactic inoculant on fermentation and aerobic stability of alfalfa silage

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**Keywords:** alfalfa, silage, inoculant

**Introduction** Ensiling alfalfa can be challenging because of the natural high buffering capacity of the forage, and the amount of fermentable sugars may also be a limiting factor; in addition, field and weather conditions during forage wilting can worsen this situation. Microbial inoculants are a safe, natural and an environmentally friendly tool to help in this process. Specifically, classical (homofermentative) inoculants contain bacteria that efficiently produce lactic acid during the phase of active ensiling to preserve most of the nutrients and dry-matter (DM) from the ensiled crop (Kung et al. 2003). The hypothesis of this study was that adding a commercial microbial inoculant would improve the fermentation characteristics, forage quality and aerobic stability of alfalfa ensiled in a farm scale system.

**Material and Methods** Alfalfa was harvested at UW-Arlington Agriculture Research Station under cloudy skies, at 14$^{o}$C, and wilted to approximately 40% DM. Six bales weighing approximately 750 kg were prepared without additive (control), while six other bales were treated with Sil-All 4×4 (Lallemand Animal Nutrition, Milwaukee, WI) at the rate of 200,000 colony-forming units (CFU) per gram of fresh forage. Bales were wrapped with at least 7 layers of plastic film with a line wrapper and stored for 108 days. All samples were analysed for nutrient composition, and silage samples were tested for fermentation profile, microbial counts and aerobic stability. For the aerobic stability challenge, all samples were aerated and a data logger (HOBO Pendant® Temperature/Alarm Data Logger 8K - UA-001-08), programmed to record temperatures once every 15 minutes for 10 days, was placed in the geometric center of each treatment replicate to monitor temperature of the silages. Aerobic stability was defined as the amount of time it took until silage temperature increased by 2°C above ambient temperature. All other analyses were done at Dairyland Labs (Arcadia, WI). Chemical composition and fermentation profile data were analysed using Proc Mixed (SAS v.9.2; Cary, NC). The model used was a one-way analysis of variance that compared nutrient composition and fermentation profile means of control to treated silage. Yeast and mould counts were evaluated by Chi-square analysis using Proc Freq (SAS v.9.2; Cary, NC). Significance was declared for $P \leq 0.05$ and tendency to significance when $0.10 \geq P > 0.05$.

**Results and Discussion** The fresh alfalfa was very high quality at the time of harvest as indicated by the contents of crude protein (21.2% DM) and fibre (NDFom, 29.2% DM). However, the low content of water soluble carbohydrates (1.30% DM) was a reflection of the challenging weather conditions during harvest (cool temperature, cloudy skies), as well as plant respiration during the wilting process, depleting the levels of sugars and starch. There were significant differences in the fermentation profiles of the fermented samples: the pH was significantly lower for inoculated silage (5.23 vs. 6.00), while the concentration of lactic acid was elevated (2.06% vs. 0.13% DM), as confirmed by others (Oliveira et al., 2017). Contents of acetic acid were similar for both silages. Ethanol and ammonia-CP levels were significantly higher in control silages than in inoculated silages, which again suggests a more desirable and effective fermentation pattern due to inoculation. Inoculation also resulted in no detectable populations of yeasts and moulds in the silages (<1,000 CFU/g fresh forage) and significantly enhanced aerobic stability compared to control silages. Control silages were stable for 3 days and then temperature quickly rose; treated silages were stable for the entire 10-day test period, even though this effect from homolactic inoculation is not expected (Muck and Kung, 1997).

**Table 1**. Composition and fermentation profile of control alfalfa and alfalfa inoculated with Sil-All 4x4 Water Soluble Inoculant after 108 days of storage.

| | Control | Treated | SEM | *P*-value |
|---|---|---|---|---|
| DM (%FM) | 41.36[b] | 42.65[a] | 0.34 | 0.0292 |
| CP (%DM) | 22.87 | 22.95 | 0.10 | 0.7321 |
| ADF (%DM) | 26.58 | 26.34 | 0.25 | 0.6874 |
| aNDFom (%DM) | 29.84 | 29.26 | 0.24 | 0.2625 |
| Lignin (%DM) | 6.40 | 6.24 | 0.20 | 0.7224 |
| uNDFom240* (%DM) | 15.45 | 15.56 | 0.35 | 0.8975 |
| AD-ICP* (%DM) | 2.27 | 1.36 | 0.32 | 0.1698 |
| Ash (%DM) | 9.32 | 8.41 | 0.38 | 0.271 |
| NFC* (%DM) | 37.75 | 38.80 | 0.37 | 0.1732 |
| pH | 6.00[a] | 5.23[b] | 0.02 | <.0001 |
| Lactic acid (%DM) | 0.13[b] | 2.06[a] | 0.03 | <.0001 |
| Acetic acid (%DM) | 0.31 | 0.26 | 0.03 | 0.1404 |
| Ethanol (%DM) | 1.03[a] | 0.85[b] | 0.06 | 0.0338 |
| Ammonia-CP (%CP) | 2.27[a] | 1.95[b] | 0.02 | 0.0002 |
| RFQ* (%DM) | 231.90[b] | 240.42[a] | 2.29 | 0.0409 |

* uNDFom240 – 240-h undigested neutral detergent fiber corrected for ash; AD-ICP – acid-detergent insoluble crude protein; NFC – nonfibrous carbohydrates; RFQ – relative forage quality
Means within the same row with different superscript letters differ ($P < 0.05$).

**Conclusion** The fermentation process in alfalfa harvested late in the season can be challenging; harvest weather conditions likely restricted the fermentation process, especially in control silages. Inoculation with Sil-All 4×4 at recommended rate improved the fermentation process and aerobic stability, maintaining higher nutritional quality during feed-out.

**References**

Kung Jr., L., Stokes, M.R. & Lin, C.J. **(**2003) Silage additives. In: Silage Science and Technology. Buxton, D.R., Muck, R.E., & Harrison, J.H. (ed.). ASA/CSSA/SSSA, Madison, Wisconsin, USA. p. 305-360.

Muck, R.E., & Kung Jr., L. (1997) Effects of silage additives on ensiling. In: Fendrick-Jaynes, J., Hyde, H. & Popow, J.S. (eds.). Proceedings of the Silage: Field to Feedbunk North American Conference. NRAES-99. Northeast Regional Agricultural Engineering Service, Ithaca, NY, USA, 1997, pp. 187-199.

Oliveira, A.S., Weinberg, Z.G., Ogunade, I.M, Cervantes, A.A.P., Arriola, K.G., Jiang, Y., Kim, D., Li, X., Goncalves, M.C.M., Vyas, D., & Adesogan, A.T. (2017) Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. Journal of Dairy Science, 100 (6), 4587-4603.

# Effects of a mixture of lactic acid bacteria containing *Lactobacillus diolivorans* on aerobic stability of grass silage after short time of storage

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**Keywords:** aerobic stability, *Lactobacillus diolivorans*, storage time

**Introduction** Heterofermentative lactic acid bacteria (LAB), e.g. *Lactobacillus buchneri* are widely used to improve aerobic stability in silages. Nevertheless, these strains need a minimum time of storage of at least 6 to 8 weeks for a significant effect on aerobic stability (Driehuis et al. 1996).

*Lactobacillus diolivorans* has not been used as silage inoculant in the past. However, it is commonly known as a scarce native lactic acid bacteria strain occurring in silages with long time of storage. Usually, the fermentation pattern is described by forming propanol and propionic acid from 1.2-propandiol (Krooneman et al. 2002). Recent research has shown specific characteristics of the fermentation pattern during the first 14 to 30 days . During these initial days lactic acid, acetic acid and n-propanol are formed as consequence of the activity of *L. diolivorans*. Therefore, the aim of the present study was to assess the short-term effect of a LAB mixture containing *L. diolivorans* on aerobic stability of grass silage after 14 and 28 days of storage compared to 49 and 90 days of storage.

**Material and Methods** First cut grass material (28.2% DM, 20.4% CP, 6% WSC, 3.2 g/100g DM BC (Buffering capacity), 53.2% NDFom, 29.9.% ADFom, 6.0 MJ NEL/kg DM, 43 FC (Fermentation Coefficient = DM(%)+8(WSC/BC)) from an intensively managed site was ensiled in laboratory silos. Treatments consisted of an untreated control and a treated variant with a mixture[1)] of homo – and heterofermentative LAB containing *Lactobacillus diolivorans* DSM 32074, *Lactobacillus buchneri* DSM 12856, *Lactobacillus plantarum* DSM 21762 at an application rate of 250.000 CFU/ g fresh matter (FM) in a total of three replications resulting in 24 mini silos. After 14, 28, 49 and 90 days of fermentation at a constant temperature of 20°C, the silages were evaluated and analyzed for aerobic stability and fermentation pattern. Within the storage period air stress was simulated at day 7, 21, 28 and 42 for a total of 24 h each. On each sampling day, forage samples were analyzed to determine forage quality including the pH-value, fermentation pattern and ammonia-N content as well as aerobic stability according to Honig (1990).

For statistical evaluation, means and standard deviations were calculated for each parameter. The data were examined by SAS evaluation including T-test for significant differences ($P<0.05$) between the control and the treatment group.

**Results** The LAB mixture containing *L. diolivorans* led to significantly higher ($p< 0.05$) amounts of lactic acid, acetic acid, n-propanol and a lower pH after 14 and 28 days of storage (Table 1). At day 49 and 90 the LAB mixture treatment showed a reduced amount of lactic acid and a higher pH ($p< 0.05$). The amounts of acetic acid, propanol, 1,2 propandiol, however, were increased on both respective samplings while on day 90 additionally propionic acid was increased.

$NH_3$-N was significantly reduced by LAB mixture at day 14, whereas it did not influence $NH_3$-N at days 28, 49 and 90, respectively. Dry matter (DM) losses at day 90 were not significantly higher in the treated variants. The LAB mixture significantly ($p < 0.05$) prolonged the aerobic stability at all dates beginning from the earliest sampling (Figure 1).

**Discussion** The results indicate a special fermentation pattern of the LAB mixture containing *L. diolivorans*. In contrast to Krooneman et al. (2002) a rapid production of lactic acid, acetic acid and n-propanol was observed - even after 14 and 28 days of storage. Furthermore a significant improvement

of aerobic stability was observed without a subsequent increase of DM losses during ensiling, which has not been observed for heterofermentative LAB mixtures before (e.g. Driehuis et.al 1996).

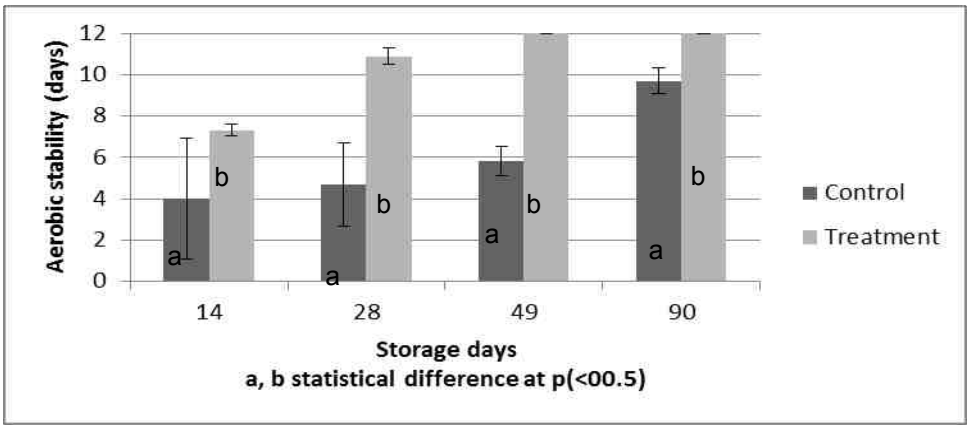
[1)] The individual proportion of the stems is not indicated due to company policy

**Table 1** Silage parameters after different days of storage for control (a) and treated LAB mixture (b)

| Item | Days of storage 14 | | 28 | | 49 | | 90 | |
|---|---|---|---|---|---|---|---|---|
| | a | b | a | b | a | b | a | b |
| DMc (%) | 29.2 | 28.1 | 29.5 | 27.9 | 28.8 | 27.1 | 30.6 | 27.1 |
| LA (% DM) | 6.9 | 7.4 | 7.3 | 7.5 | $7.7^{a}$ | $4.5^{b}$ | $7.6^{a}$ | $3.8^{b}$ |
| AA (% DM) | 1.6 | 2.0 | $1.9^{a}$ | $2.7^{b}$ | $1.8^{a}$ | $4.0^{b}$ | $1.9^{a}$ | $4.8^{b}$ |
| PD (% DM) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | $0.02^{a}$ | $0.4^{b}$ |
| Pol (% DM) | $0.0^{a}$ | $0.14^{b}$ | $0.0^{a}$ | $0.39^{b}$ | $0.0^{a}$ | $1.20^{b}$ | $0.0^{a}$ | $1.12^{b}$ |
| PA (% DM) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | $0.0^{a}$ | $0.31^{b}$ |
| pH | $4.44^{a}$ | $4.25^{b}$ | 4.36 | 4.27 | $4.32^{a}$ | $4.46^{b}$ | $4.34^{a}$ | $4.52^{b}$ |
| AS (days) | $4.0^{a}$ | $7.3^{b}$ | $4.7^{a}$ | $10.9^{b}$ | $5.8^{a}$ | $>12^{b}$ | $9.7^{a}$ | $>12^{b}$ |
| $pH_{out\ AS}$ | $4.6^{a}$ | $4.3^{b}$ | $4.7^{a}$ | $4.3^{b}$ | $4.9^{a}$ | $4.5^{b}$ | $5.0^{a}$ | $4.5^{b}$ |

$DM_c$ = DM corrected, LA=Lactic acid; AA= Acetic acid; PD= 1,2-Propandiol; Pol= n-Propanol; PA= Propionic acid; AS= Aerobic stability (max. length: 12 days); $pH_{out\ AS}$ = pH after aerobic stability test;[a, b] symbolize significant differences ($p < 0.05$)

**Figure 1** Aerobic stability after 14, 28, 49 and 90 days of storage for control and treated LAB mixture




*Max duration of the test: 12 days

**Conclusion** Compared to other commercial heterofermentative LAB, such as *L. buchneri*, the results of the present study indicate the potential of LAB mixtures, containing *L. diolivorans,* for faster opening after short time of storage. The mixture offers a clear benefit for farmers due to increased flexibility in terms of feeding the forage, because the inoculation can help to minimise the gap of scarce forage quantities on farm level.

**References**

Driehuis, F., Spoelstra, S.F., Cole, S C.J. & Morgan, R.(1996) Improving aerobic stability by inoculation with Lactobacillus buchneri, In: Jones, D.I.H., Jones, R., Dewhurst, R., Merry, R. & Haigh, P.M. (eds). Proceedings of the XI International Silage Conference, Aberystwyth, Wales, 1996, pp. 106-107.

Honig, H. (1990) Evaluation of aerobic stability. In: Lindgren, S. & Pettersson, K. (eds.), Proceedings of the EUROBAC Conference, Uppsala, Sweden, 1990, special issue, pp. 76-82.

Krooneman, J., Faber, F., Alderkamp, A.C., Elferink, S.J., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C., Vancanneyt, M. & Nousiainen, J. (2002) *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal System Evolution Microbiology. , 52, 639-646.

# Effects of different formic acid/salt containing additives on stabilisation of TMRs

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**Keywords:** aerobic stability, formic acid, propionic acid, solid application, TMR

**Introduction** High quality silages and wet feed stuff are quite often aerobically instable, depending on the number of yeasts and ambient temperature. In silages, a temperature difference of more than 3.0 °C compared to ambient temperature defines aerobic instability (Honig 1990). This affects the final stability of total mixed rations (TMR). Yeasts and moulds are both controlled by propionic, benzoic, sorbic or acetic acid (Kung et al. 1998, Nussbaum 2005, Seppälä et al. 2012, Thaysen 2007). Aerobic bacteria and some yeast may not be covered by these acids and the use of formic acid may widen the range or effectiveness of yeast control. We therefore analysed the effect of additives containing formic acid/formates on TMR stabilisation.

**Material and Methods** A TMR based on silages not treated with any additives was mixed on 7$^{th}$ of November 2017 and its aerobic stability was tested over 46 hours with and without different preservatives. The TMR had a pH of 4.55, a dry matter content of 50.01 % and was composed of 3.19 % wheat-straw, 0.43 % hay, 30.36 % concentrate, 17.04 % alfalfa silage, 13.84 % pressed sugarbeet pulp, 6.39 % grass silage and 28.75 % maize silage based on fresh matter (FM). Six different preservatives were tested for their effect on TMR stabilisation: AXF composed of propionic acid and formic acid, calcium salt by 3 kg/t FM (ADDCON XF Superfine); ACP consisting of calcium propionate by 2 kg/t FM (ADDCON CalPro); MKG composed of potassium sorbate, calcium propionate, calcium formiate and sodium benzoate by 3 kg/t FM (MAIZE KOFASIL GRANULAR); KGG composed of propionic acid and formic acid by 3 kg/t FM (KOFA GRAIN GRANULAR); KTM composed of propionic acid, sodium propionate, sorbic acid and 1,2-propandiol by 2 L/t FM (KOFA TMR); and KTP composed of propionic acid and its potassium salt by 2 L/t FM (ADDCON KTP). The treated TMR's were compared to an untreated one. One thousand one hundred gram of TMR mixed with the additives was filled into aluminium trays with a capacity of 2500 mL and stored at 17.5 °C ambient temperature. The random block design was three times replicated. The temperature in the core of the samples was logged every hour by a 10K NTC thermistor data logger over the experimental period of 46 h, the number of yeasts and moulds was determined at hour 0 and hour 46 by plate count method on agar plates (VDLUFA, 2012). Statistical analysis was done with the program "R". When the overall P–value was significant ($p < 0.05$) using ANOVA, pair wise comparisons between LSMEANS of variants were done using Tukey´s test.

**Results and Discussion** The untreated control showed a significant (p=0.031) temperature increase compared to the KGG treated variant after 27 hours. The temperature-difference between ambient temperature and the control is significant (p=0.028) after 36 hours. After 42 hours the temperature of the control was 3.0 °C higher than the ambient temperature. At the end of the experiment after 46 hours the temperature of the control was 8.0 °C higher than ambient temperature and 7.7 °C higher than the treated samples. The formic acid containing additives (AXF and KGG) showed a 1.1 °C lower temperature than those additives not based on formic acid (KTM, MKG and KTP) after 46 hours. The temperature difference of KGG was significant to KTM (p=0.040) and to KTP (p=0.049). Only the control became aerobically instable during the experimental period of 46 hours. The yeast number increased from $2.2 \times 10^6$ to $1.0 \times 10^8$ CFU/g in the control after 46 hours. The additives limited the yeast count increase to between 1.3 and $3.0 \times 10^7$ CFU/g. In the control the yeasts grew by a factor of 45.5, whereas in the treated samples there was only a growth by a factor of between 5.9 and 13.6.

The moulds were almost eliminated in the treated samples. The high number of yeasts corresponds with the higher temperature increase compared to the treated versions. In the case of the number of yeasts and moulds determined at hour 46 by plate count method on agar plates (VDLUFA 2012) there was no clear additive effect between the treated variants.

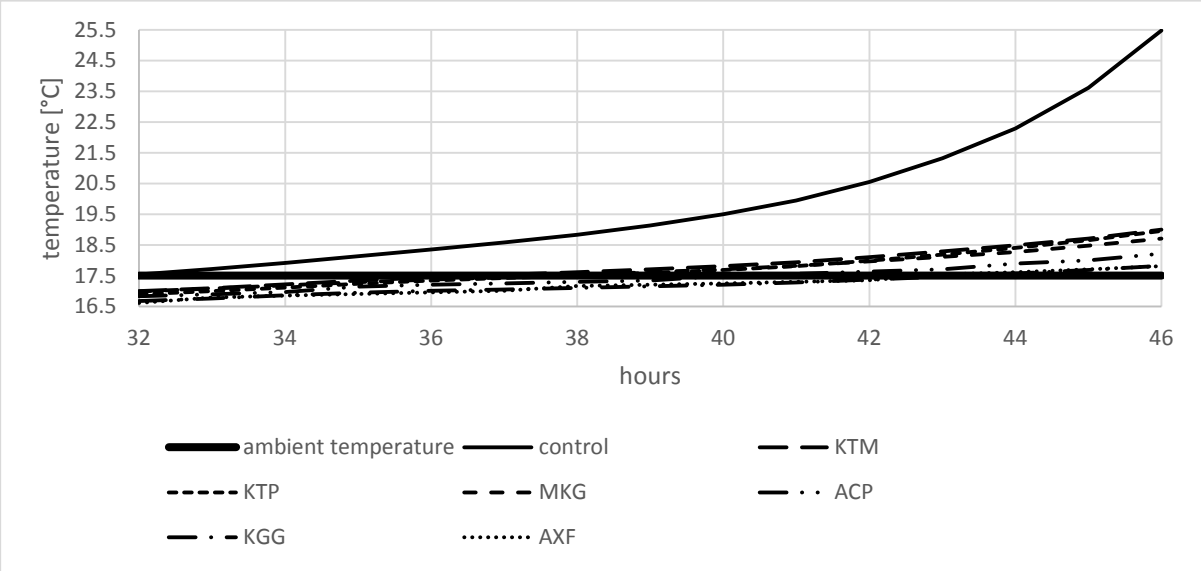


**Figure 1.** Temperature course of TMR treated with different additives in the temperature affecting period between 32 and 46 hours of the experimental period under aerobic conditions

**Conclusion** Non-treated TMR's tend to become aerobically instable due to yeast growth. Additives may control the aerobic instability. There might be a slight advantage of using additives containing formic acid/salt.

**References**

Honig, H. (1990) Evaluation of aerobic stability. Proceedings of the Eurobac Conference 1986, Grass and Forage Reports, Uppsala, Sweden, pp. 76-82.

Kung Jr, L., Sheperd, A.C., Smagala, A. M., Endres, K. M., Bessett, C. A., Ranjit, N. K. & Glancey, J. L. (1998) The effect of preservatives based on propionic acid on the fermentation and aerobic stability of corn silage and a total mixed ration. Journal of Dairy Science, 81, pp.1322-1330.

Nussbaum, H. (2005) Stabilisierung von Total-Misch-Rationen. Landinfo, 6, LEL, Schwäbisch Gmünd, pp. 37-39.

Honig, H., Kalzendorf, K., Spiekers, H. & Staudacher, W., Thaysen, J (2007) Siliermittel: Rechtliche Rahmenbedingungen, Wirksamkeit DLG-geprüfter Produkte und Einsatzempfehlungen. Übersichten zur Tierernährung, 35, pp. 55-91.

Heikkilä, T., Mäki, M. & Rinne, M., Seppälä, A (2012) The aerobic stability of total mixed ration can be managed by silage additive, In: Kuoppala, K., Rinne, M. & Vanhatalo, A. (eds.). Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 416-417.

VDLUFA (2012) Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln. no. 28.1.1–28.1.2, VDLUFA-Verlag, Darmstadt.

# Effects of four organic acids known as key intermediates in citric acid cycle on fermentation quality of lucerne silage

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**Keywords:** lucerne silage, fermentation quality, organic acids

**Introduction** Extensive research over recent years has explored the potential of using organic acids (fumaric, malic, citric, and succinic acids, etc.) as alternatives to feed antibiotics to improve performance and feed efficiency. As important intermediates in citric acid cycle, these four acids play crucial roles in microorganisms. Previous studies have also shown that malic and citric acids could accelerate the growth of lactic acid bacteria (Branen and Keenan, 1970; Passos et al., 2003). However, little information is available on effects of these four organic acids on silage fermentation, and whether these four organic acids have different effects on promoting the fermentation quality of ensiled lucerne is unclear. Thus, the objective of this study is to investigate the effects of these four acids on fermentation quality during ensiling of lucerne.

**Material and Methods** Chopped lucerne was ensiled in vacuum-sealing polyethylene plastic bags with treatments of control, 0.5% DL-malic acid (MA), 0.5% citric acid (CA), 0.5% succinic acid (SA), 0.5% fumaric acid (FA). All the application rates of the organic acids were based on fresh weight of forage and each treatment was prepared in triplicate. The silos were then stored at ambient temperature (22–25°C) and sampled on days 3, 7, 14, 30, 60 and 90 d for fermentation profile. The effects of these four acids on fermentation quality and proteolysis of lucerne silage were investigated after 90 d of ensiling. The water-soluble carbohydrates (WSC) was analyzed using the method of Thomas (1977). Crude protein (CP) was calculated as Kjeldahl N × 6.25. Non-protein nitrogen (NPN) content of forage samples was analyzed by using TCA as described by Licitra et al (1996). Data from silages of each sampling time were subjected to one-way ANOVA of the Statistical Package for the Social Science (SPSS 17.0, SPSS, Inc., Chicago, IL). The differences among treatment means were tested by using the Tukey's multiple range test, and significance was declared at $P < 0.05$.

**Results and Discussion** With prolonged ensiling time, the pH in different lucerne silages decreased while the lowest pH was observed in the control silage after 30 d (Figure 1). The pH of all silages was above 5, and the result was probably due to the low lactic acid bacteria in fresh lucerne and high content of CP in silages (Table 1). The population of lactic acid bacteria (LAB) in each group increased at first 30 days and then decreased except for FA-treated silage (Figure 2). Generally, application of the four organic acids did not promote the growth of LAB during the whole procedure of silage fermentation except for FA at 3 d fermentation and CA at 7 d fermentation in which higher populations of LAB were found compared to the control silage. After 90 d of ensiling, application of organic acids increased the lactic acid concentration in silages when compared to the control except for FA. It have been proved that MA and CA could accelerate the growth of lactic acid bacteria, succinic acid might have similar function. Malic acid, CA and SA promoted lactic acid fermentation while SA and FA increased the acetic acid concentrations in ensiled lucerne.

As key intermediates in citric acid cycle, these four acids could be utilized as a carbon and energy source. Because of the additional fermentation substrate provided by these four organic acids, the treated silages had greater DM and less WSC consumption when compared to the control silage. The greatest water soluble carbohydrate (WSC) was observed in SA-treated group. It might due to the

relative lower NDF in SA-treated silages for the degraded NDF was converted into extra carbohydrates and/or more SA was used by silage microbes as compared to the rest 3 organic acids. Compared with the control silage, acid-treated silages had lower non-protein N (NPN) and ammonia N ($NH_3$-N). The results of Mckersie (1985) showed that the optimal pH of proteolysis was about 6 in lucerne. In this study, FA and SA-treated silages had relative lower pH when compared to other treatments which might lead lower NPN in silages. Even though the lowest NPN was found in SA and FA-treated silages, FA-treated silage had greater $NH_3$-N than other acid-treated silages. However, the reason is still unclear. It might relate to the lower lactic acid in FA-treated silage.

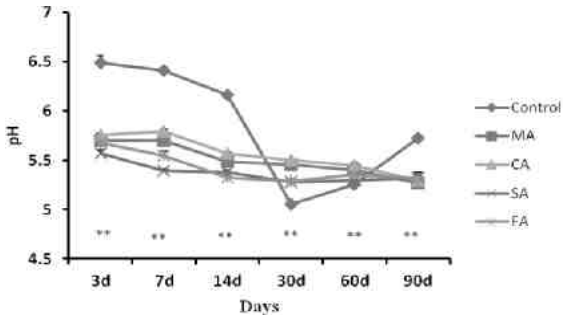


**Figure 1**. The pH of silages for different days.

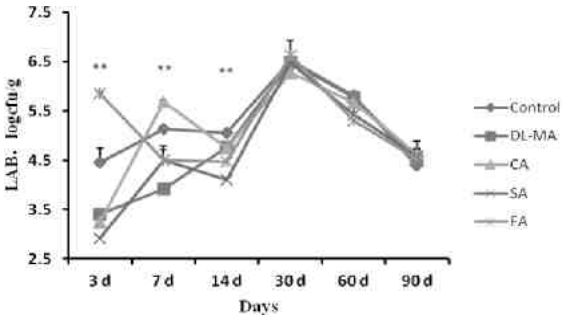


**Figure 2**. The LAB in silages for different days.

MA, malic acid; CA, citric acid; SA, succinic acid; FA, fumaric acid.
LAB, lactic acid bacteria; cfu, colony-forming unit.

**Table 1**. The effects of four acids on the fermentation quality and proteolysis of lucerne silage after 90 d

| Item | Control | MA | CA | SA | FA | SEM | P-value |
|---|---|---|---|---|---|---|---|
| Lactic acid g/kg DM | 33.00$^{c}$ | 42.20$^{a}$ | 38.64$^{b}$ | 39.24$^{ab}$ | 19.11$^{d}$ | 2.212 | < 0.001 |
| Acetic acid g/kg DM | 25.41$^{c}$ | 28.60$^{b}$ | 29.49$^{b}$ | 34.04$^{a}$ | 33.52$^{a}$ | 0.887 | < 0.001 |
| DM g/kg | 327.21$^{d}$ | 345.49$^{ab}$ | 337.45$^{c}$ | 343.97$^{b}$ | 350.47$^{a}$ | 2.266 | < 0.001 |
| WSC g/kg DM | 9.89$^{e}$ | 21.60$^{b}$ | 17.31$^{d}$ | 45.50$^{a}$ | 19.16$^{c}$ | 3.228 | < 0.001 |
| CP g/kg DM | 246.85 | 237.23 | 241.60 | 241.26 | 246.63 | 1.753 | < 0.407 |
| NPN g/kg TN | 699.10$^{a}$ | 610.17$^{bc}$ | 646.96$^{b}$ | 578.48$^{c}$ | 552.25$^{c}$ | 15.427 | < 0.002 |
| $NH_3$-N g/kg TN | 42.20$^{a}$ | 22.23$^{c}$ | 21.53$^{c}$ | 24.23$^{c}$ | 27.86$^{b}$ | 2.074 | < 0.001 |
| NDF g/kg DM | 246.61$^{a}$ | 255.50$^{a}$ | 239.73$^{ab}$ | 229.75$^{b}$ | 239.99$^{ab}$ | 2.944 | 0.042 |
| ADF g/kg DM | 175.20$^{b}$ | 191.12$^{a}$ | 176.37$^{b}$ | 169.79$^{b}$ | 175.27$^{b}$ | 2.519 | 0.053 |

$^{a-e}$Within a row, means without a common superscript letter differ ($P < 0.05$).
MA, malic acid; CA, citric acid; SA, succinic acid; FA, fumaric acid.
SEM, Standard error of the mean; DM, dry matter; WSC, water soluble carbohydrates; CP, crude protein; NPN, non-protein N; $NH_3$-N, ammonia N; NDF, neutral detergent fiber; ADF, acid detergent fiber.

**Conclusion** Application of these four acids could effectively improve lucerne silage fermentation quality with greater DM, WSC and limit proteolysis during ensiling. Malic acid, CA, SA promoted lactic acid fermentation while SA and FA increased the acetic acid concentrations in ensiled lucerne. Among all the additives, treatments of FA had better performance in decreasing silage NPN while SA-treated silages had better performance in preserving more WSC, and lower NDF were also observed in SA-treated silages when compared to the control.

**References**

Branen, A.L. & Keenan, T.W. (1970) Growth stimulation of *Lactobacillus casei* by sodium citrate. Journal of Dairy Science, 53, 593-597.

Licitra, G., Hernandez, T.M., & Van Soest. P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology, 57, 347-358.

Mckersie, B.D. (1985) Effect of ph on proteolysis in ensiled legume forage. Agronomy Journal, 77, 81-86.

Passos, F.V., Fleming, H.P., Hassan, H.M. & Mcfeeters, R.F. (2003) Effect of malic acid on the growth kinetics of *Lactobacillus plantarum*. Applied Microbiology and Biotechnology, 63, 207-211.

Thomas, T.A. (1977) An automated procedure for the determination of soluble carbohydrates in herbage. Journal of the Science of Food and Agriculture, 28, 639-642.

# Effects of intermediate storage and additive use on the formation of volatile organic compounds in sugar beet pulp silage pressed in plastic bags

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**Keywords:** alcohols, esters, silage, sugar beet pulp, volatile organic compounds, VOC

**Introduction** Pressed sugar beet pulp (PSBP) silage represents an important component of ruminant feed rations. Although PSBP ferment well (Auerbach et al. 2016), silages produced from it may contain high concentrations of ethanol and be prone to rapid deterioration after silo opening (Weber et al. 2006). Results on the formation of volatile compounds (VOC), e. g. ethyl ethers, have not been reported yet. Under practical conditions, PSBP, sometimes, need to be stored on-site before dispatch to farms. Therefore, this study aimed at elucidating the effects of intermediate storage before ensiling and silage additive treatment on VOC formation in PSBP silage stored in plastic bags.

**Material and Methods** Three batches of about 50 t of PSBP (DM 22%) were obtained from the sugar factory of Südzucker AG, Zeitz, Germany. Before dispatch to the trial location, one batch was stored on-site in a loose pile for 36 hrs (DEL), another one was treated with 2 L $t^{-1}$ of a chemical additive, containing potassium sorbate (154 g $L^{-1}$), sodium benzoate (257 g $L^{-1}$) and ammonium propionate (57 g $L^{-1}$) before storage for 36 hrs (DELADD) and the third batch was freshly produced (FRESH). Within 3 hours after dispatch, the material was pressed into plastic bags (diameter: 2.7 m, plastic thickness: 215 µm) by a rotor bagger (BUDISSA Bag, RT 8000) and stored for 223 days outside. On the day of opening of the three bags, three samples from the top layer at 20 cm below the surface and three samples at 1 m above the ground were taken across the whole width of the bag by a drill. Alcohols, acids and esters were determined by gas-chromatography. Data were submitted to statistical analyses by SAS 9.4, using the procedures MIXED and REG without consideration of the effect of sampling location. Comparisons among treatment means were made at $P<0.05$ by Tukey's test, or by non-parametric ANOVA-type statistics (ANOVAF-option), when values were not normally distributed.

**Results and Discussion** The PSBP silages contained a range of alcohols of which ethanol was the primary alcohol detected, followed by n-propanol, methanol and 2-butanol (Table 1). The concentrations of ethanol, 2-butanol and n-propanol were highest in intermediately stored, untreated PSBP silage when compared with DELADD and FRESH. Methanol remained unaffected by treatment.

**Table 1.** Effects of treatment on fermentation products and VOC in pressed sugar beet pulp silages (data given as LSmeans in mg $kg^{-1}$ DM unless stated otherwise, n=6)

| Parameter | DEL[1] | | DELADD[2] | | FRESH[3] | | *P* |
|---|---|---|---|---|---|---|---|
| | LSM | SEM | LSM | SEM | LSM | SEM | |
| Methanol | 659 | 50.3 | 564 | 43.1 | 612 | 87.8 | ns |
| Ethanol | $14767^{y}$ | 1869.9 | $1915^{x}$ | 349.7 | $4475^{x}$ | 1629.0 | ** |
| 2-Butanol | $100^{y}$ | 24.5 | $10^{x}$ | 10.1 | $8^{x}$ | 8.0 | *** |
| n-Propanol | $2956^{y}$ | 1007.6 | $691^{x}$ | 283.5 | $88^{x}$ | 49.2 | ** |
| Lactic acid[4] | $24.9^{b}$ | 3.00 | $23.6^{b}$ | 3.78 | $11.6^{a}$ | 0.74 | ** |
| Acetic acid[4] | $20.7^{c}$ | 1.99 | $15.7^{b}$ | 1.13 | $10.8^{a}$ | 0.32 | *** |
| Ethyl lactate | $295^{y}$ | 26.3 | $38^{x}$ | 17.2 | $84^{x}$ | 55.7 | ** |
| Ethyl acetate | $375^{b}$ | 16.5 | $309^{ab}$ | 14.5 | $264^{a}$ | 27.2 | ** |
| Sum ethyl esters | $670^{b}$ | 26.5 | $347^{a}$ | 30.8 | $348^{a}$ | 82.5 | *** |

[1]intermediate storage, [2]intermediate storage and additive treatment, [3]immediate bagging, [4]g $kg^{-1}$ DM, ns not significant, [x,y]LSmeans in rows bearing unlike superscripts differ, non-parametric test, ANOVAF-option, [a,b]LSmeans in rows bearing unlike superscripts differ, Tukey`s test.

Propyl acetate was not detected in any of the silage despite the occurrence of the alcohol n-propanol. In maize silage, Weiss et al. (2015) reported propyl acetate concentrations of up to 676 mg $kg^{-1}$ DM, but n-propanol was much higher than in our study. Apparently, the formation of propyl acetate depends on the presence of a minimum content of n-propanol. The formation of total ethyl esters and of ethyl acetate was reduced by treatments DELADD and FRESH. Immediately bagged PSBP contained the lowest concentration of ethyl acetate, and a trend was observed that additive use decreased its formation in intermediately stored material (*P*=0.085). This study supports observations on other silage types by Weiss (2017), highlighting the prominent role of ethanol in the accumulation of ethyl esters. Also in PSBP silage, there existed a strong correlation between ethanol and its esters of lactate and acetate (Figure 1). Moderate positive relationships were found between the concentrations of ethanol and ethyl lactate (y=12.95+0.019x, $R^2$=0.66, *P*<0.001) and ethyl acetate (y=261.82+0.008x, $R^2$=0.58, *P*<0.001), respectively. There was no significant relationship detected between the concentrations of lactic acid and ethyl lactate, whereas acetic acid and ethyl acetate were moderately correlated (y=174.54+92.67x, $R^2$=0.50, *P*<0.001).

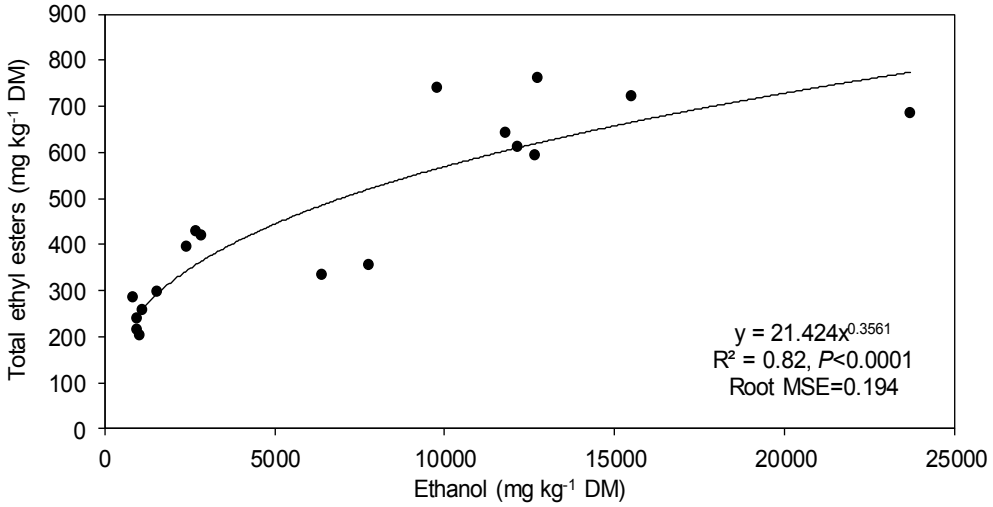


**Figure 1.** Relationship between the concentrations of ethanol and total ethyl esters (sum of ethyl esters of lactate and acetate) in sugar beet silage pressed in plastic bags, n=18.

**Conclusion** This study confirmed that also PSBS contained a range of VOC, including less frequently analysed alcohols, e. g. 2-butanol. Any measure to decrease ethanol concentrations, especially the use of the chemical additive, will result in reduced ethyl ester formation in PSBP silage even if the material is subjected to intermediate storage before bag filling.

**References**

Auerbach, H., Weber, G., Nadeau, E., Weber, U., Weiss, K. & Potthast, C. (2016) Effects of delayed filling and additive use on the quality of pressed sugar beet pulp ensiled in plastic bags. In: Rajcakova, L. (ed). Proceedings of the 17th International Conference Forage Conservation, Stary Smokovec, Slovakia, 2016, pp. 179-185.

Weber, U., Kaiser, E. & Steinhöfel, O. (2006) Studies on ensiling pressed sugar beet pulp in plastic tubes. Part 2: Effect of storage length, addition of ensiling aids and extraction end closure on the aerobic stability of pressed pulp silage. Sugar Industry, 131 (12), 857-862.

Weiss, K. (2017) Volatile organic compounds (VOC) in silages - Effects of silo management factors on its formation. In: Nussio, L. G., de Sousa, D. O., Gritti, V. C., Salvati, G. G., Santos, W. P. & Salvo, P. A. R. (eds.). Proceedings of the V International Symposium on Forage Quality and Conservation, Piracicaba, SP, Brazil, 2017, pp. 181-210.

Weiss, K., Olbrich, C. & Thaysen, J. (2015) Volatile organic compounds (VOC) in maize silages at German dairy farms. In: Daniel, J. L. P., Morais, G., Junges, D. & Nussio, L. G. (eds.). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 98-99.

# Effects of lactic acid bacteria isolated from cow rumen fluid and feces on quality and *in vitro* digestibility of alfalfa silage

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**Keywords:** feces, *in vitro* digestibility, lactic acid bacteria, rumen fluid, silage

**Introduction** Silage quality is improved by the addition of lactic acid bacteria (LAB). Feeding animals with silages fermented by LAB will effectively reduce the number of pathogenic bacteria and promote the digestion and absorption of nutrients in intestine, increase the conversion rate of feeds, and thereby improve production performance of animals. Because of the homology, LAB from animal's gastrointestinal tract are more likely to have a good environmental adaptation and thus colonize the host digestive tract and exert their function. The effects of screened LAB isolated from gastrointestinal tract of dairy cows as a kind of additive on quality, gas production and *in vitro* digestibility of alfalfa silage were studied to provide reference for application of homologous LAB on silage.

**Materials and Methods** 104 strains of lactic acid bacteria were isolated from rumen fluid and feces of six middle-aged Holstein cows in good condition. Four strains (*L. plantarum* F1 and F50, *L. salivarius* L100 and *L. fermentum* L120) were selected by their fast growth rate, high acid productivity, and excellent physiological and biochemical characteristics. Alfalfa (DM 418.29 g/kg, at early bloom stage of second cutting) was treated with no additive as control (CK), a commercial inoculant (GFG, Sichuan Gao Fuji Biotechnology Co., Ltd., Sichuan, China) or with one of the four selected strains, respectively (n=3). Fermentation quality, chemical composition, gas production and *in vitro* digestibility (Zhang et al. 2011) of samples were tested after fermented 60 day. Datas were processed using SAS V8 software with a multiple comparison test (Tukey/Kramer) used for comparisons at 5% significant level.

**Results and Discussion** The silages inoculated with selected strains presented different levels of improvement in fermentation quality and *in vitro* digestibility, as shown in Table 1. Lactic acid is usually the main reason for low pH (Wang et al. 2006). After 60 days ensilage, a reduction of the pH value compared to CK was observed for all silage samples ($P<0.01$), especially for F1 treatment, where the pH value decreased to 4.60, which was even lower than GFG treatment ($P<0.01$). That might be coincided with its excellent acid production performance. Higher contents of lactic acid and the ratio of lactic acid to acetic acid (L:A) in F1, F50 and L100 treatments were observed compared to CK ($P<0.01$). Microorganisms in rumen have been found to ferment a wide range of endogenous and exogenous substrates to produce fermentation gases (Zhang et al. 2017). Silages inoculated with F1 showed higher contents of cumulative gas production of 72h ($GP_{72h}$) than CK and silages inoculated with GFG ($P<0.01$), indicating a higher potential content of substrates in F1 silage for rumen microorganism to degrade. F1 silage increased *in vitro* digestibility (IVDMD) as compared to others ($P<0.01$), maybe due to the degradation from cellulose into soluble sugar that LAB can easily use as fermentable substrate. Therefor, F1 displayed the best effect on alfalfa silage among all additives.

**Table 1.** Fermentation quality, chemical composition, gas production and *in vitro* digestibility of alfalfa silage after 60 day

| Items | CK | GFG | F1 | F50 | L100 | L120 | *P* |
|---|---|---|---|---|---|---|---|
| DM ( g/kg ) | 417.97 | 426.14 | 428.62 | 405.11 | 424.17 | 447.65 | 0.10 |
| NDF ( g/kg DM ) | 436.41 | 412.84 | 404.88 | 416.74 | 422.32 | 414.20 | 0.48 |
| ADF ( g/kg DM ) | 332.96 | 325.90 | 320.11 | 322.29 | 327.00 | 322.21 | 0.65 |
| HC ( g/kg DM ) | 103.45 | 86.94 | 84.77 | 94.45 | 95.32 | 91.99 | 0.54 |
| pH | $5.34^{a}$ | $4.81^{bc}$ | $4.60^{d}$ | $4.71^{cd}$ | $4.70^{cd}$ | $4.89^{b}$ | <0.01 |
| LA ( g/kg DM ) | $20.60^{b}$ | $32.73^{ab}$ | $40.78^{a}$ | $37.97^{a}$ | $43.40^{a}$ | $31.32^{ab}$ | 0.04 |
| AA ( g/kg DM ) | 27.32 | 28.81 | 33.99 | 38.28 | 31.74 | 37.16 | 0.38 |
| L:A | $0.76^{e}$ | $1.15^{bc}$ | $1.20^{b}$ | $0.99^{cd}$ | $1.37^{a}$ | $0.84^{de}$ | <0.01 |
| PA ( g/kg DM ) | 0.00 | 3.75 | 0.13 | 0.14 | 0.00 | 0.43 | 0.49 |
| BA ( g/kg DM ) | 3.45 | 3.14 | 1.21 | 1.12 | 3.97 | 1.11 | 0.60 |
| $NH_3$-N (% TN) | 7.18 | 8.60 | 6.53 | 4.36 | 5.39 | 7.23 | 0.33 |
| $GP_{72h}$ (mL/ g DM) | $35.83^{d}$ | $38.50^{bc}$ | $40.47^{a}$ | $37.80^{c}$ | $39.90^{ab}$ | $39.00^{abc}$ | <0.01 |
| IVDMD (g/kg DM) | $559.33^{b}$ | $566.97^{b}$ | $602.67^{a}$ | $544.23^{b}$ | $531.10^{b}$ | $486.53^{c}$ | <0.01 |

DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; TN, total nitrogen; L:A, the ratio of lactic acid to acetic acid; $GP_{72h}$, cumulative gas production of 72 h; IVDMD, *in vitro* dry matter digestibility; Different letters indicate significant differences ($P < 0.05$).

**Conclusions** *L. plantarum* F1 and F50, *L. salivarius* L100 and *L. fermentum* L120 were selected by their fast growth rate, high acid productivity, and excellent physiological and biochemical characteristics from 104 LAB strains isolated from cow rumen fluid and faeces. Low pH value, high content of L:A and IVDMD can be obtained by 60 day ensiling alfalfa with additive of strain F1, which could thus be considered a promising inoculant for alfalfa silage, revealed the benefits to gastrointestinal tract of animals and the potential application value of LAB isolated from cow rumen fluid and feces on silage.

**References**

Wang, X., Haruta, S., Wang, P., Ishii, M., Igarashi, Y. & Cui, Z. (2006) Diversity of a stable enrichment culture which is useful for silage inoculant and its succession in alfalfa silage. FEMS Microbiology Ecology, 57, 106-115.

Zhang, D. F. & Yang, H. J. (2011) *In vitro*, ruminal methanogenesis of a hay-rich substrate in response to different combination supplements of nitrocompounds; pyromellitic diimide and 2-bromoethanesulphonate. Animal Feed Science & Technology, 163, 20-32.

Zhang, Q., Zhao, M., Wang, X., Yu, Z. & Na, R. (2017) Ensiling alfalfa with whole crop corn improves the silage quality and *in vitro* digestibility of the silage mixtures. Grassland Science, 63, 211-217.

# Effects of molasses and exogenous enzymes on the fermentation, aerobic stability and nutrient composition of ensiled maize cob and potato hash mixtures

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**Keywords:** exogenous enzymes, fibre, fermentation, maize cob, potato hash

**Introduction** Affordability of conventional feeds has gone beyond the reach of smallholder pig farmers in South Africa due to declining grain production, increasing competition with humans for feed ingredients and rise in feed prices. Availability of agro-industrial by-products has compelled smallholder pig farmers to use alternative energy sources to replace cereals in pig diets (Thomas *et al*. 2010). These by-products contain essential nutrients and might make excellent pig feed if further processed. Maize is a major cereal grown in South Africa and one of its by-products at harvest, maize cobs (MC), can mitigate against the high pig feed prices. Presently, the MC are being thrown away or burnt for fuel. Potato hash (PH), a by-product of the potato food producing industry is available in Gauteng, South Africa. An estimated amount of 50 ton of potato hash is produced per day. However, its high moisture content makes it difficult to incorporate it in animal feed. Ensiling can be considered as an efficient way of preserving PH. The aim of the present study was to assess the effects of silage additives (molasses and exogenous enzymes) on the fermentation, aerobic stability and nutrient composition of ensiled maize cob and potato hash mixtures.

**Materials and Methods** Potato hash (150 g kg/DM), 105g crude protein (CP) kg/DM, 58.5 g crude fibre kg/DM, and 700 g starch kg/DM) was collected from Simba (Pty) Ltd (Isando, Gauteng Province, South Africa) and brought to the Animal Production (ARC-Irene, Pretoria) for analysis and silage making. Maize cobs (920 g kg/DM and 168 g crude fibre kg/DM) were collected from the Agricultural Research Council - Animal Production fields (ARC-AP, Irene, Gauteng, South Africa), and ground to pass through a 5 mm sieve. Sugarcane molasses syrup was obtained from Obaro®, a local dealer in South Africa. An amount of 800 g PH was mixed with 200 g of MC to produce silage (MCPHS). Sugarcane molasses syrup was diluted with warm water at a ratio of 1:2 and sprayed evenly over the MCPHS at a rate of 100 mL/kg and exogenous xylanase enzyme (Natugrain TS L®) was added at an inclusion rate of 100 g/t. The treatments were: i) control (no additive), ii) molasses (MOL) and iii) exogenous xylanase enzyme (ENZ). These mixtures were ensiled in 1.5 L airtight glass jars (J. Weck, GmbH u. Co. KG, Wehr-Öflingen, Germany) equipped with lids, rubber rings and steel clamps to enable gas release and keep the jars airtight. Jars were stored out of sunlight and kept at 24 - 28 ºC for 32 days. After 32 days of ensiling, three jars per treatment were opened and samples were collected and analysed for chemical composition and fermentation characteristics. In addition, silage samples were subjected to an aerobic stability test by exposing samples to air for 7 days following the procedure of Ashbell *et al*. (1991). Data for fermentation, aerobic stability and nutrient composition of the silage was analysed for effects of treatment using GLM procedures of SAS (2012).

**Results and discussion** After 32 days of ensiling, ENZ inoculation had higher ($P<0.05$) residual WSC and LA leading to poor aerobic stability of the silage as indicated by higher ($P<0.05$) $CO_2$ production (Table 1). This concurs with other studies (Weinberg *et al*. 1993, Nkosi *et al*. 2015) that reported reduced silage aerobic stability with higher residual WSC. In addition, ENZ inoculation improved ($P<0.05$) DM and CP contents and reduced aNDF, ADF and ADL contents compared to other treatments, which was consistent to Sucu and Filya (2006). The MOL inoculation increased ($P<0.05$) the content of acetic acid and improved silage aerobic stability compared to other treatments.

**Table 1.** Fermentation characteristics and chemical composition of maize cob silage after 32 days of ensiling (n=3).

| | Treatments | | | | |
|---|---|---|---|---|---|
| Parameter | Control | ENZ | MOL | SEM | P-value |
| **Fermentation characteristics** | | | | | |
| WSC, g/kg DM | 2.7[b] | 8.3[a] | 7.5[a] | 0.365 | 0.001 |
| pH | 4.4[b] | 4.0[b] | 5.0[a] | 0.108 | 0.001 |
| LA, g/kg DM | 64.8[b] | 79.7[a] | 48.2[c] | 1.317 | 0.001 |
| AA, g/kg DM | 51.0[b] | 35.4[c] | 60.1[a] | 1.771 | 0.001 |
| PA, g/kg DM | 6.9[b] | 4.8[c] | 30.1[a] | 0.624 | 0.001 |
| BA, g/kg DM | 30.4[a] | 6.2[c] | 13.8[b] | 0.557 | 0.001 |
| $NH_3$-N, g/kg DM | 12.3[a] | 7.5[c] | 13.5[b] | 0.144 | 0.001 |
| **Chemical composition** | | | | | |
| DM, g/kg | 402.7[b] | 413.4[a] | 338.3[c] | 0.284 | 0.001 |
| CP, g/kg DM | 144.6[b] | 152.7[a] | 122.5[c] | 1.185 | 0.001 |
| GE, MJ/kg DM | 18.5 | 18.5 | 18.1 | 0.186 | 0.348 |
| EE, g/kg DM | 52.1[b] | 57.7[b] | 69.8[a] | 0.875 | 0.014 |
| aNDF, g/kg DM | 485.1[a] | 398.5[c] | 453.4[b] | 1.028 | 0.001 |
| ADF, g/kg DM | 396.9[a] | 314.7[b] | 399.9[a] | 1.048 | 0.001 |
| ADL, g/kg DM | 69.1[a] | 52.0[b] | 66.9[a] | 0.359 | 0.001 |
| **Aerobic stability** | | | | | |
| $CO_2$, g/kg DM | 23.6[b] | 27.9[a] | 7.0[c] | 0.512 | 0.001 |

[a-c] Means with different letters in a row differ significantly (P<0.05).
DM, dry matter; CP, crude protein; GE, gross energy; EE, ether extract; aNDF, amylase-treated neutral detergent fibre; ADF, acid detergent fibre; WSC, water soluble carbohydrates; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; $NH_3$-N, ammonia-N

**Conclusions** It was concluded that good quality silage of PH and MC mixtures was produced with exogenous enzymes inoculation. More studies need to be carried out to determine the optimum dietary inclusion level of this silage on pig growth performance and the cost benefit.

**References**

Ashbell, G., Weinberg, Z.G., Azriel, A., Hen, Y. & Horev. B. (1991) A simple system to study the aerobic deterioration of silages. Canadian Agricultural Engineering, 33, 391-393.

Nkosi, B.D., Meeske, R., Langa, T., Motiang, M.D., Mutavhatsindi, T.F., Thomas, R.S., Groenewald, I.B. & Baloyi, J.J. (2015) The influence of ensiling potato hash waste with enzyme/bacterial inoculant mixtures on the fermentation characteristics, aerobic stability and nutrient digestion of the resultant silage by rams. Small Ruminant Research, 127, 28-35.

Sucu, E. & Filya, I. (2006) Effects of homofermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of low dry matter corn silages. Turkish Journal of Veterinary and Animal Science, 30, 83-88.

SAS (2012) SAS for Windows. Version 9.3. SAS Inst. Inc., Cary, NC., USA.

Thomas, R.S., Nkosi, B.D., Umesiobi, D.O., Meeske, R., Kanengoni, A.T. & Langa., T. (2010) Evaluation of potato hash silage from two bacterial inoculants and their effects on the growth performance of grower pigs. South African Journal of Animal Science, 40, 488-490.

Weinberg, Z.G., Ashbell, G., Azrieli, I. & Brukental, I. (1993) Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria (LAB) and cell wall degrading enzymes. Grass and Forage Science, 48, 70-78.

# Effects of sucrose and lactic acid bacteria inoculant on quality and characteristics of protein fractions of mulberry silage

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**Keywords:** additives, Mulberry, silage

**Introduction** Mulberry *(Morus alba L.)* is not only adaptable and high-yielding but also providing high-protein feed, having widely been paid attention in the tropics and subtropics of Southern China. It has been reported that mulberry could be widely used in the farm animals to become a good source for feeding and supplementing ruminant animals (Trabi et al. 2017). The aim of the study was to evaluate the effect of *Lactobacillus plantarum*, *Lactobacillus casei* and sucrose on ensiling characteristics, nutrient compositions and protein fractions by the Cornell Net Carbohydrate and Protein System (Sniffen, C.J et al. 1992) of mulberry silage.

**Materials and Methods** Mulberry leaves *(Morus alba L.)* were collected from a field in South China Agricultural University, Guangdong Province, China. Freshly harvested mulberry leaves and twigs (chopped with lengths of 1 to 1.5 cm by a hand forage chopper) were assigned to different inoculants, including no inoculant(CK), *Lactobacillus plantarum*(LP, $1\times10^6$ CFU/g), *Lactobacillus casei* (LC, $1\times10^6$ CFU/g) and sucrose(S, 20g/kg) with or without S with three replications, using plastic film bags. Silages stored indoors at ambient temperature around 29 °C. The material BC content was measured by lactat (Heinritz et al. 2012). After 60 d bags were opened for later examination of all conventional index and protein fractions (Zhang et al. 2017; Tao et al. 2017). All statistical analyses were performed using the general linear model procedure of SAS 9.0 (2002; SAS Institute, Cary, NC, USA).

**Results and Discussion** Table 1 shows the tested chemical contents from raw mulberry leaves before ensiling. The fresh material DM content was measured 386.72 g/kg FM and BC content was measured 83.55g LA/kg DM. CP of fresh leaves was 193.83 g/kg DM.

**Table 1**. Chemical composition of mulberry leaves before ensiling.

| Items | Value |
|---|---|
| DM (g/kgFM) | 386.7 |
| CP (g/kg DM) | 193.8 |
| NDF (g/kg DM) | 184.3 |
| ADF (g/kg DM) | 127.7 |
| ADL (g/kg DM) | 26.2 |
| BC (g LA/kg DM) | 83.6 |

DM, dry matter; FM, fresh matter; BC, buffering capacity; WSC, water soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein.

As presented in Table 2, the fermentation quality of mulberry leaves silage after two months under room temperature was good. All additive treatments decreased the pH value of mulberry leaves silage as compared with CK, and the lowest was in LC+S ($P<0.001$). Silages treated with LC or LC+S increased LA content($P<0.001$), the addition of LP or LC decreased ammonia nitrogen/total nitrogen ($P<0.001$) whether adding S or not.

**Table 2**. Fermentation quality of mulberry leaves silages.

| Items | CK | LP | LC | S | LP+S | LC+S |
|---|---|---|---|---|---|---|
| pH | 5.1$^{a}$ | 4.3$^{c}$ | 4.2$^{d}$ | 4.7$^{b}$ | 4.1$^{d}$ | 4.0$^{e}$ |
| LA (g/kg DM) | 70.1$^{e}$ | 117.3$^{cd}$ | 147.5$^{a}$ | 98.9$^{d}$ | 123.8$^{bc}$ | 137.5$^{ab}$ |
| AA (g/kg DM) | 17.1$^{bc}$ | 23.8$^{a}$ | 14.5$^{cd}$ | 10.6$^{d}$ | 20.8$^{ab}$ | 11.4$^{d}$ |
| PA (g/kg DM) | 15.1$^{a}$ | 0.5$^{c}$ | 7.6$^{b}$ | 15.0$^{a}$ | 7.3$^{b}$ | 5.0$^{b}$ |
| BA (g/kg DM) | ND | ND | ND | ND | ND | ND |
| LA/AA | 4.1$^{b}$ | 4.93$^{b}$ | 10.0$^{a}$ | 9.5$^{a}$ | 6.0$^{b}$ | 12.7$^{a}$ |
| AN (g/kg TN) | 66.9$^{a}$ | 28.58$^{b}$ | 18.2$^{c}$ | 59.1$^{a}$ | 16.5$^{c}$ | 10.2$^{c}$ |

C, control; LP, *Lactobacillus plantarum* (1×10$^{6}$ CFU/g); LC, *Lactobacillus casei* (1×10$^{6}$ CFU/g); S, sucrose (20 g/kg on fresh matter basis); pH, pH value; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; LA/AA, lactic acid/ acetic acid; AN, ammonia nitrogen; TN, total nitrogen; ND, not detected. Means within the same row with different superscripts differ significantly from each other ($P < 0.05$).

**Table 3**. Chemical composition and protein fractions of mulberry leaves silage.

| Items | CK | LP | LC | S | LP+S | LC+S |
|---|---|---|---|---|---|---|
| DM (g/kgFM) | 327.5$^{b}$ | 334.0$^{b}$ | 334.5$^{b}$ | 337.1$^{b}$ | 347.1$^{b}$ | 413.2$^{a}$ |
| WSC (g/kg DM) | 10.1$^{b}$ | 12.5$^{b}$ | 11.4$^{b}$ | 10.2$^{b}$ | 24.7$^{a}$ | 14.8$^{b}$ |
| NDF (g/kg DM) | 211.2$^{a}$ | 187.9$^{bc}$ | 188.8$^{bc}$ | 201.0$^{ab}$ | 169.2$^{d}$ | 174.4$^{cd}$ |
| ADF (g/kg DM) | 149.6$^{a}$ | 141.7$^{ab}$ | 134.5$^{bc}$ | 141.2$^{ab}$ | 127.6$^{cd}$ | 122.7$^{d}$ |
| ADL (g/kg DM) | 45.3$^{a}$ | 33.0$^{ab}$ | 40.2$^{ab}$ | 44.9$^{a}$ | 28.2$^{b}$ | 30.6$^{ab}$ |
| CP (g/kg DM) | 191.2$^{a}$ | 189.0$^{a}$ | 191.0$^{a}$ | 191.2$^{a}$ | 175.7$^{c}$ | 182.6$^{b}$ |
| protein fractions | | | | | | |
| Fraction A (%CP) | 37.3$^{a}$ | 31.9$^{bc}$ | 35.1$^{ab}$ | 38.3$^{a}$ | 29.4$^{c}$ | 32.1$^{bc}$ |
| Fraction B1 (%CP) | 4.0b$^{c}$ | 2.7$^{c}$ | 7.4$^{a}$ | 5.4$^{ab}$ | 2.9$^{c}$ | 4.0$^{bc}$ |
| Fraction B2 (%CP) | 42.4$^{ab}$ | 48.6$^{a}$ | 32.7$^{c}$ | 33.9$^{bc}$ | 45.5$^{a}$ | 41.9$^{ab}$ |
| Fraction B3 (%CP) | 8.7$^{c}$ | 10.1$^{c}$ | 17.5$^{b}$ | 17.0$^{c}$ | 18.0$^{b}$ | 17.7$^{a}$ |
| Fraction C (%CP) | 7.6$^{ab}$ | 6.7$^{bc}$ | 7.3$^{a}$ | 5.4$^{cd}$ | 4.2$^{d}$ | 4.3$^{d}$ |

DM, dry matter; FM, fresh matter; WSC, water soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein. Means within the same row with different superscripts differ significantly from each other ($P < 0.05$).

The chemical composition of mulberry leaves after ensiling is shown in Table 3. Ensilage transformed the contents of protein compared to fresh material. Additive inoculants decreased NDF of mulberry leaves silage as compared with CK, and the lowest was in LP+S ($P<0.001$). There was a lower fraction C content but a higher fraction fraction B3 in silages treated with LP+S or LC+S ($P<0.001$).

**Conclusion** There is a positive potential of producing well preserved and high nutritional silage from mulberry leaves. In this study, we can summarize that well fermented mulberry silages were obtained by adding LP or LC, and S can be recommended as an additive to improve the protein fractions.

**References**

Heinritz, S. N., Martens, S. D., Avila, P. & Hoedtke, S. (2012) The effect of inoculant and sucrose addition on the silage quality of tropical forage legumes with varying ensilability. Animal Feed Science and Technology, 174, 201-210.

Tao, L., Zhou, H., Zhang, N.F., Si, B.W., Tu, Y., Ma, T. & Diao, Q.Y. (2017) Changes in carbohydrate and protein fractions during ensiling of alfalfa treated with previously fermented alfalfa juice or lactic acid bacteria inoculants. Animal Production Science, 58 (3) , 577-584.

Trabi, E. B., Yuan, X. J.; Li, J. F., Dong, Z., Shah, A A., & Shao, T. (2017) Effect of glucose and lactic acid bacteria on the fermentation quality, chemical compositions and *in vitro* digestibility of Mulberry (Morns alba) leaf silage. Pakistan Journal of Zoology, 49, 2271-2277.

Sniffen, C.J., Oconnor, J. D., Vansoest, P.J., Fox, D. G. & Russell, J. B. (1992) A net carbohydrate and protein system for evaluating cattle diets. 2. carbohydrate and protein availability. Journal of Animal Science,70 (11) 3562-3577.

Zhang, Q., Yang, H. & Yu, Z. (2017) Effects of sucrose, formic acid and lactic acid bacteria inoculant on quality, *in vitro* rumen digestibility and fermentability of drooping wild ryegrass (*Elymus nutans* Griseb.) silage. Journal of Animal and Feed Sciences, 26, 26-32.

# Effects of three strains of heterofermentative bacteria on the conservation of sugarcane silage

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**Keywords:** aerobic stability, gas loss, *Lactobacillus buchneri*, *Lactobacillus hilgardii*

**Introduction** At maturity, sugarcane crop has a high fermentability coefficient, especially because of the great content of soluble sugars. Despite the good ensilability, sugarcane conserved by natural fermentation has high fermentative losses [up to 30% of dry matter (DM) loss], due to the formation of carbon dioxide and ethanol by yeast metabolism. Hence, treating sugarcane with heterolactic bacterial inoculants would increase the formation of antifungal compounds and inhibit yeast activity during fermentation and aerobic exposure. The objective of this work was to compare the effectiveness of three strains of heterofermentative bacteria on the conservation of sugarcane silage.

**Material and Methods** A plot of sugarcane (variety CTC-25) with 10 months of regrowth (2$^{nd}$ cut) was manually harvested, mechanically chopped (8 mm) and divided in five piles (8 kg fresh matter per pile). Piles were treated with: distilled water (5 mL/kg; Control), *Lactobacillus hilgardii* CNCM I-4785 [3 × $10^5$ colony-forming units (cfu)/g; LH], *L. buchneri* NCIMB 40788 (3 × $10^5$ cfu/g; LB40788), *L. buchneri* CNCM I-4323 (3 × $10^5$ cfu/g; LB4323), and LH+LB40788 (1.5 × $10^5$ cfu/g of LH + 1.5 × $10^5$ cfu/g of LB40788). Afterwards, treated forages were packed (0.40 porosity) into 1.96 L gas-tight silos (4 replicates per treatment). The internal pressure of the silos was measured by using a pressure transducer and converted to volume (volumetric method, Daniel and Nussio 2015). Accumulated gas production per kg DM was fitted with an exponential 1-pool model ($Gt = G \times [1 - e^{-(k \times t)}]$ to estimate the fractional rate of gas production (k) and gas pool (G). After 70 d of storage, silages were sampled for measuring microbial counts, pH, DM content, fermentative losses and aerobic stability. Data were analyzed using the MIXED procedure of SAS. Means were compared by Tukey's test ($\alpha = 0.05$).

**Results and Discussion** Silages treated with heterolactic bacterial inoculants had higher DM content, and lower fermentative losses, measured by both gravimetric and volumetric methods. Overall, gas loss of treated silages was reduced by 295% (13 vs. 4.4 % of DM, Table 1). Interestingly, control silage was still producing gas all along the 70-d of storage (Figure 1). Based on the 1-pool exponential model, 90% of the gas volume was produced within 13 d in treated silages (on average), whereas in untreated silage it was delayed by 58 d. Hence, the potential to preserve nutrients during fermentation of sugarcane silages was similar among the three tested strains.

Yeast counts were reduced from 4.42 log cfu/g in control to approximately zero in inoculated silages, except for LH (2.06 log cfu/g). Therefore, sugarcane silages treated with LB40788, LB4323 and LH+LB40788 had higher aerobic stability.

**Conclusion** All heterolactic bacterial inoculants applied at 3 × $10^5$ cfu/g were effective to inhibit yeast metabolism and mitigate gas formation during sugarcane silage fermentation. Additionally, the two strains of *L. buchneri* and combination of *L. buchneri* and *L. hilgardii* were capable to extend the aerobic stability.

**Table 1**. Fermentative losses and aerobic stability of sugarcane silage treated with heterolactic inoculants.

| Item | Control | LH | LB40788 | LB4323 | LH+ LB40788 | SEM | *P*-value |
|---|---|---|---|---|---|---|---|
| $DM_{oven}$, % as fed | 21.3$^{c}$ | 26.4$^{b}$ | 28.4$^{a}$ | 29.5$^{a}$ | 28.9$^{a}$ | 0.35 | <0.01 |
| pH | 3.57$^{a}$ | 3.46$^{cd}$ | 3.48$^{c}$ | 3.51$^{b}$ | 3.45$^{d}$ | 0.006 | <0.01 |
| $DM_{oven}$ loss, %DM | 33.5$^{a}$ | 15.3$^{b}$ | 8.92$^{c}$ | 5.20$^{c}$ | 6.95$^{c}$ | 1.098 | <0.01 |
| Gas loss, %DM | 12.9$^{a}$ | 4.60$^{b}$ | 4.39$^{b}$ | 4.39$^{b}$ | 4.13$^{b}$ | 0.209 | <0.01 |
| Gas emission[1], %DM | 13.0$^{a}$ | 4.59$^{b}$ | 4.46$^{b}$ | 4.40$^{b}$ | 4.08$^{b}$ | 0.174 | <0.01 |
| Gas pool, mL/kg DM | 73.8$^{a}$ | 23.1$^{b}$ | 22.7$^{b}$ | 22.4$^{b}$ | 20.6$^{b}$ | 1.28 | <0.01 |
| Fractional rate of gas production, /h | 0.040$^{c}$ | 0.169$^{ab}$ | 0.167$^{b}$ | 0.193$^{a}$ | 0.177$^{ab}$ | 0.0058 | <0.01 |
| Lactic acid bacteria, log cfu/g | 4.87$^{a}$ | 2.65$^{b}$ | 4.84$^{a}$ | 4.27$^{a}$ | 3.50$^{ab}$ | 0.324 | <0.01 |
| Molds, log cfu/g | <2 | <2 | <2 | <2 | <2 | - | - |
| Yeasts, log cfu/g | 4.42$^{a}$ | 2.06$^{b}$ | <2$^{c}$ | <2$^{c}$ | <2$^{c}$ | 0.447 | <0.01 |
| Aerobic stability, h | 144$^{b}$ | 143$^{b}$ | 163$^{a}$ | 163$^{a}$ | 158$^{ab}$ | 4.3 | 0.02 |

Control: without additive, LH: *Lactobacillus hilgardii* CNCM I-4785 at 3 × $10^5$ cfu/g, LB40788: *L. buchneri* NCIMB 40788 at 3 × $10^5$ cfu/g; LB4323: *L. buchneri* CNCM I-4323 at 3 × $10^5$ cfu/g, LH+LB40788: *Lactobacillus hilgardii* CNCM I-4785 at 1.5 × $10^5$ cfu/g + *L. buchneri* NCIMB 40788 at 1.5 × $10^5$ cfu/g. SEM: standard error of the mean.
[1]Estimated from the gas volume.
[a,b,c,d]Means within a row with different superscripts differ (Tukey test, α = 0.05).

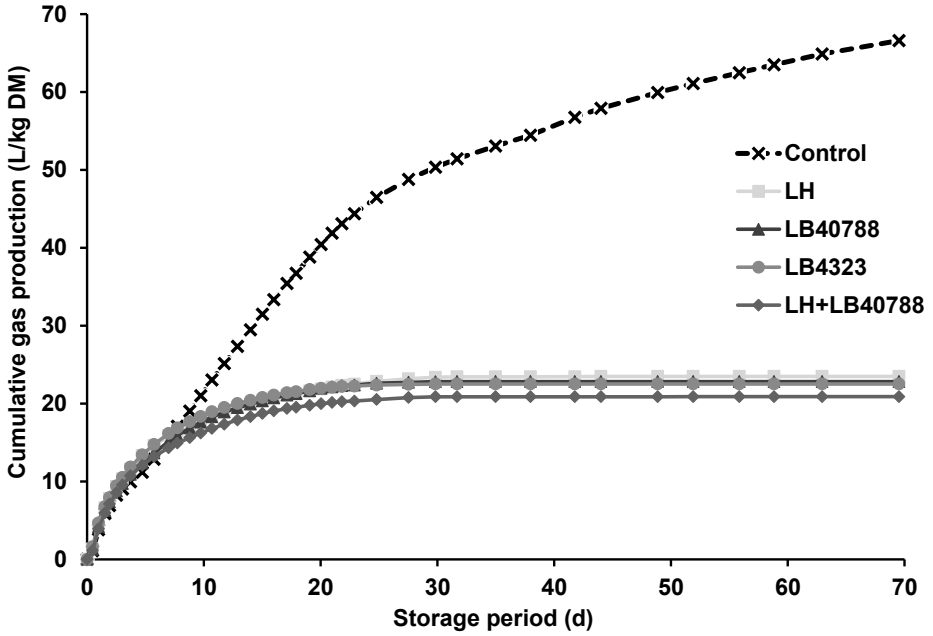


**Figure 1.** Gas production during fermentation of sugarcane silage treated with heterofermentative inoculants. Control: without additive, LH: *Lactobacillus hilgardii* CNCM I-4785 at 3 × $10^5$ cfu/g, LB40788: *L. buchneri* NCIMB 40788 at 3 × $10^5$ cfu/g; LB4323: *L. buchneri* CNCM I-4323 at 3 × $10^5$ cfu/g, LH+LB40788: *Lactobacillus hilgardii* CNCM I-4785 at 1.5 × $10^5$ cfu/g + *L. buchneri* NCIMB 40788 at 1.5 × $10^5$ cfu/g.

**References**

Daniel, J.L.P. & Nussio, L.G. (2015) A simple and reliable system for measuring gas production kinetics during silage fermentation in lab scale silos, In: Daniel, J.L.P., Morais, G., Junges D., & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 576-577.

# Effects of viable lactic acid bacteria inoculants on perennial ryegrass silage fermentation and aerobic stability

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**Keywords:** aerobic stability, fermentation, lactic acid bacteria, moulds

**Introduction** The DLG Guidelines for the testing silage additives takes into account the two main actions, which are improving the fermentation process on the one hand and improving aerobic stability on the other hand (Weiss et al. 2011). The companies producing inoculants expect that new strains and mixtures will be highly competitive to achieve a rapid pH drop through organic acid production, and some strains will demonstrate their efficacy to improve aerobic stability and increase microbial quality of silages by inhibiting spoilage moulds and yeasts. Homofermentative lactic acid bacteria (LAB) are used as inoculants to secure preservation during storage, while heterofermentative LAB are applied to silage to combat outgrowth of aerobic spoilage strains at feed-out (Milora et al., 2015). The objective of this trial was to test the effect of two silage LAB blends on fermentation characteristics and aerobic stability of perennial ryegrass silage according to the DLG (Deutsche Landwirtschafts-Gesellshaft e.V./ internationally acknowledged German Agricultural Society) Guidelines for the testing of silage additives).

**Material and Methods** This study was a randomized block design (2×1×10) to analyze two silage inoculants (*L.buchneri, L.plantarum, P.acidilactici* (T1) and *L.buchneri, L.plantarum* (T2) on one silage. A negative control (C) was included for each tested product. The first cut from a 2-year old perennial ryegrass (*Lolium perenne* L.) sward was used. Inoculants were applied at an application rate of 300 000 cfu $g^{-1}$ forage. For Objective 1in DLG to test improvements in fermentation the 3-litre mini silos were fully filled at a density of 1 kg DM/5 L (1.89 kg DM at 31.8% DM content) and for Objective 2 in DLG to test aerobic stability mini silos were fully filled in so much that the density of the silage in the silos was 2/3 of the recommended for Objective 1 (1.27 kg DM at 31.8% DM content). The mini silos were incubated for a period of 90 d for Objective 1 and for a period of 49 d for Objective 2 at a constant temperature of 20°C. For Objective 2 the silos were exposed to air stress for one d at d 28 and d 42 after ensiling. In Objective 1 and Objective 2 the counts of yeasts and moulds at the time of silage unloading and the pH value upon completion of the aerobic stability test were determined. Silages were analyzed for DM content, lactic acid and volatile fatty acids (VFA), ethanol, ammonia-N, counts of yeasts and moulds and aerobic stability (AS). AS was defined as the number of hours the silage remained stable before rising more than 3$^{o}$C above the ambient temperature. Data were analysed in PROC GLM of SAS, version 8.02, with treatment as a fixed factor. Five replications per treatment were used. Significance was declared at $P<0.05$.

**Results and Discussion** The DM (corrected for volatiles) concentrations were by 2.9 % and 2.7 % higher ($P<0.05$) and DM losses were reduced by 56.6 % and 51.0 % ($P<0.05$) for the inoculated T1 and T2 silages compared to C silages for Objective 1 (Table 1). The increased DM concentration and reduced DM losses in inoculated silages can be related to improved silage fermentation (Li et al., 2016). The T1 inoculant produced significantly more lactate and significantly less acetate than inoculant T2. The pH values after 3 days and 90 days of fermentation were significantly lower in the T1 silage. The alcohol concentration was lower ($P<0.05$) in T1 than in T2 silage. Yeast and mould counts were significantly decreased by application of T1 and T2 inoculants.

For Objective 2, the treatments increased the fermentation rate of perennial ryegrass silages, resulting in a more rapid pH drop 3 days and 49 days after ensiling. Additives T1 and T2 reduced ammonia-N concentration and decreased alcohol concentration and did not allow butyric acid formation compared to the control (C). T1 and T2 treatments produced 98.0 and 89.9 %, respectively ($P<0.05$) higher

lactate concentration than the control. The T1 treatment reduced the amount of lactate-reducing yeasts by 35.2 % (P<0.05) and reduced the amounts of moulds by 57 % (P<0.05) compared to the control silage. The T2 treatment reduced the amount of lactate-reducing yeasts by 51 % (P<0.05) and reduced the amounts of moulds by 66 % (P<0.05) compared to the control silage. Compared to the untreated silage, aerobic stability was improved (P<0.05) for T1 and T2 by 49 h and 79 h, respectively, (Objective 1), and (P<0.05) aerobic stability was improved for T1 and T2 by 34 h (1.4 days) and 46 h (1.9 days), respectively (Objective 2).

**Table 1**. Means for the fermentation, in-silo losses and microbial variables of perennial ryegrass silages untreated or treated with *L.buchneri, L.plantarum, P.acidilactici* (T1) and *L.buchneri, L.plantarum* (T2) 90 d after ensiling (Objective 1) and 49 d after ensiling (Objective 2).

| | Objective 1 | | | | Objective 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | C | T1 | T2 | $LSD_{.05}$ | C | T1 | T2 | $LSD_{.05}$ |
| Corr. DM, g $kg^{-1}$ FM | 303.3$^{b}$ | 312.1$^{a}$ | 311.4$^{a}$ | 2.899 | 299.9$^{c}$ | 310.0$^{a}$ | 307.0$^{b}$ | 2.374 |
| Lactic acid, g $kg^{-1}$ DM | 41.81$^{c}$ | 61.87$^{a}$ | 55.83$^{b}$ | 5.413 | 27.30$^{b}$ | 54.06$^{a}$ | 51.85$^{a}$ | 7.067 |
| Acetic acid, g $kg^{-1}$ DM | 22.19$^{a}$ | 17.24$^{c}$ | 20.47$^{b}$ | 1.509 | 19.99$^{a}$ | 18.78$^{a}$ | 20.30$^{a}$ | 1.933 |
| Butyric acid, g $kg^{-1}$ DM | 0.93$^{a}$ | 0.12$^{b}$ | 0.15$^{b}$ | 0.385 | 6.78$^{a}$ | 0.92$^{b}$ | 0.59$^{c}$ | 2.255 |
| Propionic acid g $kg^{-1}$ DM | 0.18$^{a}$ | 0.19$^{a}$ | 0.26$^{a}$ | 0.122 | 0.07$^{a}$ | 0.09$^{a}$ | 0.08$^{a}$ | 0.140 |
| Alcohols, g $kg^{-1}$ DM | 7.26$^{a}$ | 3.93$^{c}$ | 5.07$^{b}$ | 0.515 | 8.16$^{a}$ | 4.20$^{b}$ | 4.96$^{b}$ | 1.190 |
| pH after 3 d. | 4.99$^{a}$ | 4.32$^{c}$ | 4.44$^{b}$ | 0.035 | 5.20$^{a}$ | 4.63$^{c}$ | 4.84$^{b}$ | 0.103 |
| pH after 90 or 49 d. | 4.50$^{a}$ | 4.11$^{c}$ | 4.21$^{b}$ | 0.059 | 4.65$^{a}$ | 4.11$^{c}$ | 4.21$^{b}$ | 0.040 |
| pH after AS | 7.74$^{a}$ | 4.67$^{b}$ | 4.44$^{b}$ | 0.433 | 8.80$^{a}$ | 8.28$^{b}$ | 8.13$^{c}$ | 0.134 |
| Ammonia-N, g $kg^{-1}$total N | 58.02$^{a}$ | 36.11$^{c}$ | 42.08$^{b}$ | 2.850 | 62.36$^{a}$ | 40.79$^{b}$ | 43.75$^{b}$ | 5.508 |
| DM loss, g $kg^{-1}$ | 53.04$^{a}$ | 23.04$^{b}$ | 26.00$^{b}$ | 10.00 | 66.88$^{a}$ | 31.34$^{c}$ | 40.89$^{b}$ | 7.635 |
| Yeast, log cfu $g^{-1}$FM | 3.29$^{a}$ | 1.31$^{b}$ | 1.12$^{b}$ | 0.393 | 6.13$^{a}$ | 3.97$^{b}$ | 3.00$^{c}$ | 0.551 |
| Mould, log cfu $g^{-1}$FM | 3.01$^{a}$ | 1.22$^{b}$ | 1.06$^{b}$ | 0.399 | 4.49$^{a}$ | 1.94$^{b}$ | 1.51$^{c}$ | 0.387 |
| Aerobic stability, hours | 79.2$^{c}$ | 128.4$^{b}$ | 158.4$^{a}$ | 12.45 | 54.0$^{b}$ | 87.6$^{a}$ | 99.6$^{a}$ | 13.99 |
| | $t_{0.05}$=2.179; Error df=12 | | | | | | | |

Dry matter, calculated dry matter losses and fermentation parameters are corrected for volatiles (Weißbach, 2005)

$^{a, b, c}$ – Means with different superscripts within a row indicate significant differences of P<0.05*;*

T1-T2 – See text in the Materials and methods for explanation of treatments T1-T2; AS – aerobic stability

**Conclusion** The inoculants T1 and T2 generally had a positive effect on the characteristics of perennial ryegrass silages (in both Objective 1 and Objective 2) in terms of a more efficient fermentation, resulting in lower pH, improved fermentation product profile and a lower dry matter loss. The inoculants were effective in limiting the degradation of protein, lowering growth of moulds and enhancing silage aerobic stability.

**References**

Li, P., Bai, S., You, M., & Shen, Y. (2016) Effects of maturity stage and lactic acid bacteria on the fermentation quality and aerobic stability of Siberian wildrye silage. Food Science and Nutrition, 4 (5), 664-670.

Milora, N., Hindrichsen, I.K., Richelieu, M., & Geppel, A. (2015) Developing a novel dual purpose silage inoculant. In: Daniel J.L.P. et al. (eds.) Proceedings of the XVII International Silage Conference. Piracicaba, Brazi, 2015, pp. 352-353.

Weißbach, F. (2005) A simple method for the correction of fermentation losses measured in laboratory silos. Proceedings of the XIV International Silage Conference. Belfast, Norhern Ireland, 2005, p. 278.

Weiss, K., Gerlach K., & Südekum, K.-H. (2011) Flüchtige Substanzen in Maissilagen in Abhängigkeit von Silierbedingungen und aerober Lagerungsdauer. Kongressband 2011, VDLUFA-Schriftenreihe 67, p. 534–540 (in German).

# Ensiling of crimped faba beans decreased selected antinutritional factors

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**Keywords:** convicine, fermentation, formic acid, lactic acid bacteria, tannin, *Vicia faba*, vicine

**Introduction** Faba bean (*Vicia faba*) is a grain legume, which can be used for both human and animal nutrition. In Northern Europe, it may not fully ripen due to a short growing season. Further, humid autumns result in low dry matter (DM) of the beans resulting in high energy demand for drying them. Crimping and ensiling provides an alternative method of preserving the beans instead of drying. The antinutritional factors present in faba beans may limit the use of them particularly for single stomached animals. Fermentation has been used as a means to reduce their concentrations (Gefrom et al. 2013). The main objective of the present study was to compare the effects of different additives on the fermentation quality and aerobic stability of ensiled crimped high moisture faba beans. In addition, the effects of different types of fermentation induced by additives on the concentrations of antinutritional factors present in faba beans were evaluated. As DM concentration is a major factor affecting the fermentation quality and via the extent of fermentation potentially also the modification of antinutritional factors, an additional factor of increased moisture was included.

**Material and Methods** Faba bean (cv. Kontu) was combine harvested on 7 October 2015 in Tammela, Finland and ensiled in pilot scale in three replicate silos using 7 treatments: 1) control (C) without any additive, 2) combination of two commercial lactic acid bacteria inoculants (LABmix; Josilac® Classic, Josera, Kleinheubach, Germany and Bonsilage, Schaumann, Pinneberg, Germany; $6.25\times10^5$ CFU/g), 3) Luke in-house produced LAB inoculant (LABLuke, $1\times10^6$ CFU/g), 4) Formic acid based product (FA; AIV2 Plus, Eastman Chemical Company, Oulu, Finland; 6 l/ton), 5) Antimicrobial chemical additive (AMC; SafeSil, Salinity Agro, Västra Frölunda, Sweden; 4 l/t), 6) Control with tap water addition (C+water) and 7) LABmix with tap water addition (LABmix+water). The silos were opened after a 106 day ensiling period and sampled for fermentation quality, antinutritional factors and aerobic stability using routine methods of Luke (for details, see Seppälä et al. 2016).

**Results and Discussion** The faba beans prior to ensiling had a DM concentration of 557 g/kg, and the ash, crude protein, starch and NDF concentrations were 41, 294, 352 and 189 g/kg DM, respectively. The average DM of ensiled faba beans without water addition was 562 and with water addition 508 g/kg and it is obvious that even the treatments without water addition were exceptionally moist (Table 1). The beans ensiled without additive showed elevated pH, butyric acid and ethanol concentrations and $NH_3$-N proportion and can be described as poorly preserved. Addition of water decreased the fermentation quality even further. The LAB inoculants improved the fermentation quality of the beans compared to C, and FA efficiently restricted the fermentation. Use of both LAB and chemical additives improved aerobic stability compared to C. Microbiological quality was improved during ensilage, especially the counts of moulds decreased considerably in all treatments (4.8 log CFU/g in raw material vs. <2.0-<2.6, 3.0-3.3, 2.4-<3.6, <2.0-<2.6, 2.4-<2.6, <2.0-<2.6 and 2.5-2.7 log CFU/g for C, C+water, LABmix, LABmix+water, LABLuke, FA and AMC, respectively). AMC was especially effective in improving the aerobic stability.

The vicine and convicine concentrations of the original faba beans were 9.76 and 5.69 g/kg DM, respectively. No vicine could be detected in the ensiled faba beans and also the concentration of convicine was greatly reduced. The proportion of convicine remaining after the ensiling process was 0.17, 0, 0.18, 0.07, 0.22, 0.41 and 0.24 for treatments C, C+water, LABmix, LABmix+water, LABLuke, FA and AMC, respectively. It seems that promoting microbial activity in the silo either by LAB inoculation or by increased moisture promoted the degradation of convicine, while chemical additives

decreased the degradation of it simultaneously as they restricted fermentation. Also tannin was degraded to some extent during the ensiling period. The original tannin concentration in the faba beans was 10.9 g/kg DM and in treatments C, LABmix and FA ca. 0.67 of the original amount of tannin could be detected while in AMC the proportion was as low as 0.32. This may indicate improved protein digestibility and palatability especially for single stomached animals.

**Conclusion** Crimping and ensiling is an alternative preservation method for faba beans which eliminates the energy consumption and investments required for drying. The fermentation quality of moist beans may be poor, but it can be improved by using additives. In addition, reduced concentrations of antinutritional factors may be expected during the ensiling process.

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**Table 1**. Fermentation quality, concentrations of antinutritional factors and aerobic stability of crimped faba beans ensiled using different additive treatments.

| | DM, g/kg | pH | $NH_3$-N, g/kg N | In DM, g/kg | | | | | | Aerobic stab., h |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ethanol | LA | AA | BA | Convicine | Tannins | |
| Additive treatment | | | | | | | | | | |
| Control (C) | 552 | 5.38 | 117 | 18.2 | 27.5 | 7.3 | 0.34 | 0.97 | 7.6 | 61 |
| C+water | 505 | 5.10 | 154 | 15.3 | 35.1 | 10.1 | 1.48 | 0 | 10.3 | 66 |
| LABmix | 563 | 4.29 | 80 | 8.0 | 48.3 | 7.4 | 0.07 | 1.00 | 7.1 | 79 |
| LABmix+water | 512 | 4.27 | 100 | 7.4 | 53.8 | 6.9 | 0.07 | 0.40 | 9.4 | 65 |
| LABLuke | 560 | 4.29 | 67 | 5.9 | 57.3 | 5.7 | 0.07 | 1.25 | 9.3 | 100 |
| FA | 572 | 4.92 | 34 | 3.3 | 7.3 | 1.8 | 0.09 | 2.32 | 7.2 | 83 |
| AMC | 564 | 4.61 | 61 | 9.1 | 33.8 | 7.8 | 0.09 | 1.34 | 3.5 | 210* |
| SEM | 1.0 | 0.048 | 1.7 | 0.6 | 1.65 | 0.25 | 0.119 | 0.07 | 0.36 | 8.8 |
| Statistical significance of contrasts | | | | | | | | | | |
| Water | <0.001 | 0.008 | <0.001 | 0.011 | 0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.594 |
| C vs all | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| C vs LAB | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.038 | 0.084 | 0.089 | 0.197 | 0.021 |
| C vs chem | <0.001 | <0.001 | <0.001 | <0.001 | 0.004 | <0.001 | 0.106 | <0.001 | <0.001 | <0.001 |
| LAB vs chem | <0.001 | <0.001 | <0.001 | 0.228 | <0.001 | <0.001 | 0.871 | <0.001 | <0.001 | <0.001 |
| FA vs AMC | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.979 | <0.001 | <0.001 | <0.001 |

DM = dry matter; LA = lactic acid; AA = acetic acid; BA = butyric acid; LABmix = mixture of two commercial lactic acid bacteria (LAB) inoculants; LABLuke = LAB isolated by Luke; FA = formic acid based additive; AMC = antimicrobial chemical additive; SEM = standard error of the mean

*Difference between ambient and sample temperature did not reach 2 °C during the measuring period of 210 h.

Description of contrasts: Water: C and LABmix vs. C+water and LABmix+water; C vs all: C and C+water vs. all other treatments; C vs LAB: C vs LABmix and LABLuke; C vs chem: C vs FA and AMC; LAB vs chem: LABmix and LABLuke vs FA and AMC; FA vs AMC: FA vs AMC.

## References

Gefrom, A., Ott, E.M., Hoedtke, S. & Zeyner, A. (2013) Effect of ensiling moist field bean (*Vicia faba*), pea (*Pisum sativum*) and lupine (*Lupinus spp*.) grains on the contents of alkaloids, oligosaccharides and tannins. Animal Physiology and Animal Nutrition, 97, 1152-1160.

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M. (2016) Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass and Forage Science, 71, 458-471.

# Ensiling wet lucerne with biological or formic acid based silage additives

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**Keywords:** alfalfa, fermentation, quality

**Introduction** Restricted silage fermentation can improve silage intake (Huhtanen et al. 2007), ruminal microbial protein synthesis (Jaakkola et al. 2005) and raise milk protein and fat concentration (Huhtanen et al. 2003). In silo fermentation can be restricted by prewilting or by using a strong chemical silage additive. Effective prewilting is sometimes challenged by variable rainy weather or a combination of coldness and high air humidity. The aim of the present work was to investigate the effect of biological and chemical silage additives on the fermentation quality and aerobic stability of slightly prewilted lucerne.

**Material and Methods** A budding stage lucerne was manually harvested 19 October close to Rostock, Germany. After a brief prewilting indoors to 20.6 % dry matter (DM), lucerne was chopped (chop length 4 cm) and treated with additive treatments in quadruplicate. Treatments were a control without additive, a treatment with inoculation of *Lactobacillus plantarum* and four treatments with buffered formic acid based silage additives (Table 1). Treated forage (880 g) was packed into 1.5 L glass jars. Silos were stored in 25°C and opened after 60 days. The silage extracts were analysed for pH, lactic acid (HPLC), volatile fatty acids, ethanol (GC) and $NH_3$ (Conway method). The aerobic stability was determined by means of temperature measurement. Statistical test was conducted by analysis of variance and using either Student-Newman-Keuls or Dunnett's test.

**Results** Prior to ensiling the lucerne had DM 20.6 %, sucrose 31 g/kg DM and buffering capacity 43 g lactic acid/kg DM. Control and Inoculant silages showed a low fermentation quality with high contents of undesirable products from secondary fermentation (Table 1). Formic acid treatments improved silage fermentation quality by lowering silage pH and reducing sum of volatile fatty acids from above 147 to below 30 g/kg DM. Only high application (4.8 kg/t) of formic acid could restrict lactic acid fermentation. All investigated silages were aerobically stable for 14 d, presumably due to the high occurrence of organic acids.

**Table 1**. Fermentation quality of the lucerne silages and weight losses during ensiling.

| | Control | Inoculant | FA1 | FA2 | FA3 | FA4 |
|---|---|---|---|---|---|---|
| Applied on previlted lucerne | | | | | | |
| total formic acid[1], kg/t | | | 3.1 | 3.4 | 4.4 | 4.8 |
| propionic acid, kg/t | | | 0.7 | 0.6 | 1.0 | 0.0 |
| losses during ensiling[2], % | 2.57$^{a}$ | 2.63$^{a}$ | 0.62$^{b}$ | 0.63$^{b}$ | 0.29$^{b}$ | 0.43$^{b}$ |
| Silage quality | | | | | | |
| Dry matter, g/kg | 19.3$^{b}$ | 19.4$^{b}$ | 20.7$^{a}$ | 20.5$^{a}$ | 21$^{a}$ | 20.5$^{a}$ |
| pH | 5.83$^{b}$ | 5.76$^{c}$ | 4.71$^{d}$ | 4.63$^{d}$ | 4.38$^{d}$ | 4.61$^{d}$ |
| In dry matter, g/kg DM | | | | | | |
| Sugars | ND | ND | ND | ND | ND | 21±0.4 |
| Formic acid | ND | ND | 14.4$^{b}$ | 16.8$^{b}$ | 21.3$^{a}$ | 23.5$^{a}$ |
| Lactic acid g/kg DM | ND | ND | 72.3$^{a}$ | 64$^{a}$ | 77.3$^{a}$ | 40.2$^{b}$ |
| Acetic acid g/kg DM | 68.4$^{a}$ | 64.5$^{a}$ | 13$^{c}$ | 12$^{c}$ | 8.4$^{c}$ | 8.8$^{c}$ |
| Propionic acid g/kg DM | 33.4$^{a}$ | 30.6$^{a}$ | 3.7$^{c}$ | 3.5$^{c}$ | 4.3$^{c}$ | 0.8$^{d}$ |
| Butyric acid g/kg DM | 45.9$^{c}$ | 54.1$^{b}$ | 12.4$^{d}$ | 11.1$^{d}$ | 1.9$^{d}$ | 8.2$^{d}$ |
| Ethanol | 15.6$^{a}$ | 11.2$^{b}$ | 4.2$^{c}$ | 3.7$^{c}$ | 2.9$^{c}$ | 3.5$^{c}$ |
| Ammonium-N g/kg total N | 366.4$^{a}$ | 361$^{a}$ | 97.6$^{b}$ | 104.5$^{b}$ | 81.7$^{b}$ | 86.3$^{b}$ |

Tested treatments were Inoculant *Lactobacillus plantarum* $10^{9}$ *cfu/g;* FA Treatments were commercial formic acid based blends FA1 and FA3 = AIV Pro NC, FA2 = AIV 2000 Plus Na and FA4 = AIV 2 Plus Na (Eastman Chemical Company, Oulu, Finland). [1]Sum of free formic acid and salts of formic acid calculated as formic acid. [2]calculated as % of the initial plant material weight before ensiling. ND = not detected. VFA= sum of acetic, propionic and butyric acids. Means with different superscripts within a row differ with $P < 0.05$ (n = 4).

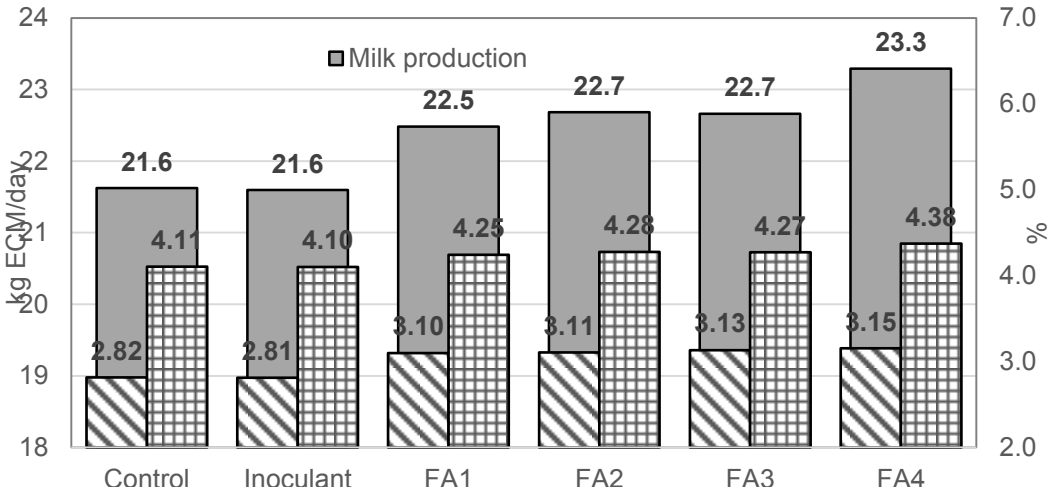


**Figure 1**. Effects of fermentation quality on milk production parameters calculated based of review of Huhtanen et al. (2003). Linear effect of silage total fermentation acids on milk production (Energy corrected milk ECM kg/day) and milk fat concentration and multiple regression between silage fermentation parameters (sugar and volatile fatty acid concentration) and milk protein concentration were used in calculations. Treatments see Table 1.

**Discussion** Lucerne is a valuable forage but difficult to ensile especially if weather is not suitable for proper prewilting. In these situations, strong silage additive is needed to prevent secondary fermentation. While formic acid (100 %) application was 3.1 - 4.4 kg/t fresh weight the fermentation was dominated by lactic acid production, while all the sugars were spent in fermentation. Only the strongest application level (4.8 kg/t) was strong enough to restrict the fermentation, which was shown as the lowest amount of total fermentation acids and highest amount of residual sugars present in the silage. Regression equations presented by Huhtanen et al. (2003) were used to quantify importance of the silage fermentation quality. Substantial improvements can be achieved both in milk production (1,67 kg more milk per cow per day) and in milk composition (0,27 and 0,34 % -unit higher fat and protein concentrations respectively) by restricting silage fermentation by sufficient formic acid application (Figure 1). Results of this trial clearly demonstrate, that restriction of fermentation is only achieved with sufficient application level.

**Conclusion** These results confirm that wet lucerne should be ensiled with sufficient amount of formic acid. Moderate application level helps avoiding high amounts of volatile fatty acids while application 4.8 kg/t can restrict the total amount of fermentation. Benefits of restricted fermentation are known based on literature, those include improvements in intake and milk production, ruminal microbial protein synthesis and milk composition.

**References**

Huhtanen P., Nousiainen, J.I., Khalili, H., Jaakkola, S. & Heikkilä, T. (2003) Relationships between silage fermentation characteristics and milk production parameters: analyses of literature data. Livestock Production Science, 81, 57-73.

Huhtanen P., Rinne M. & Nousiainen J. (2007) Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal, 1, 758-770.

Jaakkola, S., Kaunisto, V. & Huhtanen, P. (2006) Volatile fatty acid proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. Grass and Forage Science, 61, 282-292.

# Evaluation of silage additives and fermentation characteristics of maize forage using laboratory and field scale silo

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**Keywords:** aerobic exposure, big bale, fermentation, mini-silo's

**Introduction** Successful preservation of forage crops as silage depends on the production of sufficient amount of organic acids in the right ratio to inhibit the activity of undesirable microorganisms under anaerobic conditions (Muck, 2012). During feed out silage exposed to air would cause rapid yeast proliferation, the rapid rise in temperature and decline in sensory and nutritive quality (Dolci et al., 2011). This study was aimed to determine and to compare fermentation characteristic, microbial population and aerobic stability of inoculated maize forage ensiled in laboratory-scale and big bale silos.

**Material and Methods** Whole crop maize (*Zea mays* L.) was harvested at dough line stage adjusted to achieve a 10 mm theoretical cut length and packed immediately into 1.2 m diameter × 1.2 m height round bales using equipment Göweil LT-Master (GÖWEIL Maschinenbau GmbH, Germany**)** and into a 3 L laboratory mini silo.Maize forage was either untreated (T1) or treated with the bacterial silage inoculant SiloSolve FC containing *Lactococcus lactis* DSM 11037/1k208 and *Lactobacillus buchneri* DSM 22501/1k20738 (50:50) at $1.5 \times 10^{-5}$ colony forming units $g^{-1}$ (cfu $g^{-1}$) of herbage (T2). Big bales and laboratory silos remained undisturbed for120 din farm and laboratory conditions, respectively. For each treatment five big bales chosen at random and five laboratory silo were sampled for chemical and microbial analyses and five were designated for aerobic stability test. Samples were analyzed for the DM content, nutrient content and fermentation parameters as well as pH, silage acids (lactic, acetic, butyric and propionic acids), alcohols and ammonia, and lactobacilli, yeast and mould number. From bales designated for exposure to air for 39 d were removed plastic cover and each bale was fitted with two thermocouple wires. For aerobic stability test on the laboratory scale, a 1000 g sample from each mini-silo was loosely placed into a polystyrene box fitted with temperature sensor and allowed to aerobically deteriorate for 19 d at constant room temperature (~20ºC). Aerobic stability was defined as the number of hours the silage remained stable before rise more than $3^{o}$ C above the ambient temperature.

**Results**The use of viable LAB lead to a significantly lower pH, a reduction in protein breakdown and ammonia N concentrations and an increase of the concentration of lactic and acetic acids in both big bale and laboratory silages compared with control silages. Regardless of silo types, a significant reduction in DM losses during fermentation and aerobic exposure period was found in the T2B and T2L silagescompared to the T1B and T1L silages (Table 1). Lower yeast and mould counts in the inoculated silages at opening and after aerobic exposure of silos correlated with a higher aerobic stability of LAB treated silages compared with control silages.The application of the silage inoculant improved the aerobic stability in the laboratory silos and the round bales and gave a significant temperature response to treatment. The silages without additives heated up earlier and temperature increase was stronger in comparison to the bales or laboratory silos with inoculant (Figure 1, Table 1). Results suggest that the aerobic metabolism of inoculated silages reduced nutrient oxidation due to significantly less mould and yeast number in big bale and laboratory silages and significantly reduced visible mould cover being present on big bales surface (Table 1). All the parameters used to measure aerobic deterioration, pH rise, maximum temperature above ambient and sum of temperature rise, yeast and mould population showed a good correlation with dry matter loss during air exposure.

**Table 1.**Chemical composition, DM loss, fermentation pattern and microbial counts of big bale and laboratory silages after a120 day ensiling and after exposure to air.

| Treatment | Big bale silage | | | Laboratory mini silage | | |
|---|---|---|---|---|---|---|
| | T1B | T2B | SE | T1L | T2L | SE |
| | | | After a120 day of ensiling | | | |
| DMC, g $kg^{-1}$ | 315.6 | 323.4* | 1.722 | 315.9 | 320.7** | 0.587 |
| DM loss, g $kg^{-1}$ | 85.9 | 54.7** | 3.142 | 67.7 | 49.4** | 1.536 |
| Crude protein, g $kg^{-1}$ DM | 80.0 | 88.8** | 1.208 | 70.8 | 80.9* | 2.231 |
| $NH_3$-N, g $kg^{-1}$ total N | 72.15 | 55.65** | 2.154 | 81.43 | 61.54** | 3.412 |
| Alcohols, g $kg^{-1}$ DM | 16.86 | 11.21** | 0.949 | 20.63 | 11.45** | 0.747 |
| Lactic acid, g $kg^{-1}$ DM | 22.92 | 39.98** | 1.202 | 37.75 | 65.69** | 2.403 |
| Acetic acid, g $kg^{-1}$ DM | 8.54 | 17.47** | 0.456 | 19.01 | 33.73** | 1.063 |
| Butyric acid, g $kg^{-1}$ DM | 2.20 | 0.37** | 0.241 | 1.19 | 0.15** | 0.119 |
| Propionic acid, g $kg^{-1}$ DM | 0.56 | 0.70** | 0.080 | 0.82 | 1.27** | 0.076 |
| pH | 4.07 | 3.91** | 0.009 | 3.91 | 3.79** | 0.013 |
| LAB, $log_{10}$cfu $g^{-1}$ FM | 5.60 | 7.27** | 0.162 | 6.69 | 8.14** | 0.174 |
| Yeast, $log_{10}$cfu $g^{-1}$ FM | 4.55 | 2.36** | 0.169 | 3.79 | 1.56** | 0.097 |
| Mould, $log_{10}$cfu $g^{-1}$ FM | 2.56 | 1.22** | 0.091 | 2.05 | 1.06** | 0.051 |
| Visual mould score | 0.40 | 0.00** | 0.000 | - | - | - |
| | | | After aerobic exposure | | | |
| DM loss during AE, g $kg^{-1}$ | 24.2 | 12.4** | 1.523 | 29.5 | 20.5** | 0.980 |
| pH | 4.93 | 4.31** | 0.028 | 7.27 | 4.05** | 0.121 |
| LAB, $log_{10}$ cfug$^{-1}$ FM | 5.35 | 7.16** | 0.184 | 6.27 | 8.02** | 0.099 |
| Yeast, $log_{10}$cfu $g^{-1}$ FM | 8.09 | 3.76** | 0.111 | 8.52 | 4.47** | 0.124 |
| Moulds, $log_{10}$cfu $g^{-1}$ FM | 6.11 | 3.68** | 0.233 | 7.80 | 4.34** | 0.102 |
| Aerobic stability,h | 406.8 | 715.2** | 33.670 | 86.40 | 338.40** | 5.724 |

DM – dry matter, DMC – dry matter corrected for volatiles, AE – aerobic exposure, LAB – lactic acid bacteria, cfu – colony forming units, FM - fresh matter, T1B – control big bale, T2B – inoculated big bale, T1L - control laboratory silos, T2L – inoculated laboratory silos,* and ** statistically significant difference P<0.05 and P<0.01.

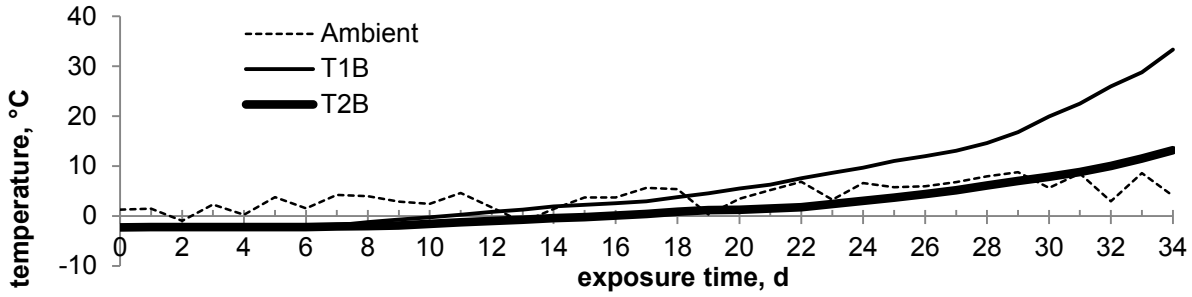


**Figure 1.** Temperature inside big bales inoculated (T2B) and control (T1B) during air exposure period.

**Conclusion** Applying viable bacteria inoculant containing *Lactococcus lactis* DSM 11037/1k208 and *Lactobacillus buchneri* DSM 22501/1k20738 affected fermentation pattern, yeast and mould count, and aerobic stability of maize ensiled in big bales and laboratory silos in a directional manner.The similarities observed between the big bale and laboratory silages showed that small-scale silages can resemble specific silo and can serve as a model for big bale silage and when testing silage additives.

**References**

Muck, R. (2012) Microbiology of ensiling.In: Kuoppala, K., Rinne, M., & Vanhatalo, A. (eds). Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 75-86.

Dolci, P., Tabacco, E. & Borreani, G. (2011) Microbial dynamics during aerobic exposure of corn silage stored under oxygen barrier or polyethylene films. Applied and Environmental Microbiology, 77, 7499-7507.

# Fermentation and aerobic stability of grass and grass-legume silages ensiled for 14 days

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**Keywords:** aerobic stability, early opening, *Lactobacillus buchneri*, silage inoculant, spoilage

**Introduction** During the silage making process, air present in the forage is undesirable. Specifically, oxygen facilitates the growth of aerobic spoilage organisms such as yeast and mold that may compromise preservation and hygienic quality of the silage. Additionally, when the silo bunker is opened and exposed to air, it needs to remain stable in order to preserve its nutritive quality and to minimize negative effects when fed to livestock. Silage inoculants can help to reduce levels of yeast and mold and further suppress their growth, thereby enhancing aerobic stability of silage when exposed to air (Kung et al., 2003). The objective of this study was to evaluate the effectiveness of using a silage inoculant on microbial composition and aerobic stability of two different crops after only two weeks of fermentation.

**Material and Methods** Six mini-silos for each treatment (three vacuum bags, 502 ± 2.1 g; three plastic buckets, 508 ± 161 g) were filled with two different wilted crops: either pure grass ensiled at 120 kg DM/$m^3$ (46.5% DM; Exp.1) or grass/legume mixture (8% Red clover, 9% White clover, 23% Hybrid ryegrass, 60% Perennial ryegrass) ensiled at 190 kg DM/$m^3$ (28.9% DM; Exp.2). Control (CTR) with no inoculant or SiloSolve® FC (FC), containing 50:50 of *Lactobacillus buchneri* (LB 1819; DSM22501/1k20738) and *Lactococcus lactis* (LL O224, DSM11037/1k2081) was applied at a target rate of 150,000 cfu/g of forage. Mini-silos were stored at 25°C for 14 days. Forages were analysed for pH, DM, lactic acid bacteria, yeast and mold count before ensiling (day 0, data not shown). Vacuum bags were used for total lactic acid bacteria, yeast and mold count, pH and DM measurement after 14 days of fermentation. The other three bucket mini-silos were used to evaluate aerobic stability (AS). After fermentation, each mini-silo was challenged aerobically for 7 days. The temperature inside the mini-silo was monitored with a data logger. By definition, AS is determined by the amount of time it takes the silage temperature to exceed the ambient temperature by 3°C (EFSA, 2018). Total yeast and mold were enumerated on wilted material prior to ensiling (day 0), after fermentation when vacuum bags were opened and at the end of the AS test. For each experiment, data were analysed separately before and after AS test by adopting a completely randomized design using the GLM procedure in SAS. The main tested effect was treatment (CTR vs. FC) and the mini-silo was the experimental unit.

**Results** Using a FC inoculant resulted in an increase of lactic acid bacteria present in the treated silages after two weeks of fermentation, and before and after AS (Exp. 1 and 2). FC significantly reduced the level of yeast after two weeks of fermentation and was more effective in Exp.2 (P=0.0002). Reduction of yeast (P=0.003) and mold (P=0.04) growth was observed after aerobic challenge in Exp.2. The stability of the silage face was enhanced by three days when using the silage inoculant only in Exp.1 (data not shown) despite no clear reduction of yeast and mold in this experiment (table 1).

**Discussion** These results clearly indicate that hetero-fermentative *L. buchneri* LB1819 in combination with homo-fermentative *L. lactis* LL-O224 was able to control yeast and mold growth after a short period of fermentation in two different crops. Further, the present results are in agreement with several previous studies that reported improvement in aerobic stability of different silages treated with the same combination of hetero- and homo-fermentative bacteria (Jatkauskas et al. 2013; Witt et al., 2015; Copani et al. 2017). Yeast generally drives the aerobic deterioration of silage and mold completes this process leading to rising temperature of the unstable silage. However, König et al. (2012) showed that using pure *L. buchneri* as inoculant in a crop with low dry matter content did not

result in an increase in aerobic stability. Yet, despite FC not yielding enhanced aerobic stability in Exp. 2, pH was numerically lower than untreated, and yeast and mold counts significantly lower, suggesting that the combination of *L. buchneri* and *L. lactis* may have another mode of action.

**Table 1**. Silage characteristics and microbiological status, before and after aerobic exposure (AS), on grass (Exp.1) or grass/legume mixture (Exp.2) inoculated with SiloSolve® FC (FC) or without additive (Control, CRT) after two weeks of fermentation.

| Items | Exp. 1 | | | | Exp. 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Treatment | | s.e.m. | P-value | Treatment | | s.e.m. | P-value |
| | CTR | FC | | | CTR | FC | | |
| *Before AS* | | | | | | | | |
| DM, % | 46.3 | 46.8 | 0.21 | 0.212 | 29.19 | 28.39 | 0.24 | 0.0784 |
| pH | 4.60 | 4.45 | 0.047 | 0.086 | 4.1 | 4.1 | 0.02 | 0.3331 |
| *Total count* ($log_{10}$ cfu/g) | | | | | | | | |
| Lactic acid bacteria | 9.14 | 9.88 | 0.060 | 0.001 | 8.8 | 9.7 | 0.08 | 0.0012 |
| Yeast | 4.87 | 4.10 | 0.251 | 0.096 | 6.5 | 3.9 | 0.14 | 0.0002 |
| Mold | 3.55 | 3.00 | 0.389 | 0.374 | 4.0 | 2.0 | 0.00 | <0.0001 |
| *After AS* | | | | | | | | |
| DM, % | 47.5 | 46.8 | 0.83 | 0.589 | 26.03 | 26.89 | 0.45 | 0.2506 |
| pH | 8.03 | 7.09 | 0.474 | 0.233 | 5.4 | 4.7 | 0.347 | 0.2082 |
| *Total count* ($log_{10}$ cfu/g) | | | | | | | | |
| Lactic acid bacteria | 9.81 | 10.24 | 0.081 | 0.020 | 10.0 | 10.7 | 0.053 | 0.0006 |
| Yeast | 8.80 | 8.92 | 0.053 | 0.200 | 9.1 | 7.9 | 0.075 | 0.0003 |
| Mold | 9.19 | 9.02 | 0.068 | 0.148 | 9.1 | 8.6 | 0.108 | 0.0451 |

**Conclusion** The present studies further confirm the efficacy of combining specific strains of *L. buchneri* and *L. lactis* as a silage inoculant to control yeast and mold growth after a short period of fermentation. Additionally, the use of FC could provide more flexibility in silage face management due to its ability to mitigate growth of spoilage microorganisms and maintain enhanced silage stability.

**References**

Copani, G., Nielsen, N. G., Bryan, K. A., Vrotniakiene, V., Jatkauskas, J. & Witt, K.L. (2017) Effects of a dual strain inoculant on alfalfa silage fermentation and aerobic stability through 32 days of ensiling, In: Nussio, L.G, De Sousa, D.O., Gritti, V.C., De Souza Salvati, G.G., Dos Santos, W., P. & Salvo, P.A.R. (eds). Proceedings of the V International symposium on forage quality and conservation, Piracicaba, Sao Paulo, Brazil, 2017.

EFSA Scientific Opinion (2018) Guidance on the assessment of the efficacy of feed additives, EFSA Journal, 16(5):5274, 6-7.

Jatkauskas, J., Vrotniakiene, V., Ohlsson, C. & Lund, B. (2013) The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agricultural and Food Science, 22 (1), 137-144.

König, W., Puhakka, L. & Jaakkola, S. (2002) The effect of lactic acid bacteria-based additives and wilting on grass silage fermentation characteristics, In: Kuoppala, K., Rinne, M., & Vanhatalo, A. (eds). Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 378-379.

Kung, L. Jr, Stokes, M.R. & Lin, C.J. (2003) Silage additives, In: Buxton, D.R., Muck, R.E., & Harrison, J.H. (eds) Silage Science and Technology. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. Publications, Madison, Wisconsin, USA, 2003, pp. 305-360.

Witt, K.L., Jatkauskas, J., Vrotniakiene, V. & Voss, M.T. (2015) Effects of a novel dual purpose silage additive on aerobic stability and fermentation characteristics of whole crop maize silage after a short time of ensiling, In: Daniel, J.L.P., Morais, G., Junges, D., & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Sao Paulo, Brazil, 2015, pp. 82-83.

# Fermentation profile and aerobic stability of sugar cane silage inoculated with *Lactobacillus buchneri* NCIMB 40788

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**Keywords:** ethanol, *Lactobacillus buchneri*, losses, silage, sugar cane

**Introduction** Sugar cane (*Saccharum officinarum L.*) silage is an interesting option for farmers in the Northwest of Argentina, because of the advantages of this technique compared to the traditional fresh consumption of the sugar cane. One of the factors which still inhibit the expansion of sugar cane silage as a conservation technique in northwestern Argentina is its alcoholic fermentation, due to the intense activity of yeasts that turn the sugars to ethanol, $CO_2$ and water. Ethanol levels in the order of 8 to 17% of dry matter (DM) have been reported for sugar cane silage without additives, with total dry matter losses (DML) of up to 29% (Pedroso et al. 2004). Lower values in ethanol production and in DML, together with an increase in hours of aerobic stability (AS), were found in sugar cane silages inoculated with urea and with bacterial additives containing *Lactobacillus buchneri* (Kung 2001, Queiroz 2006, Schmidt 2006, Pedroso et al. 2007 and 2008). The objective of this experiment was to evaluate the effect of chemical and bacterial additives with *Lactobacillus buchneri* NCIMB 40788 on ethanol production, DML and AS of sugarcane silage.

**Material and Methods** Mature sugar cane, variety Lcp 85-384 (13 months of vegetative growth, second cut), was hand harvested, processed and ensiled in 20 L plastic buckets provided with valves for gas release and a device for effluent collection, according to the methodology proposed by (Queiroz, 2006; Pedroso et al., 2007 and 2008). The following treatments were applied to the fresh forage before ensiling: 1) untreated (control), 2) urea ($CO(NH_2)_2$) at 1% of fresh forage (FF), 3) bacterial inoculant with heterolactic bacteria *Lactobacillus buchneri* (LAB) strain NCIMB 40788 ($1 \times 10^5$ CFU $g^{-1}$ of FF). Laboratory silos were kept at ambient temperature and sampled 60 and 120 days of ensiling (D.E.). Four repetitions were made for each combination of treatments and D.E. For AS evaluation, samples of approximately 3.5 kg of each replicate were transferred to plastics buckets kept at room temperature. Temperature was recorded every three hours through thermometers positioned at the centre of the forage mass. Also in relation to AS, was calculated the cumulative daily average difference between silage and room temperature, after five and ten days of air exposure (CDAD-5 and CDAD-10). Data were analyzed as a completely randomized design and subjected to ANOVA by InfoStat® software (Di Rienzo et al. 2011). Differences among means were tested using Tuckey's test ($p < 0.05$).

**Results and discussion** LAB treated silage showed the lowest ethanol production with respect to the other treatments, with values with highly differences between treatments ($p<0.001$). The AS results obtained 60 D.E. show differences ($p<0.05$) between the three treatments, obtaining the best result in the treatment with LAB. The AS results obtained 120 D.E., show no differences between the urea treatment and the LAB treatment ($p>0.05$). CDAD-5 and CDAD-10 were also lowest in LAB treated silage with highly differences between the three treatments ($p<0.001$) in results obtained 60 D.E., indicating that even after deterioration started microbial activity was less intense. The conversion of lactic acid to acetic acid by LAB and the fungicidal effect of this acid is probably the main explanation for these results. This difference between treatments in CDAD-5 and CDAD-10 values had lower significance in silo opened later (120 D.E.). LAB treatment showed the lowest values of DML, in silos opened 60 and 120 D.E., with highly significant differences between treatments ($p<0.001$). This is in agreement with the results found previously by other authors, and is supported by the data of lower ethanol production in the LAB treatment, which would cause a lower loss of dry matter in the form of gases.

**Table 1.** Ethanol production, aerobic stability and dry matter losses of sugar cane silages, 60 and 120 days of ensiling (D.E.) with and without additives.

| | Treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| | 60 D.E. | | | 120 D.E. | | |
| Parameter | Control[2] | Urea[3] | LAB[4] | Control[2] | Urea[3] | LAB[4] |
| Ethanol (% p/p) | $9.79^{aaa}$ | $7.21^{bbb}$ | $0.84^{ccc}$ | $6.93^{aaa}$ | $6.76^{aaa}$ | $0.85^{bbb}$ |
| AS (h) | $60^{a}$ | $39^{b}$ | $207^{c}$ | $63^{a}$ | $93^{b}$ | $78^{b}$ |
| CDAD-5 (°C) | $21.2^{aaa}$ | $58.2^{bbb}$ | $-0.13^{ccc}$ | $20.9^{a}$ | $5.0^{b}$ | $11.8^{b}$ |
| CDAD-10 (°C) | $43.7^{aaa}$ | $65.1^{aaa}$ | $4.3^{bbb}$ | $45.6^{a}$ | $48.9^{b}$ | $48.1^{b}$ |
| DML (%) | $22.32^{aaa}$ | $19.22^{bbb}$ | $15.76^{ccc}$ | $22.01^{aaa}$ | $22.26^{aaa}$ | $13.68^{bbb}$ |

$^{aaa, bbb, ccc,}$ Means in the same row with unlike superscripts differ ($p<0.0001$). $^{a,b,c,}$ Means in the same row with unlike superscripts differ ($p<0.05$). [1] 60 and 120 days of ensiling (D.E). [2] Sugar cane silages with no additive (control). [3] urea ($CO(NH_2)_2$ - 1 % FF). [4] inoculant with heterolactic bacteria *Lactobacillus buchneri* NCIMB 40788 ($1 \times 10^5$ CFU $g^{-1}$ of FF).

**Conclusion** The use of additives in sugar cane silages improves their fermentative profile, increases their aerobic stability and decreases the losses of dry matter along the storage period of the silo. The results found in this essay point to *Lactobacillus buchneri* NCIMB 40788 as better treatment than urea to reduce ethanol production and decrease the losses of dry matter in sugar cane silage.

**References**

Di Rienzo, J.A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M. & Robledo, C.W. (2011) InfoStat version 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba (ed.), Argentina. URL: http://www.infostat.com.ar/.

Kung, L.J. (2001). Silage fermentation and additives. Direct-fed Microbial, Enzyme & Forage Additive Compendium, Miller Publishing Co. (ed.), Minnetonka, MN. pp. 145-160.

Pedroso, A., Rodrigues, A.A., Schmidt, L. & Nussio, P. (2004). Suplementación y Engorde de Bovinos con caña de azúcar y Silaje de Caña de azúcar. CEA. 69-83.

Pedroso, A.F., Nussio, L.G., Loures, D.R.S., Paziani, S.D.F., Igarasi, M.S., Coelho, R.M., Horii, J. & Rodrigues, A.D.A. (2007). Efeito do tratamento com aditivos químicos e inoculantes bacterianos nas perdas e na qualidade de silagens de cana-de-açúcar. Revista Brasileira de Zootecnia, 36, 558-564.

Pedroso, A.F., Nussio, L.G., Loures, D.R.S., Paziani, S.D.F., Ribeiro, J.L., Mari, L.J., Zopollatto, M., Schmidt, P., Mattos, W.R.S. & Horii, J. (2008). Fermentation, losses, and aerobic stability of sugarcane silages treated with chemical or bacterial additives. Scientia Agricola. 65, (6), 589-594.

Queiroz, O. C. M. (2006). Associação de aditivos microbianos na ensilagem e o desempenho de vacas em lactação recebendo silagem de cana-de-açúcar comparada a volumosos tradicionais. Disertação de mestrado. Área de concentração Ciência Animal e Pastagens. 99 p.

Schmidt, P. (2006). Perdas fermentativas na ensilagem, parâmetros digestivos e desempenho de bovinos de corte alimentados com rações contendo silagens de cana-de-açúcar. Tese apresentada para obtenção do título de Doutor em Agronomia. Área de concentração: Ciência Animal e Pastagens, Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo (ed.). 229 p.

# Fermentation quality and *in vitro* gas production of corn stover silage inoculated with or without *Lactobacillus plantarum* and *Enterococcus faecium*

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**Keywords:** *Enterococcus faecium*, fermentation quality, *in vitro* gas production, *Lactobacillus plantarum*

**Introduction** Approximately 172 million tons of corn stover are produced annually in China. Corn stover is an important feed resource for ruminants, but its utilization is low. About 40% proportion of corn stover was used as forage for ruminant, and the rest was burned and discarded as agricultural waste. Therefore, the main problem is rational processing and utilization of corn stover for livestock production. This problem can be solved by ensiling, which is an effective technique to improve nutritive value and palatability of stover. In addition, Inoculation of lactic acid bacteria could effectively enhance the fermentation quality of silage and promote livestock production. Therefore, the objective of this study was to examine the effects of *Lactobacillus plantarum* and *Enterococcus faecium* on fermentation characteristics, subsequent *in vitro* gas and methane production of corn stover silage.

**Materials and methods** Corn stover was immediately reaped after corn grain harvested. The silage treatments were designed as follow: control without inoculant, *L. plantarum*, and *E. faecium.* About 650 grams of chopped fresh corn stover was immediately packed into triplicate plastic laboratory silos (1L), followed by being sealed with a screw top and stored at ambient temperature for 45 days. After ensiling, *in vitro* gas production of fresh silage samples were determined according to the method of Menke et al. (1979) using100 ml glass syringes. Readings of gas production were recorded before incubation and after 4, 8, 12, 24, 48 and 72 h. The potential gas production was calculated according to the method of Ørskov and McDonald(1979). The gas of each syringe was collected in a gas sampling bag and methane content was determined by gas chromatography. Data on the silage fermentation quality and *in vitro*gas and methane production were analyzed by one–way analysis of variance (ANOVA), the significance was declared at $P< 0.05$.

**Results** Corn stover silage inoculated with *L. plantarum* and *E. faecium* had lower ($P<0.05$) ammonia nitrogen and acetic acid concentration and higher ($P<0.05$) ratio of lactic acid to acetic acid (Table 1). The lactic acid concentration and potential gas production did not show differences ($P>0.05$) among treatments. Methane production was significant decreased ($P<0.05$) for silage inoculated with *L. plantarum* compared with that of others (Figure 1).

**Table 1.** Effect of *L. plantarum* and *E. faecium* inoculation on fermentation quality of corn stover silage.

| Item | Treatment | | | SEM |
|---|---|---|---|---|
| | Control | *L. plantarum* | *E. faecium* | |
| pH | 3.62 | 3.56 | 3.55 | 0.01 |
| Dry matter (g/kg FW) | 258$^{b}$ | 288$^{a}$ | 269$^{ab}$ | 2.55 |
| Ammonia /total nitrogen (g/kg) | 82.6$^{a}$ | 46.4$^{b}$ | 57.1$^{b}$ | 1.09 |
| Water soluble carbohydrates (g/kg DM) | 25.1 | 27.8 | 27.0 | 1.88 |
| Lactic acid (g/kg DM) | 70.6 | 76.9 | 73.5 | 1.00 |
| Acetic acid (g/kg DM) | 15.80$^{a}$ | 4.98$^{b}$ | 5.15$^{b}$ | 1.21 |
| Lactic acid/acetic acid | 4.47$^{b}$ | 15.44$^{a}$ | 14.27$^{a}$ | 1.48 |

FW=fresh weight; DM=dry matter; Butyrate and propionate were not detectable.
$^{abc}$Means within a row with different superscripts differ ($P< 0.05$).

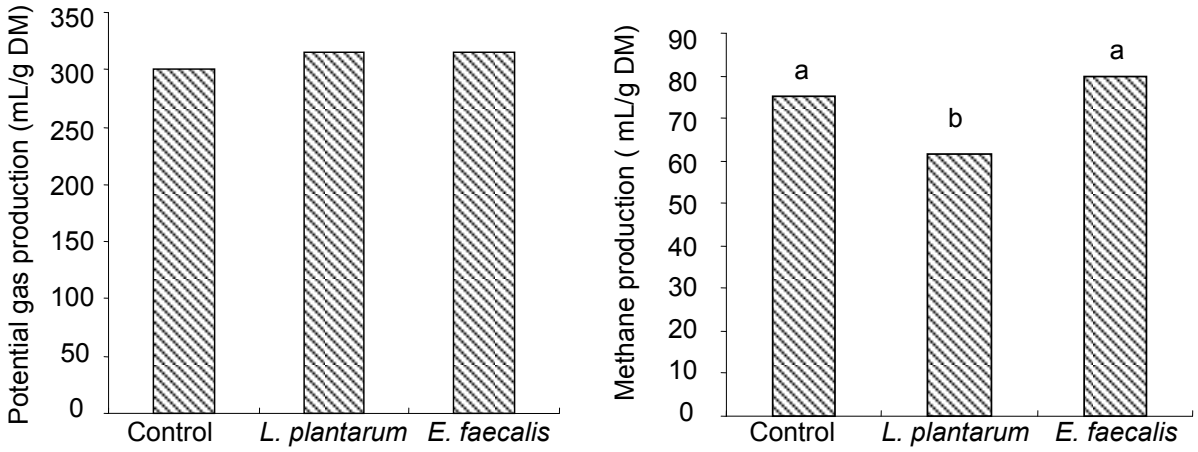


**Figure 1.** Potential gas production and methane production of fresh corn stover silage after 72 h incubation. [ab]Means with different superscripts differ ($P < 0.05$).

**Discussion** The results indicate that the lactic acid fermentation efficiency was improved by inoculation with homofermentative lactic acid bacteria. The dry matter content was higher for silage inoculated with *L. plantarum* as compared to that of control silage. This is due to *L. plantarum* can quickly produce large amounts of lactic acid and reduce dry matter loss (Cai et al. 1999). The lower dry matter loss could provide more organic matter for ruminants. In addition, the lower methane production for silage inoculated with *L. plantarum* as compared to that of control. This might explain lactic acid bacteria added to silage have high nutrient digestion efficiency, although it did not change the fermentation characteristics (Weinberg et al. 2007). Reduction of methane emissions could increase the energy utilization efficiency of silage for ruminant.

**Conclusions** The results suggested that *L. plantarum* not only improved fermentation quality of corn stover silage, but also increased their energy utilization efficiency for ruminant.

**References**

Cai,Y. M., Kumai, S., Zhang, J. G. & Benno, Y.(1999) Comparative studies of Lactobacilli and Enterococci associated with forage crops as silage inoculants. Animal Science Journal, 70, 188-194.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W. (1979) The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. Journal of Agricultural Science, 93, 217-222.

Ørskov, E.R. & McDonald, I. (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, 92, 499-503.

Weinberg, Z.G., Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D. & Miron, J. (2007) Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. Journal of Dairy Science, 90, 4754-4762.

# Fermentation suitability of Moso bamboo silage prepared with sake cake and lactic acid bacteria

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**Keywords:** Moso bamboo, sake cake, silage

**Introduction** In recent years, the area of abandoned Moso bamboo (*Phyllostachys heterocycla* var. *pubescens*) forest in subtropical regions of Japan has been rapidly increasing. As such, its influence on the natural ecosystem is of concern. Since bamboo contains various potentially physiologically active substances such as fibre and amino acids, it can be used as livestock feed. In order to effectively use the regional resources and improve feed self-sufficiency rate, Moso bamboo silage was prepared with lactic acid bacteria (LAB) and sake cake, which is a biproduct of rice wine production, and their fermentation suitability were studied.

**Materials and methods** Moso bamboo was grown in the Sasayama area (Hyogo, Japan) and harvested in January 2016. Stems and Leaves were separated and crushed to 5 mm with a bamboo crusher (Ohashi Co., Ltd, Saga, Japan). Three replicates per treatment of silage were prepared as intended for a small-scale fermentation method, and $1.0\times10^6$ colony-forming units of selected LAB and 5 grams of sake cake were used as additives in samples of 100 grams of bamboo chips that had been adjusted for moisture. The silage mixtures were contained in bags of laminated ethylene, nylon, and vinylidene and closed with a vacuum sealer. The silo was opened after 60 days of fermentation, after which the chemical composition and fermentation quality were analysed using the methods described by Cai (2004).

**Results** The dry matters (DM) of bamboo stem and leaves were 54.88 and 51.25%, their crude protein (CP) contents were 1.05 and 9.29% DM, and their NDF contents were 87.54 and 75.35% on a DM basis, respectively (Table 1). As shown in Table 2, in bamboo stem silage, sake cake treatment was good quality, with a lower ($P<0.05$) pH and higher ($P<0.05$) lactic acid content than control and RO50 treatment. However, in leaf silage no treatments were of any great difference. After 60 days of fermentation, all silages were preserved well without any deterioration. The CP and ether extract contents of stem and leaf silages treated with sake cake were higher ($P<0.05$), but the NDF content was lower than that of control and RO50 treatment (Table 3). The results confirmed that sake cake could improve bamboo stem silage quality, and that the bamboo can be well preparing a silage as good quality and it has good potential as a feed source for livestock diets.

**Table 1.** Chemical composition of ensiled Moso bamboo and sake cake.

| Material | DM | OM | CP | EE | NDF | ADF |
|---|---|---|---|---|---|---|
| | (%) | % of DM | | | | |
| Stem | 54.88±0.06 | 98.91±0.03 | 1.05±0.16 | 1.04±0.17 | 87.54±0.77 | 65.08±0.80 |
| Leaf | 51.25±0.12 | 91.87±0.66 | 9.29±0.29 | 2.81±0.19 | 75.35±1.95 | 44.92±1.38 |
| Sake cake | 53.67±0.08 | 98.89±0.16 | 27.7±0.44 | 2.46±0.23 | 3.88±0.13 | 4.88±0.73 |

OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre. Values are means of three samples.

**Table 2.** Fermentation quality of bamboo silage.

| Silage | Treatment | DM | pH | Lactic acid | Acetic acid | Propionic acid | Butyric acid |
|---|---|---|---|---|---|---|---|
| | | % | | (% of FM) | | | |
| Stem | Control | 54.74 | 4.25a | 0.37b | 0.20 | nd | 0.01 |
| | Sake cake | 55.48 | 3.99b | 0.83a | 0.19 | nd | nd |
| | RO50 | 54.38 | 4.32a | 0.37b | 0.08 | nd | nd |
| | Sake cake+RO50 | 54.25 | 3.92b | 0.86a | 0.18 | nd | nd |
| Leaf | Control | 49.92 | 4.49 | 1.24 | 0.37 | 0.01 | 0.02 |
| | Sake cake | 52.35 | 4.45 | 1.47 | 0.38 | nd | 0.10 |
| | RO50 | 50.08 | 4.33 | 1.47 | 0.33 | 0.01 | nd |
| | Sake cake+RO50 | 50.92 | 4.44 | 1.14 | 0.33 | 0.02 | nd |

DM, dry matter. FM, fresh matter. Values are means of three silage samples. Means within a column in the same silage group with different superscripts differ (P<0.05). Please explain all abbreviations of the table.

**Table 3.** Chemical composition (% of DM) of bamboo silage.

| Silage | Treatement | OM | CP | EE | NDF | ADF |
|---|---|---|---|---|---|---|
| Stem | Control | 98.89±0.13 | 0.98±0.04a | 0.82±0.09b | 88.43±1.22a | 65.86±0.68 |
| | Sake cake | 98.96±0.07 | 2.42±0.10a | 1.15±0.08a | 86.41±0.39b | 65.14±0.42 |
| | RO50 | 99.08±0.06 | 1.01±0.04b | 0.79±0.04b | 88.57±0.26a | 66.36±0.18 |
| | Sake cake+RO50 | 99.14±0.26 | 2.69±0.05b | 0.39±0.04a | 88.34±0.29a | 65.51±0.27 |
| Leaf | Control | 90.03±0.09 | 8.93±0.27b | 3.00±0.12 | 69.80±0.41a | 42.63±0.67 |
| | Sake cake | 89.61±0.64 | 10.76±0.32a | 3.30±0.05 | 64.67±0.59b | 41.56±0.44 |
| | RO50 | 89.59±0.72 | 9.03±0.18b | 3.09±0.07 | 69.42±0.62a | 42.45±0.58 |
| | Sake cake+RO50 | 90.15±0.19 | 10.19±0.20a | 3.27±0.28 | 64.58±0.64b | 41.69±0.28 |

OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre. Values are means of three silage samples. Means within a column in same silage group with different superscripts differ (P<0.05).

**Conclusions** The expansion of neglected bamboo forests in Japan is progressing year by year. To take this abundant resource and test its more sustainable uses, in this study, bamboo cut from neglected forests in the Sasayama area was used as the main raw material to make silage, and missing proteins were supplemented with sake cake. Results showed that the bamboo silage to which sake cake was added had great potential to be used as a livestock feed, and the added sake cake was able to improve the quality of bamboo silage fermentation. The next step for this study would be to conduct chemical analysis on a large-scale fermentation method. Future studies could possibly indicate whether using this method to make silage could notably decrease the effects of expanding bamboo forests as well as decrease the amount of livestock feed that subtropical Japan must import from other countries.

**References**

Cai, Y. (2004) Analysis method for silage. In: Japanese Society of Grassland Science (ed.), Field and laboratory methods for grassland science. Tosho Printing Co., Ltd. Tokyo, Japan. pp.279-282.

# Fibrolytic enzyme enhances feed efficiency of Nellore bulls when added to ensiling on corn-based silages

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**Keywords:** beta-glucanase, enzyme, high moisture corn, performance, snaplage, xylanase

**Introduction** The use of exogenous fibrolytic enzymes (EFE) as a feed additive is well-known in the literature to improve *in vitro* dry matter digestion (Phakachoed et al. 2013). However, studies have shown inconsistent responses in growth performance when EFE were added to ruminant feed. Partially, this inconsistency can be attributed to the suboptimal condition of the rumen, 39ºC and pH 6, (Adesogan et al. 2014) where EFE could be less efficient in breaking cellulose and xylose bonds. Thus, we hypothesized that corn-based silage, high moisture corn, and snaplage could represent an environment which enzymes could act properly (50ºC and pH 4-5) and improve silage quality and digestibility. Therefore, the objective of this study was to evaluate the performance of Nellore bulls fed high moisture corn silage and snaplage with fibrolytic enzyme complex added for ensiling.

**Material and Methods** This study was carried out at Nutripura Research Center (Pedra Preta, MT, Brazil). Four hundred and sixty-seven Nellore Bulls were allocated to 16 feedlot pens, with an initial average body weight of 421 ±23 kg. The drive-over silos containing high moisture corn silage (HMC), snaplage and whole-plant corn silage (WPCS) were made between June and July of 2017 and were opened after 50, 45 and 55 days of fermentation, respectively. High moisture corn and snaplage were ensiled with or without a fibrolytic enzyme complex (β–glucanase and xylanase, Rovabio Advance P®, Adisseo, France) at the dose of 100 grams per ton of fresh matter, at the ensiling moment. A control silo (without enzymes) was made for both HMC and snaplage. Each silage had two silos, as repetition. Therefore, four treatment diets were composed by combining the silages with (EFE) or without (Control) exogenous fibrolytic enzymes: 1) Snaplage Control + HMC Control; 2) Snaplage added EFE + HMC added EFE; 3) WPCS + HMC Control; 4) WPCS + HMC added EFE. The concentrate of diets was composed of soybean meal, soybean hulls, urea, and minerals. The diets were formulated to attend the bull requirements to gain 1.5 kg per day (BCNRM, 2016). Also, they were formulated to have the same content of NDF from forage (12.58%) and crude protein (13%). The experimental period was 122 days, 27 days for adaptation and 95 days on treatment diets. The bulls' body weight was accessed on day 27 of adaptation and at 122 day of the experimental period. To monitor the daily feed intake, a feed bunk score was used, where the target was score 1 which meant, at least 300 grams of DM per animal as orts (3% of orts/day). The experimental design was a randomized complete block, with factorial treatment structure, two diets (snaplage + high moisture corn or whole-plant corn silage + high moisture corn) and two treatments (control or EFE). The data were analyzed using the PROC MIXED procedure of SAS, with random effects for the block. The experimental unit considered was the pen, for each variable, and means were considered statistically significant for $P<0.10$.

**Results** According to the results in Table 1, the initial body weight of Nellore bulls did not differ. However, after 95 days receiving the experimental diets, the final body weight was lower ($P=0.076$), for the SNAP + HMC Control in comparison to WPCS + HMC Control treatment. The bulls fed HMC and snaplage inoculated with EFE had statistically the same average daily gain in comparison to animals fed control silages. However, treatments using fibrolytic enzyme reduced ($P=0.0194$) dry matter intake when compared to silage without enzyme, improving ($P=0.0855$) feed efficiency of animals fed silages with EFE compared to control silages. Both average daily gain ($P=0.0285$) and dry matter intake ($P=0.0031$), were lower for the diets using snaplage, without altering the feed efficiency.

**Table 1.** Performance of Nellore bulls fed diets containing high moisture corn and snaplage, with exogenous fibrolytic enzymes (EFE) added for ensiling.

| Item[1] | SNAP + HMC[2] | | WPCS + HMC[2] | | SEM | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | Control | EFE | Control | EFE | | Diet | Enzyme | D*E[3] |
| Initial Body Weight (kg) | 421.97 | 420.99 | 422.44 | 418.41 | 23.27 | 0.6965 | 0.3615 | 0.5737 |
| Final Body Weight (kg) | 562.64b | 567.75ab | 575.59a | 568.46ab | 24.51 | 0.0512 | 0.7536 | 0.076 |
| Average Daily Gain (kg) | 1.40 | 1.49 | 1.56 | 1.53 | 0.05 | 0.0285 | 0.4557 | 0.1588 |
| Dry Matter Intake (kg/day) | 9.17 | 9.07 | 9.60 | 9.26 | 0.34 | 0.0031 | 0.0194 | 0.1802 |
| Feed Efficiency[1] | 0.1532 | 0.1646 | 0.1625 | 0.1651 | 0.0049 | 0.2075 | 0.0855 | 0.2587 |

[1]Feed efficiency - average daily gain / dry matter intake; [2]SNAP - snaplage; HMC – high moisture corn; WPCS – whole-plant corn silage; EFE - exogenous fibrolytic enzymes (Rovabio Advance P®); [3]SEM – standard error of the mean; D*E – interaction diet × enzyme.

**Discussion** The lack of difference in the initial body weight suggests that animals were fairly distributed into the blocks and treatments, with a low difference in weight among them. Even though the chemical composition of the diets was not presented (in progress), the animal responses pointed out that there were differences between treatments. Regarding the reduction of the final body weight in consequence of a lower average daily gain of the animals fed snaplage, this could be attributed to the reduction of the dry matter intake. This reduction is possible due to the chemical regulatory mechanism of intake of the bulls (Mertens 1994). The cob and husk fractions, presented in snaplage, are more digestible than the stem fraction of the corn plant (Mahanna 2008). Also, the increase in the feed efficiency caused by the addition of exogenous fibrolytic enzymes to the silages, could be explained by the possible improvement on the NDF digestibility (Phakachoed et al. 2013), either of the fibrous and kernel fractions.

**Conclusions** The results suggest that the inoculation with fibrolytic enzyme complex on corn-based silages is a promising technology to improve feed efficiency in beef cattle, probably due to the increase in the quality and digestibility of silages.

**References**

Adesogan, A.T., Ma, Z.X., Romero, J.J. & Arriola, K.G. (2014) RUMINANT NUTRITION SYMPOSIUM: Improving cell wall digestion and animal performance with fibrolytic enzymes. Journal of Animal Science, 92, 1317-1330.

Mahanna, B. (2008) Renewed interest in snaplage displayed. Feedstuffs, 80, 1-3.

Mertens, D.R. (1994) Regulation of forage intake. In: G.C. Fahey, Jr, M. Collins, D.R. Mertens, & L.E. Moser, (ed.) Forage Quality, Evaluation, and Utilization. Madison, Wisconsin, USA: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. pp. 450-493.

Phakachoed, N., Suksombat, W., Colombatto, D. & Beauchemin, K..A. (2013) Use of fibrolytic enzymes additives to enhance *in vitro* ruminal fermentation of corn silage. Livestock Science, 157, 100-112.

# First estimation and validation of a new model to predict dry matter loss based on temperature changes – II. Validation of maize mini silo and big scale silage

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**Keywords:** aerobic stability, dry matter loss estimation, spoilage control, temperature increase

**Introduction** It was recently demonstrated that a linear correlation between dry matter (DM) loss and temperature exists (Pires et al. 2018). The model is, however, based on a meta-analysis study and should be validated on real time temperature tracking during aerobic exposure of silage. The purpose of this study was to validate the model on continued temperature recording in a crop (maize) typically associated with aerobic stability challenges. In addition, the model was tested with different storage structures (mini silos and big bales) to examine if mini silos can be used to predict silage fermentation and aerobic stability just as well as big bales.

**Material and Methods** Five mini-silos (3 l volume) and 10 big bales (wrapped in 6 layers of 0.025 mm white stretch film, width 750 mm) for each treatment were filled with maize crop (1.76-1.86 kg and 872 kg, respectively). Two different treatments were tested: Control (C) without inoculant and SiloSolve® FC (SSFC), containing 50:50 of heterofermentative bacteria *Lactobacillus buchneri* (DSM22501/1k20738) and homofermentative bacteria *Lactococcus lactis* O224 (DSM11037/1k2081). The target application rate was 150,000 cfu/g of forage. Control forage was sprayed with sterile water and SSFC with the stated amount of inoculum. The crop was analyzed for composition before ensiling (Dry matter (DM) 387.2 g/kg; Crude protein 99.3 g/kg DM; Crude fiber 216.2 g/kg DM; Water soluble carbohydrates 78.8 g/kg DM; pH 5.82; yeast 4.72 log cfu/g; mold 4.64 log cfu/g). Mini-silos and big bales were stored 120 days at 20°C (mini silos) and ambient temperature (big bales). After fermentation, each mini-silo and big bale followed an aerobic stability (AS) test lasting for 19 days (mini silos) and 34 days (big bales). The AS test was performed by aerobically challenging the mini-silos and monitoring the temperature increase inside the forage mass. The big bales were stripped from the wrapping plastic and left exposed while temperature was recorded. By definition (EFSA, 2018), AS is determined by the amount of time it takes the silage temperature to exceed the ambient temperature +3°C. Total yeast and mold counts were measured prior to ensiling, when the silos were opened (before aerobic exposure) and at the end of the AS test. Temperature development was recorded during AS test and DM loss was determined after AS test. The temperature data was subjected to the model and estimated DM losses were correspondingly calculated and compared to the actual determined DM loss.

**Results** Adapting the linear correlation model (Pires et al. 2018) to temperature scores in mini silos and big bale silages (maize), showed a vast improvement in the estimation of dry matter loss during aerobic challenge compared to the previous model (McDonald et al. 1991), using a step logic approach of temperature delta versus ambient temperature (Table 1). In addition, it was demonstrated that compared to the untreated control, ensiling with SSFC reduced ($P$<0.01*)* the DM loss in big bales and mini silos both during fermentation (47% and 22%, respectively) and after aerobic challenge (65% and 53%, respectively). Yeast and mold were reduced ($P$<0.01) with SSFC treated silages irrespectively of storage structure (Table 2).

**Discussion** Submitting real time temperature recordings to the newly established model led to a significantly ($P$<0.001) better prediction of DM loss vs. the stepwise delta to ambient T logic (McDonald et al. 1991) previously used. The model was robust disregarding structure (mini silos or big bales [$P$<0.001]) and also disregarding treatment (untreated or control [$P$<0.001]). Interestingly it was also observed that there was no interaction between neither structure nor treatment on the model ($P$=0.1901).

**Table 1**. DM loss (recorded vs. calculated) using 2 different models based on temperature (T) in the easy to ensile maize crop, comparing 2 different structures (mini silos or big bales) and 2 different treatments (TRT) (untreated [C] and SiloSolve® FC [SSFC]), AS: Aerobic stability

| Structure | Ambient T at max T during AS test (°C) | Max T (°C) reached after [hours] of AS test | | DM loss (%) recorded after AS test (Std. dev.) | | DM loss (%) calculated using delta vs. ambient T according to McDonald (1991) | | DM loss (%) calculated using linear regression model according to Pires et al. (2018) (Std. dev.) | |
|---|---|---|---|---|---|---|---|---|---|
| TRT | | C | SSFC | C | SSFC | C | SSFC | C | SSFC |
| Big bales | 4.1 | 33.4 [816] | 13.2 [816] | 3.9 (+/-0.17) | 1.5 (+/-0.17) | 15 | 15 | 6.5 (+/-0.4) | -3.3 (+/-0.4) |
| Mini silos | 20.4 | 28.7 [276] | 24.3 [390] | 8.2 (+/-0.12) | 5.6 (+/-0.12) | 15 | 10 | 4.2 (+/-0.4) | 2.1 (+/-0.4) |

**Table 2.** Yeast and mold counts in the easy to ensile maize crop, comparing 2 different structures (mini silos or big bales) and 2 different treatments (TRT) (untreated [C] and SiloSolve® FC [SSFC]) **(*P*<0.01*)*

| Structure | Before ensiling | | After ensiling | | | | After AS test | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yeast (log cfu/g) | Mold (log cfu/g) | Yeast (log cfu/g) | | Mold (log cfu/g) | | Yeast (log cfu/g) | | Mold (log cfu/g) | |
| TRT | | | C | SSFC | C | SSFC | C | SSFC | C | SSFC |
| Big bales | 4.72 | 4.64 | 3.96 | 1.12** | 1.70 | 1.0** | 5.65 | 1.16** | 4.44 | 1.97** |
| Mini silos | | | 3.65 | 1.06** | 1.59 | 1.0** | 7.63 | 4.25** | 9.38 | 3.33** |

The response to aerobic challenge was delayed in big bales compared to mini silos, which most likely reflects the difference in mass, yet the differences between treatments were observed irrespectively of storage structure. It may preliminary be concluded that the new linear regression model tends to underestimate the DM losses (Δ1.5% pts +/-0.4), yet compared to the delta to ambient temperature step logic, we observed a vast improvement from the over estimation with this model (Δ8.2% pts +/-0.4). As discussed (Pires et al. 2018) the new model predicts that no DM loss occurs at temperatures below 20 °C (-3.3%, SSFC big bales (max T = 13.2 °C [Table 1]). The model proposed by Henderson et al. (1979) showed no DM loss at T < 9 °C, whereas Woolford (1984) suggested no DM loss occurs at T < 13 °C. With a DM loss of 1.5% after 34 days aerobic challenge in the SSFC treated big bales, the logic of a minimum threshold for DM loss should be further investigated.

**Conclusion** Based on the recently developed model we found a better fit to the DM loss when estimating this from real time temperature recording of maize silage during aerobic exposure in both mini silos and big bales. For maize silages it can be concluded that mini silos may be used equally well as big bales to discriminate treatments (inoculated vs. untreated) both in terms of fermentation parameters, yeast and mold control, aerobic stability and also the model for predicting DM loss. The method should be validated on a crop more difficult to ferment such as an alfalfa/grass mixture.

**References**

EFSA Scientific Opinion (2018). Guidance on the assessment of the efficacy of feed additives, EFSA Journal, 16(5):5274, 6-7.

Henderson, A.R., Matthew Ewart, J., & Robertson, G.M. (1979) Studies on the aerobic stability of commercial silages. Journal of the Science of Food and Agriculture, 30(3), 223-228.

McDonald, P., Henderson, A.R., & Heron, S.J E. eds. (1991) The biochemistry of silage, 2nd edition, Chalcombe Publications, 340 p.

Pires, S, Joergensen, J.N., Groenlund Nielsen, N., Bryan, K., Copani, G. & Witt, K.L. (2018) First estimation and validation of a new model to predict dry matter loss based on temperature changes – I. A meta-analysis study. In Proceedings of the XVIII International Silage Conference in press.

Woolford, M.K. (1984) The Silage Fermentation. Microbiology Series vol.14. New York: Marcel Dekker, Inc. 350 p.

# First estimation and validation of a new model to predict dry matter loss based on temperature changes – III. Validation of model in a crop with low ensilability

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**Keywords:** aerobic stability, dry matter loss estimation, spoilage control, temperature increase

**Introduction** It was recently demonstrated that a linear regression correlation exists, between dry matter (DM) loss and temperature development during aerobic stability challenge (Pires et al. 2018). The model was validated and proved useful to estimate DM loss in different storage structures (mini silos and big bales) and showed no sensitivity to different treatments (untreated and inoculated with SiloSolve® FC (Witt et al. 2018)). While it was concluded that the method could be applied to a crop with high ensilability, such as maize, it was unknown how the model could be applied to a crop with low ensilability. The purpose of this study was to test how the model could be used for DM loss prediction in alfalfa/grass silages during aerobic spoilage vs. the model described by McDonald et al. (1991), using the difference between recorded temperature in the silo and the ambient temperature.

**Material and Methods** Five mini-silos (3 l volume) for each treatment were filled with alfalfa:ryegrass (50:50) crop (fresh weight 1.82-1.92 kg). Two different treatments were tested: Control (C) without inoculant and SiloSolve® FC (SSFC), containing 50:50 of homofermentative bacteria *Lactobacillus buchneri* (DSM22501/1k20738) and heterofermentative bacteria *Lactococcus lactis* (O224 DSM11037/1K2081). The target application rate was 150,000 cfu/g of forage. Control forage was sprayed with sterile water and the SSFC treatment with the stated amount of inoculum. Mini-silos were stored 90 days at 20°C. After fermentation an aerobic stability (AS) test lasting for 10 days followed. Total yeast and mold counts were measured prior to ensiling and when the silos were opened (before aerobic exposure). Temperature development was recorded during AS test and DM loss was determined after AS test. The temperature data was subjected to the model and estimated DM losses were correspondingly calculated and compared to the actual determined DM loss.

**Results** Adapting the linear correlation model (Pires et al. 2018) to temperature scores in alfalfa/grass silage demonstrated a vast improvement in the estimation of dry matter loss during aerobic challenge compared to the model described by McDonald et al. (1991), using the difference between recorded temperature in the silo and the ambient temperature (table 1). Chemical composition of the crop prior to ensiling was DM 350.1 g/kg; Crude protein 200.0 g/kg DM; Crude fibre 251.8 g/kg DM; Water soluble carbohydrates concentration of 66.9 g/kg DM; pH 6.16; yeast 4.97 log cfu/g and mold 4.96 log cfu/g. In addition, it was demonstrated that ensiling with SSFC ($P<0.05$) reduced the DM loss in mini silos both during fermentation (40%) and after aerobic challenge (39%) as well as significantly ($P<0.05$) reducing yeast and mold (log cfu/g yeast 3.02 vs. 1.00; log cfu/g mold 2.84 vs. 1.00, after fermentation, C vs. SSFC, respectively).

**Table 1**. DM loss recorded vs. predicted using 2 different models based on temperature in the difficult to ensile alfalfa/grass crop, comparing 2 different treatments (TRT) (untreated (C) and SiloSolve® FC (SSFC)).

| Crop | Ambient T at max T during AS test (°C) | Max T (°C) reached after [hours] of AS test | | DM loss (%) recorded after AS test (std. dev.) | | DM loss (%) using delta vs. ambient T according to McDonald (1991) | | DM loss (%) using linear regression model according to Pires et al. (2018) (std. dev.) | |
|---|---|---|---|---|---|---|---|---|---|
| | TRT | C | SSFC | C | SSFC | C | SSFC | C | SSFC |
| Alfalfa/grass Mini silos | 20.6 | 25.3 [204] | 22.1 [240] | 3.1 (+/- 0.21) | 1.9 (+/- 0.21) | 10 | 10 | 2.6 (+/- 0.4) | 1.0 (+/- 0.4) |

**Discussion** Alfalfa/grass mix is a less ensilable crop that normally is not associated with aerobic stability challenges, confirmed by the present study, where untreated alfalfa/grass silage kept aerobically stable for more than 8 days. Nevertheless, inoculation with SiloSolve® FC was effective in extending aerobic stability to a minimum of 10 days (+3 °C threshold (EFSA, 2018) above ambient not crossed at time of terminating the aerobic challenge test). The step logic model, the new model and actual DM loss were evaluated, and the new model more accurately ($P<0.001$) aligned with DM loss recorded in the trial.

**Conclusion** This study confirms that the model is useful when subjecting both easy and difficult to ferment crops ensiled for 90-120 days. It should be investigated if the model works with silages fermented for a short period.

**References**

EFSA Scientific Opinion (2018). Guidance on the assessment of the efficacy of feed additives, EFSA Journal, 16(5):5274, 6-7.

McDonald, P., Henderson, A. R., & Heron, S.J.E. eds. (1991) The biochemistry of silage, 2nd edition, Chalcombe Publications, 340 p.

Pires, S., Joergensen, J.N., Nielsen, N.G., Bryan, K.A., Copani, G., Witt, K.L. (2018). First estimation and validation of a new model to predict dry matter loss based on temperature changes – I. A meta-analysis study. In Proceedings of the XVIII International Silage Conference in press.

Witt, K.L., Joergensen, J.N., Nielsen, N.G., Bryan, K.A., Copani, G., Pires, S., Vrotniakiene, V., Jatkauskas, J. (2018). First estimation and validation of a new model to predict dry matter loss based on temperature changes – II. Validation of maize mini silo and big scale. In Proceedings of the XVIII International Silage Conference in press.

# First estimation and validation of a new model to predict dry matter loss based on temperature changes – IV. Validation of model in a short fermentation regime

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**Keywords:** aerobic stability, dry matter loss estimation, spoilage control, temperature increase

**Introduction** It was recently demonstrated that a linear regression correlation exists, between dry matter (DM) loss and temperature development during aerobic stability challenge (Pires et al. 2018). The model was validated and proved useful to estimate DM loss in different storage structures mini silos and big bales (Witt et al. 2018a) and across different crops (maize and alfalfa/grass) (Witt et al. 2018 b). Based on this, it was concluded that the method could be applied to both easy and difficult to ensile crops when silages had fermented for up to 120 days. While it was demonstrated that aerobic stability could be improved using SiloSolve® FC in both maize (Witt et al. 2015) and alfalfa silage (Copani et al. 2017) after short fermentation time. It was unknown if the model could be applied to crops when ensiled for a shorter time. The purpose of this study was to test how the model could be used for DM loss prediction in maize and alfalfa silages during aerobic spoilage when ensiled for 8, 16 and 32 days vs. the model described by McDonald et al. (1991), using the difference between recorded temperature in the silo and the ambient temperature.

**Material and Methods** Five mini-silos (3 l volume) for each treatment were filled with maize or alfalfa crop. Two different treatments were tested: Control (C) without inoculant and SiloSolve® FC (SSFC), containing 50:50 of homofermentative bacteria *Lactobacillus buchneri* (DSM22501/1k20738) and heterofermentative bacteria *Lactococcus lactis* (O224 DSM11037/1K2081). The target application rate was 150,000 cfu/g of forage. Control forage was sprayed with sterile water and the SSFC treatment with the stated amount of inoculum. Mini-silos were stored for 8, 16, and 32 days at 20°C. After fermentation an aerobic stability (AS) test followed lasting for 10 days (maize) and 30 days (alfalfa), respectively. Total yeast and mold counts were measured prior to ensiling, when the silos were opened (before aerobic exposure) and at the end of the AS test. Temperature development was recorded during AS test and DM loss was determined after AS test. The temperature data was subjected to the model and estimated DM losses were correspondingly calculated and compared to the actual determined DM loss.

**Results** Adapting the linear correlation model to temperature scores in alfalfa and maize silage at short fermentation regime demonstrated a vast improvement in the estimation of dry matter loss during aerobic challenge compared to previous step logic model (table 1). In addition, it was demonstrated that ensiling with SSFC ($P<0.01$) reduced the DM loss in both maize and alfalfa silages at all fermentation times both during fermentation and after aerobic challenge. It was also demonstrated that yeast and mold counts were significantly ($P<0.01$) reduced compared to control silages in both crops at all fermentation times before and after aerobic stability challenge. The old model, the new model and actual DM loss were evaluated, and the new model more accurately aligned with DM loss recorded in the trial across both crops and ensiling times.

**Discussion** Submitting real time temperature recordings to the newly established model (Pires et al. 2018), yielded a significantly ($P<0,001$) better prediction of DM loss vs. the step logic (McDonald et al. 1991) previously used also at shorter fermentation regimen (8, 16, and 32 days of fermentation). In contrast to what was reported by Witt et al. (2018a) the mini silo assessment at short fermentation (8, 16, and 32 days, respectively) of the easy to ensilage Maize forage was consistently overestimated vs. real recording using the model proposed by Pires et al. (2018). However, as Witt et al. (2018b)

reported on the less ensilable alfalfa/grass forage, in the case of short fermentation (8, 16, and 32 days, respectively) of the difficult to ferment Alfalfa forage, the DM loss was underestimated using the model proposed by Pires et al. (2018).

**Table 1**. DM loss (recorded vs. calculated), comparing two different crops (maize and alfalfa), two different treatments (TRT) (untreated (C) and SiloSolve® FC (SSFC)) at 3 different fermentation times (8, 16 and 32 days).

| Crops | Ambient T at max T during AS test (°C) | Max T (°C) reached after [hours] of AS test | | DM loss (%) recorded after AS test (std. dev.) | | Calculated DM loss (%) using delta vs. ambient T * | | Calculated DM loss (%) using linear regression model** (std. dev.) | |
|---|---|---|---|---|---|---|---|---|---|
| Mini silo | TRT | C | SSFC | C | SSFC | C | SSFC | C | SSFC |
| Maize 8 d | 21.0 | 28.9 [120] | 27.3 (144] | 3.8 (+/- 0.14) | 2.6 (+/- 0.14) | 15 | 10 | 4.3 (+/- 0.4) | 3.5 (+/- 0.4) |
| Maize 16 d | 21.1 | 29.1 [126] | 28.1 [168] | 3.3 (+/- 0.17) | 2.2 (+/- 0.17) | 15 | 10 | 4.4 (+/- 0.4) | 3.9 (+/- 0.4) |
| Maize 32 d | 20.8 | 30.6 [120] | 26.9 [168] | 2.9 (+/- 0.18) | 1.8 (+/- 0.18) | 15 | 10 | 5.1 (+/- 0.4) | 3.3 (+/- 0.4) |
| Alfalfa 8 d | 20.2 | 36.9 [420] | 25.9 [696] | 10.8 (+/- 0.19) | 5.7 (+/- 0.19) | 15 | 10 | 8.2 (+/- 0.4) | 2.8 (+/- 0.4) |
| Alfalfa 16 d | 20.8 | 31.8 [516] | 24.5 [684] | 7.5 (+/- 0.15) | 4.9 (+/- 0.15) | 15 | 10 | 5.7 (+/- 0.4) | 2.2 (+/- 0.4) |
| Alfalfa 32 d | 30.1 | 30.1 [588] | 25.2 [720] | 7.3 (+/- 0.17) | 5.02 (+/- 0.17) | 10 | 3 | 4.9 (+/- 0.4) | 2.5 (+/- 0.4) |

* according to McDonald (1991) ** according to Pires et al. (2018)

**Conclusion** Although silages are usually fermented for a minimum of 60 days to become stable, ensiling maize and alfalfa with SiloSolve® FC for 8, 16, and 32 days resulted in a significantly enhanced fermentation and stability. Even at such short fermentation regime it was demonstrated that the recently developed model gave a better fit to the DM loss when estimating this from real time temperature recording during aerobic exposure

**References**

Copani, G., Nielsen, N.G., Bryan, K A., Vrotniakiene, V., Jatkauskas, J. & Witt, K.L. (2017) Effects of a dual strain inoculant on alfalfa silage fermentation and aerobic stability through 32 days of ensiling, In: Nussio, L.G, De Sousa, D.O., Gritti, V.C., De Souza Salvati, G.G., Dos Santos, W., P. & Salvo, P.A.R. (eds). Proceedings of the V International symposium on forage quality and conservation, Piracicaba, Sao Paulo, Brazil, 2017.

McDonald, P., Henderson, A.R., & Heron, S.J.E. eds. (1991) The biochemistry of silage, 2nd edition, Chalcombe Publications, 340 p.

Pires, S., Joergensen, J. N., Nielsen, N. G., Bryan, K.A., Copani, G. & Witt, K.L. (2018) First estimation and validation of a new model to predict dry matter loss based on temperature changes – I. A meta-analysis study. In Proceedings of the XVIII International Silage Conference in press.

Witt, K.L., Jatkauskas, J., Vrotniakiene, V. & Voss, M.T. (2015) Effects of a novel dual purpose silage additive on aerobic stability and fermentation characteristics of whole crop maize silage after a short time of ensiling. In: XVII International Silage Conference, July 1-3, 2015, Piracicaba, Sao Paulo, Brazil p. 82-83.

Witt, K.L., Joergensen, J.N., Nielsen, N.G., Bryan, K.A., Copani, G., Pires, S., Vrotniakiene, V. & Jatkauskas & J. (2018a) First estimation and validation of a new model to predict dry matter loss based on temperature changes – II. Validation of maize mini silo and big scale. In Proceedings of the XVIII International Silage Conference in press.

Witt, K.L., Joergensen, J.N., Nielsen, N.G., Bryan, K.A., Copani, G., Pires, S., Vrotniakiene, V. & Jatkauskas, J. (2018b) First estimation and validation of a new model to predict dry matter loss based on temperature changes – III. Validation of model in a difficult to ferment crop. In Proceedings of the XVIII International Silage Conference in press.

# Grass silage fermentation characteristics and aerobic stability as affected by type of silage additive

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**Keywords:** additive, grass, silage

**Introduction** Grass is harvested for silage all over the world but the choice of additive type by farmers can differ both within and between continents of various reasons. Biological and chemical additives affect silage fermentation characteristics and aerobic stability in different ways depending on their active substances chosen for specific modes of action. This study aimed at comparing different types of additives on fermentation characteristics and aerobic stability of grass silage from Northern Europe.

**Material and Methods** Grass was wilted overnight to 33% DM and chopped to 20 mm theoretical length. The following additives were manually applied to the herbage: homofermentative lactic acid bacteria (LAB) *Lactobacillus (Lb.) plantarum* DSM 16627 and *Lb. paracasei* NCIMB 30151 ($LAB_{ho}$) at 150,000 cfu $g^{-1}$, *Lb. plantarum* DSM 16627, *Lb. paracasei* NCIMB 30151 and heterofermentative *Lb. brevis* DSM 21982 ($LAB_{hohe}$) at 210,000 cfu $g^{-1}$, a salt-based additive containing sodium nitrite, hexamethylene tetramine and sodium benzoate at 2.0 mL $kg^{-1}$ and an acid-based additive containing formic acid, sodium formate, propionic acid, lactic acid and sodium benzoate at 3.0 mL $kg^{-1}$ (Konsil Scandinavia AB, Tvååker, Sweden). Water was added to the untreated control (CON) and to the additive treatments to a total volume of 10 mL $kg^{-1}$. Herbage was packed into 1.7-L glass jars, which were replicated three times per treatment and equipped with fermentation traps to enable gas release. After 107 days of storage at 22°C, silage fermentation characteristics were analysed according to conventional methods. Aerobic stability was tested for 14 days according to Honig (1990). Data were analysed statistically by using the mixed model procedure of SAS 9.4. Least-square means were separated by Tukey´s adjustment, and significance was declared at $P<0.05$.

**Results and Discussion** When compared with untreated silage and $LAB_{hohe}$ (Table 1), chemical additives and $LAB_{ho}$ improved the efficiency of fermentation as reflected by lowest dry matter (DM) losses during storage (4.7% *vs* 6.3%, $P<0.001$) and highest concentrations of water-soluble carbohydrates (WSC; 1.9% *vs* 0.7% of DM, $P<0.001$). The $LAB_{ho}$ increased lactic acid production while decreasing the acetic acid concentration, inhibiting butyric acid formation and decreasing ammonia-N concentration compared to the control ($P<0.001$). The chemical additives restricted fermentation and inhibited butyric acid formation ($P<0.001$). Silage treated with these additives also had lower ammonia-N concentration compared to the control ($P<0.001$). As butyric acid was present and proteolysis was low in untreated silage, it seems likely that a lack of nitrate in the fresh herbage and a too slow acidification rate (pH after 3 days of fermentation = 5.01) allowed clostridia to grow in the early phases of fermentation producing butyrate from WSC (Weiss 2001). Propionic acid was highest in untreated silage and may have been caused by anaerobic degradation of 1,2-propandiol to form this product and n-propanol by *Lb. diolivorans* (Krooneman et al. 2002), although these metabolic end-products were not analysed in this study. Silage treated with the acid-based additive contained larger concentrations of formic acid than any other silage (0.39 *vs* 0.02% of DM, $P<0.001$) and can be attributed to the addition of this acid with the additive. Unlike $LAB_{ho}$, the chemical additives decreased ethanol formation and produced silage that was as aerobically stable as the butyric acid containing control silage. The highest concentration of ethanol was detected in silage treated with $LAB_{hohe}$ containing the obligate heterofermentative *Lb. brevis*, which produces acetic acid and may also form ethanol from WSC, depending on the used sugar type (Rooke and Hatfield 2003). Silage that received

$LAB_{ho}$ had the highest yeast count of all treatments (log cfu $g^{-1}$ 4.0 *vs* 1.8, *P*<0.001), resulting in the shortest time for the silage to become aerobically unstable (5.7 *vs* 10.5 days, *P*<0.001). These findings substantiate previous observations that inoculation with homofermentative LAB increases the risk for heating of silage upon air exposure and that combining homofermentative LAB with heterofermentative LAB can alleviate this effect (Auerbach et al. 2013).

**Table 1.** Effects of different additive types on fermentation pattern, DM loss, yeast count and aerobic stability (ASTA) of grass silage (data given as least-square means in % of DM unless stated otherwise, n=3).

| Item | Control[1] | $LAB_{ho}$[2] | $LAB_{hohe}$[3] | Salt[4] | Acid[5] | SEM | *P* |
|---|---|---|---|---|---|---|---|
| WSC | 0.75$^{c}$ | 1.79$^{b}$ | 0.64$^{c}$ | 1.64$^{b}$ | 2.40$^{a}$ | 0.091 | *** |
| pH | 4.07$^{b}$ | 4.01$^{c}$ | 4.10$^{a}$ | 4.11$^{a}$ | 4.02$^{c}$ | 0.003 | *** |
| Lactic acid | 9.35$^{b}$ | 9.83$^{a}$ | 9.52$^{b}$ | 8.73$^{c}$ | 8.27$^{d}$ | 0.059 | *** |
| Acetic acid | 1.26$^{b}$ | 0.71$^{d}$ | 1.44$^{a}$ | 1.20$^{b}$ | 1.11$^{c}$ | 0.013 | *** |
| Propionic acid | 0.24$^{a}$ | 0.10$^{b}$ | 0.07$^{c}$ | 0.07$^{c}$ | 0.12$^{b}$ | 0.005 | *** |
| Butyric acid | 0.26$^{a}$ | 0.0$^{b}$ | 0.0$^{b}$ | 0.0$^{b}$ | 0.0$^{b}$ | 0.006 | *** |
| Formic acid | 0.02$^{b}$ | 0.02$^{b}$ | 0.02$^{b}$ | 0.02$^{b}$ | 0.39$^{a}$ | 0.006 | *** |
| Ethanol | 0.92$^{b}$ | 0.85$^{b}$ | 1.35$^{a}$ | 0.25$^{d}$ | 0.35$^{c}$ | 0.015 | *** |
| 2,3-butanediol | 0.28$^{a}$ | 0.14$^{b}$ | 0.06$^{d}$ | 0.08$^{c}$ | 0.04$^{e}$ | 0.004 | *** |
| $NH_3$-N (% of total N) | 5.8$^{a}$ | 3.2$^{d}$ | 5.4$^{b}$ | 3.1$^{d}$ | 4.1$^{c}$ | 0.084 | *** |
| DM loss (%) | 6.4$^{a}$ | 5.0$^{b}$ | 6.1$^{a}$ | 4.9$^{b}$ | 4.3$^{b}$ | 0.18 | *** |
| ASTA (days) | 12.5$^{a}$ | 5.7$^{c}$ | 8.6$^{b}$ | 9.4$^{ab}$ | 11.6$^{a}$ | 0.59 | *** |
| Yeast (log cfu $g^{-1}$) | 1.8$^{b}$ | 4.0$^{a}$ | 1.7$^{b}$ | 1.7$^{b}$ | 2.0$^{b}$ | 0.13 | *** |
| pH after ASTA | 3.98$^{c}$ | 8.33$^{a}$ | 7.77$^{b}$ | 7.34$^{b}$ | 4.18$^{c}$ | 0.099 | *** |

[1]untreated, [2]*Lb. plantarum* DSM 16627 and *Lb. paracasei* NCIMB 30151, [3]*Lb. plantarum* DSM 16627, *Lb. paracasei* NCIMB 30151 and *Lb. brevis* DSM 21982, [4]liquid blend of sodium nitrite, hexamethylene tetramine and sodium benzoate, [5]liquid buffered mixture of formic acid, sodium formate, propionic acid, lactic acid and sodium benzoate. [abcd]Means with different superscripts within a row differ significantly (*P*<0.05).

**Conclusions** All additives inhibited butyric acid formation and proteolysis during ensiling and total DM losses during storage were decreased by use of $LAB_{ho}$ and chemical additives of which the latter also reduced yeast counts. Use of $LAB_{hohe}$ inhibited yeast growth and improved aerobic stability compared to silage treated with $LAB_{ho}$.

**References**

Auerbach, H., Weiss, K., Theobald, P., & Nadeau, E. (2013) Effects of inoculant type on dry matter losses, fermentation pattern, yeast count and aerobic stability of green rye silages. In: Mair, C., Wetscherek, W., & Schedle, K. (eds). 12. BOKU-Symposium, Vienna, Austria, pp. 179-185.

Honig, H. (1990) Evaluation of aerobic stability. Grass and Forage Reports Special issue, 3, 76-82.

Krooneman, J., Faber, F., Alderkamp, A. C., Oude Elferink, S. J. H. W., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J. C., & Vancanneyt, M. (2002) *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 52, 639–646.

Rooke, J. A. & Hatfield, R. D. (2003) Biochemistry of ensiling. In: Buxton, D. R, Muck, R. E., & Harrison J. H. (eds). Silage Science and Technology. Agronomy Monograph No. 42, ASA, CSSA, SSSA, Madison, WI, USA, pp. 95-139.

Weiss, K. (2005) Gärungsverlauf und Gärqualität von Silagen aus nitratarmem Grünfutter [Course and quality of fermentation in forages low in nitrate]. Doctoral thesis, Humboldt University, Berlin, Germany, 159 p.

# Growth of lactic acid bacteria in the presence of various tannins

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**Keywords:** growth, lactic acid bacteria, MRS-bouillon, tannin extracts

**Introduction** Hydrolysable and condensed tannins are able to reduce the ruminal degradation of plant proteins and are used as ensiling additive to improve the nutritional value of feedstuffs for ruminants. Tannins form insoluble complexes with macromolecules, including the plant proteins as well as the bacterial proteins and other compounds of the bacterial cell wall in the gut (Scalbert 1999). Costabile et al. (2011) examined the influence of different tannins on the survival rate of *Salmonella typhimurium* and demonstrated various toxic properties. Bossi et al. (2007) identified in *Lactobacillus hilgardii* with high-resolution proteomic analyses a series of proteins with different expression rates, which depend on the tannic acid concentration. The objective of this study was to identify the influence of commercially available products of condensed tannins on the growth of various lactic acid bacteria used as silage additives in growth medium in vitro.

**Material and Methods** MRS-bouillon, supplemented with 0.17% or 1% of tannin containing products, was applied as complex medium to study the growth rate of different lactic acid bacteria. In this study we used industrial tannins, manufactured from *Acacia maernsii* (Mimosa), *A. catechu* (Catechu), *Caesalpinia spinosa* (Tara) and *Schinopsis lorentzii* (Quebracho, ByproQ) with different polymerization degree, proportion of soluble and condensed tannins, residual sugars and organic solvents. As part of the macromolecules of the nutrient medium precipitated by tannin addition after autoclaving, the solutions were sterile-filtered. Ten ml of these solutions were inoculated with 500 µl of an overnight culture of the corresponding microorganisms with about $2.5\times10^5$ cfu and incubated at 30°C. We have measured the optical density at 600 nm after 19 and 27 hours of incubation. The uninoculated solution of the respective nutrient medium was taken into account as blank. Nutrient broth acc. to De Man, Rogosa and Sharpe (MRS) (1960) without tannin supplement served as control medium (100% growth). Lactic acid bacteria (LAB) of different strains of *Lactobacillus plantarum* (DSM 8862, DSM 8866, MA 18/5U, MTD 1, DSM 4784), *L. fermentum* (DSM 14638), *L. buchneri* (DSMZ 20057) and *Enterococcus faecium* (DSM 4788) have been chosen. All Statistical analysis was computed by using the SPSS statistical package (ver. 21.0; SPSS, IBM USA). Differences between the growth rates of the lactic acid bacteria influenced by the different tannins were tested with the nonparametric Kruskal-Wallis or Mann-Whitney test, respectively. In all tests, the probability was P = 5 %.

**Results and Discussion** Table 1 shows the growth of the LAB in the presence of 0.17 % tannins in comparison to untreated MRS-bouillon after 19 hours. The growth repression after 19 hours in presence of 0.17% tannin is insignificant low. The industrial product Tara (*Caesalpinia spinosa*) has the strongest influence on the growth. The growth depression is significantly stronger at a concentration of 1.0% tannin (Table 2). The heterofermentative LAB grow with delay and are most inhibited. The growth inhibition of the *Lactobacillus plantarum*-strains is least by the used tannins. Similar results were obtained after 27 hours incubation. The growth of the studied lactic acid bacteria is influenced by tannins of different plant origins and their processing technology. Both the strains of one species (*L. plantarum*) and also different species are differently sensitive to the used tannin products. The homofermentative organisms grow best in the presence of Quebracho, while the heterofermentative LAB show the highest growth in Catechu but in no case reach the level of homofermentative LAB. One explanation could be that the technical products contain not only useful sugars, but also a proportion of hydrolysable tannins which could be splitted by tannases into inactive monomers. Schweigmann and Gierus (2011) also showed that different tannin extracts have a pH-dependent precipitation behavior towards proteins.

**Conclusions** The results demonstrate that at low tannin-supplementation, only small differences in the growth between the tested strains can be seen, whereas at higher tannin-concentrations large differences between the strains and the strain combinations were observed. The tested low concentration of tannins is not relevant for the ensiling process, but the higher concentration is used in praxis. Using simple microbiological tests, it is possible to predict the suitability of different LAB in combination with tannins for ensiling. With regard to several properties of ensiling material, it is additionally necessary to test the growth and the acidification activity of the microbiological additives in presence of tannins at various osmolalities.

**Table 1.** Influence of the tannin containing products Mimosa, Catechu, Tara, Quebracho and ByproQ as supplement in nutrient broth acc. to De Man, Rogosa and Sharpe (MRS) on the growth of different lactic acid bacteria (supplemented with 0.17 % tannin).
Percentage of growth in comparison to MRS (growth in MRS-bouillon without tannin = 100 %)
Incubation 19 hours (N=3, MV-Mean value, SD-Standard deviation).

| | Percentage growth (% of MRS) *(MV±SD)* | | | | |
|---|---|---|---|---|---|
| | Supplement | | | | |
| Strain | Mimosa | Catechu | Tara | Quebracho | ByproQ |
| *L. plantarum* DSM 8862 | 95 ± 3 | 105 ± 3 | 95 ± 1 | 94 ± 2 | 92 ± 3 |
| *L. plantarum* DSM 8866 | 92 ± 1* | 98 ± 1 | 91 ± 1* | 90 ± 1* | 88 ± 2* |
| *L. plantarum* DSM 8862+8866 | 93 ± 3 | 90 ± 7 | 92 ± 2 | 86 ± 3 | 87 ± 4 |
| *L. fermentum* DSM 14638 | 81 ± 3* | 82 ± 2* | 70 ± 2* | 83 ± 3* | 80 ± 4* |
| *L. plantarum* MA 18/5U + *P. acidipropionici* MA 26/4U | 99 ± 2 | 99 ± 4 | 97 ± 3 | 90 ± 3 | 92 ± 3 |
| *L. plantarum* MTD 1 | 99 ± 2 | 99 ± 2 | 97 ± 2 | 95 ± 1 | 94 ± 1 |
| *L. buchneri* DSMZ 20057 | 100 ± 10 | 104 ± 20 | 43 ± 6* | 100 ± 11 | 109 ± 7 |
| *L. plantarum* DSM 4784 + *E. faecium* DSM 4788 | 104 ± 2 | 89 ± 5 | 92 ± 2 | 99 ± 3 | 104 ± 3 |

* = means significant difference to control version ((100 % growth rate of LAB on MRS; P=5 %)

**Table 2.** Influence of the tannin containing products Mimosa, Catechu, Tara, Quebracho and ByproQ as supplement in nutrient broth acc. to De Man, Rogosa and Sharpe (MRS) on the growth of different lactic acid bacteria (supplemented with 1.0 % tannin).
Percentage of growth in comparison to MRS (growth in MRS-bouillon without tannin = 100 %)
Incubation 19 hours (N=3, MV-Mean value, SD-Standard deviation).

| | Percentage growth (% of MRS) *(MV±SD)* | | | | |
|---|---|---|---|---|---|
| | Supplement | | | | |
| Strain | Mimosa | Catechu | Tara | Quebracho | ByproQ |
| *L. plantarum* DSM 8862 | 85 ± 3 | 79 ± 3* | 71 ± 2* | 103 ± 6 | 64 ± 2* |
| *L. plantarum* DSM 8866 | 85 ± 3* | 91 ± 3 | 82 ± 4* | 109 ± 8 | 80 ± 3* |
| *L. plantarum* DSM 8862+8866 | 99 ± 4 | 97 ± 7 | 87 ± 5 | 131 ± 6* | 77 ± 2* |
| *L. fermentum* DSM 14638 | 13 ± 2* | 12 ± 1* | 0* | 5 ± 1* | 52 ± 4* |
| *L. plantarum* MA 18/5U + *P. acidipropionici* MA 26/4U | 85 ± 6 | 98 ± 4 | 44 ± 3* | 103 ± 7 | 68 ± 2* |
| *L. plantarum* MTD 1 | 96 ± 7 | 122 ± 10* | 101 ± 11 | 122 ± 11 | 87 ± 3 |
| *L. buchneri* DSMZ 20057 | 26 ± 4* | 41 ± 2* | 8 ± 2* | 37 ± 4* | 12 ± 2* |
| *L. plantarum* DSM 4784 + *E. faecium* DSM 4788 | 9 ± 1* | 30 ± 2* | 0* | 42 ± 2* | 6 ± 2* |

* = means significant difference to control version (100 % growth rate of LAB on MRS; P=5%)

**References**

Bossi, A., Rinalducci, S., Zolla, L., Antonioli, P., Righetti, P. G. & Zapparoli, G. (2007) Effect of tannic acid on *Lactobacillus hilgardii* analysed by proteomic approach. J. Appl. Microbiol., 102, 787-795.

Costabile, A., Sanghi, S., Martin-Palaez, S., Mueller-Harvey, I., Gibson, G. R., Rastall, R. A. & Klinder, A. (2011) Inhibition of Salmonella Typhimurium by tannins in vitro. Journal of Food, Agriculture & Environment, 9 (1). 119-124.

De Man, J.C, Rogosa, M. & Sharpe, M. (1960) A medium cultivation of Lactobacilli. J. Appl. Microbiol. 23, 130-135

Scalbert, A. (1991) Antimicrobial properties of tannins. Phytochemistry, 30, 3875-3883.

Schweigmann, M. & Gierus, M. (2011) Tanninhaltige Pflanzenextrakte und ihre

Proteinfällungskapazität in vitro. Mitteilungen der Arbeitsgemeinschaft Grünland und Futterbau, 12, 185-190.

# Identification of lactic acid bacteria isolated from mulberry (*Morus alba* L.) to improve tannin degradation and silage quality

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**Keywords:** lactic acid bacteria, mulberry, silage, tannin

**Introduction** Mulberry (*Morus alba* L.) is a popular new type forage resources in China characterised by a high yield and crude protein (CP), which is perennial plant (Hejcman et al.2016). However, hinder of feeding mono-gastric animal is high hydrolysable tannin content, and the high-quality silage processing is still limited. In order to solve these problems, lactic acid bacteria (LAB) inoculants which can both improve the fermentation quality and tannin degradation may adopt. The results in this paper will be valuable for the future design of appropriate inoculants.

**Material and Methods** Mulberry (Tianyuan NO.3-high protein forage mulberry) used to isolate LAB was harvested at the Luoyang Academy of Agriculture and Forestry Science in Henan, China on 24$^{th}$ June 2016 and wilted for 14h. The LAB were isolated from pre-ensiled mulberry and after ensiling 60days.Then, the catalase activity, gas production from glucose, temperature tolerance, pH tolerance, salt tolerance and carbohydrate fermentation of isolated LAB were determined as described by Zhang et al. (2015). The LAB used to improve the silage quality was selected by the rapid acidification ability test (Ni et al. 2016). The LAB used to tannin degradation was selected by the method of tannin transparent zone according to the procedure described by Osawa et al. (2000). In brief, the LAB separated from mulberry was inoculated onto tannin-treated infusion agar. The inoculated plates were incubated in an anaerobic incubator at 37℃ for 72 h. The strains with distinct transparent extending zone beyond their edges were identified as tannin degradation LAB. Then, the tannase enzyme activity was determined by solid fermentation and the enzyme unit was defined as the needed enzyme content which can product 1μmol gallic acid per minute under the condition of pH5.0 and 30 ℃. The species of isolated strains were identified by the method of 16S rRNA gene sequencing (Cai et al. 1999)**.** Finally, the isolated strains which can produce acid and digest tannin fast were selected for further experiment.

**Results and Discussion** In total, 103 strains were considered to be LAB, which were gram-positive and catalase-negative. All isolates could grow at pH ranged from 6.5 to 9.0 and in 3.0% (W/V) NaCl. Among them, 6 strains were selected due to their high growth rate, acidification ability and tannin degradation ability. The tannase enzyme activity of six strains was range from 0.82 to 1.25 μmol/min· ml (Table 1). And the 24 pH of these isolates were almost under 4.00. The isolated strains (ML11, ML16, ML37, ML47, ML56 and ML58) were all identified as *Lactobacillus* (*L.*) *plantarum*, which is consistent with Ueda et al. (2014) reported that tannase can be produced from *L. plantarum*, *L. paraplantarum* and *L. pentosus*.

**Table 1** Tannase activity and pH value (24h) in LAB strains isolated from mulberry

| Strains | Tannase activity (μmol/min· ml) | pH(24h) |
|---|---|---|
| ML11 | 1.05 | 4.02 |
| ML16 | 0.82 | 3.74 |
| ML37 | 1.25 | 3.75 |
| ML47 | 1.20 | 3.77 |
| ML56 | 0.91 | 3.77 |
| ML58 | 1.17 | 3.75 |

**Conclusions** In this study, a total of 6 LAB strains were considered to be potential inoculants for ensiling mulberry and degrade tannin, and they were all identified as *L*. *plantarum*. However, further research is warranted to investigate their effect on the silage quality of mulberry under small-scale fermentation conditions.

**References**

Cai Y., Benno Y., Ogawa M. & Kumai S. (1999) Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. Journal of Dairy Science, 82, 520-526.

Hejcman M., Hejcmanova P., Pavlu V. & Thorhalls T. (2016) Forage quality of leaf fodder from the main woody species in Iceland and its potential use for livestock in the past and present. Grass and Forage Science, 71, 649-658.

Ni K., Yang H., Hua W., Wang Y. & Pang H. (2016) Selection and characterisation of lactic acid bacteria isolated from different origins for ensiling *Robinia pseudoacacia* and *Morus alba* L. leaves. Journal of Integrative Agriculture, 15, 2353-2362.

Osawa R., Kuroiso K., Goto S. & Shimizu A. (2000) Isolation of tannin-degrading Lactobacilli from humans and fermented foods. Applied and Environmental Microbiology, 66, 3093-309.

Ueda S. Nomoto R., Yoshida K. & Osawa R. (2014) Comparison of three tannases cloned from closely related *lactobacillus* species: *L. plantarum*, *L. paraplantarum*, and *L.pentosus*. BMC Microbiology, 14, 1-9.

Zhang Q., Li X., Zhao M. & Yu Z. (2015) Lactic acid bacteria for enhancing the fermentation quality and aerobic stability of *Leymus chinensis* silage. Grass and Forage Science, 71, 472-481.

# Impact of grass silage with high levels of propylene glycol on ketosis prophylaxis during transition phase and early lactation

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**Keywords:** Dairy cattle**,** ketosis, *Lactobacillus buchneri*, propylene glycol

**Introduction** During early lactation, energy requirement and energy intake of dairy cattle are almost never balanced. To prevent ketosis, a common consequence of negative energy balance (Nielsen and Ingvartsen, 2004), dietary addition of glucogenic compounds (e.g., Propylene glycol (PG)) is frequently recommended. High amounts of PG (> 4% in DM) can be observed in *Lactobacillus buchneri* treated grass silages after 120 d of ensiling (Nishino et. al, 2003). Assuming dairy cows consume 8.0 kg grass silage per day containing 4.0% PG (all DM basis), total daily intake would be 320 g PG, which meets common recommendations (DLG, 2005; DLG, 2012). The aim of this study was to evaluate the impact of different grass silages with/without high levels of PG on ketosis relevant metabolic parameters, milk yield and feed intake of dairy cattle.

**Material and Methods** In May 2016, grass from first cut was harvested with two separate harvesters, which chopped in parallel (total 120 ha). Grass silage materials were ensiled in two different silos and stored over 90 d. The following treatments were applied: (1) inoculant containing a mixture of homofermentative and heterofermentative lactic acid bacteria $3.0 \times 10^5$ colony forming units [CFU] per g fresh forage (hoheLAB – *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus buchneri*) and (2) inoculant containing a mixture of homofermentative lactic acid bacteria $3.0 \times 10^5$ CFU/g fresh forage (hoLAB – *Lactobacillus plantarum, Lactobacillus rhamnosus*). During a three-month feeding trial, two groups of 60 dairy cows were fed a total mixed ratio (TMR) with either hoheLAB or hoLAB inoculated grass silages. Our study was performed in the Gut Huelsenberg (Wahlstedt). The cows (Holstein-Friesian breed) were high yielding with a preceding lactation of over 12000 kg of milk. The TMR of the two treatments (hoheLAB and hoLAB) differed only in grass silage and was composed as follows: 25.7% corn silage, 34.1% grass silage, 39.5% concentrates and 0.7% Silostar Liquid (Na-benzoate and Na-acetate) (all DM basis) (NEL: 7.12 MJ/kg DM; CP: 175 g/kg DM). Cows within each of the two treatment groups were blocked according to lactation number, milk yield and days in milk (N = 120) (cLAB group: average lactation number: 2.0 ± 1.1; average days in milk: 206 ± 96; hLAB group: average lactation number 2.1 ± 1.3; average days in milk: 204 ± 118). Freshly calved cows (hoheLAB: n = 12 and hLAB n = 11) were randomly distributed into the experimental groups on day 3 after calving according to lactation number and milk yield in the previous lactation. Measured parameters included daily dry matter intake of the group (DMI) and milk yield which were recorded every day. Milk composition was recorded five times for each cow during the trial period. Measured parameters of freshly calved cows included: beta-hydroxbutyrate (**BHB**) and non-esterified fatty acids (**NEFA**). Blood samples were collected six weeks before calving, on the day of calving and weekly for the following 6 weeks and submitted to a commercial laboratory (Biocheck GmbH, Leipzig). Blood samples were collected from the vena jugularis 1 hour before morning feeding. Measured parameters of the silages and the TMR included: nutrient contents (according to VDLUFA, 2012), enzyme soluble organic substance (**ELOS**), and concentration of fermentation acids (high performance liquid chromatography (HPLC)) and counts of molds, lactic acid bacteria (**LAB**), yeast and Clostridia. Data were analyzed using the mixed model procedure (PROC MIXED) of SAS 9.4 (SAS, 2013). Differences in BHB between treatment groups (hoLAB vs. hoheLAB) were tested via ANOVA. Differences between least squares means were determined by the PDIFF option with the Tukey`s adjustment. The model included the fixed effect of treatment, week and the interaction of treatment × week. Cow was included as random effect. Treatment differences were declared significant at $P < 0.05$.

**Results and Discussion** Grass silage inoculated with hoheLAB had an average PG content of 3.89 ± 0.07%; silage inoculated with hoLAB contained 0.06 ± 0.08% PG (both DM basis). TMR I (hoheLAB grass silage) had an average PG content of 1.7 ± 0.13%; TMR II (hoLAB) contained 0.4 ± 0.06% PG (both DM basis). Average milk yield of freshly calved cows in hoheLAB group was 41.2 ± 3.74 kg/d and in the hoLAB group 40.7 ± 3.63 kg/d. The milk composition over six weeks post-partum was as follows: hoheLAB group – protein: 3.4 ± 0.29% and fat 4.8 ± 0.49%; hoLAB group – protein: 3.2 ± 0,31% and fat 5.1 ± 0.24%. Average DMI of cows in hoheLAB group was 23.6 ± 1.03 kg/d and of those in hoLAB group 22.7 ± 0.58 kg/d. Compared to the hoLAB group (96 ± 13.9 g), daily PG intake of the hoheLAB group was more than four times higher (401 ± 41.9 g). Compared to the hoLAB group, feeding microbially produced PG trough hoheLAB-inoculated grass silage decreased level of BHB (average 43%) in postpartum cows over the first six weeks of lactation. Subclinical ketosis is defined as a serum BHB concentration of 1.0 – 1.4 mmol/l (Van Saun, 2007, Oetzel, 2004). In the hoheLAB group, no increase in the average NEFA concentration was observed in the first six weeks after calving, whereas in hoLAB NEFA concentration increased (hoLAB: 0.61 ± 0.18 mmol/l; cLAB: 0.25 ± 0.09 mmol/l).

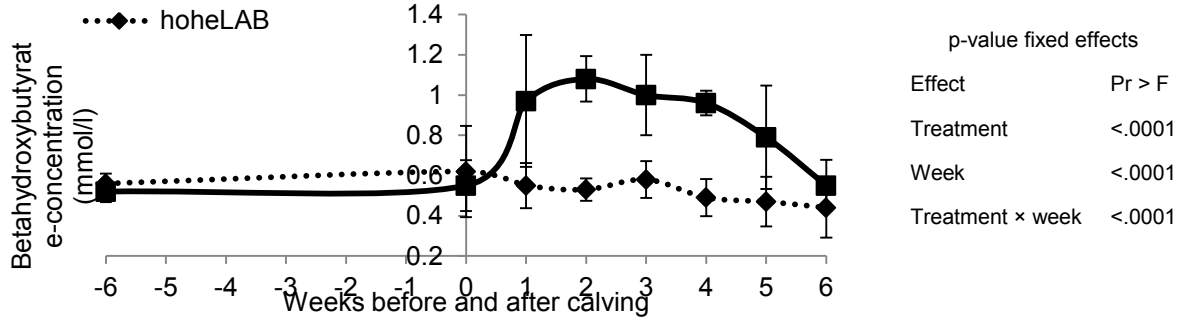


**Figure 1**. Betahydroxybutyrate-concentration (mmol/l; all in average ± SD) of freshly calved cows (hoheLAB: n = 12; hoLAB: n = 11); *Treatment differences were declared significant at P < 0.05.

**Conclusion** Although the energy concentration of the TMR of both groups was not different (energy content of diets 7.12 MJ NEL/kg DM for both groups), the hoheLAB group had considerably lower BHB concentrations. Microbially produced propylene glycol had the capacity to reduce ketone bodies in the blood of dairy cows after calving.

**References**

DLG (2005) Fachinfos Rinder: Zum Einsatz von Propylenglykol in der Milchviehfütterung. DLG-Verlag, Frankfurt.

DLG (2012) Fütterungsempfehlungen für Milchkühe im geburtsnahen Zeitraum. DLG-Verlag, Frankfurt.

Nielsen, N.I. & Ingvartsen, K.L. (2004) Propylene glycol for dairy cows. A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Animal Feed Science Technology 115, 191-213.

Nishino, N., Yoshida, M., Shiota, H. & Sakaguchi, E. (2003) Accumulation of 1,2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with *Lactobacillus buchneri*. Journal of Applied Microbiology 94, 800-807.

Oetzel, G.R. (2004) Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice 20 (3), 651-674.

SAS Institute. (2013) System for Windows Release 9.4. SAS Inst. Inc., Cary, NC, USA.

Van Saun, R.J. (2007) Application of a pooled sample profile for use as a herd screening tool. Proceeding Danske Kvaegfagdyrlaegers Årsmøde (Danish Bovine Practitioner Seminar) Middelfart.

Weissbach, F., Kuhla, S. (1995) Stoffverluste bei der Bestimmung des Trockenmassegehaltes von Silagen und Grünfutter. Entstehende Fehler und Möglichkeiten der Korrektur. Übersichten Tierernährung 23, 189-214.

# Impact of various silage additives on propylene glycol content of grass silages

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**Keywords:** Ensiling, Ketosis, Propylene glycol, silage additives

**Introduction** Various strains of homofermentative and heterofermentative lactic acid bacteria (LAB) are used as silage inoculants. Homofermentative LAB convert sugar to lactic acid, while heterofermentative LAB convert sugar to lactic and acetic acid. Certain LAB strains (e.g., *Lactobacillus buchneri*) additionally form alcohol and propylene glycol (PG; Driehuis et al. 2001). However, the metabolic pathway of heterofermentative LAB commonly results in a greater loss of dry matter (DM) and energy compared to homofermentative LAB (Driehuis et al. 2001). Synthetic PG is commonly used as glucogenic supplement for postpartum dairy cows to compensate for an energy deficit at the onset of lactation (Nielsen and Ingvartsen 2004) and to serve as a precursor for gluconeogenesis (Emery et al. 1967). This suggests that consumption of silage with elevated PG content could positively affect milk performance and reduce risk of ketosis in dairy cattle (Nishino et al. 2002). The present study aims to investigate whether inoculation with *L. buchneri* leads to the formation of PG in grass silage of varying DM, sugar and neutral detergent fiber content ($aNDF_{om}$).

**Material and Methods** This study consisted of small batch silage experiments (n = 19). To test the effect of silage inoculant on young and more mature grass, first, second, third, and fourth cuts were chosen in two different years (2016 and 2017). Fresh grass was ensiled in quadruplicate. Following treatments were applied: (1) control (no additive), (2) inoculant containing a mixture of homofermentative LAB at $3.0 \times 10^5$ CFU/g fresh grass (hoLAB – *Lactobacillus plantarum, Lactobacillus rhamnosus*) and (3) inoculant containing a mixture of homofermentative and heterofermentative LAB $3.0 \times 10^5$ CFU/g fresh grass (hoheLAB – *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus buchneri*). All fresh grass samples varied in DM (23.6% to 78.5%), sugar (6.6 ± 0.14% to 23.2 ± 0.13%) and NDF content (42.2 ± 0.18% to 55.5 ± 0.12%; all DM basis). The grass was ensiled in preserving mini silos (1500 mL) for 90 d. Measured parameters included: chemical composition of the fresh and ensiled grass (Methods according to VDLUFA 2012) and determination of enzyme soluble organic matter (ELOS) in the DaisyII incubator (Stadler and Henkelmann 2012). Fermentation gas losses (FGL) were determined based on the mass difference of the mini silos before and after ensiling and expressed as percentage fresh matter (Weissbach 1998). According to Weissbach and Kuhla (1995), DM losses (DML) were determined from the mass difference before and after ensiling, the DM content and a proportion of dissolved $CO_2$ in the silage. Silage DM were corrected for losses of ethanol, PG, volatile fatty acids, and lactic acid due to drying (Weissbach and Kuhla 1995).Concentration of fermentation acids, ethanol and PG from each vessel was measured by high performance liquid chromatography (HPLC). Colony counts of LAB, mold, yeast, and clostridia are always quantitative. For enumeration of LAB, samples were plated on MRS agar. Yeasts and mold counts were determined on YGC agar. Differences among treatments (control, hoheLAB, hoLAB) were tested via ANOVA. A comparison of means was subsequently performed using the Tukey Test. The model included the fixed effect of treatment. Mini silo was included as random effect. Treatment differences were declared significant at $P < 0.05$ (SAS, 2013).

**Results and Discussion** Loss of DM was higher for hoheLAB (4.98%) compared to hoLAB (4.34%; $P< 0.05$). In contrast, DML between hoheLAB and the control (4.83%) was similar ($P=0.78$). As expected, pH after 90 d was lower in hoLAB (4.21) compared to cLAB (4.42) and the control respectively (4.36; $P< 0.05$). No differences in pH were detected between the control and hoheLAB

(P=0.57). Inoculation with hoheLAB lead to substantially higher concentration of PG (1.90% DM) compared to the control (0.10% DM) and hoLAB (0.04% DM; P<0.001), while there was no difference in PG content between hoLAB and the control (P=0.85). However, DM and sugar content of the grass used in our study varied more substantially and the DM (Figure 1) and sugar content (Figure 2) of the ensiled forage seems to have an impact on the formation of PG. Markedly higher concentrations of PG can be expected when grass was ensiled with < 40% DM and a sugar content of ≥ 12% DM. The PG content varied between 0.09% DM (DM content of fresh grass: 53.4%; $aNDF_{om}$ content 42.4% DM; sugar content 20.1% DM) and 4.1% DM (DM content of fresh grass 24.2%; $aNDF_{om}$ content 43.5% DM; sugar content 14.7% DM). The results of the fermentation acids and fermentation products confirm that activity levels of LAB are low in dry grass (> 50% DM). The results justify the claim that inoculation with hoheLAB led to increased formation of PG. The results of this study show that the PG content differed depending on level of DM content (I) 20-30% DM on average 3.2 ± 0.94% of DM (n = 4 ); (II) 30-40% DM on average 2.2 ± 0.94% of DM (n = 9); (III) >40% on average 0.60 ± 0.41% of DM (n=6) after 90 days of storage.

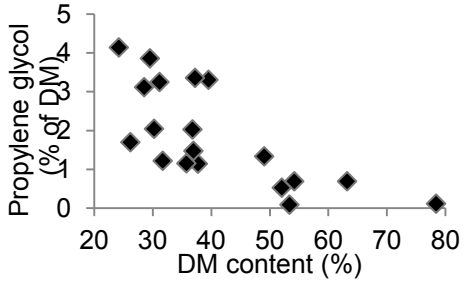


**Figure 1.** Propylene glycol content in ensiled grass (supplemented with a mixture of homo- and heterofermentative lactic acid bacteria) in relation to the DM content of fresh grass

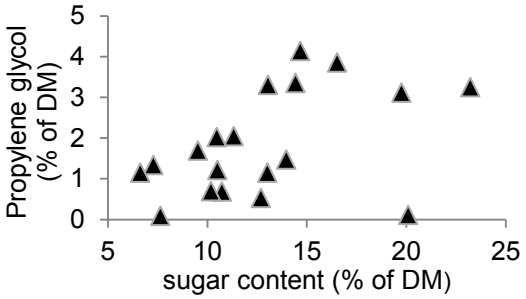


**Figure 2**. Propylene glycol content in ensiled grass (supplemented with a mixture of homo- and heterofermentative lactic acid bacteria) in relation to the sugar content of fresh grass

**Conclusion** The concentration of PG in grass silage is foremost dependent on the choice of inoculant. Additional factors influencing the formation of PG in silage are the DM and sugar content of the fresh grass.

**References**

Driehuis, F., Oude Elferink, S.J.W.H. & Van Wikselaar, P.G. (2001) Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass and Forage Science 56, 330-343.

Emery, R.S., Brown, R.E. & Black, A.L.. (1967) Metabolism of DL-1,2-propanediol-2-$^{14}$C in a lactating cow. Journal of Nutrition, 92, 348-356.

Nielsen, N.I. & Ingvartsen, K.L. (2004) Propylene glycol for dairy cows. A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Animal Feed Science Technology, 115, 191-213.

Nishino, N., Yoshida, M., Shiota, H. & Sakaguchi, E. (2002) Accumulation of 1,2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with *Lactobacillus buchneri*. Department of Animal Science, Faculty of Agriculture, Okayama University, and Futaba Feed Ltd, Okayama, Japan.

Stadler, B. & Henkelmann G. (2012) Moderne Analyse von ELOS in pflanzlichen Rohstoffen im Bioenergie- und Futtermittelbereich. VDLUFA Schriftenreihe 68, pp. 855-864.

Weissbach, F. & Kuhla, S. (1995) Stoffverluste bei der Bestimmung des Trockenmassegehaltes von Silagen und Grünfutter: Entstehende Fehler und Möglichkeiten der Korrektur. Übersichten zur Tierernährung, 23, 189-214.

Weissbach, F. (1998) Über die Bestimmung der Gärverluste in Silierversuchen unter Laborbedingungen. VDLUFA Schriftenreihe 49, 110. VDLUFA Meeting, pp. 461-464.

# Influence of chemical pesticides on the survival of lactic acid bacteria in silage inoculants

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**Keywords:** additive, *Lactobacillus buchneri*, *Lactobacillus plantarum*

**Introduction** Inoculants have been used worldwide to improve the fermentation and aerobic stability of silages (Muck 2010). During the application of inoculants, the survival of lactic acid bacteria is essential for their acting and can be influenced by several factors, such as environmental conditions (e.g. high temperature) and the contact with chemical contaminants (Kung et al. 2003).

In developing countries, the number of small farms is significant. In these small properties, the application of silage inoculants is generally done with hand sprayers (e.g., costal backpack pumps). In some instances, the same sprayer is used for applying pesticides, such as parasiticides (in animals) and herbicides (in crops). Even if the operator empties the sprayer, one might have cross-contamination of products and, depending on the molecule, the residual solution would impair the survival and effectiveness of the bacteria. Thus, the objective of this work was to evaluate the survival of bacterial inoculants exposed to pesticides.

**Material and Methods** The survival of two commercial strains of bacterial inoculants [*Lactobacillus plantarum* (LP) and *Lactobacillus buchneri* (LB)] were evaluated after simulated contamination with three herbicides (glyphosate, 2,4-Dichlorophenoxyacetic acid and atrazine) and three ectoparasiticides (amitraz, cypermethrin and trichlorphon). Falcon tubes with 9 mL of ringer solution containing the respective bacteria were prepared. To evaluate the effect of chemical contaminants, commercial pesticides containing those chemical compounds were diluted in distilled water following the instructions in the label. Afterwards, work solutions were further diluted to obtain final concentrations of 0%, 0.1% and 1% v/v of contaminant solution after transferring 1 mL to the tubes containing the bacteria (final volume = 10 mL). Then, the tubes were vortexed and exposed at room temperature (24°C) for 0, 8 and 24 h inside the lab (3 replicates per treatment). Inoculant survival was expressed as percentage of the 0% dose (control) at time 0 h, obtained by plating in selective media for lactobacilli [De Man, Rogosa, and Sharpe (MRS) agar]. Agar plates were counted after incubation at 30°C for 48 h.

Data were analysed separated for LP and LB as repeated measures using the MIXED procedure of SAS. Model included the fixed effects of chemical, dose, time and their interactions. The effect of tube nested within chemical × dose was used as an error term. Covariance structures were chosen based on the corrected Akaike information criterion (AICc).

**Results and Discussion** There was a three-way interaction (chemical × dose × time) for the survival of both LP and LB (Figure 1). The susceptibility of LP and LB was fairly similar. However, all tubes lost viability over time, including the control (e.g., 70% of viability after 8 h of exposure). Considering that our tubes were exposed inside the laboratory at 24 °C, and in field conditions higher temperatures would represent a stressor factor to bacterial inoculants (Windle and Kung 2016), it is recommended to refill the inoculant applicator tank at least twice daily during the ensiling period.

Overall, herbicides were more toxic than parasiticides, but again, the effect was dependent on chemical, dose and exposure time. Glyphosate at 1% of contaminant solution was very toxic and killed virtually all bacteria immediately after exposure (0 h). Atrazine was also harmful, decreasing inoculant viability to less than 30% after 8 h of exposure, for both LP and LB. Among parasiticides, amitraz and trichlorphon significantly decreased the counts of bacteria after 8 h of exposure, whereas cypermethrin had no effect compared with the control.

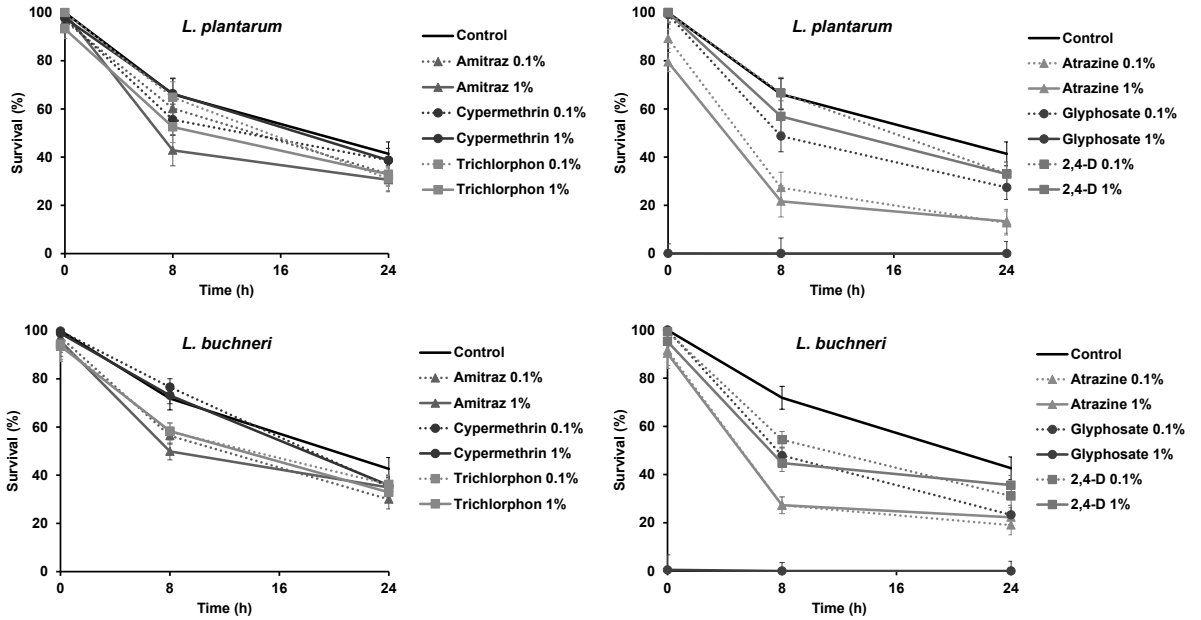


**Figure 1.** Influence of pesticides on the survival of lactic acid bacteria in silage inoculants (ectoparasiticides on left, herbicides on right). $P < 0.01$ for chemical × dose × time interaction.

**Conclusion** Depending on the chemical compound, dose and exposure time, pesticides impair the survival of bacterial inoculants. Hence, the sprayer used for applying silage inoculants should be exclusive for this purpose.

**References**

Kung Jr., L., Stokes, M.R. & Lin, C.J. (2003) Silage additives. In: Buxton, D.R., Muck, R.E., & Harrison, J.H. (eds.). Silage Science and Technology. Madison, Wisconsin, USA: American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. p. 305-360.

Muck, R.E. (2010) Silage microbiology and its control through additives. Revista Brasileira de Zootecnia, 39, 183-191.

Windle, M.C. & Kung Jr., L. (2016) Factors affecting the numbers of expected viable lactic acid bacteria in inoculant applicator tanks. Journal of Dairy Science, 99, 9334-9338.

# Inoculant effects on mycotoxins, fermentation characteristics, and nutritive value of bermudagrass silage

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**Keywords:** bermudagrass, inoculants, nutritive value, silage

**Introduction** Bermudagrass (*Cynodon dactylon* L.) is the most planted warm-season grass species in the southeast USA and Jiggs bemudagrass has been an ecotype of interest by dairy producers, primarily due to the tolerance to poorly drained soils, commonly found in South Florida. Mycotoxins are secondary metabolites produced by over 100 different molds that can cause reduced feed intake and milk production, disease infestation, reproductive problems and death in livestock. Mycotoxins can occur at lower levels that result in interactions with other stressors to cause subclinical losses in performance, increases in incidence of disease and reduced reproductive performance. These subclinical losses are of greater economic importance than losses from acute effects. According to Adesogan (2006), using proven additives and inoculants to minimize mold growth may be a feasible management practice to decrease the incidence of mycotoxins in bermudagrass silage. The addition of inoculants to silage has been an effective management practice to enhance fermentation and conservation of silage; however, most of the silage inoculants available in the market were designed and extensively tested for corn silage and there is limited information on the effects of these inoculants on bermudagrass silage. Therefore, there is a need to test the effects of commercial inoculants on mycotoxins and nutritive value of bermudagrass silage in Florida. The objective of this study was to evaluate the effects of commercial silage inoculants on mycotoxins, fermentation characteristics, and nutritive value of bermudagrass silage.

**Material and Methods** The experimental area was located at the Range Cattle Research and Education Center, Ona, FL. A Jiggs bermudagrass hayfield was subdivided in 36 plots of 5 x 5 m each plot. The plots were staged on June 15 2014 at 10-cm stubble height and fertilized with 80 kg N/ha. The harvest occurred from July 15 to July 17 2014 with target regrowth interval of 4 wk. Treatments were 7 commercial inoculants and control (untreated treatment) in a randomized complete block design with 6 replicates. The inoculants tested were B500 (BN, *Pediococcus pentosaceous* and *Lactobacillus buchneri*), Biotal Plus II (BP, *Pediococcus pentosaceous* and *Propionibacteria freudenreichii*), Early Sile Advance (ESA, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Lactobacillus plantarum*, and *Lactobacillus brevis*), Promote HQ (HQ, *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *Pediococcus pentosaceus*), Promote VS-3 (VS3, *Lactobacillus plantarum* and *Pediococcus acidilactici*), F20 (FL, *Lactococcus lactis, Pediococcus pentosaceous*, *Enterococcus facium*, and *Lactobacillus plantarum*), and F600 (FP, *Lactobacillus buchneri* and *Lactobacillus plantarum*). The mini-silos (PVC pipes with rubber caps with capacity of 1 kg of green forage) were filled immediately after harvest with the target 20-30% DM concentration. The inoculants were applied with a hand sprayer before ensiling. The silos were opened on October 22 2014. Forage samples were dried at 55$^{o}$C for 72 h and ground in an Udy mill (Udy Corporation, Fort Collins, CO.) to pass a 1-mm screen. Samples of the ensiled forage were analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent fiber digestibility (NDFD), in vitro true digestibility (IVTD), $NH_3$-N, pH, and volatile fatty acids (VFA) concentrations. Silage samples destined for microbial analysis will be placed in bags, heat sealed, and analyzed for yeast and mold counts (AOAC, 2005) at the Dairyland Laboratory Inc. (Sauk Rapids, MN). Aerobic stability was measured by placing 0.5 kg of silage in an open-top plastic bag. Temperature sensors (Onset Computer Corporation, Bourne, MA) were placed at the center of bags and set to record the temperature every 30 min for 14 d. Aerobic stability was

denoted by the time (h) that elapsed before silage temperature increased by at least 2°C above the room temperature of 23°C. In addition, the presence and concentration of aflatoxin, zearalenone, T-2 toxin, and fumonisin were measured according to the methods described by Turner at al. (2009).

**Results** There were no effects ($P$>0.05) of the inoculants on silage, DM and DM recovery, and CP, NDF, ADF, IVTD, and NDFD concentrations in the DM (Table 1). The treatments VS3 and Control had lower ($P$<0.01) pH than BN, BP, and FP and similar to the other treatments (Table 2). Lactic acid was greater ($P$=0.01) in silage treated with VS3 and Control than FP but similar among the other treatments (Table 2). BN had greater ($P$=0.04) acetic acid concentration than VS-3 and similar among other treatments (Table 2). There were no differences ($P$>0.05) in propionic and butyric acids and ammonia concentrations among treatments (Table 2). Aerobic stability (240 h) and mold and yeast counts measurements did not differ among treatments ($P$>0.05). In addition, there was no presence of aflatoxin (<5 ppb), zearalenone (<500 ppb) and T2 (<500 ppb) on the silage in any of the treatments.

**Table 1.** Nutritive value and DM recovery of Jiggs bermudagrass silage treated with different inoculant treatments.

| Item | Inoculant | | | | | | | | *P* value | SE |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | BN | BP | ESA | FL | FP | HQ | VS3 | | |
| DM, g kg$^{-1}$ | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 0.87 | 20 |
| DM recovery, g kg$^{-1}$ | 898 | 878 | 860 | 866 | 886 | 866 | 888 | 880 | 0.56 | 23 |
| CP, g kg$^{-1}$ DM | 107 | 102 | 101 | 103 | 104 | 107 | 105 | 109 | 0.11 | 4.5 |
| ADF, g kg$^{-1}$ DM | 397 | 404 | 398 | 406 | 404 | 395 | 394 | 391 | 0.33 | 46 |
| NDF, g kg$^{-1}$ DM | 720 | 736 | 728 | 730 | 730 | 728 | 726 | 721 | 0.64 | 9.9 |
| IVTD, g kg$^{-1}$ DM | 522 | 507 | 507 | 504 | 509 | 509 | 513 | 531 | 0.49 | 19 |
| NDFD, g kg$^{-1}$ DM | 300 | 295 | 277 | 284 | 287 | 291 | 289 | 311 | 0.82 | 19 |

DM = dry matter, CP = crude protein, ADF, acid detergent fiber, NDF = neutral detergent fiber, IVTD = in vitro true digestibility, NDFD = neutral detergent fiber digestibility.
BN = B500, BP = Biotal Plus II, ESA = Early Sile Advance, HQ = Promote HQ, VS3 = Promote VS-3, FL = F20, FP = F600.

**Table 2.** Fermentation characteristics of Jiggs bermudagrass silage treated with different inoculants.

| Item | Inoculant | | | | | | | | *P* value | SE |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | BN | BP | ESA | FL | FP | HQ | VS3 | | |
| pH | 4.6$^{b}$ | 4.95$^{ab}$ | 4.9$^{a}$ | 4.8$^{ab}$ | 4.8$^{ab}$ | 4.91$^{ab}$ | 4.7$^{ab}$ | 4.6$^{b}$ | 0.007 | 0.1 |
| Lactic acid, g kg$^{-1}$ DM | 223$^{a}$ | 60$^{ab}$ | 91$^{ab}$ | 164$^{ab}$ | 159$^{ab}$ | 47$^{b}$ | 164$^{ab}$ | 197$^{a}$ | 0.01 | 6.8 |
| Acetic acid, g kg$^{-1}$ DM | 245$^{ab}$ | 332$^{a}$ | 235$^{ab}$ | 246$^{ab}$ | 241$^{ab}$ | 184$^{ab}$ | 203$^{ab}$ | 32$^{b}$ | 0.04 | 3.2 |
| Propionic acid, g kg$^{-1}$ DM | 3.5 | 3.9 | 2.9 | 4.0 | 3.5 | 5.7 | 3.1 | 2.7 | 0.40 | 1.5 |
| Butyric acid, g kg$^{-1}$ DM | 32.4 | 39.0 | 31.9 | 47.8 | 41.1 | 47.3 | 42.2 | 38.8 | 0.64 | 11 |
| Isobutyric acid, g kg$^{-1}$ DM | 1.5 | 1.7 | 2.0 | 2.5 | 1.1 | 1.1 | 1.4 | 0.9 | 0.28 | 3.0 |
| Ammonia, g kg$^{-1}$ CP | 212 | 280 | 296 | 261 | 186 | 206 | 216 | 198 | 0.09 | 61 |

$^{a,b}$ Means within rows with different superscripts differ ($P$ < 0.05).
BN = B500, BP = Biotal Plus II, ESA = Early Sile Advance, HQ = Promote HQ, VS3 = Promote VS-3, FL = F20, FP = F600.

**Discussion** The inoculants VS-3, BN, and FP affected the fermentation characteristics of the silage with greater effects on pH, lactic acid, and acetic acid, likely due to the composition of the bacteria species in the inoculant. However, the inoculants did not improved fermentation when compared to the control silage. The lack of response of the inoculants may be related to decreased concentration of WSC and reduced DM concentration of the bermudagrass silage. Similar results were found by Vendramini et al. (2015), which did not find improvements in nutritive value and fermentation of bermudagrass silage treated with inoculants. The lack of presence of mycotoxins in the control silage did not allow this study to make inferences about the effects of inoculants on mycotoxins.

**Conclusions** The decision to add inoculants to bermudagrass silage must be done carefully due to inconsistent results and unlike economic return. It is necessary to develop management practices to enhance the efficiency of inoculants in bermudagrass silage.

**References**

Adesogan, A.T. (2006) Mycotoxins in ensiled forages. In: Charley R (ed). Key Silage Management Topics. Milwaukee, WI: Lallemand Animal Nutrition North America; p. 44-51.

Tuner, N.W., Subrahmanyam, S & Piletsky, S.A. (2008) Analytical methods for determination of mycotoxins: A review. Analytica Chimica Acta, 632, 168-180.

Vendramini, J.M.B., Aguiar, A.D., Adesogan, A.T., Sollenberger, L.E., Alves, E., Galzerano, L., Salvo, P., Valente, A.L., Arriola, K.G., Ma, Z.X. & Oliveira, F.C.L. (2016) Effects of genotype, wilting, and additives on the nutritive value and fermentation of bermudagrass silage. Journal of Animal Science, 94, 3061-3071.

# *Lactobacillus hilgardii* as inoculant for corn silage in Italy

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**Keywords:** aerobic stability, dry matter content, ensiling duration, *Lactobacillus hilgardii*.

**Introduction** In the last 20 years, heterofermentative lactic acid bacteria (LAB), and *L. buchneri* in particular, have been used as inocula to prevent the aerobic deterioration of silages, because of their ability to increase the production of acetic acid, which inhibits yeasts and increases aerobic stability. Silages that have a high dry matter (DM) content (>35%) have lower concentrations of acetic acid than wetter silages and thus are often more prone to aerobic spoilage. *L. buchneri*-based inocula need a long conservation period (>90 d) to be efficacious (Driehuis et al. 1999) and have resulted to be less efficient in high DM content corn silages (Comino et al. 2014). Owing to the farmers' need to quickly open corn silages made in late summer, due to the feeding needs of the herd or to compensate high DM losses that may have occurred in the previous year's silages, early acting inocula are required to prevent aerobic deterioration. The aim of this study was to evaluate the effect of a new strain of *L. hilgardii* on the aerobic stability of whole crop corn silage affected by different DM contents and different ensiling durations.

**Material and Methods** The effect of *L. hilgardii* was evaluated in 5 different trials. Five corn fields (from 27 to 42% DM) were harvested as whole plants and not treated (C) or inoculated with *L. hilgardii* CNCM I-4785 (LH) [theoretical application rate of 300,000 cfu/g fresh matter (FM)]. The fresh forages were ensiled in 20-L plastic silos with five replications for each treatments at a density ranging from 669±31 kg FM/$m^3$ to 529±21 kg FM/$m^3$ and opened after 15, 30, 100 and 250 days of conservation. At opening the silages were analyzed for DM content, pH, fermentative profile and microbial count. The DM content was determined at 60°C for 72 h, fermentative profile was characterized in the acid extract by HPLC. Yeast and mold counts were obtained using the pour plate technique on Yeast Extract Glucose Chloramphenicol agar. After each opening, the silages were subjected to an aerobic stability test by continuously measuring the temperature during air exposure. Aerobic stability was defined as the number of hours the silage temperature remained stable before increasing more than 2°C above room temperature. Data were analyzed via analysis of variance, utilizing inocula and DM content as the fixed factors, with their significance reported at a 0.05 probability level, using the General Linear Model of the Statistical Package for Social Science (v 24.0, SPSS Inc., Chicago, Illinois, USA).

**Results and Discussion**

The LH inoculum influenced the fermentation profile by reducing the lactic acid content and by increasing the acetic acid content of silages after 100 and 250 d of conservation (data not shown). At 100 d of ensiling, the lactic-to-acetic acid ratio decreased in LH silages, except for the wetter silage (Figure 1). The 1,2-propanediol level was under the detection limit (<0.01 g/kg DM) in 4 out of 5 control silages, whereas it was detected in 3 out of 5 LH silages as already reported by Assis et al. (2014). The aerobic stability of the silages increased as the ensiling duration increased, regardless of the treatments (Table 1). This effect is mainly explained by the reduction of yeast count as illustrated in Figure 2, where the higher the yeast count, the lower the aerobic stability. Furthermore, Figure 2 showed the effect of lactic-to-acetic acid ratio and ensiling duration on yeast count, and the higher the ratio the higher the yeast count. According to our results, Reis et al. (2018) found that, after 19, 60 and 103 d, the lactic-to-acetic acid ratio was always smaller in LH treated than in the control silages, due to the higher production of acetic acid by the inoculum.Yeast count under detection level were only observed after 100 d of ensiling, especially in LH silages. Interestingly, the yeast count was reduced by LH starting from 15 d onwards in all trials. After 30 and 100 d of fermentation, LH increased the aerobic stability of the silages, by a mean value of 15 h (+18.6%) and 29 h (+27.2%), respectively. The

yeast count was reduced by LH, from 15 d onwards in all the trials. Dry matter content of the forages influenced this pattern as reported by Comino et al. (2014).

**Table 1.** Aerobic stability and yeast count after 4 ensiling periods for different DM corn silages

| DM (%) | Aerobic stability 2°C (h) | | | | | | | | Yeast count ($log_{10}$ cfu/g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 d | | 30 d | | 100 d | | 250 d | | 15 d | | 30 d | | 100d | | 250 d | |
| | C | LH | C | LH | C | LH | C | LH | C | LH | C | LH | C | LH | C | LH |
| **27** | 56 | 57 | 137 | 154 | 182 | 209 | 221 | 247 | 3.47 | 3.05 | 2.37 | 2.04 | 1.73 | 1.05 | 0.50 | 0.50 |
| **34** | 65 | 81 | 72 | 96 | 97 | 119 | 102 | 124 | 3.90 | 3.57 | 3.72 | 2.56 | 3.17 | 1.16 | 2.17 | 1.41 |
| **36** | 69 | 66 | 83 | 97 | 76 | 77 | 184 | 200 | 4.26 | 3.96 | 3.50 | 3.22 | 3.71 | 3.78 | 0.88 | 1.02 |
| **40** | - | - | 48 | 58 | 89 | 91 | 52 | 103 | - | - | 4.04 | 4.28 | 4.29 | 4.10 | 4.72 | 3.41 |
| **42** | 51 | 53 | 58 | 67 | 96 | 191 | - | - | 5.11 | 5.00 | 4.49 | 4.23 | 4.00 | 2.80 | - | - |
| *Treat* | NS | | ** | | *** | | NS | | ** | | ** | | ** | | NS | |
| DM | *** | | *** | | *** | | *** | | *** | | *** | | *** | | *** | |
| Treat*DM | * | | NS | | ** | | NS | | NS | | ** | | * | | NS | |

C = control; DM = Dry matter; LH = *L. hilgardii*

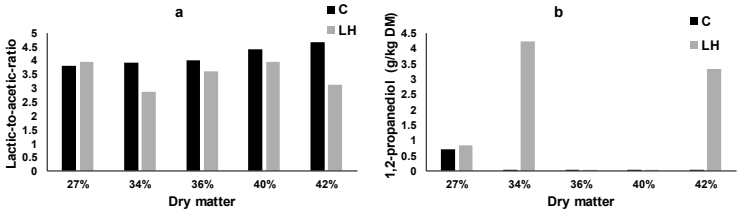


**Figure 1.** Lactic-to-acetic acid ratio (a) and 1,2-propanediol (b) of the treated and untreated corn silages with different DM contents after 100 d of ensiling (C = control; LH = *L. hilgardii*).

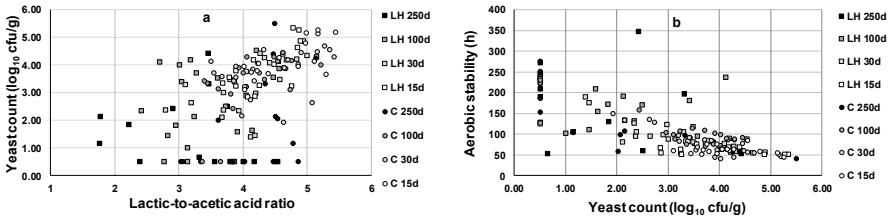


**Figure 2.** Scatter plot of lactic-to-acetic ratio and yeast count (a) and scatter plot of the yeast count and aerobic stability (b), as affected by the treatments and ensiling duration (C = control; LH = *L. hilgardii*).

**Conclusion** The new strain of *L. hilgardii* had positive effects on improving the aerobic stability of early opening corn silages as it increases the acetic acid content and reduces the yeast count. However, the effect of *L. hilgardii* is influenced by the DM content of the silages.

**References**

Assis, F.G.D., Avila, C.L.D., Pinto, J.C. & Schwan, R.F. (2014) New inoculants on maize silage fermentation. Revista Brasileira de Zootecnia, 43, 395-403.

Comino, L., Tabacco, E., Righi, F., Revello-Chion, A., Quarantelli, A. & Borreani, G. (2014) Effects of an inoculant containing a *Lactobacillus buchneri* that produces ferulate-esterase on fermentation products, aerobic stability, and fibre digestibility of maize silage harvested at different stages of maturity. Animal Feed Science and Technology, 198, 94-106.

Driehuis, F., Elferink, S. & Spoelstra, S.F. (1999). Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. Journal of Applied Microbiology, 87, 583-594.

Reis, C.B., Santos, A.O., Carvalho, B.F., Schwan, R.F. & Ávila, C.L.S. (2018) Wild *Lactobacillus hilgardii* (CCMA 0170) strain modifies the fermentation profile and aerobic stability of corn silage. Journal of Applied Animal Research, 46, 632-638.

# *Lactobacillus plantarum* TAK 59 as a silage additive to improve the silage quality.

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**Keywords:** fermentation, inoculant, *Lactobacillus plantarum*, silage

**Introduction** Ensiling is a complex of several different chemical and microbiological processes and of their concurrence. Ensiling is considered to be anaerobic lactic acid fermentation. Under favourable conditions, the fermentation process produces predominantly lactic acid. The consequent decrease in pH inhibits the growth and activity of undesirable microorganisms such as clostridia and enterobacteria. The aim of this study was to evaluate the effect of a novel *Lactobacillus plantarum* strain on the fermentation parameters of difficult-to-ensile forage.

**Material and Methods** The ensiling experiment comprised a mixture of unwilted red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.) forage, at a ratio of 75:25. The water soluble carbohydrates content in the harvested fresh material (FM) was 9.1 g/kg, which is regarded as characteristic for a difficult-to-ensile forage (according to OJEU, 2008). The control silage was made without silage additives. The second silage was inoculated with *L. plantarum* strain TAK 59 (NCIMB 42150). Strain TAK 59 is a facultatively heterofermentative, gram-positive, rod-shaped non-spore forming bacterium (Olt et al., 2017). The inoculant was diluted in chlorine-free tap water and sprayed evenly onto ensiled forage. The third silage was prepared with chemical additive (formic acid 42.5 %, ammonium formate 30.3 %, propionic acid 10.0 %, benzoic acid 1.2 %, ethyl benzoate 1.0 % and water 15.0 %). The addition rate of chemical additive was five litres per ton of FM. The biological inoculant was added in the same volume of solution, such that the concentration of inoculant in the FM was $1.2x10^5$ CFU/g. The same volume of chlorine-free tap water was used in the control treatment which had no additional additives. All treatments were made in triplicate. The control and treatment silages were stored in a dark room under constant temperature conditions (20 °C), and opened after 100 days of ensiling. Silage samples were analysed for chemical composition and pH according to generally accepted methods (AOAC, 2005). Ethanol and acids produced during fermentation were determined by gas chromatography. Statistical differences between treatments were analysed with the Kruskal-Wallis test.

**Results and Discussion** Forage that is difficulttoensile is characterised by a low dry matter (DM) and sugar and a high protein contents. Difficult-to-ensile forage prepared without additive can show very poor fermentation quality. The control silage contained high concentrations of undesirable butyric acid and ammonia nitrogen (Figure 1, Table 1), which are indicative of extensive proteolysis during fermentation and clostridial activity. The butyric and lactic acid concentration in the control silage were 30.5 g/kg in DM and 34.2 g/kg in DM respectively. The silage treated with *L. plantarum* TAK 59 contained twice as much lactic acid (69.3 g/kg in DM) as the control, and the butyric acid content was very low. *L. plantarum* TAK 59 reduced the pH of the silage to 4.4, while the pH of the control silage was 5.1. Similarly, when compared to the control silage, the silage prepared with *L. plantarum* TAK 59 had better quality parameters for ammonia nitrogen, propionic acid and ethanol. Silages inoculated with *L. plantarum* TAK 59 were uniform in terms of quality, whereas the corresponding parameters of the control and chemical additive treated silages varied greatly. *L. plantarum* TAK 59 improved lactic acid fermentation and reduced propionic acid content compared to the chemical additive treated silage ($p<0.05$).The lowest ethanol content was found in the chemical additive treated silage ($p<0.05$) compared to the untreated and inoculated silages. The high ammonia concentration in the silage treated with chemical additive, 114.0 g/kg (Table 1), may be partly related to the ammonium formate content in the additive. Chemical additive treated silage reduced the silage pH, and the butyric acid content was lower compare to the untreated control silage, Nevertheless, the silage pH did not reach a low enough level sufficient to inhibit clostridial activity and proteolysis during fermentation. Ammonia

nitrogen, acetic-, propionic- and lactic acid concentrations were similar between the control and chemical treatment silages.

**Table 1.** Chemical composition and fermentation quality of silages.

| Parameter | Control | | TAK 59 | | Chemical | |
|---|---|---|---|---|---|---|
| | Mean | s.d. | Mean | s.d. | Mean | s.d. |
| Dry matter, g/kg | 132.7 | 5.0 | 136.4 | 1.2 | 138.5 | 5.9 |
| In dry matter, g/kg | | | | | | |
| Crude protein | 153.1[a] | 8.3 | 171.0[a] | 2.1 | 177.2[b] | 3.3 |
| Crude ash | 97.4[a] | 3.0 | 93.2[a] | 1.1 | 89.2[b] | 3.5 |
| NDF | 544.4 | 23.5 | 499.7 | 4.1 | 517.1 | 23.2 |
| ADF | 383.3[a] | 18.1 | 362.2 | 8.3 | 362.3[b] | 10.7 |
| pH | 5.1 | 0.4 | 4.4 | 0.1 | 4.6 | 0.4 |
| $NH_3$-N/total N, g/kg | 109.7 | 19.3 | 79.7 | 2.5 | 114.0 | 40.0 |

[a], [b] - Least square means within a row with different superscript letters differ significantly ($p<0.05$); s.d. – Standard deviation.

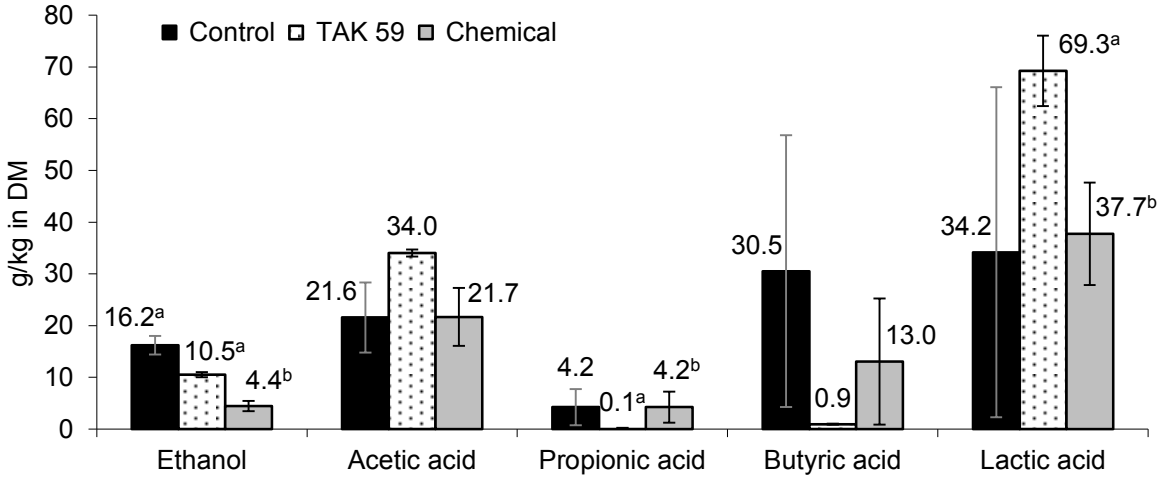


**Figure 1.** Concentration of fermentation products in silages with different treatments. Numbers above the columns are mean concentrations, whiskers show the standard deviation of each parameter. [a], [b] - Least square means within a parameter with different superscript letters differ significantly ($p<0.05$).

**Conclusions** The results demonstrate that inoculation of silage with an *L. plantarum* strain minimized the activity and spoilage effects of detrimental organisms in difficult-to-ensile forage, and improved the silage fermentation quality. *L. plantarum* TAK 59 increased the concentration of lactic acid, reduced pH, and decreased the concentrations of ammonia nitrogen, butyric- and propionic acid in silage.

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**References**

AOAC (2005) Official methods of analysis of AOAC International, 18th ed. Association of Official Analytical Chemists International, Gaithersburg, MD, USA.

OJEU. (2008) Commission regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. Official Journal of the European Union, 51, L 133/1-65.

Olt, A., Kaldmäe, H., Kärt, O., Ots, M., Songisepp, E., Rätsep, M., Kokk, K., Stsepetova, J., Kõljalg, S. (2017) Isolated microorganism strain Lactobacillus plantarum TAK 59 NCIMB42150 and its use. European patent EP3027734. Patent owner BioCC LLC (previous name Tervisliku Piima Biotehnoloogiate Arenduskeskus OÜ).

# Long or short shredded corn silage with additives - differences in fermentation quality parameters

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**Keywords:** additives, aerobic stability, maize, shredded maize silage

**Introduction** For maize silage preparation in Germany, a harvesting regime using increased cutting length plus intense conditioning instead of the general short cutting length has been increasingly discussed. The length of shreds and fiber may affect the bacterial fermentation during ensiling (Nußbaum 2013). Hence the question arose, whether length of shreds and the interaction with different silage additives may have an effect on quality parameters of the fermentation process such as feed quality and aerobic stability. Those parameters were measured after different lengths of ensiling.

**Material and Methods** The laboratory scale silage tests compared the silage of maize material with 8 mm (variant=”short”) and 22 mm shredding length (variant=”long”). The harvesting included intense conditioning. Maize was harvested in 2016 with a John Deere 8500i forage harvester. The speed difference between the cracker rollers was 50% and gap clearance was 1 mm. The samples were taken from two consecutive transport vehicles. Silage treatments consisted of an untreated control, three biological (one homo- and heterofermentative lactic acid bacteria ($LAB_{ho+he}$ (1.0 g/t FM (fresh matter)), two heterofermentative lactic acid bacteria ($LAB_{he}$ 1 (1.0 g/t FM) and $LAB_{he}$ 2 (2.4 g/t FM)) and one chemical silage additive (Chem (3.0 l/t FM), based on sodium benzoate and sodium propionate). Silage additives were applied to the fresh maize and ensiled in 1.5 l mason jars (n=3). The experiment was conducted in accordance with the “DLG Testing Guidelines for the award and use of the DLG Quality Mark for ensiling agents” (DLG 2013). The length of ensiling was 3, 21, 49 (with air stress) and 90 days. Following those periods, samples were tested for aerobic stability (ASTA) for 14 days according to the method outlined in Honig (1990). A temperature difference of +3 K in the silages compared to room temperature (20 °C.) was considered aerobically unstable. After 90 days of ensiling the feed value in the samples was determined. Here only the energy contents (MJ NEL/kg FM) and crude protein (g CP/kg DM) are listed. Statistical analysis was conducted for the quality parameters and dry matter losses (SAS 9.4, procedure ANOVA, t-Test).

**Results and Discussion** The low pH values of silages on day 3 (Table 1) suggests rapid acidification. The silages of treatments with "short" cutting length had lower pH values at 3 and 21 days compared to treatments using “long” cut material. In contrary, after 90 days, the pH values of treatments with "long" cut material were lower compared to “short” cut material. The ethanol content of silages prepared from “long“ cut material was significantly higher than samples prepared from "short" cut material, regardless of silage additives. The treatments using "long" cut material showed the highest dry matter losses, irrespective of length of ensiling or silage additive. Lowest dry matter losses were measured in silages using chemical silage additives.

The harvested material “short” had 7.41 MJ NEL/kg FM and “long” 7.23 MJ NEL/kg FM. After 90 days of ensiling, the control silages from "long" cut material showed an energy content of 7.25 MJ NEL/kg FM which was significant the highest (Table 1). The treatment LABhe 2 "short" resulted in the lowest energy content. Basically, heterofermentative LAB reduce the energy content in silages. Here, the effect occurred only in the long variant. The mean CP content of the control treatment "short" (73.2 g CP/kg DM) was significantly higher than all other treatments, the lowest CP was found in the long variants from control and LABhe 1-silages. The silages of the "short" cutting length had in general higher CP contents compared to treatments with a "long" cutting length. This may be related to the slower ph-value waste of the “long” maize.

The control treatment with "long" cut material showed higher aerobic stability than the control with "short" cut material, irrespective of days of ensiling (Table 2). Silage additives improved aerobic stability of silages prepared from “long” or “short” cut material. There were only exceptions in the “short” variants after 21 days of ensiling. Biological silage additives increased aerobic stability of the "short" cut material. In treatments based on “long" cut material this effect was less pronounced. In contrary, chemical silage additives increased aerobic stability of “long” cut material measurably.

**Table 1.** The ph-value, ethanol and dry matter losses of maize silage after 3, 21 and 90 days of ensiling and CP and NEL after 90 days of ensiling (n=3)

| Additive | days | pH-value | | ethanol | | DM loss | | CP | | NEL | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | g/kg DM | | | | | | MJ / kg FM | |
| | | short | long | short | long | short | long | short | long | short | long |
| Control | 3 | 4,21$^{c}$ | 4,28$^{a}$ | 4,0$^{d}$ | 17,7$^{b}$ | 35$^{d}$ | 44$^{b}$ | | | | |
| $LAB_{ho+he}$ | 3 | 4,21$^{c}$ | 4,28$^{a}$ | 4,0$^{d}$ | 18,0$^{ab}$ | 34$^{de}$ | 46$^{a}$ | | | | |
| $LAB_{he}$ 1 | 3 | 4,20$^{c}$ | 4,29$^{a}$ | 4,3$^{d}$ | 18,3$^{ab}$ | 34$^{de}$ | 46$^{a}$ | | | | |
| $LAB_{he}$ 2 | 3 | 4,20$^{c}$ | 4,27$^{a}$ | 4,3$^{d}$ | 19,3$^{a}$ | 34$^{de}$ | 47$^{a}$ | | | | |
| Chem | 3 | 4,23$^{b}$ | 4,27$^{a}$ | 3,0$^{d}$ | 9,7$^{c}$ | 33$^{e}$ | 38$^{c}$ | | | | |
| P | | <0.0001 | | <0.0001 | | <0.0001 | | | | | |
| Control | 21 | 4,06$^{e}$ | 4,10$^{c}$ | 14,0$^{c}$ | 18,3$^{ab}$ | 42$^{c}$ | 48$^{a}$ | | | | |
| $LAB_{ho+he}$ | 21 | 4,08$^{cd}$ | 4,12$^{b}$ | 12,7$^{cd}$ | 19,0$^{a}$ | 44$^{b}$ | 49$^{a}$ | | | | |
| $LAB_{he}$ 1 | 21 | 4,07$^{de}$ | 4,15$^{a}$ | 11,0$^{de}$ | 17,7$^{ab}$ | 43$^{bc}$ | 49$^{a}$ | | | | |
| $LAB_{he}$ 2 | 21 | 4,07$^{de}$ | 4,15$^{a}$ | 10,0$^{e}$ | 16,3$^{b}$ | 41$^{c}$ | 48$^{a}$ | | | | |
| Chem | 21 | 4,06$^{e}$ | 4,10$^{c}$ | 6,3$^{f}$ | 12,7$^{cd}$ | 36$^{d}$ | 42$^{c}$ | | | | |
| P | | <0.0001 | | <0.0001 | | <0.0001 | | | | | |
| Control | 90 | 3,97$^{d}$ | 3,82$^{f}$ | 13,8$^{b}$ | 22,5$^{a}$ | 44$^{e}$ | 50$^{d}$ | 73.2$^{a}$ | 69.6$^{e}$ | 7.14$^{b}$ | 7.25$^{a}$ |
| $LAB_{ho+he}$ | 90 | 4,25$^{a}$ | 4,04$^{c}$ | 15,7$^{b}$ | 21,3$^{a}$ | 60$^{a}$ | 60$^{a}$ | 71.2$^{bcd}$ | 70.0$^{de}$ | 7.12$^{b}$ | 7.07$^{b}$ |
| $LAB_{he}$ 1 | 90 | 4,19$^{b}$ | 3,94$^{de}$ | 11,4$^{c}$ | 22,6$^{a}$ | 51$^{d}$ | 56$^{bc}$ | 71.1$^{bcd}$ | 69.6$^{e}$ | 7.06$^{b}$ | 7.06$^{b}$ |
| $LAB_{he}$ 2 | 90 | 4,03$^{c}$ | 3,93$^{e}$ | 14,7$^{b}$ | 22,8$^{a}$ | 53$^{c}$ | 55$^{b}$ | 71.6$^{bc}$ | 70.5$^{cde}$ | 6.94$^{c}$ | 7.05$^{bc}$ |
| Chem | 90 | 3,96$^{d}$ | 3,82$^{f}$ | 8,1$^{d}$ | 15,7$^{b}$ | 39$^{f}$ | 44$^{e}$ | 72.0$^{ab}$ | 70.9$^{bcd}$ | 7.06$^{b}$ | 7.06$^{b}$ |
| P | | <0.0001 | | <0.0001 | | <0.0001 | | 0.0005 | | 0.004 | |

$^{ab}$ means depicted with the same letter in claims of similar ensiling days are not significant different
DM loss=dry matter loss; CP=crude protein; NEL=net energy lactation

**Table 2.** Aerobic stability (ASTA) of maize silages (in hours) after 21, 49 and 90 days of ensiling

| | | ASTA 21 | | ASTA 49 | | ASTA 90 | |
|---|---|---|---|---|---|---|---|
| additive | n | short | long | short | long | short | long |
| Control | 3 | 175 | 206 | 38 | 80 | 190 | 201 |
| $LAB_{ho+he}$ | 3 | 287 | 300 | 224 | 134 | cold | 295 |
| $LAB_{he}$ 1 | 3 | cold | cold | 223 | 116 | 327 | 227 |
| $LAB_{he}$ 2 | 3 | cold | 333 | 212 | 92 | 335 | 235 |
| Chem | 3 | 92 | cold | 78 | 87 | 259 | cold |

Cold=no temperature difference of +3 K compare to room temperature during the 14 days of test

**Conclusion** The difference in cutting length caused differences in fermentation attributes. The longer cut resulted in slower fermentation, lower protein levels and higher DM losses. The control silages “long” had a higher stability than the “short” variant. Biological additives tend to improve the stability at “short” and less so of long cut material. Possibly the dosage has to be adjusted for long cutting length.

**References**

Nußbaum, H. 2013. Futterkonservierung. In: Lütke-Entrup, N., Schwarz, F., Heilmann, H. (eds.). Handbuch Mais. Deutsches Maiskomitee, Bonn, Germany. pp. 264-287

DLG, 2013. DLG-Richtlinie für die Prüfung von Siliermitteln auf DLG-Gütezeichen-Fähigkeit

Honig, H. (1990) Evaluation of aerobic stability. Proceedings of the Eurobac Conference 1986, Grass and Forage reports, Uppsala, Sweden, 1990, pp. 76-82

# Protein degradation during ensiling comparing tannin extracts to conventional additives exemplified by lucerne (*Medicago sativa*)

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**Keywords:** *Medicago sativa,* protein degradation, protein fractions, silage additives, tannin extracts

**Introduction** During wilting and ensiling true protein of forages is partly solubilized and degraded to smaller fractions of peptides, free amino acids and ammonia. While ruminal microflora can incorporate part of these components into microbial protein which is highly digestible, excessive soluble N has to be discharged by the animal. On the other hand, rumen undegradable protein is needed for high performing dairy cows. Tannins are known to bind to protein. Thus, the objective of our study was to evaluate these alternative silage additives for their potential to decrease protein degradation in a forage legume.

**Materials and Methods** From 2013-2016 lucerne was ensiled in eight trials at two locations in East Germany in three or four replicates on laboratory scale. Dry matter ranged from 170-510 g/kg (mean and median 380 g/kg). Tannin extracts from mimosa (*Acacia mearnsii*) and quebracho (*Schinopsis balansae)* were applied in varying concentrations from 0, 0.5, 1.5 to 3 % of forage dry matter (Control K 0; T 0.5, T 1.5, T 3.0). Other treatments comprised a biological silage additive (B) (*Lactobacillus plantarum*) (6 trials), partly buffered formic acid (C) (6 trials), a combination of *L. plantarum* and formic acid (BC) (4 trials) and a combination of $NaNO_2$ + hexamethylenetetramine (NA) (5 trials). Silos were opened after varying ensiling duration (partly staggered opening days between 3 to 180 d). All trials included a treatment of tannin extract (3 % of forage DM) and an ensiling duration of ≥49 d. Fermentation quality was determined chemically. Protein fractions were analyzed according to Licitra et al. (1996). For the evaluation, increasing A-fraction of crude protein (CP) (i.e. non-protein nitrogen) served as main indicator for proteolysis. To describe the course of proteolysis, descriptive statistics was used including 538 datasets. To evaluate the effects of silage additives, the dataset was reduced to silages with an ensiling duration of ≥24 d, leaving 251 datasets for ANOVA and LSD-Test (P<0.05) (RStudio – © 2009-2017 RStudio, Inc).

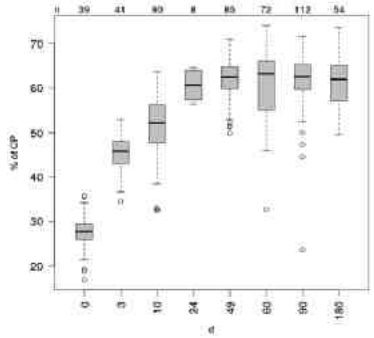

**Figure 1.** Changes of A-fraction of CP over ensiling duration

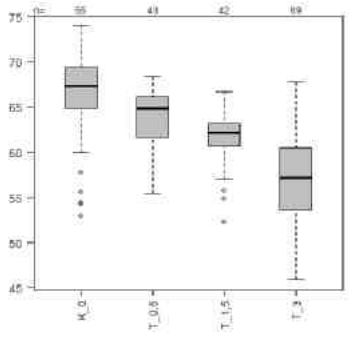

**Figure 2.** A-fraction of CP in silages ensiled at different tannin concentrations (K_0 Control, T_0.5/1.5/3.0)(≥24 d of ensiling).

**Results and Discussion** Protein was degraded stepwise over time, the A-fraction starting with an average of 28%, with 53% at day 10 and reaching a plateau of around 61% of CP at day 24 (Figure 1),

thus doubling within the first 4 weeks in the silo. No significant difference between mimosa and quebracho applied at 3% of DM was detected considering the A-fraction in silages ≥24 d. Thus they were regarded together as “tannin” treatment (T). When contrasting the control against the three levels of T, it (A-fraction 66% of CP) was similar to T at 0.5% while T levels of 1.5 and 3.0% of DM significantly decreased proteolysis by 4 and 9% points respectively (Figure 2), which confirmed the expected direction of action. When assessing all additives against each other none of them inhibited proteolysis more than tannin at 3% (Table 1). However, treatments BC and C were equally effective. The soluble B1-fraction had a maximum content of 3.7% of CP. There was more variation in the B2 fraction where true protein was much more fixed in T 3.0 compared to the control (Table 1).The indigestible C-fraction of CP was <7.0% and thus within an acceptable limit. Although tannin extract limited proteolysis when applied at a minimum of 1.5% of forage DM it was not more effective than conventional chemical silage additives which are approved by the European Food Safety Authority. This might be partly explained by tannin degradation which occurs during ensiling (Martens et al., 2014).

**Table 1.** Crude protein fractions (% of CP) of lucerne silage (≥24 d of ensiling) comparing all additives

| Treatment | A | B1 | B2 | B3 | C | *n* |
|---|---|---|---|---|---|---|
| Control | 65.97 $^{a}$ | 1.97 $^{b}$ | 23.92 $^{d}$ | 3.07 $^{c}$ | 4.92 $^{ab}$ | 66 |
| B | 64.30 $^{ab}$ | 1.54 $^{b}$ | 25.76 $^{cd}$ | 3.89 $^{abc}$ | 4.52 $^{b}$ | 22 |
| C | 60.48 $^{bc}$ | 2.30 $^{ab}$ | 27.69 $^{bc}$ | 4.43 $^{abc}$ | 5.1 $^{ab}$ | 23 |
| BC | 57.18 $^{c}$ | 3.68 $^{a}$ | 27.48 $^{bc}$ | 5.84 $^{a}$ | 5.51 $^{a}$ | 37 |
| NA | 60.67 $^{bc}$ | 2.28 $^{b}$ | 26.23 $^{bc}$ | 4.91 $^{ab}$ | 5.38 $^{ab}$ | 29 |
| T 0.5 | 64.02 $^{ab}$ | 2.21 $^{b}$ | 26.01 $^{c}$ | 2.41 $^{c}$ | 5.29 $^{ab}$ | 43 |
| T 1.5 | 61.61 $^{b}$ | 1.76 $^{b}$ | 28.36 $^{b}$ | 3.06 $^{c}$ | 5.26 $^{ab}$ | 42 |
| T 3.0 | 56.82 $^{c}$ | 2.78 $^{ab}$ | 31.20 $^{a}$ | 3.81 $^{bc}$ | 5.39 $^{ab}$ | 49 |
| *SEM* | 0.322 | 0.101 | 0.207 | 0.151 | 0.065 | |
| *P* | *<0.001* | *<0.001* | *<0.001* | *<0.001* | *0.025* | *311* |

Different letters within a column mean significant differences between treatments (P<0.05)

B biological additive, C formic acid, BC combination of B and C, NA $NaNO_2$+ hexamethylenetetramine, T 0.5, 1.5, 3.0 tannin extract at respective concentrations of forage DM

**Conclusion** Ensiling provokes intensive proteolysis. Some silage additives can preserve true protein up to 9% points more than the control; formic acid combined or not with a biological additive, and tannin extracts at 3% of forage DM take the lead. However, to supply dairy cattle with high-quality protein via forage, alternative presentation forms such as fresh or dried lucerne with > 70% true protein of CP (Martens et al., 2018) should be considered.

**References**

Licitra, G., Hernandez, T.M. & Van Soest, P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358.

Martens, S.D., Thate, E., Zeyner, A. & Steinhöfel, O. (2018) True protein conservation in a forage legume comparing drying to ensiling. In: Proceedings of the XVIII International Silage Conference, Bonn, Germany, 24-26 July 2018.

Martens, S.D., Hoedtke, S., Avila, P., Heinritz, S.N. & Zeyner, A. (2014) Effect of ensiling treatment on secondary compounds and amino acid profile of tropical forage legumes, and implications for their pig feeding potential. J. Sci. Food Agric. 94, 1107-1115.

# Silage fermentation of sugarcane plants prepared in Mozambique

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**Keywords:** cellulase, lactic acid bacteria, silage fermentation, sugarcane top

**Introduction** Sugarcane (*Saccharum officinarum*) plants are one of the main by-products of sugar production and represent 15-25% of the aerial part of the plant. Sugarcane tops are generally discarded in the field, where they are often burned and used as fertilizer. In addition, sugarcane tops can be used fresh or dried as livestock feed, and are cheap and abundant material in the tropics when other green fodder is unavailable. Because they are not available year around, an ensiling method to create good quality and provide long-term storage of the resulting silage is needed in tropics (Khota et al. 2016; Pholsen et al. 2016). Therefore, the objective of this study was to determine the chemical population and silage fermentation of sugarcane top. To improve silage fermentation, we studied the effect of lactic acid bacteria (LAB) and cellulase enzymes on fermentation quality of sugarcane top silage in Mozambique.

**Materials and methods** Sugarcane top is obtained from an agricultural field in an industrial sugar-production region (Maputo, Mozambique) on September 1, 2017. Sugarcane tops without wilting were immediately cut into 1-2 cm lengths using a chopper machine. The LAB inoculant (*Lactobacillus plantarum* Chikuso-1; Snow Brand Seed Co., Ltd, Sapporo, Japan) and *Acremonium* cellulase (AC; Meiji Seika Pharma Co., Ltd, Tokyo, Japan) were used as additives based on the manufacturer's guideline. Then, 6-8 kg of material was packed into 20-L polyethylene drum can silos. The silos were kept at an ambient temperature (25-38°C), three silos per treatment were opened after 60 d of ensiling to analyze the fermentation quality.

**Figure 1**. Sugarcane top and silage prepared in Mozambique.

Silage fermentation end-products were analyzed from cold water extracts as described by Cai (2004). Silage (10 g FM) was added to 90 ml of sterilized distilled water. The pH was measured using a glass electrode pH meter (FiveGo; Mettler Toledo, Greifensee, Switzerland). Ammonia nitrogen content was determined using a spectrophotometer (UV/VIS Spectrometer, PG Instruments Ltd., London, UK). The organic acid and WSC contents were measured by HPLC methods as described by Cai (2004).

**Results** Before ensiling, sugarcane tops contained $10^5$ LAB colony-forming units (cfu)/g of fresh matter (FM), $10^7$ aerobic bacteria, $10^4$ coliform bacteria and yeasts. Mould (<$10^3$ cfu/g) was not detected. During ensiling, the lactic acid and acetic acid contents increased in all silages, while the pH decreased. After 60 d of fermentation, the control silage was of poor quality, containing 0.17% lactic acid of FM and pH 4.7. However, the LAB, AC, and LAB+AC-treated silages had similar fermentation

patterns; more than 0.90% lactic acid of FM was produced and the pH reduced below 4.0. All 60-d silages were well preserved with a golden yellow color, good smell, and without any mold growth as deterioration.

Sugarcane is an important crop for sugar production in Mozambique and worldwide. When sugarcane is harvested, the top is typically left in the field, even though it is a good source of nutrients and is suitable for feeding ruminants. The major constraint for livestock production in Africa is a shortage of good-quality feed, where native grass hay is the major source of roughage for livestock in dry season. Sugarcane top silage may become the major component of forage for dairy or beef cows under most dietary regimes, and has greater potential to improve ruminant production system performance than the more conventional conserved forages used in sugarcan production areas.

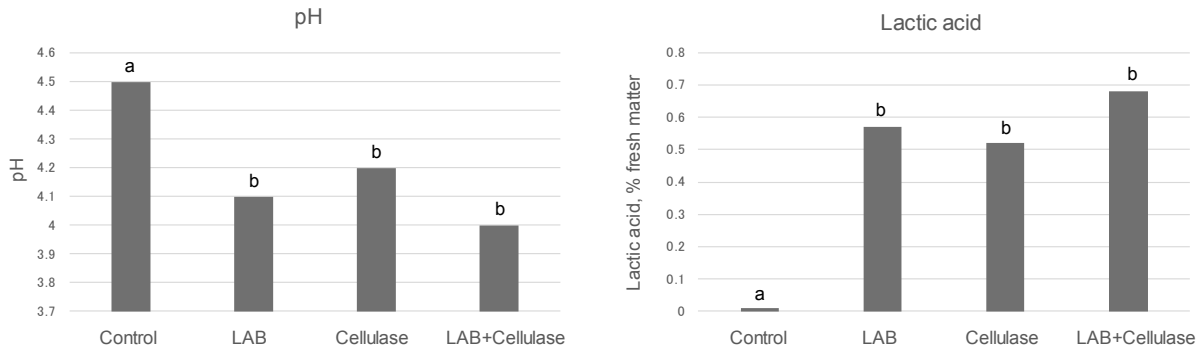


**Figure 2**. Fermentation quality of sugarcane top silage (Means with difference superscript letters differ at $P < 0.05$).

**Conclusions** The results suggest that sugarcane tops can be used to prepare relatively good-quality silage that can be used as a preserved feed resource for local livestock production in Mozambique.

**References**

Cai Y. (2004) Analysis method for silage. *In* Japanese Society of Grassland Science (ed.), Field and Laboratory Methods for Grassland Science. Tosho Printing Co. Ltd., Tokyo, Japan. 279-282.

Khota W., Pholsen, S., Higgs, D. & Cai, Y. (2016) Natural lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant. Journal of Dairy Science 99: 9768-9781.

Pholsen, S., Khota W., Pang H., Higgs, D. & Cai, Y. (2016) Characterization and application of lactic acid bacteria for tropical silage preparation. Animal Science Journal 86, 842-848.

# Sugar-rich grass: effect of two inoculants on silage fermentation characteristics and nutritional value

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**Keywords:** grass, inoculant, lactic acid bacteria, silage, sugar

**Introduction** A microsilo experiment with sugar-rich perennial ryegrass (170 g water-soluble carbohydrates (WSC) per kg dry matter (DM)) was conducted to determine the effect of two commercial silage inoculants on silage fermentation characteristics and nutritional value parameters: Lalsil Combo HC and Lalsil Dry HC (Danstar Ferment) were compared to a negative control.

**Material and Methods** Perennial ryegrass (first cut) was mown, prewilted and chopped to 5-8 cm particles with a New Holland precision chopper. Microsilos with a content of 2.75 liter, equipped with a Bunsen valve, were used for an ensiling experiment (Wambacq 2017). A negative control object (treated with sterile physiological water) was compared to two inoculated objects, *i.e.* Lalsil Combo HC and Lalsil Dry HC (Danstar Ferment) diluted in sterile physiological water. Both inoculants were dosed at one gram per ton fresh matter (FM), corresponding to resp. $1.9*10^5$ and $1.3*10^5$ cfu of lactic acid bacteria (LAB) per gram FM. The high-concentrate (HC) inoculants both contain *Lactobacillus buchneri* NCIMB40788. *L. plantarum* MA18/5U is also present in Lalsil Combo HC, while Lalsil Dry HC contains *Pediococcus acidilactici* MA18/5M as well as beta-glucanase and xylanase. All treatments were sprayed with an equal volume of treatment solution using different sprayers, in a ratio of 10 l $ton^{-1}$ FM. Dry matter content of the grass at ensiling was 352 g DM $kg^{-1}$ FM. Per treatment, five microsilos were ensiled at an average silo density of 156±2 kg dry matter (DM) per cubic meter. After an ensiled period of 90 days, all microsilos were sampled. DM content was determined by air drying at 65 °C, ammonia by Kjeldahl, pH on an 1:10 (w/w) aqueous extract, fermentation acids by HPLC (Ohmomo et al 1993), and ethanol by NIR absorption on an aqueous extract (Sørensen 2004). The 1,2-propanediol content was determined by GC: monopropylene glycol was extracted with water and quantified by addition of ethylene glycol. Crude protein, crude ash, crude fat, *in vitro* digestibility of the organic matter (DOM), NDF and ADF were determined by ISO 17025 as follows: crude protein by NF ISO 15670, crude ash by 71/250/EG, crude fat by 71/393/EG – 98/64-EG, DOM by De Boever et al. (1986), and NDF and ADF by Van Soest et al. (1991). Net energy for lactation was calculated with the formula for grass silage. WSC were analysed by Luff-Schoorl's method. The obtained data were statistically analysed with SAS 7. Outliers were removed per treatment. Normality was tested by Kolmogorov-Smirnov and homogeneity of variances by Levene's test. Normally distributed, homoscedastic data were subjected to two-sided one-way ANOVA with Tukey as *post hoc* test. Otherwise, data were subjected to a non-parametric test according to Kruskal-Wallis, without Bonferroni correction. Significance was declared at $p < 0.05$.

**Results and Discussion** The fermentation characteristics and some nutritional value parameters per treatment are summarized in Table 1. Butyric acid and propionic were below the level of detection. Despite the fact that Lalsil Combo HC and Lalsil Dry HC both contain homofermentative LAB (HoLAB, *i.e.* resp. *L. plantarum* and *P. acidilactici*) and heterofermentative LAB (HeLAB, *i.e. L. buchneri*), their effect on the silage fermentation characteristics was very different. The significantly lowest pH values were observed after Lalsil Combo HC application, associated with the highest lactic acid levels. Lalsil Combo HC application increased acetic acid levels, but these were even further increased by Lalsil Dry HC. After Lalsil Dry HC application, a more intense formation of 1,2-propanediol by *L. buchneri* was observed compared to Lalsil Combo HC. Ethanol contents on the other hand were lowered by Lalsil Dry HC application, but even further decreased by Lalsil Combo HC.

**Table 1**. Fermentation characteristics and nutritional value of grass silage: mean value per treatment.

| Parameters | Negative control | | Lalsil Combo HC | | Lalsil Dry HC | | Sign. |
|---|---|---|---|---|---|---|---|
| DM at desiling (g/kg FM)° | 337 | a | 360 | b | 341 | a | ** |
| Ammonia (g/kg DM) | 2.88 | a | 2.05 | b | 3.21 | c | *** |
| Ammonia-nitrogen / total nitrogen (%) | 7.88 | a | 5.83 | b | 8.17 | a | * |
| pH | 4.73 | a | 4.07 | b | 4.38 | c | *** |
| Lactic acid (g/kg DM) | 41.4 | a | 88.2 | b | 44.9 | a | *** |
| Acetic acid (g/kg DM) | 5.25 | a | 25.20 | b | 51.70 | c | *** |
| 1,2-Propanediol (g/kg DM) | 0.00 | a | 2.67 | b | 47.76 | c | ** |
| Ethanol (g/kg DM) | 46.0 | a | 17.3 | b | 29.3 | c | *** |
| WSC (g/kg DM) | 28.4 | a | 19.4 | b | 5.74 | c | *** |
| NEL (MJ/kg DM) | 6.04 | a | 6.33 | b | 6.21 | b | ** |

° DM corrected for volatile compounds according to Dulphy and Demarquilly (1981)

The amount of residual WSC was significantly lowered by inoculant application compared to the negative control, being even significantly lower for Lalsil Dry HC than for Lalsil Combo HC. This suggests a more intense fermentation after Lalsil Dry HC application. This could also be attributed to the action of the cellulolytic enzyme complex, releasing sugars from cellulose. Low levels of residual WSC decrease the risk of heating during the feed-out period (Wilkinson and Davies 2012), as well as the risk of rumen acidosis (Krause and Oetzel 2006). Lalsil Combo HC as well as Lalsil Dry HC application significantly increased the net energy for lactation (NEL) of the grass silage compared to the negative control, as a result of differences in the parameters based on which the NEL is calculated.

**Conclusion** Both Lalsil Combo HC and Lalsil Dry HC altered the silage fermentation compared to untreated silage. Although they both contain a HoLAB as well as a HeLAB strain, the fermentation pattern differed significantly between both inoculants. NEL was significantly increased by inoculant application, which is very interesting for practical on-farm application.

**References**

De Boever, J.L., Cottyn, B.G., Buysse, F.X., Wainman, F.W. & Vanacker, J.M. (1986) The use of an enzymatic technique to predict digestibility, metabolizable and net energy of compound feedstuffs for ruminants. Animal Feed Science and Technology, 14, 203-214.

Dulphy, J.P. & Demarquilly, C. (1981) Problèmes particuliers aux ensilages – Prévision de la valeur nutritive des aliments des ruminants. Versailles: INRA Publications. 577 p. (in French).

Krause, K. M. & Oetzel, G. R. (2006) Understanding and preventing subacute ruminal acidosis in dairy herds: a review. Animal Feed Science and Technology, 126, 215-236.

Ohmomo, S., Tanaka, O. & Kitamoto, H. (1993) Analysis of organic acids in silage by high-performance liquid chromatography. Bulletin of the National Grassland Research Institute, 48, 51-56.

Sørensen, L.K. (2004) Prediction of fermentation parameters in grass and corn silage by near infrared spectroscopy. Journal of Dairy Science, 87, 3826-3835.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991) Method for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

Wambacq, E. (2017) *Penicillium roqueforti s.l.*: growth and roquefortine C production in silages. PhD thesis, Ghent University, Belgium.

Wilkinson, J.M. & Davies, D.R. (2012) The aerobic stability of silage: key findings and recent developments. Grass and Forage Science, 68, 1-19.

# Temperature track and spoilage microbes affected by different additives in wet hay

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**Keywords:** formic acid, sodium benzoate, sodium propionate, spoiling microorganisms, wet hay

**Introduction** The risk-free harvest period for haymaking has a limited time-frame and is strongly dependent on weather conditions. Hay with low dry matter content, not suitable for safe storage is subjected to the risk of heating and offers good conditions for the growth of spoilage microbes. High counts of spoilage microbes are quite often found in wet hay (Meisser 2001). This is undesirable for a hygienic, clean feed because microbial growth can also cause livestock diseases (Sommer and Möllering 2007, Nebe et al. 2009). The use of additives may control the number of spoilage microbes so that wet harvested hay might be stored long-term without loss of feed quality (Wyss 2012). Agar diffusion tests according to the modified method of Kirby & Bauer (Bauer et al.1966) have shown positive results concerning the inhibition of spoilage microbes by using specific blends of organic acids and their corresponding salts. We have also achieved similar results with treated hay stored under compaction in 50 l wire baskets (in-house method). Following these positive results at laboratory and pilot plant scales, experiments were carried out under practical conditions in the summer of 2016.

**Materials and Methods** Hay of the first cut of a mixed grass sward, cut on 3$^{rd}$ of June, was compacted on 8$^{th}$ of June 2016 during unsafe weather conditions in Lower Saxony (Germany). The time of baling was determined according to the dry matter (DM) content, as measured by the microwave method (Carlier and Hee 1971) and the conductivity method (SuperTech Agroline 2016, humidity and temperature measuring rod). Hay of a DM between 735 g/kg and 815 g/kg was compacted into square bales with a Krone Big Pack 120-80 baler. Two different additive mixtures (KOFA GRAIN -pH5- and KOFA FEED Plus) were used, as well as an untreated control, each replicated 3 times. KOFA GRAIN -pH5- is based on sodium propionate, sodium benzoate and propionic acid and KOFA FEED Plus contains sodium propionate, benzoic acid, propionic acid and formic acid. The additives were applied to the top of the swath by a commercial spraying system with one nozzle in front of the pick-up. The high target dosage of 10 l/t was chosen because of the low and uneven degree of wilting, the anticipated high numbers of spoilage microbes due to delayed harvest and the expected wide range of the additive distribution. After determination of weight, the square bales were stored dry and at ambient temperature. The storage period lasted until 8$^{th}$ of November 2016. Temperature was logged in the core of the square bales over a period of seven days after baling with the temperature measuring rod. Samples of starting material were taken immediately before compaction and the drill core samples after a storage time of five months. The analyses of the feed ingredients and the determination of the spoilage microbes (VDLUFA 2012) were done by LKS-Laboratory in Lichtenwalde (Saxony, Germany). Orientation values of product typical and spoilage indicating microorganisms for the estimation of the hygienic quality in hay were taken of VDLUFA 2012. The real applied dosage of the tested additives was determined by measuring of the non-volatile benzoate with HPLC-UV “Determination of benzoate and sorbate in E700” (ADDCON EUROPE GmbH 2010).

**Results and Discussion** The hay used in this experiment contained 80-102 g crude protein/kg DM, 309-341g crude fibre/kg DM and 72-79 g sugar/kg DM. The energy density was 4.5-5.0 MJ NEL per kg DM and reflected a late harvest. In the samples taken directly from the swath just before pressing, no spoilage indicating moulds of germ-group 5 (VDLUFA 2012) were found and the DM content was 799 g/kg on average. After five months of storage, moulds of germ-group 5 were found at $4.9 \times 10^6$ – $1.2 \times 10^7$ CFU/g hay in the square bales of the control. A value of $10 \times 10^4$ moulds per g is the threshold for germ-level 1 (VDLUFA 2012). This shows that the wet hay of the untreated control offers optimal growing conditions for moulds. However, there was only a low rise in temperature during

the first 48h of storage in all of the three bales, possibly caused by their relative low DM-density of 127.9 kg/m$^3$. In contrast, the bales treated with KOFA FEED Plus contained only 4 x 10$^3$ - 5 x 10$^4$ spoilage-indicating moulds of germ-group 5 at the end of storage. The usage of this additive showed a very high effect in controlling the development of spoilage microbes. No rise in temperature rise was observed. The application of KOFA GRAIN -pH5- led to mould-values between 1.5 x 10$^4$ and 1.1 x 10$^7$ per g hay. One of the three bales of this group contained nearly as many moulds as the untreated bales of the control and its temperature increased. However, this bale also showed a measured application rate of only 1.4 l/t, instead of the target dose of 10 l/t. This low dosage might be induced by the application system using only one nozzle. A three-fold higher application rate of 4.2 l/t reduced the moulds to 35% compared to the less treated bale. The measured application rate of the additive in the bale containing only 1.5 x 10$^4$ CFU of spoilage indicating moulds per g hay was 19.1 l/t. The target dosage of 10l/t that should keep the number of spoilage microbes below the guide-value was confirmed by this experiment, when the regressions curve ($r^2$=0.9659) is considered (Figure 1).

**Table 1.** Parameters of bales

| Variant | Do-sage [l/t] | Aver. dos. [l/t] | Moulds GG5 [CFU/g] | Bale-weight [kg] | Bale-DM [%] |
|---|---|---|---|---|---|
| KGpH5 | 4.2 | 8.2 | 3.95x10$^6$ | 360 | 74.4 |
| KGpH5 | 1.4 | | 1.14x10$^7$ | 440 | 73.5 |
| KGpH5 | 19.1 | | 1.5x10$^4$ | 400 | 75.8 |
| KFPlus | 14.5 | 9.1 | 4x10$^3$ | 380 | 80.4 |
| KFPlus | 4.0 | | 5x10$^4$ | 360 | 77.4 |
| KFPlus | 8.8 | | 5x10$^3$ | 380 | 73.3 |
| Control | 0 | 0 | 1.20x10$^7$ | 320 | 78.5 |
| Control | 0 | | 4.9x10$^6$ | 340 | 81.5 |
| Control | 0 | | 9x10$^6$ | 300 | 79.7 |

Control (without additive), KFPlus (KOFA FEED Plus), KGpH5 (KOFA GRAIN –pH 5-), GG 5 (germ-group 5)

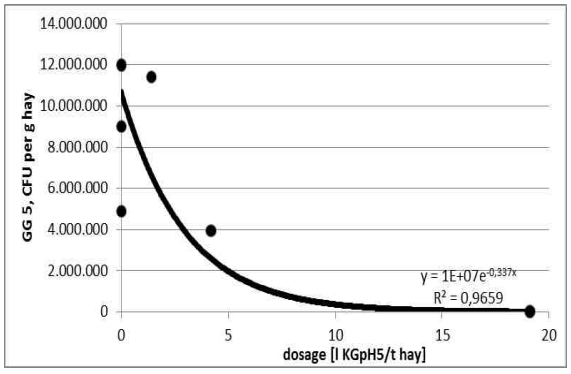


**Figure 1.** Moulds of GG 5 vs. dosage of KGpH5

**Conclusions** The risk of a high content of spoilage microorganisms is a real problem in hay harvested under unfavorable weather conditions. The application of both the additives tested at the advised dosage keeps the development of spoilage microbes below the guide-values of the VDLUFA. The use of additives can control spoilage processes and results in a hygienic feed quality, when homogenous distribution of the additives is ensured.

**References**

ADDCON EUROPE GmbH (2010) Determination of benzoates and sorbates in preservative E 700, premixtures, cereals, complete feeds and silages by HPLC-UV. EURL Evaluation Report on the Analytical Methods: FAD-2010-0356, CRL/100049.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. & Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45: 493-496.

Carlier, L.A. & van Hee, L.P. (1971) Microwave drying of lucerne and grass samples. J. Sci. Food Agric., 22, 306-397.

Meisser, M. (2001) Konservierung von Feuchtheu. AGRARForschung 8 (2) 87-92

Nebe, H.-D., Kühl, J. & Strauß ( 2009) Mikrobiologische Grundfutterqualität in der Pferdehaltung – Landesprojekt Rheinland Pfalz.

Sommer, W. & Möllering (2007) Pferdeheu oft mit hygienischen Mängeln. https://www.landwirtschaftskammer.de/landwirtschaft/tierproduktion/pferdehaltung/fuetterung/pferdeheu-2007.htm.

Supertech Agroline (2016) The portable moisture analyzer for Hay, Straw and Silage http://supertechagroline.com/products/portable-moisture-analyzers/superpro-combi/.

VDLUFA (2012) Versuchs- und Untersuchungsmethodik -Methodenbuch Bd. III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt.

Wyss, U. (2012) Wirkung eines Konservierungsmittels bei Feuchtheu. Agrarforschung Schweiz 3 (6), 314-321.

# The aerobic stability of avocado (*Persia Americana*) pulp silage treated with microbial additives

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**Keywords:** carbon dioxide**,** fermentation, temperature, yeasts

**Introduction** Shortages of animal feed has compelled animal nutritionists to focus on non-conventional feed resources to mitigate this problem. Avocado (*Persea Americana*) is one of the available resource that is produced in the oil producing industries in South Africa. This by-product contains 332 g dry matter (DM)/kg, 20 gross energy MJ/kg DM, 119 g ether extract/kg DM and 5.7 pH. The high moisture content in avocado pulp (AP) makes it difficult to feed it to ruminants at its fresh form, hence it should be ensiled with dry resources. The ensiling method involves the use of additives to improve the fermentation process and the aerobic stability of the silage. The aerobic stability of silage is a problem since well fermented silages lose their nutritional value during the feed-out period. Consequently, different silage additives have been used to improve the aerobic stability of silage (Wilkinson and Davies 2013). The present study was therefore conducted to evaluate the influence of microbial additives on the aerobic stability of avocado pulp silage.

**Materials and Methods** Avocado pulp (AP) was collected from Westfalia and brought to ARC-Irene for nutrient analyses and silage production. The AP contains 242 g DM/kg, Gross energy (GE) 21 MJ/kg DM, 60 g crude protein/kg DM, 119 g ether extract/kg DM, 712 g neutral detergent fibre/kg DM and 482 g acid detergent fibre/kg DM. An amount of 80 kg AP was mixed with 15 g grape pomace plus 5 g sugarcane molasses. The mixture was treated with: i) no additive (control), ii) Emsilage (microbial inoculant, contains *Bacillus subtilis, Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus fermentum, Lactobacillus plantarum, Lactococcus lactis subsp. Lactis, Rhodopseudomonas palustris, Saccharomyces cerevisiae, Streptococcus salivarius subsp. Thermophiles*) and Sil-All 4x4 (microbial inoculant, contains > 1.2 x $10^{10}$ CFU/g *Lactobacillus plantarum CNCM I-3235*, > 1.5 x $10^{9}$ CFU/g *Enterococcus faecium CNCM I-3236* , > 1 x $10^{8}$ CFU/g *Lactobacillus salivarius CNCM I-3238*, > 6 x $10^{9}$ CFU/g *Pediococcus acidilactici CNCM I-3237*, > 30 CMC/g cellulase from *Trichoderma longibrachiatum ATCC 74252*, > 500 IU/g *Beta glucanase from Aspergillus niger* MUCL 39199, and > 750 IU/g xylanase from *Trichoderma longibrachiatum MUCL 39203*). The inoculants were applied at 5 x $10^{5}$ colony-forming units (cfu) $g^{-1}$. The treatments were ensiled in 1.5 L anaerobic jars and kept in room temperature for 90 days. After 90 days of ensiling, 3 jars per treatment were opened and subjected to an aerobic stability test that lasted for 7 days (Ashbell et al. 1991). Data on effects of treatments on aerobic stability of silage were analysed in a completely randomized design by ANOVA using Genstat (2011). Differences among treatment means were compared with least significant difference (LSD) and significance was declared at the 0.05% probability level.

**Results** After 7 days of aerobic exposure (Table 1), the dry matter of the silage was not affected by treatments. However, there was a rise in pH, carbon dioxide production, temperature and the population of yeast and moulds in the silage treated with LAB inoculation compared to the control treatment.

**Discussion** The reduced silage aerobic stability with treatment Sil-All 4x4 is inconsistent with Uriate-Archundia et al. (2002) and Baah et al. (2011) who reported improved silage aerobic stability in corn and barley silages respectively. This reduced aerobic stability in avocado silage treated with inoculants might be attributed to the high residual sugar content in the inoculant treated avocado silage, which is known to negatively affect the aerobic stability in silages (Weinberg et al. 1993).

**Table 1**. Effects of treatments on the aerobic stability of avocado pulp silage (n=3)

| Parameter | Treatments | | | SEM | P-value |
|---|---|---|---|---|---|
| | Control | EMS | Sil-All | | |
| **DM g/kg** | 338.6 | 337.1 | 330.3 | 3.54 | 0.282 |
| **pH** | 5.88$^{b}$ | 5.4$^{c}$ | 6.5$^{a}$ | 0.119 | 0.002 |
| **WSC g/kg DM** | 17.9$^{c}$ | 38.0$^{c}$ | 70.4$^{a}$ | 0.695 | 0.001 |
| **$CO^2$ g/kg DM** | 13.84$^{b}$ | 8.09$^{c}$ | 20.55$^{a}$ | 0.478 | 0.001 |
| **Yeast & moulds $log^{10}$ CFU/kg** | 7.57$^{b}$ | 7.10$^{b}$ | 8.87$^{a}$ | 0.159 | 0.001 |
| **Temperature changes (hrs)** | 39$^{a}$ | 27$^{b}$ | 42$^{a}$ | 0.833 | 0.005 |

$^{a-c}$ Means with different superscripts within rows differed significantly at $P < 0.05$
DM dry matter; $CO^2$ carbon dioxide
WSC, water-soluble carbohydrates
EMS Emsilage

**Conclusions** The aerobic stability of avocado pulp silage was reduced with inoculation. Further work to evaluate the nutrient intake and digestibility of AP silage by ruminants is needed.

**References**

Ashbell, G., Weinberg, Z.G., Azriel, A., Hen, Y. & Horev, B. (1991) A simple system to study the aerobic deterioration of silages. Canadian Agricultural Engineering, 33, 391-393.

Baah, J., Wddah, W., Okine, E.K. & McAllister, T.A. (2011) Effects of homolactic inoculant alone or combined with an anionic surfactant on fermentation, aerobic stability and in situ degradability of barley silage. Asian-Australasian Journal of Animal Science, 24, 369-378.

Genstat (2011) GenStat for Windows (14th ed.) (Payne, R.W., Murray, D.A. & Harding, S.A.). VSN International, Hemel Hempstead, UK.

Uriarte-Archundia, M.E., Bolsen, K.K. & Brent, B.E. (2002) A study of the chemical and microbial changes in whole-plant corn silage during exposure to air: effects of biological additive and sealing technique. Kansas Agricultural Experiment Station Research reports, vol. 0: issue 1. https://doi.org/10.4148/2378-5977.17SS.

Weinberg, Z.G., Ashbell, G., Hen, Y. & Azreli, A. (1993) The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. Journal of Applied Bacteriology, 75, 512-518.

Wilkinson, J.M. & Davies, D.R. (2013) The aerobic stability of silage: key findings and recent developments. Grass and Forage Science, 68, 1-19.

# The effect of *Lactobacillus buchneri* and *Pediococcus acidilactici* inoculants on pH and microbial population of wilted alfalfa silage.

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**Keywords:** Alfalfa silage, *Lactobacillus buchneri*, microbial population*, Pediococcus acidilactici*

**Introduction** Due to high buffering capacity, undesirable amount of nitrogen and low water soluble carbohydrates (WSC) in alfalfa, using an additive which on one hand promotes fermentation and on other hand limits the number of undesirable microorganisms is highly recomended(Zheng, 2017). The latest generation of bacterial silage additives are a combination of different species (Jatkauskas,2013). The objective of this study was to evaluate the effects of using a combination of twobacterial inoculantson pH and microbiological populations of wilted alfalfa ensiled at 30 percent dry matter content over a 45 days period.

**Material and Methods** Fresh alfalfa was harvested at 10% bloom stage and chopped into 2-4 cm length, then wilted until 30% DM. About 40 kg of wilted alfalfa was taken and divided into two 20 kg piles of forage and used to prepare the following treatments: I. Untreated group (UT) on which 50 ml of tap water was sprayed and contained no inoculants and II. Treated group (LBPA): 0.2 g of a bacterial additive including minimum 7.5 x $10^{10}$ CFU/g *L. buchneri* and 5 x $10^{10}$ CFU/g *P. acidilactici* was dissolved in 50 ml of tap water then sprayed uniformly on 20 kg pile of wilted forage and mixed thoroughly by hand to achieve $3.75\times10^6$ and $2.5\times10^6$ CFU/g fresh forage of *L. buchneri* and *P. acidilactici* respectively. Triplicate samples of wilted alfalfa were ensiled in two series (UT and LBPA) of cylindrical laboratory silos for 1, 3, 5, 10, 15, 30, and 45 days period. Fresh and ensiled alfalfa samples from each day were taken and subjected to analyse for pH, the number of total mesophilic and lactic acid bacteria, yeast and mould population by standard plate count. To enumerate lactic acid bacteria (LAB), samples were cultured in Man, Rogosa and Sharpe agar (MRS, Merck). Yeasts and moulds were cultured in malt extract agar (Wort agar-Merck, Germany) and mesophilic bacteria colonies were counted in brain heart infusion (BHI) agar. Cultured BHI plates incubated at 37˚C for 24-48 and MRS and Wort plates at 25˚C for 48-72 h in aerobic condition. Data were analysed by one way analysis of variance to compare the means at 95% confidence level.

**Results** Based on chemical analysis of alfalfa: DM=31±0.4%, WSC=2.2±0.06%, Buffering capacity=65±5 mEq/ kgDM. The changes in the pH and microbial populations in both UT and LBPA are presented in Table 1. pH of fresh forage was 7.4 and 7.67 for treated and control which then decreased drastically to 5.47 and 5.34 on day one and reduced at a lower speed until reached to 4.61 and 4.52 on day 45. No significant changes were observed between the pH of treated and untreated silage groups although, except day 3 and 30, the pH of treated silage was higher than that of control during the ensilage period. The initial number of mesophilic bacteria was log 6.13 CFU/g and 6.21 CFU/g for control and treated silage respectively. It showed an increase on day 1 then decreased until day 45 and reached to log 4.08 CFU/g and 3.13CFU/g for control and treated silage respectively. Except day 0 and 1, the numbers of bacteria in inoculated silage were lower than that in control from day 3 to 45 ($p<0.05$). The bacterial inoculation resulted in a higher population of lactic acid bacteria in treated groups as compared to control and the differences was statistically significant on day 0 and 45 ($p<0.05$). During the ensilage the number of LAB were log 6.82 and 7.07 CFU/g for spontaneously ensiled and treated forages before ensiling and increased to 7.84 and 8.14 on day 45. The population of moulds and yeasts in both series which were initially log 6.51 and 6.56 CFU/g in untreated and treated silage had also increased on day 1 then decreased by the end of ensilage period. However, the number of these two undesirable organisms was significantly lower in inoculated group (log 2.79 CFU/g) than that of control (log 1.77 CFU/g) on day 45.

**Table 1.** pH and number (log10) of Mesophilic bacteria, Lactic acid bacteria and moulds and yeasts in fresh forage, untreated alfalfa silage and treated alfalfa silages with Lactobacillus buchneri ($3.75\times10^6$ CFU/g) and Pediococcus acidilactici ($2.5\times10^6$ CFU/g fresh forage) ensiled at 30% dry matter contents from day 1 through day 45

| | pH | | | Mesophilic Bacteria | | | Lactic acid bacteria | | | Moulds and Yeasts | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | UT | LBPA | SEM | UT | LBPA | SEM | UT | LBPA | SEM | UT | LBPA | SEM |
| d0 | 7.40 | 7.67 | 0.134 | 6.13 | 6.21 | 0.160 | $6.82^a$ | $7.07^b$ | 0.049 | 6.51 | 6.56 | 0.060 |
| d1 | 5.34 | 5.47 | 0.055 | 7.34 | 7.26 | 0.158 | 9.21 | 9.43 | 0.102 | 7.02 | 6.94 | 0.161 |
| d3 | 5.03 | 5.00 | 0.041 | $6.87^a$ | $6.13^b$ | 0.142 | 9.36 | 9.53 | 0.160 | 6.62 | 6.52 | 0.057 |
| d5 | 4.92 | 4.95 | 0.020 | $6.49^a$ | $5.63^b$ | 0.154 | 9.33 | 9.41 | 0.088 | 6.5 | 6.33 | 0.161 |
| d10 | 4.78 | 4.81 | 0.022 | $6.29^a$ | $5.19^b$ | 0.282 | 9.12 | 9.22 | 0.153 | 6.16 | 5.98 | 0.209 |
| d15 | 4.69 | 4.73 | 0.044 | $6.05^a$ | $4.86^b$ | 0.256 | 8.85 | 9.05 | 0.106 | 5.71 | 5.61 | 0.185 |
| d30 | 4.68 | 4.65 | 0.041 | $4.6^a$ | $3.83^b$ | 0.257 | 8.08 | 8.17 | 0.065 | 4.59 | 4.46 | 0.119 |
| d45 | 4.52 | 4.61 | 0.061 | $4.08^a$ | $3.13^b$ | 0.329 | $7.84^a$ | $8.14^b$ | 0.126 | $2.79^a$ | $1.77^b$ | 0.140 |

d: day of ensiling, $^{a,b}$ Data points for specific days with unlike letters differ ($P < 0.05$), UT: untreated silage, LBPA: Treated silages

**Discussion** The addition of *P. acidilactici* has decreased silage pH(Silva, 2016) but in our study, pH did not affected by inoculation. The probable reason is the high buffering capacity (65 mEq/KgDM alfalfa silage which doesn't let homolactic bacteria to change pH drastically(Hu 2009). Also, *L. buchneri* may produce acetic acid which leads to higher pH than lactic acid which is the most important volatile fatty acid in decreasing pH. The number of LAB reached to the highest number on day 3then decreased gradually. Decrease in the number of LAB over time was expected due to low pH and shortage of water soluble carbohydrates which leads to the LABs death which are not resilient to low pH (Pahlow, 2003). In our study the LAB number was higher in treated than that of untreated silages on day 45 ($p<0.05$) which probably caused by *L. buchneri* and *P. acidilactici* resistance to acid conditions compared to epiphytic bacteria of alfalfa. The similarity in the number of LAB in both treated and untreated groups except for day 45 may indicates that inoculant didn't promote fermentation at the beginning of the ensiling but led to keep silage quality later via less yeasts and moulds because of more (*L. buchneri*) included in the additive which increased the number of acid producing bacteria on day 45. The significant reduction in the number of yeasts and moulds on day 45 can be declared with the high number of *L. buchneri* in treated groups and subsequent production of acetic acid (Reich 2010).

**Conclusion** It can be concluded that the inoculation of wilted alfalfa with *L. buchneri* and *P. acidilactici*, may improve lactic acid bacterial population and cause a significant reduction in the population of undesirable microorganisms.

**References**

Hu, W., Schmidt, R., McDonell, E., Lingerman & C. Kung, L. (2009) The effect of Lactobacillus buchneri 40788 or Lactobacillus plantarum MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. Journal of dairy science 92(8), 3907-3914.

Jatkauskas, J., Vrotniakiene, V., Ohlsson, C.& Lund, B. (2013) The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agricultural and Food Science 22(1), 137-144.

Pahlow, G., Muck, R.E., Driehuis, F., Elferink, S. & Spoelstra, S.F. (2003). Microbiology of ensiling Agronomy 42, 31-94.

Reich, L.J. & Kung, L. (2010) Effects of combining Lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage Animal Feed Science and Technology 159(3), 105-109.

Silva V., Pereira O., Leandro E., Da Silva T., Ribeiro K., Mantovani H. & Santos S. (2016) Effects of lactic acid bacteria with bacteriocinogenic potential on the fermentation profile and chemical composition of alfalfa silage in tropical conditions Journal of dairy science 99(3), 1895-1902.

Zheng M., Niu D., Zuo S., Mao P., Meng L. & Xu C. (2017) The effect of cultivar, wilting and storage period on fermentation and the clostridial community of alfalfa silage Italian Journal of Animal Science, 1-11.

# The effect of adding fibrolytic enzymes and lactic acid bacteria on fermentation quality and *in vitro* digestibility of Napier grass silage

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**Keywords:** cellulase, fermented juice of epiphytic lactic acid bacteria (FJLB), Napier grass, silage, xylanase

**Introduction** Tropical crops are known to be difficult to ensile and the resulting fermentation quality, intake and digestibility are quite low. Successful silage requires epiphytic lactic acid bacteria (LAB) and water soluble carbohydrate (WSC) to produce sufficient lactic acid for rapid pH reduction. Napier grass (*Pennisetum purpureum*) is one of the most promising grasses available for ruminant production in tropical areas because of its high potential dry matter (DM) yield. However, both the DM content and the concentration of WSC are considered too low for successful ensiling. Furthermore, the number of epiphytic LAB in Napier grass may be too low to ensure an uncomplicated process of fermentation. Indeed, it has been shown by Bureenok et al. (2006) that the addition of fermented juice of epiphytic LAB (FJLB) to Napier grass improved the quality of the silage. Fibrolytic enzymes such as cellulase and hemicellulase have also been used and improved the silage quality (Weinberg et al. 1993, Nadeau et al. 2000). Previous research has also shown that addition of cell wall degrading enzymes to forage at ensiling can improve the silage quality and chemical composition (Stokes et al. 1996). The aim of this experiment was to evaluate the effect of fibrolytic enzymes and FJLB on fermentative quality and in vitro digestibility of Napier grass silages.

**Table 1.** Fermentation quality of Napier grass silages treated with enzyme (ENZ), fermented juice of epiphytic lactic acid bacteria (FJLB) and its combination at 30 day of ensiling.

| Item | Control | ENZ | FJLB | ENZ+FJLB | SE | P-value |
|---|---|---|---|---|---|---|
| pH | 3.98$^{ab}$ | 4.26$^{a}$ | 3.70$^{b}$ | 3.71$^{b}$ | 0.08 | 0.024 |
| Lactic acid bacteria ($log_{10}$ cfu/g FW) | 6.31 | 6.33 | 6.16 | 6.14 | 0.07 | 0.748 |
| Dry matter (DM) (%) | 19.01 | 18.35 | 18.63 | 19.52 | 0.19 | 0.166 |
| Lactic acid (% DM) | 3.95$^{bc}$ | 3.70$^{c}$ | 5.25$^{a}$ | 4.95$^{ab}$ | 0.12 | 0.001 |
| Acetic acid (% DM) | 2.17$^{a}$ | 2.36$^{a}$ | 1.07$^{b}$ | 1.56$^{ab}$ | 0.10 | 0.003 |
| Propionic acid (%DM) | 0.21$^{ab}$ | 0.23$^{a}$ | 0.09$^{b}$ | 0.13$^{ab}$ | 0.02 | 0.025 |
| Butyric acid (%DM) | 0.36 | 0.47 | 0.36 | 0.27 | 0.03 | 0.263 |
| Lactic acid: Acetic acid | 1.86$^{c}$ | 1.60$^{c}$ | 4.98$^{a}$ | 3.41$^{b}$ | 0.16 | <0.001 |
| Ammonia-nitrogen (%Total nitrogen) | 8.85$^{a}$ | 8.65$^{ab}$ | 6.14$^{c}$ | 6.47$^{bc}$ | 0.26 | 0.009 |
| Water soluble carbohydrates (%DM) | 0.84$^{a}$ | 0.77$^{a}$ | 0.41$^{b}$ | 0.46$^{b}$ | 0.07 | <0.001 |
| Crude protein (%DM) | 5.67$^{b}$ | 6.03$^{ab}$ | 6.16$^{a}$ | 6.04$^{ab}$ | 0.53 | 0.032 |
| Neutral detergent fiber (%DM) | 77.59 | 76.56 | 75.69 | 78.09 | 0.43 | 0.224 |
| Acid detergent fiber (%DM) | 47.34 | 46.79 | 45.40 | 44.63 | 0.40 | 0.05 |

Means with different superscripts within columns significantly differed (P<0.05). SE = Standard error.

**Table 2.** *In vitro* degradability coefficients of the dry matter of Napier grass silages at day 30 of ensiling.

| Item | Control | ENZ | FJLB | ENZ+FJLB | SE | P-value |
|---|---|---|---|---|---|---|
| Soluble fraction (a) | 3.29$^{b}$ | 8.92$^{a}$ | 5.67$^{ab}$ | 5.93$^{ab}$ | 0.67 | 0.010 |
| Insoluble fraction (b) | 86.03 | 86.47 | 78.58 | 76.33 | 2.50 | 0.392 |
| Degradation rate of fraction (c, mL/h) | 0.04 | 0.04 | 0.04 | 0.04 | 0.001 | 0.198 |
| Potential gas production (a+b) | 89.32 | 95.39 | 84.26 | 82.26 | 2.76 | 0.363 |
| Effective degradability (ED,% DM) | 44.66 | 47.70 | 42.13 | 41.13 | 1.38 | 0.363 |
| Organic matter digestibility (% DM) | 42.74 | 47.06 | 42.93 | 38.21 | 1.74 | 0.386 |
| Metabolizable energy (MJ/kg DM) | 6.38 | 7.03 | 6.42 | 5.71 | 0.26 | 0.393 |

Means with different superscripts within columns significantly differed (P<0.05). SE = Standard error.

**Material and Methods** In this experiment, fibrolytic enzymes (xylanase and cellulase enzymes, ENZ) were produced by *Aspergillus niger* by solid state fermentation using dried tomato pomace as solid

substrate. Cellulase and xylanase activities were 60,998.11±2,419 and 37,815.69±10,487 unit/g dry weight, respectively. FJLB was prepared from 200 g of fresh Napier grass, which was immediately macerated in 1,000 ml of sterilized distilled water with a home blender. The juice was filtered through a double layer of cheesecloth; the filtrate was transferred to a glass bottle and 2% glucose was added. Then, the bottle was capped and incubated anaerobically at 30 ºC for 2 days before using as silage additive. The LAB counts on the FJLB was 5.5 x $10^8$ colony forming unit/ml. Napier grass was ensiled without additive (control), or with 0.05% ENZ, 1% FJLB and 0.05% ENZ plus 1% FJLB. Triplicated silages per treatment were opened in 3, 7, 11, 15 and 30 day of ensiling for chemical analysis. Kinetics of degradation and gas production were evaluated in the samples of 30-d of fermentation by the *in vitro* gas production (Menke and Steingass 1988). All data were analyzed using the SPSS (SPPS 16.0, Chicago, IL). Tukey test was used to determine the significant difference between means.

**Results and Discussion** The pH value of silage treated with ENZ were higher than the other treated silages during the ensiling process (Table 1). Comparing with the ENZ treated silage, the lactic acid content of all FJLB treated silages were significantly ($P<0.05$) higher. The residual WSC content of all silages were likely to decrease during fermentation in all silages. However, at 30 day of ensiling, the addition of ENZ resulted in higher WSC content when compared to the other treated silages. The crude protein of FJLB treated silage was higher than the control. The silage treated with ENZ had higher gas production of soluble fraction (a) compared with the control (Table 2). The effective degradability of DM and organic matter digestibility, metabolizable energy tented to increase in silage treated with ENZ, but the values were not different among the treatments. Li et al. (2017) confirmed that LAB or LAB plus cellulase treatment could improve the fermentation quality, chemical composition and ruminal degradation of stylo (*Stylosanthes guianensis*) silage. In our study, the combination of ENZ and FJLB did not improve the nutrient digestion. A possible reason for this is that fiber-degrading enzymes predigest the readily digestible fiber leaving a slower and less degradable fraction. Moreover, the LAB also used WSC to produce the lactic acid. Therefore, the left of readily digestible fraction were smaller than the others.

**Conclusion** Applying FJLB showed a high content of lactic acid and the ratio of LA:AA. Addition of fibrolytic enzymes produced by *Aspergillus niger* increased WSC content and tended to improve the *in vitro* digestibility of Napier grass silages.

**References**

Bureenok, S., Namihira, T., Mizumachi, S., Kawamoto Y. & Nakada, T. (2006) The effect of epiphytic lactic acid bacteria with or without different byproduct from defatted rice bran and green tea waste on napier grass (*Pennisetum purpureum* Schumach) silage. Journal of the Science of Food and Agriculture, 86, 1073-1077.

Li, M., Zhou, H., Zi, X. & Cai, Y. (2017) Silage fermentation and ruminal degradation of stylo prepared with lactic acid bacteria and cellulase. Animal Science Journal, DOI: 10.1111/asj.12795.

Menke, K.H. & Steinggass, H. (1988) Estimation of energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development, 28, 7-55.

Nadeau, E.M.G., Buxton, D.R., Russell, J.R., Allison, M.J. & Young, J.W. (2000) Enzyme, bacterial inoculant, and formic acid effects on silage composition of orchardgrass and alfalfa. Journal of Dairy Science, 83, 1487-1502.

Stokes, M.R., Wang, Y. & Doherty, J. (1996) Effects of enzyme silage additives at different fermentation temperatures. Proceeding of the XI international silage conference. Aberystwyth, Wales. p. 124-125.

Weinberg, Z.G., Ashbell, G., Azrieli, A. & Brukental I. (1993) Ensiling peas, ryegrass and wheat with additives of lactic acid bacteria and cell wall degrading enzymes. Grass and Forage Science, 48, 70-78.

# The effect of cellulase and/or *Lactobacillus plantarum* on fermentation quality of napier grass silage

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**Keywords:** cellulase, *Lactobacillus plantarum*, *napier grass*, fermenation quality

**Introduction** Grasses are the largest renewable natural resources for the production of animal feed and bioproduct production. Ensiling is a traditional conservation method aimed at preserving fresh forages and grains at their optimum stage of growth to be used as year round supply of nutritious and palatable feed for livestock (Darrab and Shaha 2012 ). Furthermore, ensiling may function as a beneficial treatment for lignocellulosic materials before further processing to bioenergy or bioproduct production (Liu et al. 2017). However, most tropical and subtropical forages are found to be very difficult to be ensiled due to their low water soluble carbohydrates and high cell wall contents. This study examined the effect of cellulase and *Lactobacillus plantarum* on fermentation quality and carbohydrate composition of napier grass.

**Materials and methods** First cut of napier grass was ensiled with no additive (control, C), *L. plantarum* (Lp, 1.0 × $10^6$ cfu/g FW); cellulase (E, 0.3%), cellulase+*L. plantarum* (E + Lp) for 3, 14, 30, 60 and 90 days. Each additive treatment was applied to 760 g of grass per silo in quintuplicates. After treating and mixing well, the grass was filled into silo (1-L polyvinyl chloride bottle, Lantian Biological Experimental Instrument Co., Ltd, Jiangsu, China), followed by sealing with two screw tops (internal and external). The fermentation quality and structural carbohydrates degradability and cellulose convertibility of napier grass silage were determined, according to experimental procedures and analytical methods desribed by Wen et al. (2017). The napier grass silage fermentation quality, structural carbohydrates degradability and enzymatic hydrolysis data were subjected to two-way analysis of variance with treatments and storage periods as main factors (SAS 9.3).

**Results** Addition of cellulase or/and *L. plantarum* improved fermentation qualityof napier grass silage by reducing pH, ammonia nitrogen, acetic acid and butyric acid contents and increasing lactic acid and ratio of lactic acid to acetic acid as compared to the control. The structural carbohydrate contents were reduced significantly ($P<0.05$) in E and E+Lp silages as compared to C silage except for acid detergent lignin. All of the additives increased the contents of water soluble carbohydrates as compared with the control. The combined addition of cellulase and *L. plantarum* significantly ($P<0.05$) improved fermentation quality, water soluble carbohydrates and reduced compositions of structural carbohydrate compared to cellulase, *L. plantarum* and control silages. The water soluble carbohydrates and structural carbohydrate reduced during ensiling in all silages. The declined for water soluble carbohydrates was rapid during the first 7 days of ensiling with higher rate recorded in cellulase and control silages. Additive treated silages released significantly($P<0.05$) higher xylose and glucose as compared to control after treated with 0.5, 1 and 1.5% sulfuric acid for 60 and 90 min at 120oC. Cellulase+ *L. plantarum* treatment released higher xylose and glucose compared to other silages at all sulfuric acid concentrations and treatment time.

**Conclusions** Additives improved the fermentation quality of Napier grass silage by increasing LA content and reducing pH.

**Table1.** Effects of cellulase and/or lactic acid bacteria on fermentation quality of napier grass during ensiling**.**

| D | T[1] | DM[2] (g/kg FW) | pH | AN (g /kg TN)[3] | Organic acid (g/kg DM)[4] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | LA | AA | PA | BA |
| 3 | C | 278$^{a}$ | 5.31$^{a}$ | 6.51$^{aC}$ | 10.0$^{cA}$ | 4.5$^{aC}$ | 0.87$^{aC}$ | 0.57$^{aE}$ |
| | Lp | 275$^{a}$. | 4.19$^{b}$ | 3.08$^{b}$ | 26.1$^{aE}$ | 2.55$^{bE}$ | 0.75$^{bD}$ | 0.51$^{b}$ |
| | E | 273$^{aA}$ | 4.98$^{a}$ | 4.48$^{bB}$ | 19.3$^{bBC}$ | 2.79$^{bE}$ | 0.97$^{aD}$ | 0.53$^{abE}$ |
| | E+Lp | 271$^{aA}$ | 4.01$^{b}$ | 2.62$^{bC}$ | 28.8$^{aC}$ | 2.23$^{bE}$ | 0.69$^{bC}$ | 0.44$^{cF}$ |
| 14 | C | 274$^{a}$ | 5.22$^{a}$ | 11.7$^{aBC}$ | 4.04$^{dB}$ | 8.15$^{aC}$ | 3.50$^{aB}$ | 3.91$^{aD}$ |
| | Lp | 270$^{a}$ | 4.1$^{c}$ | 5.24$^{b}$ | 37.6$^{bA}$ | 6.51$^{bC}$ | 1.38$^{cC}$ | 0.91$^{c}$ |
| | E | 270$^{aAB}$ | 4.89$^{b}$ | 5.77$^{bB}$ | 19.6$^{cBC}$ | 7.73$^{aD}$ | 2.72$^{bC}$ | 1.88$^{bCD}$ |
| | E+Lp | 265$^{aAB}$ | 3.96$^{c}$ | 4.88$^{bBC}$ | 44.5$^{aA}$ | 5.50$^{bC}$ | 1.04$^{cBC}$ | 0.64$^{cD}$ |
| 30 | C | 277$^{a}$ | 5.16$^{a}$ | 12.3$^{aBC}$ | 3.89$^{dB}$ | 14.2$^{aB}$ | 4.01$^{aAB}$ | 7.11$^{aC}$ |
| | Lp | 271$^{ab}$ | 4.05$^{c}$ | 5.77$^{b}$ | 29.4$^{bCD}$ | 6.99$^{cC}$ | 1.55$^{bBC}$ | 1.19$^{b}$ |
| | E | 268$^{abAB}$ | 4.79$^{b}$ | 9.52$^{aAB}$ | 21.4$^{cABC}$ | 10.0$^{bC}$ | 3.63$^{aA}$ | 2.75$^{bBC}$ |
| | E+Lp | 266$^{bAB}$ | 3.86$^{c}$ | 5.42$^{bBC}$ | 32.9$^{aC}$ | 5.88$^{cC}$ | 1.33$^{bAB}$ | 0.89$^{cC}$ |
| 60 | C | 276$^{a}$ | 5.15$^{a}$ | 18.3$^{aAB}$ | 2.08$^{dB}$ | 18.8$^{aB}$ | 4.26$^{aAB}$ | 10.2$^{aB}$ |
| | Lp | 270$^{ab}$ | 4.12$^{c}$ | 9.08$^{b}$ | 31.1$^{bBC}$ | 9.12$^{cB}$ | 2.01$^{bAB}$ | 2.94$^{b}$ |
| | E | 267$^{abAB}$ | 4.84$^{b}$ | 13.8$^{abA}$ | 23.1$^{cAB}$ | 12.2$^{bB}$ | 3.46$^{aAB}$ | 3.55$^{bAB}$ |
| | E+Lp | 265$^{bAB}$ | 3.97$^{c}$ | 7.57$^{bAB}$ | 38.4$^{aB}$ | 8.82$^{cB}$ | 1.73$^{bA}$ | 1.11$^{bB}$ |
| 90 | C | 275$^{a}$ | 5.11$^{a}$ | 19.6$^{aA}$ | 2.51$^{dB}$ | 25.5$^{aA}$ | 4.85$^{aA}$ | 13.0$^{aA}$ |
| | Lp | 267$^{b}$ | 4.14$^{c}$ | 11.0$^{bc}$ | 33.7$^{bB}$ | 11.4$^{bA}$ | 2.22$^{cA}$ | 2.06$^{c}$ |
| | E | 261$^{Cb}$ | 4.91$^{b}$ | 16.3$^{abA}$ | 24.4$^{cA}$ | 13.8$^{bA}$ | 3.45$^{bAB}$ | 4.30$^{bA}$ |
| | E+Lp | 257$^{cB}$ | 3.93$^{d}$ | 9.03$^{cA}$ | 35.6$^{aB}$ | 10.7$^{bA}$ | 1.38$^{cAB}$ | 1.35$^{cA}$ |
| SEM[5] | | 0.72 | 0.06 | 0.59 | 1.45 | 0.64 | 0.15 | 0.37 |
| Significance[6] | | | | | | | | |
| T | | * | * | * | * | * | * | * |
| D | | * | NS | * | * | * | * | * |
| T x D | | NS | NS | * | * | * | * | * |

$^{a-d}$ Values with different small letters show significant differences among treatments in the same ensiling day ($P$<0.05).$^{A-D}$ Value with different capital letters in different ensiling day show significant differences ($P$<0.05).[1]T, treatment; C, control; E, cellulase; Lp, *Lactobacillus plantarum;* E+LP*,* cellulase+ *L. plantarum.* [2]DM, dry matter; FM, fresh material. [3]AN, ammonia nitrogen; TN, total nitrogen. [4]LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; LA/AA, the ratio of lactic acid to acetic acid; %LA, (LA/organic acid )*100.[5]SEM, standard error of mean.[6]D, ensiling days; *, significant at $P < 0.05$; NS, no significantat$P< 0.05$.

**References**

Liu S., Ge X., Liu Z. & Li Y. (2016) Effect of harvest date on *Arundo donax* L. (giant reed) composition, ensilage performance, and enzymatic digestibility. Bioresource Technology, 205, 97-103.

Darrab M.J., & Shaha A. (2012) Biomass storage: an update on industrial solutions for baled biomass feedstocks. Biofuels, 3, 321-332.

Wen, A., Yuan, X., Wang, J., Desta S.T. & Shao T. (2017) Effects of four short-chain fatty acids or salts on dynamics of fermentation and microbial characteristics of alfalfa silage. Animal Feed Science and Technology, 223, 141-148.

# The effect of cellulolytic bacteria isolated from Tibetan yak (*Bos Grunniens*) on fermentation quality and cellulose convertibility of *Pennisetum sinese* silage

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**Keywords:** cellulolytic bacteria, ensiling, *Lactobacillus plantarum*, *Pennisetum sinese*

**Introduction** Ensiling as a promising technology for a prospective biofuel production from plant biomass, has benefits of low-cost storage simultaneously with a pretreatment effect. *P. sinese* is an attractive renewable resource for the production of biofuel due to a low energy input and high yield potential, however, it is difficult to ensiling due to low WSC and high fiber contents. Therefore, the objective of this study was to evaluate the effects of cellulolytic bacteria isolated from yak rumen on fermentation quality, structural carbohydrates degradability and cellulose convertibility of *P. sinese* silage.

**Materials and methods** Strain isolation, identification and enzyme assays were conducted according to Nyonyo et al. (2014). *P. sinese* grass were treated without additive (control, C), or with *Lactobacillus plantarum* (Lp, $10^6$ cfu/g FW), *E. casseliflavus* JFL12 (JFL12, $1.2\times10^6$ cfu/g FW), *E. faecalis* JF85 (JF85, $1.1\times10^6$ cfu/g FW), JFL12 combined with Lp (JFL12+ Lp), and JF85 combined with Lp (JF85+Lp). The fermentation quality, structural carbohydrates degradability and cellulose convertibility of *P. sinese* silage were determined, experimental procedures and analytical methods following the procedures of Desta *et al*. (2016). The *P. sinese* silage fermentation quality, structural carbohydrates degradability, and enzymatic hydrolysis data were subjected to two-way analysis of variance with treatments and storage periods as main factors (SAS 8.0).

**Results and discussion** Two cellulolytic bacteria (JFL12 and JF85) were isolated from Tibetan yak rumen and were identified as *Enterococcus casseliflavus* and *Enterococcus faecalis* by 16S rDNA sequencing analysis. Two strains alone or combined with Lp significantly ($P< 0.05$) increased lactic acid (LA) concentration as compared to control or Lp silage, respectively. The lowest pH and highest LA contents were observed in JF85+Lp silages (3.91 and 86.78 g/kg DM). The combined addition of isolates with Lp significantly lowered structural carbohydrates concentrations as compared to control silage (Table 1). Strains JFL12 and JF85 with CMCase (carboxymethyl cellulase) and FPase (filter-paper cellulase) activities could biodegrade structural carbohydrates into mono-saccharides for LAB fermentation. The cellulases are inducible enzymes synthesized by microorganisms, the cellulolytic activity of bacteria may be promoted in the presence of the LAB during the silage process, resulted in more efficiently de-structuring of lignocellulosic biomass. Isolates alone or in combination with Lp significantly increased ($P< 0.05$) WSC, mono- and disaccharides contents as compared to control. Higher levels of residual WSC in the isolates treated silages was attributed to incomplete fermentation of the WSC released by added cellulolytic bacteria. Combination addition efficiently improved enzymatic hydrolysis efficiency of *P. sinese* silage, indicated by higher glucose yield and cellulose convertibility.

**Conclusions** Combined application of isolates with Lp had synergistic effects on accelerating the degradation of structural carbohydrates and enhancing enzymatic hydrolysis efficiency.

**Table 1** The effect of isolates on the concentrations of structural carbohydrates of *P. sinese* silages during ensiling

| Items | Treatment | Ensilage time (d) | | | | SEM | Significance | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 7 | 14 | 30 | 90 | | T | D | T×D |
| NDF | C | 593.18$^{a}$ | 590.98$^{a}$ | 587.74$^{a}$ | 575.34$^{a}$ | 1.817 | *** | *** | * |
| (g/kg DM) | JFL12 | 572.78$^{ab}$ | 569.94$^{b}$ | 561.99$^{c}$ | 562.17$^{a}$ | | | | |
| | JF85 | 575.45$^{ab}$ | 573.27$^{b}$ | 568.66$^{bc}$ | 561.96$^{a}$ | | | | |
| | Lp | 582.95$^{a}$ | 579.00$^{ab}$ | 578.07$^{ab}$ | 576.02$^{a}$ | | | | |
| | JFL12+Lp | 562.11$^{bc}$ | 544.82$^{c}$ | 538.30$^{d}$ | 533.89$^{b}$ | | | | |
| | JF85+Lp | 553.33$^{c}$ | 543.04$^{c}$ | 534.59$^{d}$ | 531.63$^{b}$ | | | | |
| ADF | C | 354.58$^{a}$ | 349.74$^{a}$ | 352.30$^{a}$ | 344.88$^{a}$ | 0.906 | *** | *** | ** |
| (g/kg DM) | JFL12 | 344.83$^{b}$ | 342.10$^{ab}$ | 339.15$^{b}$ | 335.75$^{b}$ | | | | |
| | JF85 | 346.83$^{b}$ | 343.10$^{ab}$ | 338.15$^{b}$ | 336.00$^{b}$ | | | | |
| | Lp | 345.33$^{b}$ | 341.38$^{b}$ | 342.11$^{b}$ | 343.27$^{ab}$ | | | | |
| | JFL12+Lp | 335.72$^{c}$ | 328.20$^{c}$ | 325.77$^{c}$ | 323.86$^{c}$ | | | | |
| | JF85+Lp | 335.69$^{c}$ | 331.75$^{c}$ | 327.49$^{c}$ | 321.09$^{c}$ | | | | |
| ADL | C | 23.24 | 24.15 | 24.24 | 22.44 | 0.227 | *** | ns | ns |
| (g/kg DM) | JFL12 | 20.95 | 20.48 | 19.47 | 19.58 | | | | |
| | JF85 | 20.40 | 20.11 | 19.91 | 20.63 | | | | |
| | Lp | 24.24 | 24.15 | 21.57 | 21.44 | | | | |
| | JFL12+Lp | 21.35 | 20.40 | 20.28 | 20.06 | | | | |
| | JF85+Lp | 20.21 | 19.87 | 19.53 | 19.32 | | | | |
| Hemicellulose | C | 238.60$^{a}$ | 241.24$^{a}$ | 235.44$^{a}$ | 230.46$^{a}$ | 1.036 | *** | ** | ns |
| (g/kg DM) | JFL12 | 227.95$^{ab}$ | 227.84$^{ab}$ | 222.84$^{ab}$ | 226.42$^{ab}$ | | | | |
| | JF85 | 228.61$^{ab}$ | 230.17$^{ab}$ | 230.51$^{a}$ | 225.96$^{ab}$ | | | | |
| | Lp | 237.61$^{a}$ | 237.62$^{a}$ | 235.96$^{a}$ | 232.75$^{a}$ | | | | |
| | JFL12+Lp | 226.39$^{ab}$ | 216.62$^{bc}$ | 212.53$^{bc}$ | 210.03$^{b}$ | | | | |
| | JF85+Lp | 217.63$^{b}$ | 211.29$^{c}$ | 207.10$^{c}$ | 210.54$^{b}$ | | | | |
| Cellulose | C | 331.34$^{a}$ | 325.59$^{a}$ | 328.06$^{a}$ | 322.44$^{a}$ | 0.844 | *** | ** | ns |
| (g/kg DM) | JFL12 | 323.88$^{ab}$ | 321.62$^{ab}$ | 319.68$^{a}$ | 316.17$^{ab}$ | | | | |
| | JF85 | 326.44$^{ab}$ | 322.99$^{ab}$ | 318.24$^{a}$ | 315.37$^{ab}$ | | | | |
| | Lp | 321.09$^{ab}$ | 317.23$^{bc}$ | 320.54$^{a}$ | 321.83$^{a}$ | | | | |
| | JFL12+Lp | 314.37$^{b}$ | 307.80$^{d}$ | 305.49$^{b}$ | 303.80$^{bc}$ | | | | |
| | JF85+Lp | 315.48$^{b}$ | 311.88$^{cd}$ | 307.96$^{b}$ | 301.77$^{c}$ | | | | |

Values in the same column (a~d) with different following letters are significantly different.
ns, not significant; *$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001.

**References**

Desta, S.T., X.J. Yuan, J.F. Li, & Shao, T. (2016) Ensiling characteristics, structural and nonstructural carbohydrate composition and enzymatic digestibility of Napier grass ensiled with additives. Bioresour. Technol. 221: 447-454.

Nyonyo, T., Shinkai, T. & Mitsumori, M. (2014) Improved culturability of cellulolytic rumen bacteria and phylogenetic diversity of culturable cellulolytic and xylanolytic bacteria newly isolated from the bovine rumen. Fems Microbiol. Ecol. 88: 528-537.

# The effect of inoculation on fermentation characteristics and nutritional value of grass silage at farm scale

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**Keywords:** crude protein, grass silage, inoculants

**Introduction** Sugar-rich grasses are likely to favour the development of a bad opportunistic epiphytic microflora during fermentation, which can damage the quality of the silage. Dual purpose inoculants containing homofermentative and heterofermentative bacteria were developed to overcome the limitations of inoculants containing either type of bacteria alone (Huisden *et al*. 2009). The aim of this study is to evaluate the effect of inoculation with homofermentative and heterofermentative bacteria, on fermentation characteristics, and nutritional value of grass silages at farm level.

**Materials and Methods** The trial involved 23 farms located in the eastern part of the Netherlands. Farms were randomized between two groups: 11 farms represented the control group (C) with grass not treated with inoculants and 12 farms represented the treated group (T) where grass was inoculated with Lalsil Combo ® (Lallemand SAS – France; application rate : *Lactobacillus plantarum* CNCM MA 18/5U, >200'000 CFU/g of forage, and *Lactobacillus buchneri* NCIMB 40788, >100'000 CFU/g of forage). Ensiling occurred during spring 2016 and silos (bunker and drive over pile) were sampled at opening 6 weeks later. Samples were analyzed for nutritional value (dry matter (DM), ash, crude protein (CP), crude fiber, NDF, ADF, WSC) and fermentation parameters (pH, lactic acid (LA), acetic acid (AA), butyric acid (BA)). A conservation index (CI) as well as a heat sensitivity index (HSI) was calculated according to BLGG (2010). Both indexes are proprietary based equations of this Dutch reference laboratory for forage analysis: CI is a score taking into account the end products of fermentation, whereas HSI is an indicator of the sensitivity of the silage to heat. Data were subjected to univariate ANOVA with treatment (C or T) and grass DM class at feed-out (A≤45% DM, 45<B≤50% DM, and C>50% DM. C and T were evenly distributed between 3 DM-classes (Chi2 P=0.9)) and chosen as fixed effect (GLM procedure in SPSS v.19.0). Significance was declared at $P<0.05$ and all values are reported as estimated marginal means (EMM) ± standard error of mean (SEM).

**Results and Discussion** For the nutritional value no significant differences were detected neither for the treatment nor the DM class factors. Results on fermentation characteristics (Table 1) show that: pH and LA content were significantly improved for the treated silages ($P<0.05$). On average, pH was lower for T (5.0±0.1) than C (5.3±0.1). The average LA content was greater for T (37.3±4.4 g/kg DM) than C (21.3±4.4 g/kg DM). Zhang et al. (2009) also reported lower pH and higher LA content for alfalfa silage treated with a combination of *L.buchneri* and *L.plantarum*. BA content tended to be lower for treated silage compared to control. This finding are in line with results reported by Resch (2008), who described that content of BA can be decreased using lactic acid bacteria silage inoculants. The CI was better for the treated silage ($P<0.05$). Silage from T tended to have a higher level of CP (T: 181.3±3.4 g/kg DM vs C: 168.3±3.1 g/kg DM; $P<0.1$), suggesting a lower proteolysis. This could be explained by a faster acidification of the silage in T, which is confirmed by observation on the fermentation parameters pH and LA. The HSI was lower for treated silages, indicating lower sensitivity to aerobic spoilage. DM class had a significant effect on pH which was improved for silage belonging to groups A (4.8±0.1) and B (5.1±0.1) compared to group C (5.6±0.1) ($P<0.01$). LA content was also improved in groups A (39.2±6.0 g/kg DM) and B (30.6±6.0 g/kg DM), with a lower DM, compared to the group C (16.4±6.0 g/kg DM) ($P<0.05$). Silages having a DM <50% seem to have improved fermentation. However, higher DM shows significant lower BA content. Resch (2008) reported a similar negative correlation between DM content and BA content in grass silage. CI values tended to be lower for class C compared to class A ($P<0.1$). The average HSI was significant lower for silages

with DM ≤ 50% DM compared to silages with DM >50%. There was no significant interaction between Treatment and DM classes neither a trend for any of the described parameters.

**Table 1**. Fermentation characteristics in function of inoculation and DM class (g/kg DM when applicable) (EMM ± SEM)

| Parameters | CP | pH | LA | AA | BA | CI | HSI |
|---|---|---|---|---|---|---|---|
| Inoculant | | | | | | | |
| Control | 168.3±3.1 | 5.3±0.1 | 37.3±4.7 | 10.4±1.4 | 1.74±0.14 | 81.3± 2.3 | 42.5±4.0 |
| Treatment | 181.3±3.4 | 5.0±0.1 | 21.3±5.0 | 13.7±1.4 | 1.35±0.14 | 89.4± 2.7 | 32.2± 4.8 |
| *P* | *<0.1* | *<0.05* | *<0.05* | *NS* | *<0.1* | *<0.05* | *NS* |
| DM class | | | | | | | |
| A | 173.1±4.7 | 4.8±0.1$^{a}$ | 39.2±6.0$^{a}$ | 15.2±1.7$^{A}$ | 2.35±0.17$^{a}$ | 91.0±3.2$^{A}$ | 25.4±5.8$^{a}$ |
| B | 178.1±4.7 | 5.1±0.1$^{a}$ | 30.6±6.0$^{ab}$ | 12.3±1.7$^{AB}$ | 1.64±0.17$^{b}$ | 85.4± 2.9$^{AB}$ | 33.0±5.1$^{a}$ |
| C | 171.2±4.7 | 5.6±0.1$^{b}$ | 16.4±6.0$^{b}$ | 8.7±1.7$^{B}$ | 0.68±0.17$^{c}$ | 79.7±3.1$^{B}$ | 53.7±5.4$^{b}$ |
| *P* | *NS* | *<0.01* | *<0.05* | *<0.1* | *<0.01* | *<0.1* | *<0.05* |

**Conclusions** Results of this field study show that the addition of a *Pediococcus acidilactici* MA18/5M and *Lactobacillus buchneri* NCIMB 40788 based silage inoculant promotes a rapid acidification of grass silage, resulting in an improved fermentative profile. The associated higher CP content illustrates the benefit of such inoculation on silage quality by preserving silage nutritional value.

**References**

BLGG (2010) Aanpassingen conserveringsindex 2010. Accessed March 15, 2018. http://eurofins-agro.com/nl-nl/index/conserveringsindex.

Huisden C.M., Adesogan, A.T., Kim, S.C. & Ososanya T. (2009) Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 92:690-697.

Resch, R. (2008) Description of significant influencing factors on butyric acid content of grass silage by means of a multifactorial linear model. In: 13th Int. Conference „Forage Conservation, Nitra, Slovak Republic, pp 102-103.

Zhang, T., Li, L., Wang, X., Zheng, Z., Hu, Y. & Cui, Z. (2009) Effects of *Lactobacillus buchneri* and *Lactobacillus plantarum* on fermentation, aerobic stability, bacteria diversity and ruminal degradability of alfalfa silage. World Journal of Microbioly and Biotechn.

# The effect of two heterofermentative bacteria (*L. hilgardii* CNCM I-4785 and *L. buchneri* NCIMB 40788) and their combination on fermentation and aerobic stability of corn silage at different opening times

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**Keywords:** *Lactobacillus hilgardii*, *Lactobacillus buchneri*, corn, aerobic stability, early opening

**Introduction** Aerobic stability remains a challenge for corn silages, especially at early opening times (before 60 days of ensiling). *L. buchneri* is well documented to improve the aerobic stability of silages (Kleinschmit and Kung 2006). More recently a new heterofermentative strain *L. hilgardii* is reported to increase the aerobic stability of sugar cane (Ávila *et al*. 2014 and Carvalho *et al.* 2014). A mini-silo trial was performed to document the effect of a novel strain *L.hilgardii* CNCM I-4785 (LH) alone or in combination with *L. buchneri* NCIMB 40788 (LB) on fermentation characteristics and aerobic stability of corn silage.

**Material and Methods** Corn of 39.2% dry matter (DM) (cut off = 40-50 cm and chop-length =0.9 cm; Table 1) was divided over 4 groups: Negative Control (NC); Treatment 1 (T1): LB at 300 000 colony-forming units (CFU)/ g fresh matter (FM), Treatment 2 (T2): LH at 300 000 CFU/g FM, Treatment 3 (T3): LB and LH both at 150 000 CFU/g FM. Fifteen micro-silos (airtight glass jar of 1.7L) per treatment were prepared (Density=264 kg DM/$m^3$). The micro-silos were stored at ambient temperature (20-22°C). After 15, 30 and 90 days, 5 micro silos / treatment were opened. Fermentation characteristics (pH, acetic (AA), propionic and lactic acid (LA)), NH3-N, ethanol, 1,2-propanediol), nutritional value (DM, CP, NDF, ADF, WSC, ash) and aerobic stability (ambient temperature +3°C and pH at end of test (pHout)) were measured at each time point. Analysis of Variance was applied for all data except aerobic stability; non parametric Kruskal-Wallis test was applied in the latter case.

**Table 1**. Chemical composition fresh forage.

| | % DM |
|---|---|
| Dry matter | 39.21 %FM |
| Crude protein | 7.80 |
| NDF | 46.39 |
| ADF | 21.30 |
| WSC | 6.96 |

**Results and Discussion** Aerobic stability of the NC improved over time. For all treatments, the aerobic stability was improved at all opening times (Table 2). At early opening (15 days), pHout was improved for the T1, T2 and T3 compared to NC (NC=5.66a, T1=3.85b, T2=3.92b and T3=4.38b, P<0.05), indicating again a higher stability for the treated silages compared to NC.

**Table 2**. Aerobic stability (ambient + 3°C; h)

| | 15 | 30 | 90 |
|---|---|---|---|
| NC | 36 a | 22 a | 122 a |
| T1 | 168 b | 168 b | 168 b |
| T2 | 168 b | 168 b | 168 b |
| T3 | 160 b | 168 b | 168 b |

Table 3 summarizes the fermentation characteristics of the silage at the different opening times. Compared to NC, the addition of the silage additives resulted in more LA and significantly higher AA

content for all treated silages at day 90 (P<0.05). pH was significantly lower in NC group compared to treated silages at day 90. This is in line with the fermentation profile of a inoculation with heterofermentative lactic acid bacteria such as LB and LH as reviewed by Muck *et al.* (2018). At all opening times, treated silages showed significant lower amount of NH3-N indicating reduced proteolysis (P<0.01). Ethanol content of the treated silages was significantly lower than the negative control at all time points, except for T1 at 30 days (P<0.01). After 30 and 90 days, a significantly greater amount of WSC remained in treated silages (P<0.05). When inoculated alone (T2), LH keeps more WSC after 30 and 90 days. After 90 days, the 1,2-propanediol content of all inoculated silages was significantly higher than the NC (0.05 % FM, P<0.05). Results from the current study on corn silage are in line with the observations from Carvalho et al. (2014) and Avila et al. (2014) on sugar cane silage: higher AA, higher 1,2-propanediol and lower ethanol content.

**Table 3**. Fermentation characteristics of corn silage at 15, 30 and 90 days (FM basis)

| Days | pH | LA % | AA % | N-NH3 % of total N | Ethanol % | WSC % |
|---|---|---|---|---|---|---|
| 15 | | | | | | |
| NC | 3.74±0.02$^{a}$ | 2.09±0.43 | 0.22±0.06 | 2.53±0.06$^{a}$ | 0.65±0.19$^{b}$ | 2.53±0.06$^{b}$ |
| T1 | 3.78±0.01$^{b}$ | 2.26±0.30 | 0.15±0.03 | 2.21±0.06$^{b}$ | 0.16±0.09$^{a}$ | 2.21±0.15$^{a}$ |
| T2 | 3.74±0.01$^{a}$ | 2.30±0.27 | 0.21±0.03 | 2.27±0.06$^{b}$ | 0.18±0.04$^{a}$ | 2.27±0.08$^{a}$ |
| T3 | 3.75±0.02$^{a}$ | 2.24±0.71 | 0.19±0.08 | 2.11±0.06$^{b}$ | 0.09±0.01$^{a}$ | 2.11±0.07$^{a}$ |
| 30 | | | | | | |
| NC | 3.74±0.02$^{a}$ | 2.24±0.08$^{a}$ | 0.24±0.03 | 4.56±0.50$^{c}$ | 0.37±0.06$^{b}$ | 0.80±0.07$^{a}$ |
| T1 | 3.79±0.03$^{b}$ | 2.71±0.14$^{b}$ | 0.20±0.03 | 3.79±0.53$^{b}$ | 0.29±0.02$^{ab}$ | 1.64±0.17$^{bc}$ |
| T2 | 3.76±0.01$^{ab}$ | 2.47±0.13$^{ab}$ | 0.26±0.03 | 3.55±0.18$^{b}$ | 0.26±0.05$^{a}$ | 2.01±0.06$^{c}$ |
| T3 | 3.77±0.01$^{ab}$ | 2.65±0.25$^{b}$ | 0.27±0.07 | 2.74±0.09$^{a}$ | 0.25±0.04$^{a}$ | 1.63±0.37$^{b}$ |
| 90 | | | | | | |
| NC | 3.76±0.00$^{a}$ | 2.23±0.18$^{a}$ | 0.38±0.03$^{a}$ | 6.09±0.38$^{c}$ | 0.91±0.06$^{a}$ | 0.47±0.07$^{a}$ |
| T1 | 3.86±0.02$^{c}$ | 2.81±0.46$^{b}$ | 0.57±0.02$^{b}$ | 4.49±0.34$^{a}$ | 0.46±0.07$^{b}$ | 1.48±0.08$^{bc}$ |
| T2 | 3.80±0.03$^{b}$ | 2.60±0.29$^{ab}$ | 0.63±0.11$^{b}$ | 5.19±0.35$^{b}$ | 0.49±0.12$^{b}$ | 1.71±0.11$^{c}$ |
| T3 | 3.82±0.02$^{b}$ | 2.71±0.24$^{ab}$ | 0.61±0.11$^{b}$ | 4.41±0.20$^{a}$ | 0.48±0.03$^{b}$ | 1.26±0.25$^{b}$ |

**Conclusion** Results of this mini-silo study suggest that *L.hilgardii* CNCM 4785 alone or in combination with *L.buchneri* NCIMB 40788 reduces ethanol and NH3 content and preserves more fermentable sugars. Moreover, it allows a greater aerobic stability of corn silage at several opening times. The details of the mode of action of this new silage inoculant remain to be described.

**References**

Ávila, C.L., Carvalho, B.F., Pinto, J.C., Duarte, W.F. & Schwan R.F. ( 2014) The use of Lactobacillus species as starter cultures for enhancing the quality of sugar cane silage. Journal of Dairy Science, 97, 940-951.

Carvalho, B.F., Ávila, C.L.S., Miguel, M.G.C.P., Pinto, J.C., Santos, M.C. & Schwan. R.F. (2014) Aerobic stability of sugar cane silage inoculated with tropical strains of lactic acid bacteria. Grass Forage Science, 70,308-323.

Kleinschmit D.H. & Kung Jr. L. (2006) A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science, 89(10), 4005-4013.

Muck, R.E., Nadeau, E.M.G., McAllister, T.A., Contreras-Govea, F.E., Santos, M.C. & Kung Jr., L. (2018) Silage review: Recent advances and future uses of silage additives. Journal fo Dairy Science, 101, 3980-4000.

# The effects of inoculation of grass with either homo-fermentative or hetero-fermentative lactic acid bacteria on silage quality, diurnal variation in rumen pH, lactic and volatile fatty acids

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**Introduction** In recent years the use of heterofermentative forage inoculants has increased (Kleinschmit and Kung, 2006) to address the problem of aerobic spoilage of silages which have been poorly consolidated at filling. The inoculant species *L. buchneri* has been used as the main solution. However, animal studies have not convincingly shown a gain in production from this approach. Numerous studies using homofermentative bacterial inoculants such as *L. plantarum* have more consistently improved animal production (Kung and Muck, 2015). The exact mechanism(s) for the positive effects on production have not been fully elucidated. The aim of this study was to examine the effect different forage additive treatments at ensiling on grass silage quality, and when fed, on the rumen acidotic potential and diurnal variation in rumen pH post feeding. The hypothesis is that silages with a higher lactic:acetic acid ratio will retain more WSC, reduce NH3 and promote a more stable rumen for microbial growth.

**Materials and Methods** First cut *Lolium multiflorum* was collected immediately after precision chopping of the forage at harvest and split into 3 piles. From each pile 71 kg FW(Fresh weight) was treated with 1 litre/71 kg FW of one of 3 treatments, which were either Control (Water), *L.plantarum* (NCIMB 40027) delivering 1 x$10^6$ cfu/g FW of forage or *L. buchneri* (NCIMB 30139 ) delivering 200,000 cfu/g of forage as per manufacturers guidelines. Immediately after treatment application, the grass was packed into 120 litre drums, sealed and stored for 4 months. The ensiling process was repeated with each pile of grass to give 3 replicates per treatment. Three individually penned fistulated mature wethers were fed *ca.* 1.5 kg DM/d of each of the grass silage treatments for a period of 2 weeks in a change-over design, such that over the three experimental periods, each of the 3 sheep were fed each treatment. Rumen fluid was removed via the rumen cannula every 3h from feeding on day 13 to feeding on day 14 to determine the effect of treatment on the diurnal variation of pH, lactic acid, VFA, and ammonia. Representative samples of the silage being fed were analysed for DM, pH, lactic and acetic acids, (butryric acid and alcohols were measured with 0% and <1.1%DM detected respectively) water soluble carbohydrates and ammonia-N using the methods as described by Davies *et al. (*1998). The biogenic amines cadaverine and putrescine were analysed by the methods of Steidlova and Kalac (2003). Statistically analysis was carried out using Genstat 18, (VSN International).

**Results** The silage fermentation analyses at the end of the storage period are shown in Table 1.

**Table 1.** DM, pH, fermentation characteristics, ammonia-N and biogenic amines of silages.

| Treatment | DM g/Kg | pH | Lactic g/kg DM | Acetic g/kg DM | WSC g/kg DM | $NH_3$-N g/kg TN | Cadaverine mg/Kg DM | Putrescine mg/Kg DM |
|---|---|---|---|---|---|---|---|---|
| Control | 256.9 | 3.96 | 136.3 | 9.4 | $^{a}$41.4 | $^{a}$63.6 | $^{a}$769 | 83 |
| *L. plantarum* | 251.5 | 3.81 | 135.8 | 8.5 | $^{b}$58.9 | $^{b}$36.1 | $^{b}$381 | 2 |
| *L. buchneri* | 250.4 | 3.85 | 134.6 | 9.0 | $^{a}$46.1 | $^{a}$51.5 | $^{a}$771 | 112 |
| s.e.d. | 4.14 | 0.064 | 2.564 | 1.492 | 4.88 | 9.599 | 89.87 | 52.8 |
| P value | 0.344 | 0.156 | 0.793 | 0.822 | **0.050** | **0.107** | **0.019** | 0.176 |

Data within columns with highlighted superscripts differed significantly.

The silage treated with the homofermentative inoculant *L.plantarum* had a significantly higher WSC concentration and significantly lower concentrations of ammonia-N and cadaverine, thus indicating better protein preservation in the silage.

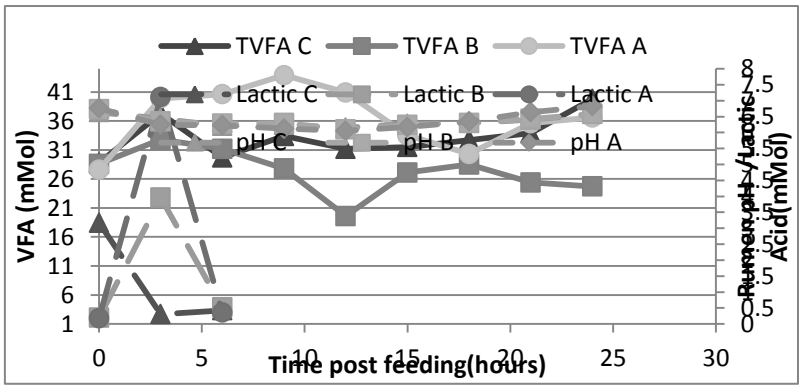


**Figure 1.** Effect of inoculant type on the diurnal rumen pH, total VFA and Lactic acid (A=Control, B=L.plantarum, C= L.buchneri).

The 3 hourly assessed diurnal variation in rumen pH after feeding indicates that there was no significant difference between silage treatments. In this study the biggest range in rumen pH was in sheep fed the control treated silage with a 0.75 pH unit amplitude. There was also no significant difference in rumen lactic acid production and this was below detectable levels post 6 hours. Total rumen VFA production was significantly lower with homo-fermented inoculated silage ($P<0.007$).

**Discussion** The results for silage fermentation indicators follow the expectations of improved protein and sugar preservation when using homofermentative lactic acid bacteria (Davies et al. 1998). There were no significant differences in silage pH but the L.planatarum silage reached terminal pH more quickly as there were significantly less protein breakdown products to buffer fermentation and significantly more WSC was retained. The relatively high levels of total acid in excess of 140 g/kg DM with a lactic:acetic ratio greater than 14:1 in all treatments did not results in a depression of rumen pH to below 5.6 at any point over the 24 h period that would cause concern for rumen acidosis, in agreement with results by Navarro-Villa et al. *(*2012). In this study all treatments indicated a high degree of homofermentation in the silo suggesting that the hetero-fermentative lactic acid bacteria species did not have an active role in the silage fermentation. It would have been interesting to see how this would have changed if the L.buchneri had been inoculated at the same rate of 1 $x10^6$ cfu/g, but the rate used was the recognised industry standard.

**Conclusions** The homofermentative inoculant improved silage quality in terms of key markers of nutrition, namely WSC and markers of protein breakdown. The relatively high acid concentration produced in all silages did not invoke rumen acidosis when fed to sheep. Measurement of total microbial yield could have provided some crucial answers for the above findings.

**References**

Davies, D.R., Merry, R.J., Williams, A.P., Bakewell, E.L., Leemans, D.K. & Tweed, J.K.S. Proteolysis During Ensilage of Forages Varying in Soluble Sugar Content. Journal of Dairy Science 81: 444-453.

Kleinschmit D.H & Kung,L.,Jr. (2006) A Meta-Analysis of the Effects of *Lactobacillus buchneri* on the Fermentation and Aerobic Stability of Corn and Grass and Small-Grain Silages .Journal of Dairy Science 89:4005-4013.

Kung, L., Jr. & Muck, R.E. (2015) Silage Additives: Where are we going? XVII International Silage Conference July 1-3 Piracicaba, Sao Paulo Brazil. Pp 72-81.

Navarro-Villa, A., O'Brien, M., Lopez, S., Boland, T.M. & O'Kiely, P. (2012). In vitro rumen methane output of grasses and grass silages differing in fermentation characteristics using the gas-production technique (GPT). Grass and Forage Science 68:228-244.

Steidlova, S. & Kalac S. (2003) The effects of using lactic acid bacteria inoculants in maize silage on the formation of biogenic amines. Archives in Animal Nutrition 57(5): 359-368.

# Use of straw like absorbent to ensiling lettuce and broccoli by-products

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**Keywords:** broccoli, by-product, lettuce, straw

**Introduction** The habits acquired in the diets of European consumers mean that more and more food is produced: prepared, processed or with industrial presentation, which, together with modern intensive agriculture, causes the appearance of an excess of by-products that have to be discarded, needing a high economic cost. Many of them could be used in animal feed (Cao et al., 2011). Lettuce and broccoli by-products are a very perishable food of rapid degradation, whose by-products, if not removed quickly produce serious environmental problems. The accumulation of these by-products, together with the high environmental temperatures,accelerate the degradability of biomass. By the other hand, the southeast of Spain produces a large amount of vegetables but no forages for animal feeding due to the scarce rains and high price of irrigation water. The objective of this study is to know the capacity of silage as a method of conservation of the raw lettuce and raw broccoli by-products, both with large production and low dry matter content, when different treatments are carried out to reduce the production of effluents and to increase the availability time to be used in ruminant feed.

**Material and Methods** The evolution of fermentative and chemical-nutritive indicators were compared after silage in two of the most important agroindustrial by-products of southeast of Spain, raw romaine lettuce *(Lactuca sativa L., var. Longifolia*) (L) and raw broccoli (*Brassica oleracea L. var. Itálica*) (B) obtained in the industries producing vegetables of the IV category. Each test plot was chopped and packed into plastic bag, in a shading place after vacuum sealing using a vacuum heat-sealer. Three replications were made for both by-products of the following four batches: lettuce or broccoli (L and B, respectively). T1: 100% by-product. T2: by-product plus barley straw (75 + 25%, respectively). T3: by-product plus sodium chloride (NaCl) (97.5 + 2.5%, respectively). T4: by-product plus barley straw plus mandarin byproduct (70 + 20 + 10%, respectively). Adjusting to 150 or 600g, respectively, of final weight and sealing in vacuum. All samples were stored for 28 days at 9 °C temperature (usual conditions for lab trials for high moisture by-product silages, in small plastic bags). The data were analyzed for their statistical significance and compared by one-way ANOVA. Statistical significance was declared at P.

**Results and discussion** Fermentative and nutrition-chemical characteristics of the by-products are shown in Table 1. The results of DM were very low in both by-products indicated that are wet silages (53.8 and 68.6 g/kg DM for lettuce and broccoli, respectively). It should be noted that the initial composition of these by-products do not have good conditions for silage. The silos made with barley straw and the lettuce by-product did not produced any effluent, presenting a better water retention capacity (Oladosu et al. 2016), but these produced an acetic fermentation. By the other hand, high effluents levels were released in trials without absorbent (94.9 and 192.6 L/tm, for LT1 and LT3, respectively). For the fermentation parameters, the LT3 batch obtained good levels, being statistically significant ($P<0.001$) its content in lactic acid, higer than acetic acid, and the pH had a good value for high moisture by-products (Megías et al. 2014) although this batch produced the largest effluent amount. No effluents were collected in any broccoli silages and similar results were obtained in BT4, with acetic fermentation. Significant differences were found between treatments BT1/ BT3 and BT2/ BT4 ($P <0.01$) on WSC content. The lactic acid content in the lots BT1, BT2 and BT3 was hihger than the acetic acid content, indicating that there was a lactic fermentation, althout the pH is very high to the BT1 and BT2. BT4 produced a acetic fermentation. So, it is necessary to look for alternatives to improve the fermentative quality of these treatments because the pH level is to high. On the one hand, for the chemical-nutritional parameters, it can be observed that the treatments that included straw presented significant results of higher contents of the fibrous fractions and dry matter, and lower in

crude protein. Crude protein shows significantly higher differences between samples LT1/BT1 and LT3/BT3 with samples LT2/BT2 and LT4/BT4 (P <0.001 and 0.01, respectively). The results obtained showed that not all the lots of the lettuce by-product can be considered suitable for good silage. In this sence, barley straw is a good absorbent for silage when the materials have high moisture content and salt was also a good additive in those silages that they were used.

**Table 1.** Chemical and fermentative composition of lettuce and broccoli by-products silage according to the different treatments.

| | L T1 | L T2 | L T3 | L T4 | Significance level |
|---|---|---|---|---|---|
| Dray matter (g/kg) | 53.8$^{a}$ | 254.6$^{b}$ | 79.9$^{a}$ | 242.3$^{b}$ | *** |
| Effluents (ml) | 156.7$^{b}$ | 0.00$^{a}$ | 383.3$^{c}$ | 0.00$^{a}$ | *** |
| pH | 4.41 $^{a}$ | 5.04 $^{b}$ | 4.56 $^{a}$ | 4.46 $^{a}$ | ** |
| Lactic acid (g/kg DM) | 39.6$^{b}$ | 8.6$^{a}$ | 129.4$^{c}$ | 22.1$^{b}$ | *** |
| Acetic acid (g/kg DM) | 32.9$^{b}$ | 27.8$^{b}$ | 19.4$^{a}$ | 58.4$^{c}$ | *** |
| WSC (g/kg DM) | 16.4 $^{b}$ | 9.1 $^{a}$ | 14.6 $^{b}$ | 7.0 $^{a}$ | *** |
| CP (g/kg DM) | 210.2$^{b}$ | 66.4$^{a}$ | 165.5$^{b}$ | 65.0$^{a}$ | *** |
| NDF (g/kg DM) | 175.0$^{a}$ | 605.2$^{b}$ | 149.6$^{a}$ | 571.1$^{b}$ | *** |
| | B T1 | B T2 | B T3 | B T4 | |
| Dray matter (g/kg) | 68.6$^{a}$ | 260.9$^{d}$ | 108.7$^{b}$ | 207.1$^{c}$ | * |
| Effluents (ml) | 0 | 0 | 0 | 0 | - |
| pH | 6.30$^{c}$ | 6.07$^{c}$ | 4.19$^{a}$ | 5.05$^{b}$ | ** |
| Lactic acid (g/kg DM) | 68.1$^{c}$ | 49.8$^{b}$ | 45.9$^{b}$ | 25.2$^{a}$ | * |
| Acetic acid (g/kg DM) | 3.8$^{a}$ | 26.0$^{b}$ | 27.8$^{b}$ | 38.5$^{b}$ | * |
| WSC (g/kg DM) | 85.0$^{b}$ | 34.9$^{a}$ | 75.8$^{b}$ | 48.2$^{a}$ | ** |
| CP (g/kg DM) | 293.2$^{b}$ | 109.4$^{a}$ | 270.5$^{b}$ | 124.4$^{a}$ | ** |
| NDF (g/kg DM) | 192.8$^{a}$ | 575.4$^{b}$ | 133.6$^{a}$ | 565.7$^{b}$ | * |

L: lettuce; B: broccoli; T1: 100% by-product; T2:by-product plus barley straw (75 + 25%, respectively); T3: by-product plus NaCl (97.5 + 2.5%, respectively); T4: by-product plus barley straw plus mandarin byproduct (70 + 20 + 10%, respectively).
*: P <0.05; **: P<0.01; ***: P<0.001

**Conclusion** This study showed that lettuce and broccoli by-products had a limited fermentation capacity to be ensiled without treatment. On lettuce silage, when the barley straw was included, the capacity for water retention rose, for which they are a good option to control the impact on the environment. The results obtained showed that of lettuce and broccoli by-products can be considered suitable for silage when the salt is added to the process. While, it is necessary to look for alternatives to improve more the silages fermentative quality of these by-products and decreasing the release of effluents.

**References**

Cao, Y., Cai, Y., Takahashi, T., Yoshida, N., Tohno, M., Uegaki, R., Nonaka, K. & Terada, F. (2011) Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. Journal of Dairy Science. 94: 3902–3912.

Megías, M.D., Meneses, M., Madrid, J., Hernández, F., Martínez-Teruel A. & Cano, J.A. (2014) Nutritive, fermentative and environmental characteristics of silage of two industrial broccoli (Brassica oleracea italica) by-products for ruminant feed. International Journal of Agriculture and Biolology, 6: 307-31.

Oladosu,Y., Rafii, M.Y., R., Abdullah, N., Magaji, U., Hussin, G., Ramli, A. & Miah, G. (2016). Fermentation quality and additives: a case of rice straw silage. BioMed Research International ID 7985167, 14 pages. http://dx.doi.org/10.1155/2016/7985167

# Production and utilization of silages in tropical areas

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**Keywords:** Brazil, forage conservation, silage, South America, warm climate

## Introduction

Tropical climates are characterized by constant high temperatures all-year-round (at sea level and low elevations). A few decades ago, there was a claim that lower crop yield and high human population would explain the level of poverty in the tropics. Presently, the adoption of technological resources has resulted in a steep increase in food production, turning tropical countries into important players to supply the global market. Indeed, a substantial portion of the food produced in tropical and subtropical zones is exported. Tropical regions are also producing biofuels (e.g., ethanol and biodiesel). In the beginning, biofuels were developed to help countries achieve sustainable fuel consumption but with increased production in tropical areas, efforts are being made to export the commodity to wealthy countries.

Climate represents an uncontrollable component that affects crop and animal production. Weeds, insects and diseases are major constraints to agricultural production (Burdon et al. 2006), especially in warm climates. Additionally, it has been claimed that climate modifications due to anthropogenic greenhouse effects might provoke variations in food production, mainly in tropical and subtropical regions (IPCC 2001). Hence, professional farming, capable of producing food in a sustainable fashion without damaging the environment, is imperative to avoid jeopardizing domestic food production and agricultural exports (Cerri et al. 2007). For instance, agriculture comprises the largest single sector of the economy in Brazil. In 2017, agribusiness represented 23.5% of the gross domestic product and 44.1% of Brazilian exports. Agribusiness has sustained the Brazilian economy, and it is not by chance that Brazil has been recognized as an important barn in the world.

In South America, most agriculture and animal production are located in Aw (tropical savanna) and Cfa (humid subtropical) climates (Köppen classification), with a ‘rainy season’ and a ‘dry season’. Because most beef and dairy farms are based on grazing systems, there is a pasture shortage four to six months of the year. Consequently, forage conservation is a key strategy to increase stocking rate and animal performance during the dry season. Moreover, a significant number of dairy farms are moving from grazing to housed systems (e.g., free stall and compost barns), which will require supply of conserved forage year round. Likewise, the number of beef cattle finished in feedyards is increasing in Brazil, which also requires conserved forage throughout the year. Hence, on-farm production of high-quality forage has been a key tool to succeed during periods of high grain prices. Because forage growth occurs mainly during the humid rainy season, silage has been by far the major conserved forage.

The International Silage Conference began in 1970, but offered papers addressing ‘tropical forage’ were seldom published (e.g., Martins et al. 1993, Ott et al. 1999) until the 13th Conference in Scotland in 2002, when an entire section was dedicated to “The role of silage in tropical, subtropical and arid systems”. Afterwards, a comprehensive review on tropical grass silages was presented during the 14th Conference in Northern Ireland (Nussio 2005). The last (17th) edition of the International Silage Conference was held in Brazil and included a keynote presentation (Bernardes et al. 2015b) and dozens of offered papers on tropical silage production and utilization.

This manuscript brings an overview of the current challenges and opportunities for silage production and utilization in tropical and subtropical areas, with particular reference to Brazil.

## Silage in dairy and beef operations in Brazil

Whole-plant corn silage (or corn silage) has been the main forage source in ruminant diets in intensive operations or during part of the year in grazing-based systems. A survey involving two hundred sixty dairy farmers in Brazil showed that 82.7% used corn silage alone or in combination with other forage sources in the ration (Bernardes and Do Rêgo 2014). Other crops mentioned in that survey were sorghum, tropical grasses (*Brachiaria* and *Panicum* genera), and sugarcane. A recent assessment of silage feeding programs currently utilized by high-producing dairy herds across the top six milk-producing Brazilian states showed that corn silage was fed on all farms (n = 146), whereas whole-plant sorghum silage was fed by 6.9% of farms (Bernardes et al. 2018a). Other types of silage from corn crop were: high-moisture corn (HMC; 24.8%), reconstituted corn grain silage (16.6%), and earlage/snaplage (3.4%). Reconstituted sorghum grain silage was included in 7.6% of the diets, and haylage was used by 28.3% of the farms surveyed (Bernardes et al. 2018a). Ryegrass and Bermuda grass were the main species for haylage production.

In the beef industry, corn silage has also been the primary source of forage in finishing diets, followed by tropical grass silage and whole-plant sorghum silage (Oliveira and Millen 2014, Pinto and Millen, 2016). Brazilian beef feedlots have also invested efforts to produce corn grain silages (HMC, reconstituted corn and ear corn silages). Recently, there has been great interest in snaplage. The logistic benefits, the stalk remaining in the field for no-till systems (direct drilling) and the presence of cob and husks capable of stimulating chewing activity (‘built-in-roughage’), have been the main reasons for the use of snaplage in Brazilian feedyards. Certainly, the greater starch digestibility of ensiled grain compared to dry ground shelled corn has led feedlots to adopt corn grain silages (Benton et al. 2005). It has been estimated that approximately 40% of Brazilian feedlots have used corn grain silages in finishing diets (Rafael Cervieri, personal communication).

## Corn silage

***Agronomic aspects*** Currently, Brazil is the world’s third largest corn grain producer and the second largest exporter (USDA 2018). Following soybean, corn is the second largest crop in the country, with a 20% share of planted area on 15.8 million hectares (CONAB 2018). Due to the tropical climate, Brazil has a long growing season, which allows the adoption of the double-cropping system. Traditionally, soybean is first-cropped in summer, followed by corn in the fall. Brazil is also one of the world’s largest consumers of corn grain. Use of corn has grown substantially over the last 15 years, driven mostly by the livestock industry, as Brazil is the world’s leading meat exporter (USDA 2017). The development and adoption of no-till farming have been a key factor in the success of the Brazilian corn industry. This technology is currently practiced on more than 50% of the annual crop area (Freitas and Landers 2014). No-tillage practices cause less soil disturbance, decrease erosion and often result in significant accumulation of soil C (Sá et al. 2001). Numerous factors interfere against achieving high yields when corn is produced in hot environments, especially pests and diseases. In this way, genetically modified corn has been used by farmers and, beneficially, it has reduced the use of pesticides. To avoid resistant insects due to the usage of genetically engineered corn, at least 10% of the area must be planted with conventional (non-Bt) corn (refuge area). Regarding the sustainability of corn production, unlike what many people think, Brazil has strict environmental preservation laws, and thus all farmers must maintain at least 20% of preserved vegetation. Therefore, Brazilian producers are making less money from grains on behalf of preserving the environment, as demonstrated by a recent report published by NASA (National Aeronautics and Space Administration).

In terms of silage, Brazil has grown approximately 4 million hectares of corn (T. Bernardes, unpublished data). Currently, about 400 corn hybrids are registered for sale in the Brazilian market. Among these, there are no specific hybrids for silage making, and thus a dual-purpose strategy has been used by seed companies (Bastos et al. 2018). Recently, forty-two cornfields were visited in three

regions of Brazil (South, Southeast, and Midwest) with the objective of assessing corn hybrids for silage production. Considering the first and second growing seasons (from September to February and from March to June, respectively), corn forage yields varied from 28.2 to 17.4 tons of DM/ha, respectively (Bastos et al. 2018). This study also showed that greater silage yield was associated with higher ear dry weight, taller plants, and greater plant population. Paziani et al. (2018) compiled a data set of silage DM and grain yields in five experimental stations in São Paulo State (Brazil) during the 2000/01 to 2013/14 growing seasons. Positive correlations were found among grain yield at maturity and DM yield at ensiling (r = 0.77), grain yield at ensiling (r = 0.84) and digestible DM yield at ensiling (r = 0.72). Hence, maximizing DM yield appears to maximize both grain and stover yield. Thus far, high yields of silage DM can be obtained by utilizing tropical hybrids, especially in the first growing season.

***Machinery*** In Brazil, pull-type forage harvesters are still common in dairy systems, mainly due to the high cost of self-propelled harvesters. In 2014, Bernardes and Do Rêgo reported that 90.4% of dairies used pull-type forage harvesters, followed by 9.6% who adopted self-propelled. In beef systems, however, the majority of farmers use self-propelled forage harvesters by hiring custom services. The number of contractors with self-propelled harvesters has significantly increased in all Brazilian regions. In addition to the low filling rate of silos (i.e., on average 10-15 tons/h), pull-type harvesters also impact both forage particle length and kernel processing, since it does not have a precision cutting system. Pull-type forage harvesters often result in longer particles (especially husks) with poor uniformity (Bernardes et al. 2012). This cutting pattern affects silage fermentation, increases the risk of silage deterioration (greater silage porosity) (Muck et al. 2003) and may affect the nutritional management in dairy herds due to feed sorting in the feedbunk (Leonardi and Armentano 2003). Furthermore, poor chopping by traditional machinery results in fewer kernels being cracked. To overcome this issue, some farmers have set the machine to chop finely and damage kernels. However, a minimal length of chop (> 8 mm) has been recommended to improve rumen function and health in dairy cows (Zebeli et al. 2010). A recent study reported that a pull-type harvester set at 3-5 mm of theoretical length of cut had more than 30% of particles shorter than 8 mm, and this would compromise chewing activity and rumen function in high-producing dairy cows (Pereira 2017), depending on diet composition.

***Sealing*** Tropical environments can have adverse effects on the physical properties of plastic films used to cover silos. In particular, tropical conditions reduce airtightness and resistance of the film to radiation and high temperatures (Paillat and Gaillard 2001). Thus, in warm climates, plastic films can readily become more permeable to air, especially if they are used to cover silages for long storage periods (Bernardes et al. 2018b).

A plastic film used to cover silage has to fulfill three essential functions, and they are independent of where the silo is located (tropical or temperate region). The film should prevent damage caused by rain, hail or birds. The film should be UV resistant in order to remain intact after prolonged exposure to sunlight. Finally, the third function of the silo film is that it must ensure anaerobic conditions during ensiling (Bernardes 2016). In addition to plastic quality, the way the film covers the silage is also important. Recently, studies were undertaken in a tropical area to examine the use of oxygen barrier film on the walls and top regions of bunker silos compared to only a single layer of standard polyethylene film on top of the bunker (Lima et al. 2017). Lining the inside of the bunker walls before filling is an alternative approach to ensuring more complete exclusion of oxygen during ensiling and storage (Bernardes 2016). Thus, the use of high-quality plastic film and ensuring close contact of the film with the silage surface are essential for producing high-quality tropical silages (Lima et al. 2017). Placing materials on top of the plastic cover also markedly affects silage quality (Bernardes 2016). This strategy avoids direct incidence of sunlight on the film and keeps the plastic tight to the silage. By keeping the plastic cover weighed down, the oxygen penetration in the peripheral areas can be reduced during the unloading phase, and this may avoid losses caused by aerobic microorganisms (Bernardes et al. 2018b).

***Additives*** In hot climates, cereal silages are even more likely to deteriorate because aerobic yeasts are more active at high temperatures. Chemical or microbial additives with antifungal capacity applied at appropriate dosages can reduce aerobic spoilage, as has also been reported for silages in cold

areas. Among chemical additives, salts of weak acids (e.g., sodium benzoate) have proven benefits on aerobic stability, if either applied onto the total forage mass (Bernardes et al. 2015a) or to the top layer before sealing (Da Silva et al. 2014).

In Brazil, approximately one-fourth of the farmers use inoculants (Bernardes et al. 2012, Novinski 2013, Bernardes and Do Rêgo 2014). Several studies have shown that inoculants containing heterolactic bacteria (e.g., *L. buchneri*) can decrease the aerobic deterioration of silages (Queiroz et al. 2013). However, when bacterial inoculants are used in warmer environments, care should be taken with the temperature of the applicator tank and the length of time that the inoculant solution is held in the tank. In the field, inoculant tanks may absorb heat from the harvester and solar radiation. In Argentina, for instance, temperatures up to 44°C have been observed in inoculant-water mixes, measured in applicator tanks (Oscar Queiroz, personal communication). Mulrooney and Kung (2008) reported that the viability of several microbial inoculants was markedly decreased when the temperature of the solution increased above 35°C. Recently, Windle and Kung (2016) showed a negative correlation between time in the applicator tank and loss of inoculant viability in the field. Placing tanks to minimize absorption of heat from machines and the use of thermal insulation materials to protect the tank should be considered by the silage machinery industry worldwide. Additionally, refilling the applicator tank with a higher frequency (e.g., twice daily) will reduce the amount of time spent in the tank and help to achieve the proper amount of viable organisms that must be applied to the forage.

***Unloading*** Losses during unloading depend on silage density, aerobic stability (i.e., fermentation end-products), and feed-out rate, which determines the duration that the silage is exposed to air (Muck et al. 2003). The recommended minimum density of silages is 705 kg/m$^3$ (as-fed basis), with a porosity of less than 0.4 (Holmes 2009). Aerobic stability can be prolonged by application of additives with antifungal capacity. Meanwhile, the unloading rate has been based on linear feed-out (cm/d or m/week), which was developed through empirical observations (Borreani and Tabacco 2012) or mathematical models (Pitt and Muck 1993). However, linear feed-out may fail since silage densities vary among silos. Thus, researchers located in tropical (Brazil) and temperate (Italy) areas studied an unloading rate based on daily silage consumption per face area (De Oliveira et al. 2018). They concluded that a daily removal rate of 250−375 kg of silage/m$^2$ reduced the risk of spoilage in corn silages. Removing more than 375 kg of silage/m$^2$ per day can prevent unspoiled silage in the ration (De Oliveira et al. 2018).

***Nutritive value*** The composition of the harvested crop has a great impact on the amount and type of nutrients available for animals. Because fiber and starch are the main fractions in corn crop, under good management, corn silages with higher content of starch and greater NDF digestibility are nutritionally superior. However, the feeding value of corn silage is further affected by kernel processing and fermentation pattern.

Although corn silage is the most energy dense forage source used in dairy and beef rations in tropical areas, corn silage produced in hot areas is sometimes poorer in quality than corn silage produced in cold areas. Adesogan et al. (2010) observed that corn silage produced in Florida/USA had a greater concentration of fiber and lower content of starch than corn silage samples from New York/USA. Crasta and Cox (1996) reported that corn cultivated at a warmer location had lower DM digestibility compared with corn produced at a cool location. The influence of high temperature on cell wall deposition and lignification is well established (Moore and Jung 2001). Beyond the negative effect of temperature on fiber digestibility, high temperature (> 30°C) may also decrease grain and total DM yield (Schlenker and Roberts 2009). Rationally, in hot weather, lower grain yields might result in lower starch concentration. A summary of corn silage samples from Brazil and USA, analyzed by the 3rlab/Rock River laboratory, is shown in Table 1. Overall, Brazilian samples had more NDF and less starch. Digestibility of NDF and starch measured by laboratory methods was approximately 10% lower in samples from Brazil. Additionally, Brazilian samples had a lower lactic acid:acetic acid ratio due to the greater content of acetic acid.

**Table 1**. Composition of corn silage (mean ± SD) sampled in Brazil and United States in 2017 (% DM, unless otherwise stated).

| Item | Brazil | USA |
|---|---|---|
| NDF | 42.8 ± 6.0 | 37.9 ± 6.0 |
| Total-tract NDF digestibility, % | 38.8 ± 5.4 | 43.0 ± 5.3 |
| Starch | 30.4 ± 6.6 | 33.8 ± 6.8 |
| In vitro starch digestibility-7 h, % | 75.7 ± 8.7 | 78.5 ± 10.3 |
| pH | 3.93 ± 0.17 | 4.00 ± 0.23 |
| Lactic acid | 3.62 ± 1.39 | 3.24 ± 1.76 |
| Acetic acid | 2.26 ± 0.91 | 1.56 ± 1.03 |
| Lactic:Acetic ratio | 1.60 | 2.08 |

Source: 3rlab® / Rock River®

A comparison of corn silages produced in two Brazilian states (Paraná and São Paulo), analyzed by the ESALQLab, is presented in Table 2. Overall, Paraná state has a milder weather and higher altitudes, whereas São Paulo state has a warmer climate. On average, corn silages sampled in São Paulo had lower starch and higher NDF, although NDF digestibility did not differ substantially. Of note, there was a wide variation in both USA and Brazilian data sets (Tables 1 and 2), suggesting that several factors such as altitude and location, soil type and nutrient status, agronomic and ensiling management, and other climatic attributes have great impacts on the final quality of the silage. Hence, it is possible to produce high-quality corn silages in tropical areas.

**Table 2**. Starch, NDF and NDF digestibility (mean ± SD) in corn silages from Paraná and São Paulo farms

| Item | São Paulo State | Paraná State |
|---|---|---|
| Starch, % DM | 25.0 ± 7.1 | 29.5 ± 5.0 |
| NDF, % DM | 45.9 ± 5.6 | 42.5 ± 5.0 |
| In vitro NDF digestibility-30 h, % | 49.8 ± 4.5 | 50.3 ± 3.4 |

Source: ESALQLab - Department of Animal Science, University of São Paulo (March, 2018).

In addition to the chemical composition, corn endosperm hardness may affect the nutritive value of corn silage. Philippeau and Michalet-Doureau (1997) observed that increased kernel vitreousness was associated with decreased ruminal starch degradation in unfermented grains. Corn hybrids grown in Brazil predominantly have a greater proportion of vitreous endosperm (Correa et al. 2002, ABIMILHO 2018). Therefore, kernel processing is paramount to improve the nutritive value of whole-plant corn silage. In the USA, Ferraretto and Shaver (2012) carried out a meta-analysis on the effect of corn silage kernel processing and reported an increase of 1.7 kg of fat-corrected milk (per cow per day) when the cracker was set at 1 to 3 mm versus 4 to 8 mm of roll clearance. In Brazil, Salvati et al. (2017) examined the effects of kernel processing and length of cut on the nutritive value of flint corn silage for lactating dairy cows. They contrasted a pull-type forage harvester without a kernel processor set for a 6-mm length of cut and a self-propelled forage harvester with a kernel processor (1-mm roll clearance) set either for 6-mm, 12-mm or 18-mm length of cut. Compared with the pull-type harvester, the self-propelled harvester set for 6-mm or 12-mm length of cut increased milk yield by 1.2 kg/d (29.4 vs. 28.2 kg/d), due to higher starch digestibility, as suggested by the lower content of fecal starch. Corn silage harvested with 18-mm length of cut tended to reduce milk yield (28.6 kg/d).

Although kernel processing improves starch availability, most Brazilian farmers use pull-type harvesters without kernel processors (Bernardes and Rêgo 2014), which would at least partially explain the lower starch digestibility reported in Table 1. However, when corn is ensiled (whole plant or grain), the decline in starch digestibility associated with vitreousness endosperm is attenuated during storage (Macken et al. 2003, Benton et al. 2005). Correa et al. (2003) did not observe any differences in starch digestibility, DM intake and milk yield in dairy cows fed diets containing flint versus dent whole-plant corn silages. Bueno et al. (2017) examined the effect of maturity (30 vs. 40% of DM) and length of storage (0 to 180 d) on the fermentative losses and in situ degradability of corn silage harvested with a pull-type forage harvester without a grain processor. Flint corn silage harvested at 30% of DM had higher DM loss during storage, compared with silage harvested at 40% of DM. On the

other hand, early-harvested plants had higher ruminal disappearance of starch, crude protein (CP) and NDF. Length of storage had minimal influence on NDF digestibility but increased ruminal disappearance of CP up to 30 d of storage. Differences in ruminal disappearance of DM and starch were large in unfermented forage but smaller or null in silages fermented for long periods. Contrary to the traditional statement "*…ensiling can only preserve those nutrients already present in crops, it does not improve the feeding value of a crop…*", storage for longer periods before feeding (e.g., 90 d) is a strategy to improve corn silage digestibility, especially if flint corn is harvested with a pull-type machine, at higher DM content. Since starch and NDF are major nutrients in corn silage, and considering that starch digestibility typically increases during ensiling (Daniel et al. 2015) and that kernels can be processed at harvesting (Salvati et al. 2017), starch content and NDF digestibility appear to be more important than endosperm vitreousness among nutritional traits for selecting corn hybrids for silage production (Carvalho and Carbonare 2017).

In addition to differences in fiber and starch fractions, corn silage produced in warm climates often undergoes a more heterolactic fermentation (Kim and Adesogan 2006, Daniel et al. 2018a). Heterolactic fermentations often lead to higher DM losses during ensiling but are usually associated with improved aerobic stability because of their greater concentration of antifungal compounds, such as acetic acid (see Table 1). Nonetheless, under hot conditions, silages are more prone to aerobic spoilage (Ashbell et al. 2002, Koc et al. 2009, Zhou et al. 2016) despite spontaneous heterolactic fermentations. This is because high temperatures are often more conducive to the growth of spoilage organisms. Wang and Nishino (2013) examined the effects of storage temperatures on fermentation and aerobic stability of TMR silages. The experimental silos were stored at 5, 15, 25 and 35°C for 10, 30 and 90 d. Afterwards, silages were exposed to air for 7 d at 25°C or the same storage temperature. Silage stored at 5°C fermented slowly, but acceptable lactic acid concentration (>4% of DM) was observed on d-90. The ethanol content was higher than the acetic acid content when silages were stored at 15 and 25°C, whereas the ethanol content was lower when stored at 35 than at 25°C. Aerobic deterioration did not occur when silage was exposed to air at the same temperature at which it was stored. Although silages stored for 10 d at 5 and 15°C deteriorated when the aerobic stability test was conducted at 25°C, heating was not observed in silages stored at 25 or 35°C or in any silage fermented during 90 d, regardless of storage and air exposure temperatures. Therefore, aerobic deterioration appears to be exacerbated in silages fermented during the winter and fed during the summer, especially if fermented by short periods (< 90 d). Fortunately, most farmers make silage during the summer for feeding during the winter or year round. Storing corn silages for longer periods (e.g., 90 d) may also improve the aerobic stability (Daniel et al. 2015).

It is commonly observed that silages with heterofermentative patterns have more complex headspace profiles than silages dominated by lactic acid fermentation (Kristensen et al. 2010), leading to a debate among researchers and field consultants on the possible negative effects of silages with high concentrations of volatile compounds (e.g., acids, alcohols and esters) on the performance of dairy cows (Raun and Kristensen 2010; Weiss et al. 2016). Kleinshmitt et al. (2013) demonstrated that silages inoculated with high doses of *L. buchneri* (≥ $5 \times 10^5$ cfu/g) and stored in farm-scale silos during the summer had high concentrations of acetic acid (i.e., > 4% of DM) and depressed the DM intake and milk yield of lactating dairy cows. Of course, the negative effect of acetic acid on feed intake will depend on its concentration in silage and the amount of silage in the ration. A dietary content of acetic acid higher than 1.73% of DM appears to have a greater impairment on DM intake (Daniel et al. 2018b).

**Grain silages**

***High moisture corn and reconstituted corn grain silage*** The Brazilian production of corn grain for the 2017/18 harvest is estimated to be 87.3 million tons (ABIMILHO 2018), distributed between the first (25.1 million of tons) and the second crop (62.2 million of tons). In the last decade, Brazil was responsible for approximately 8% of world corn production (CONAB 2018). The large supply makes corn the main cereal fed to animals in South America. This scenario favors the use of high-concentrate diets for finishing beef cattle in feedlots. Because net energy cost is frequently lower for

grains than forages, high-grain diets often lead to least costly weight gain ($/weight gain) in feedlots. In recent surveys, the average content of concentrates in finishing diets was approximately 80% (Pinto and Millen 2016).

In Brazil, grain silage technology began in the 1980s, with few farmers using HMC for feeding pigs and dairy cattle. Currently, ensiled grains have expanded significantly as a method of harvesting, storing and processing. Due to the predominance of flint hybrids in Brazil, ensiling grains with high moisture content is an effective processing method to improve corn nutritive value and feed efficiency in both beef and dairy cattle. Fernandes (2014) ensiled flint and dent corn grains harvested at different maturities. Although kernel vitreousness and maturity affected starch digestibility prior to ensiling, no differences were observed in starch digestibility after 60 d of storage. Junges et al. (2017) demonstrated that bacterial activity was the main contributor to proteolysis (60%) during fermentation of reconstituted corn grain silages. Hence, ensiling grains with high moisture is an effective method of reducing prolamin concentration and increasing starch digestibility (Fernandes, 2014).

Besides the nutritional benefit, farmers have highlighted additional advantages. Compared to drying, ensiling has lower storage costs. Insect and rodent damages typically observed in dry grains are virtually eliminated in silages. Lower field losses accompanied by early harvesting are also recognized to increase farming efficiency, allowing a larger window for planting subsequent crops (second maize crop or winter crops). For reconstituted corn grain silage, there is the possibility of purchasing corn at favorable price periods.

In the last decade, the rehydration and ensilage of mature dry grains have been adopted in dairy and beef operations. This technology gained popularity mainly due to the obstacles commonly observed for HMC. Harvesting and ensiling corn grain with original moisture require machine availability and high labor during the short harvesting window (often less than a week). Recent studies have devoted efforts to fine-tuning reconstituted corn grain silages. For instance, among feedlot nutritionists, there is no consensus on particle size of dry ground corn (Pinto and Millen, 2016) and reconstituted corn grain silage. Gomes et al. (2018) evaluated the effects of processing (grounding to pass a 5-mm screen or rolling), moisture content (30, 35 or 40%) and length of storage (0 to 180 d) on ruminal in situ degradation of reconstituted corn grain silage. They hypothesized that higher moisture and longer storage periods would at least partially offset the effect of particle size in reconstituted corn grain silage. As expected, ruminal degradability increased with the fermentation progress in all the treatments, especially during the first 60 d of storage. In both rolled and ground grains, 35% and 40% moisture led to higher ruminal degradation than did 30% moisture. Nonetheless, contrary to the hypothesis, rolled corn did not achieve the level of ruminal degradability of ground corn regardless of the moisture content and storage period. The authors concluded that ground grain might be used more efficiently by animals, but the decision on rolling or grinding before ensiling will depend on the animal physiological status and diet composition (e.g., starch and effective fiber contents). In both cases, they recommended storing grain silages for at least 2 months before feeding.

Da Silva (2015) examined the effect of particle size (ground to pass a 2- or 6-mm sieve) on the nutritive value of reconstituted corn grain silage for finishing young bulls. There was no effect of particle size on average daily weight gain (1.53 kg/d), whereas ensiled grain decreased DM intake, fecal starch concentration and increased feed efficiency by 16%. Overall, rehydrated and ensiled grain increased feedlot profit in comparison with dry grain. Since both particle sizes were relatively small (2- or 6-mm sieve), further studies are warranted to optimize particle size of reconstituted corn grain silage for finishing cattle fed high-concentrate diets. For dairy cows, Castro (2017) evaluated the effect of particle size (3- or 9-mm screen) and dietary level of reconstituted corn grain silage (29.2 vs 23.5% of starch). Treatments had no effect on milk yield (31.0 kg/d), whereas fine grinding tended to decrease DM intake and increase feed efficiency when the high starch diet was fed. The authors concluded that coarse grinding reduced the ruminal acidogenic capacity of reconstituted corn grain silage and increased the grinding rate (3.9 to 11.7 ton/h), decreasing labor and energy during kernel processing.

***Snaplage*** Various terms are used to describe the different products from corn plants depending on which plant parts are present as well as the harvest method employed (Lardy and Anderson 2010).

Snaplage contains corn grain, cobs, husks and shank, harvested and processed with a self-propelled forager equipped with a corn snapper head. In terms of morphological composition, it diverges from earlage (grain + cobs), mainly by the presence of husks. Moreover, earlage requires at least two separate operations (harvesting and grinding), whereas snaplage allows harvesting and processing the whole corn ear in a unique operation, with large logistic advantages. Due to the presence of cobs and husks (approx. 20%, DM basis), snaplage has significant amount of NDF (20 to 25% of DM) and starch (50% of DM). With approximately 80% of total-digestible nutrients, snaplage is a convenient feedstuff, especially in diets for finishing beef cattle ('built-in-roughage'). Furthermore, harvesting snaplage instead of HMC plus whole-plant corn silage permits an increase in stocking rate by ~10% in a comparable area.

At harvesting, cobs and husks are moister than grain; therefore, the moisture content of snaplage is typically 5 to 8-percentage units higher than that of grain, depending on the corn hybrid and environment. As in other silages, maturity/moisture is critical in snaplage. In conventional hybrids, NDF digestibility of snaplage is relatively high (compared with corn stalk) but tends to decrease when harvest is delayed (Soderlund et al. 2006). Moreover, harvesting snaplage with low moisture might lead to low-quality silage and compromise the conservation process because the restricted fermentation will decrease the load of fermentation products capable of protecting the mass against spoilage. On the other hand, harvesting snaplage with higher moisture (e.g., 37 to 45% of moisture) might benefit the fermentation process and the nutritive value without sacrificing yield. Higher moisture assists with grain processing at harvesting, increases the breakdown of the endosperm-protein matrix during fermentation, and ultimately enhances starch digestibility in the animal. Lastly, early harvesting compared with dry corn grain is an option widely welcomed by corn growers, custom harvesters, and cattle feeders.

In some farms, snaplage has been haunted by a bad reputation associated with excessively late harvests, poorly adjusted equipment, and poor silage-making practices. Planning and following good ensiling practices might result in high-quality snaplage.

***Sorghum grain silage*** Sorghum grain silage is also used in ruminant diets in tropical areas. Compared to corn, the amount of sorghum produced in Brazil is small (2.14 million tons in 2017). However, sorghum has been a good option for second crop where there is limited rainfall or greater risks for the corn crop. Traditionally, sorghum is a cereal with lower commercial value compared to corn and has been an economical option during high corn prices. Compared to other cereals, sorghum grain has a lower digestibility. Therefore, ensiling sorghum grain is a strategy to improve its nutritive value.

Ensiling sorghum with original moisture (high moisture sorghum) has been scarce due to the non-uniform maturity of grain across the panicle. Consequently, reconstituted sorghum grain silage has been adopted. Schonell (2016) demonstrated that additives with antifungal capacity improved the conservation of reconstituted sorghum grain for dairy cows (~32 kg of milk per day). Compared with dry grain, ensiled grain decreased the content of fecal starch and increased feed efficiency by 7%. Passini et al. (2002) evaluated the performance of finishing steers fed diets containing sorghum grain silage or ground corn. They did not find differences in animal performance but reported a higher dressing percentage and higher fat content in the meat of bulls fed sorghum grain silage.

***Additives for grain silages*** To exploit the benefits of grain silages, proper management is mandatory to minimize fermentative losses and prevent aerobic deterioration. A number of studies have assessed the effects of silage additives on high moisture grain silages. Morais et al. (2017) conducted a review on the effects of chemical and microbial additives on the conservation of grain silages. They concluded that the control of fermentative losses is not a big concern in properly made, high moisture grain silages. However, the use of additives is justified to improve the aerobic stability. Additives based on chemicals (e.g., weak acids) or heterofermentative bacteria have proven to be effective in preventing aerobic deterioration at the same magnitude. Aerobic stability linearly increased with the application rate of chemical additives, whereas the response to heterofermentative inoculants was modeled with a broken-line regression, whose optimal dose was $4.7 \times 10^5$ cfu/g.

As in high moisture grain silages, aerobic stability is also a concern in snaplage. Jobim et al. (1999) observed higher counts of yeasts and molds during the aerobic exposure of snaplage compared with HMC. Recently, Santos et al. (2018) examined the effects of *L. buchneri* ($4 \times 10^5$ cfu/g) and maturity (32% and 37% of moisture) on the conservation and ruminal degradability of snaplage and HMC. As expected, *L. buchneri* substantially improved the aerobic stability of HMC (32% or 37% of moisture) and snaplage with 37% of moisture. There were no differences in fermentative losses, aerobic stability and ruminal degradability between HMC ensiled with 32% or 37% of moisture, whereas harvesting HMC with 32% of moisture would be more desirable for grain threshing in the field. Snaplage harvested with 32% of moisture (grain fraction with ~25% moisture) had lower DM degradability, and it was unresponsive to inoculate application. Hence, harvesting snaplage with lower moisture is not recommended.

**Sorghum silage**

Sorghum is a crop adapted to environments with high temperatures, limited rainfall and soil fertility (Mann et al., 1983). Sorghum has been used as an alternative crop in marginal lands, especially for the second crop or even as a summer crop in areas susceptible to drought. Ensiling sorghum is quite similar to making corn silage. However, sorghum silage typically has a lower nutritive value than corn silage (Bolsen et al., 2003).

Often, sorghum production coincides with the adoption of fewer technological resources. Additionally, most farmers have focused on taller varieties (up to 4 m height) with high DM yields and low grain proportions. Taller varieties are more susceptible to lodging, which makes harvesting difficult and increases field losses. Thus, dual-purpose varieties are preferred due to a more favorable grain:stover ratio, suitable DM yield and lodging resistance. A further challenge with sorghum crop is bird damage during grain ripening (Duncan, 1980). Although sorghum varieties with higher tannin content are supposed to repel bird attack it has not been sufficient to prevent damage from high bird populations.

Grain processing is another issue of sorghum silage, especially if harvested with pull-type machines without a kernel processor. Compared with corn, sorghum kernels are more difficult to disrupt during harvesting, mainly due to the small size (approx. 10 times smaller than corn kernels). Then, a higher percentage of grains pass through the animal undigested. Using forage harvesters with a grain processing device is a strategy to improve sorghum silage digestibility. Recently, in Argentina, Abdelhadi and DiLorenzo (2017) reported that harvesting sorghum crop with a self-propelled harvester equipped with either a sorghum cracker or multicrop Shredlage processors was efficient to improve kernel processing score, and consequently, starch and organic matter digestibility of sorghum silage. Sowing sorghum genotypes with BMR genes (brown-midrib) is another option to improve silage nutritive value (Oliver et al. 2004). Even though the reduction in lignin concentration with BMR mutants brought an opportunity to improve the quality of forage sorghum, this also carried negative effects that have limited their acceptance, such as poorer plant health, lodging, and, especially, reduced DM yield (Pedersen et al. 2005).

Beyond the conventional varieties, sweet sorghum has appeared in the market for both biofuel (i.e., ethanol) and silage production. Due to the surplus of soluble sugars, sweet sorghum silage conserved without additives has higher DM losses due to the alcoholic fermentation performed by yeasts (Behling Neto 2017). Therefore, silage additives capable of inhibiting yeast activity are required to preserve sweet sorghum efficiently, as discussed for sugarcane silage (please see below).

Currently, a disadvantage of sorghum compared with corn is the lack of genetically engineered hybrids, for instance, tolerant to insects, diseases and selective herbicides.

**Tropical grass silage**

Tropical grasses have huge potential as a silage source in hot climates because of their high yield (e.g., 20 to 30 t DM/ha). Most of the yield of warm-season C4 grasses occurs during the rainy season, so ensilage is a key tool to improve their use across the year. Similar to temperate grasses, tropical

grasses have high moisture content if harvested with suitable nutritive value. Hence, wilting or application of additives is often needed to reduce the risk of butyric fermentation and effluent production (Nussio 2005). For instance, effluent volumes as high as 250 L/t, with a great biochemical oxygen demand, have been reported for direct-cut tropical grass silage (Loures et al. 2005). Although wilting is a feasible strategy to decrease moisture content in short and thin stemmed crops, several species of tropical grasses utilized for silage are tall, have thick stems, and are difficult to wilt (e.g., *Panicum maximum*, *Pennisetum purpureum*). Moreover, wilting tropical grasses increase field losses. Igarasi (2002) found a direct-cut loss of 5.3% of the total available forage (*Panicum maximum* cv. Tanzania) in summer. Wilting the forage for 5 h increased harvesting losses to 20%, thereby calling into question the benefits of wilting when the whole system is rationalized. Thus far, an ensiling system based on wilting is more susceptible to unfavorable harvesting conditions.

Ensiling grasses with dry feedstuffs can produce silages with minimal leachate, higher intake potential, and improved DM digestibility, depending on the absorbent selected (e.g., citrus pulp, soybean hulls, wheat bran, ground corn, ground sorghum). However, in regions where dry byproduct and concentrate feedstuffs are scarce or expensive (i.e., imported from other regions), this technique has been questioned. An additional issue with this strategy is the difficulty in mixing the absorbent onto the forage, although it has been used in large feed operations in the central-west region of Brazil. When absorbents are not feasible, farmers have relied on silage additives. Most additives sold in Brazilian market are based on lactic acid bacteria. Unfortunately, the effects of bacterial inoculants in wet tropical grass silages have been inconsistent (Mühlbach 2000, Coan et al. 2001, Tomaz et al. 2018).

Tomaz et al. (2018) compared several canopy heights on the ensilability of Guinea grass (*Panicum maximum* cv. Mombaça). Harvesting Guinea grass when it reached 130 cm in height resulted in the highest fermentability coefficient (FC = 31). Interesting, this height differed from that recommend for grazing (i.e., 90 cm) based on 95% interception of the incident light (Da Silva et al. 2009), suggesting that sward managed for ensilage changes the canopy structure. Ensiling Guinea grass alone or treated with bacterial (homo or heterofermentative) inoculants produced low-quality silages, with a very high content of butyric acid (i.e., 50 g/kg DM) and great effluent volumes (i.e., 50 kg/t). Mixing 100 kg of dry citrus pulp per ton of forage was the sole strategy capable of reducing butyric acid, fermentative loss and effluent production.

In addition to the problems related previously, smallholder ranchers lack information on animals' nutrient requirements and feed management practices. To overcome these issues, TMR silage combining fresh elephant grass with concentrate ingredients was assessed by Gusmão et al. (2018). The proportional inclusion of ingredients was defined to meet the nutrient requirements of lactating cows yielding approximately 15 kg of milk per day. They concluded that TMR silages were adequately preserved, and the protein source (soybean meal) did not negatively affect the fermentation process, whereas dried citrus pulp showed better results in terms of fermentation profile and effluent production than dry ground shelled corn (Gusmão et al. 2018).

**Sugarcane silage**

Sugarcane is a semi-perennial grass notorious for its high DM yield potential (e.g., 40 to 50 t DM/ha). Last year, Brazilian mills processed approximately 650 million tons of sugarcane stalks to produce sugar, ethanol and electricity (CONAB 2018). However, the utilization of sugarcane for feeding animals (silage or fresh) has been modest (~1.5% of the total area) (L. Nussio, unpublished data). Although the maximum nutritive value of sugarcane matches pasture shortage (dry season), sugarcane has been ensiled to prolong field lifespan (by uniform harvesting and better agronomic management), to prevent the occurrence of lodging, and avoid daily harvesting (logistics benefits). Due to its high content of soluble carbohydrates and large yeast population (Ávila et al. 2010), ensiling sugarcane without additives results in a substantial conversion of soluble sugars into ethanol (Daniel et al. 2013c). As in sugar beet silage, ethanol concentrations above 20% of DM are often found in sugarcane silage (Rabelo et al. 2016). Therefore, sugarcane conserved by natural fermentation loses considerable amounts of DM and net energy (Daniel and Nussio 2011). Although ethanol has a higher content of gross energy than carbohydrates (7.1 vs. 4.2 Mcal/kg), replacing carbohydrates with

ethanol has led to similar or even lower feed efficiency, without effecting DM intake (Randby et al. 1999, Daniel et al. 2013a). Ethanol is partially volatilized from silage (Daniel et al., 2013a) and partially converted into acetate and methane in the rumen (Yoshii et al. 2005). Hence, alcoholic fermentation is undesirable. Several types of additives have been examined in sugarcane silage. In brief, when chemical or microbial additives with a powerful antifungal capacity (e.g., sodium benzoate, potassium sorbate, *L. buchneri*, *L. kefiri*, *L. hilgardii*) are applied at appropriate dosages (e.g. 1 to 2 g/kg as fed of sodium benzoate), most of the soluble carbohydrates are preserved and silage nutritive value is comparable to that of freshly-chopped sugarcane (Queiroz et al. 2008). Although calcium oxide is capable of improving DM recovery and aerobic stability, it worsens the hygienic quality of sugarcane silage. Due to its alkaline nature, calcium oxide raises forage and silage pH and markedly increases enterobacterium and clostridium counts, ammonia and butyric acid concentrations (Custódio et al. 2016, Jacovaci et al. 2017). Consequently, treating sugarcane silage with calcium oxide alone might not be recommended.

Sugarcane silage is composed of three main fractions: soluble carbohydrates (mainly sucrose), fermentation end-products, and NDF. Crude protein, ether extract and ash together comprise less than 7% of DM. Since the true digestibility of the soluble fraction (soluble carbohydrates + fermentation end-products) is close to the unit, the nutritive value of sugarcane is primarily a function of the concentration and digestibility of NDF. Compared to other crops, sugarcane NDF digestibility is low (< 35%) (Oliveira et al. 2011), due to the higher proportion of indigestible NDF (~50% of NDF) and lower digestibility of the potentially digestible NDF (Daniel et al. 2013b). Puzzling, sugarcane has a relatively low proportion of lignin in NDF, but the negative impact of lignin on fiber digestibility is more pronounced in sugarcane than in other grasses. A high indigestible NDF to lignin ratio (4.55 on average) has been found in commercial genotypes of sugarcane (Daniel et al. 2017). Sugarcane fiber tends to have a high ruminal retention time and physical fill capacity, which accounts for the higher physical effectiveness of sugarcane NDF (Sá Neto et al. 2014) and lower DM intake of sugarcane based diets (Correa et al. 2003, Oliveira et al. 2011) .

Two strategies have been used to overcome the low NDFD of sugarcane silage. Chopping sugarcane into fine particles not only benefits the packing and preservation of silage, but it also decreases the sorting of TMR in the feedbunk. Moreover, sugarcane NDF retains its high physical effectiveness, even when it has a short particle length (Campos, 2015). Another strategy for improving the nutritive value of sugarcane silage-based rations is to increase the proportion of concentrates in the diet (Sá Neto et al. 2014). A plausible strategy is partially replacing sugarcane silage with byproducts. Although it has been seldom used in large feedlots (Pinto and Millen, 2016), sugarcane silage is a forage source with large potential for beef operations. Considering its high physical effectiveness, a low dietary inclusion is enough to meet the requirements for physically effective fiber. Additionally, silage moisture assists in bunk management, reducing dust and fines. Hence, a very high stocking rate is feasible in feedyards that use sugarcane silage as a roughage source.

**Wet byproducts**

Wet byproducts are available worldwide, including in tropical zones. Several wet byproducts have suitable nutritive value (e.g., brewers grains, distillers grain, corn gluten feed, okara), but because of the high and variable moisture content, many wet feedstuffs have a very short shelf life if appropriate storage conditions are not used. Compared with the drying process, ensiling wet feedstuffs would be economically efficient. Nonetheless, several wet byproducts are difficult or moderately difficult to ensile and, currently, relatively little effort has been devoted to understanding wet byproduct preservation. Thus, most wet byproducts are still stored unprotected on the ground, for short periods, limiting their utilization in many livestock operations. Based on the extensive review by Orosz and Davies (2015), chemical additives (e.g., 4 L/t) containing mixtures of acids and salts (e.g., formic, propionic, sorbic, benzoic) might improve long-term anaerobic storage and prevent aerobic deterioration after feedout. Storage of wet byproducts in plastic tubes (ag-bags) using a delivery truck bagger, which transports and dumps (bagging) the byproduct, has been successfully used mainly in Europe. So far, it is not popular in South America and represents a good opportunity for agro-industries. Total mixed ration

(TMR) silage is another strategy for storing and feeding wet byproducts. In Asia and Israel, ensiling TMR containing wet byproducts has been a common practice (Weinberg et al. 2011, Wang and Nishino 2013). Beyond the high aerobic stability, TMR silage permits the use of less palatable feedstuffs and provides a balanced and uniform feed, which would be very attractive for small farmers. Meanwhile, mixing wet corn byproducts with lower quality dry forages (e.g., hay, straws and stalks) before ensiling has been recommended in USA (Erickson et al. 2008). In many cases, this mixture of forage and wet byproduct may serve as the entire roughage source for feedlots to use in their finishing rations. In Brazil, Tres (2015) conserved okara, a byproduct of soy milk factories, by ensiling mixtures of dry ground corn and okara. When fed to lactating cows, corn grain ensiled with 30% of okara was comparable to a dry concentrate based on corn and soybean meal; thus it is a good option for small dairy farmers.

**Silage pathogenicity**

Silage quality goes beyond its proximal composition. If prerequisites for the desired fermentation are not met or if silage management is inappropriate, undesirable microorganisms may multiply and eventually lead to the accumulation of harmful products (e.g., toxins, amines). Despite its importance, this subject has been little studied in the tropics, and few data are available on pathogenicity and risks of silages for humans and animals. As reported for temperate grass silages, clostridium develops in tropical silages of wet grasses and legumes, resulting in high concentrations of butyric acid and ammonia, great nutrient loss, and lower feeding value (please see the topic 'Tropical grass silage'). Sugarcane silage treated with calcium oxide also undergoes clostridial fermentation due to the alkaline nature of calcium oxide (please see the topic 'Sugarcane silage'). In August 2017, an outbreak of botulism occurred in a beef feedlot in Brazilian Savanna. Approximately 1.1 thousand animals perished, and their carcasses were burned. Among the samples collected for laboratory tests, botulinum toxins were only confirmed in corn grain silage (IAGRO 2017). How *Clostridium botulinum* developed in that silage remains a matter of debate, but certainly, poor ensiling practices were involved. The development of clostridia in micro-niches within spoiled spots is a plausible possibility (Pahlow et al. 2003).

Mold development is another issue in silage pathogenicity, notoriously because they produce mycotoxins. Mycotoxins can be derived from the field or formed during ensilage. In 2015, Schmidt et al. carried out a survey on the occurrence of mycotoxins in whole-crop corn silage (n = 327 samples) sampled in Brazilian dairy farms. Mycotoxins were found in more than 91% of the samples, with zearalenone being the most prevalent (72.8%). The incidence of aflatoxin B1 was low (0.92%). The authors concluded that the pre-harvest field phase is the main source of mycotoxins in corn silage and that incidence of mycotoxins in Brazilian corn silages seems greater than that reported in Europe (e.g., Driehuis et al. 2008). Since the warm climate can predispose silage to spoilage and mycotoxin incidence, further studies are warranted to monitor mycotoxins in tropical silages.

**Environmental issues of silage production and utilization in tropical areas**

Emissions of volatile organic compounds (VOC) and effluent production are the main environmental issues directly associated with silage. Other concerns include on-farm burning of fossil fuels and emissions from fertilizers. Mitigating the emission of carbon dioxide from silage is key to prevent nutrient loss, but it does not affect its balance in the atmosphere, because when plants are grown they absorb carbon dioxide, which is then released during biomass oxidation (net emission is zero).

Several studies have reported the importance of silage effluent as a pollutant and its control (please see the topic 'Tropical grass silage'). On the other hand, few studies were performed on the emissions of VOC in tropical areas. Not long ago, Daniel et al. (2015b, 2015c, and unpublished data) measured the emissions of acetic acid and ethanol (main VOC in silages) from whole-plant corn and sugarcane silages in farm-scale silos. In those experiments, emissions were affected by ambient temperature, time elapsed after panel defacing, silo layer, and use of additives. Loss of VOC from the silo working face increased during the day but decreased at night, suggesting that higher temperatures had a

greater influence on VOC emission than the time elapsed after panel defacing. In corn silage, the bottom layer of a bunker silo (3 m deep) released 50% more acetic acid to the atmosphere than the top layer (0.5 m deep). Inoculation with *L. buchneri* increased the emission of acetic acid in both corn and sugarcane silages. In sugarcane silages, *L. buchneri* decreased ethanol emission by 54%, whereas sodium benzoate decreased it by 81%. In both silages, emission rates of ethanol and acetic acid were positively correlated with their concentrations in silage. Therefore, preventing the excessive formation of VOC is a strategy for mitigating VOC emissions.

**Final remarks**

Historically, much knowledge on silage making in the tropics has been transferred from traditional centers located in temperate zones. However, in recent decades, there has been significant scientific progress on tropical silages. Due to high temperatures, tropical crops have a large productive potential during the rainy season. Hence, silage has a central role in feed preservation. In the meantime, warmer climate increases the risk of silage spoliation. Therefore, silage quality has been variable, depending on location and adoption of technological resources. Policies that prioritize extension services might help farmers obtain high-quality silages in the tropics. More research on the effects of tropical silages on the environment and food safety are welcome.

**References**

Abdelhadi, L.O. & DiLorenzo, N. (2017) Shredlage technology: a key to expanding grain sorghum silage, In: Nussio, L.G., Sousa, D.O., Gritti, V.C., Salvati, G.G.S., Santos, W.P. & Salvo, P.A.R. (eds). Proceedings of the V International Symposium on Forage Quality and Conservation, Piracicaba, Brazil, 2017, pp. 107-119.

ABIMILHO. Brazilian Association of Corn Milling Industry. Accessed March 20: https://www.abimilho.com.br [website in Portuguese].

Adesogan, A.T. (2010) Corn silage quality in tropical climates, in Proceedings of the 5th Symposium on strategic management of pasture, Federal University of Viçosa, Viçosa, Brazil, pp. 311-327.

Ashbell, G., Weinberg, Z.G., Hen, Y. & Filya, I. (2002) The effects of temperature on the aerobic stability of wheat and corn silages, Journal of Industrial Microbiology and Biotechnology, 28, 261-263.

Ávila, C.L.S., Bravo Martins, C.E.C., & Schwan, R.F. (2010) Identification and characterization of yeasts in sugarcane silages, Journal of Applied Microbiology, 109, 1677-1686.

Bastos, M., Lima, L.M., Gusmão, J., Cardoso, M., Chiarini, T., Avila, C. & Bernardes T.F. (2018) A survey of maize hybrids for whole-plant silage in a hot climate, In: Proceedings of the XVIII International Silage Conference, Bonn, Germany.

Behling Neto, A., Reis, R.H.P., Cabral, L.S., Abreu, J.G., Sousa, D.P., Pedreira, B.C., Mombach, M.A., Balbinot, E., Carvalho, P. & Carvalho, A.P.S. (2017) Fermentation characteristics of different purpose sorghum silage, Semina: Ciências Agrárias, 38, 2607-2618.

Benton, J.R., Klopfenstein, T. & Erickson, G.E. (2005) Effects of corn moisture and length of ensiling on dry matter digestibility and rumen degradable protein, In: Nebraska Beef Cattle Reports, pp. 31-33.

Bernardes, T.F., Carvalho, I.Q. & Silva, N.C. (2012) A snapshot of maize silage quality on dairy farms in South Brazil. In: Kuoppala, K., Rinne, M., & Vanhatalo, A. (eds). Proceedings of the XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 322-323.

Bernardes, T.F. & Do Rêgo, A.C. (2014) Study on the practices of silage production and utilization on Brazilian dairy farms. Journal of Dairy Science, 97, 1852-1861.

Bernardes, T.F., De Oliveira, I.L., Lara, M.A.S., Casagrande, D.R., Ávila, C.L.S. & Pereira, O.G. (2015a) Effects of potassium sorbate and sodium benzoate at two application rates on fermentation and aerobic stability of maize silage, Grass and Forage Science, 70, 491-498.

Bernardes, T.F., Schmidt, P. & Daniel, J.L.P (2015b) An overview of silage production and utilization in Brazil, In: Daniel, J.L.P., Morais, G., Junges, D., & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 124-144.

Bernardes, T.F. (2016) Advances in silage sealing. In: Da Silva, T. & Santos, E. M. (ed). Advances in Silage Production and Utilization. InTech, Rijeka, Croatia. p. 53-62.

Bernardes, T.F., Cardoso, M.V.S. & Lima, L.M. (2018a) Silage feeding programs on intensive dairy farms. Journal of Dairy Science, E-Suppl. (Abstract) *in press*.

Bernardes, T.F., Daniel, J.L.P., Adesogan, A.T., McAllister, T.A., Drouin, P., Nussio, L. G., Huhtanen, P., Tremblay, G.F., Bélanger, G. & Cai, Y. (2018b) Silage review: Unique challenges of silages made in hot and cold regions, Journal of Dairy Science https://doi.org/10.3168/jds.2017-13703, *in press*.

Bolsen, K.K., Moore, K.J., Coblentz, W.K, Siefers, M.K. & White, J.S. (2003) Sorghum Silage. In: Buxton, D.R., Muck, R.E., & Harrison, J.H. (ed.) Silage Science and Technology. Madison, WI, USA: ASA, CSSA, and SSSA, pp. 609-632.

Borreani, G. & Tabacco, E. (2012) Effect of silo management factors on aerobic stability and extent of spoilage in farm maize silages. In: Kuoppala, K., Rinne, M., & Vanhatalo, A. (eds). Proceedings XVI International Silage Conference, Hämeenlinna, Finland, 2012, pp. 71-72.

Bueno, J.L., Bolson, D.C., Jacovaci, F.A., Gomes, A.L.M., Jobim, C.C. & Daniel, J.L.P. (2017) Maturity interacts with length of storage to affect ruminal disappearance of starch in mechanically unprocessed corn silage, In: Nussio, L.G., Sousa, D.O., Gritti, V.C., Salvati, G.G.S., Santos, W.P., & Salvo, P.A.R. (eds). Proceedings of the V International Symposium on Forage Quality and Conservation, Piracicaba, Brazil, 2017.

Burdon, J.J., Thrall, P.H. & Ericsson, L. (2006) The current and future dynamics of disease in plant communities, Annual Review of Phytopathology, 44, 19-39.

Campos, A.F. (2015) Effect of particle size on ensilability and nutritive value of sugarcane silage for beef cattle. PhD Dissertation, São Paulo State University, Jaboticabal, Brazil, 87 p. [in Portuguese].

Castro, L.P. (2017) Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and inclusion in the diet. Master Thesis, University of Lavras, Lavras, Brazil, 71 p.

Cerri, C.E.P., Sparovek, G., Bernoux, M., Easterling, W.E., Melillo, J.M. & Cerri, C.C. (2007) Tropical agriculture and global warming: impacts and mitigation options, Scientia Agricola, 64, 83-99.

Coan, R.M., Vieira, P.F., Silveira, R.N., Pedreira, M.S. & Reis, R.A. (2001) Effect of bacterial/enzymatic inoculant on the chemical composition, digestibility and quality of Tanzania and Mombaça grass silages. In: Proceedings of the 38th Annual Meeting of the Brazilian Society of Animal Science, Piracicaba, Brazil, 2001, p. 124. [in Portuguese].

CONAB – The National Supply Company (2018) Accessed March 21, 2018. http://www.conab.gov.br/ [website in Portuguese].

Correa, C.E.S., Shaver, R.D., Pereira, M.N., Lauer, J.G. & Kohn, K. (2002) Relationship between corn vitreousness and ruminal in situ starch degradability,Journal of Dairy Science, 85,3008-3012.

Correa, C.E.S., Pereira, M.N., Oliveira, S.G. & Ramos, M.H. (2003) Performance of Holstein cows fed sugarcane or corn silages of different grain texture, Scientia Agricola, 60, 621-629.

Crasta, O.R. & Cox, W.J. (1996) Temperature and soil water effects on maize growth, development yield, and forage quality, Crop Science, 36, 341-348.

Custódio, L., Morais, G., Daniel, J.L., Pauly, T. & Nussio, L.G. (2016) Effects of chemical and microbial additives on clostridium development in sugarcane (*Saccharum officinarum* L.) ensiled with lime, Grassland Science, 62, 135-143.

Da Silva, S.C., Bueno, A.A.O., Carnevalli, R.A., Uebele, M.C., Bueno, F.O., Hodgson, J., Matthew, C., Arnold, G.C. & Morais, J.P.G. (2009). Sward structural characteristics and herbage accumulation of *Panicum maximum* cv. Mombaça subjected to rotational stocking managements. Scientia Agricola, 66, 8-19.

Da Silva, N.C., Santos, J.P., Ávila, C.L.S., Evangelista, A.R., Casagrande, D.R. & Bernardes, T.F. (2014) Evaluation of the effects of two *Lactobacillus buchneri* strains and sodium benzoate on the characteristics of corn silage in a hot-climate environment, Grassland Science, 60, 169–177.

Da Silva, M.R.H. (2015) Processamento e ensilagem no valor nutritivo de grãos de milho para novilhos em confinamento, PhD Dissertation, State University of Maringá, Maringá, Brazil, 80 p. [in Portuguese].

Daniel, J.L.P. & Nussio, L.G. (2011) Contribution of silage volatile compounds for the animal nutrition. In: Daniel, J.L.P., Zopollatto, M., & Nussio, L.G. (eds). Proceedings of the II International Symposium on Forage Quality and Conservation, São Pedro, Brazil, pp. 279-306.

Daniel, J.L.P., Amaral, R.C., Sá Neto, A., Cabezas-Garcia, E.H., Bispo, A.W., Zopollatto, M., Cardoso, T.L., Spoto, M.H.F., Santos, F.A.P., & Nussio, L.G. (2013a) Performance of dairy cows fed high levels of acetic acid or ethanol, Journal of Dairy Science, 96, 398-406.

Daniel, J.L.P., Capelesso, A., Cabezas-Garcia, E.H., Zopollatto, M., Santos, M.C., Huhtanen, P. & Nussio, L.G. (2013b) Fibre digestion potential in sugarcane across the harvesting window, Grass and Forage Science, 69, 176-181.

Daniel, J.L.P., Weiß, K., Custódio, L., Sá Neto, A., Santos, M.C., Zopollatto, M. & Nussio, L.G. (2013c) Occurrence of volatile organic compounds in sugarcane silages, Animal Feed Science and Technology, 185, 101-105.

Daniel, J.L.P., Junges, D., & Nussio, L.G. (2015a) A meta-analysis of the effects of length of storage on starch digestibility and aerobic stability of corn silages, In: Daniel, J.L.P., Morais, G., Junges, D., & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, pp. 306-307.

Daniel, J.L.P., Junges, D., Santos, M.C., Kleinshmitt, C. & Nussio L.G. (2015b) Emissions of ethanol and acetic acid in corn silages inoculated with *Lactobacillus buchneri*, In: Daniel, J.L.P., Morais, G., Junges, D. & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, pp. 372-373.

Daniel, J.L.P., Junges, D. & Nussio, L.G. (2015c) The effects of chemical and microbial additives on the emissions of ethanol and acetic acid in sugarcane silages, In: Daniel, J.L.P., Morais, G., Junges, D. & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, pp. 444-445.

Daniel, J.L.P., Jacovaci, F.A., Junges, D., Santos, M.C., Lima, J.R., Anjos, I.A., Landell, M.G.A., Huhtanen, P. & Nussio, L.G. (2017) Fibre digestibility and its relationships with chemical and morphological traits in thirty-two sugarcane varieties, Grass and Forage Science,72,545-555.

Daniel, J.L.P., Queiroz, O.C.M., Arriola, K.G., Daetz, R., Basso, F., Romero, J.J. & Adesogan, A.T. (2018a) Homolactic bacterial inoculant effects on the performance of lactating dairy cows, Journal of Dairy Science, 101, 1-8.

Daniel, J.L.P., Jobim, C.C. & Nussio, L.G. (2018b) A data analysis on the effect of acetic acid on dry matter intake in dairy cattle, In: Proceedings of the XVIII International Silage Conference, Bonn, Germany.

De Freitas, P.L. & Landers, J.N. (2014) The transformation of agriculture in Brazil through development and adoption of zero tillage conservation agriculture. International Soil and Water Conservation Research, 2, 35-46.

De Oliveira, I.L., Tabacco, E., Ferrero, F., Borreani, G., & Bernardes, T.F. (2018) A new approach to assess feed-out rate in maize silage bunker, In: Proceedings of the XVIII International Silage Conference, Bonn, Germany.

Driehuis, F., Spanjer, M.C., Scholten, J.M. & Te Giffel, M.C. (2008) Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands, Food Additives & Contaminants, 1, 41-50.

Duncan, R.R. (1980) Methiocarb as a bird repellent on ripening grain sorghum, Canadian Journal of Plant Science, 60, 1129-1133.

Erickson, G.E., Klopfenstein, T.J., Rasby, R.J., Stalker, L.A., Plugge, B., Bauer, D., Mark, D.R., Adams, D.C., Benton, J., Greenquist, M.A., Nuttleman, B., Kovarik, L., Peterson, M., Waterbury, J. & Wilken, M. (2008) Storage of wet corn co-products, UNL Extension Publication. University of Nebraska, Lincoln, USA.

Fernandes, J. (2014) Influence of genotype, maturity and length of storage on silage corn grain quality with high moisture. Master Thesis, University of São Paulo, Piracicaba, Brazil, 97 p. [in Portuguese].

Ferraretto, L.F. & Shaver, R.D. (2012) Meta-analysis: Impact of corn silage harvest practice on intake, digestion and milk production by dairy cows, Professional Animal Scientist, 28, 141-149.

Gomes A.L.M., Bueno, J.L., Jacovaci, F.A., Bolson, D.C., Jobim, C.C. & Daniel, J.L.P. (2018) Effects of processing, moisture and length of storage on the fermentative losses and ruminal degradability of reconstituted corn grain silage, In: Proceedings of the XVIII International Silage Conference, Bonn, Germany.

Gusmão, J.O., Danés, M.A.C., Casagrande, D.R. & Bernardes, T.F. (2018) Total mixed ration silage containing elephant grass for small-scale dairy farms. Grass and Forage Science, doi: 10.1111/gfs.12357, *in press*.

Holmes, B.J. (2009) Software applications for sizing silos to maximize silage quality. In: Zopollatto, M., Muraro, G. B., & Nussio, L. G. (eds). Proceedings of the International Symposium on Forage Quality and Conservation, Piracicaba, Brazil, 2019, pp. 189-208.

IAGRO (2017) State Agency of Plant and Animal Health, Accessed March 25, http://www.semagro.ms.gov.br/wp-content/uploads/sites/157/2017/08/Joint-Technical-Note-Nr.-002.pdf.

Intergovernmental Panel on Climate Change - IPCC. Climate Change (2001) Impacts, adaptation & vulnerability. Contribution of Working Group II to the Third Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge: University Press, 2001. 1000 p.

Jacovaci, F.A., Jobim, C.C., Schmidt, P., Nussio, L.G. & Daniel, J.L.P. (2017) A data-analysis on the conservation and nutritive value of sugarcane silage treated with calcium oxide, Animal Feed Science and Technology, 225, 1-7.

Jobim, C.C., Reis, R.A., Schoken-Iturrino, R.P. & Rosa, B. (1999) Desenvolvimento de microrganismos durante a utilização de silagens de grãos úmidos de milho e de espigas de milho sem brácteas, Acta Scientiarum, 21, 671-676. [in Portuguese].

Junges, D., Morais, G., Spoto, M.H.F., Santos, P.S., Adesogan, A.T., Nussio, L.G. & Daniel, J.L.P. (2017) Short communication: Influence of various proteolytic sources during fermentation of reconstituted corn grain silages, Journal of Dairy Science, 100, 9048-9051.

Kim, S.C. & Adesogan A.T. (2006) Influence of ensiling temperature, simulated rainfall, and delayed sealing on fermentation characteristics and aerobic stability of corn silage, Journal of Dairy Science, 89, 3122-3132.

Kleinshmitt, C., Morais, G., Custódio, L., Fernandes, J., Santos, M.C., Daniel, J.L.P. & Nussio, L.G. (2013) Performance of lactating dairy cows fed maize silage with increased dosages of *L. buchneri*. In: Proceedings of the 15th International Conference on Forage Conservation, High Tatras, Slovakia, pp. 141-142.

Koc, F., Coskuntuna, L., Ozduven, M.L., Coskuntuna, A. & Samli, H.E. (2009) The effects of temperature on the silage microbiology and aerobic stability of corn and vetch-grain silages, Acta Agriculturae Scandinavica A, 59, 239246.

Kristensen, N.B., Sloth, K.H., Højberg, O., Spliid, N.H., Jensen, C. & Thøgersen, R. (2010) Effects of microbial inoculants on corn silage fermentation, microbial contents, aerobic stability, and milk production under field conditions, Journal of Dairy Science, 93, 37643774.

Lardy, G & Anderson, V. (2010) Harvesting, storing, and feeding corn as earlage. AS-1490. North Dakota State University Extension, North Dakota, USA.

Leonardi, C. & Armentano, L.E. (2003) Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. Journal of Dairy Science, 86, 557-564.

Lima, L.M., Dos Santos, J.P., Casagrande, D.R., Ávila, C.L.S., Lara, M.A.S. & Bernardes, T.F. (2017) Lining bunker walls with oxygen barrier film reduces nutrient losses in corn silages. Journal of Dairy Science, 100, 4565-4573.

Loures, D.R.S., Nussio, L.G., Paziani, S.F., Pedroso, A.F., Mari, L.J., Ribeiro, J.L., Zopollatto, M., Schmidt, P., Junqueira, M.C., Packer, I.U., & Campos, F.P. (2005) Chemical composition and effluent yield of Tanzania grass silages affected by wilting, particle size and enzymatic/microbial additive, Revista Brasileira de Zootecnia, 34, 726-735. [in Portuguese].

Macken, C., Erickson, G., Milton, T., Klopfenstein, T. & Block, H. (2003) Effects of starch endosperm type and corn processing method on feedlot performance, nutrient digestibility, and ruminal fermentation of high-grain diets, In: Nebraska Beef Cattle Report MP80-A, pp. 32-34.

Mann, J.A., Kimber, C.T. & Miller, F.R. (1983) The origin and early cultivation of sorghums in Africa, Bulletin 1454, Texas Agricultural Experiment Station, TX, USA.

Martins, J.M., Fernandes, T.H. & Ferreira, L.M.A. (1993) A note on ensiling characteristics of the tropical grass *Setaria sphacelata* var. splendida (Stapf), In: O'Kiely, P., O'Connel, M., & Murphy, J. (eds). Proceedings of the X International Silage Conference, Dublin, Republic of Ireland, 1993, pp. 110-111.

Moore K.J., & Jung, H.J.G. (2001) Lignin and fiber digestion, Journal of Range Management, 54, 420-430.

Morais, G., Daniel, J.L.P., Kleinshmitt, C., Carvalho, P.A., Fernandes, J. & Nussio, L.G. (2017) Additives for grain silages: A review, Slovak Journal of Animal Science, 50, 42-54.

Muck, R.E., Moser, L.E. & Pitt, R.E. 2003. Postharvest factors affecting ensiling. In: Buxton, D.R. Muck, R.E. & Harrison J.H. (ed). Silage Science and Technology. ASA, CSSA., and SSSA, Madison, WI. p. 251-304.

Mühlbach, P.R.F. (2000) Additives to improve the silage making process with tropical forages, In: t'Mannetje, L. (ed). Proceedings of FAO Electronic Conference on Tropical Silage, Paper 161, Food and Agricultural Organization of the United Nations, Rome, Italy.

Mulrooney, C.N. & Kung Jr., L. (2008) The effect of water temperature on the viability of silage inoculants, Journal of Dairy Science, 91, 236-240.

Novinski, C.O. (2013) Composição de micotoxinas e bromatologia de silagens de milho em silos de grande porte utilizando imagens em infravermelho. Master Thesis, Federal University of Paraná, Curitiba, Brazil, 85 p. [in Portuguese].

Nussio, L.G. (2005) Silage production from tropical forages, In: Park, R.S., & Stronge, M.D. (eds). Proceedings of the XIV International Silage Conference, Belfast, Northern Ireland, 2005, pp. 97-107.

Oliveira, A.S., Detmann, E., Campos, J.M.S., Pina, D.S., Souza, S.M., & Costa, M.G. (2011) Meta-analysis of the impact of neutral detergent fiber on intake, digestibility and performance of lactating dairy cows, Revista Brasileira de Zootecnia, 40, 1587-1595. [in Portuguese].

Oliveira, C.A. & Millen, D.D. (2014) Survey of the nutritional recommendations and management practices adopted by feedlot cattle nutritionists in Brazil. Animal Feed Science and Technology, 197, 64-75.

Oliver, A. L., Grant, R.J., Pedersen, J.F. & Rear, J.O. (2004) Comparison of brown midrib-6 and -18 forage sorghum with conventional sorghum and corn silage in diets of lactating dairy cows, Journal of Dairy Science, 87, 637-644.

Orosz, S. & Davies, D.R. (2015) Short and long term storage of wet by-products fed by ruminants, In: Daniel, J.L.P., Morais, G., Junges, D., & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 200-242.

Ott, E.M., Friedel, K., Llorente-Perez, R. & Gabel, M. (1999) Feeding value and ensilability of two sorghum varieties under tropical conditions, In: Pauly, T. (ed). Proceedings of the XII International Silage Conference, Uppsala, Sweden, 1999, pp. 201-202.

Pahlow, G., Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H., & Spoelstra, S.F. (2003) Microbiology of ensiling. In: Buxton, D.R, Muck, R.E., & Harrison, J.H. (eds). Silage Science and Technology. Madison, USA: ASA, CSSA, SSSA. p. 31-93.

Paillat, J.M. & Gaillard, F. (2001) Air-tightness of wrapped bales and resistance of polythene stretch film under tropical and temperate conditions. Journal of Agricultural Engineering Research, 79, 15-22.

Passini, R., Silveira, A.C., Titto, E.A.L., Rodrigues, P.H.M., Arrigoni, M.B., Costa, C. & Chardulo, L.A.L. (2002) Silagem de grãos úmidos de milho e de sorgo e níveis proteicos sobre desempenho e características da carcaça de novilhos superprecoces, Acta Scientiarum, 24, 1133-1140. [in Portuguese].

Paziani, S.F., Duarte, A.P., Nussio, L.G., Daniel, J.L.P., Freitas, R.S., Gallo, P.B., Ticelli, M. & Mateus, G.P. (2018) Estimates of maize forage production from grain yield data, Revista Brasileira de Zootecnia, *in press*.

Pedersen, J. F., Vogel, K. P., & Funnell, D. L. (2005) Impact of reduced lignin on plant fitness, Crop Science, 45, 812-819.

Pereira, M.N. (2017) Silagem de milho para vacas leiteiras: processamento da fração grãos e da fração fibrosa, In: Teixeira, A. M., Gonçalves, L. C., Netto, A., Silva, B., Prado, D., Oliveira, F., Gomes, G., Leão, I., Martins, L., Oliveira, L., & Marinho, M. (eds). Proceedings of the III National Conference on Dairy Cattle, Uberlândia, Brazil, 2017, pp. 140-158. [in Portuguese].

Philippeau, C. & Michalet-Doreau, B. (1997) Influence of genotype and stage of maturity of maize on rate of ruminal starch degradation. Animal Feed Science and Technology, 68, 25-35.

Pinto, A.C.J. & Millen, D.D. (2016) Situação atual da engorda de bovinos em confinamento e modelos nutricionais em uso. In: X Simpósio de Produção de Gado de Corte (Simcorte). Federal University of Viçosa, Viçosa, Brazil, pp. 103-120. [in Portuguese].

Pitt, R.E. & R.E. Muck. (1993) A diffusion model of aerobic deterioration at the exposed face of bunker silos. Journal of Agricultural Engineering Research, 55, 11-26.

Queiroz, O.C.M., Nussio, L.G., Schmidt, P., Ribeiro, J.L., Santos, M.C. & Zopollatto, M. (2008) Sugarcane silage as compared to traditional supplemental sources of forage in the performance of high production cows, Revista Brasileira de Zootecnia, 37, 358–365. [in Portuguese].

Queiroz, O.C.M., Arriola, K.G., Daniel, J.L.P, & Adesogan, A.T. (2013) Effects of 8 chemical and bacterial additives on the quality of corn silage, Journal of Dairy Science, 96, 5836-5843.

Rabelo, C.H.S., Mari, L.J. & Reis, R.A. (2016) Survey about the use of bacterial inoculants in Brazil: effects on silage quality and animal performance. In: Silva, T. & Santos, E.M. (eds). Advances in silage production and utilization. InTech Publications, Rijeka, Croatia, p. 3-37.

Randby, Å.T., Selmer-Olsen, I. & Baevre, L. (1999) Effect of ethanol in feed on milk flavor and chemical composition, Journal of Dairy Science, 82, 420-428.

Raun, B.M.L., & Kristensen, N.B. (2010) Propanol in maize silage at Danish dairy farms, Acta Agriculturae Scandinavica A, 60, 53–59.

Sá, J.C.M., Cerri, C.C., Lal, R., Dick, W.A., Venzke Filho, S., Piccolo, M.C. & Feigl, B. (2001) Organic matter dynamics and carbon sequestration rates for a tillage chronosequence in a Brazilian Oxisol, Soil Science Society of America Journal, 65, 1486-1499.

Sá Neto, A., Bispo, A.W., Junges, D., Bercht, A.K., Zopollatto, M., Daniel, J.L.P. & Nussio, L.G. (2014) Exchanging physically effective neutral detergent fiber does not affect chewing activity and performance of late-lactation dairy cows fed corn and sugarcane silages, Journal of Dairy Science, 97, 7012-7020.

Salvati, G.G.S., Santos, W.P., Silveira, J.M., Gritti, V.C., Arthur, B.A.V., Salvo, P.A.R., Ribeiro, A.P., Lima, T.A.D., Morais Jr, N.N., & Nussio, L.G. (2017) Impact of kernel processing and particle size in whole plant corn silage on the performance of dairy cows, In: In: Nussio, L.G., Sousa, D.O., Gritti, V.C., Salvati, G.G.S., Santos, W.P, & Salvo, P.A.R. (eds.) Proceedings of the V International Symposium on Forage Quality and Conservation, Piracicaba, Brazil, 2017.

Santos, R.M., Jacovaci, F.A., Garcia-Diaz, T., Scheidt, K.C., Jobim, C.C. & Daniel, J.L.P. (2018) Effects of moisture and *L. buchneri* on the conservation and ruminal degradability of high moisture corn and snaplage, In: Proceedings of the XVIII International Silage Conference, Bonn, Germany.

Schlenker, W. & Roberts, M.J. (2006) Nonlinear effects of weather on corn yields, Review of Agricultural Economics, 28, 391-398.

Schmidt, P., Novinski, C.O., Junges, D., Almeida, R. & de Souza, C.M. (2015) Concentration of mycotoxins and chemical composition of corn silage: A farm survey using infrared thermography, Journal of Dairy Science, 98, 6609-6619.

Schonell, E.P. (2015) Silagem de grãos de sorgo reconstituídos para vacas leiteiras: Efeitos da conservação da silagem e respostas de aditivos. Master Thesis, University of São Paulo, ESALQ, Piracicaba, Brazil, 105 p. [in Portuguese].

Soderlund, S.D., Uhrig, J., Curran, B. & Nuzback, L. (2006) Influence of maturity on the yield and nutritional quality of four Pioneer corn hybrids harvested as high moisture ear corn, Nutritional Insights, DuPont Pioneer, Johnston, IA, USA.

Tomaz, P.K., Araujo, L.C., Sanches, L.A., Santos-Araujo, S.N., Lima, T.O., Lino, A.A. & Ferreira, E.M. (2018) Effect of sward height on the fermentability coefficient and chemical composition of Guinea grass silage, Grass and Forage Science, DOI: 10.1111/gfs.12349.

Tres, T.T. (2015) Inclusão de okara na qualidade da silagem de grãos de milho reidratados e na alimentação de ruminantes, PhD Dissertation, State University of Maringá, Maringá, Brazil, 80 p. [in Portuguese].

USDA - Foreign Agriculture Service (2017) Livestock and poultry: World markets and trade. Accessed March 21, 2018. https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf.

USDA - Foreign Agriculture Service (2018) World agricultural production. Accessed March 21, 2018. https://apps.fas.usda.gov/psdonline/circulars/production.pdf

Wang, C. & Nishino, N. (2013) Effects of storage temperature and ensiling period on fermentation products, aerobic stability and microbial communities of total mixed ration silage, Journal of Applied Microbiology, 114, 1687-1695.

Weinberg, Z.G., Chen, Y., Miron, D., Raviv, Y., Nahim, E., Bloch, A., Yosef, E., Nikbahat, M. & Miron, J. (2011) Preservation of total mixed rations for dairy cows in bales wrapped with polyethylene stretch film - A commercial scale experiment, Animal Feed Science and Technology, 164, 125-129.

Weiss, K., Kroschewski, B. & Auerbach, H. (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage, Journal of Dairy Science, 99, 8053-8069.

Windle, M.C. & Kung Jr., L. (2016) Factors affecting the numbers of expected viable lactic acid bacteria in inoculant applicator tanks, Journal of Dairy Science, 99, 9334-9338.

Yoshii, T., Asanuma, N. & Hino, T. (2005) Effect of ethanol on nitrate and nitrite reduction and methanogenesis in the ruminal microbiota, Animal Science Journal, 76, 37-42.

Zebeli, Q., Mansmann, D., Ametaj, B.N., Steingass, H. & Drochner, W. (2010) A statistical model to optimize the requirements of lactating dairy cows for physically effective neutral detergent fiber. Archives of Animal Nutritional, 64, 265-278.

Zhou, Y., Drouin, P. & Lafreniere, C. (2016) Effect of temperature (5–25°C) on epiphytic lactic acid bacteria populations and fermentation of whole-plant corn silage, Journal of Applied Microbiology, 121, 657-671.

# Utilization of silages in the diets of high producing dairy cows: Limitations and opportunities

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**Keywords:** alfalfa silage, corn silage, dairy cows, milk production

## Introduction

Silage is an integral ingredient in the diets of lactating dairy cows in temperate regions of the world. In North America, silages formulated into the diets of high-producing dairy cows are predominantly corn silage and alfalfa silage along with smaller usage of other forages such as small grain silage and grass silage. The choice of silage used in the dairy ration is highly dependent on the climate, soil types, and growing conditions in that particular geographic region. In the Midwest portion of the United States, dairy rations are dominated by corn silage and alfalfa silage, whereas in Canada, small grain silages such a barley silage dominate. In the southern part of the United States, both temperate and tropical grasses are utilized in lactating dairy cows diets, and in California, there may be double cropping systems in place that may use corn and alfalfa silages in combination with small grain silages. All of these types of forages present some challenges in fermenting them into silage and may also limit their utilization in the high-producing dairy cow diet. While various options for forages exist in the United States, alfalfa silage and corn silage dominate most rations. Different challenges exist for different forage crops that are ensiled. These challenges affect how dairy nutritionists utilize these silages in the diets of high producing dairy cows. Challenges include finding the best way to utilize the nitrogen provided by the silage without losing it as waste and how to best utilize the carbohydrates in forages through improved ruminal digestibility. Overall, the goal as a dairy nutritionist is to utilize as much forage as possible in the diet without limiting intake, and ultimately, improve milk production.

## Formulation of high silage diets for lactating dairy cows

Forages are one of the least expensive feedstuffs to include in dairy cow diets, therefore it is a goal of most nutritionists to include as much forage into the lactating dairy cow diet without limiting intake and causing a decrease in milk production. In cold weather climates, this means that for 6 or more months of the year, cows must be fed conserved forages. While dried forages (i.e. – hays) were once very commonly included in lactating dairy cow diets throughout the United States, the use of ensiled forages has increased even as milk production per cow continues to increase. In the U.S., today's lactating dairy cow diets are typically presented to the cow as a total mixed ration and formulated with high amounts of alfalfa silage and corn silage. How can we increase silage utilization in dairy cow diets? As a nutritionist, we need to consider nutrient composition of the forages that are on hand, determine what the limits are for inclusion, and formulate diets that will optimize dry matter intake (DMI) and the conversion of nutrients into milk and milk components. Typical nutrient composition of alfalfa silages and corn silages grown in North America are presented in Table 1 (Dairy One, 2018). Alfalfa silage can be a high quality, high protein forage feedstuff [mean is 21.6% CP (over 54,000 samples)], but it also known for its high concentration of ruminally available nitrogen. Both the higher CP % and higher ruminal degradation of this nitrogen poses challenges to the nutritionist and may limit its inclusion in lactating dairy cow diets if formulating diets to minimize nutrient excretion to the environment. In addition, alfalfa has lower concentrations of total ruminally available carbohydrates needed to match up with the higher concentration of ruminally available nitrogen. This creates challenges in formulating rations to take full advantage of the nitrogen provided by alfalfa silage. Corn

silage, on the other hand, is low in crude protein [mean is 8.3% CP (over 245,000 samples)], but high in starch (mean of 31.8%) resulting in a relatively high-energy forage feedstuff (Table1). The increased energy provided by corn silage, makes it relatively easy to formulate into high production dairy cow diets. Agronomically, corn silage also has an advantage as it is a high volume crop that can be harvested once per planting, whereas alfalfa silage require multiple harvests increasing the probability of reduced quality because of weather. Ultimately, most dairy producers blend corn silage and alfalfa silage together in total mixed rations, however, because of advantages in harvest and energy concentration, inclusion of corn silage in lactating dairy rations are on the rise.

**Table 1**. Nutrient composition of alfalfa silage and corn silage[1]

| | Legume silage[2] | Corn silage[3] |
|---|---|---|
| Nutrient[4] (%) | Mean ± std. dev. | Mean ± std. dev. |
| DM (% as is) | 40.3 ± 12.0 | 33.7 ± 9.15 |
| CP | 21.6 ± 2.99 | 8.3 ± 1.06 |
| SP (% of CP) | 58.8 ± 9.32 | 54.8 ± 10.3 |
| NDICP | 3.38 ± 1.07 | 1.20 ± 0.32 |
| ADICP | 1.55 ± 0.44 | 0.60 ± 0.15 |
| NDF | 44.1 ± 5.76 | 43.6 ± 5.91 |
| ADF | 34.4 ± 4.24 | 25.7 ± 4.04 |
| Lignin | 7.71 ± 3.71 | 3.25 ± 0.68 |
| Starch | 2.05 ± 1.26 | 31.8 ± 7.41 |
| WSC | 6.10 ± 4.79 | 3.24 ± 1.56 |
| Crude fat | 3.81 ± 0.74 | 3.29 ± 0.48 |
| Ash | 11.1 ± 2.09 | 4.30 ± 1.22 |
| pH | 4.99 ± 0.61 | 3.97 ± 0.50 |
| Ammonia-N (% of total N) | 10.3 ± 9.16 | 6.93 ± 3.80 |
| Lactic acid | 5.12 ± 3.11 | 4.79 ± 2.18 |
| Acetic acid | 2.35 ± 1.72 | 2.43 ± 1.55 |
| Propionic acid | 0.14 ± 0.27 | 0.26 ± 0.41 |
| Butyric acid | 0.42 ± 1.39 | 0.03 ± 0.16 |
| $NE_L$ (Mcal/kg) | 1.37 ± 0.14 | 1.61 ± 0.14 |
| $NE_M$ (Mcal/kg) | 1.29 ± 0.17 | 1.63 ± 0.14 |
| $NE_G$ (Mcal/kg) | 0.71 ± 0.15 | 1.03 ± 1.03 |

[1]Dairy One Interactive Feed Composition Libraries (2018). Includes samples analysed from May 2000 to April 2018.
[2]Number of samples for legume silage: 3,730 to 54,237 depending on the nutrient analysed.
[3]Number of samples for corn silage: 17,717 to 288,358 depending on the nutrient analysed.
[4]Nutrients: DM = dry matter, CP = crude protein, SP = soluble protein, NDICP = neutral detergent insoluble CP, ADICP = acid detergent insoluble CP, NDF = neutral detergent fiber, ADF = acid detergent insoluble fiber, WSC = water soluble carbohydrates, $NE_L$ = net energy for lactation, $NE_M$ = net energy for maintenance, and $NE_G$ = net energy for gain.

So what is the impact of increasing forage/silage concentration in dairy cow diets? Numerous studies have been conducted evaluating the formulation of diets at differing forage to concentrate ratios. Typically, diets with increasing forage NDF concentration generally decrease DMI attributed to greater forage NDF concentration and bulk density of the diet (Allen, 2000). This does not necessarily result in decreased milk production. Schuler et al. (2013) evaluated the optimum dietary forage concentration on intake and production. Forage (formulated with 70% corn silage and 30% alfalfa silage) was included in the diet at 42, 50, 58, and 66% of the diet (DM basis). Forage replaced primarily non-forage fiber feedstuffs in the diet (corn gluten feed, soybean hulls, and beet pulp). As forage increased in the diet, DMI decreased linearly, while milk yield and energy-corrected milk (ECM) remained the same across all 4 diets. As a result, milk production efficiency (ECM/DMI) increased linearly as forage increased from 42 to 66% of the diet.

**Nitrogen utilization in silages**

Alfalfa has long been considered to be one of the highest quality forages that can be included in lactating dairy cow diets because of its higher CP content. Agronomically, alfalfa is noted for its ability to fix soil nitrogen and help sustain soil quality and health (Russelle 2004). A challenge with including high levels of alfalfa silage in dairy cow diets is that extensive proteolysis occurs during the fermentation process resulting in increased concentrations of non-protein nitrogen, in particular, ammonia-N (Pichard et al. 2006). Proteolysis is a common process that occurs during silage fermentation. The conversion of crop protein to non-protein nitrogen compounds increase as fermentation progresses (McDonald et al. 1991). Since low pH reduces plant enzyme activity, a rapid drop in pH during fermentation, reduces proteolysis.Unfortunately, the high buffering capacity of alfalfa slows this decrease in pH resulting in greater proteolysis than other forage feedstuffs (Muck and Walgenback 1985). In a recent meta-analysis utilizing 34 comparisons across 10 studies, DMI decreased linearly and milk production and milk fat yield tended to decrease linearly as ammonia-N concentration in alfalfa silages increased across diets (Sanchez-Duarte and Garcia 2017). Currently, if diets are formulated with high levels of alfalfa silage resulting in excessive concentrations of highly degraded protein, this excessive protein will be lost as urinary nitrogen (Jonker et al. 1998) and not utilized efficiently for milk production (NRC 2001). Decreasing proteolysis during the fermentation process or decreasing overall degradability of the protein would result in greater value of alfalfa silage formulated in lactating dairy cow diets. The rate and extent of proteolysis during ensiling can be affected by many factors. Some of these factors which reduce proteolysis include: wilting of forage to increase DM content at ensiling (Muck 1987), heat treatment of the forage (Charmley and Viera 1990), temperature during storage (Muck and Dickerson 1988), increasing the rate of pH change (Scallet et al. 1984), and the addition of additives and inoculants (Weinberg and Muck 1996, Nsereko et al. 1998). Future opportunities to reduce proteolysis and potentially decrease overall degradability of the protein in alfalfa silage include increasing the activity of polyphenol oxidase (Lee et al. 2006, Sullivan and Hatfield 2006) or increasing the concentration of condensed tannins (Potkański et al. 2002, Coblentz and Grabber 2013). Protecting protein from proteolysis and ruminal degradation resulting in increased flow of amino acids reaching the small intestine would allow for greater inclusion of alfalfa silage in lactating dairy cow diets, and ultimately, increase its value as a forage feedstuff.

**Fiber utilization in silages**

Another limiting factor in increasing the proportion of silages in lactating dairy cow diets is energy provided by those silages. One simple reason why corn silage has increased in its usage as a forage in lactating dairy cows is the amount of energy provided by that forage. With starch concentrations (Table 1) commonly between 30 to 35% (of DM), and sometimes higher, there is considerable amount of energy provided in a crop that is harvested once per planting. Ultimately, increased value from any forage could be obtained via increasing in the fermentability of the structural carbohydrate portion of that forage. Improvements in fiber fermentability in the rumen need to occur without sacrificing agronomic characteristics of that forage. Currently, there are some management options to improve fiber digestibility of corn silage and alfalfa silage. One such method to improve fiber fermentability of corn silage is to chop the silage at a greater height. Wu and Roth summarized 11 studies and examined the effect of increasing the cutting height from an average of 17 cm for the low cutting to 49 cm for the high cutting. For silages cut at the higher height, NDF concentration decreased about 7.4% and ADF concentration decreased 10.2% compared to silages cut at the lower height. Starch concentration, NDF digestibility (NDFD), and DM digestibility increased 5.9, 4.7, and 2.5%, respectively, for silages cut at the higher height compared to the lower height. On the other hand, yield per ha was reduced 7.4% for the high cut corn silage because more stalk was left in the field. Corn hybrids containing the brown midrib gene (BMR) result in silage with lower lignin, higher fiber digestibility, and greater intake (Holt et al. 2013). Four natural mutations were identified in the 1930-40's, but didn't become marketable until the 1990's. Historically, BMR varieties are slightly lower yielding (about 10%), have greater lodging incidence, are more stressed by drought conditions, and are more susceptible to northern corn leaf blight. Response from feeding BMR corn silage can be variable, but it's recommended to include it in the diet at inclusion levels greater than 20% of the total

diet DMI. Because it's lower in lignin, it degrades more quickly (greater NDFD), and what doesn't degrade, also passes from the rumen quickly. This creates more space in the rumen resulting in greater DMI for cows fed the BMR corn silage compared to cows fed conventional corn silage diets (Oba and Allen 1999). Greater DMI often results in greater milk production. The BMR trait can also be found in other forages including sorghum, sudangrass, and pearl millet varieties. Jung et al. (2011) demonstrated that low-ferulate corn silage had similar lignin concentration compared to its isogenic control, but had reduced lignin-arabinoxylan crosslinking by ferulates. This results in increased NDFD (neutral detergent fiber digestibility), increased DMI, and increased milk production. Relatively new to the market, are low-lignin alfalfa varieties. Research suggests that the value of the low lignin alfalfa varieties will be an extended harvest window resulting in greater tonnage per cutting, a reduced number of cuttings (3 instead of 4 in the Midwest U.S.) while maintaining or improving forage quality. A low lignin variety was planted in 6 states, and cut 3 times. Reduced lignin alfalfa was 10% lower in NDF, 18% lower in lignin, and 10% higher in NDF digestibility compared to 2 alfalfa varieties chosen for high yield or high quality (Sulc et al. 2017). Agronomic advantages are less labor, lower equipment and harvest cost, and less traffic damage while maintaining high quality. Potentially low-lignin alfalfa silages could be included in the lactating dairy cow diets at a higher inclusion rates, but care in diet formulation will need to occur to ensure that there is sufficient effective fiber in the diet to maintain normal rumen function. Feeding studies will be needed to investigate how low-lignin alfalfa silages can best be formulated in lactating dairy cows diets.

## Conclusions

With the goal of increasing silage utilization in lactating dairy cow diets, it is first important to identify some of the challenges that exist in our current silages. Two of the most important challenges that currently exist with alfalfa silages and corn silages commonly used in U.S. dairy cow diets are the following: 1) Improving N utilization - issues concerning proteolysis of true protein during the ensiling process and its high ruminal degradability of protein in alfalfa silages, and 2) Improving fiber utilization of both alfalfa and corn silages by utilizing varieties designed to improve NDF digestibility. Opportunities exist to improve on both of these challenges. If proteolysis of true protein of alfalfa silage can be reduced during the ensiling process and more amino acids flow to the small intestine, it is likely greater levels of alfalfa silage can be incorporated into high producing dairy cow diets while minimizing wasted dietary protein. Utilization of BMR corn silages and low-lignin alfalfa varieties could help improve fiber utilization of forages resulting in greater inclusion of forages in the diets of high-producing dairy cows. Combining the use of both BMR corn silage and low-lignin alfalfa into one diet may help push the upper limit of how much forage can be included while maintaining high milk production.

## References

Allen, M.S. (2000) Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83, 1598-1624.

Charmley, E. & Veira, D.M. (1990) Inhibition of proteolysis in alfalfa silages using heat at harvest: Effects on digestion in the rumen, voluntary intake and animal performance. J. Anim. Sci. 68, 2042-2051.

Coblentz, W.K. & Grabber, J.H. (2013) In situ protein degradation of alfalfa and birdsfoot trefoil hays and silages as influenced by condensed tannin concentration. J. Dairy Sci. 96, 3120-3137.

Dairy One. (2018) Interactive Feed Composition Libraries. Accessed May. 30, 2018. http://dairyone.com/analytical-services/feed-and-forage/feed-composition-library/interactive-feed-composition-library/.

Holt, M.S., Eun, J.-S., Thacker, C.R., Young, A.J., Dai, X. & Nestor, K.E. (2013) Effects of feeding brown midrib corn silage with a high dietary concentration of alfalfa hay on lactational performance of Holstein dairy cows for the first 180 days of lactation. J. Dairy Sci. 96, 515-523.

Jonker, J.S., Kohn, R.A. & Erdman, R.A. (1998) Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81, 2681-2692.

Jung, H.G., Mertens, D.R. & Phillips, R.L. (2011) Effect of reduced ferulate-mediated lignin/arabinoxylan cross-linking in corn silage on feed intake, digestibility, and milk production. J. Dairy Sci. 94, 5124-5137.

Lee, M.R.F., Olmos Colmenero, J.J., Winters, A.L., Scollan, N.D. & Minchin, F.R. (2006) Polyphenol oxidase activity in grass and its effect on plant-mediated lipolysis and proteolysis of Dactylis glomerata (cocksfoot) in a simulated rumen environment. J. Sci. Food Agr. 86, 1503-1511.

McDonald, P., Henderson, A.R. & Heron, S.J.E. (1991) The Biochemistry of Silage. 2nd ed. Chalcombe Publ., Marlow, UK.

Muck, R.E. (1987) Dry matter level effects on alfalfa silage quality: I. Nitrogen transformations. Trans. ASAE 30, 7-14.

Muck, R.E. & Dickerson, J.T. (1988) Storage temperature effects on proteolysis in alfalfa silage. Trans. ASAE 31, 1005-1009.

Muck, R.E. & Walgenbach, R.P. (1985). Variations in alfalfa buffering capacity. Am. Soc. Agric. Eng. ASAE Paper No. 85-1535.

Nsereko, V.L., Rooke, J A., Newbold, C.J. & Wallace, R.J. (1998) Influence of protease inhibitors on nitrogen distribution in ensiled perennial ryegrass and the utilisation of silage nitrogen for growth by rumen bacteria in vitro. Anim. Feed Sci. Tech. 76, 51-63.

NRC. (2001) National Research Council. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Oba, M. & Allen, M. (1999) Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J. Dairy Sci. 82, 135-142.

Pichard, G.R., Tesser, B.R., Vives, C., Solari, C., Hott, A. & Larrain, R. E. (2006) Proteolysis and characterization of peptidases in forage plants. Agron. J. 98, 1392-1399.

Potkański, A., Gulewicz, K. & Michalak, S. (2002) Effect of tannins on the degradability of protein silages in the rumen. Proc. XIIIth International Silage Conference, Auchincruive, Scotland, pp. 306-307.

Russelle, M. (2004) The environmental impact of $N_2$ fixation by alfalfa. Proceedings of the National Alfalfa Symposium. UC Cooperative Extension, UC-Davis. Accessed May 30, 2018. http://alfalfa.ucdavis.edu/+symposium/proceedings/2004/04-57.pdf.

Sánchez J.I. & García A. (2017) Ammonia-N concentration in alfalfa silage and its effects on dairy cow performance: A meta-analysis. Rev. Colomb. Cienc. Pecu. 30, 175-184.

Scallet, M., Alpi A. & Picciarelli, P. (1984) Proteolytic activities in alfalfa (Medicago sativa L.) leaves. J. Plant Physiol. 116, 133-145.

Schuler, A.M., Kalscheur, K.F., Casper, D.P. & Anderson, J.L. (2013). Determination of the optimum dietary forage concentration when using canola meal as a primary protein source in lactating dairy cow diets. J. Dairy Sci. 96 (E-Suppl. 1), 397. (Abstr.).

Sulc, R.M., Parker, A., Albrecht, K., Cassida, K., Hall, M., Min, D., Orloff, S., Xu, X. & Undersander D. (2017) Agronomic and nutritional attributes of reduced lignin alfalfa. Proceedings of the 26th Annual Tri-State Dairy Nutrition Conference, Fort Wayne, IN. p.79-86.

Sullivan, M.L. & Hatfield, R.D. (2006) Polyphenol oxidase and o-diphenols inhibit postharvest proteolysis in red clover and alfalfa. Crop Sci. 46, 662-670.

Weinberg Z.G. & Muck R.E. (1996) New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol. Rev. 19, 53-68.

Wu, Z. & Roth, G. (2003) Considerations in managing cutting height of corn silage. Penn State Extension Bulletin DAS 03-72. Accessed May 30, 2018. https://extension.psu.edu/considerations-in-managing-cutting-height-of-corn-silage.

# A data analysis on the effect of acetic acid on dry matter intake in dairy cattle

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**Keywords:** acetic acid, heterofermentation, intake, *Lactobacillus buchneri*

**Introduction** A certain amount of acetic acid is desirable in silage, in order to minimize growth of yeasts and molds. However, the effect of acetic acid on the feeding value of silages remains debatable. Some studies found that high concentrations of acetic acid have been associated with lower feed intake, but others reported that depressed intake was not consistently related with the level of acetic acid in silage. In wet silages or in silages with high buffering capacity, the negative effect of acetic acid on feed intake would be confounded with other compounds formed by a poor fermentation (e.g. proteolysis end-products) and not because of the content of acetic acid itself. On the other hand, in silages inoculated with heterolactic bacteria (e.g. *Lactobacillus buchneri*) or in diets treated with pure acetic acid (experimental conditions) the increase of acetic acid concentration occurs independently of a significant change in proximate composition (e.g. NDF content) and without the concomitant production of undesirable compounds with hypophagic effects. The objective of this meta-analysis was to evaluate the effect of the dietary content of acetic acid on the dry matter intake (DMI) in dairy cattle.

**Material and Methods** The database was created with publications that addressed the effects of acetic acid on DMI by dairy cattle (cows and heifers). Six full articles published in peer reviewed journals (five manuscripts with cows and one enrolling heifers) and three abstracts (all with cows) reported in proceedings of international events with scientific board were compiled. Data were collected from publications where corn silage or high moisture corn silage were treated with *L. buchneri* or pure acetic acid was added onto the ration. Data from trials with sugarcane silage inoculated with heterofermentative bacteria was not included in the dataset, since in that silage the inoculant changes the proximal composition significantly, by preserving soluble sugars. The DMI was expressed as proportion of body weight (kg/100 kg BW = % BW) to standardize this measurement across animal categories (i.e., cows and heifers).

Data analysis was performed with the MIXED procedure of SAS. The initial model included random effects of experiment (intercept and slope) and fixed effects of acetic acid concentration (intercept, linear and quadratic terms). The covariance structure was defined as unstructured (UN). Because experimental designs and accuracy varied across studies, observations were weighted by the inverse of the standard error of the mean using the Weight statement. The quadratic effect of acetic acid was not significant ($P$ = 0.57) and then removed from the final model. Afterwards, data were fitted with a broken-line regression with two linear segments, using the NLMIXED procedure. Model adjustments were assessed by the corrected-Akaike's information criterion (AICc; smaller is better) and adjusted-$R^2$ (larger is better).

**Results and Discussion** Diet content of acetic acid ranged from 0 to 6% of DM, where the highest level was achieved by adding acetic acid to the ration. Although the linear model had a reasonable adjustment (AICc = -94.0, $R^2$-adj = 0.67), a broken-line regression showed better goodness of fit (AICc = -102.1, $R^2$-adj = 0.77). The DMI was slightly depressed from 0 to 1.73% (slope 1 = -0.024), and markedly impaired from 1.73 to 6% of acetic acid in diet (slope 2 = -0.043) (Figure 1). Therefore, the negative effect of silage acetic acid on feed intake will depend on the concentration of acetic acid in silage and the proportion of silage in the ration. Typical concentrations of acetic acid in silages vary from 1 to 3% of DM (Kung et al. 2018), but sometimes silages present higher levels of acetic acid. Although a higher content of acetic acid will protect the silage and total mixed ration against aerobic

deterioration (Wilkinson and Davies 2012), dairy nutritionists should consider the content of acetic acid in fermented ingredients upon balancing the diet, to avoid its negative effect on feed intake.

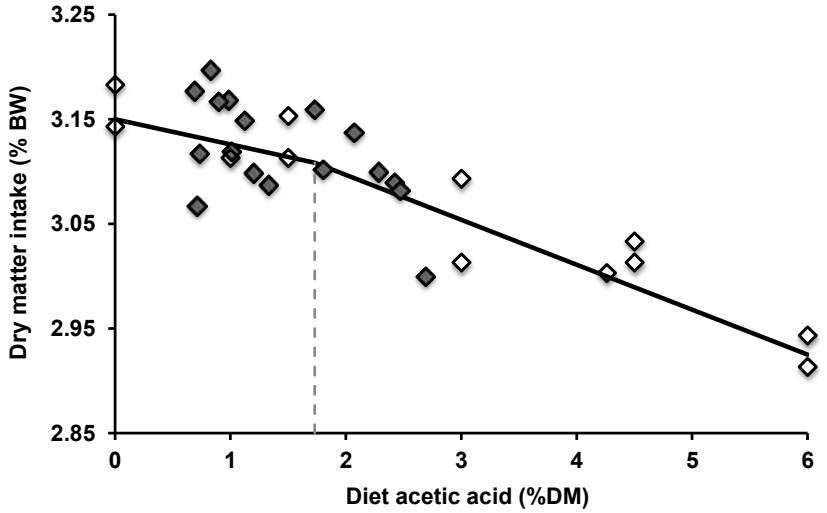


**Figure 1.** Relationship between dietary content of acetic acid (%DM) and dry matter intake (% BW) in dairy cattle (cows and heifers). If Acetic acid < 1.73, then DMI = 3.15 – 0.024 × Acetic acid, else DMI = (3.15 – 0.024 × Acetic acid) – 0.043 × (Acetic acid – 1.73). Open diamonds: acetic acid was added onto the ration. Closed diamonds: silage was inoculated with *L. buchneri*.

**Conclusion** The content of acetic acid in diet was negatively associated with DM intake in dairy cattle. However, the negative effect of acetic acid on feed intake depends on its dietary concentration, which is determined by the concentration of acetic acid in silage and the proportion of silage in the ration. A greater impairment on DM intake occurred when the content of acetic acid was higher than 1.73% of diet DM.

**References**

Kung, L. Jr., Shaver, R.D., Grant, R.J. & Schmidt, R.J. (2018) Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science, 101, 4020-4033.

Wilkinson, J.M. & Davies, D. R. (2012) The aerobic stability of silage: Key findings and recent developments. Grass and Forage Science, 68, 1-19.

# Corn silage (native vs. hybrid varieties) as forage to evaluate potential milk yield production in Mexico

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**Keywords:** Corn silage, milk yield, Mexico

**Introduction** Corn forage and milk yield production represent two of the main economic activities in Mexico (Reta et al. 2015). In the case of the cultivation of hybrid maize, greater emphasis has been given to the yield of forage per unit area, than to the nutritional quality, while native maize has greater advantages than hybrid maize as it is a rustic crop that requires low level of inputs such as fertilizers and pesticides.The objective of the present study was to determine the quality and forage yield (ton / ha) of corn silages produced in Mexico, as well as to estimate the potential production of milk with the Milk 2006 program.

**Materials and Methods** A search was made of studies focusing on the forage yield (ton / ha), density (number of plants / ha) and chemical composition, Dry matter (DM), Organic matter (OM), Crude protein (CP), Neutral detergent fiber (NDF), starch, fat, DM digestibility and NDF digestibility of corn silage produced in Mexico, according to its genetic line (native vs. hybrid). The net energy of lactation (NEL, MJ/kg DM), kilograms of milk per ton of dry matter (kg milk / ton DM) and kilograms of milk per hectare (kg milk / ha) were determined using the MILK2006 spreadsheet (Shaver 2006). The publications were obtained from searches in databases such as Elsevier, Google, SCOPUS and Web of Science. Using the terms “corn silage”, “production”, “Mexico” and “milk”, a total of 14 articles and 144 studies from 2001 to 2016 were obtained. A completely randomized design was used to consider the genetic line variables (native vs. hybrid), and a correlation analysis was performed. The effects were considered significant ($P < 0.05$), using the Tukey test for comparison of means.

**Results** Forage yield per ha was higher for the hybrids ($P < 0.001$) compared to the native silages, and the two were similar ($P > 0.05$) in terms of density of plants and their content of CP, NDF and DM and NDF digestibility. A trend ($P = 0.07$) was observed of NEL and kg milk / ton DM being higher in native’s silages than in hybrid silages; native silages were higher ($P = 0.03$) with respect to the hybrid silages in milk yield kg / ha. A positive correlation was observed ($P < 0.01$) for forage production and kg milk / ha, and Total Digestible Nutrients (TDN / kg DM )($P < 0.01$) with respect to the content of NEL (MJ / kg DM) and kg milk / ton DM.

**Discussion** The forage yield (ton DM / ha) and the % DM were higher in the hybrid maize with respect to the native silages presented in this study. This may be due to the plant density is 22% higher in hybrid maize at the time of cutting. Also, Elizondo and Boschini (2002) mention that when comparing hybrid maize against native with equal age at time of cutting, the hybrid maize surpasses the dry matter content by 400% with respect to the native silages, probably because some hybrids are early mature varieties. In addition, the increase in milk production that occurs with native maize can be explained because hybrid maize with a greater amount of forage yield evidences decreased ear production (Nuñes et al. 2003), which can decrease the amount of starch in the plant, having lower NEl causing lower milk yields per ton DM and kg milk / ha (Ferraretto and Shaver 2013; Lascano et al. 2016). The correlations obtained in this study agree with those of Shaver and Lauer (2006). Schwab et al. (2003) mentions that the Milk2006 spreadsheet model has the basic concept of a summative

energy equation, which coincides in this work, obtaining a significant correlation in terms of NEL concentration and the estimated production of kg milk / ton DM ( r = 0.99).

**Conclusions** The use of native corn silage has a greater potential for milk production / ha and milk yield/ ton DM compared to hybrid silage produced in Mexico probably by their late maturity stage. The conservation of native genetic resources such as corn is thus important in order to maintain sustainable forage resources.

**Table 1.** Forage production (ton / ha), plant density, chemical composition of the silage and the potential milk production (kg milk / ton DM and kg milk / ha) of hybrid and native corn silages sown in Mexico.

| | Native silage | Hybrid silage | | |
|---|---|---|---|---|
| | Mean | Mean | SEM | P-value |
| Forage Yield (DM/ ha) | 18.34[b] | 19.23[a] | 1.41 | 0.0018 |
| Density (plants/ha) | 65714.28 | 80492.59 | 3112 | 0.6226 |
| DM % | 20.95 | 30.34 | 2.05 | 0.0001 |
| DMd % | 65.56 | 66.56 | 1.68 | 0.1144 |
| CP% | 6.97 | 7.75 | 0.435 | 0.7266 |
| NDF % | 52.56 | 57.02 | 2.84 | 0.1261 |
| NDFd % | 58.72 | 57.46 | 1.33 | 0.3715 |
| TDN-DM % | 65.33 | 63.52 | 1.26 | 0.1058 |
| NEL MJ/kgDM | 5.82 | 5.65 | 0.026 | 0.0700 |
| Kg Milk/ton DM | 516.84 | 493.24 | 15.52 | 0.0733 |
| Kg Milk/ ha | 22471[a] | 22399[b] | 1654.97 | 0.0307 |

DM = dry matter content, DMd = dry matter digestibility, CP = crude protein, NDF = neutral detergent fiber, NDFd = neutral detergent fiber digestibility, TDN = total digestible nutrients, NEL = net energy for lactation (MJ / kg DM), kg milk / ton DM = kilograms of milk per ton of dry matter, Kg milk / ha = kilograms of milk per hectare.

**References**

Elizondo, J. & Boschini, C. (2002) Producción de forraje con maíz criollo y maíz híbrido. Agronomía Mesoamericana, 13, 13-17.

Ferraretto, L.F. & Shaver, R.D. (2013) Meta-analysis: Effects of corn silage hybrid type on intake, digestion, and milk production by dairy cows. Journal of Dairy Science, 96, 214.

Lascano, G.J., Alende, M., Koch, L.E. & Jenkins, T.C. (2016) Changes in fermentation and biohydrogenation intermediates in continuous cultures fed low and high levels of fat with increasing rates of starch degradability. Journal of Dairy Science, 99, 6334-6341.

Núñez-Hernández, G., Contreras-G, E.F. & Faz-Contreras, R. (2003) Important agronomic and chemical characteristics in high energy hybrid forage corns. Técnica Pecuaria en México, 41(1), 37-48.

Reta-Sánchez, D.G., Figueroa-Viramontes, U., Serrano-Corona, J.S., Quiroga-Garza, H.M., Gaytan-Mascorro, A. & Cueto-Wong, J. A. (2015) Forage potential and wáter productivity in alternative cropping patterns. Revista Mexicana de Ciencias Pecuarias, 6(2), 153-170.

Schwab, E.C., Shaver, R.D., Lauer, J.G. & Coors, J.G. (2003) Estimating silage energy value and milk yield to rank corn hybrids. Animal Feed Science and Technology, 109, 1-18.

Shaver, R. (2006) Corn silage evaluation: MILK 2000 challenges and opportunities with MILK 2006. http://www.uwex.edu/ces/dairynutrition/ documents/milk20062.pdf. 12-09-2017.

Shaver, R.D. & Lauer, J.G. (2006) Review of Wisconsin corn silage milk per ton models (Abstr.). Journal of Dairy Science, 89, 282-283.

# Digestion kinetics of neutral detergent fibre fraction of corn silages determined from *in vitro* gas production

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**Keywords:** Corn silage, Kinetics, Digestion, *In vitro*

**Introduction** Fibre digestion largely determines the feed value of corn silages for dairy cows. It impacts ruminal pH, volatile fatty acids production, dry matter intake and milk production. Assessment of digestion kinetics of the neutral detergent fibre (NDF) fraction of corn silages is increasingly important as ration balancing programs are designed to optimize rumen function and to predict nutrient supply (Deaville and Givens 2001). The *in vitro* gas production technique can be used as rapid tool to study feed degradation over numerous time points, allowing more accurate parameter estimation than other *in vitro* or *in situ* evaluation methods (Huhtanen et al. 2008). Our objectives were to evaluate variability in digestion kinetics of NDF fraction of corn silages, and to examine potential relationships between NDF degradability and their digestion kinetics determined from *in vitro* gas production.

**Materials and Methods** 10 conventional and 4 brown midrib (BMR) corn silage hybrids dried in 60 °C forced air ovens and ground to a 1 mm particle size on a Foss Cyclotec 1093 grinder, were selected for analysis with NDF values ranging from 32 - 45% dry matter (DM). The NDF was extracted and isolated from each sample using an ANKOM$^{2000}$ Fibre Analyzer (Ankom Technology, Macedon, NY, USA) and stored for fermentation analysis. The amount of extracted NDF run per sample was equivalent to the amount extracted from 0.5 g of corn silage. These samples were placed into 250 ml bottles equipped with ANKOM RF Gas Production Modules (Ankom Technology, Macedon, NY, USA). Prior to their morning feeding, rumen fluid was collected from two lactating, cannulated Holstein cows, being fed a conventional lactation diet, and placed in a pre-warmed container for transportation to the lab where it was filtered under $CO_2$ through 4 layers of cheese cloth. The particle free rumen fluid was then mixed with a rumen buffer in a 1:3 (v/v) ratio and 50 ml of the buffered inoculum was added to each sample bottle. Modules were set to vent at 1 psi and the gas volumes were measured every 15 minutes for 72 hours. A single-pool exponential model with a discrete lag time (Schofield et al. 1994) was used to fit these data for obtaining kinetic parameters of *in vitro* gas production from extracted NDF. The model is $Y = Vmax * (1 - e^{-K*(t-L)})$, where Y is the volume of gas produced at time t, Vmax the maximum gas production (mL/g DM), K the fractional gas production rate (1/hour), and L the discrete lag time (hours). Further, uNDF240 (i.e., undigestible NDF after incubation of 240 hours) was determined using wet chemistry method. Lignin and NDF degradability at 8, 12, 24, 30, 48, 72, and 120 hours for each of corn silage samples were determined by near-infrared reflectance (NIR) scans (FOSS NIRS DS2500). All digestion kinetic parameters, the Pearson correlation coefficients and regression relationships among selected variables were determined using the procedures of SAS (SAS Enterprise Guide 5.1).

**Results and Discussion** The one-pool exponential model fitted all the cumulative gas curves from the digestion of the extracted NDF of corn silages with $R^2$ higher than 0.97. The cumulative maximal gas production volume differed between BMR (373.0 ± 20.5) and conventional (336.3 ± 15.2) corn silages (Figure 1; $P < 0.01$). There was no significant difference in the fractional gas production rates of the extracted NDF between BMR (0.078 ± 0.0017) and conventional (0.076 ± 0.0051) corn silages. Lag times of NDF digestion from BMR (2.57 ± 0.21) and conventional (2.45 ± 0.13) corn silages did not differ.Fractional gas production rates were poorly correlated with uNDF240, lignin, and NDF degradability determined at various time points. However, maximal volume of gas production

predicted was correlated with uNDF240 (r = - 0.71; *P* < 0.01) and lignin (r = - 0.47; *P* = 0.09). In addition, a strong relationship occurred between cumulative gas production and NDF degradability determined at time of 8, 12, 24, 30, 48, and 72 hours (Figure 2; $R^2$ = 0.93; *P* < 0.01). Higher contents of uNDF240 and lignin occurred in conventional vs. BMR corn silages; however, fractional gas production rates did not reflect the variation of chemical compositions of extracted NDF from BMR and conventional corn silages. Tovar-Gómez et al. (1997) indicated that the fractional rate of *in situ* NDF degradation did not vary among stalks from different corn genotypes, and that the amount of lignin had no significant impact on NDF degradation rate but was closely related to the extent of degradation.

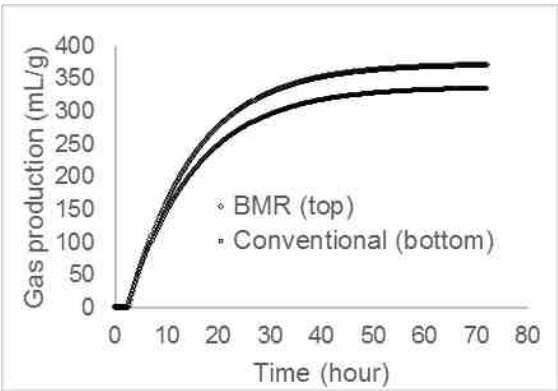


**Figure 1.** Average cumulative gas production from extracted NDF of BMR (n = 4) and conventional (n = 10) corn silages.

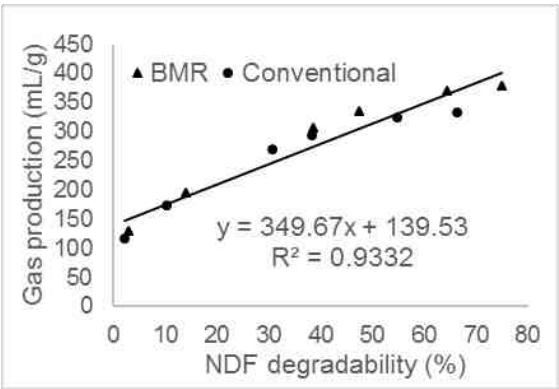


**Figure 2.** Relationship between gas production from extracted NDF and NDF degradability of corn silages determined at 8, 12, 24, 30, 48, and 72 hours. Each point represents the mean gas production and NDF degradability of BMR (n = 4) and conventional (n = 10) at a given time point.

**Conclusions** The use of the automated gas production system to estimate digestion kinetics of extracted NDF provides valuable insights into the fibre degradation of corn silages. It appeared that the fractional gas production rate did not vary between the different genotypes of corn silage. Nevertheless, the volume of gas production explained a large portion of variation in the extent of NDF digestion between BMR and conventional corn silages. Further studies, using *in vitro* gas production technique, on more corn silage samples from different genotypes are needed to confirm current observations.

**References**

Deaville, E.R. & Givens, D.I. (2001) Use of the automated gas production technique to determine the fermentation kinetics of carbohydrate fractions in maize silage. Animal Feed Science and Technology, 93, 205-215.

Huhtanen, P., Seppälä, A., Ots, M., Ahvenjärvi, S. & Rinne, M. (2008) In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen. Journal of Animal Science, 86, 651-659.

Schofield, P., Pitt, R.E. & Pell, A.N. (1994) Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science, 72, 2980-2991.

Tovar-Gómez, M.R., Emile, J.C., Michalet-Doreau, B. & Barrière, Y. (1997) In situ degradation kinetics of maize hybrid stalks. Animal Feed Science and Technology, 68, 77-88.

# Effect of differently conserved herbage on chemical composition of forages and nitrogen turnover in dairy cows

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**Keywords:** silage, hay, crude protein composition, feed intake, N balance

**Introduction** In forages derived from herbage high ruminal degradability of crude protein (CP) can result in poor nitrogen (N) utilisation by ruminal microbes and N losses to the environment. However, the mode of conservation can influence the CP fractions in forage and, thereby, the potential N utilisation by the animal. The objective of the present study was to determine the effect of three types of forage preservation on CP fractions and N turnover in dairy cows.

**Material and Methods** Herbage from a 34-d regrowth was harvested on 30th August 2016 from a ley mainly composed of *Lolium perenne*, *Trifolium repens* and *T. pratense*. After 24 h of wilting one third of the forage was baled without additives at a dry matter (DM) content of 56% (SI). A further third of the forage, after 26 h on the field, was put on the ventilation at an average DM content of 68% and dried to 88% DM (VH). The rest was harvested after 72 h drying on the field at 86% DM (FH). The three forage types were utilized in a replicated 3 × 3 Latin Square arrangement of treatments, to six multiparous Holstein cows. The experimental periods lasted 21 days and included a 14-day adaptation and a 7-day data collection period. The cows were fed for ad libitum intake either silage (SI), ventilated hay (VH) or field-dried hay (FH) and received 300 g/day of a mineral mix. During the collection periods cows received 95% of the intake of the adaptation period. During the data collection period, feed intake, milk yield and milk composition were recorded daily and excreta were completely collected. On day 2 and 5 of each collection period, ruminal fluid via stomach tube and blood were sampled. Non-protein N, buffer-soluble N and fibre-bound N were analysed according to Licitra *et al.* (1996) in the conserved forage. Subsequently, CP fractions A, B1, B2, B3 and C were calculated. These CP fractions are assumed to differ in rate and extent of ruminal degradability, with A being non-protein N, C being undegradable N and B1 to B3 being intermediate (Sniffen *et al.* 1992). Data were subjected to ANOVA using MIXED procedure of SAS 9.2 software (SAS Institute Inc., Cary, NC).

**Results and Discussion** The contents of CP (g/kg DM) and net energy for lactation (NEL, MJ/kg DM) were highest in SI (207; 5.9) followed by VH (187; 5.5) and FH (176; 5.4). All three forages were poor in energy and high in CP. The different CP fractions in the herbage and the three conserved forages are shown in Figure 1. The patterns of CP fractions for the respective treatments were similar to those described by Wyss (2018). The silage showed a very good quality. On average the pH was 5.5, with 27 g/kg DM lactic acid, 5 g/kg DM acetic acid and 1 g/kg DM butyric acid. The proportion of ammonia-N of total N amounted 4.5% and the silages attained 91 DLG-Points. Milk yield and milk components did not differ ($P>0.05$) among treatments. On average cows yielded 19.7 kg/day of milk with 4.93% fat and 3.79% protein. Cows receiving SI had a lower ($P<0.05$) DM intake (17.3 kg/d) than cows fed VH (19.2 kg/d), and cows fed FH (17.9 kg/d) were intermediate. According to Jans (1991) the DM intake of silage is often lower than DM intake of hay from the same plot and harvested at the same time. Daily N intake was greater ($P<0.05$) for VH cows compared to FH cows. The N intake of SI cows did not differ from the other cows. The N excreted with milk tended to be higher with VH (123 g/d) compared to FH (113 g/d; $P=0.08$) and SI (112 g/d; $P=0.07$).

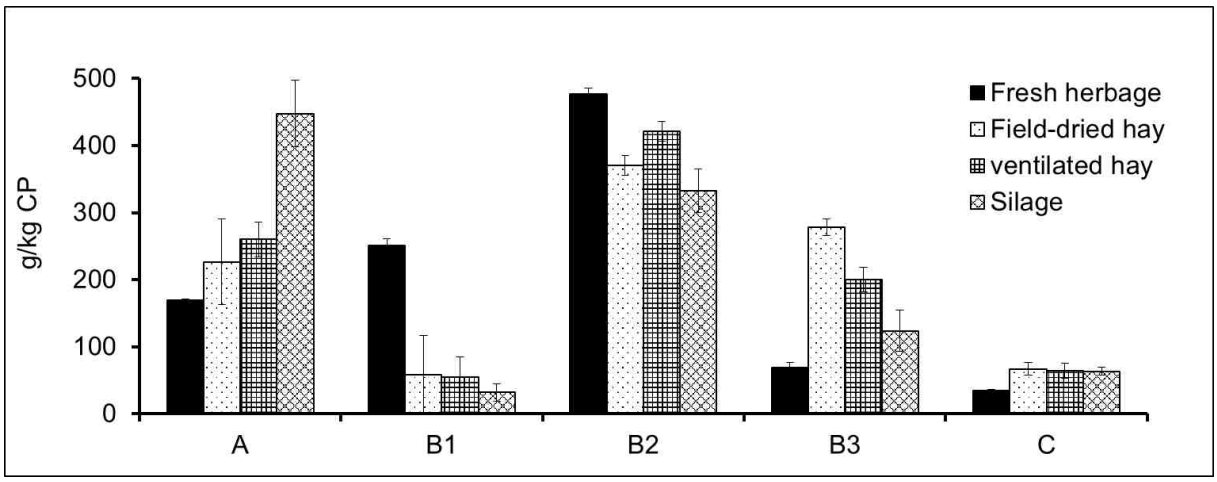


Fig. 1. Crude protein fractions in the fresh herbage and the conserved forage. CP, crude protein, A, B1, B2, B3, C, CP fractions according to Sniffen et al. (1992)

Faecal N excretion was similar (P>0.05) for all treatments (177 g/d) whereas urinary N excretion tended to be higher (P=0.06) for cows fed SI (307 g/d) compared to cows fed FH (251 g/d). Cows fed VH (295 g/d) were intermediate. Treatments had no effect (P>0.05) on the N balance which was negative (-27.2 g/d) for all cows. Presumably due to the deficit of energy the high CP quantity could not be used for the microbial synthesis in the rumen and the surplus of CP, which was degraded to ammonia had to be eliminated via the urine (Sutter 1998). Furthermore, the limitation of the intake during the collection period of 95% of the adaptation period could have influenced the N balance. Windisch et al. (1989) found a negative N balance, when the energy and protein supply was deficient. The proportion of faecal N (% of N intake) was lower (P<0.01) for cows fed SI (30.0) and VH (31.4) compared to cows fed FH (35.3). The proportion of urinary N (52.0% of N intake) did not differ among treatments. The milk urea contents were different (P<0.01). For SI, VH and FH the values amounted to 370, 351 and 306 mg/kg.

**Conclusion** Silage making had a greater impact on CP composition than haymaking. Especially the fraction A increased during the fermentation process. Feeding only forage derived from herbage in late lactation resulted in high urinary N losses and an N deficiency in dairy cows. The absolute excretion of urinary N was highest when cows were fed silage, which had more non-protein-N (fraction A) than the two other forages.

**References**

Jans, F. (1991) Grassilage oder Dürrfutter für Hochleistungskühe? Landwirtschaft Schweiz 4, 333-336.

Licitra, G., Hernandez, T.M. & Van Soest, P.J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347-358.

Sniffen, C.J., O'Connor, J.D., Van Soest, P.J., Fox, D.G. & Russell, J.B. (1992) A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562-3577.

Sutter, F. (1998) Nachhaltige Alpung von Milchkühen und Mutterkühen: Auswirkungen auf Leistung, Stoffwechsel und Stickstoffverwertung. 4. Alpenländisches Expertenforum 1998, Irdning. p.15-20.

Windisch, W., Müller, H.L. & Kirchgessner M. (1989) Stickstoffbilanz und Stickstoffverwertung bei der Milchkuh während und nach Energie- und Proteinmangel. Journal of Animal Physiology and Animal Nutrition. 61, 206-213.

Wyss, U. (2018) Influence of silage making or haymaking on different protein fractions. Proceedings of 27th General Meeting of the European Grassland Federation, Cork, Ireland. In press.

# Effect of dry matter concentration and a chemical silage additive on fermentation quality of ensiled sugar beet pulp and preference shown by goats

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**Keywords:** by-product, preference, silage additive, sugar beet

**Introduction** Beet pulp as by-product of sugar production from sugar beets can be used as feedstuff for ruminants containing considerable amounts of pectins and metabolizable energy (ME). After sugar extraction, the beet pulp typically contains low dry matter (DM) and is ensiled in bunkers or silo bags. Different technical treatments are currently implemented in sugar factories to reduce the water content of those humid by-products. There is a lack of research on the effects of increasing the DM concentration of beet pulp on fermentation quality and the acceptance when offered to ruminants. The aim of this study was to evaluate the effect of DM concentration, addition of molasses and a chemical additive on chemical composition of ensiled sugar beet pulp and on preference shown by goats.

**Material and Methods** Directly after delivery from the sugar factory, sugar beet pulp was compacted and ensiled in 120-L plastic barrels (n = 2/treatment). A total of eight different treatments was prepared: DM 23%, DM 32%, DM 45%, DM 45% + molasses, each one ensiled with and without a chemical additive based on sodium benzoate, potassium sorbate and ammonium propionate (1.5 l/t). After seven months of storage, barrels were opened, ensiled sugar beet pulp was taken out and sampled for chemical analysis (proximate constituents, fibre fractions, *in vitro* 24-h gas production (GP) and ME, pH, fermentation products and aerobic stability). A preference trial with goats (n = 8) was conducted to study the treatment effects on eating behaviour and short-time DM intake (DMI) according to Buntinx et al. (1997). After an adaptation period each possible combination of two treatments was presented as free choice for 3 h with measuring DMI in 30 min intervals. In each case, sugar beet pulp was presented as sole feed. Data were analyzed using SAS 9.4 and an analysis of variance including terms for animal and treatment. Within the treatments, means were separated using the minimum significant difference from the Waller-Duncan k-ratio t-test.

**Results** All treatments were well fermented with low pH and high sensory quality. However, fermentation pattern varied considerably in dependence of treatment and differences were most pronounced for concentrations of lactic and acetic acids and ethanol (Table 1).

**Table 1**. Chemical composition of different treatments of sugar beet pulp ensiled without (-) or with (+) a chemical additive and molasses (mol) (in g/kg dry matter (DM) unless stated)

| | P23- | P23+ | P32- | P32+ | P45- | P45+ | P45mol- | P45mol+ |
|---|---|---|---|---|---|---|---|---|
| DM (g/kg) | 230 | 234 | 315 | 320 | 445 | 427 | 458 | 484 |
| Crude protein | 97.4 | 97.3 | 92.2 | 87.5 | 90.6 | 88.3 | 87.5 | 79.6 |
| aNDFom | 461 | 468 | 522 | 500 | 517 | 495 | 471 | 397 |
| ME (MJ/kg DM) | 12.8 | 12.8 | 13.3 | 13.1 | 13.6 | 13.7 | 13.4 | 11.5 |
| pH | 3.56 | 3.53 | 3.73 | 3.58 | 3.96 | 3.78 | 3.85 | 3.89 |
| Lactic acid | 44.1 | 56.1 | 29.5 | 52.2 | 14.2 | 25.7 | 20.6 | 25.5 |
| Acetic acid | 13.5 | 23.4 | 26.6 | 23.1 | 20.5 | 20.6 | 17.2 | 14.1 |
| Propionic acid | 0.7 | 1.0 | 3.2 | 1.0 | 3.7 | 1.2 | 0.4 | 0.3 |
| Ethanol | 5.9 | 3.4 | 25.6 | 4.5 | 14.7 | 3.4 | 2 | 1.4 |
| Propanol | 1.3 | 3.6 | 5.4 | 2.8 | 4 | 2.1 | 0.2 | 0.2 |
| WSC | 14.9 | 6.4 | 5.1 | 9.1 | 13.7 | 22.2 | 42.4 | 41.3 |

aNDFom = Acid detergent fibre treated with heat-stable amylase and expressed exclusive residual ash; ME = Metabolizable energy; WSC = Water-soluble carbohydrates.

The aerobic stability was very high (> 7 d) for all treatments. The ME concentration, estimated from chemical composition and GP ranged consistently high (12.8-13.7 MJ ME/kg DM) except for one treatment. In the preference trial, goats strongly differed between treatments with highest short-time DMI (828 g/3 h) for DM 45% with added molasses and without silage additive ($P < 0.001$). Interestingly, the respective treatment ensiled with additive was the most avoided feed (194 g DM/3) ($P < 0.001$). The fermentation products of that treatment were inconspicuous; however, its GP was lower than the GP of the other silages. Other treatments that were less preferred showed low DM, low pH and slightly increased concentrations of acetic acid. In general, preference increased with increasing DM concentration.

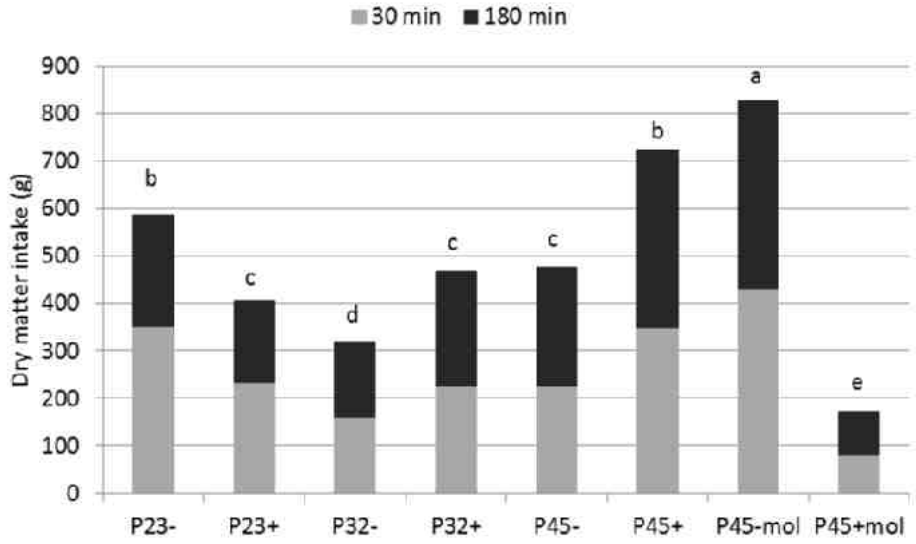


**Figure. 1**. Short-time dry matter (DM) intake of different treatments of sugar beet pulp (P) ensiled with 23, 32 or 45% DM, without (-) or with (+) a chemical additive and molasses (mol) shown by goats (n = 56)

**Discussion** In general, preference for ensiled sugar beet pulp increased with increasing DM concentration. Preparing beet pulp silages with higher DM than commonly before could therefore be a useful way to improve its palatability for ruminants. Low pH values and increased concentrations of acetic acid might have caused reductions in DM intake as also described by Eisner et al. (2006). The strong avoidance of one treatment (P45+mol) is difficult to explain and needs further research. Obviously, a negative post-ingestive feedback caused avoidance. The reduced in vitro GP might give evidence for impaired ruminal degradability. Chemical analysis alone might not provide complete information when evaluating new technical or chemical treatments for feedstuffs, as shown by the diverging intake behaviour of the animals for silages with only small differences in chemical composition.

**Conclusion** Sugar beet pulp fermented well in a big range of DM concentration (23-45% DM) and showed high aerobic stability irrespective of treatment. The preference of goats was strongly influenced by treatment and ranged from 194 to 828 g DM/3 h such that influencing the ensiling conditions; e.g. by increasing the DM concentration offers an important possibility to influence the acceptance of ensiled sugar beet pulp for ruminants.

**References**

Buntinx, S.E., Pond, K.R., Fisher, D.S. & Burns, J.C. (1997). The utilization of multidimensional scaling to identify forage characteristics associated with preference in sheep. Journal of Animal Science, 75, 1641-1650.

Eisner, I., Südekum, K.-H. & Kirchhof, S. (2006) Beziehungen zwischen Fermentationscharakteristika von Silagen und der Futteraufnahme von Milchkühen. Übersichten Tierernährung, 34, 197-221.

# Effect of maize hybrid and year on chemical composition and digestibility of nutrients

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**Keywords:** fermentation, *in vivo*, neutral detergent fibre, nutritive value, silage

**Introduction** Numerous studies have evaluated the impact of maize hybrid on chemical composition and digestibility of silage (Hetta et al. 2012). The combined effects of weather and genotypes on maize chemical composition were considered by Kruse et al. (2008), while Darby and Lauer (2002) investigated the effects of hybrids on chemical composition development as crop maturity progressed through to harvest time. The objective of our study was to confirm the declared properties of the selected two maize hybrids two years, especially in terms of amylase-treated neutral detergent fibre digestibility (aNDFD) in silage, which could strongly influence the result of the calculation of the energy value of the fodder.

**Materials and Methods** Silages from two maize hybrids were made two years in a row. Both hybrids (H1, H2) are early maturing (FAO 250 for silage) stay-green varieties. Both are flint x dent hybrids, three-way (Tc). Both hybrids were planted in the same field in randomised blocks with three replicates per hybrid and harvested by a cutter Claas Jaguar 690 with a corncracker and chopped to a theoretical length of cut (TLC) of 12 mm when grain was at 2/3 milk line. No biological or chemical additives were used in the ensiling process. The silages were stored in large plastic containers in two repetitions per hybrid/year, each with a volume of 1.2 $m^3$ and sealed with a black and white foil of thickness 250 microns. The methodology of filling, compressing and sealing was the same for all variants. After complete fermentation (90 days), the silages were opened and used for an *in vivo* balance experiment with 6 sheep. Three samples of approximately 2 kg each, taken from each container of the final silages, were used for chemical analyses (AOAC 2005). Statistical values were processed using the software package Statistica 10 (StatSoft, Tulsa, OK, USA) while utilizing the methods for calculating ANOVA, Tukey's test.

**Results** Chemical composition, fermentation quality, and nutrient digestibility of silages are presented in Table 1. Hybrid had a greater effect upon all indicators of chemical composition and digestibility than year of cultivation, but not for indicators of fermentation quality (pH, voluntary fatty acids).

**Discussion** Our objective was to harvest both hybrids for silage at the 2/3 milk line stage considered optimal for silage-making and which is recommended, for example, by Peyrat et al. (2014). So, we found no differences in DM between hybrids ($P$=0.183). Most similar research compares hybrids harvested at different DM levels, thereby reflecting different stages of maturity (Hetta et al. 2012). Along with the change in DM, there are changes also in other indicators of silage nutritional value. In our study, all measures of chemical composition differed between hybrids ($P$<0.05). Differences between years were not confirmed only in relation to aNDF and starch ($P$=0.083 and $P$=0.328, respectively). The fermentation quality can be characterized by average values, which indicate good fermentation. The good fermentation quality for all silages reflects the very careful attention we gave to producing the experimental silages. Good quality of fermentation is a major prerequisite for properly evaluating silages. No differences between hybrids or years were found for the silages according to any indicator of fermentation quality. Standard deviations of means were not very large for any parameter. The CPD and aNDFD values measured in silages of H2 hybrid were higher ($P$<0.001) than those of H1. As compared to tabulated values (Zeman et al., 1995) where ensilaged maize (harvested with 31% dry matter) shows a digestibility of crude protein at 57.5 % and the digestibility of crude fibre at 63.8 %, the CPD and aNDFD of the H2 were higher, while those of H1 were lower.

**Table 1**. Effect of hybrid (H) and year (Y) on chemical composition, fermentation quality, and digestibility of silages.

| H, Y | | H1 | H2 | Y1 | Y2 | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|
| Indicator | Unit | | | | | SEM | H | Y | H x Y |
| DM | g/kg | 327 | 321 | 318 | 331 | 3.06 | 0.183 | 0.008 | <0.001 |
| CP | g/kg DM | 84 | 90 | 89 | 86 | 0.75 | <0.001 | 0.023 | <0.001 |
| Ash | g/kg DM | 47 | 52 | 47 | 51 | 0.58 | <0.001 | <0.001 | <0.001 |
| aNDF | g/kg DM | 460 | 488 | 474 | 475 | 3.63 | <0.001 | 0.083 | 0.004 |
| ADF | g/kg DM | 274 | 252 | 270 | 256 | 2.51 | <0.001 | 0.001 | 0.272 |
| Starch | g/kg DM | 292 | 298 | 305 | 309 | 5.31 | 0.031 | 0.328 | 0.781 |
| LA | g/kg DM | 72.2 | 70.8 | 73.8 | 69.2 | 1.66 | 0.551 | 0.064 | 0.003 |
| AA | g/kg DM | 20.1 | 21.3 | 21.9 | 19.5 | 1.16 | 0.462 | 0.168 | 0.309 |
| LA/VFA | - | 3.50 | 3.29 | 3.32 | 3.47 | 0.16 | 0.358 | 0.508 | 0.514 |
| pH | - | 3.74 | 3.77 | 3.71 | 3.80 | 0.03 | 0.493 | 0.058 | 0.520 |
| DMD | % | 67.7 | 72.0 | 70.4 | 69.3 | 0.72 | <0.001 | 0.295 | 0.034 |
| CPD | % | 56.1 | 61.8 | 59.7 | 58.2 | 1.14 | 0.002 | 0.368 | 0.197 |
| OMD | % | 69.4 | 73.9 | 72.2 | 71.1 | 0.77 | 0.001 | 0.309 | 0.031 |
| aNDFD | % | 53.4 | 64.7 | 61.3 | 56.8 | 1.48 | <0.001 | 0.044 | 0.053 |
| ADFD | % | 52.2 | 59.0 | 60.2 | 51.0 | 1.35 | 0.002 | <0.001 | 0.001 |

AA – acetic acid, ADF – acid detergent fibre, ADFD – ADF digestibility, aNDF – amylase-treated neutral detergent fibre, aNDFD – aNDF digestibility, CP – crude protein, CPD – CP digestibility, DM – dry matter, DMD – DM digestibility, LA – lactic acid, OMD – organic matter digestibility, VFA – volatile fatty acids

**Conclusion** The results confirm the importance of breeding hybrids and analysing silages for aNDFD. Hybrid had a greater effect upon all indicators of chemical composition and digestibility than year of cultivation, but not for indicators of fermentation quality (pH, volatile fatty acids).

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**References**

AOAC (2005) Official Methods of Analysis. 18th Ed. AOAC International, USA.

Darby, H.M. & Lauer, J. G (2002) Harvest date and hybrid influence on corn forage yield, quality, and preservation. Agronomy Journal, 94, 3, 559-566.

Hetta, M., Mussadiq, Z., Gustavsson, A.M. & Swensson, C. (2012) Effects of hybrid and maturity on performance and nutritive characteristics of forage maize at high latitudes, estimated using the gas production technique. Animal Feed Science and Technology, 171, 1, 20-30.

Kruse, S., Herrmann, A., Kornher, A. & Taube, F. (2008) Evaluation of genotype and environmental variation in fibre content of silage maize using a model-assisted approach. European Journal of Agronomy, 28, 210-223.

Peyrat, J., Noziere, P., Le Morvan, A., Ferard, A., Protin, P. V. & Baumont, R. (2014) Effects of ensiling maize and sample conditioning on in situ rumen degradation of DM, starch and fibre. Animal Feed Science and Technology, 196, 12-21.

Zeman, L., Šimeček, K., Krása, A., Šimek, M., Lossman, J., Třináctý, J., Rudolfová, Š., Veselý, P., Háp, I., Doležal, P., Kráčmar, S., Tvrzník, P., Michele, P., Zemanová, D. & Šiške, V. (1995) Feed Catalog (Katalog krmiv). VÚVZ Pohořelice, pp. 465. (in Czech).

# Effect of maize hybrids differing by maturity and endosperm type on digestibility of silage

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**Keywords:** correlation, dent, earliness, flint, milk line

**Introduction** Improving the digestibility of forage maize through plant breeding is important for optimizing the efficiency of ruminant rations. This can be achieved in part by improving the digestibility of stem tissue, a genetically complex and diverse trait that changes during the growing season (Boon et al. 2012). The aim of this study was compared maize hybrids of the early maturity group (FAO 230–260) and the moderately early group (FAO 290–340), while comparing also the influence of endosperm type (dent or flint kernel and intermediate values for these), according to the chemical composition, ensilability, and especially *in vivo* total tract digestibility of silage.

**Materials and Methods** Eight tested hybrids were grown, harvested, and ensiled under identical conditions. No biological or chemical additives were used in the ensiling process. The silages were stored in large plastic containers each with a volume of 1.2 $m^3$, silage density 700 $kg.m^{-3}$ and sealed with a black and white foil of thickness 250 microns. After complete fermentation (90 days), the silages were opened and used for an *in vivo* experiment. Three samples of approximately 2 kg each, taken from each container of the final silages, were used for chemical analyses (AOAC, 2005). *In vivo* total tract digestibility of 8 maize silages was measured in digestion trials with 6 sheep (Homolka, 2000). Statistical values were processed using the software package Statistica 10 (StatSoft, Tulsa, OK, USA) while utilizing the methods for calculating ANOVA, Tukey's test.

**Results** The effect of earliness (early maturity group (FAO 230–260) vs. moderately early (FAO 290–340) had significant ($P$<0.05) effect on the chemical composition, fermentation quality, and digestibility of neutral and acid detergent fibre. Type of kernel endosperm (dent vs. flint) differed by digestibility of nutrients (Table 1).

**Table 1.** Digestibility (%) of maize silages by hybrids group (G, T)

| | FAO maturity group (G) | | | Type of endosperm (T) | | | | | Effect | |
|---|---|---|---|---|---|---|---|---|---|---|
| Index | E | M | SEM | F | IF | ID | D | SEM | G | T |
| DMD | 69.9 | 68.8 | 0.6 | $69.4^{ab}$ | $68.9^{ab}$ | $67.4^{b}$ | $71.7^{a}$ | 0.9 | 0.205 | 0.018 |
| OMD | 71.9 | 70.8 | 0.7 | $71.3^{ab}$ | $71.0^{ab}$ | $69.6^{b}$ | $73.7^{a}$ | 0.9 | 0.239 | 0.027 |
| aNDFD | $57.7^{B}$ | $53.1^{A}$ | 1.3 | $48.8^{a}$ | $53.2^{ab}$ | $59.5^{b}$ | $58.2^{b}$ | 1.8 | 0.049 | <0.001 |
| ADFD | $55.1^{B}$ | $47.3^{A}$ | 1.5 | $44.7^{a}$ | $46.5^{a}$ | $57.5^{b}$ | $56.2^{b}$ | 2.1 | <0.001 | <0.001 |

FAO – maturity index; E – early FAO group (FAO 230–260); M – moderately early FAO group (FAO 290–340); F – flint; IF – intermediate F; ID – intermediate D; D – dent; DMD – dry matter digestibility; OMD – organic matter digestibility; aNDFD – amylase-treated neutral detergent fibre digestibility; ADFD – acid detergent fibre digestibility; A-B, a-b within a row means with different superscripts differ ($P$<0.05) for G and T, respectively

The values of aNDF ranged from 357 to 512 g/kg DM, OMD from 63.4% to 75.7%, and aNDFD from 49.7% to 67.6%. Effect of earliness had a significant ($P$<0.05) effect on digestibility of aNDF (aNDFD) and ADF (ADFD), but not on digestibility of DM (DMD) and organic matter (OMD). By contrast the effect of kernel endosperm type (dent vs. flint vs. intermediates) was significant ($P$<0.05) for the digestibility of all nutrients. Digestibility of DM, OM and aNDF were highest ($P$<0.05) for silage of the dent hybrid FAO 250 and these digestibility values were significantly ($P$<0.05) lower for silage of the

dent hybrid FAO 320. The data presented that all correlation coefficients between the digestibility values were significantly ($P$<0.01) high (DMDxOMD 0.99, DMDxaNDFD 0.91, OMDxaNDFD 0.92).

**Discussion** The experiment was organized in such a way that plants were harvested at very similar stages of maturity at 2/3 ML at similar DM content (311 ± 12.1 g/kg; P>0.05). That had been the case, too, in our previous work (Loucka et al, 2015). All silages had good fermentation quality, as indicated by pH 3.83 ± 0.15 and ratio of lactic acid/volatile fatty acids of 3.42 ± 1.06. Conducting *in vivo* experiments with sheep over a period of 34 years, Barriere et al. (2004) had found that the digestibility of amylase-treated neutral detergent fibre (aNDF) of silages made using different maize hybrids within the range FAO 170–350 varied from 35.9% to 60.4%. Although the ranges are somewhat narrower, these values are similar to those reported by Ferret et al. (1997). They had confirmed mainly a wide range of content and OMD and aNDFD. Di Marco et al. (2002) had confirmed that *in vivo* DMD of silage remained constant with maturity because the depression in aNDFD could be compensated by starch accumulation in the grain. The objective of earlier research by Moreno-Gonzalez et al. (2000) had been to determine the potential performance and heterosis when using flint and dent populations as base germplasm for developing silage maize hybrids. In our study, the relevant differences (P>0.05) between intermediate and specialized types of hybrids were not observed.

**Conclusion** The digestibility differed mainly in dent-type hybrids. All correlation coefficients between the digestibility values were significantly ($P$<0.05) high.

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**References**

AOAC (2005) Official Methods of Analysis. 18th Ed. AOAC International, USA.

Barriere, Y., Emile, J.C., Traineau, R., Surault, F. & Briand, M. (2004) Genetic variation for organic matter and cell wall digestibility in silage maize. Maydica, 49, 115-126.

Boon, E. J. M. C., Struik, P. C., Engels, F. M., & Cone, J. W. (2012) Stem characteristics of two forage maize (Zea mays L.) cultivars varying in whole plant digestibility. NJAS-Wageningen Journal of Life Sciences, 59, 13–23.

Di Marco, O.N., Aello, M.S., Nomdedeu, M. & Van Houtte, S. (2002) Effect of maize crop maturity on silage chemical composition and digestibility in vivo, in situ and in vitro). Animal Feed Science and Technology, 99, 37-43.

Ferret, A., Gasa, J., Plaixats, J., Casanas, F., Bosch, L. & Nuez, F. (1997) Prediction of voluntary intake and digestibility of maize silages given to sheep from morphological and chemical composition, in vitro digestibility or rumen degradation characteristics. Animal Science, 64, 493-501.

Homolka, P. (2000) Digestbility of nutrients, nitrogen degradability and intestinal digestibility of rumen undegraded protein of alkali-treated barely. Czech Journal of Animal Science, 45, 447-450.

Loucka, R., Nedelnik, J., Lang, J., Jambor, V., Trinacty, J. & Tyrolova, Y. (2015) Evaluation of maize hybrids types harvested at the similar stage of maturity. Plant, Soil and Environment, 61, 560-565.

Moreno-Gonzalez, J., Martinez, I., Brichette, I., Lopez, A. & Castro, P. (2000) Breeding potential of European flint and U.S. corn belt dent maize populations for forage use. Crop Science, 40, 1588-1595.

# Effect of processing of whole crop wheat silage on digestibility by cows

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**Keywords:** digestibility, kernel processing, wheat silage, whole crop

**Introduction** Whole crop wheat silage (WCWS) may give high dry matter (DM) yields from one single cut. Partial replacement of grass silage by WCWS for cattle frequently increases total DM intake, but increases in production are often small or absent. Hard cereal grains are known to be poorly digested by cows. Previous research has revealed a large flow of whole grains at duodenum in cows offered WCWS that possibly could explain low DM and starch digestibility in the rumen (Abdallah et al. 1999). We aimed to isolate and reveal the potential effect of kernel processing on digestibility of WCWS harvested at two maturity stages.

**Material and Methods** Spring wheat, var. *Krabat*, 240 kg/ha was sown at Ås, Norway (60°N, 11°E, elevation 93 m.a.s.l.) on April 20, 2015. The crop was harvested on July 27, 2015 at the early dough stage of maturity, 330 g DM/kg, Zadoks 83 (Zadoks et al. 1974), and on August 14, at the soft-to-hard dough, 430 g DM/kg, Zadoks 86. The crop was mown as gently as possible to avoid kernel loss, and picked up without wilting by driving Orkel hiQ smartbaler in the opposite direction to the mower. The beam carrying knives was elevated to get the knives out of use. No additive was applied. Bales were wrapped in inner plastic followed by 12 layers of 0.75 m wide and 0.025 mm thick white Triowrap 750 outer plastic. No mould was detected in the wheat silage bales at opening after 5-8 months of storage. Prior to feeding, WCWS was chopped using Silo King TMR mixer. Thereafter, half of the silage from each maturity stage was processed using Murska 350 S roller mill. At feeding, median particle length of WCWS harvested at the early and soft-to hard dough, respectively, was 35 and 25 mm for unprocessed, and 21 and 19 mm for processed silage, respectively. For DM intake determination, the four silage qualities were oven dried at 59°C to constant weight and corrected for volatiles. Fresh samples were analysed for pH, $NH_3$-N, organic acids and ethanol, and freeze dried samples for ash, N, NDFom and water-soluble carbohydrates (WSC) as described by Randby et al. (2010). Samples for starch analysis were milled (0.5 mm), extracted with acetone and analysed using an enzymatic method (heat stable α-amylase, and amyloglucosidase (Megazyme, Wicklow, Ireland)), with spectrophotometric quantification. Total tract apparent digestibility of the total ration was determined using eight dairy cows in mid-to-late lactation housed in tie-up stall. Depending on days in milk, cows were allocated to one of two 4 × 4 Latin Squares with four cows and four three-week periods. Wheat silage, sprayed with 50 g/d of water soluted urea, was offered for *ad libitum* consumption as the only roughage (on average 4.8% refusals), supplemented with 6.6 kg DM/d of concentrates that included the requirements for protein, minerals and vitamins, and 200 g/d Plus Vomstabil (containing $NaHCO_3$, Ca, Mg, live yeasts). The four dietary treatments consisted of WCWS from the two maturity stages, either unprocessed or processed. Urine and faeces were collected during the last four days in each period. Data were analysed using the Proc mixed procedure of SAS, by the following model: $Y_{ilmn} = \mu + c_i + P_l + H_m + K_n + H_m \times K_n + e_{ilmn}$, where $\mu$ = general mean, $c_i$ = random effect of cow $i$, $P_l$ = effect of period $l$, $H_m$ = effect of harvest time $m$, $K_n$ = effect of kernel processing $n$, $H_m \times K_n$ = effect of interaction, and $e_{ilmn}$ = random residual error.

**Results** Wheat silage harvested at the early and soft-to-hard dough stage, respectively, contained (on average for unprocessed and processed silage) 334 and 423 g DM/kg, and per kg DM: 947 and 957 g

OM, 16.5 and 16.4 g N, 482 and 456 g NDFom, 96 and 48 g WSC, 110 and 253 g starch, 51 and 29 g lactic acid, 15 and 7 g acetic acid, 9 and 9 g propionic acid, 19 and 15 g ethanol and 2.6 and 1.9 g $NH_3$- N, and had a pH of 4.35 and 4.71. Butyric acid was not detected. Cows consumed more DM per day of

**Table 1**. Effect of maturity stage and kernel processing of whole crop wheat silage on daily intake by cows and total tract apparent digestibility of total ration.

| | Early dough | | Soft-to-hard dough | | | *P-value* | | |
|---|---|---|---|---|---|---|---|---|
| | Un-processed | Processed | Un-processed | Processed | SEM | Maturity | Processing | Interaction |
| *Total ration daily intake* | | | | | | | | |
| DM, kg | 20.4 | 20.9 | 21.0 | 21.5 | 0.389 | <0.001 | <0.001 | 0.87 |
| OM, kg | 19.1 | 19.5 | 19.8 | 20.2 | 0.375 | <0.001 | <0.001 | 0.92 |
| N, kg | 0.51 | 0.51 | 0.51 | 0.53 | 0.008 | <0.001 | <0.001 | <0.001 |
| NDF, kg | 7.61 | 7.87 | 7.54 | 7.77 | 0.175 | 0.07 | <0.001 | 0.76 |
| Starch, kg | 3.47 | 3.51 | 5.75 | 5.49 | 0.085 | <0.001 | <0.001 | <0.001 |
| WSC, kg | 1.83 | 1.84 | 1.21 | 1.21 | 0.029 | <0.001 | 0.24 | 0.34 |
| *Total ration digestibility* | | | | | | | | |
| DM | 0.673 | 0.671 | 0.665 | 0.655 | 0.0046 | 0.01 | 0.15 | 0.42 |
| OM | 0.690 | 0.689 | 0.678 | 0.669 | 0.0046 | 0.002 | 0.23 | 0.35 |
| N | 0.724 | 0.716 | 0.722 | 0.720 | 0.0057 | 0.87 | 0.35 | 0.58 |
| NDF | 0.477 | 0.490 | 0.428 | 0.424 | 0.0091 | <0.001 | 0.66 | 0.35 |
| Starch | 0.981 | 0.982 | 0.986 | 0.984 | 0.0010 | 0.002 | 0.62 | 0.05 |
| WSC | 0.993 | 0.994 | 0.988 | 0.987 | 0.0009 | <0.001 | 0.77 | 0.54 |

OM = organic matter, NDF = neutral detergent fibre, WSC = water soluble carbohydrates

WCWS harvested from the soft-to-hard dough stage than from the early dough stage, and more of processed than of unprocessed WCWS (Table 1). Total tract apparent DM, OM, NDF and WSC digestibility was higher when harvested at the early dough stage than at the soft-to-hard dough stage. Starch digestibility was slightly, but significantly, higher from the soft-to-hard dough stage than from the early dough stage. There was no effect of processing on digestibility for any nutrient.

**Discussion** Increasing intake with advancing crop maturity was in line with calculated Silage Dry Matter Intake Indexes (Huhtanen et al. 2007) and was due to reduced concentrations of acids, higher DM and lower NDF concentrations. Poorer production responses than expected when a portion of grass silage is substituted by whole crop silage may be caused by poor kernel digestibility due to later maturity (hard dough) or higher DM concentration (Sutton et al. 1997, Abdallah et al. 1999) than in the present study.

**Conclusion** Starch in wheat kernels harvested at the soft-to-hard dough stage, at Zadoks 86, or earlier, at a DM concentration up to 430 g/kg, is completely digested in dairy cows without any processing.

**References**

Abdalla, A.L., Sutton, J.D., Phipps, R.H. & Humphries, D.J. (1999) Digestion in the rumen of lactating dairy cows given mixtures of urea-treated whole-crop wheat and grass silage. Animal Science, 69, 203-212.

Huhtanen, P., Rinne, M. & Nousiainen, J. (2007) Evaluation of the factors affecting silage intake of dairy cows: a revision of the relative silage dry-matter intake index. Animal, 1, 758-770.

Randby, Å.T., Nørgaard, P. & Weisbjerg, M.R. (2010) Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red Bulls. Grass and Forage Science, 65, 273-286.

Sutton, J.D., Abdallah, A.L., Phipps, R.H., Cammell, S.B., & Humphries, D.J. (1997) The effect of the replacement of urea-treated whole-crop wheat on food intake and apparent digestibility and milk production by dairy cows. Animal Science, 65, 343-251.

Zadoks, J.C., Chang, T.T. & Konzak, C.F. (1974) A decimal code for the growth stages of cereals. Weed Research, 14, 415-421.

# Effects of shredlage and long cut maize silage on dry matter intake and performance of dairy cows

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**Keywords:** dairy cows, maize silage, particle size, physical structure, shredlage

**Introduction** In addition to appropriate quantities of energy and nutrients, ruminants need adequate levels of physically effective fibre to ensure and maintain an optimum rumen function (Steingass and Zebeli 2008). Whole-crop maize silage is a major forage ingredient in diets of high-yielding dairy cows and, as it contains considerable concentrations of starch and physically effective fibre, provides both, energy and physical structure (Südekum 2009). Because of this outstanding importance in the feeding of ruminants, there has been a discussion since the beginning of maize silage cultivation about the optimal preparation and efficient nutrient utilization of maize silage by dairy cows and beef cattle. Based on systematic research between 2005 and 2007, the current recommendation in Germany is to harvest whole crop maize silage (WCS) at a theoretical length of cut of 5 to 8 mm (Spiekers et al. 2009). The resulting particle size enables low-loss forage preservation, high feed intakes of maize silage-based rations and finally, high milk yields. A novel system of harvesting maize WSC labelled "shredlage" was developed in the United States of America. Shredlage is harvested with a self-propelled forage harvester equipped with after-market cross-grooved crop-processing rolls. Additionally in the system "Shredlage" the self-propelled forage harvester is set to a longer theoretical length of cut than commonly used, namely 26 to 30 mm. During the years 2012 till 2015 four feeding trials have been performed in the USA (Ferraretto and Shaver 2012, Vanderwerff et al. 2015, Flis 2015, Chase 2015). Contrary to the German theoretical length of cut, 19 mm theoretical length of cut are used in USA generally, which was also in the control variants in the feeding trials. Comparable results between the feeding variants were determined.

**Material and Methods** The table 1 summarises the total of five feeding trials with lactating dairy cows which were conducted in Germany in years 2016 and 2017 comparing a conventional chop length of 7 mm with shredlage WCS with a theoretical chop length of 26 mm.

**Table 1.** Experimental setup of feeding trials with shredlage and long cut maize silage in rations for lactating dairy cattle.

| Location/ Authors | VBZL Haus Riswick | | | | VBZL Haus Riswick | | Achsel-schwang | | Achsel-schwang | | D. Kampf (unpublished) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KOS | KMS | SOS | SMS | KMS | SMS | KMS | SMS | KOS | SOS | KMS | LMS |
| tHL (mm) | 7 | 7 | 26 | 26 | 7 | 26 | 7 | 26 | 7 | 26 | 7 | 26 |
| Rations | TMR | TMR | TMR | TMR | TMR | TMR | PMR | PMR | PMR* | PMR* | TMR | TMR |
| MS/ MR (% DM) | 45 | 44 | 46 | 45 | 40 | 40 | 39 | 39 | 47 | 47 | 46 | 46 |
| NEL (MJ/kg DM) | 7.3 | 7.2 | 7.3 | 7.2 | 7.1 | 7.1 | 6.8** | 6.9** | 7.1** | 7.1** | n.d. | n.d. |
| peNDF$_{>8\ mm}$ (%) | 17.8 | 17.8 | 19.6 | 19.3 | 18.1 | 21.1 | 15.1** | 17.5** | 14.3** | 15.8** | n.d. | n.d. |
| Animals (n) | 39 | 44 | 41 | 40 | 24 | 24 | 24 | 24 | 24 | 24 | 36 | 36 |
| Time (d) | 140 | | | | 188 | | 84 | | 84 | | 100 | |

KOS: conventional maize silage without straw supplement in ration, KMS: conventional maize silage with straw supplement in ration, SOS: Shredlage maize silage without straw supplement in ration, SMS: Shredlage maize silage with straw supplement in ration, LMS: Long cut maize silage (Scherer special rollers) with straw supplement, tHL: theoretical length of cut, MS: maize silage, MR: mix ration, TMR: total mix ration, PMR: partially upgraded mix ration, n.d.: not determind *straw for adlibitum intake, **inclusive concentrates

**Results** A comparative overview of the results of feeding trials with maize shredlage shows the table 2. Most of the differences are numerical and at a similar level between the different research institutes.

**Table 2.** Experimental results of feeding trials with shredlage and long cut maize silage in rations for lactating dairy cattle.

| Location/ Authors | VBZL Haus Riswick | | | | VBZL Haus Riswick | | Achsel-schwang | | Achsel-schwang | | D. Kampf (unpublished) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KOS | KMS | SOS | SMS | KMS | SMS | KMS | SMS | KOS | SOS | KMS | LMS |
| DM Intake (kg/d) | 23.2$^{a}$ | 24.1$^{ab}$ | 23.2$^{a}$ | 25.0$^{b}$ | 21.9 | 22.3 | 24.4$^{a}$ | 22.7$^{b}$ | 24.9 | 24.7 | 22.2 | 21.8 |
| Milk (kg/d) | 38.5 | 38.2 | 38.4 | 39.1 | 35.2 | 36.1 | 35.9 | 34.6 | 38.9 | 39.0 | 38.9 | 38.0 |
| Fat (%) | 3.70 | 3.73 | 3.61 | 3.67 | 3.77 | 3.81 | 3.82 | 3.83 | 3.69 | 3.72 | 3.62 | 3.67 |
| Protein (%) | 3.26 | 3.26 | 3.24 | 3.25 | 3.07 | 3.01 | 3.66 | 3.61 | 3.68 | 3.64 | 3.21 | 3.26 |
| ECM* (kg/d) | 37.2 | 36.6 | 36.2 | 37.0 | 33.8 | 34.6 | 35.7 | 34.3 | 38.2 | 38.2 | 36.9 | 36.4 |
| Rumination (min/d) | 544$^{a}$ | 623$^{ab}$ | 653$^{b}$ | 678$^{b}$ | 606 | 602 | 485 | 502 | 591 | 557 | n.d. | n.d. |

KOS: conventional maize silage without straw supplement in ration, KMS: conventional maize silage with straw supplement in ration, SOS: Shredlage maize silage without straw supplement in ration, SMS: Shredlage maize silage with straw supplement in ration, LMS: Long cut maize silage (Scherer special rollers) with straw supplement, n.d.: not determind, * energy corrected milk (kg) = ((0,38 x fat (%) + 0,21 x protein (%) + 1,05) x milk yield (kg)) / 3,28, $^{a,b}$ indicate $P < 0.05$.

**Discussion** Contrary to the results of the past German feeding trials (Spiekers et al. 2009), the increased chop length of 26 mm in shredlage WCS showed no negative influence on feed intake and milk production of dairy cattle. Similar results are known from feeding trials in the United States of America, but differences in theoretical length of cut of WCS need to be considered in the comparison.

**Conclusion** Shredlage, providing intensively processed maize kernels and long particles in the same forage, may merge two previously incompatible objectives, namely high energy density and high concentration of physically effective fibre. For this purpose, however, increased technical input (silo packing, transport capacity, diesel, wear) has to be budgeted.

**References**

Chase, L.E. (2015) Shredlage in Dairy Cattle Rations, Cornell Nutrition Conference, Cornell University, https://ecommons.cornell.edu/handle/1813/41239 (Download: 17.09.2017).

Ferraretto, L.F. & Shaver R.D. (2012) Effect of corn shredlage on lactation performance and total tract starch digestibility by dairy cows. The Professional Animal Scientist 28, 639-647.

Flis, S. (2015) What did we learn about shredlage? Dairy One, Measurement to Management, http://dairyone.com/wp-content/uploads/2016/07/What-did-we-learn-about-shredlage-2015.pdf (Download: 17.09.2017).

Spiekers, H., Ettle, T., Preissinger, W. & Pries, M. (2009) Häcksellänge und Strukturwert von Maissilage. Übersichten der Tierernährung, 37, 91-102.

Steingass, H. & Zebeli, Q. (2008) Strukturbewertung von Rationen für die Milchkuh. In: Lehr- und Forschungzentrum für Landwirtschaft Raumberg-Gumpenstein (eds.), 35. Viehwirtschaftliche Fachtagung, Raumberg-Gumpenstein, Germany, 2008, pp. 19-25.

Südekum, K.-H. (2009) Mais und Maisnebenprodukte und ihre Bedeutung für die Protein- und Aminosäurenversorgung von Wiederkäuern und Nicht-Wiederkäuern. Landbauforschung Sonderheft 331, 19-27.

Vanderwerff, L.M., Ferraretto, L.F. & Shaver, R.D. (2015) Brown midrib corn shredlage in diets for high-producing dairy cows. Journal of Dairy Science 98, 5642-5652.

# Effects of plant species and ensiling conditions on the formation of biogenic amines in silage and the preference behaviour of ruminants

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**Keywords:** biogenic amines, ensiling conditions, plant species, preference, small ruminants

**Introduction** During ensilage of high-protein forage proteolysis and desmolysis occur to a certain extent and the concentrations of the fermentation acids change. Proteolysis and desmolysis result in a loss of true protein and hence in an increase of the concentration of the soluble non-protein nitrogen (NPN) fraction including biogenic amines. The influence of amines on dry matter (DM) intake (DMI) and preference behaviour of ruminants has been rarely studied, even more so considering silages with biogenic amines that were formed during ensiling. Similarly, findings on the effects of amines on fermentation products like fermentation acids, water soluble carbohydrates and ammonia-N are rare. This study examined the impact of biogenic amines in silages on DMI and the preference behavior of goats and to find feasible relations between feed intake and silage characteristics including amines.

**Materials and Methods** Six silage treatments were produced in quadruplicate each for lucerne (*Medicago sativa* L., first cut; LUC), red clover (*Trifolium pratense*, first cut; RC) and Italian ryegrass (*Lolium multiflorum* L., second cut; IRG) including different DM (222-391 g/kg; via wilting), silage additives and intended addition of soil were applied to obtain a range of fermentation qualities (see Table 1). Sucrose was added to the forage at ensiling to ensure adequate substrate availability. After 120 days of ensiling, silages were sampled for chemical analyses (conventional chemical analysis, fermentation acid analysis including 20 variables and protein fractionation) and than vacuum-packed and refrigerated for subsequent preference trials with goats. For each plant species a separate run was conducted. During the experimental phase, each possible 2-way combination of the six silages and a standard hay (n = 21 combinations) was presented for 3 h for ad libitum intake to Saanen-type wethers (n = 8; mean (SD) body weight 104 kg (± 3.2) and 105 kg (± 2.2), respectively). Amine analyses included histamine, gamma-, beta- and alpha-amino butyric acid, tyramine, putrescine and cadaverine. Silage samples were subjected to an acid extraction procedure. Analytes were separated as N-substituted phenylthiocarbamyl derivatives via reversed phase high performance liquid chromatography and detected by UV absorbance. All data were analysed using SAS 9.4 (SAS®, 2010). Each run was tested by analysis of variance after averaging DMI of each forage (averaged across each combination, n = 8). Analysis of variance included terms for animal and forage. Within forage treatments, means were separated using the minimum significant difference (MSD) from the Waller-Duncan *k*-ratio *t*-test (*k* = 100) (Burns et al. 2001). Furthermore, correlation coefficients between silage composition and DMI were calculated. Significance was defined at $P<0.05$, and a trend towards a significant effect was noted when $0.05 \leq P \leq 0.10$. Simple linear regression was used to associate the amine concentrations with the 3 h-DMI.

**Results** Treatments led only to few differences in fermentation acids and crude protein composition, yet preference behaviour within one species was strongly divergent. Rankings were very similar for the three plant species. Fermentation acid analysis showed the most obvious differences between treatments in acetic acid (9-62 g/kg DM), but correlations with DMI were weak (-0.5 ($P<0.05$), -0.43 ($P<0.05$) and 0.00048 for lucerne, red clover and Italian ryegrass silages, respectively. The pH was in the range of 4.0-4.8. Lactic acid ranged from 42-95 g/kg DM. Butyric acid was generally below detection limit, only in some silages it was found (0.1–1.5 g/kg DM). Crdue protein fractions differed only marginally within plant species, but the degree of proteolysis was lower in red clover than other

silages. Histamine was the most widespread amine followed by gamma- and beta-amino butyric acid. The concentrations of histamine, beta-amino butyric acid and the total concentrations of analysed amines (TBA) as well as the DM, DMI and NPN are shown in Table 1. The coefficient of determination ($R^2$) for TBA (g/kg DM) and DMI (g DM/3 h) was 0.07.

**Table 1**. Dry matter (DM), DM intake (DMI), non-protein nitrogen (NPN), histamine, beta-amino butyric acid and total biogenic amines (TBA) of lucerne (LUC), red clover (RC) and Italian ryegrass (IRG) silages (n=4)

| Plant species | Variable | Treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | CON1 | CHEM1 | BIO | CON2 | CHEM2 | SOIL |
| LUC | DM (g/kg) | 374 | 384 | 382 | 283 | 264 | 276 |
| RC | | 301 | 301 | 301 | 230 | 233 | 234 |
| IRG | | 369 | 395 | 378 | 244 | 257 | 252 |
| LUC | DMI (g DM/3 h) | $748^{b}$ | $860^{a}$ | $744^{b}$ | $480^{d}$ | $622^{c}$ | $226^{e}$ |
| RC | | $858^{a}$ | $704^{b}$ | $647^{bc}$ | $559^{c}$ | $666^{b}$ | $283^{d}$ |
| IRG | | $763^{a}$ | $606^{ab}$ | $540^{bc}$ | $460^{bc}$ | $402^{cd}$ | $262^{de}$ |
| LUC | NPN (g/kg N) | 784 | 666 | 668 | 751 | 696 | 766 |
| RC | | 478 | 396 | 395 | 493 | 417 | 513 |
| IRG | | 648 | 590 | 610 | 633 | 645 | 649 |
| LUC | Histamine (g/kg DM) | 1.2 | 1.29 | 1.18 | 0.48 | 1.77 | 0.28 |
| RC | | 0.83 | 0.95 | 0.64 | 1.05 | 0.95 | 0.36 |
| IRG | | 0.64 | 0.87 | 0.71 | 0.35 | 0.68 | 0.27 |
| LUC | Beta-amino butyric acid | 0.78 | 0.59 | 0.66 | 1.12 | 0.84 | 1.84 |
| RC | (g/kg DM) | 0.4 | 0.3 | 0.34 | 0.49 | 0.29 | 0.59 |
| IRG | | 0.66 | 0.59 | 0.63 | 0.82 | 0.69 | 0.78 |
| LUC | TBA (g/kg DM) | 2.63 | 2.17 | 2.17 | 2.73 | 3.18 | 4.08 |
| RC | | 1.54 | 1.39 | 1.18 | 1.91 | 1.23 | 1.61 |
| IRG | | 1.87 | 1.86 | 1.72 | 2.47 | 1.81 | 2.17 |

Means within row with different superscripts (a-e) differ ($p < .05$).
CON1 and CON2, respectively: untreated; CHEM1: chemical silage additive (2 L/t), based on sodium nitrite and hexamine (hexamethylenetetramine); CHEM2: chemical silage additive (4 L/t), based on 75% formic acid buffered with sodium hydroxide to pH 2.5; BIO: biological additive (1 g/t), based on homofermentative lactic acid bacteria (*Lactobacillus plantarum*; $3.0 \cdot 10^{11}$/cfu); SOIL: addition of 7,600 g soil/t.

**Discussion** It was confirmed that DMI was lowest in SOIL. As the DM was not related to amine concentrations (data not shown) the lower DMI of the moist silages including SOIL did not seem to be related to amines. Thus, the reduced DMI of moist silages was not caused by amines in the observed concentration range. When analysing amine contents of silages in relation to the method of preservation and to the type of ensiled forage, Křížek (1993) observed putrescine and cadaverine to be most concentrated which was not confirmed with the current data as both amines were rarely detected (data not shown). The different preservation treatments decreased the total concentrations of amines (Křížek 1993), which is also unlike the current data since in the silages treated with additives partly higher concentrations were found compared to the controls.

**Conclusions** The similar DMI rankings of the three preference trial runs did not seem to be related to the analysed amines. Although the degree of proteolysis as measured by NPN of all silages was high the amine and butyric acid concentrations were relatively low. Therefore, it would be advisable to examine which other metabolic products with a potential negative effect on feed intake arise during proteolysis and desmolysis. The different treatments applied in this study had no obvious influence on the formation of biogenic amines.

**References**

Burns, J.C., Fisher, D.S. & Mayland, H.F. (2001). Preference by sheep and goats among hay of eight tall fescue cultivars. Journal of Animal Science, 79, 213-224.

Křížek, M. (1993). Biogenic amines in silage. 1. The occurrence of biogenic amines in silage. Archives of Animal Nutrition, 43, 169-177.

# Effects of plant species, ensiling conditions and storage duration on chemical composition and protein quality of lucerne and red clover silage

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**Keywords:** ensiling condition, fermentation, plant species, protein composition

**Introduction** The basic goals of ensiling are to maintain the feeding value of the fresh plant material and to minimize the degradation of organic matter by conserving the digestible nutrients. This study aimed to evaluate the effects of two plant species and various ensiling conditions on the chemical composition and protein quality of silage examined during the course of fermentation.

**Materials and Methods** The silages were prepared from lucerne (*Medicago sativa* L., first cut) and red clover (*Trifolium pratense*, first cut) with different dry matter (DM) (224-409 g/kg), silage additives and intended addition of soil aiming at obtaining a range of qualities concerning the protein composition. Per plant species six silage treatments each in triple repetition were prepared including an untreated variant per DM content (control). Detailed information about the DM concentrations and the used treatments are summarised in Table 1. Refined sugar (sucrose) was added to the plants to ensure adequate substrate availability during fermentation. After the appropriate treatment the plant material was compacted in 1.5 litre preserving jars and then stored in a cool and dark place. Immediately after harvest the forages were sampled for comprehensive chemical analysis including fermentation acid analysis and crude protein fractionation as well as after 30, 60 and 120 days (d) of ensiling. For each sampling day separate silos (n = 3) were used.

**Table 1**. Treatments of lucerne and red clover, the associated dry matter (DM, in %) and the abbreviations of treatments.

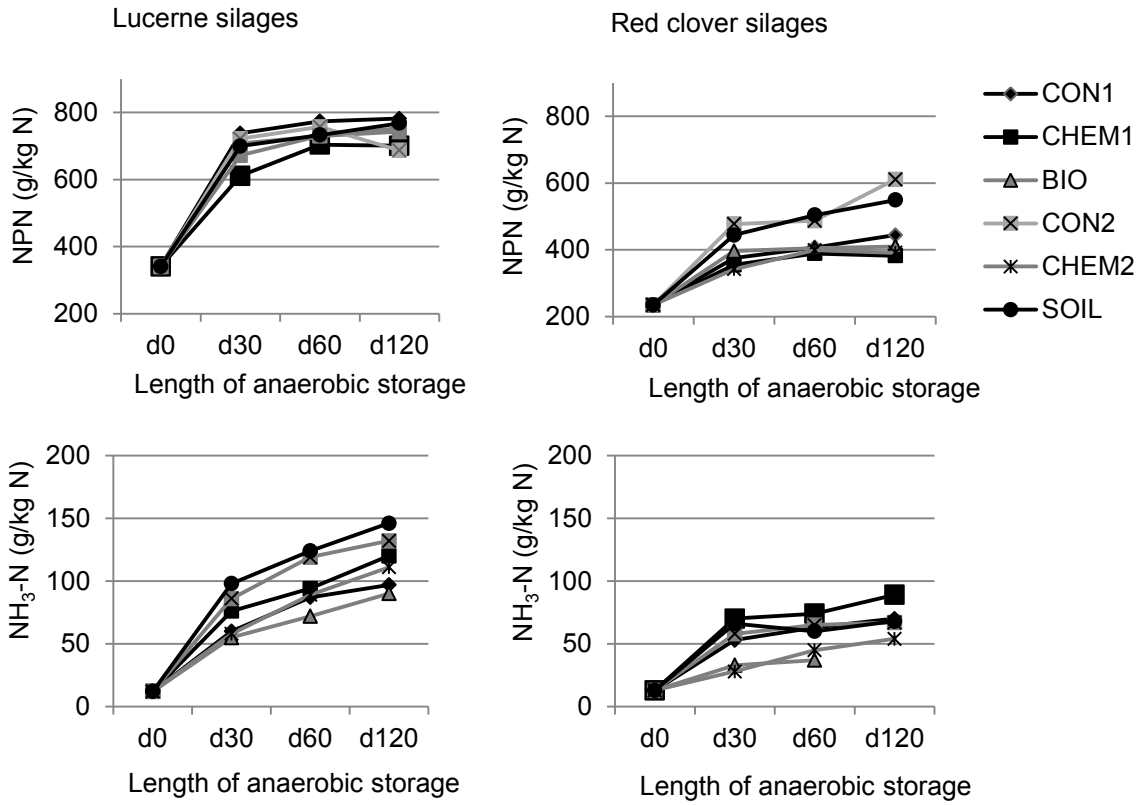
| Substrate | | |
|---|---|---|
| Lucerne | Red clover | |
| DM and abbreviation of treatment | | Treatment |
| 37 (L37CON1) | 30 (C30CON1) | Untreated control |
| 37 (L37CEM1) | 30 (C30CHEM1) | Chemical silage additive (2.5 L/t) based on sodium nitrite and hexamine (hexamethylenetetramine) |
| 37 (L37BIO) | 30 (C30BIO) | Biological additive (1 g/t) based on homofermentative lactic acid bacteria (*Lactobacillus plantarum*; $3.0 \cdot 10^{11}$ colony-forming units/g) |
| 27 (L27CON2) | 22 (C22CON2) | Untreated control |
| 27 (L27CHEM2) | 22 (C22CHEM2) | Chemical silage additive (4 L/t) based on 75% formic acid buffered with sodium hydroxide to pH 2.5 |
| 27 (L27SOIL) | 22 (C22SOIL) | Addition of 7,600 g soil/t |

All data were analysed using SAS 9.4 (SAS®, 2010) by a two factorial (plant species, length of storage and their interaction) analysis of variance and treatment means were separated with the Tukey test. Significance was defined at $P<0.05$.

**Results** Immediately after harvest crude protein (CP) in lucerne accounted for 193 g/kg DM and in red clover for 188 g/kg DM. Non-protein nitrogen (NPN) and $NH_3$-N in lucerne were at 341 g NPN/kg N and 12 g $NH_3$-N/kg N, respectively, in red clover they were at 235 g NPN/kg N and 13 g $NH_3$-N/kg N, respectively. After 120 days of ensiling CP in lucerne silages ranged between 160 and 172 g/kg DM and NPN between 611 and 782 g/kg N. In red clover silages CP ranged from 117 to 162 g CP/kg DM and from 342 to 611 g NPN/kg N, respectively. Lucerne silages showed high levels of $NH_3$-N: 58-146 g $NH_3$-N/kg N. In red clover silages they were lower with 33-89 g $NH_3$-N/kg N (see Figure 1). Both storage time and plant species had a significant impact on NPN and $NH_3$-N. There was no significant

interaction between the storage time and the plant species for NPN, but for $NH_3$-N an interaction was found. The proportion of $NH_3$-N in NPN increased in the most extreme cases (L27CON2d120 and L27SOILd120) six-fold between harvest and d120. The increase in the proportion of $NH_3$-N in NPN between d30 and d60 was 20% for both lucerne and red clover silages.

**Figure 1**. Non-protein nitrogen (NPN) (g/kg N) and $NH_3$-N (g/kg N) in lucerne and red clover silages during the course of fermentation (day (d) 0-d120).




**Discussion** From d30 on there were no major changes in the concentrations of NPN. This was particularly true for lucerne silages. Thus, proteolysis seemed to occur mostly in the first 30 days of ensiling. The $NH_3$-N concentrations, which steadily increased until d120 of the anaerobic storage period, indicate that the composition of NPN has continuously changed between d30 and d120. It is plausible that proteolysis turned into desmolysis resulting in cleaving oligo- and dipeptides in amino acids, amines, butyric acid and $NH_3$. The degree of proteolysis was more distinctive in lucerne silages than in red clover silages, which was also reflected in the lower $NH_3$-N concentrations. It might be due to the action of polyphenol oxidase in red clover, which protects against protein degradation (Lee et al., 2004). Concerning the protein quality as reflected by NPN and $NH_3$-N there were tendencies between treatments and some similarities among plant species. For example, NPN was lowest for CHEM1 and CHEM2 and highest for CON2. Contrary to expectations, SOIL had only the fourth and the second highest NPN concentrations in lucerne and red clover silages, respectively. The $NH_3$-N concentrations were lowest in BIO, L37CON1 and C22CHEM2 and the highest in L27SOIL and C30CHEM1. Altogether, the additives seemed to provide some protection again proteolysis and desmolysis.

**Conclusions** Although the rate of proteolysis decreases after d30 compared to the initial period of ensiling, further degradation processes still take place. The treatments resulted in small differences in NPN concentrations with the chemical silage additives showing the strongest effects.

Lee, M.R.F., Winters, A. L., Scollan, N.D., Dewhurst, R.J., Theodorou, M.K. & Minchin, F.R. (2004) Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities. Journal of the Science of Food and Agriculture, 84, 1639-1645.

# Effects of two various chopping lengths and crop processing conditions of maize silage on silage quality, nutrient digestibility and performance of high yielding dairy cows

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**Keywords:** Chopping length, corn silage, dairy cow, nutrient digestibility, particle size

**Introduction**

Increasingly, longer chopping lengths of maize silage have been utilised to increase fibre efficacy in the forestomachs of ruminants. Trials performed in the USA indicate the combination of longer chopping lengths with a special crop processing technology (e.g. John Deere [JD] kernel star processor; where the stem is also processed in longitudinal direction), show similar fibre supplying effects to dietary straw (Ferreira and Mertens 2005). However, the compression of the silage material associated with this new technology is reduced, and it can be argued that there is a higher risk from this of reduced aerobic stability. Furthermore, it gives rise to the question of whether a longer chopping length may completely replace dietary straw, and if it supplies sufficient physically effective fibre to ensure undisturbed rumen motility and fermentation.

**Material and Methods**

In the present study one variety of maize was harvested from a single field and processed into two chopping lengths and with different crop processing conditions, namely:

- Treatment 1: 8 mm chopping length, JD kernel star processor, standard rollers, reversed sawtooth;
- Treatment 2: 26 mm chopping length, JD kernel star processor, standard rollers, reversed sawtooth, spiral groove.

Maize was harvested at 35% dry matter and ensiled according the procedure described by Thaysen et al. (2018), stocking density and aerobic stability was monitored by regular sampling of the maize silage prior to feeding. The treatments were used in two feeding trials with high-yielding Holstein dairy cows and nutrient digestibility and performance was measured. The experimental setup of both trials was identical and differed only in the type of silage preparation or the dietary inclusion of straw. Trials were carried out at the Education and Research Centre Futterkamp using 72 cows, which were evenly distributed (by lactation number, lactation stage, lactation performance) to one of the two experimental groups, the first two weeks of the respective trial were used as adaption period:

Trial 1:

- Feeding group 1: standard diet with maize silage as 'short cut' (treatment 1)
- Feeding group 2: standard diet with maize silage as 'long cut' (treatment 2)

Trial 2:

- Feeding group 1: standard diet with maize silage as 'long cut' (treatment 2) and now straw addition
- Feeding group 2: standard diet with maize silage as 'long cut' (treatment 2) and a typical straw addition (500 g per head per day)

Maize silage proportion was about 46% in total mixed ration dry matter and was freshly mixed twice a day. Other ration ingredients were grass silage, chopped straw, protein supplement, compound feed and mineral feed. Dietary composition as well as the ingredients used were identical between experimental groups of the respective trials. Feed and water were offered for *ad libitum* consumption and experiments lasted for approximately 100 days for each trial (13.03.2017-30.06.2017 respectively 19.07.2017-26.10.2017).

**Results** Greater length of cut trended towards a reduced compressibility in the silo (Figure 1) resulting in greater reheating in this treatment towards the end of the trial. In both feeding trials, no significant differences in feed intake or milk yield were determined between the experimental groups, both diets

led to identical results (Table 1). No differences in the other variables were found, which suggests that the treatments investigated do not significantly affect feeding behaviour or milk yield. Slightly higher concentrations of milk fat and protein in the long cut treatment could be seen as an indication for a more stable rumen fermentation, however total amounts of milk fat and protein remained the same, which is contrary to this hypothesis.

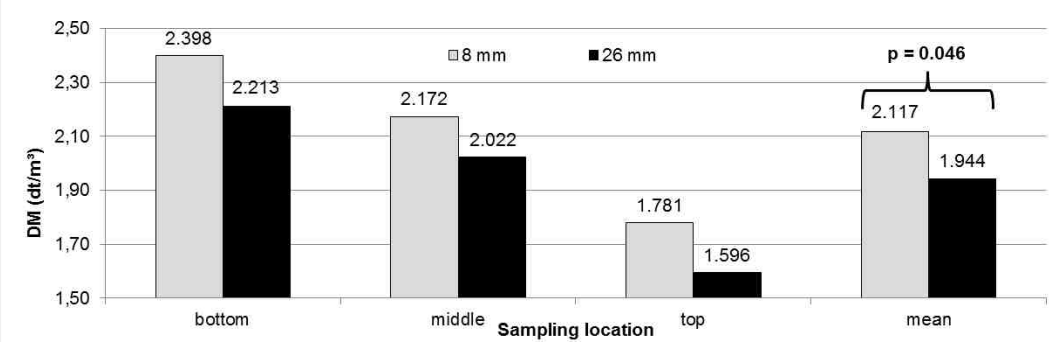


**Figure 1.** Stocking density of short- and long-cut silage in the silo (average of 4 measuring dates and 3 measuring points each)

**Table 1**. Dry matter intake, live weight, milk yield and composition depending on cutting length, processing condition or the addition of feed straw in dairy cows feeding trials

| | | | **Trtmt. 1** | **Trtmt. 2** | | |
|---|---|---|---|---|---|---|
| **Parameter** | Unit | F-Test | LSM | LSM | LSM difference | SE |
| **Feeding trial 1 (short vs. long)** | | | Short cut | Long cut | | |
| **Dry matter intake** | kg | 0.246 | 22.2 | 21.8 | 0.45 | 0.39 |
| **Live weight** | kg | 0.680 | 696 | 691 | 5.4 | 12.9 |
| **Milk fat** | % | 0.543 | 3.61 | 3.67 | -0.05 | 0.08 |
| **Milk protein** | % | 0.256 | 3.21 | 3.26 | -0.05 | 0.04 |
| **Energy corrected milk** | kg | 0.510 | 37.0 | 36.5 | 0.55 | 0.83 |
| **Milk urea** | mg/kg | 0.226 | 232 | 240 | -7.5 | 6.2 |
| **Somatic cell count** | log10 | 0.899 | 1.42 | 1.38 | 0.03 | 0.25 |
| **Feeding trial 2 (low vs. high straw)** | | | Low straw | High straw | | |
| **Dry matter intake** | kg | 0.221 | 23.1 | 23.7 | -0.58 | 0.47 |
| **Live weight** | kg | 0.632 | 691 | 696 | -5.1 | 10.7 |
| **Milk fat** | % | 0.271 | 3.73 | 3.85 | -0.12 | 0.10 |
| **Milk protein** | % | 0.391 | 3.36 | 3.4 | -0.04 | 0.05 |
| **Energy corrected milk** | kg | 0.721 | 35.5 | 35.8 | -0.35 | 0.97 |
| **Milk urea** | mg/kg | 0.112 | 218 | 229 | -10.8 | 6.8 |
| **Somatic cell count** | log10 | 0.961 | 1.69 | 1.68 | 0.01 | 0.28 |

**Summary** In contrast to conventional short-cut silage, longer silage chop length reduces stocking density of maize silage in silo and increases the risk for reheating. In contrast to findings in literature, no differences in feed intake or milk yield were found in the present study. Additionally, a further straw-saving effect could not be shown. This is contrary to other studies, and therefore further investigation is needed to further determine the reasons behind this.

**References**

Ferreira G., Mertens D.R. (2005): Chemical and physical characteristics of maize silages and their effects on in vitro disappearance. Journal of Dairy Science 88, 4414-4425.

Thaysen J., Gerighausen H.-G., Maack C., Richardt W., Ewen A., Kellner K., Sierts H.-P. (2018) Effects of stage of maturity, rollers and chopping length on starch availability, losses and aerobic stability of maize (Zea mays L.) silage. Proc. Int. Silage Conference. Bonn, 24-26 July 2018, submitted.

# Ensilage characteristics of three tropical grasses fertilized with different animal manures, each harvested at four dates

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**Keywords:** Ensilage, growth, tropical

**Introduction** Manure is an important resource in plant production, as it supplies the soil with both nutrients and organic matter. Large quantities of animal manure are generated daily from a high volume of animal production activities on varying scales (Osuhor et al., 2002; Fasae et al. 2009). Manures not well managed and utilized result in environmental hazards, they are usually source of water pollution as they are sometimes discharged into water bodies and underground waters. In some cases, the manures have built up over the years and are occupying considerable spaces that could otherwise be profitably used. Onifade et al. (2005) and Sodeinde et al. (2009) have shown that some of these manures can be profitably used for improved production of some forage grasses. Utilization of manures from various animal species for increasing production of pasture may provide a means of recycling these “animal waste” for environmental sustainability and may, on the long run, revolutionize ruminant livestock production in Nigeria as it has been reported in other parts of the world (Rammer and Lingvall, 1997; Wróbel and Jankowska-Huflejt, 2010; Coblentz et al., 2014). It is therefore needful to hypothesize the need to use animal manures for fertilizing grassland for ruminant livestock productivity. The aim of this study was to investigate if the application of animal manures as fertilizer on grasses affects the quality of silage produced from such grasses.

**Materials and Methods** Triplicate plots of three tropical grasses (*Panicum maximum* var. Local, *P. maximum* var. Ntchisi and *Pennisetum purpureum*) were grown under five animal manures (cattle, swine, poultry, sheep and control) and harvested at four dates (at four weeks intervals). At each harvest date, appropriate plots were precision-chopped with wilting for four hours and representative samples were ensiled in laboratory silos of 960 ml. After 30 days of ensilage, representative silage samples were oven dried at 105°C for 12 h to estimate dry matter (DM) concentration, while aqueous extracts were used to determine pH, fermentation products (lactic acid, acetic acid, propionic acid and butyric acid) and ammonia-N ($NH_3$-N) according to Novozamsky et al. (1974). Data were analyzed as a split-split-plot design with manure as the main plot, and grass specie as the sub plot and harvest date as sub-sub-plot. SAS computer package was used for the analysis and significant difference was tested at 5% probability level.

**Results and Discussion** The DM concentration of the silages was affected ($p<0.05$) by the animal manure applied but not affected by the species and harvest date. The pH of the silages was affected with pH of silage produced from unfertilized grasses being the highest and pH of the silages increase with increase in the harvest date. The high pH (>4.5) in this study is in line with report of Rammer et al. (1994). The concentration of the $NH_3$-N increased with increase in the harvest date and silages produced from unfertilized grasses had the highest concentration of $NH_3$-N. Lactic acid concentration of silages produced from swine manured grasses was the highest ($p<0.05$) and it decreased with increased date of harvest is in line with the findings of Xie et al. (2012).

**Conclusions** *Panicum maximum* var. Ntchisi Silage had the highest lactic acid concentration and the lowest pH value, and silage produced from swine manure fertilized grasses is recommended for its highest lactic acid concentration.

**Table 1.** Effects of manure type and age at harvest on the DM (g/kg DM), pH and fermentative characteristics (%) of silage produced from the three grass species

| Factors | DM | Ph | $NH_3$-N | Lactic Acid | Acetic Acid | Propionic Acid | Butyric Acid |
|---|---|---|---|---|---|---|---|
| Manure type | | | | | | | |
| Cattle | 228$^{c}$ | 4.64$^{b}$ | 5.11$^{d}$ | 6.32$^{c}$ | 2.43$^{c}$ | 1.17$^{c}$ | 0.35$^{c}$ |
| Swine | 218$^{d}$ | 4.72$^{b}$ | 5.69$^{c}$ | 7.40$^{a}$ | 2.40$^{c}$ | 1.43$^{b}$ | 0.37$^{b}$ |
| Poultry | 220$^{d}$ | 4.75$^{b}$ | 6.37$^{b}$ | 6.23$^{c}$ | 2.38$^{c}$ | 1.16$^{c}$ | 0.32$^{d}$ |
| Sheep | 242$^{a}$ | 4.72$^{b}$ | 5.64$^{c}$ | 6.84$^{b}$ | 2.52$^{b}$ | 1.37$^{b}$ | 0.36$^{bc}$ |
| Control | 236$^{b}$ | 6.09$^{a}$ | 8.57$^{a}$ | 4.27$^{d}$ | 2.99$^{a}$ | 1.54$^{a}$ | 0.53$^{a}$ |
| SEM | 1.7 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.0 |
| Species | | | | | | | |
| *P. maximum* (Local) | 229 | 4.98 | 6.32$^{b}$ | 6.04$^{c}$ | 2.52$^{b}$ | 1.37 | 0.43$^{a}$ |
| *P. maximum* (Ntchisi) | 229 | 4.91 | 5.90$^{c}$ | 6.35$^{a}$ | 2.45$^{c}$ | 1.29 | 0.36$^{b}$ |
| *P. purpureum* | 228 | 5.06 | 6.60$^{a}$ | 6.24$^{b}$ | 2.65$^{a}$ | 1.33 | 0.38$^{b}$ |
| SEM | 1.8 | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 | 0.0 |
| Age at harvest (weeks after planting) | | | | | | | |
| 4 | 229 | 4.76$^{c}$ | 5.07$^{c}$ | 5.95$^{c}$ | 2.44$^{c}$ | 1.09$^{b}$ | 0.41$^{a}$ |
| 8 | 227 | 4.98$^{b}$ | 5.15$^{c}$ | 6.99$^{a}$ | 1.99$^{d}$ | 1.01$^{c}$ | 0.39$^{ab}$ |
| 12 | 229 | 4.99$^{b}$ | 7.31$^{b}$ | 6.13$^{b}$ | 2.68$^{b}$ | 1.58$^{a}$ | 0.36$^{c}$ |
| 16 | 231 | 5.20$^{a}$ | 7.58$^{a}$ | 5.79$^{d}$ | 3.06$^{a}$ | 1.64$^{a}$ | 0.38$^{bc}$ |
| SEM | 2.0 | 0.1 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 |

$^{a, b, c, d}$: Means with different superscripts along same column are significantly ($p<0.05$) different

DM=Dry matter; SEM=Standard Error of Mean

## References


Coblentz, W.K., Muck, R.E., Borchardt, M.A., Spencer, S.K., Jokela, W.E., Bertram, M.G. & Coffey, K.P. (2014) Effects of dairy slurry on silage fermentation characteristics and nutritive value of alfalfa. Journal of Dairy Science, 97(11), 7197-7211.

Fasae, O.A., Emiola, O.S. & Adu, I.F. (2009) Production of manure from West African dwarf sheep. Archivos Zootecnia, 58, 601-604.

Novozamsky. I., Van, E.R., Van Schouwenburg, J.C. & Walinga I. (1974) Total nitrogen determination in plant material by means of the indophenol-blue method. Netherland Journal of Agricultural Science, 22, 3-5.

Onifade, O.S., Olanite, J.A., Jolaosho, A.O., Arigbede, O.M. & Tijani, N.K. (2005) Response of guinea grass (*Panicum maximum*) to application of cow dung in South West, Nigeria. In: F. P. O'Mara, R. J. Wilkins, L. t'Mannetje, D. K. Lovett, P. A. M. Rogers and T. M. Boland (eds). Proceedings of the XX International Grassland Congress, Dublin, Ireland, 2005, pp. 396.

Osuhor, C.U., Alawa, J.P. & Akpa, G.N. (2002) Research note: Manure production by goats grazing native pasture in Nigeria. Tropical Grasslands, 36, 123-125.

Rammer, C, Ostling, C, Lingvall, P. & Lindgren, S. (1994) Ensuing of manured crops - effects on fermentation. Grass and Forage Science, 49, 343-351.

Rammer, C. & Lingvall, P. (1997) Influence of farmyard manure on the quality of grass silage. Journal of the Science of Food and Agriculture, 75, 133-140.

Sodeinde, F.G., Akinlade, J.A., Aderinola, O.A., Amao S.R., Alalade, J.A. & Adesokan, A.T. (2009) The effect of poultry manure on proximate composition and *in vitro* gas production of *Panicum maximum* cv T 58 in the derived savanna zone of Nigeria. Pakistan Journal of Nutrition, 8, 1262-1265.

Xie, Z.L., Zhang, T.F., Chen, X.Z., Li, G.D. & Zhang, J.G. (2012) Effects of Maturity Stages on the Nutritive Composition and Silage Quality of Whole Crop Wheat. Asian-Australasian Journal of Animal Sciences, *25*, 1374-1380.

Wróbel, B. & Jankowska-Huflejt, H. (2010) The influence of natural fertilisation on quality and nutritive value of grass silage. In H. Schnyder J. Isselstein F. Taube K. Auerswald J. Schellberg M. Wachendorf A. Herrmann M. Gierus N. Wrage A. Hopkins (eds). Proceedings of the 23th General Meeting of the European Grassland Federation Kiel, Germany August 29th - September 2nd 2010, pp. 581-583.

# Effect of ensiling reconstituted corn grains with whole soybeans on the performance of finishing beef cattle

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**Keywords:** corn, feeding behavior, performance

**Introduction** Ensiling high moisture or reconstituted corn grains is a farm-storage method which improves starch digestibility, due to the proteolysis of the protein matrix surrounding the starch granules during fermentation (Kung et al. 2014). Hence, fermented corn grains typically have higher energy and lower true protein content than dry corn.

The objective of this work was to evaluate animal performance and ingestive behavior of finishing beef cattle fed diets based on dry or ensiled concentrates. We hypothesized that ensiling reconstituted corn grains with whole soybeans (WS) would increase both energy and protein supply and enhance the performance of finishing beef cattle.

**Material and Methods** Corn kernels and a mixture of corn kernels (82%) and WS (18% as fed) were dry rolled and stored both as dry concentrates or rehydrated with tap water to 35% moisture and ensiled in Ag-bags for 90 d (ensiled concentrates). Thirty-two Nellore bulls [382 ± 38 kg of body weight (BW)] were blocked by BW (4 bulls per block) and housed in individual pens to receive one of four treatments, based on concentrate source: DRC = dry rolled corn, DRCWS = dry rolled corn plus WS, RCS = reconstituted corn silage, and RCSWS = reconstituted corn and WS silage. Experimental diets were iso-nitrogenous (13% of crude protein) and contained 25% of whole plant corn silage [dry matter (DM) basis]. The crude protein of the diets containing DRC and RCS was balanced with urea (2.15% on DM basis). The feeding period lasted 70 d.

**Results and Discussion** Both silages were well conserved. There was no interaction between storage method and protein source for animal outcomes (Table 1). It is often reported in the literature that ensiling high moisture or reconstituted corn grains improves feed efficiency by decreasing DM intake without affecting average daily gain (ADG), in high-concentrate diets (Owens et al. 1997). At the end of the day, DM intake is a result of meal size and number of meal bouts. It is well documented that high moisture corn may decrease meal length, meal size or, sometimes, meal bouts, and consequently, decrease DM intake (hepatic oxidation of fuels; Allen et al. 2009). In this trial, ensiled grains decreased meal size and meal length, but increased the number of meal bouts resulting in a longer eating time. Therefore, ensiled grains did not affect DM intake, whereas there was a tendency to increase ADG in comparison with dry grains ($P$ = 0.08). Hence, feed efficiency was improved by ensiling corn alone or combined with whole soybeans. As expected, dry shelled flint corn had lower content of total digestible nutrients (TDN) than tabular values for yellow dent corn (82 vs. 88%). Nonetheless, ensiling rehydrated flint corn increased grain TDN by 14%, supporting the better feed efficiency. Including WS either dry or ensiled led to a numerical increase in ADG ($P$ = 0.12) and feed efficiency ($P$ = 0.16).

**Conclusion** Ensiled corn grains improved the feed efficiency of finishing cattle. Also, ensiling corn grains with whole soybeans is an alternative method for storing concentrates within the farm and increases the energy content of finishing diets.

**Table 1.** Performance and ingestive behavior of finishing feedlot cattle fed diets based on dry or ensiled concentrates.

| Item | Dry | | Ensiled | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | Urea | Soybean | Urea | Soybean | | S[3] | P[4] | E x P |
| DM intake, kg/d | 9.25 | 9.10 | 8.87 | 8.83 | 0.480 | 0.52 | 0.84 | 0.92 |
| ADG[1], kg/d | 1.26 | 1.52 | 1.53 | 1.77 | 0.142 | 0.08 | 0.12 | 0.86 |
| Gain:Feed[2] | 0.132 | 0.171 | 0.188 | 0.210 | 0.020 | 0.04 | 0.16 | 0.65 |
| Grain TDN[5] | 82.2 | 88.5 | 93.3 | 100.9 | 5.44 | 0.07 | 0.20 | 0.89 |
| Meal bouts, /d | 7.32 | 5.64 | 11.1 | 9.13 | 0.96 | <0.01 | 0.08 | 0.85 |
| Meal length, min | 16.6 | 22.0 | 13.3 | 14.8 | 2.16 | 0.02 | 0.15 | 0.34 |
| Meal size, kg | 1.96 | 2.42 | 1.22 | 1.44 | 0.30 | <0.01 | 0.25 | 0.65 |
| Eating, min/d | 121 | 112 | 155 | 140 | 17.8 | 0.05 | 0.48 | 0.85 |
| Ruminating, min/d | 279 | 299 | 319 | 291 | 35.4 | 0.65 | 0.93 | 0.49 |
| Chewing, min/d | 397 | 411 | 470 | 430 | 38.0 | 0.23 | 0.74 | 0.46 |

[1]Average daily gain; [2]Feed efficiency; [3]Effect of storage method (dry or ensiled); [4]Effect of protein source (urea or whole soybeans); [5]Total nutrients digestible from grain (calculated according to Zinn and Shen 1998).

**References**

Allen, M.S., Bradford, B.J. & Oba, M. (2009) Board-invited review: the hepatic oxidation theory of the control of feed intake and its application to ruminants. Journal of Animal Science, 87, 3317-3334.

Kung Jr, L., Windle, M.C. & Walker, N. (2014) The effect of an exogenous protease on the fermentation and nutritive value of high-moisture corn. Journal of Dairy Science, 97, 1-6.

Owens, F.N., Secrist, D.S., Hill, W.J. & Gill, D.R. (1997) The effect of grain source and grain processing on performance of feedlot cattle: a review. Journal of Animal Science, 75, 868-879.

Zinn, R.A. & Shen, Y. (1998) An evaluation of ruminally degradable intake protein and metabolizable amino acid requirements of feedlot calves. Journal of Animal Science, 76, 1280-1289.

# Evaluation of stylosanthes silage with varying concentrate levels in diets for beef cattle: intake and digestibility

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**Keywords:** indigestible neutral detergent fiber, legume silage, ruminal digestibility

**Introduction** Feeding beef cattle with tropical legumes is a common practice around the world, but the use of these crops preserved as silage has not been evaluated as the other crops like corn and grasses, at least in Brazil. Recent studies have shown that it is possible to obtain well-fermented silages with tropical legumes, like *Stylosanthes sp.* and *Arachis pintoi*. In a previous study, our research group concluded that *Stylosanthes* cv. Campo Grande silage (StS), when included at 500 g/kg of dietary DM, could replace corn silage (CS) in diets of feedlot beef cattle without affecting feed intake or performance (Souza et al. 2014). However, there is a lack of information about the ideal proportion of concentrate that should be included in diets containing StS as a forage source. The hypothesis was that StS as the exclusive source of forage would result in animal performance values like those with corn silage, depending on the dietary concentrate level. In this context, the present study was conducted to evaluate the nutrient intake, ruminal and intestinal digestion in beef cattle fed diets containing StS with different concentrate levels.

**Materials and Methods** Four Holstein × Zebu bulls with ruminal and abomasal cannulas, and with an average initial body weight (BW) of 429 ± 15 kg, were used. A 4 × 4 Latin square design was used, with four treatments, represented by the experimental diets. Each experimental period (total of four periods) lasted for 16 d, with 10 d of adaptation to the diets and five days for collecting samples and data. Animals were housed in 10 $m^2$ individual stalls with feeders and a drinking water system and were fed twice a day at 0800 h and 1500 h, allowing for a maximum of 10% of orts. Indigestible neutral detergent fibre (iNDF) was used as a marker to estimate the abomasal flow and fecal excretion of DM. Samples of the feedstuffs and orts were collected daily during the feeding time from the 11th to 15th day of each experimental period. Samples of feces and abomasal digesta were collected within a 26-hour interval. Treatments consisted of diets containing StS with different concentrate levels (C), based on dry matter (DM, g/kg): 200 (StS-200), 400 (StS-400), 60 (StS-600), and a control treatment with 600 g/kg CS and 400 g/kg concentrate (CS-400). Results were subjected to ANOVA and StS diets were compared with control diet by contrasts (α=0.05).

**Results and discussion** Nutrient intake was not affected by the treatments (P>0.05), except for the intake of non-fiber carbohydrates (NFC) and total digestible nutrients of diets StS-200 and StS-400 were lower than the control diet (Table 1). The StS-200 and StS-400 diets had lower values (P < 0.05) for total apparent digestibility of DM, organic matter (OM), and crude protein (CP) in relation to the control diet. Only the StS-200 diet showed lower (P < 0.05) total digestibility of NDF compared to the diet containing CS. The total apparent digestibility values of the StS-600 diet did not differ from the control diet (P > 0.05). Ruminal digestion of NDF and NFC was lower and intestinal digestion of NDF and NFC was higher for StS-200 than for CS-400 (P < 0.05). The diet StS-200 showed lower values of digestibility of OM and NDF because of the high lignin content of StS which increases the dietary proportion of iNDF. The concentration of lignin is known to limit the digestion of fiber carbohydrates in the rumen because it has a protective role in cell wall components by becominghydrophobic and impermeable (Van Soest 1994). Furthermore, the composition of lignin in legumes differs in relation to grasses, because it has low lignin content in relation to legumes, although negative correlations with digestibility are stronger for grasses because of its higher concentration of hemicellulose (Carvalho and Pires 2008**).**

**Table 1.** Total, ruminal, and intestinal apparent digestibility in bulls fed diets containing *Stylosanthes* cv. Campo Grande silage (StS) or corn silage (CS) with different concentrate levels (g/kg DM).

| | CS | StS | | | SEM[1] | Contrasts[2] | | |
|---|---|---|---|---|---|---|---|---|
| Concentrate | 400 | 200 | 400 | 600 | | 1 | 2 | 3 |
| | | | Total (g/kg) | | | | | |
| Dry matter | 676 | 574 | 590 | 655 | 15.4 | 0.01 | 0.03 | 0.54 |
| Organic matter | 690 | 603 | 616 | 678 | 14.3 | 0.02 | 0.04 | 0.70 |
| Crude protein | 627 | 506 | 455 | 555 | 21.5 | 0.02 | <0.01 | 0.13 |
| Ether extract | 708 | 687 | 676 | 682 | 24.0 | 0.73 | 0.60 | 0.67 |
| NDF[3] | 568 | 474 | 514 | 513 | 17.3 | 0.04 | 0.20 | 0.19 |
| NFC[4] | 791 | 776 | 725 | 780 | 13.1 | 0.67 | 0.07 | 0.75 |
| | | | Ruminal (g/kg) | | | | | |
| Dry matter | 691 | 693 | 713 | 684 | 25.5 | 0.97 | 0.64 | 0.88 |
| Organic matter | 741 | 721 | 750 | 714 | 13.8 | 0.53 | 0.76 | 0.39 |
| Crude protein | 328 | 271 | 298 | 310 | 14.2 | 0.15 | 0.44 | 0.64 |
| Ether extract | 217 | 191 | 171 | 153 | 22.5 | 0.63 | 0.49 | 0.34 |
| NDF | 836 | 729 | 801 | 831 | 15.8 | 0.01 | 0.35 | 0.87 |
| NFC | 798 | 689 | 754 | 790 | 20.1 | 0.04 | 0.36 | 0.86 |

[1]Standard error of mean; [2]1- StS-200 vs. CS-400; 2- StS-400 vs. CS-400; 3- StS-600 vs. CS-400; [3]Neutral detergent fiber; [4]Non-fiber carbohydrates.

**Conclusions** Dietary StS levels of 800 and 600 g/kg decrease energy intake and nutrient digestibility compared to CS-400. Thus, StS is recommended as a forage source in diets with a high proportion of concentrate.

**References**

Carvalho G.G.P & Pires A.J.V. (2008) Organização dos tecidos de plantas forrageiras e suas implicações para os ruminantes. Archivos de Zootecnia 57, 13-28.

Souza, W.F., Pereira, O.G., Riberio, K.G., Santos, S.A. & Valadares Filho, S.C. (2014) Intake, digestibility, nitrogen efficiency, and animal performance of growing and finishing beef cattle fed warm-season legume (Stylosanthes capitata plus Stylosanthes macrocephala) silage replacing corn silage. Journal of Animal Science, 92:4099-4107.

Van Soest, P.J. (1994) Nutritional Ecology of the Ruminant. 2nd ed. (Cornell University: Ithaca NYC).

# Finding a consensus on the effects of tropical legume silages on intake, digestibility and performance in ruminants: A meta-analysis

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**Keywords:** large ruminants, silages, small ruminants, tropical legumes

**Introduction** The use of legume forages to feed ruminants has been suggested as a means to ensure a sustainable ruminant production by decreasing the dependency on imported feeds while maintaining or increasing animal production. However, in tropical regions legume forages are not fully adopted, likely due to a lack of consensus on their effects on the animal, the most suitable species to grow, and how to incorporate them into the production system (i.e. fresh, hay, silage). Fresh feeding and hay making of tropical legumes may be restricted by trampling sensitivity, seasonality, and high nutrients losses (Titterton and Bareeba 1999). These limitations are minimized when legumes are conserved as silage, making the latter the most suitable option to feed tropical legumes to ruminants. Contrasting results found in *in vivo* studies evaluating tropical legume silages (TLS) make it hard to conclude on the effects of these forages. Reviews on commercially important legumes in temperate regions summarize the effects of those forages on dry matter intake (DMI), milk yield (MY) and apparent total tract digestibility (aTTd) (e.g. Dewhurst 2013), but due to the different environmental, animal and plant characteristics found in the Tropics, a similar evaluation for TLS is still needed.

**Material and Methods** Sixty-two trials (33 with large ruminants (LR), 29 with small ruminants (SR)) were included in this analysis. The data comprised 162 treatments containing TLS, and 56 control treatments (no TLS). First, a regression analysis was performed only for the diets containing TLS using the legume proportion as fixed effect. Second, an analysis of variance compared diets without legumes with diets with medium (101–400 g/kg dry matter (DM)) and high (401–800 g/kg DM) legume inclusion. In both analyses, observations were weighted by trial according to Sauvant et al. (2008), the trial was included as random effect and a set of parameters related to diet and animal characteristics were included as covariables. Analyses for LR and SR were done separately.

**Results** In total 23 legume species were found, the most common being soya bean (*Glycine max*), stylosanthes (*Stylosanthes spp.*), leucaena (*Leucaena spp.*) and lablab (*Lablab purpureus*). The TLS proportion in the diet averaged (g/kg DM, ± standard deviation) 408 ± 217 for LR and 507 ± 300 for SR.

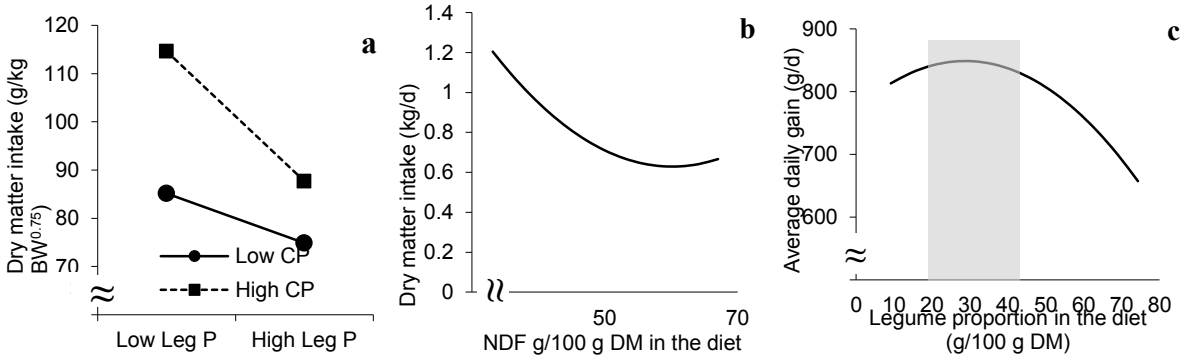


**Figure 1.** Interaction effects between tropical legume silage (TLS) (Leg P) and dietary crude protein (CP) on dry matter intake (DMI) by large ruminants (LR) (n = 69) (**a**); effects of dietary neutral detergent fiber (NDF) on DMI by small ruminants (n = 55) (**b**); effects of TLS proportion on average daily weight gain in LR (n = 25; shaded area showing the range of maximal gains) (**c**).

Regression analysis. In diets containing legumes, DMI by LR decreased with increasing proportions of TLS showing an interaction with dietary CP concentration (Figure 1a), whereas DMI by SR was

negatively affected by dietary NDF concentration with no influence of TLS (Figure 1b). Interestingly, ADG by LR was quadratically affected by TLS proportion with maximal ADG at TLS inclusion between 200 and 400 g/kg DM (Figure 1c). Milk yield by dairy cows and ADG by SR were positively affected by dietary CP with no effects of TLS proportion.

**Table 1**. Effects of level of inclusion of legume silages on dry matter intake (DMI), and apparent total tract digestibility (aTTd) of organic matter (OM) and crude protein (CP) for large (LR) and small ruminants (SR). Lsmeans ± standard deviation (number of observations).

| Variable | Legume inclusion† | | | P |
|---|---|---|---|---|
| | No legumes | Medium (101–400 g/kg DM) | High (401–800 g/kg DM) | |
| DMI (g/d) (**LR**) | 10.1 ± 5.09$^{ab}$ (32) | 10.5 ± 4.82$^{a}$ (39) | 9.53 ± 4.00$^{b}$ (24) | <0.01 |
| aTTd OM (g/kg) (**LR**) | 658 ± 74.7$^{a}$ (13) | 631 ± 60.5$^{ab}$ (27) | 591 ± 42.9$^{b}$ (11) | <0.01 |
| aTTd CP (g/kg) (**LR**) | 661 ± 94.7$^{a}$ (16) | 625 ± 80.2$^{b}$ (31) | 577 ± 77.6$^{c}$ (11) | <0.01 |
| DMI (g/d) (**SR**) | 0.642 ± 0.33 (22) | 0.676 ± 0.156 (38) | 0.680 ± 0.370 (16) | 0.42 |
| aTTd OM (g/kg) (**SR**) | 658 ± 125$^{a}$ (16) | 625 ± 94.7$^{a}$ (30) | 591 ± 95.1$^{b}$ (16) | 0.02 |
| aTTd CP (g/kg) (**SR**) | 681 ± 120$^{a}$ (14) | 648 ± 94.2$^{ab}$ (26) | 604 ± 58.3$^{b}$ (15) | 0.09 |

†Different superscript within rows denote differences (P < 0.05) between categories

Analysis of variance. For LR, no differences were found for DMI between the medium inclusion and diets without legumes, but DMI decreased with TLS inclusions above 400 g/kg DM (Table 1). The same trend was observed for MY and ADG. For SR DMI was not affected by TLS (Table 1), with a similar effect observed for ADG. For both LR and SR aTTd of OM and CP decreased with increasing proportion of TLS inclusion in the diet, with more pronounced effects for aTTd of CP (Table 1).

**Discussion** The lower DMI observed when increasing proportions of TLS are fed to LR may be linked to the lower nutrients aTTd of those forages, in contrast to temperate legumes (Dewhurst 2013). Additionally, high concentrations of NDF and antinutritive compounds in TLS may contribute to the lower intake, a thesis supported by the lessened effects observed in SR, typically more tolerant to both antinutritive compounds and NDF-rich diets. The lower aTTd of CP in TLS may be associated to a high fiber bound nitrogen due to both, a high NDF concentration in TLS, and excessive heating during ensiling (Weiss & Conrad 1986), a common occurrence in the Tropics. Despite the decreased DMI and aTTd, performance was not negatively affected by TLS. Reasons for this apparent paradox remain unclear, but factors like an increase in microbial protein synthesis, supply of dietary amino acids to the duodenum, or a better synchronization of energy and nitrogen in the rumen upon TLS feeding may play a role in the observed phenomenon.

**Conclusion** Tropical legume silages do not share all the attributes given to temperate legumes, as TLS elicit negative effects on DMI and aTTd, whith large ruminants being more sensitive to these effects than small ruminants. Nevertheless, TLS seem to promote a more efficient utilization of feed, particularly at inclusion levels below between 100 and 400 g/kg DM. However, the mechanisms by which tropical legume silages maintain or increase performance despite lower intake and digestibility could not be clarified and should be subjects of further research with well designed feeding trials.

**References**

Dewhurst, R. (2013) Milk production from silage: Comparison of grass, legume and maize silages and their mixtures. Agricultural and Food Science, 22, 57-69.

Sauvant, D., Schmidely, P., Daudin, J.J. & St-Pierre, N. R. (2008) Metaanalyses of experimental data in animal nutrition. Animal, 2, 1203-1214.

Titterton, M. & Bareeba, F. (1999) Paper 4.0: Grass and legume silages in the tropics. FAO, Rome, Retrieved from http://www.fao.org/docrep/ 005/x8486e/x8486e0c.htm [Cited 11 March 2018].

Weiss, W.P. & Conrad, H.R. (1986) Digestibility of nitrogen in heatdamaged alfalfa. Journal of Dairy Science, 69, 2658-2670.

# Improving nitrogen utilization of alfalfa protein by co-ensiling with red clover

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**Keywords:** alfalfa, co-ensiling, protein quality, red clover.

**Introduction** Extensive proteolysis is extremely prevalent for alfalfa silage, leading to low efficient capture of protein by animals. In contrast, red clover exhibits dramatic potential in preservation of forage protein. It is demonstrated that the low extent of protein breakdown is related with the presence of polyphenol oxidase (PPO) in red clover ( Winters et al. 2008). PPO is a diphenol oxidizing enzyme and is particularly active in red clover. The PPO is hypothesized to catalyze the oxidation of endogenous phenols to quinones, which can then form covalent bonds with the proteins. Proteolysis thus would be reduced either directly, by active inhibition of proteases, or a reduction in general protein solubility through protein-phenol binding (Lee, 2014). In consideration of the leading role of alfalfa in forage production, effective approaches to confer alfalfa the same properties of reducing proteolysis as red clover would be of economic importance. The objective of this study was to characterize, through co-ensilling, whether red clover could supply protein protection for alfalfa protein compared with alfalfa ensiling alone.

**Materials and Methods** Alfalfa and red clover were harvested at the stage of early flowering, chopped and then assigned to treatments as follows: (1) alfalfa ensiled alone (A); (2) alfalfa ensiled with red clover at a ratio of 1:1 (M); (3) red clover ensiled alone (R). For preparation of each silo, about 780 g of forage material was packed into a laboratory silo (polyethylene bottle, 1 L capacity) and compacted manually with uniform intensity (190 kg DM/$m^3$). Each treatment with five replicates were stored at room temperature (20±5.0°C) and opened on day 3, 5, 7, 14, and 30 days. The fermentation quality and nitrogen (N) fractions including non-protein (NPN), free amino acid (FAA-N), peptides and ammonia N (AN) were analyzed after silos opening. Data related to fermentation qualities and N fractions were analyzed by two–way analysis of variance (ANOVA). Tukey's tests were used for means separation.

**Results and discussion** Retarded nitrogen transformation of true protein to non-protein fractions were observed in R and M silages as opposed to A silage, as indicated by lower concentration of NPN ($P$<0.05) and FAA-N ($P$<0.05) during the entire 30 days of ensiling, lower peptides N ($P$<0.05) during the first 14 days ensiling, plus lower AN ($P$<0.05) at the end of ensiling. No signifiant difference between R and M were found at early intervals of the ensiling, representing that red clover may have conferred alfalfa the same properties of inhibiting proteolysis in the mixed silage. Plausible explanation for this phenomenon could be due to transfer of PPO's protein protection system between red clover and alfalfa in the mixed silage. However, there are arguments questioning whether the PPO from PPO–containing forage could be available for non–PPO containing forage when combined in the same silo, since oxidized phenol (i,e.quinones), produced by PPO, would react with PPO and quickly deactivate PPO itself in red clover. A study on tea (*Camellia sinensis*) leaves by Ölmez and Yilmaz (2010) showed a depression effect of 10% on PPO activity per unit increase in DM content, implying that DM content plays an essential role in the level of PPO activity. In this study, we consider the successful transfer of PPO's protein protection was probably benefited by the high moisture of the two forages (271 g/kg FM for alfalfa and 258 g/kg FM for red clover) at ensiling, which not only favored the sufficient mixing of the two plant juices but also maintained PPO activity.

**Conclusions** Co-ensiling with red clover conferred alfalfa the same properties of reducing proteolysis as red clover. The results of this study potentially provide an effective strategy for controlling protein loss during silage production.

**Table 1** Nitrogen fractions contents (g $kg^{-1}$ TN) of the alfalfa, red clover and their mixture during ensiling

| Item | Days of ensiling | | | | | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 5 | 7 | 14 | 30 | | T | D | T×D |
| Peptide N | | | | | | | | | | |
| Alfalfa | 88.0[b] | 246[b] | 232[b] | 228[b] | 212[b] | 134[a] | 2.71 | 0.006 | <0.001 | <0.001 |
| Mixture | 83.1[a] | 206[a] | 201[a] | 181[a] | 178[a] | 152[b] | | | | |
| Red clover | 78.4[a] | 198[a] | 192[a] | 186[a] | 180[a] | 170[b] | | | | |
| Free amino acid N | | | | | | | | | | |
| Alfalfa | 62.1[b] | 168[b] | 179[b] | 183[b] | 204[c] | 249[c] | 2.20 | <0.001 | <0.001 | <0.001 |
| Mixture | 49.2[a] | 98.8[a] | 115[a] | 121[a] | 144[b] | 165[b] | | | | |
| Red clover | 38.7[a] | 86.3[a] | 95.1[a] | 103[a] | 116[a] | 139[a] | | | | |
| Ammonia N | | | | | | | | | | |
| Alfalfa | 10.6 | 47.4 | 62.6 | 68.1 | 85.9[b] | 106[b] | 1.41 | <0.001 | <0.001 | 0.035 |
| Mixture | 9.54 | 46.0 | 61.0 | 63.2 | 72.2[a] | 85.5[a] | | | | |
| Red clover | 9.10 | 44.1 | 61.4 | 66.7 | 67.6[a] | 79.3[a] | | | | |
| Non-protein N | | | | | | | | | | |
| Alfalfa | 170[b] | 417[b] | 489[b] | 509[b] | 544[b] | 539[c] | 5.13 | 0.030 | <0.001 | 0.002 |
| Mixture | 159[a] | 332[a] | 378[a] | 392[a] | 422[a] | 432[b] | | | | |
| Red clover | 150[a] | 289[a] | 346[a] | 361[a] | 386[a] | 397[a] | | | | |

TN, total nitrogen; SEM, standard error of the mean; T, treatment; D, days of ensiling. Means within the same column with different letter differ ($P$<0.05).

**References**

Lee M.R. (2014) Forage polyphenol oxidase and ruminant livestock nutrition. Frontiers in Plant Science, 5, 694.

Ölmez H., Yilmaz A. (2010) Changes in chemical constituents and polyphenol oxidase activity of tea leaves with shoot maturity and cold storage. Journal of food processing and preservation, 34, 653-665.

Winters A.L., Minchin F.R., Michaelson-Yeates T.P.T., Lee M.R.F. & Morris P. (2008) Latent and active Polyphenol oxidase (PPO) in red clover (*Trifolium pratense*) and use of a low PPO mutant to study the role of PPO in proteolysis reduction. Journal of Agricultural and Food Chemistry, 56, 2817-2824.

# *In vitro* ruminal fermentation of lucerne silages differing in nitrogen fractions and fermentation quality

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**Keywords:** crude protein, lucerne silage, nitrogen, rumen fermentation

**Introduction** Lucerne (*Medicago sativa* L.) silages contain high crude protein (CP) contents, but the vast majority of this fraction consists of non-protein-nitrogen (NPN). Together with the low concentration of water-soluble carbohydrates, this may lead to poor ruminal nitrogen (N) fixation. However, different pre-ensiling treatments were used to manipulate the CP composition of lucerne silages (LS). Wilting lucerne with high intensity (on black plastic in the sun) or low intensity (on white plastic in the shade) to lower (25%) or higher (35%) dry matter (DM) contents and with or without the supplementation of sugar before ensiling, resulted in 8 LS differing in CP composition and fermentation quality (Hartinger et al. 2017). With an additional focus on variables of the ruminal N turnover, the aim of the present study was to evaluate *in vitro* ruminal fermentation of these LS.

**Material and Methods** The 8 LS were tested in quadruplicate using the Rusitec system (Czerkawski and Breckenridge 1977). Each of the 6 experimental runs lasted 17d. Ruminal fluid and solid digesta were obtained from 3 rumen-cannulated Holstein steers fed a hay and concentrate diet (70:30) on maintenance level. The first 8d, during which hay and concentrate (70:30) were incubated in the vessels, were used for adaption of the system. The subsequent 9d were used for incubating the LS on an isonitrogenous level. Gas production, effluent production and pH in the vessels were measured daily. Ammonia-N and volatile fatty acids (VFA; acetate, propionate, butyrate, valerate, i-butyrate, i-valerate, capronate) in the vessel fluid were measured 2, 4, 12 and 23h after feedbag exchange on days 7, 10 and 15, respectively. Concentrations of VFA in the effluent were measured before feedbag exchange on days 8, 11 and 16, respectively, and were used to calculate daily VFA production. Analysis of VFA was performed by gas chromatography (GC Auto System, Perkin Elmer Inc., Waltham, MA, USA), ammonia-N by automated distillation (Vapodest 50s carousel, Gerhardt, Königswinter, Germany), pH by potentiometry (pH 315i, WTW, Weilheim, Germany), gas production by water displacement technique and the effluent using a measuring cylinder**.** Data were analysed for differences between silage treatments and effects of sugar supplementation, wilting intensity and DM content (without interactions) using PROC GLM of SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

**Results and Discussion** From the second day of LS incubation, average daily gas production was higher for sugar-supplemented LS variants (Table 1). This may be due to higher amounts of residual sugar (Belanche et al. 2013), but also higher concentrations of lactate, which can be metabolized by rumen microbes (Satter and Esdale 1968). Likewise, production of n-butyrate (+23%), propionate (+63%), n-valerate (+15%) and i-valerate (+15%) was increased for sugar-supplemented LS (Table 1).

**Table 1.** Daily production means of gas (ml/d) and specific VFA (mmol/d) for LS variants with or without sugar supplementation (respective standard error of the mean in brackets).

| LS variants | Gas production | Acetate | n-Butyrate | Propionate | n-Valerate | i-Valerate |
|---|---|---|---|---|---|---|
| With sugar supplementation | 1412 (12.9) | 29.34 (.51) | 6.62 (.19) | 10.23 (.26) | 1.81 (.05) | 3.05 (.09) |
| Without sugar supplementation | 980 (13.3) | 28.97 (.53) | 5.37 (.20) | 6.29 (.27) | 1.58 (.05) | 2.66 (.09) |
| P-value | <.0001 | .6210 | <.0001 | <.0001 | .0018 | .0030 |

Regarding N metabolism, incubating low DM LS slightly increased the average ammonia-N concentration in the vessel fluid (31.8 mmol/L vs. 30.9 mmol/L for high DM). This effect may be due to the 8% higher percentage of NPN in low DM silages as this N fraction is rapidly degraded to ammonia by rumen microbes (Sniffen et al. 1992). Production of i-valerate, a branched-chain VFA (BCVFA) formed during deamination (Carro und Miller 1999) was 32% higher for low DM LS and extensive wilting treatment (3.37 mmol/d) when compared to intensively wilted high DM LS (2.54 mmol/d). This may result from Leu and ILe degradation (Rasmussen et al. 1988) as these amino acids were more abundant in the extensively wilted low DM LS. However, although sugar supplementation decreased proportions of NPN in LS and that of Leu and ILe in particular, daily production of i-valerate was 15% higher for sugar-supplemented LS (Table 1). But irrespective of BCVFA being indicators for ruminal deamination, they also stimulate cellulolytics (Allison et al. 1962) and can positively affect ruminal fibre degradation, which may partly explain higher acetate (+5%) and n-butyrate (+21%) concentrations with sugar-supplemented LS. On the other hand, acetate and n-butyrate are also formed during deamination (Sirotnak et al. 1953) and may not only originate from fibre but amino acid degradation, too. Thus, sugar supplementation prior to ensiling may stabilize true protein in the LS, but could also have a contrary effect in the rumen by stimulating microorganisms that ferment both carbohydrates and CP, e.g. Prevotella (Rubino et al. 2017).

**Conclusion** Variations in fermentation quality and CP composition of LS result in specific in vitro rumen fermentation patterns. Higher gas and VFA production along with lower ammonia concentration for sugar-supplemented high DM LS indicate improved ruminal fermentation and energy provision to the ruminant (Bergman 1990). Intensive wilting seems to be favourable in regards of ruminal N catabolism as it reduced NPN in LS and i-valerate concentration during in vitro incubation. The present findings clearly illustrate the beneficial effects of pre-ensiling lucerne treatment on ruminal fermentation and may be used to improve ruminants' energy and protein supply from LS.

**References**

Allison, M.J., Bryant, M.P. & Doetsch, R.N. (1962) Studies on the metabolic function of branched-chain volatile fatty acids, growth factors for ruminococci I. Incorporation of Isovalerate into Leucine, Journal of Bacteriology 83, 523-532.

Belanche, A., Lee, M.R.F., Moorby, J.M. & Newbold, C.J. (2013) Comparison of ryegrass and red clover on the fermentation pattern, microbial community and efficiency of diet utilisation in the rumen simulation technique (Rusitec), Animal Production Science 53 (10), 1052-1064.

Bergman, E.N. (1990) Energy contributions of volatile fatty acids from the gastrointestinal tract in various species, Physiological reviews 70 (2), 567-590.

Carro, M.D. & Miller, E.L. (1999) Effect of supplementing a fibre basal diet with different nitrogen forms on ruminal fermentation and microbial growth in an *in vitro* semi-continuous culture system (RUSITEC), British Journal of Nutrition 82 (02), 149-157.

Czerkawski, J.W. & Breckenridge, G. (1977) Design and development of a long-term rumen simulation technique (Rusitec), British Journal of Nutrition 38 (03), 371-384.

Hartinger, T., Gresner, N. & Südekum, K.-H. (2017) Einfluss von Trockenmassegehalt, Anwelkintensität und Zuckerzulage auf Stickstoffverbindungen in Luzernesilagen. VDLUFA-Schriftenreihe, 387-394.

Rasmussen, H.S., Holtug, K. & Mortensen, P.B. (1988) Degradation of amino acids to short-chain fatty acids in humans. An *in vitro* study, Scandinavian Journal of Gastroenterology 23 (2), 178-182.

Rubino, F., Carberry, C.M. Waters, S., Kenny, D., McCabe, M.S. & Creevey, C.J. (2017) Divergent functional isoforms drive niche specialisation for nutrient acquisition and use in rumen microbiome, The ISME Journal 11 (4), 932-944.

Satter, L.D. & Esdale, W.J. (1968) In vitro lactate metabolism by ruminal ingesta, Applied Microbiology 16, 680-688.

Sirotnak, F.M., Doetsch, R.N., Brown, R.E. & Shaw, J. C. (1953) Amino acid metabolism of bovine rumen bacteria, Journal of Dairy Science 36 (10), 1117-1123.

Sniffen, C.J., O'Connor, J.D., van Soest, P.J., Fox, D.G. & Russell, J.B. (1992) A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability, Journal of Animal Science 70 (11), 3562-3577.

# Kinetics parameters of different purpose sorghum silages at second crop

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**Keywords:** digestibility, ensiling, gas production

**Introduction** Sorghum is an alternative to corn due to lower soil fertility requirement and increased tolerance to drought (Borba et al. 2012), especially when planted at second crop in the Midwest region of Brazil. Among sorghum types there are grain, forage and sweet sorghum, which differ in plant height and nutritional value. In Brazil, grain sorghum cultivars have been suggested for the production of high quality silage due to their higher proportion of grains, whereas forage sorghum cultivars have been recommended for high dry matter yield. The sweet sorghum has a high content of water soluble carbohydrates, resulting in forage with high energy content. Besides the chemical composition, digestibility is a key parameter in the evaluation of forage quality. The *in vitro* procedure for determining the digestibility of food is the most widespread technique used in ruminant nutritional studies, owing to its low cost and quickness, as well by its high precision. Lack of information about the qualitative behaviour of sorghum hinders the recommendation of different purpose sorghum cultivars. The goal was to evaluate the kinetics parameters and *in vitro* digestibility of silages of different purpose sorghum cultivar, at second crop.

**Materials and Methods** The trial was carried out at the Plant Production Department, Federal Institute of Education, Science and Technology of Rondonia, Colorado do Oeste campus, Rondonia state. All chemical analyses and *in vitro* incubation were performed at the Laboratory of Animal Nutrition, Federal University of Mato Grosso, Cuiabá, Mato Grosso state. The experimental design was a randomized block, with four replications. Treatments consisted of six different purpose sorghum cultivars (grain sorghum: BRS 308 and BRS 310; forage sorghum: BR 655 and BRS 610; sweet sorghum: BRS 506 and CMSXS 647). Seeding was done on March 22, 2012, while the harvesting was performed when the plants showed grain at the hard dough stage, which was at 95 days after emergency (DAE) for grain sorghum cultivars and 105 DAE for cultivars of forage and sweet sorghum. For ensiling, forage was chopped to approximately 20 mm and immediately ensiled into glass jars silos, which were the experimental units. The silos remained closed for 30 days. The silages sampling was done after the silos opening, and the samples were freeze-dried at -70 °C for 48 h. After that, silage samples were ground in a Wiley mill until they could pass through a 20 mesh steel sieve. Two *in vitro* incubations were performed based on the methodology described by Pell and Schofield (1993), in which 450 mg of samples were weighed and added into 120 mL bottles, with 50 mL of inoculum/media (10:40 mL) solution. The pressure readings from gas production inside the bottles were taken at the following times: 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36, 48, 60, 72, 84, and 96 h after the incubation start. The cumulative gas production profiles were used to estimate the kinetic parameters (final gas volume, digestion rate, lag time) of sample digestion by the one-pool model, proposed by Schofield et al. (1994). After incubation, the residue from each bottle was filtered under vacuum in a glass filter crucible of porosity 2 (40 to 100 µm) in order to measure the *in vitro* digestibility of dry matter (IVDDM). Indigestible neutral detergent fiber (iNDF) data was obtained after 240 h of incubation *in situ*. Data were subjected to analysis of variance and the means were compared by the least significant difference (LSD) test, adopting the probability level of 5%, with the SISVAR statistical program, version 5.3.

**Results** Effect of cultivars was observed on all evaluated variables ($P<0.01$). Silages of sweet sorghum cultivars presented higher IVDDM and lower lag time and iNDF, while lower final gas volume was observed for the silages of grain sorghum cultivars. Silages of grain sorghum cultivars and forage

sorghum cultivar BRS 605 presented higher digestion rate (Table 1). The water soluble carbohydrates (WSC) content observed was 45.4, 52.4, 80.7, 45.5, 128.1, and 110.1 g $100g^{-1}$ DM for silages of grain sorghum cultivars BRS 308 and BRS 310, forage sorghum cultivars BRS 655 and BRS 610, and sweet sorghum cultivars BRS 506 and CMSXS 647, respectively.

**Table 1.** Kinetic parameters and *in vitro* digestibility of dry matter of different purpose sorghum cultivars. Colorado do Oeste-Ro, Brazil. 2012

| Cultivar | Grain sorghum | | Forage sorghum | | Sweet sorghum | | CV (%) |
|---|---|---|---|---|---|---|---|
| | BRS 308 | BRS 310 | BRS 655 | BRS 610 | BRS 506 | CMSXS 647 | |
| IVDDM | 58.92$^{bc}$ | 57.01$^{c}$ | 57.18$^{c}$ | 59.36$^{bc}$ | 64.45$^{a}$ | 61.51$^{b}$ | 3,21 |
| FGV | 14.75$^{c}$ | 14.42$^{c}$ | 18.00$^{a}$ | 16.62$^{b}$ | 18.58$^{a}$ | 17.38$^{ab}$ | 4,97 |
| DR | 1.85$^{ab}$ | 1.89$^{ab}$ | 2.03$^{a}$ | 1.34$^{d}$ | 1.78$^{b}$ | 1.54$^{c}$ | 7,66 |
| LAG | 5.39$^{ab}$ | 7.28$^{a}$ | 8.49$^{a}$ | 6.84$^{a}$ | 1.30$^{c}$ | 2.53$^{bc}$ | 39,90 |
| iNDF | 25.27$^{a}$ | 21.18$^{ab}$ | 22.66$^{ab}$ | 25.29$^{a}$ | 17.94$^{b}$ | 19.10$^{b}$ | 15,68 |

Means followed by the same small letters in the row do not differ among themselves by LSD test ($p > 0.05$). IVDDM: *In vitro* digestibility of dry matter (g $100g^{-1}$ DM); FGV: Final gas volume (mL 100 $mg^{-1}$ DM); DR: Digestion rate (% $h^{-1}$). LAG: Lag time (h). CV (%): Coefficient of variation.

The higher IVDDM observed for sweet sorghum cultivars silages probably is because of the higher level of WSC and lower content of iNDF, while the lower lag time obtained by these cultivars, was possibly due to the higher levels of WSC and non-protein nitrogen observed in these cultivars. The iNDF content of a feed is related to its digestibility (Huhtanen et al. 2006). Grain sorghum cultivars and forage sorghum cultivar BRS 655 presented higher digestion rate, probably due to the higher starch content, while the lower final gas volumes obtained by grain sorghum cultivars might be because starch fermentation promotes propionate production, with less $CO_2$ generation (Beuvink and Spoelstra 1992).

**Conclusion** The silages made using sweet sorghum presented higher *in vitro* digestibility of dry matter and might be recommend to the production of good quality silage for ruminant feeding.

**References**

Beuvink, J.M.W. & Spoelstra, S.F. (1992) Interactions between substrate, fermentation end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms *in vitro*. Applied Microbiology and Technology, 37, 505-509.

Borba, L.F.P., Ferreira, M.D.A., Guim, A., Tabosa, J.N., Gomes, L.H.D.S., & Santos, V.L.F.D. (2012) Nutritive value of different silage sorghum (*Sorghum bicolor* L. Moench) cultivars. Acta Scientiarum. Animal Sciences, 4, 123-129.

Huhtanen, P.; Nousiainen, J. & Rinne, M (2006) Recent developments in forage evaluation with special reference to practical applications. Agricultural and Food Science, 15, 293- 323.

Pell, A.N. & Schofield, P. (1993) Computerized monitoring of gas production to measure forage digestion *in vitro*. Journal of Dairy Science. 76, 1063-1073.

Schofield, P.; Pitt, R.E. & Pell, A.N. (1994) Kinetics of fiber digestion from *in vitro* gas production. Journal of Animal Science. 72, 2980-2991.

# Profile of chemical quality of corn silage in the Brazilian milk capital

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**Keywords:** analysis, ensiling, milk, nutrition

**Introduction** Due to the seasonality in the production of forages, corn silage is an important source of forage for dairy cows. In Brazil, the studied region is among the ten cities with the highest volume of milk production and average yield of approximately 7,000 l/cow/year, much higher than the Brazilian average of 1,709 l/cow/year (IBGE 2017). The chemical quality of silage influence the availability of nutrients for animals, the balance of the diets, the consumption of dry matter, production and milk quality (NRC 2001; Linn 2003). So, measuring the chemical composition of corn silage is fundamental for the right balance of diet and increase milk yield.

**Material and Methods** In the years 2009-2017 were visited 579 farms, in 33 cities of Campos Gerais in region (Centre East of Paraná state) and south of São Paulo state. Silages were evaluated 1509 silos that were used in animal nutrition. The chemical analysis of the silage until 2015 were dry matter (DM) by AOAC (1998), crude protein (CP) (Nelson and Sommers 1980), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Van Soest1991), in vitro organic matter digestibility (IVOMD) and in vitro digestibility of NDF (IVNDFD) (Tilley and Terry 1963), starch by Pereira and Rossi (1995) and pH (Silva and Queiroz 2006). The total digestible nutrients content (TDN) were estimated by the equation TDN = 87,84 – (0,7 x ADF) by Undersander et al. (1993) and nutritional relative value (NRV) by the equation RNV = (DMI x DMD)/1,29, where DMI = 120/NDF and DMD = 88,9 – (0,779 x ADF) by Rohweder et al. (1978). From 2016 the chemical composition of silages was determined using NIRS equipment. A descriptive statistical analysis and regression analysis between the years at 5% probability was conducted, using SAS software 9.4.

**Results and Discussion** The chemical characteristics of silage over the years are shown in Table 1. There was an increase of DM content of silage over the years ($r^2$ = 0.7749; $P$ = 0.0017), and the average was always near indicated in Brazil, which is 30 to 35% DM (Nussio et. al, 2001). In 2009, 60% of the farmers harvested green silage, after nine years in 2017, this percentage dropped to 22%. The ADF and NDF content reduced ($r^2$ = 0.0053; $P$ = 0.6939 and $r^2$ = 0.0483; $P$ = 0.4490, respectively). The NDT and NRV increased ($r^2$ = 0.0042; $P$ = 0.7133 and $r^2$ = 0.0178; $P$ = 0.5753, respectively) with the time, which means that the silages are improving their quality of fiber and energy. When the ten best silages of the year 2017, were evaluated the average values of ADF, NDF and starch, were similar to those found in silages in Europe (ADF 19 x 20%, NDF 35 x 36% and starch 37 x 37, respectively), (Corn Silage Contest and abcLab, 2017).

**Table 1** Chemical and Physical Characteristics of silage over the years (%).

| | Year | | | | | | | | | | | | | Year Regress. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Analysis** | **2009** | **2010** | **2011** | **2012** | **2013** | **2014** | **2015** | **2016** | **2017** | **Averge** | **Mín.** | **Máx.** | **SD** | **Pr > F** | **$r^2$** |
| DM | 29 | 31 | 31 | 31 | 32 | 33 | 32 | 33 | 33 | 32 | 19 | 49 | 4.2 | **0.0017** | **0.7749** |
| CP | 7 | 7 | 8 | 7 | 7 | 7 | 8 | 7 | 8 | 8 | 5 | 11 | 0.9 | 0.3869 | 0.1084 |
| ADF | 26 | 26 | 25 | 25 | 24 | 25 | 23 | 25 | 23 | 24 | 17 | 36 | 3.2 | **0.0053** | **0.6939** |
| NDF | 45 | 44 | 46 | 46 | 45 | 46 | 44 | 43 | 41 | 44 | 30 | 62 | 4.9 | **0.0483** | **0.4490** |
| TDN | 70 | 70 | 70 | 71 | 71 | 71 | 72 | 71 | 71 | 71 | 62 | 77 | 2.2 | **0.0042** | **0.7133** |
| Starch | 33 | 34 | 33 | 33 | 33 | 31 | 34 | 30 | 32 | 32 | 5 | 47 | 4.9 | 0.2548 | 0.2208 |
| pH | 3.9 | 3.9 | 3.9 | 3.8 | 3.8 | 3.8 | 3.8 | 3.9 | 3.9 | 3.9 | 3.4 | 5.3 | 0.2 | 0.8929 | 0.9282 |
| NRV | 143 | 146 | 142 | 143 | 145 | 143 | 152 | 151 | 163 | 152 | 92 | 236 | 21.4 | **0.0178** | **0.5753** |
| IVDMD | - | - | 72 | 68 | 68 | 70 | 70 | 71 | 73 | 71 | 55 | 79 | 3.4 | 0.2667 | 0.2381 |
| IVNDFD | - | - | - | 55 | 52 | 55 | 56 | 48 | 51 | 52 | 29 | 68 | 4.6 | 0.1938 | 0.3782 |
| **n** | **53** | **89** | **107** | **120** | **114** | **130** | **192** | **278** | **426** | | | | | | |

**Conclusion** Corn silages of the studied region have good chemical quality, similar to the silages of countries considered models.

**References**

abcLab – ABC Foundation (2017) Laboratório de analyses físico-químicas - Laudos. available: < https://abclaboratorios.com.br/laboratorios/labfq/bromatologia/> Access: 15 march 2018. Castro, PR.

Association of Offizial Analytical Chemists – AOAC (1998) Official methods of analysis. 16.ed. Washington, D.C.

Corn Silage Contest – ABC Foundation (2017) Melhores Silagens – TOP 10 – 2017. available: <http://fundacaoabc.org/eventos/concurso-silagem-milho/> Access: 15 march 2018. Castro, PR.

Linn, J. (2003) Making high corn silage diets work. In: Minnesota Dairy Conf. and Dairy Expo, 2003, St. Cloud. Proceeds St Cloud, MN, 2003. p.58-63.

National Research Council – NRC (2001) Nutrient requirements of dairy cattle. 7.ed. Washington, DC: National Academy Press, 2001. 381p.

Nelson, D.W. & Sommers, L.E. (1980) Total nitrogen analysis for soil and plant tissues. Journal of the Association of Official Analytical Chemists. Environ, 1980. p. 770-778.

Nussion, L.G., Campos, F.P. & Dias, F.N. (2001) Importância da qualidade da porção vegetativa no valor alimentício da silagem de milho. In: SIMPÓSIO SOBRE PRODUÇÃO E UTILIZAÇÃO DE FORRAGENS CONSERVADAS, 1., 2001, Maringá. Anais... Maringá: UEM/CCA/DZO, 2001. p.127-145.

Pereira, J.R.A. & Rossi, jr. P. (1995) Manual prático de avaliação nutricional de alimentos. 1.ed. Piracicaba: Fundação de Estudos Agrários Luiz de Queiroz, 1995. 25p.

Rohwedder, D.A., Barnes, R.F. & Jorgensen, N. (…) Proposed hay grading standarts based on laboratory analyses for evaluating quality.

Silva, D.J. & de Queiroz, A.C. (2006) Análise de alimentos: métodos químicos e biológicos. 3.ed. Viçosa: UFV, 2006. 235p.

Tilley, J.A. & Terry, R.A.A. (1963) A two-stage technique of the in vivo digestion of forage crops. Journal British of Grassland Society, v.18, p.104-111.

Undersander, D., Mertens, D.R. & Thiex, N. (1993) Forage analyses procedures. Omaha: National Forage Testing Association, p.130-131.

Van Soest, P.J. (1994) Nutritional ecology of the ruminant. Ithaca: Cornell University, 476p.

# Relationship between length of cut and mean particle length in whole-plant corn silage at two types of forage harvesters

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**Keywords:** corn silage, forage harvester, mean particle length

**Introduction** In Brazil, most dairy farmers harvest the corn crop with a Pull Type (PT) 90.4% vs. 9.6% Self Propel forage harvester (SP - Bernardes and Rego 2014). The theoretical length of cut (TLOC) setting on forage harvesters can vary as dairy farmers attempt to increase the mean particle length (MPL) of whole-plant corn silage (WPCS) with the aim of increasing its content of physically-effective fiber. The hypothesis is that TLOC and MPL has a different relationship according to the harvesters. Our objective was to evaluate the relationship between TLOC at two kinds of forage harvesters and MPL of WPCS estimated by Penn State Particle Size Separator (PSPS).

**Materials and Methods** The corn crop was harvested at 33 % of dry matter. Two types of forage harvesters and three settings of TLOC per each type were evaluated: PT without kernel processor (3, 5 and 9 mm) and SP (6, 12 and 18 mm). The objective was to promote different TLOCs. As-fed whole-plant samples were used for determination of MPL using PSPS. The PSPS procedure was conducted manually using 3 sieves (19-mm, 8-mm, and 1.18-mm) and a pan according to the method of Kononoff et al. (2003). The MPL was estimated by PSPS spreadsheet (https://extension.psu.edu/penn-state-particle-separator). Regressions to determine linear and quadratic relationships between TLOC and MPL were performed using PROC REG of SAS. Best-fit regression (linear or quadratic) was chosen using the highest coefficient of determination ($R^2$) and lowest root mean square error (RMSE) as indicators.

**Results** There was a relationship between TLOC and MPL for SP ($y = -0.02x^2 + 0.71x + 5.01$, RMSE =0.63; $R^2 = 0.81$.; $P = 0.01$, Panel A). A relationship was also observed for PT, but with lower $R^2$ ($y = 0.12x^2 - 1.20x + 12.97$, RMSE = 0.53; $R^2 = 0.66$; $P = 0.01$, Panel B).

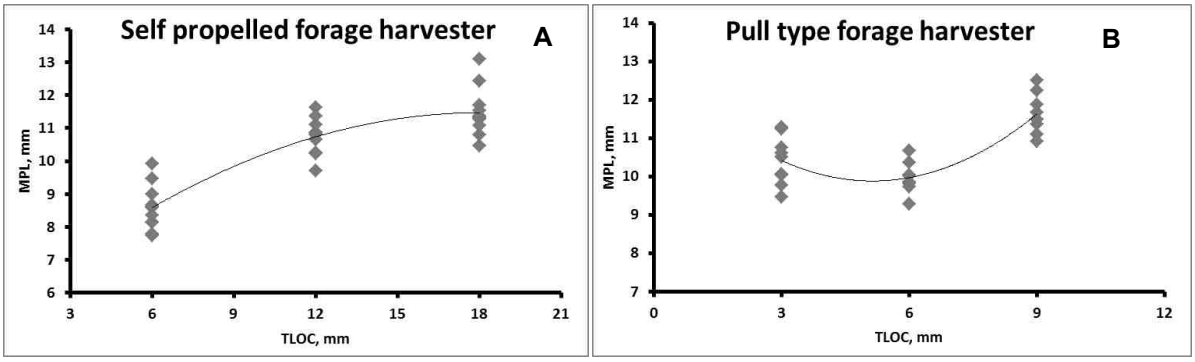


**Figure 1.** Relationship between MPL (mm) and TLOC (mm) at two different forage havesters (Panel A - SP / Panel B - PT).

**Discussion** The TLOC is controlled by the peripheral speed of the feed rolls relative to the speed of the cutter head and the number of cutter head knives (Shinners 2003) which affects MPL. The harvesters have different cutterhead assemblies, the PT has flywheel and SP has cylindrical cutterheads system. The cylindrical has room for more knives on a relatively small diameter, therefore allows higher rotational speeds than flywheel cutterheads (Shinners 2003). Other factors related to the harvesters can also affect MPL, such as kernel processing, processor roll-gap setting (Shinners et al. 2000), knife sharpness and knife to shear bar clearance (Shinner, 2003). Apparently, for PT the MPL differences between the TLOCs 3 vs. 6 mm (the means of MPL - 10.4 vs. 10 mm, respectively) were minimal and it impacted the behavior of the curve. The ratio of MPL to TLOC was 1.42, 0.82 and 0.64

for SP at TLOCs – 6, 12 and 18 mm, respectively. For PT, the ratio was 3.49, 1.45 and 1.29 at TLOC – 3, 6 and 9 mm. This ratio MPL:TLOC varies with the TLOC (Shinners 2003). For short TLOCs, the MPL is much greater than TLOC (Pitt 1987). As the TLOC increases, MPL begins to approach the TLOC (Pitt 1987).

**Conclusions** TLOC was related to MPL in both types of harvesters, however for PT harvest type the relationship showed that lower coefficient of determination probably due small differences in MPL when the TLOC was augmented from 3 to 6 mm.

**References**

Bernardes, T.F. & Rego, A.C. (2014) Study on the practices of silage production and utilization on Brazilian dairy farms. Journal of Dairy Science, 97, 1852-1861.

Kononoff, P.J., Heinrichs A.J. & Buckmaster, D.R. (2003). Modification of the Penn State forage and total mixed ration particle separator and the effects of moisture content on its measurements. Journal of Dairy Science, 86, 1858-1863.

Pitt, R.E. (1987) Theory of particle size distributions for chopped forages. Trans. ASAE, 30, 1246-1253.

Shinners, K.J., Jirovec, A.G., Shaver, R.D. & Bal, M. (2000) Processing whole-plant corn silage with crop processing rolls on a pull-type forage harvester. Applied Engineering in Agriculture, 16, 323-331.

Shinners, K.J. 2003. Engineering principles of silage harvesting equipment. In: Buxton, D. R., Muck, R. E., & Harrison, J. H. (ed). Silage science and technology. Agron. Monogr. 42. ASA, CSSA, and SSSA, Madison, WI, USA. p. 361-403.

# Relationship of the chemical composition of corn silage to milk production

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**Keywords:** corn silage, milk yield, Mexico

**Introduction** In 2013, Mexico was identified as one of the countries most affected by climate variability. Such variability can have a negative impact on maize production, which is the main peasant farming activity in Mexico, practiced by nearly 2 million farmers, 85% of whom have less than 5 ha of land. To optimize land use in this precarious situation, choice of appropriate corn silage is vital – but is complicated by the lack of information on corn silage and parameters of milk production. The objective of the present study was to characterize corn silage produced in Mexico according to chemical composition, forage yield (ton / ha), as well as potential milk production.

**Materials and Methods** A search was made for studies related to corn forage yield (ton / ha), density (number of plants / ha) and chemical composition (dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), nonfibrous carbohydrates (NFC), starch, fat, DM digestibility, NDF digestibility) of corn silage produced in Mexico. The total digestible nutrients (TDN), net energy of lactation (NEL Mcal / kg DM), kilograms of milk per ton of silage as dry matter (kg milk / ton DM) and kilograms of milk per hectare of forage silage (kg milk / ha) were determined using the spreadsheet MILK2006 (Shaver 2006). The publications were obtained from searches in databases such as Elsevier, Google, SCOPUS and Web of Science, using the terms “corn silage”, “production”, “Mexico” and “milk”, selecting a total of 14 articles and 144 studies from the years 2001 to 2016. A cluster analysis (CL) was carried out using the Proc Cluster procedure to sort the corn silage according to its chemical and productive characteristics.

**Results and Discussion** Six groups (CL) of corn silage were obtained. The first group (CL1) was made up of those with greater DMd%, CP%, NDFd%, NFC%, TDN, NEL and kg milk/ ton DM, the second (CL2) of those with the highest CP content, the third (CL3) of those with the highest plant density (number plants/ha), NDF% and the lowest NFCS, TND, NEL, kg milk/ton DM and kg milk/ha, the fourth (CL4) was made up of highest NDF% and OM% content, the fifth (CL5) had higher forage yield (ton DM / ha), DM% content and kg milk / ha, and the sixth (CL6) had higher DM digestibility and the lowest OM% content.The group that produced the highest milk production (kg milk / ton DM) was the one with the highest NDF digestibility and NFCS. This may be because one of the primary factors that determine the nutritional quality of silage is the digestibility of NDF (Khan et al. 2015). Dairy cows also require forage fiber in their diet to maintain rumen function and maximize milk production; however, the excess NDF limits the dry matter intake (CL3 and CL4) due to its contribution to rumen filling (Krämer-Schmid et al. 2016) and forage degradation. In the same way, several works have shown that when the percentage of dry matter of silage maize increases from 25 to 30%, the increase in intake and milk production is higher (Khan et al. 2015). This increase is not only related to the percentage of DM and intake, but also to the increase in nutritional value due to the higher grain content. The high concentration of NEL and CP content stimulates the production of microbial protein in the rumen, increasing the production and concentration of milk protein (Ferraretto et al. 2013; Lascano et al. 2016) and milk yield (kg milk/ton DM); this effect can be seen in CL1. The highest milk

yield (kg milk/ha) corresponds to the highest forage DM yield and DM% content which, in spite of the low TDN and NEL, produce more milk/ha derived from this higher DM yield.

**Table 1.** Corn forage production (DM yield, ton / ha), plant density (plants/ha), chemical composition (%) of the silage and its potential milk production (kg milk / ton DM and kg milk / ha) of corn silages sown in Mexico.

| Variables | CL1 | CL2 | CL3 | CL4 | CL5 | CL6 |
|---|---|---|---|---|---|---|
| DM yield | 15.6 | 18.7 | 17.4 | 21.4 | 27.7 | 20.1 |
| Plant density | 82600 | 80280 | 92400 | 62500 | 70000 | 69500 |
| DM, % | 29.6 | 29.8 | 30.2 | 25.4 | 40.0 | 22.1 |
| DMd, % | 70.5 | 67.0 | 68.8 | 62.7 | 58.9 | 64.3 |
| CP, % | 8.6 | 8.2 | 7.6 | 5.2 | 7.9 | 6.8 |
| NDF, % | 40.2 | 56.5 | 62.8 | 61.1 | 56.9 | 54.9 |
| NDFd, % | 64.9 | 57.6 | 55.4 | 55.0 | 57.5 | 57.5 |
| NFC, % | 40.8 | 24.9 | 19.2 | 24.2 | 24.8 | 25.7 |
| OM, % | 93.5 | 93.6 | 93.5 | 94.8 | 93.5 | 91.7 |
| Fat, % | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 |
| Starch, % | 22.8 | 23.0 | 23.0 | 23.0 | 23.1 | 23.1 |
| TDN, % | 68.1 | 65.1 | 60.1 | 62.9 | 61.8 | 63.6 |
| NEL(MJ/kgDM) | 1.43 | 1.38 | 1.27 | 1.34 | 1.29 | 1.34 |
| Kg milk/ tonDM | 546 | 514 | 451 | 489 | 465 | 493 |
| Kg milk/ ha | 20131 | 22702 | 18867 | 24831 | 30503 | 23397 |

DM = dry matter content, DMd = dry matter digestibility, CP = crude protein, NDF = neutral detergent fiber, NDFd = neutral detergent fiber digestibility, NFC = nonfibrous carbohydrates, OM = organic matter, TND = total digestible nutrients, NEL = net energy for lactation (MJ / kg DM), kg milk / ton DM = kilograms of milk per ton of dry matter, Kg milk / ha = kilograms of milk per hectare

**Conclusions** The CL1 corn silage in Mexico presents the highest DM digestibility and NEL, producing more milk (kg milk / ton DM); however, the best option is the silage that has a higher forage yield (ton DM / ha) and more than 35% DM (CL5), since this produces more kg milk / ha.

**References**

Ferraretto, L.F., Crump, P.M, and Shaver, R.D. (2013) Effect of cereal grain type and corn grain harvesting and processing methods on intake, digestion, and milk production by dairy cows through a meta-analysis. Journal of Dairy Science, 96, 533-550.

Khan, N.A., Yu, P.Q., Ali, M., Cone, J.W. & Hendriks W.H. (2015) Nutritive value of maize silage in relation to dairy cow performance and milk quality. Journal Science Food Agriculture. 95, 238-252.

Krämer-Schmid, M., Lund, P.& Weisbjerg, M.R. (2016) Importance of NDF digestibility of whole crop maize silage for dry matter intake and milk production in dairy cows. Animal Feed Science and Technology, 219, 68-76.

Lascano, G.J., Alende, M., Koch, L.E. & Jenkins, T.C. (2016) Changes in fermentation and biohydrogenation intermediates in continuous cultures fed low and high levels of fat with increasing rates of starch degradability. Journal of Dairy Science, 99, 6334-6341.

Shaver, R. (2006) Corn silage evaluation: MILK 2000 challenges and opportunities with MILK 2006. http://www.uwex.edu/ces/dairynutrition/documents/milk20062.pdf. 12-09-2017.

# Shredlage and its effect on particle size and digestibility

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**Keywords:** conventional silage, in vivo, separator, shredlage

**Introduction** Fine chopping during harvest improves silage packing density and fermentation, and aids in preserving nutritive value (McDonald, 1981). However, feedstuffs with small particle size may have adverse effects on digestion, e.g. decreasing ruminal pH, salivary buffer secretion, fibre degradation and dry matter digestibility reduction (Allen and Mertens, 1988). Physical effectiveness of silage-based rations can be improved by increasing forage chop length (Couderc et al. 2006). A novel method of harvesting whole-plant maize silage, shredlage, may increase kernel processing and physically effective neutral detergent fibre (peNDF). Also, potential improvements in total-tract starch digestibility by dairy cows appear to be an important benefit (Ferrareto and Shaver 2012). The objective of the study was to compare particle sizes, chemical composition and in vivo nutrient digestibility of whole plant maize silages made as conventional silage or shredlage.

**Material and Methods** Stay green hybrid Agro Vitallo (FAO 280, KWS) was harvested as conventional silage (theoretical length of cut 10 mm) and shredlage (theoretical length of cut 25 mm) by Jaguar forage harvesters (Claas Saulgau GmbH; Bad Saulgau; Germany). The silages were stored in 1 $m^3$ experimental silos. Chemical composition, particle size and in vivo nutrient digestibility were determined for both silages. Six Romanov sheep (over a year old castrated males) of similar body weight were divided into two groups. These animals were housed in individual stalls and fed either (a) conventional maize silage (CON) or (b) shredlage maize silage (SH) for 14 days. Thereafter, the sheep were individually housed in metabolic cages for five days and silages were offered in two equal meals at 7:00 a.m. and 6:00 p.m. The animals had free access to water during the whole trial. Individual sheep weight was measured at days 0 and 19. Feed refusals and excrements were sampled every day, accumulated in a plastic bag for each sheep and stored in a freezer. Feed samples and refusals were oven-dried at 55 °C and excrements were freeze-dried (Alpha 1-4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) to constant weight, then milled through a 1 mm sieve (Retsch SM 100; Retsch GmbH, Haan, Germany) and analysed for ash (6 h combustion at 550 °C), dry matter (DM; oven drying for 6 h at 105 °C), crude protein (CP; method 976.05; AOAC 2005), neutral detergent fibre (NDF; Van Soest et al. 1991), acid detergent fibre and acid detergent lignin (ADF and ADL; method 973.18; AOAC 2005), ether extract (EE; 6 h extraction with petroleum-ether), starch (method No. 920.40; AOAC 2005) and energy (calorimetric determination). All data were analysed with the GLM procedure of SAS (SAS Institute Inc. 2002). Processing of maize forage and replicates were given as fixed effects in the model for evaluation of chemical composition, fermentation profile and proportion of particle sizes. Comparison of nutrient digestibility was evaluated with processing of maize forage as a fixed effect and animals as random effect in the model. Comparisons of means were done with the Tukey-Kramer adjustment.

**Results** Chemical composition was comparable in CON and SH. A small difference was observed in dry matter concentration – CON contained 343 g/kg and SH contained 355 g/kg ($P < 0.001$). A difference was also found in CP content of the silages – CON 77.9 and SH 82.5 g/kg DM ($P < 0.01$). No differences were observed in concentrations of organic matter (OM) (CON 965 vs SH 963 g/kg DM), EE (CON 31.2 vs SH 32.6 g/kg DM), NDF (CON 438 vs SH 432 g/kg DM), ADF (CON 217 vs SH 209 g/kg DM), ADL (CON 46.3 vs SH 45.6 g/kg DM), starch (CON 331 vs 337 g/kg DM) and water soluble carbohydrates (CON 3.8 vs SH 4.0 g/kg DM). The way of maize silage harvest had no impact on fermentation profile of silages. The pH values were similar (CON 3.92 vs SH 3.94) as well as the

amount of lactate (CON 44.7 vs SH 41.2 g/kg DM), acetate (CON 16.0 vs SH 14.5 g/kg DM) and ammonia (CON 49.0 vs SH 45.1 g/kg CP) in both types of silages. Significant differences in proportion of coarse and fine particles were observed. Fraction of particles > 19 mm was higher for SH (18.8% as fed retained) compared with CON (2.2%; $P < 0.01$). The CON silage had a higher proportion of particles from 8 mm to 19 mm (62.5%) compared with SH (53.1; $P < 0.001$). A higher proportion of fine particles was observed for CON (35.3 %) compared to SH (28.1%; $P < 0.05$). Digestibility of DM was affected by maize silage processing. Higher DM digestibility was observed in SH (71.5%) compared with CON (66.2%; $P < 0.01$). There were no differences among silages in CP and EE digestibility. The digestibility of starch (CON 95.2 vs SH 97.6%) tended ($P = 0.07$) to differ between silages. The SH silage had higher NDF digestibility (56.8%) compared with CON (47.4%; $P < 0.05$). Also, the digestibility of energy was higher in SH (70.9%) compared with CON (65.9%; $P < 0.05$).

**Discussion** We observed the higher percentage of coarse particles in SH silage which could increase the risk of sorting (Chase, 2015). The higher DM and NDF digestibility were caused by lengthwise processing of maize forage. The better processing of maize kernels lead to a tendency of increasing starch digestibility in SH silage. The increasing digestibility of DM and ADF with increasing theoretical length of cut was noted in experiment of Cooke & Bernard (2005). In addition, the fibre and starch digestibility was improved by kernel processing compared with unprocessed corn silage. The significantly higher digestibility of starch was further documented by Ferraretto and Shaver, 2012.

**Conclusion** The SH had lower volume weight, which did not affect fermentation process of silage. Processing of maize forage affected particle size of silages and DM, OM and NDF digestibility.

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**References**

Allen, M.S. & Mertens, D.R. (1988) Evaluating constraints in fiber digestion by rumen microbes. Journal of Nutrition, 118, 261-270.

AOAC (2005) Official Methods of Analysis of AOAC International. 18th Ed. AOAC International, Gaithersburg, USA.

Chase, L.E. (2015) Shredlage in dairy cattle rations, In: 77th Cornell nutrition conference for feed manufactures, proceedings, 215-220.

Cooke, K.M. & Bernard, J.K. (2005) Effect of length of cut and kernel processing on use of corn silage by lactating dairy cows. Journal of Dairy Science, 88, 310-316.

Couderc, J.J., Rearte, D.H., Schroeder, G.F., Ronchi, J.I. & Santini, F.J. (2006) Silage chop length and hay supplementation on milk yield, chewing activity, and ruminal digestion by dairy cows. Journal of Dairy Science, 89, 3599-3608.

Ferraretto, L.F. & Shaver, R.D. (2012) Effect of corn shredlage on lactation performance and total tract starch digestibility by dairy cows. The Professional Animal Scientist, 28, 639-647.

McDonald, P. (1981) The Biochemistry of Silage. New York: John Wiley and Sons, 327 p.

SAS Institute Inc. (2002) SAS/STAT User´s Guide: Version 9.1 for window. SAS Institute Inc., Cary, NC, USA.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991) Methods for dietary fibre, neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

# Silage fermentation of fresh and exposed corn stover

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**Keywords:** corn stover, fermentation quality, silage

**Introduction** Corn stovers from corn after harvesting cobs are discarded on the field, where they are often burned and then used as fertilizer (Li et al. 2015). However, corn stovers are used fresh or dried for feeding livestock, and they are low cost and abundant materials in China when green grass is lacking in winter (Li et al. 2015 ;Cao et al. 2016). As they are not available year around. But, silage preserves nutritional value, and also extend the retention time, facilitating fodder provision throughout the year, regardless of the weather. The objectives of this study were to determine the silage fermentation of fresh and exposed corn stover in the field.

**Materials and Methods** A local cultivar (Jintian) of corn (*Zea mays* L.) stover was obtained from an experimental field of Inner Mongolia Agricultural University (Huhhot, China). Corn was harvested at the early dough stage of maturity in September 2nd, 2015, and silages were prepared with these stovers exposed in the field for 0, 7, 15, 30, 60, 90 and 180 d, respectively. The ensiling materials were chopped into 20 mm length and moisture was adjusted to 60%. Laboratory polyethylene silos (1 L, 10cm in diameter and 20 cm in length, Shenzhen Guanruilong Chemical Co., Ltd., China) were used for silage making, and silos were sealed with a screw top and plastic tapes, then stored at ambient temperature. Three replicates per treatment were opened at 60 d of ensiling and chemical composition and fermentation quality were determined using the method described by Cai (2004).

**Results and Discussion** Dry matter (DM) of fresh corn stover (at 0 d of exposure) was 30.7% and increased more than 3.8 to 16.1% compared to the corn stover exposed in the field for 7 to 180 days. With the extension of exposure time in the field crude protein (CP) content was decreased. At 180 d of exposure CP content was reduced to 3.3% on DM basis (Table 1).

**Table 1.** Chemical composition of corn stover during field exposure

| Exposure day | DM | OM | CP | EE | NDF | ADF | WSC |
|---|---|---|---|---|---|---|---|
| | % | ---------- %DM---------- | | | | | |
| 0 | 30.7$^{g}$ | 94.5$^{d}$ | 9.5$^{a}$ | 2.2$^{a}$ | 45.6$^{f}$ | 30.5$^{c}$ | 13.2$^{a}$ |
| 7 | 34.5$^{f}$ | 95.3$^{b}$ | 8.3$^{b}$ | 2.0$^{ab}$ | 46.0$^{ef}$ | 32.1$^{b}$ | 13.1$^{b}$ |
| 15 | 35.3$^{e}$ | 96.8$^{a}$ | 8.2$^{b}$ | 1.9$^{ab}$ | 46.3$^{e}$ | 29.1$^{d}$ | 12.8$^{c}$ |
| 30 | 46.7$^{a}$ | 95.1$^{bc}$ | 7.9$^{c}$ | 1.8$^{ab}$ | 51.7$^{c}$ | 26.3$^{e}$ | 12.2$^{d}$ |
| 60 | 39.4$^{d}$ | 94.9$^{c}$ | 7.1$^{d}$ | 1.8$^{ab}$ | 49.6$^{d}$ | 34.6$^{a}$ | 10.4$^{e}$ |
| 90 | 45.5$^{b}$ | 95.4$^{b}$ | 6.3$^{e}$ | 1.7$^{ab}$ | 52.5$^{b}$ | 29.2$^{d}$ | 9.4$^{f}$ |
| 180 | 43.7$^{c}$ | 94.6$^{d}$ | 3.3$^{f}$ | 1.6$^{b}$ | 54.7$^{a}$ | 34.7$^{a}$ | 7.2$^{g}$ |
| SEM | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 |
| *P* value | <0.0001 | <0.0001 | <0.0001 | 0.1708 | <0.0001 | <0.0001 | <0.0001 |

$^{a\ to\ g}$ Means within a column with different superscripts differ (*P*<0.05). DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrate; SEM, standard error of the mean.

The corn stover silages prepared from corn stover exposed in the field for 0/7 days were well preserved, with lower ($P$<0.05) pH and ammonia-N contents while higher ($P$<0.05) lactic acid contents than that of silages prepared from corn stover exposed in the field for 15/30/60/90/180 days. In addition, the pH of the silage dropped below 4.0, which inhibited butyric fermentation and ammonia-N production by clostridia (Table 2). Silage prepared from stover that had been exposed for 180 d exhibited poor fermentation quality. Reduction in sugar was a factor contributing to poor fermentation, as evidenced by the fact that the fresh stover contained abundant sugars as a substrate for LAB to produce lactic acid.

**Table 2.** Fermentation quality of corn stover silages at 60 days of ensiling

| Exposure | pH | Lactic acid | Acetic acid | Propionic acid | Butyric acid | Ammonia-N |
|---|---|---|---|---|---|---|
| 0 | 3.84$^{d}$ | 2.53$^{a}$ | 0.42$^{c}$ | 0.09$^{d}$ | 0.01$^{ab}$ | 0.37$^{d}$ |
| 7 | 4.07$^{c}$ | 2.43$^{a}$ | 0.86$^{b}$ | 0.11$^{d}$ | 0.02$^{a}$ | 0.42$^{d}$ |
| 15 | 4.25$^{b}$ | 0.83$^{d}$ | 0.42$^{c}$ | 0.37$^{b}$ | 0.02$^{a}$ | 0.67$^{c}$ |
| 30 | 4.35$^{b}$ | 0.58$^{e}$ | 0.53$^{c}$ | 0.20$^{c}$ | ND | 1.41$^{b}$ |
| 60 | 4.28$^{b}$ | 1.32$^{c}$ | 1.17$^{a}$ | 0.53$^{a}$ | 0.01$^{ab}$ | 1.30$^{b}$ |
| 90 | 4.32$^{b}$ | 1.52$^{b}$ | 1.20$^{a}$ | 0.09$^{d}$ | 0.01$^{ab}$ | 2.33$^{a}$ |
| 180 | 5.64$^{a}$ | 0.85$^{d}$ | 0.41$^{c}$ | 0.51$^{a}$ | ND | 2.40$^{a}$ |
| SEM | 0.04 | 0.04 | 0.05 | 0.03 | 0.0048 | 0.06 |
| $P$ value | <.000 | <.0001 | <.0001 | <.0001 | 0.0527 | <.0001 |

[a to d]Means within a column with different superscripts differ ($P$<0.05). FM, fresh matter; ND, not detected; SEM, standard error of the mean.

**Conclusions** The fresh corn stover had a higher CP content, silage prepared from fresh corn stover had better fermentation quality than other silages of exposed material. Therefore, the corn stover silage should be prepared immediately after harvesting.

**References**

Cai, Y. (2004) Analysis method for silage. In: Japanese Society of Grassland Science (ed.), Field and laboratory methods for grassland science. Tokyo, Japan: Thsho Printing Co., Ltd. p.279-282.

Cao, Y., Zang, Y., Jiang, Z., Han, Y., Hou, J., Liu, H., Zhong, R., Fang, J., Zhang, A. & Yoshida, N. (2016) Fermentation quality and nutritive value of fresh and fermented total mixed rations containing Chinese wildrye or corn stover. Grassland Science, 62:213-223.

Li, D., Ni, K., Pang, H., Wang, Y., Cai, Y. & Jin, Q. (2015) Identification and antimicrobial activity detection of lactic acid bacteria isolated from corn stover silage. Asian-Australasian Journal of Animal Sciences, 28:620-631.

# Silage preparation and fermentation qualtiy of Napier grass treated with lactic acid bacteria and cellulase in Mozambique

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**Keywords:** Napier grass, silage, lactic acid bacteria, cellulase

**Introduction** Napier grass (*Pennisetum purpureum*) commonly referred to as elephant grass is popular fodder crop for dairy farmers in tropics including Mozambique. This grass shows high dry matter yield, drought tolerant, growing in wide range of soils and long life cycle, but low crude protein and high neutral detergent fiber. Tropical silages are usually difficult to make high quality because of their high moisture and less sugar content. Technologies to create good quality animal feed from tropical grasses and to provide long-term storage of the resulting silage need to be developed.

In order to establish the animal feed production system to cover the shortage of animal feed in dry season in tropics, the microbial population, chemical composition of of Napier grass and the effects of lactic acid bacteria (LAB) and cellulase on silage fermentation was studied in Mozambique.

**Materials and Methods** Napier grass was obtained from a farm field (Xai-xai, Gaza, Mozambique) on 30 August, 2017. The grass was cut into about 10 mm, and their silage were prepared as following treatments: control; LAB inoculant (*Lactobacillus plantarum* Chikuso-1, Snow Brand Seed Co., Ltd, Sapporo, Japan); *Acremonium* cellulase (AC, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) for fresh grass as recommended by the inculant manufacturer. The silages were stored at outdoor temperature (25-38$^{o}$C) and the silos were opened after fermentation of 60 days. The pH, organic acids and ammonia-N of silage samples were analyzed.

**Results** Epiphytic LAB in the Napir grass before ensiling was relatively low ($3.2x10^4$) but coliform bacteria ($3.2x10^7$) and aerobic bacteria ($6.4x10^7$) were higher. Silage inoculated with LAB, cellulase or their conbination exhibited reduced counts of aerobic bacteria and coliform bacteria, increased lactic acid concentration, lower pH, butyric acid and ammonia nitrogen compared with the control.

**Conclusions** The results confirmed that LAB and cellulase were suitable as potential additives for silage preparation, and they could improve fermentation quality of Napier grass silage in Mozambique.

# Substitution of soybean meal and cotton seed with whole crop soybean silage in dairy cow diets to increase feed self-sufficiency of dairy farms in Italy

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**Keywords:** dairy cows, milk yield, protein self-sufficiency, soybean silage

**Introduction** Finding new sources of home-grown protein is crucial for the future profitability and environmental sustainability of the dairy livestock sector in Italy (Borreani et al., 2013). Soybean meal is the source of protein used most frequently in dairy cow diets, but its overseas origin poses several threats for the economic and environmental impact of milk production. In Italy, in order to increase the protein and energy concentrations in cow diets, many farmers utilize cottonseed, which is generally imported from non-European countries and can be contaminated by aflatoxins. The European Union is concerned that such a massive dependency on protein imports makes the livestock sector extremely vulnerable to price volatility, which can cause an increase in production costs and a reduction in the profitability of the milk production sector. Soybean could be produced in Italy, and direct harvesting as a whole crop silage could offer greater flexibility and opportunities to increase the amount of protein and energy as fat produced on farm, to reduce or eliminate the use of soybean meal and cottonseed and to make the milk production chain more traceable. The aim of this research was to evaluate whether whole-crop soybean silage could replace soybean meal and cottonseed in the mid- to late-lactation rations of Holstein cows in Italy.

**Materials and Methods** The research was conducted on a commercial dairy farm in northern Italy. Soybean crop with low trypsin inhibitor activity (Ascasubi, SIS, San Lazzaro di Savena, Bologna, Italy) was sown in a 10-ha field at the end of May 2015 (after wheat harvested as a whole-crop silage). Soybean was harvested, at the R7-8 stage of growth (October 2015), by chopping it to a theoretical length of 10 mm with a self propelled forage harvester (Claas Jaguar 960, equipped with a direct disk whole-crop header) and ensiled in a bunker silo. The feeding case study was conducted from January to April 2016 (3 periods lasting 28 d, 14 d for adaptation and 14 d for sampling, no turnout). A total of 88 mid-lactation Holstein cows, housed in a naturally ventilated free-stall barn, were used in each period. The cows (272 ± 72 d in milk, parity 2.3 ± 1.0, mean ± SD) were blocked by parity, stratified by days in milk (DIM), and randomly assigned to 4 pens. Each pen contained 4 primiparous and 18 multiparous cows, and the mean DIM and milk yield were similar across pens. The treatments consisted of either a conventional diet based on corn silage, grass and legume silages, a high moisture ear corn silage, soybean meal and cottonseed diet (**CON**; n = 2 pens; n = 44 cows) or a similar diet reformulated using whole crop soybean silage to replace soybean meal and cottonseed (**SBS**; n = 2 pens; n = 44 cows). The compositions of the diets are presented in Table 1. The cows were fed once a day at 110% of the expected intake. The amounts of feed offered and orts were weighed from day 15 to 28 of each period, and the dry matter intake (DMI) was calculated at a pen-level. Milk weights were recorded (days 15-28) and milk samples were collected for component analysis during each milking for three consecutive days (days 18-20 and 25-27). Samples of TMR and dietary ingredients were also collected once a week for nutrient analysis. The dry matter (DM) content of the TMR and orts was determined each day. Energy corrected milk (ECM) and 3.5% fat corrected milk (3.5%FCM) were calculated. The milk production and quality parameters were analysed, with treatment as a fixed effect and pen and period as random effects, and significance was declared at $P < 0.05$.

**Results and Discussion** The whole crop soybean silage had a DM content of 53% and was well fermented (pH = 4.64; lactic acid = 44.1 g/kg DM; acetic acid = 14.8 g/kg DM; butyric acid = 0.3 g/kg DM; and $NH_3$-N = 75 g/kg TN). The chemical characteristics (% of DM) of the

soybean silage were 22.8% crude protein, 10.4% ether extract, 42.1% NDF and 6.2% lignin, and the estimated Net Energy of Lactation ($NE_L$) was 6.09 MJ/kg DM. The obtained values were consistent with data reported by Vargas-Bello-Perez et al. (2008) and Mustafa and Seguin (2003). Nutrient compositions of the two diets are reported in Table 2. Replacing soybean meal (2.7% of the DMI) and cottonseed (2.0% of the DMI) with soybean silage (8.7% of the DMI) decreased the milk yield, but not the ECM or 3.5%FCM yields (Table 3). The fat and protein contents were higher for cows fed the SBS diet, but the fat and protein yields did not differ between treatments. The DMI was higher in the cows fed SBS, but the conversion of feed to milk was higher for the cows fed the CON diet.

**Conclusions** Under the conditions considered in this study, whole crop soybean silage could be used to substitute soybean meal and cottonseed in diets fed to mid-lactation cows, as it was found to lead to similar ECM and 3.5% FCM yields, even though the feed conversion to milk was lower in the cows fed SBS. The inclusion of soybean silage in the total mixed ration for dairy cows can benefit the producer as it contributes to increasing farm self-sufficiency, in terms of crude protein and energy, and to improving the traceability of the milk production chain, without penalizing the milk production potential of mid-to late-lactation cows.

**Table 1.** Diet ingredients.

| Item, % on the DM | CON | SBS |
|---|---|---|
| Corn silage | 29.9 | 29.2 |
| Alfalfa silage | 17.3 | 16.9 |
| Grass silage | 17.9 | 15.7 |
| High moisture corn | 12.4 | 12.1 |
| Soybean silage | - | 8.7 |
| Corn grain | 5.7 | 5.5 |
| Rapeseed meal | 7.7 | 7.5 |
| Soybean meal | 2.7 | - |
| Cotton seed | 2.0 | - |
| Molasses | 2.8 | 2.7 |
| Mineral and vitamin premix | 1.6 | 1.5 |

**Table 2.** Nutrient composition of the diets.

| Item | CON | SBS |
|---|---|---|
| DM (%) | 43.1 | 42.5 |
| Crude protein (% DM) | 13.9 | 13.3 |
| Ether extract | 3.1 | 3.2 |
| Neutral Detergent Fibre (% DM) | 36.4 | 37.1 |
| Acid Detergent Fibre (% DM) | 22.9 | 24.3 |
| Lignin (% DM) | 4.3 | 4.1 |
| Ash (% DM) | 7.2 | 7.7 |
| Starch (% DM) | 20.9 | 21.7 |
| Non-fibre carbohydrate (% DM) | 39.3 | 38.8 |
| Metabolizable Energy (MJ/kg DM) | 9.7 | 9.6 |
| Net Energy of Lactation (MJ/kg DM) | 6.0 | 5.9 |

**Table 3.** Dry matter intake (DMI), milk production and composition when replacing soybean meal and cottonseed with whole crop soybean silage in mid-lactation dairy cow diets.

| Item | CON | SBS | SE | *P-value* |
|---|---|---|---|---|
| Milk yield (kg/d) | 29.5 | 28.5 | 0.116 | ** |
| Fat (%) | 4.22 | 4.50 | 0.054 | ** |
| Fat yield (kg/d) | 1.25 | 1.28 | 0.016 | NS |
| Protein (%) | 3.45 | 3.60 | 0.020 | ** |
| Protein yield (kg/d) | 1.02 | 1.02 | 0.007 | NS |
| Lactose (%) | 4.82 | 4.83 | 0.005 | NS |
| Milk urea nitrogen (mg/dl) | 14.7 | 14.3 | 0.236 | NS |
| Energy-corrected milk (ECM, kg/d) | 33.8 | 33.7 | 0.271 | NS |
| 3.5% fat-corrected milk (3.5%FCM, kg/d) | 33.2 | 33.0 | 0.286 | NS |
| Dry matter intake (kg/d) | 22.3 | 23.2 | 0.107 | *** |
| Milk:DMI | 1.32 | 1.23 | 0.008 | *** |
| ECM:DMI | 1.52 | 1.45 | 0.015 | * |
| 3.5%FCM:DMI | 1.49 | 1.42 | 0.016 | * |

**References**

Borreani, G., Coppa, M., Revello-Chion, A., Comino, L., Giaccone, D., Ferlay, A. & Tabacco, E. (2013). Effect of different feeding strategies in intensive dairy farming systems on milk fatty acid profiles, and implications on feeding costs in Italy. Journal of Dairy Science, 96, 6840-6855.

Mustafa, A. F. & Seguin, P. (2003). Characteristics and in situ degradability of whole crop faba bean, pea, and soybean silages. Canadian Journal of Animal Science, 83, 793-799.

Vargas-Bello-Perez, E., Mustafa, A. F. & Seguin, P., (2008) Effects of feeding forage soybean silage on milk production, nutrient digestion, and ruminal fermentation of lactating dairy cows. Journal of Dairy Science, 91, 229-235.

# Sugar beet-straw mixed silage and its effect on milk production and feed intake of dairy cows

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**Keywords:** concentrates, ECM, energy intake, mixed silage, sugar beet

**Introduction** For some years now, ensiled sugar beets (SB) have successfully been used as feedstock for anaerobic digesters. However, limited information is available about the effect of SB feeding on animal performance. Sugar beets, an energy-rich feed, can replace cereal based concentrate (CO) in ruminant nutrition. The aim of the present study was to evaluate the effect of SB mixed silage (SBMS) fed to dairy cows on milk production, feed intake (FI) and energy intake (EI).

**Materials and Methods** In the present study, two SBMS were produced and ensiled in silage bags. The two bags contained chopped SB mixed with ground wheat straw at a ratio of 12% or 15% based on fresh matter (FM). Corn silage emphasized partial mixed ration (PMR) was fed ad libitum, additional CO was fed performance-related via an electronic CO feeder. The trial design consisted of two control periods (C-1 and C-2) without SBMS in the PMR and two feeding periods (bag 1, F-1 and bag 2, F-2) where SBMS replaced CO in the PMR. The ration formulations of total mixed ration are shown in table 1. On average 165 cows with an average energy corrected milk yield (ECM) of 34.9 kg/cow/d and average FI of 22.1 kg dry matter (DM). Data was analysed with PROC MIXED (SAS).

**Table 1:** Ration formulation of total mixed ration during feeding periods (PMR and CO)

| | C-1 | | F-1 | | F-2 | | C-2 | |
|---|---|---|---|---|---|---|---|---|
| | % FM | % $DM_{cor.}$ | % FM | % $DM_{cor.}$ | % FM | % $DM_{cor.}$ | % FM | % $DM_{cor.}$ |
| Grass Silage | 28,9 | 22,2 | 24,9 | 22,2 | 25,0 | 21,5 | 31,3 | 20,7 |
| Corn Silage | 44,8 | 31,7 | 38,8 | 31,1 | 38,9 | 31,7 | 43,1 | 32,5 |
| Concentrate | 20,7 | 36,2 | 7,7 | 15,4 | 7,7 | 15,7 | 20,5 | 37,4 |
| Soybean meal | 3,2 | 5,5 | 7,4 | 14,8 | 7,2 | 14,6 | 3,0 | 5,4 |
| Minerals | 0,9 | 1,8 | 0,7 | 1,6 | 0,7 | 1,6 | 0,7 | 1,5 |
| Straw | 1,5 | 2,6 | - | - | - | - | 1,4 | 2,5 |
| SBMS bag 1 | - | - | 20,5 | 14,9 | - | - | - | - |
| SBMS bag 2 | - | - | - | - | 20,5 | 14,9 | - | - |
| | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

**Results** The selected straw contents of 12% to 15% prevented silage effluent formation. The acetic acid contents of 0.91% and 1.09% respectively, and ethanol contents of 0.52% and 0.59% respectively, of the FM in SBMS are in the mid to low ranges in the literature comparison. The residual sugar contents were 7.3% and 9.5% of FM. At pH levels of 3.6 and 3.5, and lactic acid levels of 1.51% and 1.72% of the FM, the silages produced were stable. During the feeding of the SBMS, the FM intake of the PMR increased compared with the control periods, but DM and EI declined significantly. The results of the attributes investigated concerning the FI, EI and milk ingredients are listed in Table 2. Both the CO FI, as well as the EI from the CO, fell significantly during feeding the SBMS compared with the control periods. Although the EI from soybean meal increased during the SBMS feeding periods, the combined EI from CO and soybean meal were significantly lower, compared with EI during the control periods. With a reduced quantity of milk, but a higher fat content of the milk during the SBMS feeding periods, the ECM values in the SBMS feeding periods of 34.0 kg and 33.4 kg were

very similar to the control periods values of 34.0 kg and 33.6 kg - across all test periods. The protein contents of the milk during the SBMS feeding periods were slightly lower than during the control periods. Despite theoretically-identical crude protein contents across all periods, the urea levels in the milk increased during the SBMS feeding periods, but remained in the target range.

**Table 2:** Influence of the feeding period on traits of daily feed- and energy intake and on dairy traits, urea content and the somatic cell score, significance level of F-test and LS-means

| | Unit | F-Test | C-1 | F-1 | F-2 | C-2 |
|---|---|---|---|---|---|---|
| Feed intake | kg FM | <.0001 | 48.3 [a] | 51.4 [b] | 50.6 [c] | 45.7 [d] |
| Feed intake | kg DM | <.0001 | 23.6 [a] | 21.7 [b] | 20.8 [c] | 21.0 [d] |
| Concentrate intake | kg DM | <.0001 | 8.7 [a] | 3.3 [b] | 3.2 [c] | 8.0 [d] |
| Soybean meal intake | kg DM | <.0001 | 1.3 [a] | 3.4 [b] | 3.2 [c] | 1.2 [d] |
| Concentrate and soybean meal intake | kg DM | <.0001 | 10.0 [a] | 6.7 [b] | 6.4 [c] | 9.2 [d] |
| Energy intake | MJ NEL | <.0001 | 171.56 [a] | 156.67 [b] | 152.92 [c] | 156.12 [b] |
| Energy intake CO | MJ NEL | <.0001 | 70.48 [a] | 26.00 [b] | 25.73 [b] | 62.41 [c] |
| Energy intake SBM | MJ NEL | <.0001 | 11.47 [a] | 28.81 [b] | 27.63 [c] | 10.19 [d] |
| Energy intake CO+SBM | MJ NEL | <.0001 | 81.82 [a] | 54.76 [b] | 53.29 [c] | 72.76 [d] |
| Feed efficiency | ECM/kg DM | <.0001 | 1.54 [a] | 1.67 [b] | 1.73 [c] | 1.74 [c] |
| Energy efficiency | ECM/MJ NEL | <.0001 | 0.21 [a] | 0.23 [b] | 0.23 [b] | 0.23 [b] |
| Body Condition Score | - | <.0001 | 3.06[a,c] | 3.08[a,b] | 3.12[b,d] | 3.04 [c] |
| Milk yield | kg | <.0001 | 34.9 [a] | 33.6 [b] | 33.4 [c] | 34.2 [d] |
| Energy corrected milk | kg | <.0001 | 34.0 [a] | 34.0 [a] | 33.3 [b] | 33.6[a,b] |
| Fat content | % | <.0001 | 3.79[a] | 4.11[b] | 4.04[b,c] | 3.81[a] |
| Protein content | % | <.0001 | 3.41[a] | 3.35[b] | 3.32 [c] | 3.48[d] |
| Urea content | mg/kg | <.0001 | 211 [a] | 266 | 256 [c] | 199 [d] |

**Discussion** The pH values of the sugar beet mixed silages measured in the present study were below the average pH values described by Weißbach and Strubelt (2008) for sugar beet mono silages, but on the other hand, the residual sugar contents of the mixed silages were higher. No clinical rumen aciodosis was observed. this can be explained by the rapid reabsorption of volatile fatty acids at low pH values in the rumen, and the increased chewing activity (Schmidt et al. 2001). Including SBMS in the ration leads to equal production levels for ECM in the feeding groups. The fat content in the milk is higher in F-1 and F-2 in comparison to C-1 and C-2. Especially the elevated production of milkfat is based on higher concentration of butyric acid in the rumen fluid (Oba, 2011). The calculated usage of energy for ECM is comparable between groups. In conjunction with lower CO intake in comparison with control groups indicate that energy from SBMS compensates the reduction in CO level.

**Conclusions** Straw inclusion effectively absorbed occurred effluent in SBMS. SBMS showed a good silage quality. The data indicates that SBMS can be used to replace CO in the ration of lactating dairy cows, having no adverse effects on lactation performance.

**References**

Oba, M. (2011). Review: Effects of feeding sugars on productivity of lactating dairy cows. Canadian Journal of Animal Science, 94, 37-46.

Schmidt, S.; Steingass, H.; Jungbluth, T. & Drochner, W. (2001) Sugar beet mash silage as a component of a total-mixed-ration for dairy cows – effects on parameters of digestion and animal performance. Archives of Animal Nutrition, 54, 47-59.

Weißbach, F. & Strubelt, C. (2008). Correction of Dry Matter Content in Sugar Beet Silages as a Substrate for Biogas Production. Landtechnik 63.

# The effect of additives (formic acid or molasses) on the protein composition of Virginia fanpetals (*Sida hermaphrodita* Rusby L.) silage

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**Keywords:** additive, CNCPS, formic acid, protein, silage, Virginia fanpetals

**Introduction** Due to its low soil requirements, cold stress and water stress tolerance, and chemical composition similar to that of alfalfa, Virginia fanpetals (*Sida hermaphrodita* Rusby L.) can be a valuable high-protein roughage for ruminants. During the ensiling of Virginia fanpetals, extensive proteolysis decreases the quality of protein for ruminants (Purwin et al., 2016) It is therefore important to determine the response of Virginia fanpetals protein to additives that inhibit or stimulate the fermentation process. The aim of this study was to evaluate the protein composition of Virginia fanpetals silage made without additives or with the addition of formic acid or molasses.

**Materials and Methods** The experimental material was first-cut Virginia fanpetals herbage grown in North-West Poland (53º 67' N, 15º 67' W) harvested in the bud formation stage, on 15 June 2016, with a forage harvester equipped with a crusher. The herbage was ensiled in 220 l drums without additives (CS), with addition of formic acid (5l/t, FS) and with addition of molasses (30 kg/t, MS). After 90 days, silage samples were collected and analyzed for chemical composition (AOAC 2005), the content of water soluble carbohydrates (WSC; Thomas, 1977), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL; Van Soest et al., 1991) using the ANKOM 220 fiber analyzer. The pH of silage was measured with the HI 8314 pH-meter (Hanna Instruments), lactic acid content was determined using the Shimadzu HPLC system (Shimadzu, Kyoto, Japan) with a VARIAN MetaCarb 67H column, the concentrations of volatile acids were determined using the **Varian 450-GC** gas chromatograph (Varian Inc., Palo Alto, CA), and N-$NH_3$ content was determined by direct distillation using the 2100 Kjeltec Distillation unit (Foss Analytical A/S, Hilleröd, Denmark) after increasing the pH of the samples by adding MgO. The contribution of nitrogen compounds to total nitrogen was determined according to Licitra et al. (1996). Crude protein was divided into fractions (A, $B_1$, $B_2$, $B_3$, C) depending on its fate in the digestive system of ruminants, according to the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992). The results were analyzed by one-way (effect of additives) ANOVA.

**Results** The addition of formic acid to the ensiled Virginia fanpetals herbage restricted fermentation (Tab. 1), whereas the addition of molasses led to an increase in the content of fermentation products ($P<0.001$). During the ensiling of Virginia fanpetals biomass (Tab. 1.), the share of the non-protein nitrogen fraction (A) increased by approximately 50%. An increase was also noted in the percentages of fractions $B_3$ and (minor) C. The share of soluble-protein and rumen-degradable protein (fractions $B_2$ and $B_3$) decreased during ensilage. The tested additives affected the proportions of soluble ($B_1$) and insoluble ($B_3$) protein fractions, which were highest in silage with the addition of formic acid and lowest in silage with the addition of molasses.

**Discussion** The ensiling of Virginia fanpetals biomass considerably deteriorated protein quality for ruminants. The changes in total protein fractions observed in ensiled Virginia fanpetals were similar to those observed in ensiled alfalfa (Purwin et al. 2016). A decrease in pH values in silage with additives (FS and MS) reduced the rate of proteolysis, as indicated by a lower contribution of non-protein nitrogen to total nitrogen (fraction A) in silage with additives compared with control silage (CS) (Fijałkowska et al. 2015).

**Table 1.** Chemical composition ($g \cdot kg^{-1}$DM, unless otherwise stated) and protein composition ($g \cdot kg^{-1}$TN) of herbage, silage without additives (CS), silage with the addition of formic acid (FS) and silage with the addition of molasses (MS)

| Item | Herbage | CS | FS | MS | SEM | *P* - value |
|---|---|---|---|---|---|---|
| Dry matter ($g \cdot kg^{-1}$) | 182 | 164 | 170 | 172 | 7.59 | 0.927 |
| Crude protein | 208 | 189 | 189 | 191 | 8.50 | 0.995 |
| NDF | 381 | 390 | 373 | 365 | 17.5 | 0.870 |
| ADF | 275 | 314 | 288 | 287 | 14.3 | 0.746 |
| ADL | 29.8 | 28.2 | 29.0 | 26.7 | 1.31 | 0.821 |
| WSC | 88.2 | 8.43 | 10.6 | 78.1 | 12.1 | 0.001 |
| pH | | 4.49 | 3.84 | 3.94 | 0.21 | 0.472 |
| $N\text{-}NH_3$ ($g \cdot kg^{-1}$ TN) | | 61.2 | 27.3 | 43.8 | 0.54 | 0.006 |
| Lactic acid | | 65.9 | 28.5 | 102 | 10.9 | 0.003 |
| Acetic acid | | 11.0 | 1.71 | 9.42 | 1.49 | 0.003 |
| Butyric acid | | 0.07 | 0.08 | 0.34 | 0.04 | <0.001 |
| *Crude protein fractions* | | | | | | |
| A | 408 | 613 | 532 | 594 | 18.7 | 0.194 |
| $B_1$ | 120 | 67.6 | 106 | 32.4 | 11.1 | 0.009 |
| $B_2$ | 437 | 278 | 319 | 324 | 15.2 | 0.467 |
| $B_3$ | 16.7 | 23.7 | 37.8 | 17.9 | 3.21 | 0.004 |
| C | 35.0 | 41.0 | 43.1 | 50.4 | 2.40 | 0.275 |

TN – total nitrogen; SEM – standard error of the mean

**Conclusions** Silage additives can modify the protein composition of Virginia fanpetals. Silage with the addition of formic acid (FS), compared with control silage (CS) and silage with the addition of molasses (MS), can supply greater amounts of protein to the rumen ($B_1$) and the small intestine ($B_3$).

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**References**

Fijałkowska, M., Pysera, B., Lipiński, K. & Strusińska, D. (2015) Changes of nitrogen compounds during ensiling of high protein herbages – a review. Annals of Animal Science, 15, 289-305.

Purwin, C., Fijałkowska, M., Nogalski, Z., Lipiński, K., Michalski, J. P. & Starczewski, M. (2016) Protein fractions composition of herbage and silage made of Virginia fanpetals (*Sida hermaphrodita* Rusby L.) Ogólnopolska Konferencja Naukowa, Puławy IUNG-PIB, 19-20.05.2016.

Licitra, G., Hernandez, T. M. & Van Soest, P. J. (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology, 57, 347-358.

Sniffen, C. J., O`Connor, J. D., Van Soest, P. J., Fox, D. G. & Russel, J. B. (1992) A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. Journal of Animal Science, 70, 3562-3577.

# The effect of technological factors on the concentrations of carotenoids and tocopherols in Virginia fanpetals (*Sida hermaphrodita*) herbage and silage

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**Keywords:** β-carotene, herbage, silage, tocopherols

**Introduction** The content of α-tocopherol and β-carotene in ruminant diets, which is determined by plant species, harvest date, maturity stage (leaf/stem ratio or the ratio between vegetative and generative shoots), wilting degree and preservation method, is a biomarker of nutritional status in livestock. The research hypothesis postulated that Virginia fanpetals (*Sida hermaphorodita* Rusby L.) herbage and silage could be a rich source of carotenoids and tocopherols in ruminant nutrition. The aim of this study was to determine the effect of technological factors on the vitamin content of Virginia fanpetals herbage and silage.

**Materials and Methods** The experimental material was first-cut Virginia fanpetals herbage harvested in the bud formation stage, on 11, 18 and 25 June 2017. Herbage was cut at 15, 25, 35 and 45 cm above the ground, and it was chopped with an electric chopper to a theoretical chaff length of 10 mm. Each batch of the plant material (600 g) was ensiled in vacuum bags in triplicate. Fresh herbage samples and silage samples collected after 90 days of ensiling were subjected to chemical analyses. The content of β-carotene (Rodriguez-Bernaldo de Quirós et Costa 2006) and tocopherols (α-, β-, γ-, δ-) (PN-EN ISO 6867: 2002) was determined in samples of fresh herbage and silage using the SHIMADZU HPLC system, RP, column: Nukleosil $C_{18}$ 250x4.6 mm, 5µm; β-carotene determination: mobile phase: acetonitrile-dichloromethane (75:25, v/v), flow rate: 1 $cm^3$ $min^{-1}$, 20 µl loop, UV-vis detector - 450 nm, external standard: β-carotene type I, synthetic, Sigma-Aldrich; tocopherol determination: mobile phase: methanol:$H_2O$ (95:5 v/v), flow-rate 1 $cm^3$ $min^{-1}$, loop 20µl, RF-20A fluorescence detector, $E_x$ 293 nm and $E_m$326 nm, external standards: (±)-α-tocopherol (DL-all-rac α-tocopherol), β-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol, Sigma-Aldrich). The results were analyzed statistically with the use of STATISTICA ver.13.0 software. The significance of differences between means was verified by Duncan's test.

**Results and Discussion** The average content of β-carotene (85.8 mg/kg DM), α-tocopherol (70.4 mg/kg DM) and total tocopherols (95.0 mg/kg DM) was higher in *Sida* herbage than in silage by 49, 96 and 79%, respectively (Table 1). According to Nozière et al. (2006), Dunne et al. (2009) and Larsen et al. (2012), grass and legume herbage contains 33-700 mg/kg DM of β-carotene and 6-169 mg/kg DM of α-tocopherol, and their looses during ensilage can reach 20-80%. In the present study (Table 1), α-tocopherol loss during the ensiling of Sida biomass was somewhat higher than that determined in grass and legume silage by the cited authors. Herbage cut at a height of 45 cm and silage made from herbage cut at a height of 25 cm had a higher content of β-carotene, α-tocopherol ($P \leq 0.01$, $P \leq 0.05$, respectively) and total tocopherols ($P \leq 0.05$, $P \leq 0.01$, respectively) (Table 1). At successive harvest dates, (11, 18 and 25 June), an increase ($P \leq 0.05$) was noted in the content of β-carotene and total tocopherols in herbage (Table 2). Our results (Table 2) are consistent with the findings of Nozière et al. (2006) who demonstrated that the concentrations of β-carotene and total tocopherols increased during the growth and development of plants, and decreased towards the end of the growing season. In a study by Dunne et al. (2009), silage made after successive cuts of grasses and legumes was characterized by decreasing concentrations of β-carotene and α-tocopherol. The concentrations of β-carotene and α-tocopherol were higher ($P \leq 0.05$, $P \leq 0.01$, respectively) in silage made from first-cut herbage (11 June) than in silage made from herbage harvested on 18 and 25 June (Table 2).

According to Fredriksson Eriksson and Pickova (2007), the ensiling process reduces the content of total tocopherols, mostly α-tocopherol, in the ensiled material.

**Table 1**. Concentrations of β-carotene and tocopherols (mg/kg DM) in Virginia fanpetals silage and herbage cut at a height of 15, 25, 35 and 45 cm

| Item | Cutting height | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Herbage | | | | | Silage | | | | |
| | 15 cm | 25 cm | 35 cm | 45 cm | SD | 15 cm | 25 cm | 35 cm | 45 cm | SD |
| | n = 9 | n = 9 | n = 9 | n = 9 | | n = 9 | n = 9 | n = 9 | n = 9 | |
| β-carotene | $47.9^{C}$ | $69.3^{B}$ | $116.4^{A}$ | $109.4^{A}$ | 7.64 | $35.8^{Bb}$ | $44.9^{A}$ | $27.6^{C}$ | $42.6^{Ba}$ | 4.27 |
| α-tocopherol | $77.5^{AB}$ | $43.6^{CC}$ | $73.3^{B}$ | $87.2^{A}$ | 4.45 | $3.1^{a}$ | $3.5^{a}$ | $1.5^{b}$ | 2.1 | 0.35 |
| β-tocopherol | $11.6^{B}$ | $8.0^{C}$ | $11.9^{B}$ | $15.6^{A}$ | 0.77 | 0.40 | 0.48 | 0.32 | 0.31 | 0.08 |
| γ-tocopherol | $2.0^{B}$ | $19.7^{A}$ | $4.3^{B}$ | $2.5^{B}$ | 2.30 | $12.5^{b}$ | $15.5^{a}$ | $8.2^{b}$ | $10.7^{b}$ | 1.25 |
| δ-tocopherol | $5.2^{b}$ | $6.3^{a}$ | $6.1^{a}$ | $5.1^{b}$ | 0.20 | $5.2^{A}$ | $6.0^{A}$ | $2.8^{B}$ | 4.2 | 0.44 |
| Total tocopherols | $96.3^{a}$ | $77.6^{b}$ | $95.6^{a}$ | $110.4^{a}$ | 6,83 | 21.2 | $25.5^{A}$ | $12.8^{B}$ | 17.3 | 1.95 |

Significance of differences: a, b – P ≤0.05; A, B - P≤0.01

**Table 2**. Concentrations of β-carotene and tocopherols (mg/kg DM) in Virginia fanpetals silage and herbage depending on harvest date

| Item | Harvest date | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 11.06. | 18.06. | 25.06. | SD | 11.06. | 18.06. | 25.06. | SD |
| | Herbage | | | | Silage | | | |
| | n=12 | n=12 | n=12 | | n=12 | n=12 | n=12 | |
| β-carotene | $82.19^{b}$ | $86.81^{b}$ | $88.26^{a}$ | 6.63 | $45.24^{a}$ | $32.73^{b}$ | $35.22^{b}$ | 3.96 |
| α- tocopherol | 64.55 | 69.03 | 77.73 | 4.44 | $3.19^{A}$ | $1.83^{Bb}$ | $2.68^{Ba}$ | 0.36 |
| β-tocopherol | 11.36 | 12.50 | 11.34 | 0.74 | $0.61^{A}$ | $0.11^{C}$ | $0.41^{B}$ | 0.05 |
| γ-tocopherol | $6.38^{b}$ | $6.04^{b}$ | $8.95^{a}$ | 2.35 | 10.31 | 12.40 | 12.49 | 1.05 |
| δ- tocopherol | $4.93^{b}$ | 5.22 | $6.88^{a}$ | 0.26 | $5.10^{a}$ | $3.85^{b}$ | 4.67 | 0.48 |
| Total tocopherols | $87.22^{b}$ | $92.79^{b}$ | $104.9^{a}$ | 6.92 | 19.21 | 18.19 | 20.25 | 2.05 |

Significance of differences: a,b – P ≤0.05; A, B - P≤0.01

**Conclusions** The yield of Virginia fanpetals herbage cut at a height of 45 cm was lower, but the biomass had higher concentrations of carotenoids and tocopherols due to a higher leaf/stem ratio. Silage made from early-harvested Sida biomass (11 June), including herbage cut at a height of 15 and 25 cm, was characterized by lower losses of β-carotene and α-tocopherol during fermentation.

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**References**

Dunne, P.G., Monahan, F.J., O'Mara, F.P. & Moloney, A.P. (2009) Colour of bovine subcutaneous adipose tissue: A review of contributory factors, associations with carcass and meat quality and its potential utility in authentication of dietary history. Meat Science, 81, 28-45.

Fredriksson Eriksson, S.& Pickova J. (2007) Fatty acids and tocopherol levels in *M. longissimus dorsi* of beef cattle in Sweden – A comparison between seasonal diets. Meat Science, 76, 746-754.

Larsen, M.K., Fratté, X.C., Kristensen, T., Eriksen, J., Søegaard, K. & Nielsen, J.H. (2012) Fatty acid, tocopherol and carotenoid content in herbage and milk affected by sward composition and season of grazing. Journal of Science Food Agricultural, Published online in Wiley Online Library, wileyonlinelibrary.com/jsfa.

Nozière, P., Graulet, B., Lukas, A., Martin, B., Grolier, P. & Doreau, M. (2006) Carotenoids for ruminants: From forages to dairy products. Animal Feed Science and Technology, 131, 418-450.

# The increment of iron solubility through ensiling soil contaminated grass and its effect on growing goats

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**Keywords:** soil, goats, grass silage, iron solubility

**Introduction** Soil contamination of forages can cause excessive iron entries (Martens et al. 2015). Whitehead, 2000, described the reduction of ferric iron to ferrous iron through ensiling which increases the absorbability of iron. In general, an over-supply of iron may decrease feed intake and growth in animals (Suttle 2010). The objective of the study was to figure the effect of ensiling on the absorbability of iron entered in ryegrass by soil contamination and its consequences for the fed growing ruminant.

**Materials and Methods** In an ensiling experiment on laboratory scale in 2010, Italian ryegrass (*Lolium multiflorum*) was cut, wilted, and chopped. Mineral soil from ten different farm locations with iron contents ranging from 1,600 to 11,300 mg $kg^{-1}$ DM, dried and milled, was added at 40 g or 80 g per kg forage DM, respectively, and mixed thoroughly. The resulting twenty treatments were ensiled in triplicate in 1 l-Weck® jars for 96 d. Sensory quality of silages was assessed. Crude ash and iron content were determined before and after ensiling, the latter by atomic absorption spectroscopy. As indicator for bioaccessibility, iron solubility before and after ensiling was assessed according to Hansen and Spears, 2009 (modified). For the feeding experiment, Italian ryegrass was cut, wilted to a DM content of about 405 g $kg^{-1}$, chopped and baled for the control treatment and for the Soil-Post treatment. For the Soil-Pre treatment, fresh topsoil was applied at 14 g $kg^{-1}$ wilted forage FM on the swath before baling. Three feeding groups of 8 kids each (6 week old) were adapted to grass silage for two weeks. Then for 86 d they were fed either the control silage, the Soil-Pre or Soil-Post silage, where soil was added to the feed immediately before feeding. Feed intake was determined daily, live weight gain on a weekly base. At the end of the experiment, all goats were slaughtered and body tissue was sampled to analyse for Fe, Zn, Cu and Mn by atomic absorption spectroscopy. Data obtained in the feeding trial were subjected to the procedure Univariate by SPSS and Tukey-HSD post hoc (IBM® SPSS® Statistics Version 19).

**Table 1.** Crude ash, iron content and solubility before and after ensiling when 40 g/80 g $kg^{-1}$ soil per kg forage DM were added from the different locations (SD standard deviation)

| Location# | Crude ash g $kg^{-1}$ DM mean fresh/ensiled | Iron mg $kg^{-1}$ DM mean fresh/ensiled | % Fe solubility fresh | SD | ensiled | SD |
|---|---|---|---|---|---|---|
| 1 | 131/151 | 1541/3714 | 4.8 | 0.18 | 37.6 | 2.66 |
| 2 | 132/141 | 1044/1591 | 6.8 | 0.27 | 36.9 | 1.46 |
| 3 | 125/137 | 1161/1541 | 5.7 | 0.54 | 39.0 | 4.60 |
| 4 | 119/150 | 1004/1969 | 6.4 | 0.39 | 33.8 | 5.19 |
| 5 | 121/142 | 1099/1707 | 5.1 | 0.42 | 33.5 | 3.98 |
| 6 | 125/142 | 666/1391 | 5.0 | 1.38 | 34.4 | 4.15 |
| 7 | 134/166 | 531/812 | 12.1 | 1.48 | 24.8 | 8.39 |
| 8 | 124/137 | 674/987 | 7.9 | 1.44 | 35.6 | 3.96 |
| 9 | 123/159 | 619/1174 | 6.8 | 1.13 | 37.2 | 2.72 |
| 10 | 129/156 | 639/1174 | 7.7 | 0.93 | 29.1 | 13.00 |
| Mean | 136.4±0.8 | 1254±733 | 6.8±2.2 | | 27.3±13.7 | |
| P (ensiling) | 0.818 | 0.225 | - | | <0.001 | |
| P (soil level) | <0.001 | <0.001 | - | | 0.278 | |

**Results and Discussion** Apart from increasing levels of crude ash and iron with increasing addition of soil, there was a significant effect of ensiling on iron solubility (Table 1). There was about a

quadruplication of solubility at the end of ensiling on average compared to the initial material. In the feeding trial feed intake diminished significantly with Soil-Pre despite a good fermentation quality, and even though total iron intake was lower in this treatment daily live weight gain was less than half compared to the other two treatments. In the storage organs, iron content was significantly increased in Soil-Pre compared to the control and also in the duodenal wall (Figure 1). The feeding trial confirmed the difference which is consisting in whether soil is ingested as ensiled with forage or added to the feed just before consumption. There was no indication of iron antagonism to other trace elements such as zinc, copper or manganese.

**Table 2.** Feed and iron intake ~~(DMI)~~ per animal*d and growth performance from experimental week 2 to 12 (mean ± standard deviation)

| | Treatment[1] | | | *P*-value |
|---|---|---|---|---|
| | Control | Soil_Pre | Soil_Post | |
| Feed intake, g DM | 1306$^{a}$ ±312 | 987$^{b}$ ±238 | 1264$^{a}$ ±326 | < 0.001 |
| Iron intake, mg | 434$^{c}$ ±104 | 1607$^{b}$ ±387 | 2479$^{a}$ ±640 | < 0.001 |
| LWG[2] | 62 | 26 | 69 | < 0.001 |
| Feed conversion, kg DMI kg LWG$^{-1}$ | 33.4 | 52.0 | 34.9 | - |

[1]Control, without soil contamination, Soil-Pre, soil contamination before ensiling, Soil_Post, soil contamination after ensiling, just before feeding; [2]LWG, live weight gain [g animal$^{-1}$ d$^{-1}$];
Different superscripts within a row indicate significant difference at $P < 0.05$ (Tukey-HSD)

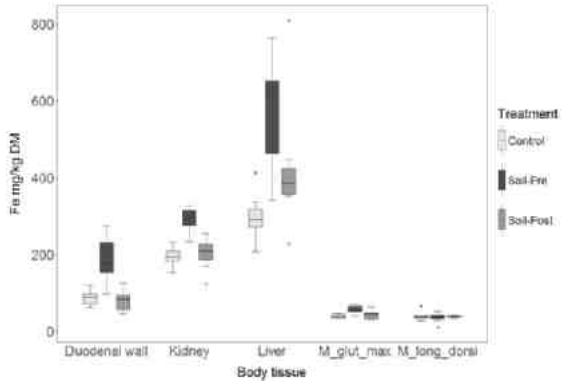


**Figure 1.** Iron content in the lyophilized body tissue (the latter two on the right hand: *Musculus glutaeus maximus, M. longissimus dorsi*)

**Conclusions** Apart from fermentation quality issues and energy dilution, ensiling of soil adherent to forage should be minimized for animal performance and health reasons.

**References**

Hansen, S.L. & Spears, J.W. (2009) Bioaccessibility of iron from soil is increased by silage fermentation. Journal of Dairy Science 92**,** 2896-2905.

Martens, S.D., Steinhöfel, O. & Majewska-Pinda, A. (2015) Mineral composition and quality of grass silages contaminated with various types of soil with different iron level,Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 1-3 July 2015. pp. 480-481.

Suttle, N. (2010) Mineral nutrition of livestock, 4th ed. CABI, Wallingford.

Whitehead, D.C. (2000) Micronutrient cations: iron, manganese, zinc, copper and cobalt, in: D.C. Whitehead (Ed.), Nutrient Elements in Grassland: Soil-Plant-Animal Relationships. CABI, Wallingford, pp. 220-254.

# The use of maize straw as alternative substrate for biogas production

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**Keywords:** aerobic stability, biogas production, *Lactobacillus diolivorans*, maize straw

**Introduction** Biogas production based on energy crops is very common in Germany (Ohl & Hartung, 2015). Nevertheless high ecological constraints, uncertain political circumstances and generally increasing costs for cropland are strong challenges in agriculture, especially in biogas production. Therefore farmers are permanently looking for an improvement of efficiency in biogas production and/or alternative substrates to maize silages. In this regard the use of maize straw has encountered more interest in research and industry although there are large differences in quality of maize straw depending on climate, dry matter, breed, ash content and field retention time. Usually maize straw remains as residual on the field after harvesting the maize grain. New techniques to easily harvest these residuals from the field are now available on the market. The aim of the present study was to assess the potential of maize straw for biogas production and, in addition, to clarify the effect of a commercially sold silage additive containing *Lactobacillus diolivorans* on fermentation, aerobic stability and methane yield.

**Material and Methods** Maize straw was immediately harvested after threshing the maize grain and ensiled in laboratory mini silos. Treatments consisted of an untreated control and a treated variant with a commercially sold mixture of homo- and heterofermentative lactic acid bacteria (*SILASIL ENERGY.XD: L. diolivorans, L. rhamnosus and L. buchneri)* at an application rate of 200.000 CFU/ g fresh matter (FM) in a total of three replications. Forage samples were analyzed to determine forage quality (plant nutrients, pH-value, fermentation pattern) as well as aerobic stability according to Honig (1990) after 28 and 90 days of storage. Both yeasts and moulds were counted according to VDLUFA method (III 28.1.2.) with the basic material and the ensiled material. Evaluation of biogas and methane yield was carried out in compliance to German Standard Procedure VDI 4630 (2006) after 90 days of storage. For statistical evaluation, means and standard deviations were calculated for each parameter. The data were examined by SAS evaluation using ANOVA with the fixed effect of treatment (SAS 9.4).

**Results** The basic material (Table. 1) was characterised in a dry matter (DM) of 30.8 %, an amount of water soluble carbohydrates of 5.7 % of DM and low ash and protein content (3.4 and 3.0 % DM).

**Table 1** Forage quality parameters of the basic material

| DM % | CA (DM %) | CP (DM %) | WSC (DM, %) | NDForg (DM, %) | ADForg (DM, %) | LAB (log cfu/ g FM) | Yeasts (log cfu/ g FM) | Molds (log cfu/ g FM) |
|---|---|---|---|---|---|---|---|---|
| 30.8 | 3.4 | 3.0 | 5.7 | 74.2 | 39.7 | 5.1 | 5.0 | 4.7 |

DM = Dry Matter, CA=Crude ash, CP= Crude protein, WSC= Water soluble carbohydrates, LAB= Lactic acid bacteria

Silage treated with lactic acid bacteria (LAB) mixture showed a significantly higher amount of acetic acid, a significantly lower amount of yeasts (day 28) and molds (day 28 and 49) and thus an improved aerobic stability after 28 and 90 days of storage (Table 2). Furthermore maize straw (Figure 1) yielded 614 $L_n$ biogas/kg $_o$DM and 319 $L_n$ methane/kg $_o$DM for the control, whereas 646 $L_n$ biogas/ kg $_o$DM and 336 $L_n$ methane/kg $_o$DM were observed for the LAB treatment ($p<0.05$).

**Table 2** Silage quality parameters at day 28 and 90 for control (a) and treated variant (b)

| Days of storage | 28 | | 90 | |
|---|---|---|---|---|
| | a | b | a | b |
| DMc (%) | 29.5 | 29.9 | 26.8* | 27.1 |
| LA (% DM) | 4.9 | 4.4 | 2.8* | 0.7* |
| AA (% DM) | 1.9* | 3.3* | 3.7* | 5.5* |
| PD (% DM) | 0.0 | 0.1 | 0.0 | 0.1 |
| Pol (% DM) | 0.0* | 0.6* | 0.1* | 0.8* |
| EA (% DM) | 1.5* | 1.1* | 1.2 | 0.9* |
| pH | 4.0 | 4.1 | 4.2 | 4.3 |
| Yeasts (log cfu/g FM) | 4.9* | 3.2* | <2 | <2 |
| Molds ((log cfu/g FM) | 4.1* | 2.4* | 2.6* | <2* |
| AS (days) | 1.2 | 4.6 | 9.2* | >12* |

$DM_c$ = DM corrected, LA=Lactic acid; AA= Acetic acid; PD= 1,2-Propandiol; Pol= n-Propanol; EA= Ethanol; AS= Aerobic stability (max. length: 12 days); * symbolize significant differences ($p < 0.05$)

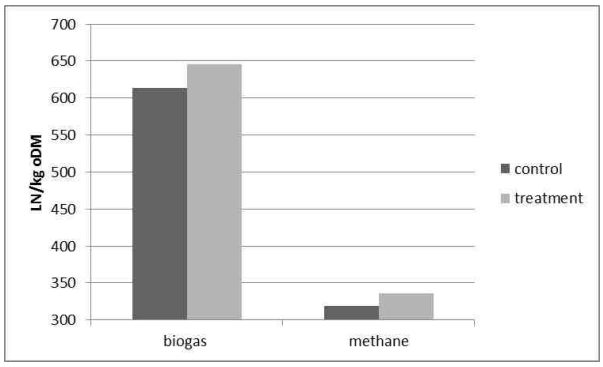


**Figure 1** Biogas and methane production of maize straw after 90 days of storage for control and lactic acid bacteria mixture treatment

**Discussion** If being harvested quickly after threshing and without lots of adherent dirt from soil, ensiling of maize straw is possible. Due to the high native amount of ash and undesired microorganisms on the fresh maize straw and a subsequent risk for failed fermentation and reheating after opening, adequate silage additives should be used. Biogas and methane yield were higher than expected and only 5-10 % less compared to maize silage of good quality (650-680 $L_n$ biogas/kg $_o$DM).

**Conclusion** The results of this study indicate the potential of maize straw as an alternative substrate for biogas production.

**References**

Honig, H. (1990) Evaluation of aerobic stability. In: Lindgren, S. & Pettersson, K. (eds.), Proceedings of the EUROBAC Conference, Uppsala, Sweden, 1990, special issue, pp. 76-82.

Ohl, S. & Hartung, E. (2015) Co-fermentation of energy crop silages in batch fermentation test. In: Daniel, J.L.P., Morais, G., Junges, D. & Nussio, L.G. (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil 2015, pp 570-571.

VDI 4630. (2006) Fermentation of organic materials. Characterisation of the substrate, sampling, collection of material data, fermentation tests. Düsseldorf. Beuth-Verlag GmbH.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) (2012)

Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt (Germany).

# Virginia fanpetals (*Sida hermaphrodita* Rusby L.) silage can be fed to young bulls

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**Keywords:** beef, daily gain, feed efficiency, Holstein-Friesian

**Introduction** The efficiency and profitability of beef production can be increased by decreasing feed cost per kg weight gain. The group of fodder crops includes Virginia fanpetals (*Sida hermaphrodita*). Virginia fanpetals is a perennial plant which has low habitat requirements and yields an annual average of 10-20 t dry matter (Ţitei 2015). It is grown mainly as an energy crop, but it could also be used for forage production. It may therefore be assumed that silage made from Virginia fanpetals biomass could replace other types of silage in cattle diets. The aim of this study was to determine the efficacy of Virginia fanpetals silage for finishing young bulls.

**Table 1.** Chemical composition ($g \cdot kg^{-1}$ DM) of experimental diets (mean ± standard error) MS – maize silage; GS – grass silage; SS – Sida silage

| Specification | MS | GS | SS | Concentrate I | Concentrate II |
|---|---|---|---|---|---|
| Dry matter $g \cdot kg^{-1}$ | 324±0.96 | 271±0.95 | 198±0.89 | 875±1.12 | 878±1.05 |
| Organic matter | 964±2.12 | 905±2.31 | 901±1.56 | 956±1.25 | 951±1.58 |
| Crude protein | 88.5±1.32 | 132±1.39 | 174±1.62 | 122±1.21 | 158±1.32 |
| aNDF | 336±5.32 | 540±4.32 | 429±5.12 | 142±1.36 | 162±1.98 |
| ADF | 194±1.32 | 317±1.69 | 315±2.03 | 37.1±0.68 | 59.7±0.84 |
| ADL | 12.4±0.65 | 26.7±0.62 | 31.5±0.85 | 11.7±0.35 | 24.3±0.54 |
| NFC | 503±3.25 | 199±3.29 | 278±4.12 | 674±4.31 | 611±4.36 |
| pH | 3.54±0.09 | 4.21±0.12 | 4.57±0.17 | | |
| Lactic acid | 27.8±7.32 | 43.8±11.3 | 65.8±9.25 | | |
| Acetic acid | 6.3±1.32 | 12.6±2.62 | 20.3±2.98 | | |
| Butyric acid | 0.08±0.03 | 0.09±0.03 | 4.4±0.56 | | |
| N-NH3 ($g\ kg^{-1}$ TN) | 33.6±11.4 | 75.6±14.3 | 105±15.2 | | |

aNDF - neutral detergent fiber; ADF - acid detergent fiber; ADL, acid detergent lignin; NFC - non fiber carbohydrate; N-NH3 - ammonia nitrogen; TN – total nitrogen

**Materials and Methods** Silage was made in bunker silos from Virginia fanpetals biomass cut in the bud formation stage with a forage harvester equipped with a crusher. Forty Holstein-Friesian bulls (4 groups of 10 animals each) were fattened for the last 7 months before slaughter. The dietary treatments were as follows: GS - grass silage + concentrate II, SS - Sida silage + concentrate I, GS+SS - grass + Sida silage (1:1) + concentrate I, SS+MS - Sida and maize silage (1:1) + concentrate II. Concentrate was added in the amount of 3 kg per animal per day. Silage was dosed from a self-propelled feed cart. Feed intake was recorded individually using the Roughage Intake Control System (Insentec BV, Marknesse, Netherlands). Samples of diets, collected once a week, were analyzed to determine their chemical composition, and the content of NDF, ADF, ADL, lactic acid, butyric acid and acetic acid using the ANKOM 220 fiber analyzer. The pH of silage was measured with the HI 8314 pH-meter, lactic acid content was determined using the Shimadzu HPLC system, the concentrations of volatile acids were determined using the **Varian 450-GC** gas chromatograph, and $N\text{-}NH_3$ content

was determined by the Conway method. The results were analyzed statistically by one-way ANOVA for orthogonal designs using STATISTICA 12.0 software.

**Results** Sida silage had a higher content of total protein and NFC and lower NDF content compared with grass silage (Table 1). Sida silage had higher concentrations of the analyzed acids and ammonia nitrogen than grass silage. The dietary treatments had no significant effect on feed intake, body weight (BW) gain or feed utilization. The animals fed Sida and maize silage achieved the highest average BW gain, whereas bulls receiving Sida silage in addition to concentrate were characterized by the lowest average BW gain.

**Table 2**. The results of fattening bulls phf (mean ± standard error)

| Parameter | GS | SS | GS+SS | SS+MS |
|---|---|---|---|---|
| Initial body weight, kg | 455±19.7 | 456±22.3 | 477±27.1 | 464±12.6 |
| Initial age, days | 407±6.32 | 398±8.69 | 414±9.21 | 406±5.87 |
| Final body weight, kg | 696±10.1 | 687±19.1 | 715±21.0 | 708±11.3 |
| Daily gain, kg | 1.126±0.04 | 1.079±0.05 | 1.129±0.04 | 1.151±0.07 |
| Silage intake | 6.04 ±.0,24 | 5.70±0.16 | 6.11±0,18 | 6.25±0.56 |
| Total intake | 9.04 | 8.70 | 9,11 | 9.25 |
| Crude protein intake | 1271 | 1357 | 1302 | 1295 |
| DM/kg BW gain | 8.03±0.18 | 8.06±0.15 | 8.07±0.17 | 8.04±0.15 |

No significant differences in all traits; GS – grass silage; SS – Sida silage; MS – maize silage

**Discussion** Virginia fanpetals biomass harvested at an early date had high protein content and low NDF content. Harvest delayed by 2 weeks contributed to an increase in NDF content and stem lignification (Kwiatkowski et al. 2014). A high moisture content of ensiled herbage deteriorated the quality of Sida silage and insignificantly reduced its intake. Sida plants have thick stems and delicate leaves, which makes it difficult to wilt biomass before ensiling (Tarkowski 2006) and improve fermentation conditions. In a previous study Virginia fanpetals silages were characterized by similar organic matter and NDF digestibility to alfalfa silage in the phase of budding (Purwin et al. 2016). Due to its high protein content, Sida silage is complementary to maize silage, which resulted in the best fattening performance of bulls. Bulls fed exclusively Sida silage were characterized by lower BW gains at a similar feed conversion ratio.

**Conclusions** Good fattening results can be obtained in bulls fed Sida silage, particularly when it is combined with maize silage. Cattle fattening based exclusively on Sida silage contributes to lower average BW gains, but dietary supplementation with expensive high-protein feeds is not required.

**References**

Kwiatkowski J., Purwin C., Sienkiewicz S., Tworkowski J., Pysera B., Wierzbowska J., Graban Ł., Lajszner W., Wyżlic I., Krzebietke S.& Żarczynski P. (2014) The Virginia fanpetals *Sida hermaphrodita* (L.) Rusby used as feedstock for an agricultural biogas plant. In: Conversion of waste from agri-food industry to biogas – a system approach; 2014; chapter 2; 37-62

Purwin C., Fijałkowska M., Nogalski Z., Lipiński K., Antoszkiewicz Z. & Starczewski M. (2016) Digestibility of ensiled Virginia fanpetals (*Sida hermaphrodita* Rusby L.) biomass. XLV Scientific Session Group of Animal Nutrition, Polish Academy of Science, Olsztyn, 21-22.06.2016, pp. 110-111.

Tarkowski, A. (2006). Nutritional and feed value of Sida (*Sida hermaphrodita* Rusby). Habilitation dissertation AR Lublin, 308: 1-81.

Titei, V. (2015). Agro Biological Peculiarities and Economical Value of Sida Hermaphrodita in Republic of Moldova. ProEnviroment 8, 485-489.

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# New technologies to monitor and improve silage quality from field to feed-out

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## Introduction

More than ever, the agriculture and food production industries are facing the challenge of establishing a permanent link between consumer and environmental protection, animal welfare, quality control and economic sustainability. These complex requirements need a suitable systematic approach such as the concept of “Precision Livestock Farming” (Schulze et al., 2007). An integral part to this is precision silage production and utilisation, and many questions require answers before proceeding on the role of new tools and technologies in improving silage management, quality and feeding. Precision crop husbandry has become a very important tool for use on arable farms. Advances have been made with the use of farm vehicles with global position systems (GPS) through to unmanned aerial vehicles (UAV) fitted with various imaging technologies. All advances have the sole purpose of improving the precision with which many operations are performed on-farm, with the overall aim of increasing production whilst reducing inputs. Such approaches have the potential to improve profitability and reduce the environmental impact.

Livestock agriculture has, to some extent, been slow to utilise technological advances in many aspects of their operations. The production, ensiling and feeding of forage crops is central to most dairy production systems and is important in many beef production systems. Without high quality ensiled forages these ruminant production systems cannot maximise the use of home grown forages in their total mixed rations.

This paper aims to investigate monitoring technologies that could have the potential to enhance the quality of ensiled forages for feeding to ruminants and the pros and cons of each approach will be assessed. The paper will be split into 3 subsections, namely growing, harvesting and ensiling, and feeding. The goal of this review is to highlight technologies that are available now or through further development could be available in the future to give forage managers practical ‘Silage Decision Support Tools (SDST)’ to aid in the efficient production of ensiled forages that meet the quality (both nutritive and hygiene) requirements of the livestock they will be fed to. To close the circle, new perspectives of silo design for automated feeding systems will be discussed. Throughout this review we will examine other industries with related challenges and assess their technologies for application to ensilage. In some situations there is no current SDST available so we will highlight the need and ‘throw down the gauntlet’ to developers to provide solutions. However at all times we must bear in mind the quote from Stephen Hawking ‘the greatest enemy of knowledge is not ignorance; it is the illusion of knowledge’. In other words we must only promote these technologies if they are providing accurate information on which informed management decisions can be based.

## 1. The role of technology in growing silage crops

The key to successful growth of quality forage for ensiling is a chain of decisions that starts with sowing in the optimal conditions, applying fertiliser and agro-chemicals appropriately with minimal

wastage, promoting good chemical composition of the plant for ensiling and feeding, and achieving a high biomass yield. In addition, long-term sustainability goals should be considered, such as building soil fertility and maintaining local biodiversity. In these regards, there are many technologies available, such as those utilised in precision arable production, which could be applied within the livestock industry to create a 'precision forage' approach to silage-making (Schellberg et al. 2008). We begin this review, by considering technologies that show promise for forage monitoring and how those may be combined with bespoke machinery solutions in the future with the ultimate goal of creating fully-automated forage production systems.

***Current technology for forage assessment***

Walking fields to observe crop health, presence of weeds, and plant maturity has historically been a necessary but time-consuming duty for the livestock farmer. Some tools already exist to aid the farmer in making predictions of biomass yield during this process. The earliest examples being rising plate meters that measure compressed sward height (Earle and McGowan 1979) and electronic capacitance probes (Michell and Large 1983); however, it has been shown that predicting yield using these devices has a high margin of error (Kallenbach, 2015), and is labour intensive. More recently, ultrasonic devices for biomass yield measurement have been developed (Fricke and Wachendorf 2013), some of which are designed to be mounted on All Terrain Vehicles (ATVs) for more rapid pasture assessment over large areas although the accuracy of a moving sensor is lower than that of a static unit (Safari et al. 2016). Perhaps the most promising technology that is only just beginning to be utilised in practice for forages, but is already well accepted in arable practice, is to use the reflectance of electromagnetic wavelengths to form images or spectra. When combined with global positioning systems (GPS) these can be analysed to create spatial mapping of crop traits (Wachendorf et al. 2018). The earliest examples of crop phenotyping using image analysis, relied upon the ratio of reflectance of two wavelengths to compute vegetation indices (VIs), the most common example being Normalised Difference Vegetation Index (NDVI) which was shown to be a predictor of crop biomass (Hill et al. 1999). Development of higher resolution equipment over time has allowed for analysis of a broader range of wavelengths, and predictions for many crop phenology traits have been developed based on a range of VIs (Perez-Sanz et al. 2017).

***Future technology for forage assessment***

***Remote vegetation sensing.*** Remote sensing using satellite offers a solution to traditional labour-intensive, *in-situ* data collection methods (Ali et al. 2016), and furthermore, data can be delivered directly to the farm office on demand without the need for capital purchase of sensors. Satellite images also enable monitoring of large geographical areas simultaneously so that national and global trends in land use can be mapped (Crowther et al. 2015); however this review will focus on application at the individual farm-scale. The launch of Sentinel-2 satellites in 2015 offered upgraded multi-spectral resolution, increased frequency of coverage, and the ability to record data from red-edge wavelengths (Lemmens 2015) in comparison to older LandSat, Satellite Pour l'Oberservation de la Terre (SPOT), and Moderate-resolution Imaging Spectroradiometer (MODIS) images, although cloud contamination remains a challenge. The study of Punalekar et al. (2018) has been one of the first to successfully utilise Sentinel-2A data for yield mapping of productive agricultural grasslands in the UK and showed that this approach could also be applied to multi-species leys (incorporating herbs and legumes) with good accuracy. However, there is a need for sensing technologies to deliver greater functionality than yield mapping alone as the feed quality of silage is primarily determined by its chemical composition. Important traits to measure over time in a growing silage crop include protein, fibre, and energy content that are strongly related to plant growth stage and leaf:stem ratio (Fick and Mueller 1989). A further consideration for silage making is the water soluble carbohydrate concentration of the plant as this is an important determinant of fermentation potential (Duniere et al. 2013). The prediction of chemical composition of plant material using high-resolution hyperspectral sensors *in-situ* or under controlled laboratory conditions has been shown to be possible with good accuracy, e.g. in maize and soybean plants (Pandey et al. 2017) and in permanent pasture (Punalekar et al. 2016). However, it has not yet been proven whether the same would be possible using the lower resolution multi-spectral images that are currently available via satellite. At present, the commercial use of close range

hyperspectral sensors is limited by the complexity, cost and sensitivity of the equipment (Mishra et al. 2017).

***In-situ vegetation sensing.*** Whereas satellite data are limited by wavelength resolution, cloud cover, and equipment capability, ground-based sensing solutions can offer increased precision and broader functionality. One example is the recognition of weeds within a crop using algorithms that can detect differing leaf shape of desired and undesired species based on images from low-cost Red, Green, Blue (RGB) cameras (Berge et al. 2012). The specificity of such algorithms has historically limited its application to arable crops; however, advanced machine-learning approaches have proven that weed recognition within grassland is also possible (Sadgrove et al. 2017). Prototype robots fitted with this capability have previously been manufactured (van Evert et al. 2011) although weed recognition capability has been limited to a small number of common species. Another potential function of *in-situ* sensing is to recognise and provide warning of crop disease (Bock et al. 2010). Software that can diagnose plant diseases and disease severity based on an image taken using a smartphone (Hallau et al. 2018) or tablet (Pethybridge and Nelson 2018) are now available but are not yet optimised for forage crops.

Thermal imaging also has the potential to provide additional crop monitoring data. The temperature of vegetation is closely correlated to heat stress. Demonstrating this, Shimoda and Oikawa (2006) used thermal imaging to distinguish between $C_3$ and $C_4$ grassland species and monitor their differential responses to hydrological conditions. Responding to heat stress is likely to become increasingly important in temperate climates as global temperatures rise and the frequency of extreme weather conditions increase.

A natural progression in the use of imaging is to move from 2D to 3D images of crop structure. Complex 3D data pertaining to sward structure can be gathered using laser scanning or structure-from-motion photogrammetry (the coupling of two dimensional image sequences with motion). Using such equipment to predict grassland biomass yield has already proven to have a greater accuracy than that of a conventional rising plate meter (Cooper et al. 2017), and potential applications go beyond yield mapping alone. Analysis of 3D plant images can enable automated detection of plant physiology, an example being the quantification of ear number and size in cereal crops (Paulus et al., 2013). Experimentally, the combination of multiple sensors, including 3D laser imaging, into one observation device can result in a wealth of plant physiological data of this kind (Virlet et al. 2017). Applying such technologies to grassland might enable prediction of plant digestibility based on numbers of plants heading and leaf structure, or quantify the value of crops to pollinators through identification of flowers in swards containing legumes. These would be important in the prediction of harvest-readiness to optimise feed value. At present, there is a lack of research adapting and validating these functions for forage crops, especially for forage crops such as maize, legume, and mixed crops (e.g. herbal leys) whose agronomic requirements are more complex than those of monoculture grassland. These technologies also have significant applications in plant breeding to allow rapid assessment of large test populations for more rapid development of new plant varieties (Tardieu et al. 2017). This is particularly needed for the forage crop industry where there are fewer varieties to choose from than for arable crops, and genetic improvement in yield and nutritional variables over time has been slow when compared with the improvement seen in wheat yield over a comparable time period (McDonagh et al. 2016).

***In-situ soil sensing.*** Promoting soil health and structure and ensuring minimisation of nutrient loss due to leaching is crucial to the long-term sustainability of forage systems. At present, measurement techniques are crude, often based simply on observation of soil structure through digging a pit or laboratory analysis of soil samples. There is a need for real-time data to enable the farmer to respond rapidly to changing soil conditions in the short term, as well as monitoring longer-term trends in soil fertility, in order to optimise efficiency. Electrochemical sensors have been developed that are able to measure a number of important soil variables (Warudkar and Dorle 2016). The study of Shaw et al. (2016) is one of the first to consider the development of a nitrogen sensor network designed to be buried in grassland to provide real-time prediction of plant-available nitrogen and leaching risk. Further improvement of such sensors in combination with robotic fertiliser dispensers or irrigation equipment would allow for a much greater level of input efficiency into forage production (Amrutha et al. 2016).

***Adoption of in-field technology***

Whilst a number of technologies exist that are either already available for forage crops or likely to be available in the near future, a key challenge is to ensure technologies can be integrated into forage systems in an intuitive way. For *in-situ* field sensing the attachment of sensors to existing machinery is a logical first step. For example, mounting biomass, crop weed recognition, or crop disease diagnostic sensors to the arms of agro-chemical sprayers is one way in which precision inputs can be achieved in forage crops (Serrano et al. 2016). Tractor mounted spectral imaging which assesses crop nitrogen content and through immediate feed-back control to variable rate fertiliser spreaders in a 'one-pass' operation are now in practical use (http://www.yara.co.uk/crop-nutrition/Tools-and-Services/n-sensor). The system alters nitrogen application rates according to spectral image assessment of the grass crop to maximise yield and protein quality by applying nitrogenous fertiliser to requirement. The system claims reduced lodging at harvest which is obviously a further benefit. Ultimately, the development of robotic machines has the potential to optimise field operations by cutting down on the requirement for labour and fuel and also reduces the risk of soil compaction, although potentially incurring higher capital equipment cost (Shafiekhani et al. 2017). Attaching sensors to UAVs shows promise as a mechanism of collecting close-range, high resolution imaging data without the farmer being physically present in the field (Holman et al. 2016). Combining the output from multiple sensing techniques will allow for the greatest modelling of crop performance and provide the highest level of decision-support. A challenge exists in delivering data to the farmer in a way which is useful and not overwhelming (Adrian et al. 2005); in this regard cross-disciplinary work with software engineers may be required in the future.

## 2. Harvesting and Ensiling

***Timing of cutting*** The technologies discussed in section 1 on growing the crop will enable better assessment to be made by the forage manager on when the optimal point of harvest is reached in relation to the question, 'is the quality correct and is there sufficient yield?' Additional information about crop quality in terms of crude protein, digestibility/energy/fibre content and starch will be at the fore. Mobile NIRS instrumentation (Aunir [*https://www.abvista.com/Products/GB/NIR-4-Farm.aspx*]; Dynamica Generale, *[https://www.dinamicagenerale.com/en-ww/agrinir.aspx*]; Scio [https://www.consumerphysics.com/business/]) with wet pre-ensiled crop calibrations have the potential to immediately assess these quality parameters, but they have two important potential drawbacks. Firstly accuracy of sampling of large field areas and sample preparation especially for seed bearing whole-crop forages such as maize and cereals is required to give a precise answer. Secondly, the accuracies of the NIRS predictions need to be validated and regularly updated for a wide variety of geographic, climatic and species/cultivar conditions. Thus technologies based on those discussed earlier will ultimately give a better whole field assessment of both yield and quality. These technologies need to capture the yield and quality data to enable simple calculation of energy and/or crude protein yield/hectare measurements and thus help break the farmers' perception that DM yield is the most important criterion.

Simpler systems based on well-established morphological/phenotypical characteristics linked to mobile phone photography and associated applications (Apps) to predict likely digestibility and energy content are likely in the short term to be more accurate and more widely available to forage managers and should be relatively easy to link to yield estimations as well.

With these technologies to aid the decision making process the only unknown is the effect the weather could have, especially with forage crops such as grasses and legumes on the wilting time and thus the DM content at harvest. With the ever increasing reliance on mobile Apps, the development of one that takes existing and developing technologies that provide yield data, alongside the physiological crop maturity and the DM content of the standing crop in combination with meteorological forecasts of rainfall, wind speeds, temperature and sunlight to predict rates of wilting would be beneficial. Futhermore density and depth of the mown crop could add further information to improve predicted optimum wilt time. Scientific input will be required to provide the wilting data but such an App would provide the forage manager with not only a risk factor for mowing at a specific time on getting to the

ideal forage DM level for ensiling before rainfall but also the wilting time required to hit the target DM content for ensiling, thus enabling more controlled management of the harvesting process and greater control of the DM variability of the ensiled forage. Such an App would not only improve management but would also help reduce DM variability across a given feed-out face of a single silo and thus improve the nutritional balance of forage:concentrate in the total mixed ration (TMR), which ultimately affects diet formulation probably more than any other single factor.

***Disease status at harvest.*** Rapid methods that assess disease and microbial status in the growing crop could also assist in forage management. Two technologies of potential use are the measurement of chlorophyll remotely using hyperspectral imaging as a measure of healthiness alongside the development of rapid fluorescence scanning techniques currently used in human health assessments of diseases caused by various fungal infection (Graham 1983) or in the rapid assessment of meat contamination during processing (Aït-Kaddour et al. 2011). Such rapid pre-ensiling methodologies will enable more precise management decisions to be made about the ensiling technology to be used. It may be more appropriate to ensile a more infected region of a field into bales to provide the opportunity to feed this portion of silage to less health vulnerable livestock. In addition pre-ensiled forages with high yeast populations are more likely to require additives designed to control aerobic spoilage.

***Mowing, tedding and raking.*** One of the most common problems in silage production is one of soil and therefore *Clostridia* contamination. Initially employing too low a cutting height results in soil contamination, but consequentially all subsequent field based forage manipulations such as tedding, raking and even pick-up with the forage harvester cause further soil contamination. The road construction industry routinely uses GPS assisted technologies. Integral to earth moving machinery are automatic levelling controls whereby the machinery buckets/shovels automatically adjust to a predefined height above the existing surface to ensure the construction is conducted in a human-proofed manner (eg Mouazen *et al.* 2004; Trimble [http://www.trimble.com/Industries/Construction/index.aspx]). With some adjustments development of in-built systems to control mower and rake height above soil level constantly as it traverses the field should be easily achievable. Additional benefits of implementing such feedback control mechanisms during field operations will enable improved total forage yield/hectare/annum to be achieved as the regrowth of the crop will be quicker. Also the more consistent, and in some cases higher stubble height, will improve silage quality by leaving in the field and not harvesting the dead and stemmier, low digestible material which in addition contains the higher population density of undesirable silage microorganisms.

***Monitoring of the mown crop before ensiling.*** The most important factor to be able to monitor instantaneously is the DM content and in particular how this is changing with wilting time, alongside this the change in water soluble carbohydrate content relative to the DM would be a useful indicator of efficiency of wilting. Currently sampling and analysis by mobile NIRS with the appropriate calibration is the most imminent approach. Whilst this does require a good field sampling regime, the results are instantaneous once analysis has been conducted. The issue with remote sensing by spectral imaging of the entire field is that this image is likely to only see the top of the laying sward which can have a considerably higher DM content than the forage hidden beneath.

***Forage harvesting*** Modern forage harvesters, particularly those from Claas and John Deere have embraced technologies in an attempt to provide farmers with more information. Sensors based on conductivity can measure DM content and temperature of the forage as it passes through the spout. Temperature assessments of forage as it leaves the spout of the forage harvester, we believe are of increasing importance in enabling the forage manager to better manage harvesting in a climate changing world. In an increasing number of countries, forage harvesting is occurring at temperatures in excess of 40$^{o}$C. The efficacy of standard inoculant silage additives becomes highly questionable at these temperatures (Marley 2017), yet the likelihood of a malodorous silage fermentation increases (Adesogan 2009) due to the temperature being nearer the optimum of the undesirable epiphytic Enterobacterial and Clostridial populations than the lactic acid bacteria. Once forewarned of the higher temperature risk factor the forage manager can make an informed decision to switch from an inoculant to a chemical preservative.

More advanced methodologies involve the use of spout mounted NIRS instrumentation that are calibrated for DM, crude protein and starch content. This instrumentation does need to be properly calibrated which is an underlying question with regard to the accuracy of the results. The latest forage harvesters have feedback control based on DM content, reducing the theoretical length of chop as DM content increases. In addition to this as the forage passes from the pick-up reel into the chopping chamber the yield can be predicted by the force exerted during the process. The manufacturers even suggest that this prediction is more accurate if on 2-3 occasions per field the trailers are weighed and the collected data are input into the prediction model on the forage harvester. However the development of weigh cells that can be directly fitted to specially adapted trailers removes the need for any predictions, as data can be collected directly and accurately from each and every load harvested. These data are invaluable to precisely measure yield and if linked to GPS for each load can combine with yield, fertiliser application and protein content maps used in part 1 crop growing, to improve the accuracy of the prediction models used in those technologies.

In addition the use of the trailers fitted with weigh cells has two further advantages. A patent (Strzelecki 2016) enables remote communication between trailer weigh cells and the forage harvester silage additive applicator allowing instantaneous adjustment of the flow rate of application of an additive to ensure correct application. An additional advantage of this system is that the operator of the forage harvester can observe the trailer weight in real-time and can visualise when forage is leaving rather than entering the trailer, and thus operators using this approach have stopped over filling trailers and thus reduced field losses of forage.

***Clamp filling.*** Packing density and moisture content of silage are of great significance in improving silage quality. A lower packing density means a higher porosity, which could result in more oxygen remaining in the silage and leads ultimately to lower silage quality with higher storage losses and greater propensity to problems of aerobic spoilage. The traditional method for determining the packing density of silage is to calculate its gross density from mass divided by volume. The disadvantage of this method is that it is unable to show density differences at specific sites, unless measured in different regions of the clamp by the coring based methodology (Ruppel et al. 1995). The $\gamma$-ray scanner is an effective tool to analyze the packing density in two dimensions with a relative error of measurement around 1% (Fuerll et al. 2008; Mumme et al. 2008). However, the $\gamma$-ray scanner is not widely applied due to its high cost and the potential danger to health (Sun et al. 2010). In recent years, the penetrometer technique has been used to measure the packing density of silage because it is straightforward to calibrate and can provide reliable data (Perumpral, 1987). Meng et al. (2018) proposed a compound sensor design based on American Society of Agricultural and Biological Engineers (ASABE) standards as a novel technique for evaluating the moisture content and density of silage. In this compound sensor, the moisture electrode and strain gauges were embedded in an ASABE standard small cone, which made the compound sensor capable of measuring the packing density and moisture content of silage simultaneously. The results appear promising as the packing density and moisture content of the silage are linearly related to the outputs of the compound sensor and both coefficients of determination were greater than 0.93. Mobile NIRs has also been investigated as a technique to measure silage density (Davies et al. 2018). The initial developments look promising; however its assessment has been on open clamp faces and not during clamp filling, and more datasets are required to ensure its validity.

Until direct measurement of density while packing is possible, it may be necessary to develop tractor/operator-based solutions, as with the field operations of mowing, tedding and raking. Filling layer depth could be controlled by similar systems developed from the construction industry. Holmes (2006) paved the way for this approach using web based excel sheets to improve silo filling (https://fyi.uwex.edu/forage/harvest/ (Floor Length to Achieve Bunker/Pile Silo Filling Layer Thickness Calculator). Further development of an interactive app that utilises input data of trailer forage weight, trailer volume and forage DM content alongside loading shovel width and volume dimensions would be able to compute the distance each loading shovel/trailer should be spread over the clamp surface to maintain the optimum layer depth and by linking to the tractor GPS could ensure it is accurately achieved.

**3. Feeding out**

The efficiency and the silage quality of the feeding phase are greatly influenced by the previous phases from the field to silo opening. Since high quality conserved forages are crucial to maximizing efficiency and sustainability of livestock farms, the objective is to feed animals a uniform high quality silage over the whole profile of the silo for the entire year (Wilkinson and Davies 2013; Borreani et al. 2018). Utilisation of the technologies discussed in parts 1 and 2 will hopefully have enabled a silage clamp with uniform ideal quality to have been produced. As previously stated, silage quality is composed of four pillars: nutritive (macro and micro), fermentative, microbial and contaminants. Properly informed management practices and decisions from the harvesting stage, through wilting and ensiling phase to feed-out phase are the only means to achieve consistently high quality silage during progression both across a clamp and from front to back.

Possibly the most important factor affecting silage diets are the contents of dry matter, crude protein and utilisable energy, and variability within a single feed-out face of the silo can be large. Thus rapid methods to accurately assess this for every feed mixer load are vital as these have a major effect on the accuracy of diet formulation and DM intake. Reference laboratory methods for DM assessment are time-consuming and cannot be applied to the daily changes in diet composition; therefore better moisture sensing techniques for modern agricultural with on-line moisture monitoring are needed (Nelson and Trabelsi 2004). Currently, new promising real-time technologies, based on microwave free-space measurements involving attenuation and phase-shift determination and density independent functions, are available to permit reliable moisture sensing applicable to moving grain in which bulk density variation occurs (Nelson and Trabelsi, 2004). These advantages, along with promise for a universal moisture calibration, should encourage the development of microwave measurement systems to be applied to silages (Perricone et al. 2017).

One of the most important factors influencing silage quality and animal performance during feed-out phase is the control of aerobic deterioration, probably the most significant problem for farm profitability and feed quality worldwide. It is now recognized that the changes during the feed-out phase are equally as important as those in the closed silo from the viewpoint of preserving nutrients and maintaining good hygienic quality of the silage (Wilkinson and Davies 2013). Several factors affect silage quality and nutritional losses during feed-out, many of which should be controlled in the field during crop growing and during harvesting and ensiling. Key factors affecting silage quality at feed-out are the daily feed-out rate, the fermentation profile and the incorporation of top and side (waste) deteriorated silage. By planning the silo size to achieve the correct daily feed-out rate, which depends on the season and latitude, and by properly sealing and covering the silo to minimize oxygen penetration during the conservation phase, many of these issues can be solved. Feeding either aerobically or anaerobically spoiled silage can have numerous impacts on the animal from increased risks of contaminants in the feed to reduced intake and production (Borreani et al. 2018).

During feed out, different stages of aerobic deterioration can be present at the same time at the silo face. Mouldy silage can be easily appraised visually; unfortunately, there may be other parts of the silage face that are rapidly spoiling but appear no different than stable silage. The ideal would be to detect all spoiled and spoiling silage and avoid its inclusion in the feed ration. The accurate evaluation of the microbiological and chemical quality of the whole working face is currently impossible in an accurate, timely and cost efficient way to be performed routinely on the farm during the feed-out phase. Hence, simple methods of enabling the forage manager to quickly and accurately assess silage quality and the extent of aerobic deterioration at the silo face are essential.

The first step in determining what analytical approach to use in silage evaluation is to define the objectives of testing. Silage testing can be summarized by the three objectives: a) Providing nutritional inputs and DM content to formulate ruminant diets; b) Evaluating potential hazards to safety and quality of milk and dairy products (mycotoxins, *Listeria*, yeasts and moulds and spore-formers); c) Evaluating conservation efficiency (DM losses and spoiled silage to be discarded). If these can be done quickly and precisely, then selective feeding actions can be employed to improve farm silage efficiency.

Unfortunately, with on-farm silages, most microbial deterioration is invisible initially and may only be detected by a temperature rise in the forage (Borreani and Tabacco 2010). Since the oxidation process is accompanied by the evolution of heat, an increase in temperature is a convenient indicator of the extent and intensity of aerobic deterioration and can have application in alerting farmers to the onset of aerobic deterioration. To monitor aerobic deterioration during silage production, the internal temperature of silage is often used as an indicator or alarm (Borreani and Tabacco 2010). Several systems to measure temperature of the working face of a silo can describe the areas involved in aerobic microbial activity that are otherwise invisible. One method consists of burying temperature loggers inside the silo at the time of silo filling and retrieving them at feed out. Green et al. (2009) proposed burying wireless sensor nodes to precisely monitor and measure the temperature inside a silage stack. These sensors reliably transmit the data to a network model to predict the normal temperature variations of the silage using the air and soil temperature as inputs, and could detect the abnormal temperature variations inside the silage caused by silage deterioration.

A first attempt to establish which area of the silo was subjected to aerobic deterioration at the farm level by means of a probe thermometer was made by Ruppel et al. (1995). Alternatively infrared thermography is a non-invasive technique capable of detecting thermal radiation from the surface of any object; heat-sensing digital cameras can capture in a single picture all temperatures of the working face, and may reduce costs associated with personnel and chemical reagents used for conventional assessment of silage aerobic stability (Addah et al. 2012). However, temperature measurement by infrared thermography is highly influenced by weather (sunny, cloudy, rainy, etc.), the time of the day, the exposure of the silo face to direct sunlight, wind, and the homogeneity of the feed-out face. To overcome these issues, Clemente et al. (2015) proposed that reliable data could be obtained by removing about 0.1 m from the silo face before shooting the image.

Monitoring silage $O_2$ concentration and temperature can provide a critical insight regarding silage quality. Bochtis et al. (2011) suggested the need for the development and implementation of dedicated decision systems for the prediction of quality parameters in ensiled biomasses, for example, by predicting the occurrence of oxygen entering the silage, by monitoring the temperature and the outside weather conditions, and incorporating such measures into designated decision support systems and by extension into farm management information systems. They showed that the system can provide the forage manager with information about biomass quality parameters by graphical visualization as a first step and, as a second step, the system issues alerts depicting real deviations between actual and predicted values of the monitored properties. Sun et al. (2015) proposed the use of the Clarke oxygen electrode as an *in situ* biosensor for simultaneous monitoring of $O_2$ and temperature to monitor maize silage aerobic deterioration.

Oxygen in silage is central to the deterioration of silage quality and thus measurement of it could provide useful decision-making information. Even though the gas chromatography measurement is very accurate, the available methods are time consuming and unable to monitor silage $O_2$ dynamics. Shan et al. (2016) focused on assessing three types of commercial $O_2$ sensors, including Clark oxygen electrodes (COE), galvanic oxygen cell (GOC) sensors and the Dräger chip measurement system (DCMS). They concluded that in terms of measurement quality, the calibrated COE and the GOC sensors reported similarly high accuracies in maize silage. The reduced accuracy of the DCMS, especially at low $O_2$ concentration, was probably caused by the relatively high levels of $CO_2$ in the silage. They concluded that the GOC provided the least costly option but was unable to simultaneously measure $O_2$ and temperature, whereas the COE had a high performance to cost ratio (Shan et al. 2016).

Some companies propose the use of a portable electronic nose to quickly identify traces of organic, biological and chemical compounds with accuracy in corn and wheat silages (http://www.estcal.com/blog/quality-assessment-corn-silage). The idea is to identify mouldy silage by quantifying the musty odour. Preliminary analyses were performed on corn grain to verify the capability of portable electronic nose to identify sour, musty and good corn samples. The chromatogram results of testing all types of corn samples displayed the capability of quantification and difference among the samples: the lactic acid peak in the sour samples was clearly visible; the

distinctive compound peaks of musty corn were different from other samples and were not present to any large extent in other samples; the relatively odour free chromatograms of good corn samples were distinct as well. These systems have been tested for silage (Masoero et al., 2007) and the use of volatile odours are used widely in the cheese and wine industries as methods to assess fermentation products of microbial origin (Liu et al., 2004; Bartowsky, 2012). Thus the potential of these approaches warrants significant further investigation for detecting not only markers of aerobic spoilage but also pathogenic bacterial contamination of silage. The use of hand-held instruments such as portable NIRS increases the potential for farmers to take more samples because analysis is quick and relatively cheap (Modroño et al. 2017). The NIRS analysis allows the characterisation of concentrate and forage feed sample components of the total mixed ration accurately and precisely (providing the prediction model is appropriate). Construction of new dairy cow farms and modernization has begun over the last few years. Modernization of dairy production has resulted in rapid growth of herds being handled on farms and the need to reduce labour have brought the development of integrated automatic solutions, such as administration of concentrates, automatic milking systems, cleaning of manure, loose handling of cows, and management of the microclimate (Saliņš et al. 2012; Bisaglia and Brambilla 2017). With this in mind some companies have produced fully automated feeding and feed mixing robots (Saliņš et al. 2012). These automated systems move forages and feeds into the feed kitchen, in which the silages and other feeds are stored. Saliņš et al. (2012) assessed, on several commercial farms, forage distribution robots in comparison with tractor mixers-distributors. They concluded that the feeding robot can compete with mobile distributors even if concentrates are included in the total mixed ration and even when they are not stored in specific feed stations. Robotic systems have opened up new perspectives and requirements with regard to technologies to monitor and improve silage quality and aerobic stability for implementation and successful application at farm level, only time will tell whether these improve or reduce the risks of aerobic stability at feed-out. However by removing the human element it is our belief that they will reduce both aerobic spoilage and variability in forage feed-out management.

The current trend is to identify integrated automatic solutions, which interact with each other and can represent the start of what will be the true development of automation in the future. The modern farm of the future with up-to-date technological solutions are already being implemented with: loose handling of cows, milking in halls with high-productivity milking equipment or automatic milking systems (AMS), TMR or use of precise feeding technology and automatic manure removal. Introduction of all these different technological solutions is possible and will become the norm on progressive large dairy farms; thus it is essential that silage quality, both nutritive and hygienic quality, is maximised for the livestock it is being fed to.

## Summary

The success of the automated livestock farm using new technologies will be enhanced if similar robust technological approaches for silage production can be developed and implemented but it is essential that these technologies provide accurate and useful information to enable the forage manager to adapt the system from field to feed-out to account for the unforeseen variables such as weather and climate that can vary year to year. Thus the monitoring and feedback technologies reviewed here need to be robust, cost effective and provide rapid information in an easily digestible form for the forage manager to make decisions on factors from: field crop nutrition and growth yields, optimal harvesting time through to harvesting and ensiling quality, finishing with feed-out quality and monitoring of aerobic deterioration. If successful, this will help ensure that the highest quality silage is being consumed and utilised within the most precisely formulated TMR by the livestock on each farm utilising such approaches.

## References

Addah, W., Baah, J., Okine, E.K., & McAllister, T.A., (2012) Use of thermal imaging and the in situ technique to assess the impact of an inoculant with feruloyl esterase activity on the aerobic stability and digestibility of barley silage. Canadian Journal of Animal Science 92:381-394.

Adesogan, A.T. (2009) Challenges of tropical silage production. In: Proc. XVth International Silage Conference, Madison USA, July 12th-14th 2009. Eds Broderick et al. Pp139-154.

Adrian, A.M., Norwood, S.H., & Mask, P.L. (2005) Producers' perceptions and attitudes toward precision agriculture technologies. Computers and Electronics in Agriculture 48:256-271.

Aït-Kaddour, A., Boubellouta, T. & Chevallier, I. (2011) Development of a portable spectrofluorimeter for measuring the microbial spoilage of minced beef. Meat Science 88:675-681.

Ali, I., Cawkwell, F., Dwyer, E., Barrett, B. & Green, S. (2016) Satellite remote sensing of grasslands: from observation to management. Journal of Plant Ecology 9:649-671.

Amrutha, A., Lekha, R. & Sreedevi, A. (2016) Automatic soil nutrient detection and fertilizer dispensary system. *In*: Proc. 2016 International Conference on Robotics: Current Trends and Future Challenges. 19-20th December. Thanjavur, India.

Bartowsky, E. (2012) Lactic acid bacteria (LAB) in grape fermentations-an example of LAB as contaminants in food processing. In: Lactic Acid Bacteria – Microbiological and functional aspects Eds Lahtinen *et al.* 2004. CRC Press Florida USA. Pp 343-359.

Bisaglia, C. & Brambilla, M. (2017) Automazione dell'unifeed: casi concreti in Italia. (Total mixed ration: concrete cases in Italy). L'Informatore Agrario 73(36):35-38.

Bochtis, D.D., Sørensen, C.G., Green, O., & Bartzanas, T. (2011) A diagnostic system for improving biomass quality based on a sensor network. Sensors 11: 4990-5004.

Bock, C.H., Poole, G.H., Parker, P.E. & Gottwald, T.R. (2010) Plant disease severity estimated visually, by digital photography and image analysis, and by hyperspectral imaging. Critical Reviews in Plant Sciences 29:59-107.

Borreani, G. & Tabacco, E. (2010) The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science 93:2620-2629.

Borreani, G., Tabacco, E., Schmidt, R.J., Holmes, B.J. & Muck, R.E. (2018) Factors affecting dry matter and quality losses in silages. Journal of Dairy Science 101: in press.

Berge, T.W., Goldberg, S., Kaspersen, K. & Netland, J. (2012) Towards machine vision based site-specific weed management in cereals. Computers and Electronics in Agriculture 81:79-86.

Clemente, G., Monge, J.L., Clemente, F.M. & Cervigni, K.D. (2015) Use of thermographic images for evaluation of superficial and subsurface temperature in the exposed area of corn silos. In: Proc. 17th International Silage Conference, Piracicaba, Sao Paulo, Brazil, Pp. 308-309.

Cooper, S.D., Roy, D.P., Schaaf, C.B. & Paynter, I. (2017) Examination of the potential of terrestrial laser scanning and structure-from-motion photogrammetry for rapid nondestructive field measurement of grass biomass. Remote Sensing 9: 531.

Crowther, T.W., Glick, H.B., Covey, K.R., Bettigole, C., Maynard, D.S., Thomas, S.M., Smith, J.R., Hintler, G., Duguid, M.C., Amatulli, G., Tuanmu, M.N., Jetz, W., Salas, C., Stam, C., Piotto, D., Tavani, R., Green, S., Bruce, G., Williams, S.J., Wiser, S. K., Huber, M. O., Hengeveld, G. M., Nabuurs, G.J., Tikhonova, E., Borchardt, P., Li, C.F., Powrie, L.W., Fischer, M., Hemp, A., Homeier, J., Cho, P., Vibrans, A.C., Umunay, P.M., Piao, S.L., Rowe, C.W., Ashton, M.S., Crane, P.R. & Bradford, M.A. (2015) Mapping tree density at a global scale. Nature 525(7568):201-205.

Davies, D.R., Davies, G.K. & Piotrowski, C. (2018) Development of Calibrations for hand-held NIRs instrumentation to measure silage density from the open face of grass silage clamps. This proceedings International Silage Conference

Duniere, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I. & Thevenot-Sergentet, D. (2013). Silage processing and strategies to prevent persistence of undesirable microorganisms. Animal Feed Science and Technology 182:1-15.

Earle, D.F. & McGowan, A A. (1979) Evaluation and calibration of an automated rising plate meter for estimating dry-matter yield of pasture. Australian Journal of Experimental Agriculture 19:337-343.

Fick, G.W. & Mueller S.C. (1989) Alfalfa: Quality, maturity, and mean stage of development. *In:* Information Bulletin 217. Cornell Cooperative Extension, Ithaca, NY, USA.

Fricke, T. & Wachendorf, M. (2013) Combining ultrasonic sward height and spectral signatures to assess the biomass of legume-grass swards. Computers and Electronics in Agriculture 99:236-247.

Fuerll, C., Schemel, H. & Koeppen, D. (2008) Principles for measuring density in silages. Landtechnik 63:94-95.

Graham, A.R. (1983). Fungal autofluorescence with ultraviolet illumination. American Journal of Clinical Pathology 79: 231-234.

Green, O., Nadimi, E.S., Blanes-Vidal, V., Jørgensen, R.N., Storm, I.M.L.D. & Sørensen, C.G. (2009) Monitoring and modeling temperature variations inside silage stacks using novel wireless sensor networks. Computers and Electronics in Agriculture 69:149-157.

Hallau, L., Neumann, M., Klatt, B., Kleinhenz, B., Klein, T., Kuhn, C., Rohrig, M., Bauckhage, C., Kersting, K., Mahlein, A.K., Steiner, U. & Oerke, E.C. (2018) Automated identification of sugar beet diseases using smartphones. Plant Pathology 67:399-410.

Hill, M.J., Donald, G.E., Vickery, P.J., Moore, A.D. & Donnelly, J.R. (1999) Combining satellite data with a simulation model to describe spatial variability in pasture growth at a farm scale. Australian Journal of Experimental Agriculture 39:285-300.

Holman, F.H., Riche, A.B., Michalski, A., Castle, M., Wooster, M.J. & Hawkesford, M. J. (2016) High throughput field phenotyping of wheat plant height and growth rate in field plot trials using UAV based remote sensing. Remote Sensing 8:1031.

Holmes, B.J. (2006) Density in silage storage. In: Proccedings of Silage for Dairy Farms: Growing, Harvesting, Storing and Feeding. Northeast Regional Agricultural Engineering Service 181.

Kallenbach, R.L. (2015) Describing the dynamic: measuring and assessing the value of plants in the pasture. Crop Science 55:2531-2539.

Lemmens, M. (2015) Sentinel-2A in Orbit. Gim International-the Worldwide Magazine for Geomatics 29:22-25.

Liu, S.-Q., Holland, R. & Crow, V.L. (2004) Esters and their biosynthesis in fermented dairy products: a review. International Dairy Journal 14:923-945.

Marley, G. (2017). Global warming and forage preservation – forages and treatments. In: Proc. 70th International Conference on Forage Conservation. Slovak Republic 27$^{th}$-29$^{th}$ September 2017. Ed. L. Rajcakova. Pp105-106.

Masoero, G., Sala, G. & Peiretti, P.G. (2007) Development of near infrared (NIR) spectroscopy and electronic nose (EN) techniques to analyse the conservation quality of farm silages Journal of Food, Agriculture & Environment 5:172-177.

McDonagh, J., O'Donovan, M., McEvoy, M. & Gilliland, T.J. (2016) Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013. Euphytica 212:187-199.

Meng, D., Meng, F., Sun, W., & Deng, S. (2018). A compound sensor for simultaneous measurement of packing density and moisture content of silage. Sensors, 18, 73.

Michell, P. & Large, R.V. (1983) The estimation of herbage mass of perennial ryegrass swards - a comparative-evaluation of a rising-plate meter and a single-probe capacitance meter calibrated at and above ground-level. Grass and Forage Science 38:295-299.

Mishra, P., Asaari, M.S.M., Herrero-Langreo, A., Lohumi, S., Diezma B. & Scheunders, P. (2017) Close range hyperspectral imaging of plants: A review. Biosystems Engineering 164:49-67.

Modroño, S., Soldado, A., Martínez-Fernández, A. & Roza-Delgado, B. (2017) Handheld NIRS sensors for routine compound feed quality control: Real time analysis and field monitoring. Talanta 162:597-603.

Mouazen, A.M., Anthonis, J., Saeys, W. & Ramon, H. (2004) An automatic depth control system for online measurement of spatial variation in soil compaction. Part 1: Sensor design for measurement of frame height variation from soil surface. Biosystems Engineering 89:139-150.

Mumme, M. & Katzameryer, J. (2008) Mobile test station for the radiometric measurement of density distribution in bales. Landtechnik, 63: 341-343.

Nelson, S.O. & Trabelsi, S. (2004) Principles for microwave moisture and density measurement in grain and seed. Journal of Microwave Power Electromagnetic Energy 39: 107-117.

Pandey, P., Ge, Y.F., Stoerger, V. & Schnable J.C. (2017) High throughput in vivo analysis of plant leaf chemical properties using hyperspectral imaging. Frontiers in Plant Science 8:1348.

Paulus, S., Dupuis, J., Mahlein, A.K. & Kuhlmann, H. (2013) Surface feature based classification of plant organs from 3D laserscanned point clouds for plant phenotyping. Bmc Bioinformatics 14:238.

Perez-Sanz, F., Navarro, P.J. & Egea-Cortines, M. (2017) Plant phenomics: an overview of image acquisition technologies and image data analysis algorithms. Gigascience 6:1-18.

Perricone, V., Agazzi, A., Costa, A., Lazzari, M., Savoini, G., Calcante, A. & Tangorra, F.M. (2017). Real-time dry matter content of corn silage by a microwave sensor. In: Proc. Veterinary and Animal Science Days 2017, 6-8 June, Milan, Italy.

Perumpral, J.V. (1987) Cone penetrometer application: A review. Transactions of the American Society of Agricultural Engineers, 30: 939-944.

Pethybridge, S.J. & Nelson, S.C. (2018) Estimate, a new iPad application for assessment of plant disease severity using photographic standard area diagrams. Plant Disease 102:276-281.

Punalekar, S., Verhoef A., Tatarenko, I.V. van der Tol, C., Macdonald, D.M.J., Marchant, B., Gerard, F., White, K. & Gowing, D. (2016) Characterization of a highly biodiverse floodplain meadow using hyperspectral remote sensing within a plant functional trait framework. Remote Sensing 8:112.

Punalekar, S.M., Verhoef, A., Quaife, T.L., Humphries, D.J., Bermingham, L. & Reynolds, C.K. (2018) Application of Sentinel-2A data for pasture biomass monitoring using a physically based radiative transfer model. Remote Sensing of Environment [In press].

Ruppel, K.A., Pitt, R.E., Chase, L.E., Galton, D.M. (1995) Bunker silo management and its relationship to forage preservation on dairy farms. Journal of Dairy Science 78:141-153.

Sadgrove, E.J., Falzon, G., Miron, D. & Lamb, D. (2017) Fast object detection in pastoral landscapes using a Colour Feature Extreme Learning Machine. Computers and Electronics in Agriculture 139:204-212.

Safari, H., Fricke, T., Reddersen, B., Mockel, T. & Wachendorf, M. (2016) Comparing mobile and static assessment of biomass in heterogeneous grassland with a multi-sensor system. Journal of Sensors and Sensor Systems 5:301-312.

Saliņš, A., Freimanis, M., Truhanovs, R. & Priekulis, J. (2012) The most efficient solutions of forage distribution on modern dairy cow farms. Agron. Res. (Biosystem Engineering Special Issue 1):219-226.

Schellberg, J., Hill, M.J. Gerhards, R., Rothmund, M. & Braun, M. (2008) Precision agriculture on grassland: Applications, perspectives and constraints. European Journal of Agronomy 29:59-71.

Schulze, C., Spilke, J. & Lehner, W. (2007) Data modeling for precision dairy farming within the competitive field of operational and analytical tasks. Computers and Electronics in Agriculture 59:39-55.

Serrano, J.M., Shahidian, S. & da Silva, J.R.M. (2016) Monitoring pasture variability: optical OptRx (R) crop sensor versus Grassmaster II capacitance probe. Environmental Monitoring and Assessment 188:17.

Shafiekhani, A., Kadam, S., Fritschi, F.B., DeSouza, G.N. (2017) Vinobot and vinoculer: two robotic platforms for high-throughput field phenotyping. Sensors 17:214.

Shan, G., Sun, Y., Li, M., Jungbluth, K.H., Maack, C., Buescher, W., Schütt, K.-B., Boeker, P., Lammers, P.S., Zhou, H., Cheng, Q. & Ma, D. (2016) An assessment of three different in situ oxygen sensors for monitoring silage production and storage. Sensors (Basel), 16: 91.

Shaw, R., Lark, R.M., Williams, A.P., Chadwick D.R. & Jones, D.L. (2016) Characterising the within-field scale spatial variation of nitrogen in a grassland soil to inform the efficient design of in-situ nitrogen sensor networks for precision agriculture. Agriculture Ecosystems and Environment 230:294-306.

Shimoda, S. & Oikawa, T. (2006) Temporal and spatial variations of canopy temperature over a C-3-C-4 mixture grassland. Hydrological Processes 20:3503-3516.

Strzelecki, A. (2016) Patent No. GB2527046, Devise and method for the treatment of crops.

Sun, Y., Buescher, W., Lin, J., Schulze Lammers, P.S., Ross, F., Maack, C., Cheng, Q. & Sun, W. (2010) An improved penetrometer technique for determining bale density. Biosystems Engineering 105:273-277.

Sun, Y.R., Li, M.H., Cheng, Q., Jungbluth, K.H., Buescher, W., Ma, D.K., Zhou, H.Y. & Cheng, H. (2015). Tracking oxygen and temperature dynamics in maize silage novel application of a Clack oxygen electrode. Biosystems Engineering 139:60-65.

Tardieu, F., Cabrera-Bosquet L., Pridmore T. & Bennett, M. (2017) Plant phenomics, from sensors to knowledge. Current Biology 27:R770-R783.

van Evert, F.K., Samsom, J., Polder, G., Vijn, M., van Dooren, H.J., Lamaker, A., van der Heijden, G., Kempenaar, C., van der Zalm, T. & Lotz, L.A.P. (2011) A Robot to detect and control broad-leaved dock (*Rumex obtusifolius* L.) in grassland. Journal of Field Robotics 28:264-277.

Virlet, N., Sabermanesh, K., Sadeghi-Tehran, V. & Hawkesford M.J. (2017) Field Scanalyzer: An automated robotic field phenotyping platform for detailed crop monitoring. Functional Plant Biology 44:143-153.

Wachendorf, M., Fricke, T. & Moeckel, T. (2018) Remote sensing as a tool to assess botanical composition, structure, quantity and quality of temperate grasslands. Grass and Forage Science 73:1-14.

Warudkar, G. & Dorle, S. (2016). Review on sensing the fertility characteristics of agriculture soils. In: Proc. International Conference on Information Communication and Embedded Systems (Icices). 25-26th February. Chennai, India.

Wilkinson, J.M. & Davies, D.R. (2013) The aerobic stability of silage: key findings and recent developments. Grass and Forage Science 68:1-19.

# 1968 – 2018: 50 years Silopress. A German idea for conservation and storage of agricultural products in large plastic bags: a review

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**Introduction** The silopress was invented in 1968 by the German company Eberhardt. Thereby, it was created a completely new technology of conservation and storage of agricultural products in large plastic bags. Over the years, several technical and technological developments increased the worldwide use of the bagging technology. Fundamental research has been directed towards the determination of fermentation losses and measurement of packing density in comparison with other storage systems as well as temperature development, and of aerobic stability during bag feed-out. This paper reviews some of those developments and summarizes the results obtained in bagging trials carried out by the research facilities the authors of this article are affiliated with.

**Material and Methods** In order to measure dry matter (DM) losses and silage characteristics in plastic bags under standardized field conditions, the “buried bag” (BB)-method was adapted to the bagging technology by placing nylon-mash nets inside of the bag while packing without damaging the plastic. Three flaps (centre, top, shoulder) of 400 x 400 mm size were installed in the tunnel wall of the silo press through which the BB, containing about 3 kg of forage and a temperature logger, could be placed at different positions with about 1 m distance between them (three replicates per position). The BB were collected during feed removal. This BB method was successfully employed in studies on by-products (pressed sugar beet pulp (PSBP), brewer`s and wet distiller´s grains, silage from whole-plant maize grass and alfalfa, crimped grains at varying moisture level). Packing density was also measured at different positions. Additionally, a method for accurate and homogeneous silage additive application was developed by spraying of the additive through five nozzles on a pipe covering the whole width of the bagging machine. It was mounted on the back wall of the rotor press over the rotor. This enabled treating of hot by-products and studying additive effects under practical conditions as laboratory-scale ensiling do not reflect typical cooling pattern in plastic bags stored outside. The BB method also allowed the continuous measurement of temperature in the bag to evaluate aerobic stability (AS) during unloading when the material is exposed to air. Finally, a method for automated measuring, steering and controlling of silage compaction in bags was developed and tested under field conditions to minimize the influence of the operator on compaction and, thus, silage quality.

**Results and Discussion** Losses of DM during fermentation measured by the BB method were generally low in bagged silage and packing density was sufficiently high (table 1). However, both DM losses and packing density may vary significantly depending on other factors. For example, Muck and Holmes (2005) reported strong relationships between DM content and particle size and packing density and stated that <10% DM losses in whole-crop maize and lucerne silage can be regularly achieved in silo bags. When compared with bunker or clamp silos, we consistently measured much lower DM losses in bags filled with grass/whole-plant maize/lucerne silage (5.3 *vs* 11.3%), crimped grain silage (3.5 *vs* 6.0%) and by-products (4.3 *vs* 100%). Muck et al. (2009) not only confirmed these observation, but also found better nutritional quality in bagged silage. Moreover, we frequently found differences in packing density between sampling sites, with silage in the core having greater packing density than that in the shoulder or top areas. The development of an automated brake pressure

adjustment generally improved packing density and can partially alleviated the effect of location in the bag leading to higher compaction also in the upper and shoulder areas. Valuable information by the BB method were also obtained regarding the course of temperature development in different locations in bags filled with PSBP. It could be shown that silage temperature in the core is less affected by ambient temperature and remains at higher levels even if ambient temperature drops in winter. This demonstrates that lab-scale experiments at constant temperature cannot be used to simulate storage conditions on farms.

**Table 1**. DM losses during fermentation of various agricultural products stored in plastic bags

| Product | | DM losses (%)[1] | Packing density (kg DM $m^{-3}$) |
|---|---|---|---|
| Pressed sugar beet pulp | <25% DM | 0.05 - 2.1 | 162 - 195 |
| | 25 - 28% DM | 1.1 - 2.4 | 184 - 213 |
| | 45% DM[2] | nd | 299 |
| Brewer`s grains | | 2.2 | nd |
| Whole-crop maize | | 4 - 8 | 170 - 220 |
| Grass | | 5 - 8 | 150 - 200 |
| Lucerne[3] | | 7 - 13 | nd |
| High-moisture grains | maize | 3 | 380 - 560 |
| | barley | <1 | 443 - 503 |
| | wheat | <1 | 524 - 754 |

[1]depending on stage of maturity at harvest, chop length, DM level, location, [2]Schneider et al., 2018, [3]up to 21% in heavily wilted, late-cut lucerne silage, nd not determined.

Although feed-out rate can be adjusted to a certain extent to the daily demand by using different bag diameters, evidence was provided that the use of silage additives may be necessary to safeguard high aerobic stability under challenging conditions, e.g. summer feeding, particularly in by-product silage and crimped cereals. In numerous trials using chemical additives we have consistently observed enhanced stability of treated silage exposed to air but the magnitude of the effects may be affected by sampling location, DM level, storage length, length of air exposure and additive composition. For example, additive use improved ASTA over that of untreated silage by 6.0 days in crimped barley (n=3), by 7.6 days in crimped maize (n=2), by 4.3 days in PSBP (n=5). Typically, higher application rates resulted in further improved ASTA. This was recently confirmed by Schneider et al. (2018) in PSBP silage who observed improved ASTA by 4.1 days and 5.7 days, respectively, when the application rate of the same chemical additive was increased from 1.5 to 2.0 L $t^{-1}$.

**Conclusion** Since the invention of the bagging technology for agricultural products 50 years ago, numerous technological and technical improvements have been made and implemented. The results clearly show that bagging minimizes losses during fermentation compared with other storage systems, e. g. bunker and clamp silos. Bagging is an efficient, flexible and environmentally-compatible technology to conserve and to store various feed materials. Chemical additives are strongly recommended to maintain high aerobic stability of silages fed-out under challenging conditions.

**References**

Muck, R.E. & Holmes, B.J. (2005) Factors affecting bag silo densities and losses. In: Park, R.S. & Stronge, M.D. (eds). XIV$^{th}$ International Silage Conference, Belfast, UK, 2005, pp. 245-246.

Muck, R.E., Broderick, G.A. & Brink, G.E. (2009) Effects of silo type on silage quality and losses. In: Broderick, G.E., Adesogan, A.T., Bocher, L.W., Bolsen, K.K., Contreras-Govea, F.E., Harrison, J.H. & Muck R.E. (eds). XV$^{th}$ International Silage Conference, Madison, WI, USA, 2009, pp. 275-276.

Schneider, M., Auerbach, H., Eklund, M., Rössle, G. & Spiekers, H. (2018) Effects of dry matter, silage additive and bagging technology on fungal counts and aerobic stability of pressed sugar beet pulp silage. Accepted by XVIII$^{th}$ International Silage Conference, Bonn, Germany.

# A new approach to assess feed-out rate in maize silage bunker

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**Keywords:** aerobic deterioration, silage management, unloading

**Introduction** There is an increasing recognition that many of the problems associated with feeding silage arise during the unloading phase. Thus, it is imperative to identify a correct feed-out rate (FR), which can mitigate aerobic deterioration in this phase. To date, all proposed FR are based on linear feed-out (cm/d or m/week), which were developed through empirical observations and mathematical models (Pitt and Muck 1993). However, this recommendation may fail, since silage densities vary among silos. We hypothesised that a parameter, which consider the silage density, can be more suitable. The aim of this study was to identify an unloading rate based on daily silage consumption per face area to reduce spoilage in farm maize silages.

**Materials and Methods** Forty-four dairy farms in South, Southeast, and Midwest of Brazil and 44 dairy farms in North of Italy, agreed to participate in this project. Farm size ranged from 6 to 1,800 lactating cows. Farms performing well, moderately well, and poorly in terms of silage management were selected. On the day of sampling, one maize silage bunker that had been open for at least 20 d was assessed in detail on each farm. Three samples from the peripheral area and one sample from the central core were taken from the face to determine microbial counts and fermentation profile. Before coring, the temperatures were measured at the same four locations. The temperature and pH of silages located in the central core were used as references. The difference between the silage sample and the reference temperature and pH were used as indices of aerobic deterioration (dT and dpH, respectively). The silage density profile was characterised according to D'Amours and Savoie (2005). The width and height of both entire face and visibly spoiled layer were recorded in order to calculate face area ($m^2$) and spoiled area (%; area visibly spoiled/face area). The silage consumption was recorded to determine the FR (kg/$m^2$/d; kg of daily silage/face area). The daily length of silage removed was also assessed. To identify silage spoilage, the following parameters were considered: dT ≥ 5°C, dpH ≥ 0.25, spoiled area ≥ 2%, and yeast and mould counts at the top ≥ 5 log colony-forming units (cfu)/g and ≥ 2 log cfu/g, respectively (described as dT5, dpH25, SA2, Y5, and M2, respectively). Each parameter was tested within four ranges of FR, as follows: < 125, 125-250, 250-375, and > 375 kg/$m^2$/d (described as FR1, FR2, FR3, and FR4, respectively). The statistical analyses were conducted using the PROC FREQ of SAS (2004). The Fisher's exact test was used to calculate *P*-values of the tested parameter. Significance was declared when the *P*-value was < 0.05. The mean and standard deviation for the linear removal were calculated using the PROC MEANS procedure of SAS (2004).

**Results** The daily FR and length of silage ranged from 29 to 990 kg/$m^2$ and from 0.05 to 1.4 m, respectively. The occurrence of Y5 was in 47.1, 44.1, 27.3, and 0% of silages for FR1, FR2, FR3, and FR4, respectively (Figure 1; $P = 0.03$). The occurrence of M2 was in 58.8, 44.1, 18.2, and 0% of silages for FR1, FR2, FR3, and FR4, respectively ($P = 0.03$). The dT5 was found in 67.7, 42.4, 15.2, and 0% of silages for FR1, FR2, FR3, and FR4, respectively ($P < 0.01$). The dpH25 was found in 47.1, 35.3, 9.1, and 0% of silages for FR1, FR2, FR3, and FR4, respectively ($P = 0.01$). The SA2 was found in 69.7, 33.3, 45.5, and 22.2% of silages for FR1, FR2, FR3, and FR4, respectively ($P = 0.01$). The number of farms for each FR was of 34, 34, 11, and 9 for FR1, FR2, FR3, and FR4, respectively, for all parameters tested. The mean linear removal (± SD) for FR1, FR2, FR3, and FR4 was 0.13 ± 0.05, 0.3 ± 0.09, 0.47 ± 0.11, and 0.8 ± 0.3 m/d, respectively.

**Discussion** Losses during unloading depend on the silage density, the aerobic stability of the silage (i.e. fermentation end-products and microbial count), the ambient temperature, the feed-out rate, and other management practices (Muck et al. 2003, Borreani and Tabacco 2010). Although the ambient temperature has been pointed out as a factor that affect silage deterioration during unloading, in our study there was a negative relationship between the ambient temperature and the parameters, which indicate spoilage silage (data not shown). Thus, the data from Brazil and Italy were combined. Conversely, the fermentation profile, the density, and the removal rate decisively affected losses during the feed-out phase. The five parameters used in this study to identify silage spoilage occurred mainly when the FR was lower than 250 kg of silage/$m^2$/d. There was no spoiled silage when the FR was greater than 375 kg silage/$m^2$/d. Several silages had FR lower than 250 kg silage/$m^2$/d and did not presented signals of deterioration (Figure 1). Most of these silages had good management practices such as materials weighing down silage cover and high concentration of organic acids and/or 1,2 propanediol. Therefore, these factors attenuated the effects of aerobic deterioration when silages had lower FR (250 kg of silage/$m^2$/d).

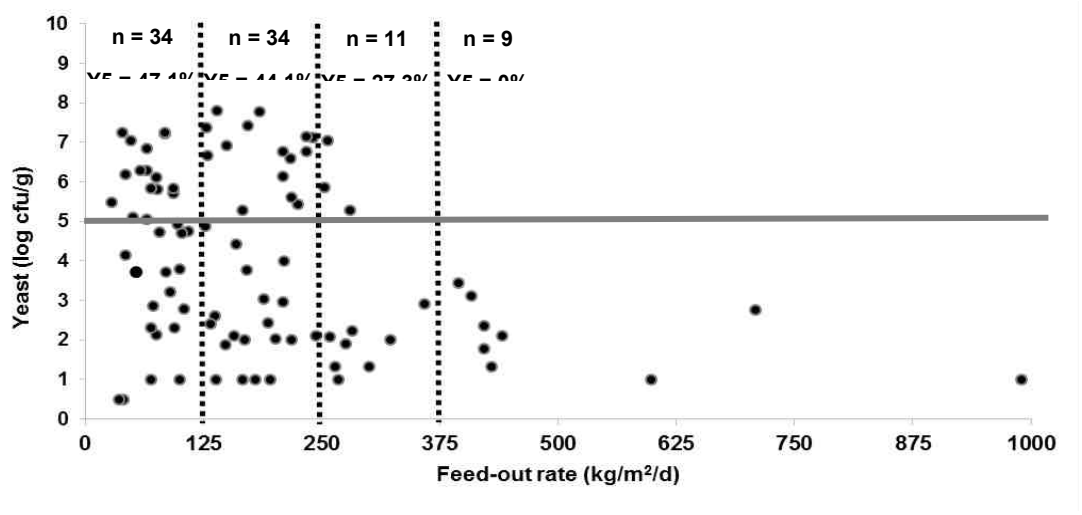


**Figure 1** Yeast counts in maize silages (n = 88) located at the top of the bunkers as influenced by feed-out rates. The dotted lines divide four ranges of feed-out rates. Y5 = silages with yeast counts ≥ 5 log cfu/g. The statistical significance was obtained using the Fisher's exact test. *P* = 0.03.

**Conclusions** The feed-out rate recommendation based on daily amount of silage per square meter is more reliable, since it considers silage density. A removal rate between 250−375 kg of silage/$m^2$/d reduces the risk of spoilage in maize silages. Removing more than 375 kg of silage/$m^2$/d the farm can guarantee unspoiled silage in the ration.

**Acknowledgements** This project was partially funded by CNPq and FAPEMIG.

**References**

Borreani G. & Tabacco, E. (2010) The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science, 93, 2620-2629.

D'Amours, L. & Savoie P. (2005) Density profile of corn silage in bunker silos. Canadian Biosystems Engineering. 47, 2.21-2.28.

Muck, R.E., Moser, L.E. & Pitt, R.E. (2003) Postharvest factors affecting ensiling. In: Buxton, D.R. Muck, R.E. & Harrison J.H. (ed). Silage Science and Technology. ASA, CSSA., and SSSA, Madison, WI. p. 251-304.

Pitt, R.E. & R.E. Muck. (1993) A diffusion model of aerobic deterioration at the exposed face of bunker silos. Journal of Agricultural Engineering Research, 55, 11-26.

SAS Institute Inc. 2004. SAS/STAT 9.1 User's Guide. Version 9.1 ed. SAS Inst. Inc., Cary, NC.

# A pH index as a method to identify aerobic deterioration in farm maize silage

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**Keywords:** farm silage, silage management, spoilage, unloading

**Introduction** There is a field method to detect silage spoilage, which relates the temperature in the central core (reference) with that measured in other locations of the silo face (dT), in particular at the peripheral areas (Borreani and Tabacco 2010). However, practical experiences have shown that more variables should be measured to confirm spoilage in farm conditions. We hypothesised that pH measurements may be useful to identify aerobic deterioration in maize silages, since its variations are linked with lactate-assimilating yeasts (Pahlow et al., 2003). The aim of this study was to correlate silage pH at the silo face with parameters that show aerobic deterioration in silages, such as temperature, yeast and mould counts.

**Materials and Methods** Forty-four dairy farms in South, Southeast, and Midwest of Brazil and 44 dairy farms in North of Italy, agreed to participate in this project. Farm size ranged from 6 to 1,800 lactating cows. Farms that performed well, moderately well, and poorly in terms of silage management were selected. On the day of sampling, one maize silage bunker that had been open for at least 20 d was assessed on each farm. Samples from the central core (defined as the reference pH; n = 1) and peripheral areas (n = 3) were taken from the operating silo face. The difference between the sample pH sample and the reference sample pH was used as an index (dpH). Before coring, the temperatures were measured at the same locations to determine the dT (Borreani and Tabacco 2010). Dry matter (DM) concentration and yeast and moulds counts were also determined. To identify silage spoilage, the following parameters were considered: dT ≥ 5°C (dT5), yeast counts at the top ≥ 5 log colony-forming units (cfu)/g (Y5), and mould counts at the top ≥ 2 log cfu/g (M2). Each parameter was tested within four ranges of dpH, as follows: < 0.15, 0.15−0.19, 0.20−0.24, and ≥ 0.25 (described as dpH1, dpH2, dpH3, and dpH4, respectively). The statistical analyses were conducted using the PROC FREQ of SAS (2004). The Fisher's exact test was used to calculate *P*-values of the tested parameter. Significance was declared when the *P*-value was < 0.05. The mean, minimum, and maximum values were calculated using the PROC MEANS procedure of SAS (2004).

**Results** The silages located in the central core showed a mean (minimum and maximum) temperature, DM concentration, pH value, and yeast and mould counts of 26.1°C (17.5-38.0), 34.1% (22.2-43.9), 3.80 (3.45-4.15), 1.60 log cfu/g (1.00-5.18), and 1.10 log cfu/g (1.00-3.08), respectively. The temperature, pH, yeast and mould counts of the silages from the peripheral areas ranged from 18.9 to 49.4°C, from 3.64 to 7.54, from 1.00 to 7.80 log cfu/g, and from <1.00 to 7.66 log cfu/g, respectively. The dpH ranged from –0.19 to 3.76. Regarding the parameters used to identify silage spoilage (Figure 1), the Y5 was found in 12.5, 22.2, 60.0, and 77.8% of silages for dpH1, dpH2, dpH3, and dpH4, respectively. The occurrence of M2 was in 20.0, 44.4, 50.0, and 74.1% of silages for dpH1, dpH2, dpH3, and dpH4, respectively. The dT5 was found in 18.0, 33.3, 40.0, and 92.6% of silages for dpH1, dpH2, dpH3, and dpH4, respectively.

**Discussion** During the unloading, detecting aerobic deterioration in the early stages, when moulded areas are not visible, is crucial for farmers and nutritionists. Borreani and Tabacco (2010) created a method to identify aerobic deterioration in silages through temperature measurements (dT). However, when this method is used, the heat from the forage respiration at the time of silo filling can be mistaken for heating from the aerobic deterioration, especially in silos, which had low filling rates. In order to overcome this issue, the dpH was tested in this study. Yeasts are responsible for starting the aerobic deterioration process because some genera are acid tolerant and use lactic acid for their

aerobic metabolism, and this leads to an increase in silage pH (Pahlow et al., 2003). When the yeast count increases to more than 5 log cfu/g, the silage becomes prone to aerobic deterioration (Woolford 1990). Our findings showed that almost 80% of silages had ≥ 5 log cfu/g when the dpH was ≥ 0.25 (Figure 1). This dpH value also showed that 74.1% and 92.6% of silages had M2 and dT5. Therefore, the dT and dpH should be combined when detecting silage spoilage on the farm, since they are quick and easy methods and improve the reliability of results.

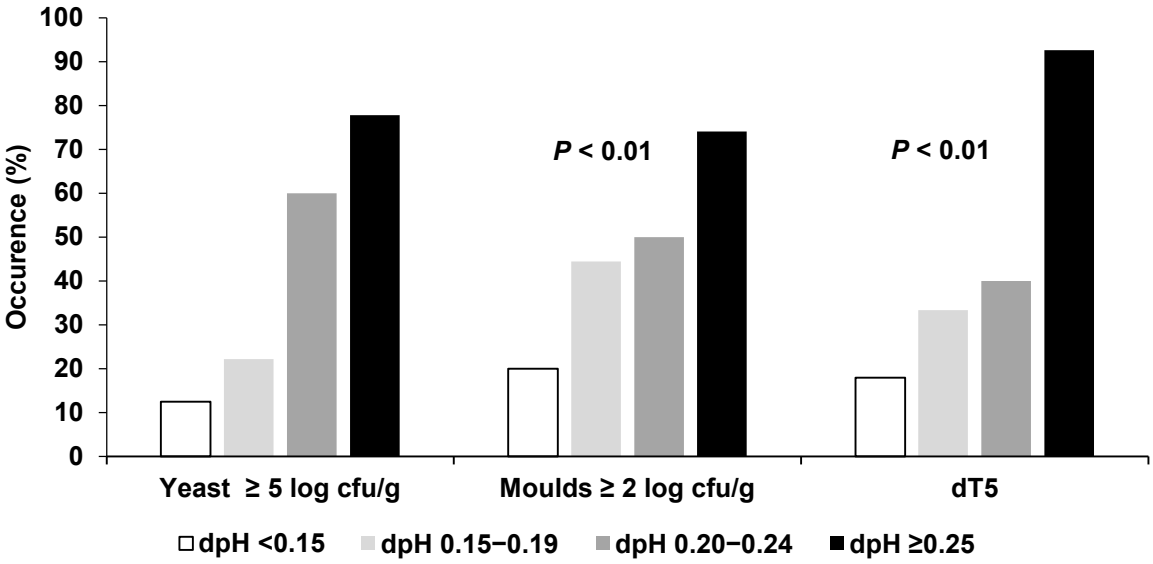


**Figure 1** Occurrence of yeast counts ≥ 5 log cfu/g and mould counts ≥ 2 log cfu/g in maize silages located at the top of bunkers and the difference between the temperature of the silage at the top and the reference temperature more than 5°C (dT5) according to different ranges of dpH. dpH = the difference between the pH sample and the reference pH (pH value from the central core of the silo). The statistical significance was obtained using the Fisher's exact test.

**Conclusions** The dpH can be used in association with dT to detect aerobic deterioration in maize silages at farm level. A dpH greater than 0.25 shows that the aerobic deterioration may be starting and thus good feeding management practices should be adopted.

**Acknowledgements** This project was partially funded by CNPq and FAPEMIG.

**References**

Borreani, G. & Tabacco, E. (2010) The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science, 93, 2620-2629.

Pahlow G., Muck R.E., Driehuis, F., Oude Elferink, S.J.W.H. & Spoelstra, S.F. 2003. Microbiology of ensiling. In: Buxton, D. R. Muck, R. E. & Harrison J. H. (ed). Silage Science and Technology. ASA, CSSA., and SSSA, Madison, WI. p. 31-94.

SAS Institute Inc. (2004) SAS/STAT 9.1 User's Guide. Version 9.1 ed. SAS Inst. Inc., Cary, NC.

Woolford, M.K. (1990) The detrimental effect of air on silage. Journal of Applied Bacteriology, 68, 101-116.

# A survey of maize hybrids for whole-plant silage in a hot climate

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**Keywords:** corn silage, corn varieties, silage yield

**Introduction** Currently, about 400 maize hybrids are registered for sale on Brazilian market. Among them, there are no specific hybrids for silage making and thus the dual-purpose strategy has been used by the seed companies. As variations exist in terms of yield and nutritive value, it is fundamental to identify varieties with a combination of these two traits. The aim of this study was to characterise maize hybrids grown in the main dairy regions on some agronomic and nutritional traits.

**Materials and Methods** From January to July 2017, livestock farms were visited in three regions (South, Southeast, and Midwest). Samples were taken from 42 maize fields, totalizing 31 different hybrids. In Brazil there are two growing seasons (GS), hybrids collected from January to March and from May to July were grouped in the first season (FGS; n = 24) and the second season (SGS; n = 18), respectively. Latitude for the FGS and SGS seasons ranged from 20º34'55'' to 25º01'08'' and from 17º38'58'' to 22º17'09'', respectively. Altitude for the FGS and SGS seasons ranged from 692 to 1060 m and from 480 to 1030 m above sea level. All samplings were performed when farmers were harvesting their fields. On the day of harvest, aerial images were obtained by flying a drone over the maize field. Four random spots (3 linear meter each) were chosen in the area, and plants from rows were cut by hand at 25 cm above ground. The plants of each row were weighed and then split into leaves, stem, and ear to determine the proportions of these components. Fertilization program, plant population, disease rating, forage yield, plant height, and ear height were also recorded. Samples were dried at 55°C for 72 h in a forced-air oven and ground to assess dry matter (DM), ash, and neutral detergent fibre (NDF). Forage DM yields were standardised at 35% DM by using the following equations: y = 0.0916 x + 23.084 and y = 0.1551 x + 13.603 for FGS and SGS, respectively, where x = 35 – the obtained DM (%). The average, maximum, minimum, standard deviation, and 95% confidence interval (CI) of all data were calculated using the PROC MEANS procedure of SAS (2004). Correlation analyses were performed through Pearson's correlation at 5% probability using the PROC CORR procedure of SAS (2004).

**Results** Forage yield for FGS and SGS were, on average, 26.3 and 19.0 tons DM/ha, respectively. This parameter showed a CI of 24.4–28.2 tons DM/ha and 17.4–20.6 tons DM/ha for FGS and SGS, respectively. In the FGS, forage yield was positively correlated with ear weight (Figure 1; $r^2 = 0.56$; $P < 0.01$), latitude ($r^2 = 0.23$; $P = 0.02$), altitude ($r^2 = 0.30$; $P = 0.01$), and plant population ($r^2 = 0.27$; $P = 0.01$). In the SGS, forage yield was related with ear weight (Figure 1; $r^2 = 0.47$; $P < 0.01$) and plant height ($r^2 = 0.31$; $P = 0.02$). A negative correlation occurred with disease index ($r^2 = 0.22$; $P = 0.08$). With respect to NDF concentration, whole-plants had, on average, 47.7 and 51.1% DM for FGS and SGS, respectively. The CI for NDF concentrations were 45.2–50.2% DM and 49.0–53.2% DM for FGS and SGS, respectively. In the FGS, NDF concentration was positively correlated with leaves proportion ($r^2 = 0.20$ $P = 0.03$) and it was negatively correlated with ear proportion ($r^2 = 0.20$; $P = 0.03$). In the SGS, NDF concentration was positively related with plant height ($r^2 = 0.32$; $P = 0.02$).

**Discussion** In Brazil, grain yields have increased an average of 4% per year since 1998 (Conab 2018). Yet recommendations concerning silage making generally have not changed during this time period. Our data for both GS showed that greater silage yield was associated with higher ear dry weight (Figure 1). Dry matter yield was also associated with more plants per area and with larger plants. It seems that maximising DM yield appears to maximise both grain and stover yield. Certainly, exceptions to this relationship might occur at an extremely high plant population (Cusicanqui and Lauer 1999). Regarding GS, there was an average reduction of 38% in DM yield for maize grown in

the SGS than FGS. The growing environment can explain this result, since in the SGS there is a reduction in light incidence, precipitation and temperature (the transition from rainy summer to dry autumn). The chemical composition of the forage was also altered by grain yield. In the FGS, NDF concentration decreased as grain yield increased.

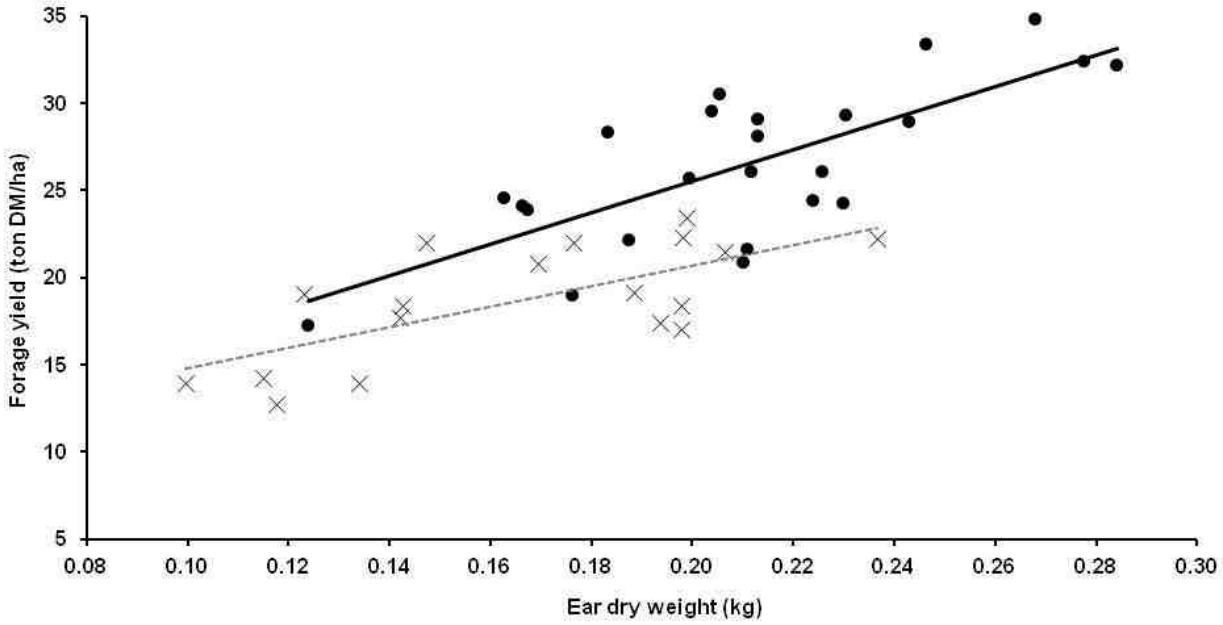


**Figure 1** The relationship between forage yield and ear weight for the first (n = 24; y = 90.656x + 7.3893; r² = 0.56; *P* < 0.001) and second growing season (n = 18; y = 58.821x + 8.9034; r² = 0.47; *P* < 0.01) in South, Southeast, and Midwest of Brazil. First growing season (●); Second growing season (X)

**Conclusions** High maize yields for silage making with intermediate NDF concentration can be obtained by utilising tropical hybrids, especially in the FGS. Available data indicates that selection for high grain yield increases total maize yield. Fibre concentration of silage is lower when grain yield is greater. More data will be collected in 2018 and 2019 for more robust data.

**Acknowledgements** This project was partially funded by Limagrain, Dow AgroSciences, 3r lab, FAPEMIG, and CNPq.

**References**

SAS Institute Inc. (2004) SAS/STAT 9.1 User's Guide. Version 9.1 ed. SAS Inst. Inc., Cary, NC.

Conab (Companhia Nacional de Abastecimento/National Supply Company). (2018) Accessed Mar. 2, 2018. http://www.conab.gov.br/conteudos.php?a=1252&Pagina_objcmsconteudos=3#A_objcmsconteudos.

Cusicanqui, J.A. & Lauer, J.G. (1999) Plant density and hybrid influence on corn forage yield and quality, Agronomy Journal, 91, 911-915.

# An interpretation of gas pressure dynamics based on the observation of multi-parameter during silage production

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**Keywords:** $CO_2$, gas pressure ($\Delta P$), $O_2$, pH, temperature

**Introduction** Silage is an important preservation technique and provides supplemental forage months after harvest (Weinberg and Ashbell 2003). The silage-making process is dominated by anaerobic processes to avoid aerobic deterioration, which can spread quickly and result in spoilage and loss of dry matter and nutrients (Borreani and Tabacco 2014). Fick's law (Pitt 1986, Rees et al. 1983, Ruxton and Gibson 1994) and Darcy's law (McGechan and Williams 1994, Williams et al. 1997) are the major physical mechanisms to describe the $O_2$ transport to the silage interior during ensilage and storage, but which one is the dominant mechanism remains unresolved. The positive pressure ('balloon phenomenon') has been reported previously during silage production, but the fundamental processes and causative factors were not explored (McGechan and Williams 1994, McEniry et al. 2011). The first multi-sensor-based, simultaneous measurements of internal $O_2$, $CO_2$, $\Delta P$, pH, and silage temperature ($T_{si}$) has been made to reveal the previously unknown dynamic interactions of the key parameters governing silage development and quality during the fermentation and storage phases of maize and ryegrass.

**Material and Methods** Table 1 lists general information of all sensors used for this study. Five different principle sensors were used to measure $O_2$, $CO_2$, $\Delta P$, pH, and silage temperature ($T_{si}$) in this study respectively. Two PVC gas-tight cylinders together with a lid which has five access ports to connect the sensor inside and the data logger placed outside were used as the experimental silos. The Test-A was an analogue to the ensiling phase using fresh chopped whole shoots of maize (*Zea mays*) and fresh-cut ryegrass (*Lolium perenne*). Test-B used previously ensiled maize that was obtained from 20 cm behind the freshly cut silage face in a concrete bunker silo and ryegrass that was obtained from a silage bale wrapped in four layers of plastic film.

**Table 1**. Sensor specifications

| Parameter | Sensor-Type | Range/Accuracy | Response (s) |
|---|---|---|---|
| Oxygen | KE-50 | 0–100% (vol.)/ ±2 % of full scale | 60 |
| Carbon Dioxide | Prime 3 | 0–100% (vol.)/ ±3 % of full scale | 60 |
| Pressure | FDA612SR | ±1000 (mb)/ ±0.5% of final value | 1 |
| Temperature | FTA15NiCr-Ni ZA9020FS | -40–160 (°C)/ ±0.1 °C | 2 |
| pH | BlueLine 21 Ph | 2-13/±0.3 | < 60 |

**Results** The testing period consisted of the initial aerobic phase (about 3 h) and the anaerobic fermentation period (about 100 h) in Test-A. During the aerobic period, $O_2$ was rapidly depleted to near zero in each of two fresh materials (maize and ryegrass), the increase in $CO_2$ was minimal. Most of the $CO_2$ was produced during the anaerobic period. The rate of $CO_2$ production was linearly related to pH for two fresh materials. The $\Delta P$ had a modest initial decrease to a negative peak and then increased to the positive values. Piecewise regression analysis between $CO_2$ and $\Delta P$ revealed strong

linear relationships in both phases for maize and ryegrass. An increase in $T_{si}$ was observed in both systems during the aerobic phase. In Test-B, the aerobic period for ryegrass was longer than for maize and the $CO_2$ concentration in both ensiled materials was lower than in the same material in the fresh state. *ΔP* only exhibited negative values throughout the re-ensiled period. The $CO_2$ concentration in the ensiled ryegrass was greater than in the ensiled maize. The simultaneous measurements of $O_2$ and $CO_2$ over time indicate that the respiratory quotient was significantly below 1.0 for both ensiled materials, which means that partial $CO_2$ dissolution in interstitial silage water.

**Discussion** In Test-A, $\Delta P < 0$ was observed over a quite short period for the fresh plant material, and it reflects the sequestration of $CO_2$. The positive *ΔP* indicated that the $CO_2$ production exceeded the capacity for $CO_2$ dissolution, therefore *ΔP* is a potentially useful indicator of $CO_2$. Here we characterize the variation in *ΔP* ($\Delta P = 0$) → ($\Delta P < 0$) → ($\Delta P > 0$) → ($\Delta P < 0$) throughout the silage production process. Aerobic deterioration is unavoidable for a field-scale bunker silo during feed-out phase as $O_2$ penetrates into the silage. With the two ensiled plant materials, Test-B provided an analog of biochemical and physical processes of aerobic respiration and partial dissolution of $CO_2$ in the interstitial silage water. The rigorous vacuum sealing of our test silos allowed *ΔP* to vary over a range of −7.5 to + 45.4 kPa for maize and of −3.1 to 19.1 kPa for ryegrass. Our experiments provide the first evidence of the physical and chemical interactions involved in the production of $CO_2$, its partial dissolution in silage water, and resulting negative and positive pressures, depending on two common plant materials at the early ensiling stage and in the ensiled state.

**Conclusion** Multiple sensors tracking of $O_2$ depletion, $CO_2$ production and dissolution, pH lowering-processes and *ΔP* dynamics has been realized during early and late phases of silage production. The positive and negative *ΔP* values indicated the net values of $CO_2$ production and dissolution. The negative *ΔP* allowed more $O_2$ entry through the silo face, risk of aerobic deterioration in the feed-out phase could be potentially higher than anticipated. These results showed that both gas diffusion (based on Fick's law) and advective transfer (Darcy's law) play equally important roles in processing models of gas transport in silage production. The results may lead to improved production practices and management techniques to reduce costly silage spoilage.

**References**

Borreani, G. & Tabacco, E. (2014) Improving corn silage quality in the top layer of farm bunker silos through the use of a next-generation barrier film with high impermeability to oxygen. Journal of Dairy Science, 97, 2415-2426.

McEniry, J., Forristal, P.D. & O' Kiely, P. (2011) Gas composition of baled grass silage as influenced by the amount, stretch, colour and type of plastic stretch‐film used to wrap the bales, and by the frequency of bale handling. Grass and Forage Science, 66, 277-289.

McGechan, M.B. & Williams, A.G. (1994) A model of air infiltration losses during silage storage. Journal of Agricultural Engineering Research, 57, 237-249.

Pitt, R.E. (1986) Dry matter losses due to oxygen infiltration in silos. Journal of Agricultural Engineering Research, 35, 193-205.

Rees, D.V.H., Audsley, E. & Neale, M.A. (1983) Some physical properties that affect the rate of diffusion of oxygen into silage. The Journal of Agricultural Science, 100, 601-605.

Ruxton, G.D. & Gibson, G.J. (1994) Self-Compaction and aerobic deterioration of bunker silage: a simulation study. Journal of agricultural engineering research, 59, 189-193.

Weinberg, Z.G. & Ashbell, G. (2003) Engineering aspects of ensiling. Biochemical Engineering Journal, 13, 181-188.

Williams, A.G., Hoxey, R.P. & Lowe, J.F. (1997) Changes in temperature and silo gas composition during ensiling, storage and feeding-out grass silage. Grass and Forage Science, 52, 176-189.

# Assessment of on-farm NIRS methodologies for predicting grass silage quality; A comparison of face measurements with cored mixed sample analysis

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**Keywords:** Grass silage, Hand-held NIRS

**Introduction** The measurement of silage quality by laboratory based Near Infra-Red Spectroscopy (NIRS) has become the standard analytical tool for predicting silage quality parameters globally. The development of hand-held NIRS makes routine on-farm prediction of silage quality a realistic option. A number of commercial pieces of equipment are now available. However, the on-farm sampling technique could have a significant effect on the prediction output. Some companies advocate placing the NIRS scanner on the clamp face to obtain a prediction, while others recommend sub sampling and mixing before using NIRS. The current study assessed both methodologies and compared the output predictions from a commercially available hand-held device (NIRS4FARM, Aunir).

**Materials and Methods** Clamp silos, filled with grass silage and open for feed-out on 20 livestock farms in England, were visited in January 2017. Nine points across each clamp face were assessed for silage quality, these were 3 each in a vertical line taken from 0.5 m from the left-hand side wall and right-hand side wall respectively and 3 from the middle of the clamp. At each vertical sampling point the 3 samples were taken at 0.5 m from the top, in the middle and 1 m from the floor. At each sampling point a hand-held NIRS device (NIRS4FARM, Aunir) was held directly on the face and the scan performed. Immediately post scanning and at exactly the same point a core sample to a depth of *ca.* 0.4 m was removed, placed in a plastic bowl, mixed well by hand and re-scanned with the same NIRS device. The NIRS predicted silage analyses were compared.

**Results** The mean, minimum, maximum and $r^2$ regression correlations for key silage analytes are shown in table 1.

**Table 1** Mean, maximum, minimum and $r^2$ values for NIRS predicted results measured directly from the face of the silage clamp or from an exact same cored mixed sample.

| | % DM | | % Digestibility | | Crude Protein % DM | | pH | | Lactic acid g/kg DM | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cored | Face | Cored | Face | Cored | Face | Cored | Face | Cored | Face |
| Mean | 31.27 | 36.38 | 63.78 | 67.48 | 11.77 | 11.97 | 4.13 | 4.11 | 65.43 | 82.15 |
| Min | 18.51 | 15.51 | 49.75 | 49.76 | 5.20 | 6.50 | 3.53 | 3.11 | 0.00 | 0.00 |
| Max | 56.29 | 80.92 | 73.09 | 87.49 | 15.86 | 23.14 | 4.80 | 5.02 | 135.76 | 272.94 |
| $r^2$ | 0.43 | | 0.59 | | 0.22 | | 0.61 | | 0.50 | |

The data indicates that for each of the analytes measured that there was a large variation in predicted values for each parameter. This indicates that the dataset represents a broad range of silage samples and therefore a relevant dataset for the comparative assessment. The regression analyses indicate that the predicted values for the two sampling methods are in poor agreement with each other. Figure 1 shows Bland-Altman plots for % DM, % Digestibility and Crude protein (%DM) content.

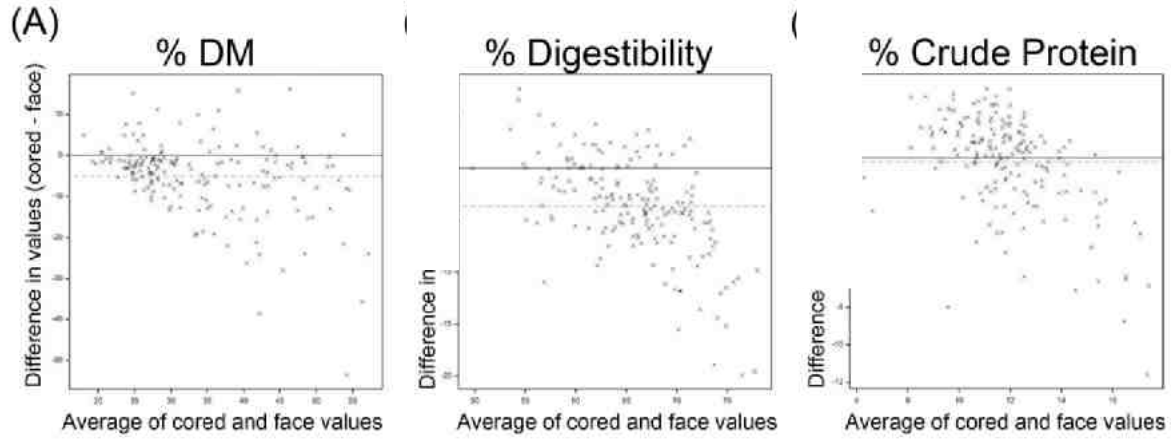


**Figure 1**. Bland-Altman plots comparing face versus cored sampling NIRS analysis prediction of A) %DM B) % Digestibility and C) % Crude Protein (on a % DM basis).The red dotted line indicates the mean difference of all measurements in the data set.

The Bland Altman (Giavarini 2015) plots indicate that although the mean differences indicate that the average discrepancy between the methods might be minimal (figure 1 C) There can still be an underlying bias as seen in all three datasets in figure 1, whereas the average of the measurements increases the differences between the values also increases, indicating that the data becomes less reliable at higher values.

**Discussion** The results clearly indicate that the hand-held NIRS scanning and sampling method can have a major effect on the predicted analytical results. The results indicate differences between face sampling and cored mixed samples. This is not surprising as NIRS can only penetrate at most 5 mm into a sample. The outer 5 mm of a silage clamp face is where most change relative to the bulk of the silage will have occurred due to weather conditions having a dramatic effect on the % DM content of the interface between silage and open air. The data suggests that other analytes are also affected. future research may result in development of a more appropriate prediction database which would enable better prediction of analytes from a face sampling approach. However, it would be preferable, even with on-farm NIRS analyses, to ensure a representative sample of the region of the clamp of interest is used for the analysis.

**Conclusion** In summary, on-farm NIRS prediction analysis, whilst rapid and relatively uncomplicated, must still be used appropriately with consistent sampling methodology otherwise misleading results are likely to be obtained which could have significant affects during ration formulation.

**References**

Giavarini, D. (2015) Understanding Bland Altman analysis. Biochemia Medica, 25,141-51.

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# Baled whole crop wheat silage: Harvesting losses, bale density and silage quality

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**Keywords:** additive, density, harvesting losses, processing, starch

**Introduction** In Norway, whole crop silage is commonly preserved in round bales. At extended processing, comprehensive harvesting losses may be expected from traditional balers, especially with late harvested, dry material. On the contrary, without processing the packing density may be low and vulnerable to spoilage due to poor fermentation (McEniry et al. 2008). However, new machinery facilitating baling of precision chopped material is now available. In a farm scale study three baling strategies were examined in order to document harvesting losses as well as the quality of the resulting silage.

**Material and Methods** The study was performed under commercial farm conditions in Orkdal, Norway (63˚N, 9˚E, 125 m a.s.l.). A mixture of whole crop wheat and grass (75:25) was cut in August at mid/hard dough stage of the wheat (Zadoks 85-87) by a rotational disc mower without conditioning. Three baling methods were objected for examination: a progressive band baler (Kuhn VB2160) operated with (B1) or without (B2) the cutting unit in use (theoretical cutting length with operating knives: 45 mm), and precision chopped material (JF FC850, theoretical chopping length: 20 mm) baled with stationary Orkel MC850 Compactor (B3). In two randomized blocks, four bales from each treatment were produced; two with and two without application of 5 L $ton^{-1}$ of an acidic additive (0.34 formic acid and 0.38 propionic acid). In B1 and B2, a tarpaulin fixed under the baler enabled recording of baling losses. All bales (24) were weighed and core samples collected from every second bale by use of a powered core sampler (40 mm inner diameter, stainless steel) before sealing with 12 polyethylene film layers. After three months of outdoor storage, core samples for chemical analyses were collected from every single bale (24). Water soluble carbohydrates (WSC) were analyzed according to Larsson and Bengtson (1983). All other analyses were performed according to the Nordic feed evaluation system (Åkerlind et al. 2011). All data were analysed statistically using the Proc GLM procedure of SAS 9.4 according to a randomized block design, baling method and acid application considered as fixed effects and block as random.

**Results and discussion** Harvesting losses were generally low in this experiment, however significantly higher when the progressive baler was operated with (B2: 1.4 kg 100 $kg^{-1}$) versus without (B1: 0.23 kg 100 $kg^{-1}$) the cutting unit in work (*P=0.0007*). No significant differences in chemical composition of fresh crop between blocks or treatments were revealed and averaged 325 g DM $kg^{-1}$, 39 g ash, 93 g crude protein (CP), 458 g NDF, 149 g starch and 109 g WSC $kg^{-1}$ DM. Taking the slightly elevated contents of ammonia-N into account, the crop was satisfactory fermented. Bales produced from precision chopped material with the Orkel Compactor (B3) were significantly more dense than bales produced with the progressive band baler (Table 1), and cutting tended to improve the density of bales produced by the latter (*P=0.06*). However, silage quality was either unaffected (B2) or reduced (depressed starch contents and elevated contents of acetic acid, propionic acid and ammonia-N in B3) by intensified processing and density. Results from a small scale experiment reported by Johansen et al. (2018), indicated that intensive processing may induce starch degradation as well as unfavourable fermentation patterns. Thus, it seems likely that the processing ahead of the baling caused the apparent loss of starch and unfavourable fermentation in B3, rather than the high degree of compaction. The overall lower content of starch revealed in samples collected immediately after baling compared to the silage are most probably due to random sampling errors.

The improved density achieved with acid treatment may be an indirect effect of reduced driving speed in order to achieve the targeted acid dosage. A more restricted fermentation with higher concentration of WSC and lower concentrations of lactic acid, acetic acid and ethanol was obtained in acid treated silage compared to untreated silage. In common with the small scale experiment (Johansen et al. 2018) the starch content was unaffected by acid treatment, whereas higher contents of ammonia-N appeared in the acid treated silage in the present experiment. The negative effect of acid treatment on ammonia concentrations was more pronounced in the processed silage in B2 and B3, compared to the unprocessed silage in B1 (*P=0.04*). Similar interactional effects were revealed for lactic acid (*P=0.003*) as well as acetic acid (*P<0.001*).

**Table 1**. Effect of processing and baling method and application of additive on chemical composition and fermentation pattern of whole crop wheat silage (g $kg^{-1}$ DM unless stated otherwise) harvested at medium/hard dough stage.

| | Baling method | | | Additive | | Level of significance | | |
|---|---|---|---|---|---|---|---|---|
| | B1 | B2 | B3 | Acid | No acid | Baling | Acid | Interact. |
| Density, kg $m^{-3}$ | 129 | 135 | 214 | 162 | 156 | *** | ** | NS |
| DM, g $kg^{-1}$ | 360 | 341 | 345 | 353 | 345 | NS | NS | NS |
| Ash | 35a | 38a | 41b | 37 | 39 | * | *P=0.1* | *P=0.1* |
| CP | 92 | 94 | 93 | 95 | 92 | NS | NS | NS |
| NDF | 443 | 439 | 437 | 434 | 446 | NS | NS | NS |
| Starch | 210 | 188 | 145 | 178 | 184 | ** | NS | NS |
| WSC | 47 | 49 | 61 | 73 | 32 | *** | *** | NS |
| pH | 4.2 | 4.1 | 4.2 | 4.2 | 4.1 | NS | NS | NS |
| Lactic acid | 31.8 | 41.2 | 32.5 | 26.3 | 44.0 | * | *** | ** |
| Acetic acid | 5.1 | 6.9 | 8.9 | 4.3 | 9.6 | *** | *** | ** |
| Propionic acid | 1.5 | 2.5 | 4.0 | 5.4 | 0 | *** | *** | *** |
| Formic acid | 1.0 | 1.7 | 3.3 | 4.1 | 0 | ** | *** | ** |
| Ethanol | 14.5 | 11.1 | 4.7 | 5.9 | 14.3 | *** | *** | NS |
| $NH_3$-N, g $kg^{-1}$ N | 92.2 | 90.7 | 127.1 | 115.2 | 91.6 | *** | *** | * |

B1=Band baler without cutting, B2=band baler with cutting, B3=Orkel Compactor, DM=dry matter, CP=crude protein, NDF=neutral detergent fibre, WSC=water soluble carbohydrates, NS=not significant

**Conclusion** It is possible to avoid comprehensive harvesting losses and to achieve satisfactory fermented, late harvested whole crop wheat silage by use of traditional band balers. Application of acid based additive had no significant impact on starch degradation in this experiment, and the effect on fermentation pattern was inconsistent. Possible impacts of the acid application on aerobic stability were not examined.

**References**

Larsson, K. & Bengtsson, S. (1983) Determination of water soluble carbohydrates in plant material (in Swedish). Method 22. National Laboratory for Agricultural Chemistry, Uppsala, Sweden.

McEniry J, O'Kiely, P., Clipson, N.J.W., Forristal, P.D., Doyle, E.M. (2008). The microbiological and chemical composition of silage over the course of fermentation in round bales relative to that of silage made from unchopped and precision-chopped herbage in laboratory silos. Grass and Forage Science. 63, 407-420.

Johansen, A., Randby, Å.T, Bakken, A.K. & Nadeau, E. (2018) Effect of kernel processing and acid treatment on fermentation characteristics and nutritive value of whole crop wheat silage. Paper in this conference.

Zadoks, J.C., Chang, T.T. & Konzak, C.F. (1974) A decimal code for the growth stages of cereals. Weed Research, 14, 415-421.

# Characteristics of *Pediococcus pentosaceus* Q6 isolated from *Elymus nutans* growing on the Tibetan Plateau and its application for silage preparation at low temperature

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**Keywords:** *Elymus nutans,* low temperature, *Pediococcus pentosaceus*, Tibetan Plateau

**Introduction** The frigid climate, heavy ultraviolet rays and short growing period of forage are considered to be the most significant limiting factors for forage yield, which lead to considerable feed shortages especially in winter on the Tibetan Plateau. Ensiling is deemed to be a desirable way to alleviate the seasonal unbalanced feed supply problem. However, the average diurnal temperature at the stage of forage harvest is about 10-15°C on the Tibetan Plateau, and the commercial inoculants can't obtain ideal effect in such harsh environment for making silage. Thus, the purposes of this study were to investigate the characteristics of a strain *Pediococcus pentosaceus* Q6 isolated from *Elymus nutans* growing on the Tibetan Plateau and to evaluate its effects on *Elymus nutans* silage fermentation stored at low temperature.

**Material and Methods** The tested strain *Pediococcus pentosaceus* Q6 was screened based on its performance of growing better in Man Rogosa Sharpe (MRS) medium and distinguished acid production ability at 10°C. Sugar fermentation pattern and growth profiles of strain Q6 and its reference strain *Pediococcus pentosaceus* APP (a commercial silage inoculant) at different temperatures and under different pH environment were characterized. The strain Q6 and APP were inoculated to *Elymus nutans* at ensiling with application rate of $1\times10^5$ cfu/g fresh forage. The untreated *Elymus nutans* was ensiled as control. The grasses (70% moisture) were ensiled at different temperatures (10, 15 and 25°C) for 30, 60 and 90 days in vacuumized polyethylene plastic bags packed with approximately 300 g of fresh forage in each bag. Silos were opened at each sampling time for fermentation profiles and biochemical composition analyses. The mean comparisons were conducted using Duncan's multiple range tests (SPSS 17.0, SPSS, Inc., Chicago, IL). The statistical significance was considered at $P < 0.05$.

**Results and discussion** The strain Q6 could grow at lowest initial pH 3.0 and at 4 °C, but APP just growth slightly at initial pH 3.5 and could not grow at 4 °C. Contrast to the reference strain APP, Q6 could ferment mannitol, saccharose, sorbitol and rhamnose (Table 1). Microorganisms have ability to adapt harsh environment, like some epiphytic LAB strains on *Kobresia littledalei* from the Tibetan Plateau had stronger resistance to temperature, acid and alkali than the strains isolated under conventional conditions (Gao et al. 2013), which were consistent with the present study. Lower pH in Q6-treated silages fermented for 60 days at 10 °C or fermented for 30 and 60 days at 15 °C was found when compared with APP-treated groups (Table 2). Among the three treatments, the greatest lactic acid concentrations were detected in Q6 inoculated silages ensiled for 30 and 60 days at 10 and 15 °C, respectively. Comparable lactic acid was found in Q6 and APP inoculated groups at 25 °C for all ensiled days. These results indicated that strain Q6 could validly promote favorable fermentation process at low temperature. When the fermentation time prolonged to 90 days, there were no differences on pH and lactic acid between Q6 and APP treated silages ensiled at 10 and 15 °C, respectively (data not shown). It is generally considered that *Pediococci*, *Lactococci*, *Enterococci* and *Leuconostocs* are starters of initial silage fermentation and then replaced by more acid-tolerant *Lactobacilli*. The result might be due to the effect of epiphytic competitive LAB, especially *Lactobacilli* dominated the process of ensiling at the late stage of fermentation (after 60 d).

**Table 1**. Characteristics of Q6 and one strain from commercial addition

| NO of strains | Q6 | APP |
|---|---|---|
| Source | lab | Commercial addition |
| Carbohydrate fermentation | | |
| Mannitol | + | — |
| Saccharose | ++ | — |
| Sorbitol | + | — |
| Rhamnose | w | — |
| Growth at pH | | |
| 3.0 | w | w |
| 3.5 | + | w |
| Growth at temperature (°C) | | |
| 4 | w | — |
| 10 | ++ | + |

Q6, *Pediococcus pentosaceus*; APP, *Pediococcus pentosaceus* from a commercial addition; in carbohydrate fermentation: +++, biochemistry kits turning color in 18-24 h ; ++, biochemistry kits turning color in 24-36 h; +, biochemistry kits turning color in 36-72 h; w, biochemistry kits turning color from blue to colourless; — , non-discolouring. In growth at temperature and pH: ++, growing better (OD > 0.5); +, growing (0.05 < OD < 0.1); —, none growing (OD < 0.01); w, growing slightly (0.01 < OD < 0.05).

**Table 2**. Fermentation characteristics of *Elymus nutans* silage for 30 and 60 d

| Strain | Temperature (°C) | 30 d pH | 30 d LA (g/kg DM) | 30 d AA (g/kg DM) | 60 d pH | 60 d LA (g/kg DM) | 60 d AA (g/kg DM) |
|---|---|---|---|---|---|---|---|
| Control | 10 | 6.42a | 5.7e | 63.1a | 6.12a | 23.0d | 29.3ab |
| | 15 | 6.03b | 7.5e | 37.8bc | 5.75b | 19.4d | 13.4c |
| | 25 | 6.08b | 9.1de | 22.2e | 5.82b | 38.7cd | 21.0bc |
| Q6 | 10 | 5.82c | 27.6a | 44.1b | 5.10de | 63.9bc | 28.0ab |
| | 15 | 5.15d | 13.9cd | 28.8cde | 5.00e | 101.1a | 38.6a |
| | 25 | 5.19d | 18.6bc | 26.9de | 5.12de | 85.1ab | 35.1ab |
| APP | 10 | 5.68c | 24.1ab | 36.3bcd | 5.28c | 40.4cd | 21.8bc |
| | 15 | 5.77c | 12.3cde | 29.6cde | 5.28c | 49.3cd | 25.9abc |
| | 25 | 5.34d | 17.2bc | 33.6cd | 5.18cd | 70.3abc | 28.6ab |
| SEM | | 0.022 | 0.850 | 0.981 | 0.016 | 0.351 | 0.146 |
| *P* value | Strain | *** | *** | ** | *** | *** | ** |
| | Temperature | *** | ** | *** | ** | NS | NS |
| | S × T | *** | NS | *** | ** | NS | NS |

DM, Dry Matter; AL, lactic acid; AA, acetic acid; LAB, Lactic acid bacteria; Means within the same line with different letters are significantly different ($P < 0.05$); NS, $P > 0.05$; *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$; SEM, error of the means; S, Strain; T, Temperature; S × T, interaction of S and T.

**Conclusion** *Pediococcus pentosaceus* Q6 could be used as starter for fermentation and improve fermentation quality at the early stage of ensiling stored at 10 °C or 15 °C, as indicated by lower pH value, more lactic acid concentration at lower temperatures. Strain Q6 can be used as a candidate strain for making silages in the Qinghai-Tibet Plateau.

**References**

Gao, J., X. S. Guo & G. C. Wang. (2013) Physiological and biochemical characteristics of lactic acid bacteria isolated from Kobresia littledalei in Tibet. Pratacultural Science 30(8),1266-1271.

# Compaction and particle size distribution of maize (*Zea mays* L.) as affected by dry matter, chop length and intensity of kernel processing

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**Keywords:** compaction, density, maize, particle size

**Introduction** Compaction of maize (*Zea mays* L.) is one main physical factor affecting silage quality and the amount of losses during the feed out at the open silo face of clamp silos. Maize silages with high contents of starch and sugar are frequently affected by heating due to intrusion of oxygen and the following microbial activity after opening the stock. Effective compaction at the filling process is necessary to limit air infiltration during the subsequent feed out period. Consequently, effective compaction is necessary to obtain maximum forage quality (Muck 2001). Main physical determinants affecting silage compaction are chop length, dry matter content (DM) and corn processing. Therefore, the present study aims to quantify the effects of chopping length, DM content and corn processing to the compaction behaviour. The differences in crop compactability in order to the above named factors were determined experimentally in 2016.

**Materials and Methods** Maize variety LG 30211 (SZ 210) was grown at the federal research station at Futterkamp Northern Germany (Schleswig-Holstein) and harvested successively at 4 stages of maturity from 30%DM at the first stage to around 40%DM at the latest stage using a self-propelled harvester (John Deere 8500i). Variation in chopping length (c.l.) was adjusted in 10 steps (3-5-8-11-14-17-20-23-26 and 29mm). All tests were done using two different roller designs (reversed saw tooth and reversed saw tooth with spiral groove) mounted in the same kernel processor housing. The ability for compaction was investigated by compacting 40 kg in 10 kg steps using an electro hydraulic press (Jungbluth et al. 2016) with 4 replications. The compaction pressure at the crop was adjusted at 0.2 MPa. The layer thickness in the bucket was between 25 and 30 cm. After filling and compacting the last 10 kg, the crop volume was measured. Particle size distribution was determined by sieving the dry sample with an electric separator from 3-6-10-15-20 and 25mm mesh diameter according to Leurs (2006). The particle mass in the different classes gives an overview about the structure of the silage crop after chopping and kernel processing.

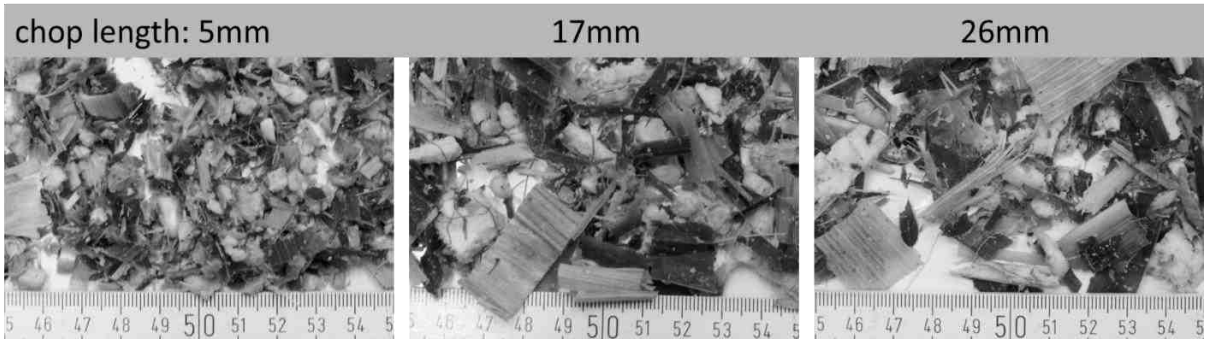


**Figure. 1.** Fresh maize after harvest at different chop length, k.p. reversed sawtooth, 40% differential speed

**Results** The maximum compaction were achieved at c.l. of 3 and 5 mm (210 kg DM $m^{-3}$ at 30% DM and 255 kg DM $m^{-3}$ at 40% DM). Increased c.l. of 8, 11, 14 and 17 mm lead to a nearly linear decrease of bulk density to 185 kg DM $m^{-3}$ at 30% DM and 225 kg DM $m^{-3}$ at 40% DM.

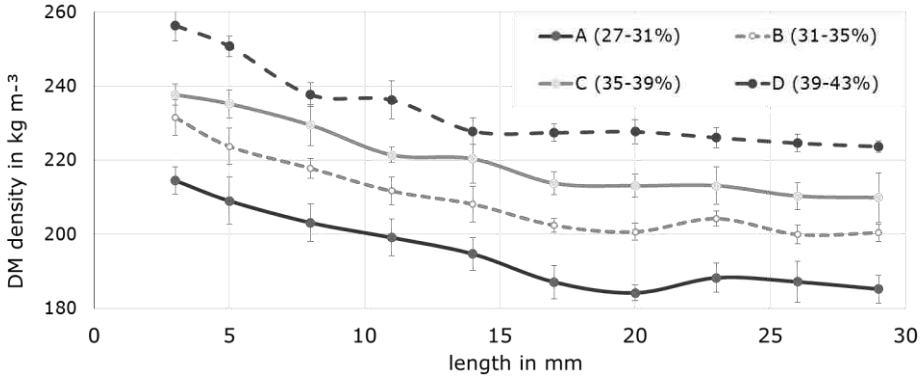


**Figure. 2.** DM Density in different DM levels on chop length from 3mm to 29mm (n=6)

No differences in compaction were observed at longer c.l. 20, 23, 26, and 29 mm. The density stayed at a similar level compared to 17mm c.l. The different kernel processors did not show a significant effect on the compaction. The sieving test showed the shift of weight percent between the size range of crop material according to the adjusted c.l.. At 5 mm, c.l. 50% of the crop mass passed the 6 mm and 90% the 10mm sieve. In case of long chopping length (23 and 29mm), 35% passed the 6mm and around 60% the 10mm sieve. The effect of 50% compared to 40% differential speed at the processor roles can be observed in the mass fraction <3mm. More kernel is totally grinded. The weight percent <3mm is about 2% higher across all c.l. Visual there is although to observe more fan out of bigger leaf and stem particles, but this has no effect in the test method of sieving.

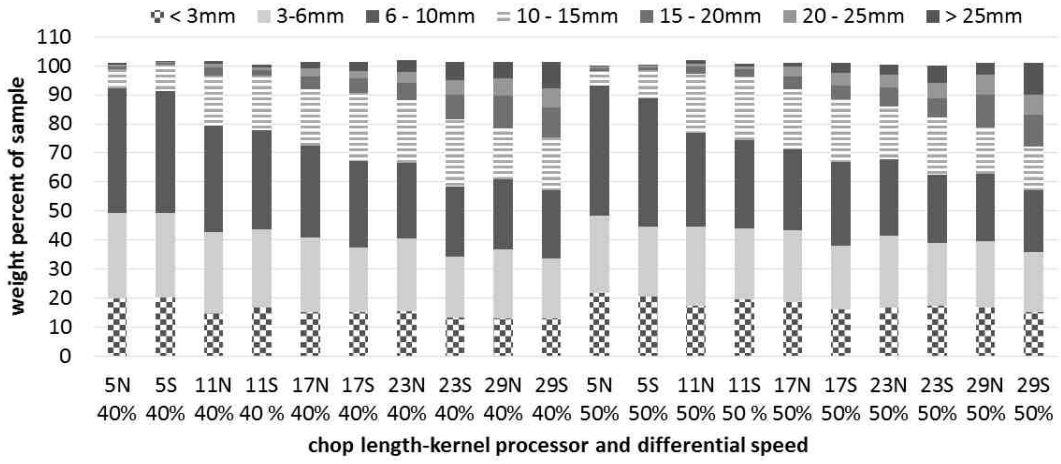


**Figure 3.** Weight percent of size range (<3- >25mm) according to chop length (5-29mm) kernel processor(N= reversed sawtooth, S= reversed sawtooth with spiral groove) at differential speed of 40% and 50%.

Conclusions: Dry matter and c.l. had a evident effect on crop compaction of maize. The different roller design had no effect on crop compaction and particle sizes in this test. The weight percent in the different? Size range shifted depending on c.l. The change of differential speed (40%-50%) caused small differences in the class <3mm.

**References:**

Muck, R.E. & Holmes, B.J. (2001) Density and Losses in Pressed Bag Silos. 2001 ASAE Annual International Meeting, Paper Number 01-1091, Sacramento.

# Comparison of whole crop triticale-pea, triticale-grass and triticale-oat blends as forage sources at six different phenological stages

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**Keywords:** oat, pea, ryegrass, triticale

**Introduction** The renaissance of winter whole crop cereals is obvious in dry continental regions of Europe due to the climate change. Triticale-based mixtures may be alternative forages complimenting maize silage for lactating dairy cows (harvested at an early stage of maturity) and heifers (at milky-waxy stage of the cereals). Therefore, we compared yield, nutrient content and digestibility of whole crop triticale (T, variety: Hungaro), triticale with pea (TP, pea variety: Szarvasi Alíz), triticale with Italian ryegrass (TG, *Lolium multiflorum*, grass variety Suxyl) and triticale with winter oat (TO, oat variety GK Impala) mixtures harvested at 6 different phenological stages.

**Materials and Methods** Experimental field was 360 $m^2$ (plot size 22,5 $m^2$ per replicate n=4, treatments= 4,). Sowing was carried out on 28 October 2016 ato Szent István University farm, Tessedik Campus, Szarvas (N 46°52'35,4'', E 20°31'38,1''). Plots were fertilized with 300 kg/ha NPK before sowing and 51 kg ammonium-nitrate in February. Sowing quantities of the species in each of the four treatments were as follows: T 210 kg/ha; TP 105 kg/ha T and 40 kg/ha P; TG 84 kg/ha T and 18 kg/ha G; TO 105 kg/ha T and 60 kg/ha O). BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale was used to identify the phenological development stages of the triticale. Yield measurements were carried out at 6 stages, at boot stage (4-6 cm head in boot - 26 April, BBCH scale code for cereal: 45 ) before heading (6-10 cm head in boot – 3 May, BBCH code: 49), at early heading (10 May, BBCH code: 51), late heading (17 May, BBCH code: 58), at milky stage (24 May, BBCH code: 74), and at early dough stage (5 June, BBCH code: 83). Cut was carried out by hand at 10 cm above ground level (randomized sampling with a quadrat : 1 $m^2$, n=4), and fresh and dry matter yield was determined. Nutritional composition and the in vitro digestibility of the treatments were determined in fresh samples at two harvesting stages (BBCH code 47 and BBCH code 83) using Near-Infra Red Spectroscopy.

**Results** There were no significant differences in dry matter yield among the treatments (Table 1) before heading (6-10 cm head in boot – 3 May 2017, BBCH code 49). The T and TO had higher dry matter yield (T DM yield 20.2 ± 1.7 Mg/ha; TO DM yield 19.8 ± 0.4 Mg/ha) compared to TP and TG (TP DM yield 15.6 ± 1.9 Mg/ha; TG DM yield 14.2 ± 2.5 Mg/ha) in early waxy stage (5 June 2017). Winter oat had no effect on yield, nutrient content, or digestibility of the mixture (TO) compared to the triticale (T) at this growth stage (Table 2). Pea reduced yield (TP), while there was no effect on nutrient content (except crude protein) or digestibility of the mixture compared to T at late cut. Otherwise, even the TP crude protein content was rather poor at this stage (86.8 ± 6.8 g/kg DM). *Brown et al. (2018)* have found negative effect, as legumes (crimson clover and hairy vetch) reduced the fiber digestibility and sugar content of winter crops (barley, ryegrass, rye, triticale and wheat) grown in monoculture.

**Table 1.** Dry matter yield of triticale (T), mixtures of triticale and winter pea (TP), triticale and Italian ryegrass (TG), triticale and winter oat (TO) harvested at different phenological stages.

| Sampling | | | T | TP | TG | TO |
|---|---|---|---|---|---|---|
| Code | Date | Phenological stage | *ton DM/ha* | | | |
| BCCH 45 | 26.04.2017. | 4-6 cm head in boot | 5.95 | 4.98 | 5.25 | 4.71 |
| BCCH 49 | 03.05.2017. | 6-10 cm head in boot | 7.52B | 6.24B | 7.13B | 6.78B |
| BCCH 51 | 10.05.2017. | early heading | 10.15C | 8.45C | 9.44C | 9.00C |
| BCCH 58 | 17.05.2017. | late heading | 10.87C | 10.57D | 10.70D | 10.37D |
| BCCH 74 | 24.05.2017. | milky stage | 10.40C | 11.70D | 11.93E | 13.12E |
| BCCH 83 | 05.06.2017. | early dough stage | 20.20aD | 15.60bE | 14.20bF | 19.83aF |

a-c Values with different letters within a row differ statistically ($P < 0.05$).
A-E Values with different capital letters within a coloumn differ statistically ($P < 0.05$).

**Table 2.** Nutritional composition (DM basis) and digestibility of fresh forage from triticale (T), mixtures of triticale and winter pea (TP), triticale and Italian ryegrass (TG), triticale and winter oat (TO) harvested at phenological stages BCCH49 and BCCH83.

| g/kg DM, signed in other cases | T | | TP | | TG | | TO | |
|---|---|---|---|---|---|---|---|---|
| | *BCCH 49* | *BCCH 83* | *BCCH 47* | *BCCH 83* | *BCCH 47* | *BCCH 83* | *BCCH 47* | *BCCH 83* |
| Dry matter, g/kg | 149.7A | 414B | 148.3A | 381.3B | 154A | 406.8B | 150.5A | 396B |
| Crude protein | 160.0A | 47.3aB | 158.8A | 86.8dB | 144.3A | 59.5bB | 143.3A | 67.8cB |
| Digestible protein | 86.7dA | 50.3aB | 75.3cA | 48.8abB | 56.3bA | 47.8aB | 54.5aA | 53.5acA |
| Soluble protein | 41.3aA | 23.7aB | 53.3bA | 42.3dB | 50.8bA | 28.4bB | 51.3bA | 36.3cB |
| Total sugar | 123.0aA | 117.8cA | 123.3aA | 84aB | 144.3aA | 103.3bB | 128.8aA | 103.5bB |
| Starch | | 192.5c | | 144.8a | | 143.5a | | 162.8b |
| Crude fiber | 276.7bA | 238.5aB | 270.8bA | 261.5bA | 257.5aA | 263.8bA | 260.5aA | 243.3aA |
| NDF | 584bA | 515.5aB | 579.3bA | 550.3bA | 548.5aA | 566.5bA | 550.3aA | 525.8aA |
| ADF | 298.3aA | 274.0aA | 310.8aA | 313.0bA | 309.3aA | 313.5bA | 313.5aA | 287aB |
| ADL | 23aA | 34.3bB | 25.3bA | 35.8cB | 23.8aA | 36.5cB | 23.5aA | 32.3aB |
| $NDFd_{48,}$% | 73.8bA | 49aB | 70.7aA | 50.4aB | 71.0aA | 49.7aB | 69.1aA | 48.9aB |
| $dNDF_{48}$ | 430.9c | 252.5aB | 409.4bA | 278.9aB | 389.1aA | 281.3aB | 386.4aA | 257.3aB |

a-c Values with different letters within a row (mixtures) differ statistically ($P < 0.05$).
A-C Values with different capital letters within a row (phenological stage) differ statistically ($P < 0.05$).
$NDFd_{48}$: *in vitro* digestibility, 48 hours incubation (NIR), OMD *in vitro* organic matter digestibility (NIR)

**Conclusions** We found no benefit in mixes of winter oat, Italian ryegrass or pea, respectively, compared to triticale grown in monoculture at an early phonological stage. The mixture with pea (TP) had a negative effect on yield, $NDFd_{48}$, and increased the lignin content at the late phenotype stage compared to the triticale (T) grown alone. Further investigations are needed with more varieties and seed-ratio combinations.

**References**

Brown. A.N., Ferreira., G., Teets. C.L., Thomason. W.E. & Teutsch, C.D. (2018) Nutritional composition and in vitro digestibility of grass and legume winter (cover) crops. J. Dairy Sci. 101:1-11.

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# Construction and calibration of a hand penetrometer to estimate crop density at the silo face

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**Keywords:** compaction, maize Silage, particle size, penetration resistance

## Introduction

Crop density is an important factor affecting silage quality and the amount of losses in bunker silos. Especially the aerobic activity of yeasts and the following heating of the silage during feed out depends on the achieved density (kg DM/m²). Investigations of many silos show reheating mostly not to be a problem at the complete silo face. Measuring of density shows mostly a relation between areas of low density and higher temperature of the silo face. High energy silages with a high DM content are more affected than wet silages of lower energy level. Quick density measuring at the silo face to estimate the risk of reheating is mostly done by sampling of cores with a constant volume. In practice the measurement error is relatively high and to do many sampling points is time consuming work. Measuring of penetration resistance using a standardized apex is a different method to estimate crop density. Electric driven penetrometer with mapping of force along the penetration way provides good results but needs costly equipment. (Menghua et al. 2016)

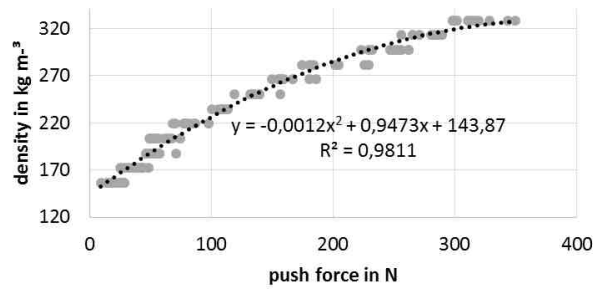
**Materials and Methods** Aim of the development was to construct a simple usable penetrometer and calibrate it first for maize silage of different crop properties in a test study. The measuring tool consists of a standard penetrometer shaft, a cylinder to integrate the changeable spring and a push bar with mm scale.

**Figure 4**. Hand pushed penetrometer with changeable spring and standard cone


The user pushes at the handle and drives the penetrometer slowly into the silo face. While pressing, the spring inside is compressed according to the hand force, and a collar is shifted on the mm scale. The position of the collar shows the maximal deflection of the spring. Using the spring rate, the max. force can be red in a table. To estimate the real bulk density, calibration tests with maize silages of different DM and chop lengths, and steps of density, were made in a test study. After statistical analyses functions to rate the density were derived. Following, the penetrometer was tested at silos in practice, and the calculated values of density compared to density data of a 104 mm core bit sampler.

**Figure 2.** Hydraulic press to compact silage in a120 l bucket, (Test volume 70 l)

**Results** The filling and compacting in the bucket provides an exact and repeatable adjustment of crop density to calibrate the penetrometer. The calibration of the penetrometer in different maize silages showed the crop properties to be an affecting factor additionally to the compaction. Therefore, it was necessary to calculate crop density with different functions according to the crop properties DM and chop length.




| Maize | DM (%) | c.l. (mm) | Polynom | R² |
|---|---|---|---|---|
| 1 | 36,1 | 10,5 | y = -0,001x² + 0,8783x + 128,96 | 0,9739 |
| 2 | 34,97 | 6,5 | y = -0,0038x² + 1,5636x + 159,11 | 0,9792 |
| 3 | 40,6 | 6,5 | y = -0,0007x² + 0,6865x + 153,96 | 0,9695 |
| 4 | 39,5 | 6 | y = -0,001x² + 0,7769x + 155,67 | 0,9679 |
| 5 | 39,7 | 10 | y = -0,0005x² + 0,5893x + 142,79 | 0,9776 |
| 6 | 41,8 | 6 | y = -0,0009x² + 0,7326x + 158,51 | 0,972 |
| 7 | 31,3 | 11,5 | y = -0,0012x² + 0,9473x + 143,87 | 0,9811 |
| 8 | 34 | 7,5 | y = -0,0007x² + 0,7009x + 157,59 | 0,9727 |

**Figure 3.** left: Example Calibration of penetrometer in Maize and Polynom fitting, right: Polynom fitting of 8 different Maize silages (N=10)

In a range from 200-300 kg DM/m³ the relative error compared to the core sampling was between 1 and 10%. In silage of very low density a softer spring has to be used because of the evidently lower penetration force. The measuring in uncompacted silage at the top leads to higher differences in repetition. The accuracy of the measured absolute values depends a lot on the comparability of the crop used in the calibration. If it fits well to the actual measured crop, the calculated density by the push force on the handle will be close to the real crop density. Obviously incorrect measurements have to be stopped and repeated.

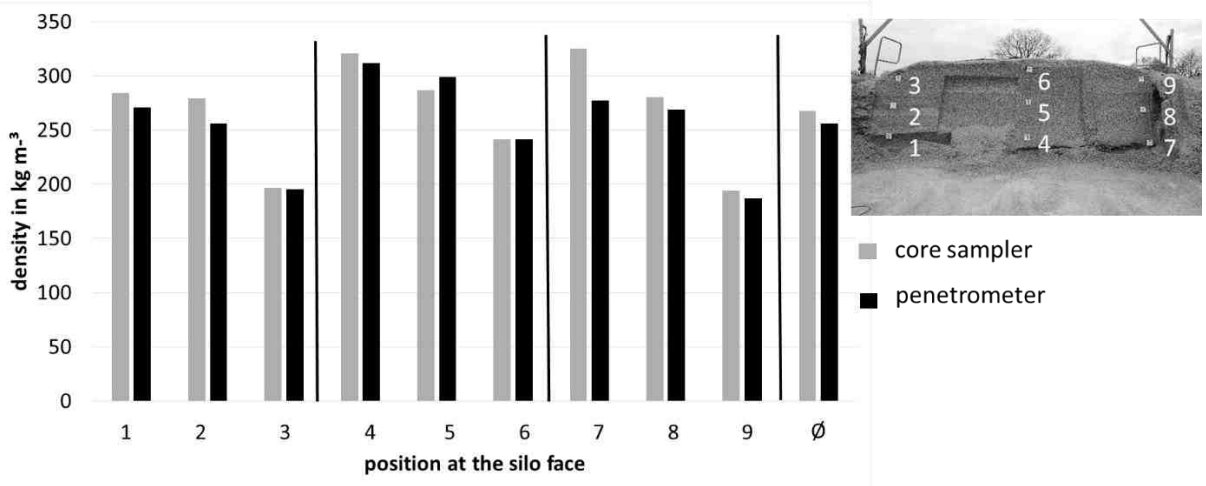



**Figure 4.** Comparison of density measured at a clamp silo face of Maize (7mm chop length, 34% DM). Ø: mean of all samples.

**Discussion** The method offers the opportunity of quick density measurement with a quite simple tool and nearly no additional equipment like it is necessary for core bit samplers. To give advice to improve crop compaction and silage quality in practice, a detailed measuring at the actual open silo is a good start.

**Conclusions** The developed hand penetrometer is an alternative tool to estimate bulk density in clamp silos. A comprehensive calibration considering the factors chop length and DM is necessary to get accurate results. The user has to make sure that his actual crop to is similar to one of the calibration set. The calibration data will be further improved in the next maize harvest.

**References**

Menghua, L. (2016) Developing a Penetrometer-Based Mapping System for Visualizing Silage Bulk Density from the Bunker Silo Face, Sensors (ISSN 1424-8220; CODEN: SENSC9).

# Determination of water-soluble carbohydrates in forages – comparison of methods

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**Keywords:** Carbohydrates, forages, water-soluble carbohydrates

**Introduction** The determination of fermentable water-soluble carbohydrates (Wsc) provides important information for the evaluation of the fermentability of forages. Carbohydrates comprise a variety of sugars, mainly lower-molecular carbohydrates, whereby mono-, di- and oligosaccharides, as well as fructans represent the major compounds of Wsc. They can be analysed by colorimetric methods, whereas single sugars (Ss) and fructans (Fruc) are usually detected by chromatography (mainly high performance liquid chromatography (HPLC)), and reducing sugars by redox/gravimetric methods. In this study, comparative analyses were carried out using different forages and methods. HPLC coupled with refraction index (RI) detection was employed to measure Ss and inulin-type Fruc. Values obtained by employing an automated colorimetric method with anthrone as reagent were compared with those detected by the redox/gravimetric method according to VDLUFA (method 7.1.3., 2012).

**Materials and Methods** A total of 135 samples of forages (cereals n=4, grass n=62, grass-legume-mixtures (GLM) n=11, tall fescues n=58) and 18 of sugar beet pulp (SBP), were freeze-dried, milled through a 1 mm sieve and analysed for Wsc with the following three methods:

1. Reducing sugars (S_reduc) according to VDLUFA 7.1.3. (2012): Aqueous extraction of 20 g in 350 mL water; clarification with Carrez reagent; hydrolysis with diluted hydrochloric acid ($H_2O$: 37% HCl =1:5) at 70°C; oxidation of reducing sugars with Fehling`s solution; gravimetric analysis of copper-I-oxide.
2. Wsc with anthrone (Wsc_A) according to Lengerken und Zimmermann (1991) and Thomas (1977): Aqueous extraction of 0.5 g in 100 mL water; clarification with Carrez reagent, hydrolysis with concentrated sulphuric acid (88%, $\rho$ 20 °c = 1,7415) and automated colorimetric analysis (both at 97°C) of blue-green furfurol derivates with continuous flow analyser (CFA San, Skalar Analytical, Breda, Netherlands).
3. Ss with HPLC according to Weiß and Alt (2017): Aqueous extraction of 0.5 g in 100 mL water; determination of glucose (Glu), fructose (Fru), sucrose (Suc), fructans as inulin (Fruc), lactose (Lac), xylose (Xyl), galactose (Gal), mannitol (Man), maltose (Mal), arabinose (Ara), ribose (Rib), stachyose or raffinose

Statistical analyses were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). The CORR and REG procedures were employed to evaluate the relationships between different methods by the simple coefficient of determination using regression analysis.

**Results and discussion** In all plant materials, the maximum amounts of Wsc_A were higher than the S_reduc and sum of Ss. Fructans were found in grass, tall fescue and, to a lesser extent, in GLM. Glu and Fru were detected in all forages, whereas Suc, Lac and Xyl were only present in grass and GLM. No tested forages contained Mal, Ara, Rib, stachyose or raffinose. The colorimetric analyses of Wsc_A in SBP showed a very high correlation ($R^2$= 0.96) with the sum of Ss measured with HPLC (table 1). The Wsc_A concentrations were higher than the sum of Ss including fructans and S_reduc, especially in grass, which was reflected by slopes of 1.40 and 1.51, respectively. This may be explained by strong acid hydrolysis with sulfuric acid of oligosaccharides and fructans in the Wsc_A analysis to release monomer sugar which can also form furfurol derivatives. The method S_reduc includes hydrolysis but with diluted acid. It can also be assumed that hydrolysis was not complete so

that not all polysaccharides were recorded. Furthermore, the calculation of S_reduc implies errors due to no clear stoichiometric reduction, especially concerning pentoses. A strong correlation was found in all tested materials between Wsc concentrations determined by redox method (S_reduc) with added fructans of the inulin-type (Ss) and by the anthrone method (Wsc_A) (figure 1 and 2).

**Table 1.** Correlations between the concentrations of Wsc_A and the sum of Ss and Wsc_A and S_reduc, respectively, as affected by forage

| Parameter | Grass (n=62) | | GLM (n=11) | | Tall fescue (n=58) | | SBP (n=18) | |
|---|---|---|---|---|---|---|---|---|
| | Ss | S_reduc | Ss | S_reduc | Ss | S_reduc | Ss | S_reduc |
| a | 1.40 | 1.51 | 0.63 | 0.99 | 1.27 | 1.36 | 1.08 | 1.36 |
| b | -2.53 | 2.27 | 16.75 | 26.06 | 46.55 | 29.82 | 21.25 | 24.14 |
| $R^2$ | 0.77 | 0.97 | 0.73 | 0.97 | 0.88 | 0.92 | 0.96 | 0.98 |
| *P* | *** | *** | ** | *** | *** | *** | *** | *** |

Wsc_A detected by anthrone/CFA, Ss determined by HPLC, S_reduc measured by redox/gravimetric method, Y=Wsc_A, f (Wsc_A) = ax+b, *** *P*<.0001, ***P*<.001

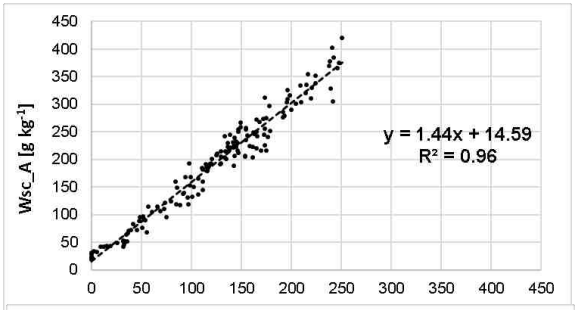


**Figure 1.** Correlations between Wsc_A determined by anthrone and S_reduce analysed by redox/gravimetric methods, n=153.

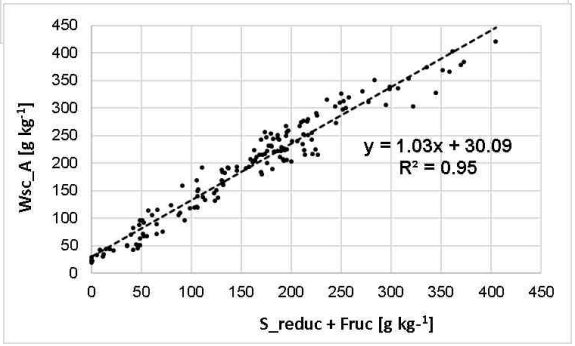


**Figure 2.** Correlations between Wsc_A determined by anthrone and S_reduce analysed by redoc/gravimetric method with additional fructans determined by HPLC, n=153.

**Conclusions** The employed methods differed regarding analytical principle, reaction and hydrolysis conditions. The concentrations of colorimetrically detected Wsc were higher than those measured by the redox/gravimetric method, and an effect of the analysed material was observed. As the determination of fermentable carbohydrates to evaluate the fermentability of forages is, historically, based on the colorimetric anthrone-method, which also detects water-soluble fructans, these should generally be analysed and added to the concentrations obtained by the redox/gravimetric method. This, in turn, would give a very good estimate of the total Wsc in forages.

**References**

VDLUFA (2012) Die chemische Untersuchung von Futtermitteln. Methodenbuch, Vol. III. Kapitel 7.1.3. Darmstadt: VDLUFA-Verlag.

Lengerken, von J. & Zimmermann, K. (1991) Handbuch Futtermittelprüfung. Berlin: Deutscher Landwirtschaftsverlag. p. 260-266.

Thomas, T.A. (1977) An automated procedure for the determination of soluble carbohydrates in herbage. Journal of the Science of Food and Agriculture, 28, 639-642.

Weiß, K. & Alt, M. (2017) Determination of Single Sugars, Including Inulin, in Plants and Feed Materials by High-Performance Liquid Chromatography and Refraction Index Detection. Fermentation, 3 (3), 36; doi:10.3390/fermentation3030036.

# Development of calibrations for hand-held NIRs instrumentation to measure silage density from the open face of grass silage clamps

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**Keywords:** Grass, silage density, Hand-held NIRs

**Introduction** Silage density is a major factor affecting the quality of silage preservation and nutrient content. Variability across the clamp can be huge and the ability to measure the variability within and between silage clamps rapidly would aid in the understanding of management factors affecting silage density and the relationship with silage quality. Laboratory based Near Infra-Red Spectroscopy has become the standard analytical tool for predicting silage nutritional quality parameters globally. The development of hand-held NIRS makes on farm prediction of silage quality a step closer, however the technique has the potential to predict other markers of silage quality such as density. The present study was conducted to assess the efficacy of hand-held NIRS to predict silage density on the open faces of silage clamps in England.

**Materials and Methods** Bunker silos, filled with grass silage and open for feed-out on 20 livestock farms in England, were visited in January 2017. Nine points across each clamp face were assessed for density, these were 3 each in a vertical line taken from 0.5 m from the left hand side wall and right hand side wall respectively and 3 from the middle of the clamp. At each vertical sampling point the 3 samples were taken at 0.5 m from the top, in the middle and 1 m from the floor. At each sampling point a hand-held NIRs device (NIRS4FARM, Aunir) was held directly on the face and the scan performed. Immediately post scanning and at exactly the same point a core sample was removed, the density was calculated by measuring the fresh weight of silage removed in the corer and the volume of the core calculated from a measurement of diameter and depth of the hole (Ruppel et al. 1995). Subsequently a dry matter determination was conducted on each sample to convert fresh matter density to dry matter density. The hand held NIR4farm instrument provides an absorbance spectrum covering 950-1650nm. The spectral and chemical data was subjected to chemometric modelling to produce a calibration equation. The spectral data was normalised using a Standard Normal Variate algorithm and the spectra were then derivatised before modelling.

**Results** Measured values for Fresh and Dry matter densities and volatile corrected oven dry matter are shown in Table 1.

**Table 1**. Showing fresh matter (FM) and dry matter density (DM) and DM

| | FM Density kg FM/$m^3$ | DM* g $kg^{-1}$ FM | DM Density Kg DM/$m^3$ |
|---|---|---|---|
| mean | 613 | 300 | 177 |
| min | 54 | 141 | 19 |
| max | 1619 | 549 | 587 |
| range | 1565 | 408 | 568 |

*DM = Volatile corrected dry matter was used as in this study a wide variation in % DM were assessed with very different fermentation products thus the correction for volatile losses during drying were important.

Large variation in density and DM was measured on the farms, thus providing a good data set for the initial establishment of a NIRS prediction database. In addition large within farm variation was also observed as shown for fresh matter observed density in figure 1. Figure 1 shows the variation in

density for all 20 farms (F1-F20). At each of the 9 sampling sites density was measured. The graph indicates the mean values of the three right hand side, three left hand side, three top and three vertically central samples. Farm 9 was the largest clamp in the study hence the central sample density is partly a result of the settling effect from the weight of silage above it. Interestingly F1 used a self-loading wagon to harvest the silage and overall had the lowest density variation and the highest mean density across all samples.

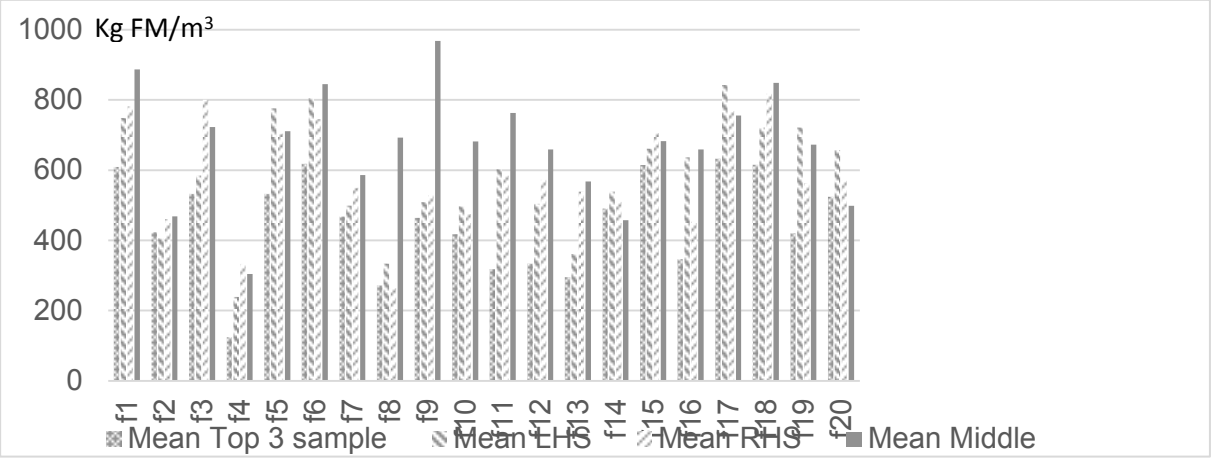


**Figure 1.** Variation in fresh matter density across clamps

For Fresh Matter density a NIRS calibration with an $r^2$ = 0.63 and Standard error of calibrations = 140 was established.

**Discussion** The data collected on density in grass silage clamps indicates that there is a wide variation both between farms but also within farms. The data in Figure 1 clearly indicates where the points of lower density are within the bunker on each individual farm and thus the position where silage feed value could be compromised. The accuracy of the NIRS silage density prediction needs to be evaluated and updated further by conducting more determinations on farms and assessing the accuracy of the prediction against measured values.

**Conclusions** Rapid methodologies to measure silage density at multiple sites across a single clamp face will enable better silage filling and removal, and could improve feeding decisions to be made on farm. The ability of farmers to rapidly assess silage density on multiple sites across the clamp face will enable them to visualise issues, connect the issues of poorer density with other silage quality parameters, such as spoiled or heating silage. As a result of the information provided they will be better placed to introduce management practices during future silage harvests to reduce variability and potentially issues associated with poor compaction density such as aerobic spoilage and mycotoxin risk. On farm NIRS methodologies have the potential to offer this solution.

**References**

Ruppel, K.A., Pitt, R.E., Chase, L.E., & Galton, D.M. (1995) Bunker silo management and its relationship to forage preservation on dairy farms. Journal of Dairy Science, 78,141-153.

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# Dry matter losses and nutrient changes in grass and maize silages stored in bunker silos

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**Keywords:** dry matter losses, grass, maize, nutrient changes, silage

**Introduction** Agricultural production faces the challenge of a good balance of economy in compliance with ecosystem services. Knowledge about the complexity of mass and nutrient flows within the dairy farm is evident in order to enhance nutrient use efficiency of the system (Misselbrook et al. 2013). Particularly the feed management offers substantial reserves for optimisation (Wilkinson 2011). To reduce losses and improve the forage use efficiency, mass and nutrient flows in the silage making system have to be measured from field to bunk. The aim of our study was to quantify the dry matter (DM) losses and the changes of nutrient contents of silages related to the figures measured before ensiling in order to identify deficits in silage production on farm scale.

**Material and Methods** The mass flow and the changes of nutrient contents from ensiling to removal were examined from silages on three experimental farms of the Bavarian State Research Centre for Agriculture (LfL), Germany. The treatments included silages from permanent grassland, maize and additionally on individual farms from grass-clover mixtures and annual ryegrass, respectively. In total 32 grass, 25 maize and 7 “sandwich” silages were conserved in side walled bunker silos on farm scale with fresh forage weights from 37 to 897 tonnes per silo as filled. Sandwich practice means to ensile grass and maize layer one upon the other. Procedures of silage making were in line with the good practice standards as described by DLG (2011). For determination of DM losses, the total-in vs. total-out method was applied according to Köhler et al. (2013). Wilted material was sampled during ensiling on the one hand and the silage material during removal time on the other hand and both materials were analysed for chemical composition and preservation quality parameters as described according to Köhler et al. (2013). The nutrient contents from ensiled and silage materials were pooled and paired to each silo. Data analysis was carried out with the statistic software R. Forage species (grass; maize) and conservation status (ensiled; silage) were determined as fix factors, while years (2008 to 2011) and farms were set random. The changes of nutrient contents and feeding values were analysed using ANOVA and the means across groups were compared with multiple contrast tests.

**Results and Discussion** All farms realised a silage production management that resulted in good fermentation qualities according to Spiekers et al. (2009). DM losses ($DM_{total\ in\text{-}total\ out}$) due to conservation status showed a trend towards losses during the silage process, however, these losses of around 9% for grass and 7% for maize were not statistically significant (figure 1). The chemical components and the feeding value (dry matter (DM), crude protein (CP), phosphorus (P), acid detergent fibre expressed exclusive of residual ash (ADFom), neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash (aNDFom) and net energy for lactation (NEL) contents) of grass and maize crops differed significantly. The DM contents of grass silages – which commonly have wide ranges – are characterized by a stronger reduction of organic matter (OM)

during the silage process. On the other hand, the fermentation does not significantly affect that material in terms of DM contents. As grass silages contain higher proportions of volatile N-components from non-protein N (NPN)-compounds it is logical that crude protein is degraded more strongly in grass compared to maize silages ending up in a tendency towards reduced figures for CP in grass silages. During the ensiling process of maize on the other hand fermentation intensifies the degradation of N-free components slightly which results in a tendency towards an increase of the crude protein contents in the silages. Phosphorus contents are affected by forage species, but not by conservation status. Interesting results are revealed when ADFom and aNDFom figures were compared for wilted material on the one hand and silages on the other hand. For both forages a significant reduction in aNDFom contents is evident, while ADFom is not affected by the ensiling process. This considerable reduction of the aNDFom contents is obviously related to the depletion of hemicellulose, which is triggered by weak organic acids. The same effect regarding reduced aNDFom contents but stable ADFom figures has been described by Nadeau et al. (2000). In the end this process might cause a compensatory effect regarding the breakdown of sugars (reduction of OM digestibility) resulting in stable figures for NEL before and after ensiling in grasses, while an overcompensation for maize (higher NEL values in silages) can be expected. This stronger aNDFom degradation explains the significant increase of the energy value of maize silage for milk production. We conclude that compared to wilted material best practise in silage making ends up in poor DM losses and stable values for CP and NEL contents, respectively and thus best silages for feeding dairy cows.

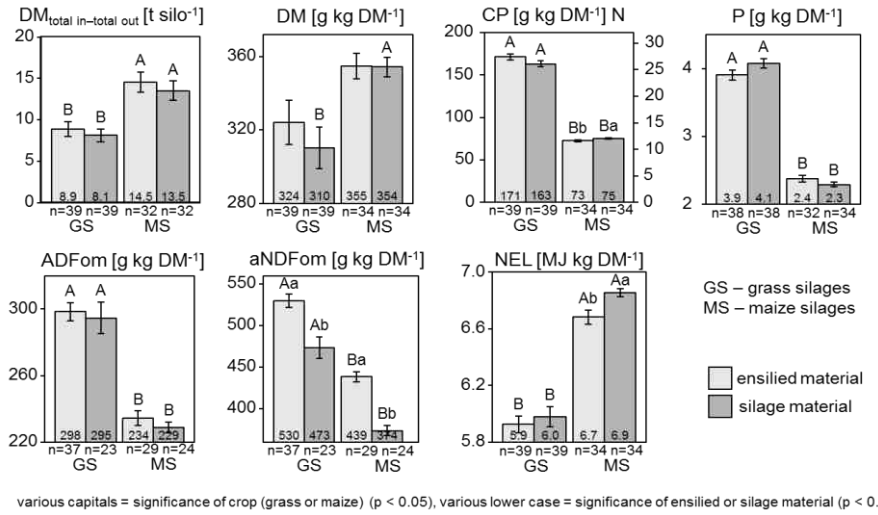


**Figure 1.** Comparison of means from grass and maize silages with the total-in vs. total-out method on farm scale

**References**

DLG (2011) Praxishandbuch Futter- und Substratkonservierung. Deutsche Landwirtschafts-Gesellschaft (DLG), DLG-Verlag, Frankfurt a.M., 416 p.

Köhler, B., Diepolder, M., Ostertag, J., Thurner, T. & Spiekers, H. (2013) Dry matter losses of grass, lucerne and maize silages in bunker silos. Agricultural and Food Science, 1, 145-150.

Misselbrook, T., del Prado, A., & Chadwick, D. (2013) Opportunities for reducing environmental emissions from forage-based dairy farms. Agricultural and Food Science, 1, 93-107.

Nadeau, E.M.G., Russell, J.R. & Buxton, D.R. (2000) Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. Journal of Animal Science, 78, 2980-2989.

Spiekers, H., Ostertag, J., Meyer, K., Bauer, J. & Richter, W.I.F. (2009) Managing and controlling silos to avoid losses by reheating of grass silage. In: Broderick et al. (eds). Proceedings of the XV International Silage Conference, Madison, USA, pp. 317-318.

Wilkinson, J.M. (2011) Re-defining efficiency of feed use by livestock. Animal, 5, 1014-1022.

# Effect of early feed out and additive treatment onto maize silage

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**Keywords:** aerobic stability, fermentation length, silage maize

**Introduction** Often, farmers open silages quite early. After a short fermentation period, spoilage microbes are still active. This may arise from the short-lasting impact of the fermentation acids and of the $CO_2$ partial pressure. In addition to the acetic acid produced by the natural epiphytic flora, inoculants based on heterofermentative lactic acid bacteria (LAB) or chemical additives may be used to control the spoilage microbes after only a short fermentation time (Pflaum et al. 2002). However, the microbes still have to develop and the effective amount of acetic acid is not available from the beginning of inoculation (Driehuis et al. 1999). This time delay of active substances in the silage does not occur in the case of chemical additives. As a result, chemical silage additives may have an advantage over inoculants and could help to stabilize an early opened bunker. Therefore, the fermentation pattern, aerobic stability and spoilage microbes after short and long-term fermentation of differently treated maize silages were analysed.

**Material and Methods** Chopped whole crop maize (29 % DM) was brought directly from the field to the laboratory for fermentation in 1.5 litre jars. The maize crop was treated with *Lactobacillus buchneri* DSM 13573 (L.b., KOFASIL S, $1x10^5$ CFU/g fresh material, ADDCON Europe GmbH) and an additive based on sodium benzoate and sodium propionate (SBSP, Maize KOFASIL liquid 4 L/t, ADDCON Europe GmbH). Each treatment was prepared from 15 kg maize. The material was treated with the L.b. inoculant, 150 ml of a suspension of 0.1 g inoculant/1000 ml or the additive SBSP, 60 ml diluted to 150 ml with water for better distribution. In the control, 150 ml of water was used. The jars were filled with 1.1 kg chopped maize on average within a two-hour period. After 14 and 60 days of fermentation, the silage was analysed in the laboratory (LKS, Lichtenwalde), for silage DM and plant ingredients as well as fermentation parameters (VDLUFA 2012). The aerobic stability was measured over a 5 day period (Honig 1990). Fermentation loss was calculated after Weißbach (2005) and the yeasts and moulds counts were done after VDLUFA (2012). The experimental design was a random block design with 3 replicates. Statistical analyses were done using the ANOVA procedure, program "R". When the overall P-value was significant at 5% level, pair-wise comparisons between LSMEANS of treatments were done using Tukey´s test.

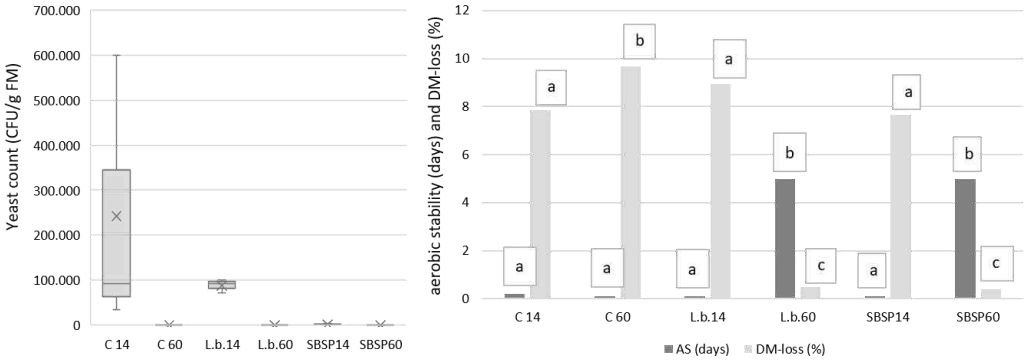
**Results and Discussion** After 14 days, the fermentation losses increased in the order chemical additive < control < *Lactobacillus buchneri* (Tab.1). Further increases could be observed with the control and the *Lactobacillus buchneri* from days 14 to 60, but not with the chemical additive. The aerobic stability was positively affected by the treatments, but only after a fermentation of 60 days

**Table 1.** Roughage and fermentation traits of whole crop maize after different length of fermentation.

| | FL14 | FL60 | starch | aNDFom | ELOS | NEL | pH | Acetic acid | Lactic acid | Ethanol | 1,2-PD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (%) | | (g/kg DM) | | (%/kg DM) | (MJ/kg DM) | | (g/kg DM) | | | |
| C 14 | 4.4[a] | | 296.3 | 365.0 | 65.6 | 6.7 | 3.9[ac] | 11.5[a] | 69.4[a] | 9.1[ac] | 0.2[a] |
| C 60 | | 4.6[a] | 272.7 | 361.3 | 65.4 | 6.8 | 3.6[b] | 12.8[a] | 99.9[b] | 17.6[b] | 0.0[a] |
| L.b. 14 | 4.5[a] | | 301.3 | 368.3 | 65.2 | 6.7 | 3.8[a] | 11.1[a] | 65.0[ac] | 13.4[a] | 0.2[a] |
| L.b. 60 | | 5.2[b] | 264.3 | 380.0 | 63.5 | 6.7 | 3.7[bc] | 22.6[b] | 85.2[abc] | 17.7[bd] | 1.6[b] |
| SBSP14 | 3.7[b] | | 314.7 | 352.3 | 66.4 | 6.8 | 3.9[a] | 11.0[a] | 65.6[c] | 7.9[c] | 0.3[a] |
| SBSP60 | | 3.7[c] | 287.0 | 352.7 | 66.8 | 6.8 | 3.7[bc] | 13.2[a] | 92.5[b] | 6.2[c] | 0.0[a] |

C=control; L.b.=*Lactobacillus buchneri*; SBSP=sodium benzoate, sodium propionate; FL=fermentation loss; ELOS=enzyme soluble organic substance; 1,2-PD=1,2-propanediol; different letters show significant differences within the test criteria

(Figure1). As a result, the dry matter losses during the air exposure were lowest in the treated silages. Obviously after 14 days of fermentation the activity of the yeasts was still given. The yeast counts were subjected to strong variation (Fig.1). However, the highest mean counts were found in the control, whereas the yeasts nearly disappeared in the SBSP treatment. It appears that the yeasts can be reduced by the SBSP treatment after 14 days, but a critical number and activity are still present and can therefore decrease aerobic stability after some hours of air ingress (Fig.1). Effects on the aerobic stability of silages inoculated with *Lactobacillus buchneri* may begin after 30 days (Oude Elferink et al. 1999). It is possible that a week later, the effects of controlling the heating could start. The growing stability of the *Lactobacillus buchneri* treatment after 60 days is in accordance with the higher acetic acid concentration in the silage (Tab.1). The increase of 1,2 propanediol indicates the acetic acid as the metabolic product of the applicated *Lactobacillus buchneri* in this storage phase. However, after 60 days the ethanol could not be reduced compared to the control. Using the SBSP additive, the ethanol content was lowest. Unexpectedly, in all treatments, no yeasts could be found after 60 days. There seems to be a tendency (p=0.10) for the SBSP treatment to affect the starch content and the enzyme soluble organic matter (ELOS). This may result from the lower fermentation losses.




Left: x = mean, - = median of the sample. Right: different letters show significant differences within the test criteria. 14/60=fermentation-length; C=control, L.b.=*Lactobacillus buchneri*, SBSP=sodium benzoate, sodium propionate

**Figure 1.** Effect of inoculum and chemical additive on yeast counts (left) as well as aerobic stability (AS) and DM-loss during the 5 days AS testing period (right) after a fermentation time of 14 and 60 days.

**Conclusions** Early feed out (14 days) bears a high risk for spoilage and heating in maize silages. The yeast count is not reduced and affected long enough to prevent their re-activity. Feed-out of silages after a short fermentation should be avoided. Chemical additives may also improve the silage quality.

**References**

Driehuis, F., Van Wikselaar, P. & Oude Elferink, S. (1999) Lactobacillus buchneri improves aerobic stability of laboratory and farm scale whole crop maize silage but does not affect feed intake and milk production of dairy cows. 9th International Conference forage conservation. Nitra, Slovakia, p. 120-121.

Honig, H. (1990) Evaluation of aerobic stability. Proceedings of the Eurobac Conference 1986, Grass and Forage Reports, Uppsala, Sweden, pp. 76-82.

Oude Elferink, S., Driehuis, F., Krooneman, J.,Gottschal, J. & Spoelstra, S. (1999) Lactobacillus buchneri can improve the aerobic stability of silage via a novel fermentation pathway: the anaerobic degradation of lactic acid to acetic acid and 1,2-propanediol. In: Pauly, T. (eds). Proceedings of the XII International Silage Conference, Uppsala, Sweden, 1999, pp. 266-267.

Pflaum J, Honig H, Pahlow G, Staudacher W. (2002) A comparison of the aerobic stability of maize silage produced in laboratory and farm silos. In: Gechie, L., Thomas, C. (eds.) Proceedings of the XIII International Silage Conference, Auchincruive, Scotland 2002, 11-13.

VDLUFA (2012) Versuchs- und Untersuchungsmethodik -Methodenbuch Bd. III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt**.**

Weißbach, F. (2005) A simple method for correction of fermentation losses measured in laboratory silos. In: Proceedings of the XIV Int. Silage Conference, Belfast, Irland, 2005, pp. 278.

# Effect of ensiling on fermentation profile and corn silage processing score in whole-plant corn

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**Keywords:** corn silage processing score, ensiling, fermentation profile

**Introduction** It is well-established that ensiling and prolonged storage improve fermentation profile and starch digestibility of whole-plant corn. However, experiments evaluating the impact of ensiling on corn silage processing score in whole-plant corn silage are scarce; but preliminary data suggest that corn silage processing score is improved with ensiling (Ferraretto et al. 2015). Perhaps proteolysis associated with disruption of the starch-protein matrix during ensiling may dissociate starch granules and thereby reduce mean particle size of kernels. Therefore, the objective of this study was to evaluate the effect of ensiling on the fermentation profile and corn silage processing score of whole-plant corn. We hypothesized that corn silage processing score would increase with ensiling.

**Material and Methods** Eleven corn hybrids (used as experimental units) were obtained at harvest at the University of Florida - Plant Science Research & Education Unit (Citra, FL). Each of the 11 samples was homogenized manually, and allocated into 4 samples of approximately 600 g each using a quartering technique. Each of the 4 samples was randomly assigned to 1 of 2 treatments (0 or 120 d of ensiling) and vacuum-sealed in nylon-polyethylene standard barrier vacuum pouches (25.4 x 35.6 cm) using an external clamp vacuum machine. Mini-silos were stored at room temperature (approximately 20ºC) and in the dark until targeted ensiling period was reached. A representative subsample of each mini-silo was collected for pH, organic acids, ammonia-N, dry matter (DM) and water-soluble carbohydrates (WSC) analysis. Subsequently, a subsample of each mini-silo was collected and analyzed for corn silage processing score (% of starch passing through a 4.75 mm sieve; Ferreira and Mertens 2005) and starch. Data were analyzed using Proc Mixed of SAS with the fixed effect of fermentation. Duplicates for each hybrid within an ensiling time were averaged and used for statistical analysis. Statistical significance and tendencies were declared at $P \leq 0.05$ and $P > 0.05$ to $P \leq 0.10$, respectively.

**Results** Effect of ensiling on fermentation profile and corn silage processing score of whole-plant corn is presented in Table 1. Concentrations of DM and starch were unaffected ($P > 0.10$) by ensiling and averaged 36.2% of as fed and 31.2% of DM, respectively. Measurements of pH were lower ($P = 0.001$) for 120 d compared with 0 d of ensiling. This is likely related to the 7.7%-, 1.0%- and 1.2%-units greater ($P < 0.02$) lactic, acetic and isobutyric acids concentrations, respectively, for 120 d compared with 0 d. Conversely, WSC concentrations were reduced ($P = 0.001$) by 9.9%-units for 120 d in comparison with 0 d. Propionate and butyrate concentrations were not detected. Concentrations of ammonia-N increased ($P = 0.001$) with ensiling, as expected. Contrary to our hypothesis, however, corn silage processing score was similar ($P = 0.97$) between ensiling treatments and averaged 28.9%.

**Discussion** Ensiling effects on pH, organic acids and ammonia-N were as expected and indicates that the fermentation process was adequate. Lack of an effect on corn silage processing score was contrary to our hypothesis and in disagreement with our previous findings. Based on the data by Ferraretto et al. (2015), a hypothesis that ensiling time would enhance corn silage processing score in poorly (< 50% of starch passing through 4.75 mm sieve) compared to adequate or optimally (> 50% or 70% of starch passing through 4.75 mm sieve, respectively) processed silage could be raised. Nevertheless, whole-plant corn was poorly processed in the present study and our findings denies this premise. Further research is warranted across a wide range of corn silage processing score to elucidate under which conditions ensiling time enhances this parameter.

**Table 1**. Effect of ensiling on fermentation profile and corn silage processing score of whole-plant corn

| Item | 0 d | 120 d | SEM | *P*-value |
|---|---|---|---|---|
| Dry matter (DM), % of as fed | 36.6 | 35.6 | 0.9 | 0.29 |
| pH | 5.74 | 4.00 | 0.03 | 0.001 |
| Lactate, % of DM | 0.03 | 7.73 | 0.74 | 0.001 |
| Acetate, % of DM | 0.00 | 1.01 | 0.09 | 0.001 |
| Isobutyrate, % of DM | 0.43 | 1.59 | 0.43 | 0.02 |
| Total acids, % of DM | 0.47 | 10.33 | 1.06 | 0.001 |
| Water-soluble carbohydrates, % of DM | 11.0 | 1.1 | 0.5 | 0.001 |
| Ammonia-N, % of DM | 0.11 | 0.57 | 0.02 | 0.001 |
| Starch, % of DM | 31.4 | 31.0 | 2.2 | 0.89 |
| CSPS[1], % of starch passing through 4.75 mm sieve | 28.8 | 28.9 | 1.9 | 0.97 |

[1]Corn silage processing score.

**Conclusion** Ensiling improved fermentation profile but did not affect corn silage processing score.

**References**

Ferraretto, L.F., Dias Junior, G.S., de Resende, L.C., & Shaver, R.D. (2015) Effect of ensiling on kernel processing score in whole-plant corn silage harvested with varied processors and settings. Journal of Dairy Science, 98, Supplement 2, 689.

Ferreira, G. & Mertens, D. R. (2005) Chemical and physical characteristics of corn silages and their effects on in vitro disappearance. Journal of Dairy Science, 88, 4414-4425.

# Effect of length of storage and sodium benzoate use on *in-vitro* parameters of sorghum grain silages

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**Keywords:** ammonia-N, degradation rate, ensiling, proteolyses

**Introduction** In Brazil, ensiling of rehydrated cereals kernels such as sorghum and corn has become a great strategy to storage grains for beef and dairy cattle. The degree of physical processing and days of ensiling are two important aspects that increase ruminal dry matter (DM) and starch digestibility. The aim of this study was to evaluate the potentially associate effect of particle size and length of storage on *in vitro* gas production rate. We hypothesized that lower mean particle size associated with prolonged time of storage might increase the rate of DM degradation.

**Materials and Methods** Sorghum grains were ground in a hammer mill through a 2 mm sieve and rehydrated to reach 35 % moisture and the experiment was set according to a randomized block arranged in a 3x2 factorial design: length of storage (0, 30, and 90 d) and additive (with or without 0.2% as fed Na benzoate). Plastic buckets of 200 L were used to ensile the grains with 4 replications for each treatment. Silos were opened with 30 and 90 days of storage and sampling were performed over next five days. Sub-samples was collected daily during unloading (fed out rate 30 cm/d) and frozen at - 20º C to form a composite sample. One composite sample of 500 g was collected for DM and ammonium nitrogen determination. Another sample of 1000 g was collected, dried in air forced oven at 55º C for 72 h and used to perform a particle size distribution using a set of 9 sieves with nominal square apertures of 9.50, 6.70, 4.75, 3.35, 2.36, 1.70, 1.18, 0.59 mm and pan. Geometric mean particle size was calculated based on the distribution across sieves. Samples were collected of each sieve to run in vitro gas production assay. Fractional degradation rate were estimated by one-pool exponential equation (Schofield et al., 1994). Data were analyzed by PROC MIXED of SAS 9.3 with model containing effects of block, storage length, additive and their interactions. Means were compared by Tukey test ($P < 0.05$).

**Results** The DM content was no different among treatments ($P > 0.05$) and the mean value was 64 %. There was an increase in ammonia nitrogen (% of DM) throughout the length of ensiling, 0.02, 0.11 and 0.15 % for zero, 30 and 90 days of storage, respectively ($P < 0.01$).There was no statistical difference between 30 and 90 days. Geometric mean particle size did not differ among treatments ($P > 0.10$). Fractional gas production rate increased with length of storage (Table 1). There was no statistical effect for additive or interactions.

**Discussion** Increasing storage time and degree of mechanical process result in greater DM digestion rate (Table 1). The combination of factors, lower particle size and longer time of storage, allowed the maximum potential of degradation rate (Table 1). The reducing of storage time will require a heavy mechanical process to target the same rate of degradation. Sodium benzoate did not affect measured variables suggesting that it can be used to increase aerobic stability and reduce DM losses (Morais et al., 2017) without compromising the digestion.

**Conclusions**: Dry matter degradation of rehydrated sorghum grain silage is increased by storage length.

**Table 1.** Chemical composition, particle size distribution, and fractional gas production rate of rehydrated sorghum grains without ensiling (Non-ensiled) or ensiled for 30 (30) or 90 (90) days with (BEN) or without (CON) sodium benzoate (0.2% as fed).

| | Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Non-ensiled | | 30 | | 90 | | | *P*-value* | | |
| Item | CON | BEN | CON | BEN | CON | BEN | SEM | T | A | T*A |
| Nutrient | | | | | | | | | | |
| DM, % fresh | 64.6 | 65.0 | 64.3 | 64.6 | 64.2 | 64.6 | 0.07 | 0.81 | 0.53 | 0.99 |
| CP, % of DM | 11.6 | 11.7 | 12.0 | 12.1 | 11.7 | 11.8 | 0.22 | 0.15 | 0.66 | 0.88 |
| $NH_3$-N, % of DM | 0.02[a] | 0.02[a] | 0.11[b] | 0.11[b] | 0.16[b] | 0.15[b] | 0.01 | 0.01 | 0.79 | 0.88 |
| ASABE MPS, µm | | | | | | | | | | |
| >2,360 | 1.7 | 1.2 | 1.8 | 1.5 | 1.8 | 1.8 | 0.02 | 0.32 | 0.18 | 0.46 |
| 1,700 | 7.6 | 6.0 | 6.7 | 7.0 | 6.7 | 7.1 | 0.07 | 0.97 | 0.60 | 0.36 |
| 1,180 | 24.3 | 22.2 | 22.2 | 23.0 | 21.9 | 23.0 | 0.10 | 0.80 | 0.99 | 0.36 |
| 600 | 50.4 | 53.5 | 50.6 | 50.1 | 50.5 | 50.5 | 0.11 | 0.27 | 0.34 | 0.20 |
| Pan | 15.9 | 17.0 | 18.5 | 18.3 | 19.0 | 17.4 | 0.18 | 0.51 | 0.88 | 0.73 |
| GMPS | 911 | 870 | 871 | 874 | 862 | 888 | 29.8 | 0.80 | 0.86 | 0.54 |
| In vitro gas production rate, h | | | | | | | | | | |
| Unprocessed | 0.037[a] | 0.036[a] | 0.038[a] | 0.038[a] | 0.046[b] | 0.048[b] | 0.0049 | 0.04 | 0.97 | 0.95 |
| >2,360 | 0.028 | 0.024 | 0.024 | 0.023 | 0.028 | 0.031 | 0.0035 | 0.18 | 0.68 | 0.54 |
| 1,700 | 0.028[a] | 0.023[a] | 0.028[a] | 0.028[a] | 0.035[b] | 0.033[b] | 0.0022 | 0.02 | 0.34 | 0.59 |
| 1,180 | 0.030[a] | 0.027[a] | 0.029[ab] | 0.032[b] | 0.038[b] | 0.036[b] | 0.0026 | 0.04 | 0.76 | 0.47 |
| 600 | 0.037[a] | 0.035[a] | 0.039[b] | 0.038[b] | 0.041[b] | 0.047[b] | 0.0027 | 0.03 | 0.29 | 0.85 |
| Pan | 0.046[a] | 0.046[a] | 0.047[a] | 0.048[a] | 0.056[b] | 0.051[b] | 0.0012 | 0.01 | 0.32 | 0.14 |

*Probabilities for time effect (T), additive effect (A) and interaction between time and additive (T*A)

**References**

Morais, G., Daniel, J.L.P., Kleinshmitt, C., Carvalho, P.A., Fernandes, J., Nussio, L.G. (2017) Additives for grain silages: A review. Slovak Journal of Animal Science, 50, 42-54.

Schofield, P., Pitt, R.E. & Pell, A.N. (1994) Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science, 72, 2980-2991.

# Effect of maturity at harvest on fermentation profile and starch digestibility of corn silage hybrids in Florida

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**Keywords:** corn silage, hybrids, starch digestibility

**Introduction** Corn silage is the most common forage used in the total mixed ration from dairy cow diets in the United States. Many current corn silage hybrids in Florida aimed to fill out the ear corn while the stalks and leaves stay green as long as possible and this characteristic could be an indicator of the presence of the stray-green trait. Thomas and Smart (1993) reported the association of stay-green with greater water and chlorophyll concentration in the leaves and stalk. Plant maturity at harvest is one of the primary factors influencing forage quality and digestibility; as plants age, their digestibility tend to decrease. Relatively little is known about the effects of maturity on the digestibility of starch in corn silage. Based on previous studies, we hypothesized that harvesting corn between 30 and 40% dry matter (DM) would improve starch digestibility and silage fermentation. The aim of this study was to examine the effect of maturity at harvest on the fermentation profile and starch digestibility of corn silage from various Florida summer corn hybrids.

**Materials and Methods** Nine corn hybrids from five seed companies were grown on irrigated field at the Plant Science Research and Education Center (Citra, FL). Hybrids were grown on four replicate plots (1.8 x 6.1 m) in each of four blocks and the planting rate was 75,683 seeds/ha, 76.2 cm row spacing and 15.24 cm between plants. Hybrids were harvested at targeted DM concentrations of 30 (M1), 35 (M2), 40 (M3), and 45% (M4) DM, chopped and ensiled in 20-L mini-silos. Silos were stored for 108 d at room temperature (22°C). After silo opening, final silo weights were recorded to estimate DM recovery, subsamples from each hybrid were collected for DM determination, juice extraction (20 g) for pH, ammonia-N, lactic and acetic acid, total VFA, and yeast counts. Additional subsamples were dried and ground to pass a 4-mm screen to determine ruminal in *vitro* starch digestibility after 7 h of incubation and at 1-mm to determine NDF concentration. Temperature sensors were placed at the center of 20-L plastic buckets containing 4 kg of silage and set to record the temperature every 20 min. Aerobic stability was determined by the time (h) elapsed before silage temperature increased at least 2ºC above room temperature. Data were analyzed using the Glimmix procedure of SAS with the fixed effects of hybrid, maturity and their interaction. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P < 0.10$.

**Results** Although the targeted DM concentrations at harvest were 30, 35, 40 and 45%, the variation between corn hybrids forages resulted in DM concentrations at harvest (day 0) of 31.9, 34.6, 40.2 and 41.4% for M1, M2, M3, and M4, respectively. Concentrations of NDF from all forage were 45.7 (M1), 41.5(M2), 39.1 M3), and 43.3(M4), respectively. Forage starch concentrations were 25.2(M1), 29.9(M2), 33.3(M3), and 28.9(M4), respectively. The pH at day 0 were 4.09, 4.09, 4.13, and 4.20 M1, M2, M3, and M4, respectively. At silo opening, DM concentrations of the silages were 28.7, 31.5, 36.4 and 39.2% for M1, M2, M3 and M4, respectively. Silage starch concentration increased with maturity ($P < 0.01$). Compared with other treatments, M4 silage had the lowest pH (3.46 vs. 3.61), highest DM recovery (93.3 vs. 89.6%), ammonia-N concentration and lactate to acetate ratio (5.9 vs. 4.3; $P < 0.01$). Recovery of DM from M3 silage was similar ($P > 0.05$) to those from M1 and M2 silage, but ammonia-N concentration was greater ($P < 0.01$) for the M3 silage. Lactic acid concentration was greater ($P < 0.01$) in M1 and M2 silages compared with other treatments. Similarly, acetic acid concentration was highest for M1 silage, intermediate for M2 silage and lowest for M3 and M4 silage ($P < 0.001$). Yeast counts were lowest ($P < 0.01$) in M1 silage compared to M3 and M4

(6.14 vs. 6.99 log cfu/g). Starch digestibility was lower ($P < 0.01$) for M3 silages compared with others (43.3 vs. 50.7%).

**Table 1**. Effect of maturity at harvest in corn hybrids ensiled for 108 d

| Items | Targeted DM at harvest | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|
| | 30 | 35 | 40 | 45 | | |
| pH | 3.59 [b] | 3.55 [b] | 3.71 [a] | 3.46 [c] | 0.022 | <0.0001 |
| DM recovery, % | 88.7 [b] | 90.0 [b] | 90.2 [b] | 93.3[a] | 0.823 | 0.0026 |
| Aerobic stability, h | 10.5 [c] | 14.7 [ab] | 11.3 [bc] | 18.6 [a] | 2.08 | 0.0002 |
| Ammonia-N, % of DM | 0.029[c] | 0.027[c] | 0.034[b] | 0.038[a] | 0.0011 | <0.0001 |
| Total VFA, % of DM | 8.7 [a] | 8.6 [a] | 7.4 [b] | 7.2 [b] | 0.265 | <0.0001 |
| Lactic acid, % of DM | 4.90 [a] | 4.63 [a] | 4.00 [b] | 3.93 [b] | 0.139 | <0.0001 |
| Acetic acid, % of DM | 1.37 [a] | 1.20 [b] | 0.79 [c] | 0.67 [c] | 0.053 | <0.0001 |
| Lactate to acetate | 3.8 [c] | 3.9 [c] | 5.3 [b] | 5.9 [a] | 0.165 | <0.0001 |
| Yeast, log cfu/g | 6.14 [b] | 6.62 [ab] | 7.15 [a] | 6.83 [a] | 0.255 | 0.0087 |
| Starch digestibility at 7 h, % | 53.1 [a] | 48.9[b] | 43.3 [c] | 50.0 [ab] | 1.228 | <0.0001 |

[a-c] means in the same row with different superscript differed ($P < 0.05$)

**Discussion** Concentrations of DM at harvest for M3 and M4 were close to each other (40.2 vs. 41.4 %) due to the variation between hybrids (range from 34 to 45% and 37 to 47% for M3 and M4 respectively) and this could be related to different hybrid relative maturity and stay-green traits among hybrids. Silages made from the present hybrids were only aerobically stable from 10 to 19 h and yeast counts were between 6 to 7 log cfu/g; these results agreed with previous results from our group where corn silage from stay-green hybrids were aerobically stable for 24 to 25 h and yeast counts were from 6 to 8 log cfu/g (Arriola et al., 2012). Reduced starch digestibility for M3 than earlier maturity treatments (M1 and M2) is in agreement with previous literature, but M4 starch digestibility values were unexpected.

**Conclusions** This study highlights that effects of maturity at harvest on quality attributes of corn silage differed. Harvesting at 41.4% DM resulted in the lowest pH, highest aerobic stability, and greatest DM recovery; however, lactic acid concentration was greater for M1 and M2 while acetic acid concentration was greater for M1 silage. Results from this study underscores that harvest maturity remains an important factor in determining the digestibility and fermentation characteristics of corn silage. Therefore, among the hybrids evaluated in the present study, it is recommended to target 35% DM at harvest to optimize nutritive value of corn silage and thereby nutrient utilization by dairy cows.

**References**

Arriola, K.G.,Kim, S.C. Huisden, C.M., Adesogan, A.T. (2012) Stay-green ranking and maturity of corn hybrids: 1. Effect on dry matter yield, nutritional value, fermentation characteristics, and aerobic stability of silage hybrids in Florida. J. Dairy Sci. 95:964-974.

Thomas, H. & Smart, C.M. (1993) Crops that stay green. Ann. Appl. Biol. 123:193-219.

# Effect of kernel processing and acid treatment on fermentation characteristics and nutritive value of whole crop wheat silage

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**Keywords:** additive, cracking, ensiling, net energy, starch hydrolysis

**Introduction** Intensive processing facilitated by cracker units mounted on the forage harvester machinery is frequently recommended in order to improve the fermentation process as well as the availability of starch by ruminants from late harvested whole crop wheat (WCW). However, it has been discussed if such processing may have unintended negative implications during fermentation, e.g. elevated losses of starch by hydrolysis, and whether application of additive in order to restrict the fermentation will moderate or intensify such effect. A small scale ensiling experiment was carried out in order to examine these questions.

**Material and Methods** Spring wheat was harvested as whole crop from three replicates at the hard dough stage of maturity (Zadoks 87-89, Zadoks et al. 1974) at a stubble height of 70 mm at NIBIO Kvithamar Research Station, Stjørdal, Norway (63°N, 11°E, 28 m a.s.l). The WCW was objected to eight combinations of treatments: processing (cracked vs intact kernels) x additive application (with vs without) x storage length (84 vs 174 days). The WCW from each block was separated into ears and straw (stem and leaves). Half of the ear fraction was processed by using a cereal roller, and the remaining part was left intact. The straw was chopped with a laboratory chopper. Thereafter, the separate fractions were mixed together in their original proportions. The relative proportion of ears averaged 0.55 on dry matter (DM) basis. Within each replicate and mechanical treatment, four polyethylene bags were filled with 500 g WCW. Two of the bags were filled with WCW treated with 5 L tonne$^{-1}$ of an acid containing formic acid, sodium formate, propionic acid, benzoic acid and sorbic acid. The other two were filled with WCW without application of additive. After filling, the bags were vacuumed, sealed and stored at 20°C. Half of the vacuum bags were opened after 84 days and the remaining after 174 days. Water soluble carbohydrates (WSC) were analysed according to Larsson and Bengtsson (1983). All other chemical analyses and further computations of nutritive values were performed according to the Nordic feed evaluation system (Volden 2011, Chapter 5), except for indigestible NDF (iNDF) that was calculated from *in vitro* organic matter digestibility (Eriksson 2010). Data of chemical composition were analysed statistically using the Proc mixed procedure of SAS 9.4 according to a randomized block design with processing-, additive- and storage length treatments and their interactions as fixed effects and block as random effect.

**Results and discussion** At silo filling, the chemical composition of the material averaged 459 g DM kg$^{-1}$, and 68 g crude protein (CP), 475 g NDF, 280 g starch and 49 g WSC kg$^{-1}$ DM. Challenging growing conditions with high precipitation during spring, however still high DM yields (8750 kg ha$^{-1}$) may explain the low CP contents. Only minor differences between storage lengths were revealed. Consequently, results averaged over time are presented and discussed. Kernel processing did not affect the DM content, nor CP or NDF concentrations in the subsequent silage (Table 1). However, more intensive and partly unfavourable fermentation seemed to have appeared in the silage with processed kernels compared to silage with intact kernels, expressed by elevated concentrations of lactic acid, acetic acid, ethanol and ammonia. The nutritive value of the WCW silage was also affected by kernel processing with increased proportions of soluble CP (sCP), WSC and iNDF, whereas the starch content decreased. Most naturally occurring lactic acid bacteria are not able to ferment starch directly (McDonald et al., 1991). However, the starch may be hydrolysed into simple sugars when the pH drops, especially in acid-treated silage (Nadeau 2007). In this experiment, the decrease in pH by

intensive processing may have facilitated hydrolysis of starch into sugars, expressed by depressed starch and elevated WSC content, both compared to the composition before ensiling and to the silage with intact kernels. A more restricted fermentation with higher concentration of WSC and lower concentrations of lactic acid, acetic acid and ethanol was obtained in acid treated silage compared to untreated silage. This effect was more pronounced in the intensive processed silage compared to silage with intact kernels, and statistically significant in respect of WSC (*P=0.003*), acetic acids (*P=0.04*) as well as total acids (*P=0.05*, results not shown). Starch and WSC are important energy sources for both animals and microbes, taken into consideration in the Norfor feed evaluation system for ruminants (Volden 2011). Consequently, the reduced content of starch in silage with processed kernels caused reduced metabolisable protein (AAT) and net energy value (NEL, *P=0.06*).

**Table 1**. Effect of kernel processing and acid treatment on fermentation characteristics and nutritive value of whole crop wheat silage (g $kg^{-1}$ DM unless stated otherwise) harvested at hard dough stage, when average over storage times.

| | Kernel processing | | Acid | | | Level of significance | | |
|---|---|---|---|---|---|---|---|---|
| | Cracked | Intact | With | Without | SEM | Processing | Acid | Interaction |
| DM, g $kg^{-1}$ | 471 | 456 | 465 | 461 | 6.6 | NS | NS | NS |
| CP | 69 | 67 | 64 | 71 | 1.8 | NS | * | NS |
| sCP, g kg $CP^{-1}$ | 782 | 628 | 714 | 696 | 12.6 | *** | NS | NS |
| NDF | 460 | 452 | 458 | 466 | 12.1 | NS | NS | NS |
| INDF, g $kg^{-1}$ NDF | 358 | 327 | 340 | 345 | 5.2 | *** | NS | NS |
| Starch | 219 | 259 | 232 | 246 | 13.0 | * | NS | NS |
| WSC | 43 | 32 | 61 | 15 | 2.2 | ** | *** | ** |
| pH | 4.3 | 4.8 | 4.5 | 4.6 | 0.22 | NS | NS | NS |
| Lactic acid | 18.5 | 6.7 | 5.5 | 19.7 | 1.75 | *** | *** | NS |
| Acidic acid | 3.8 | 1.5 | 1.1 | 4.2 | 0.43 | ** | *** | * |
| Ethanol | 9.3 | 3.2 | 3.2 | 9.3 | 1.10 | *** | *** | NS |
| $NH_3$-N, g $kg^{-1}$ N | 174 | 49 | 108 | 115 | 3.90 | *** | NS | NS |
| NEL, MJ $kg^{-1}$ DM | 4.87 | 5.09 | 4.93 | 5.04 | 0.08 | *P=0.06* | NS | NS |
| AAT | 68 | 73 | 71 | 70 | 0.6 | *** | NS | * |

DM=dry matter, CP=crude protein, sCP=soluble CP, NDF=neutral detergent fibre, INDF=indigestible NDF, WSC=water soluble carbohydrates, NEL=net energy for lactation, AAT= amino acids absorbed in the intestine.

**Conclusion** Kernel processing prior to ensiling may facilitate starch degradation and have negative impacts on the nutritional value of late harvested whole crop wheat silage. Application of acid based additive had no significant impact on starch degradation in this experiment.

**References**

Eriksson, T. (2010) In vitro methods for indigestible neutral detergent fiber (iNDF). In: Udén, P., Eriksson, T., Müller, C.E., Spörndly, R., Liljeholm, M. (eds). Proceedings of the 1st Nordic Feed Science Conference, 22-23 June, Uppsala, Sweden, pp. 41-45.

Larsson, K. & Bengtsson, S. (1983) Determination of water soluble carbohydrates in plant material (in Swedish). Method 22. National Laboratory for Agricultural Chemistry, Uppsala, Sweden.

McDonald, P., Henderson, A.R. & Heron, S.J.H. (1991) The biochemistry of silage. Second edition. Chalcombe Publication, UK, 340 p.

Nadeau, E. (2007) Effects of plant species, stage of maturity and additive on the feeding value of whole-crop cereal silage. Journal of the Science of Food and Agriculture, 87, 789-801.

Volden, H. (2011) NorFor – The Nordic feed evaluation system. EAAP publication No 130, Wageningen Academic Publishers, 180 p.

Zadoks, J.C., Chang, T.T. & Konzak, C.F. (1974) A decimal code for the growth stages of cereals. Weed Research, 14, 415-421.

# Effect of shredding on silage density and the fermentation characteristics

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**Keywords:** conservation, density, grass, legumes, shredding

**Introduction** In earlier studies, intensive physical processing (maceration, shredding) of forages such as lucerne was reported to increase digestibility of fiber fraction of the forage. Bruising the stems, tissues and breaking the fibrous structure before ensiling, will possibly improve the ability to compact the crop in silos as well. Furthermore, increased silage density will decrease initial oxygen availability, resulting in favorable anaerobic conditions. In addition, processing the crop results in cell rupturing, increasing the nutrients immediately accessible by microorganisms for the fermentation. Therefore, intensive physical processing would be expected to increase the capacity of the silos and to improve the fermentation quality of the silage. The aim of this lab scale experiment was to study the effects of shredding of different green forages before ensiling, on silage density and fermentation characteristics.

**Material and Methods** Lucerne (L), red clover (RC), perennial ryegrass (PR) and grass-clover (GC) mixture [RC, white clover, PR and hybrid ryegrass] were harvested without chopping on 5$^{th}$ and 12$^{th}$ of September 2017, and were wilted indoor for 1-2 days before processing. Harvested forages (F) were either unprocessed (0) or shredded once (1×) or four (4×) times as physical processing treatments (T), using a novel lab shredder. Shredded once (1×) meant one passage and four times (4×) meant four passages, through the lab shredder. The 4× passage was used to model a more intensive shredding. Each forage receiving each treatment had three replicates (R). Three forages [L, RC and PR] were cut on 5$^{th}$ of Sep 2017 and treated (3F× 3T × 3R; n=27) and, all four forages including GC were harvested and treated on 12$^{th}$ Sep 2017 (4F× 3T × 3R × 2 sets; n=72). Therefore, 99 vacuum bags as lab silos were made using a vacuum packer (Webomatic I22). Data of two bags were omitted as outliers. Density of vacuum bag silages was measured as bag weight over volume of water displacement. Two sets of silage bags were prepared in second harvest day, because one set was used for fermentation weight-loss measurement as a time series of weights after ensiling (n=36). This was intended for preventing possible effects on weight measures, by remaining water on the bag surface due to density measurements. Fermentation process was terminated by freezing after either two (n=63) or four (n=36) months. All the silages were extracted using a Waring blender (100 g silage in 1000 ml de-mineralized water). Extracts were analysed for pH, VFA (gas chromatography), L-lactate (YSI 7100 analyzer) and ammonia concentration enzymatically. Statistical analyses were performed using the GLM procedure of SAS (9.3), considering treatment effect, forage types, duration of ensiling, their two-way interactions and harvesting dates.

**Results and Discussion** Initial density of silages increased by shredding ($P<0.001$), and increased 57% for L, 46% for RC, and 29% for PR comparing 0 and 4× treatment, where 1× treatment was intermediary (Table 1). Weight losses after 1-day conservation were higher for 4× treatment ($P<0.001$), whereas after 113 days of conservation zero treatment resulted in the highest losses ($P<0.01$) (Table 2), indicating the treatment increased onset of fermentation, but also resulted in less intensive fermentation during the conservation process. Fermentation products of silages confirmed the improved fermentation due to physical processing (Table 1). Final pH of the ensiled PR, GC and RC were considerably lower in the 4× treatment than that of the 0 treatment ($P<0.001$) (Table 1). Ammonia concentrations in PR, L and RC silages were reduced by 29%, 25% and 44% respectively in 4× treated samples, and L-lactate concentrations of the 4× treated silages were 43%, 33% and 94% higher in PR, GC and RC silage respectively, in comparison to 0 treatment of respective silages ($P<0.001$). Butyrate concentrations were reduced by 95%, 100% and 83% in 4× treated PR, GC and RC silages in comparison to the 0 treatment ($P<0.001$). However, acetate concentrations did not differ

due to the physical treatments (P>0.05). The enhanced fermentation can be a direct result of the higher initial density thus the faster establishment of anaerobic conditions (Dunière et al., 2013) and enhanced access to cellular nutrients by lactic acid bacteria (LAB) due to physical treatment of forages. There were no favorable effects on fermentation due to the physical processing of L (Table 1). L silages had higher pH than other silages irrespective of the physical treatments (P<0.001). Furthermore, $NH_3$, butyrate and acetate concentrations were higher, and L-lactate concentration of L silages was lower (P<0.001) (Table 1). Comparatively low DM concentration in L silages (Table 1) together with inherently low fermentable carbohydrate content and high buffering capacity might result in clostridial fermentation. Parveen et al. (2010) reported that the extent of proteolysis in L silages was considerably higher than that of RC due to a high level of polyphenol oxidase enzyme in RC, which might explain the differences between fermentation parameters of L and RC.

**Table 1.** Effect of shredding at different intensities on initial silage density (g/cm$^3$) and several fermentation quality parameters (g/kg of DM) (n=97)

| | | Density[1] | DM (%) | pH | $NH_3$ | L-lactate[2] | Acetate | Butyrate |
|---|---|---|---|---|---|---|---|---|
| Forage type (F) | Trt (T) | | | | | | | |
| Perennial ryegrass (PR) | 0 | 0.67$^{a}$ | 20.6$^{a}$ | 4.5$^{a}$ | 5.2$^{a}$ | 39.2$^{a}$ | 35.5$^{a}$ | 15.2$^{a}$ |
| | 1× | 0.75$^{a}$ | 21.8$^{a}$ | 4.2$^{b}$ | 4.4$^{ab}$ | 48.4$^{ab}$ | 39.1$^{a}$ | 4.5$^{b}$ |
| | 4× | 0.87$^{b}$ | 21.7$^{a}$ | 4.1$^{b}$ | 3.7$^{b}$ | 56.0$^{b}$ | 42.7$^{a}$ | 0.8$^{b}$ |
| Grass-clover mix (GC) | 0 | 0.71$^{a}$ | 21.8$^{a}$ | 4.5$^{a}$ | 5.9$^{a}$ | 45.9$^{a}$ | 37.3$^{a}$ | 3.7 |
| | 1× | 0.79$^{ab}$ | 21.9$^{a}$ | 4.3$^{ab}$ | 4.8$^{a}$ | 57.4$^{ab}$ | 40.1$^{a}$ | ND |
| | 4× | 0.87$^{b}$ | 21.0$^{a}$ | 4.2$^{b}$ | 4.8$^{a}$ | 61.0$^{b}$ | 42.8$^{a}$ | ND |
| Lucerne (L) | 0 | 0.66$^{a}$ | 17.5$^{a}$ | 5.8$^{a}$ | 16.1$^{a}$ | 4.5$^{a}$ | 91.5$^{a}$ | 7.6$^{a}$ |
| | 1× | 0.85$^{b}$ | 18.0$^{a}$ | 5.7$^{a}$ | 13.5$^{b}$ | 8.8$^{a}$ | 93.8$^{a}$ | 5.2$^{a}$ |
| | 4× | 1.04$^{c}$ | 18.0$^{a}$ | 5.7$^{a}$ | 12.1$^{c}$ | 10.2$^{a}$ | 92.8$^{a}$ | 6.1$^{a}$ |
| Red clover (RC) | 0 | 0.72$^{a}$ | 17.2$^{a}$ | 4.7$^{a}$ | 5.9$^{a}$ | 32.4$^{a}$ | 57.7$^{a}$ | 11.0$^{a}$ |
| | 1× | 0.82$^{a}$ | 17.9$^{a}$ | 4.3$^{b}$ | 4.0$^{b}$ | 49.5$^{b}$ | 55.7$^{a}$ | 1.6$^{b}$ |
| | 4× | 1.05$^{b}$ | 17.9$^{a}$ | 4.1$^{b}$ | 3.3$^{b}$ | 62.9$^{c}$ | 54.2$^{a}$ | 1.9$^{b}$ |
| P value | F | *** | *** | *** | *** | *** | *** | *** |
| | T | *** | * | *** | *** | *** | 0.1 | *** |
| | F×T | *** | 0.2 | ** | *** | ** | * | *** |

[1]Density of the silage samples immediately after vacuum packing (n=63)
[2]Total lactate concentration approx. twice L-lactate
[a,b,c] significant difference among treatments within forage

**Table 2.** Fermentation weight loss (g/kg)[1] during the ensiling period across forage types (F) (n=36)

| Weight loss | | Day 1 | Day 4 | Day 11 | Day 28 | Day 50 | Day 113 |
|---|---|---|---|---|---|---|---|
| Treatment (T) | 0 | 0.37$^{a}$ | 3.24$^{a}$ | 5.79$^{a}$ | 8.88$^{a}$ | 12.79$^{a}$ | 15.52$^{a}$ |
| | 1× | 0.57$^{a}$ | 3.09$^{a}$ | 4.61$^{b}$ | 7.38$^{b}$ | 10.93$^{b}$ | 13.91$^{ab}$ |
| | 4× | 1.07$^{b}$ | 3.00$^{a}$ | 4.51$^{b}$ | 6.90$^{b}$ | 10.32$^{b}$ | 12.92$^{b}$ |
| P value | T | *** | 0.5 | * | ** | *** | ** |
| | F×T | * | ** | * | * | ** | * |

[a,b] significant difference among treatments within day [1] Fresh matter basis

**Conclusion** Physical processing of forages increased silage density and affected fermentation weight loss from the vacuum bag silages. Fermentation quality was also improved favorably due to shredding.

**References**

Dunière, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I. & Thévenot-Sergentet, D. (2013) Silage processing and strategies to prevent persistence of undesirable microorganisms. Animal Feed Science and Technology, 182, 1-15.

Parveen, I., Threadgill, M.D., Moorby, J.M. & Winters, A. (2010) Oxidative phenols in forage crops containing polyphenol oxidase enzymes. Journal of agricultural and food chemistry, 58, 1371-1382.

# Effect of shredlage maize harvesting technology on fermentation parameters, packing densities and aerobic stability of maize crop ensiled in bunker silos

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**Keywords:** aerobic stability, fermentation quality, packing density, shredlage

**Introduction** During the past few years, a novel system of harvesting whole plant maize silage (WPMS) labelled “shredlage” was developed and applied in the United States. “Shredlage” is harvested with a self-propelled forage harvester equipped with after-market cross-grooved kernel-processing rolls. Additionally in the system “shredlage” the self-propelled forage harvester is set to a longer theoretical length of cut of 26 to 30 mm compared to commonly used 6-10 mm. Older research (Leurs; 2006) states that increased particles length did not affect fermentation quality remarkably but decreased the ability to pack WPMS chopped at longer chop lengths. This study reports about the impact of thhis new harvest technology on fermentation quality, packing densities and aerobic stabilities of the ensiled WPMS on farm scale size bunker silos compared with conventionally processed maize silage.

**Material and Methods** In two consecutive years at two research institutes in the north-west (“Riswick”) and the south of Germany (“Achselschwang”) WPMS was harvested with two self-propelled forage harvesters parallel either as “shredlage” (Shred) or conventionally processed maize silage (Conv) with theoretical length of cut of 7 mm. Each harvesters roll clearance was set to 1 mm. The freshly harvested material at both locations and in both years was ensiled into separate bunker silos. Packing and distributing work at Riswick was done with wheel loaders of app. 15t added with a 12t weighing tractor for each bunker in 2015. In 2016 the additional packing tractor of 12t for Shred was replaced by a Claas Xerion weighing 23t. In Achselschwang in 2015 packing and distributing tractors weighing 10,5 t for Conv and 11,5 t for Shred were used. In 2016 Shred was packed with two vehicles weighing 10,8 and 19,2 t; Conv was packed with one vehicle of a weight of 13,9t. After a storage period of at least 7 weeks samples were taken from multiple points of each bunker to determine the nutrients and fermentation parameters of the different silages. During feed out phase measurements for packing densities and silage temperatures were carried out to cover aerobic stability development at the bunker surface. The following Table 1 shows averaged the fermentation products of the WPMS harvested either as Shred or Conv on the two different locations of the two years. The range of analyse conducted varied on the two research sides caused the partly missing data for $NH_3$-N and ethanol.

**Table 5** Impact of harvest technology on fermentation quality of maize WPMS (Riswick: 2015: n=1, 2016: n=1; Achselschwang: 2015 n=6, 2016: n=8)

| | | Riswick | | Achselschwang | |
|---|---|---|---|---|---|
| | | Conv | Shred | Conv | Shred |
| Dry matter (DM) | g/kg fresh matter (FM) | 350 ± 4 | 359 ± 6 | 344 ± 6 | 344 ± 5 |
| Lactic acid | g/ g DM | 54 ± 8 | 45 ± 3 | 55 ± 11 | 56 ± 12 |
| Acetic acid | g/kg DM | 11 ± 2 | 10 ± 2 | 15 ± 1 | 17 ± 2 |
| pH | | 3,9 ± 0,1 | 3,9 ± 0,1 | 3,8 ± 0,0 | 4,0 ± 0,1 |
| $NH_3$-N | % of total N | 7,0 ± 1,1 | 6,7 ± 0,4 | n.d. | n.d. |
| Ethanol | g/kg DM | 7 ± 2 | 5 ± 1 | n.d. | n.d. |

n.d. = not determined

In Table 2 the averaged packing densities depending on harvest technology and the location are displayed. With this figures it has to be considered that conclusions were taken from first years' results and packing machinery of increased weight was used for the packing work for Shred in the second year to realize sufficient packing especially for the top layer of the bunker.

**Table 6** Impact of harvest technology on packing density (Riswick: 2015: n=1, 2016: n=2; Achselschwang: 2015 n=6, 2016: n=4)

| | | Riswick | | Achselschwang | |
|---|---|---|---|---|---|
| | | Conv | Shred | Conv | Shred |
| Top | kg DM/m³ | 198 ± 23 | 156 ± 11 | 222 ± 23 | 210 ± 27 |
| Mid | kg DM/m³ | 264 ± 14 | 251 ± 3 | n.d. | n.d. |
| Bottom | kg DM/m³ | 280 ± 24 | 276 ± 14 | 237 ± 32 | 234 ± 34 |

n.d. = not determined

During the feed out phase of the ensiled WPMS the temperature development at the face of the bunkers was monitored on a regular base (see Table 3) by measuring ambient temperatures and the temperatures 40 - 50 cm behind the face in the top/middle area of the bunkers. At "Riswick" in both years of the trial a chemical additive was used to ensure aerobic stability. At "Achselschwang" only in the second year both, Conv and Shred WPMS were treated with an inoculant to improve aerobic stability.

**Table 7** Average temperatures during feed out phase of ensiled WPMS

| | | Riswick | | Achselschwang | |
|---|---|---|---|---|---|
| | | 2015 (n = 20) | 2016 (n = 13) | 2015 (n = 11) | 2016 (n = 12) |
| Ø ambient | °C | 13,3 | 8,3 | 23,8 | 10,7 |
| Ø Conv | °C | 17,1 | 15,9 | 21,0 | 9,5 |
| Ø Shred | °C | 17,8 | 14,9 | 23,0 | 9,6 |

**Results and Discussion** Independent if the WPMS was harvested as Shred or Conv the analysed parameters for fermentation quality showed comparable results for lactic- and acetic acid and nearly identical pH values were found (Ferraretto et al. 2012). Additional parameters such as $NH_3$-N and ethanol were only determined at the "Riswick" side but these too did not differ to indicate any impact of the harvest system on the fermentation process of WPMS. Packing densities showed comparable results for Shred and Con in the bottom and mid layer of the bunkers. Results for the top layers differed between the research sides but showed more or less clear a reduced density for Shred. The increase of particle length tents to decrease the ability to get maize packed properly (Leurs 2006). In the second years trials at both sides the weight of packing vehicles compacting the Shred WPMS was increased as a result from first years lower packing densities of the top layers. With a sufficient feed out rate of > 1.5m per Week in trials conducted early in the year with low ambient temperatures no differences in average temperatures of the differently harvested silages were found.

**Conclusion** Even with big differences in particle size due to the novel processing system "shredlage", this system did not affect the fermentation process and led to comparable compositions of fermentation parameters. Increased packing weight on Shred could not entirely eliminate the reduced the ability to pack the bigger particles and thus a sufficient weekly feed out is crucial to prevent aerobic deterioration especially in the warmer month of the year.

**References**

Ferraretto, L.F. & Shaver, R.D. (2012) Effect of corn shredlage on lactation performance and total tract starch digestibility by dairy cows. Professional Animal Scientist, 28, 639-647.

Leurs, K. (2006) Einfluss von Häcksellänge, Aufbereitungsgrad und Sorte auf die Siliereigenschaften von Mais. Forschungsbericht Agrartechnik des Arbeitskreises Forschung und Lehre der Max-Eyth-Gesellschaft Agrartechnik im VDI, 438, 68.

# Effects of different moisture levels on fermentation quality and aerobic stability of sweet potato residue TMR silage

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**Keywords:** aerobic stability, fermentation quality, moisture level, microorganism, TMR silage

**Introduction** Sweet potato residue (SPR) is a by-product produced in the production of SPR beverage, which contains high concentration of starch and fibre. However, its high moisture content results in undesirable fermentation and effluent production. There is a common practice to preserve high-moisture by-products with dry feeds as total mixed ration (TMR) silage in recent years. As moisture affects TMR preparation, we hypothesized that its concentration has an effect on the fermentation profile and microorganisms counts, which affect fermentation quality and aerobic stability of TMR silage. The purpose of this study was to evaluate the effects of different moisture levels on the fermentation quality and aerobic stability of TMR silage with SPR, by detecting dynamics of temperature and microorganism counts during aerobic exposure.

**Materials and Methods** TMR were formulated with alfalfa hay, SPR, corn meal, soybean meal, a vitamin-mineral supplement and molasses in a ratio of 40:30:18:9:1:2 on a dry matter (DM) basis. The hay were chopped to a length of 1 to 2 cm with a manual forage chopper prior to ensiling. The SPR was obtained from a local juice factory in Beijing and used within 12 h of production. TMR was prepared in three independent batches and the moisture level of each batch was adjusted to 450 g/kg (L), 600g/kg (M) and 750g/kg (H) to obtain the treatments of this experiment. Approximately 2 kg of TMR were packed into each plastic film bag and the bags were degassed, sealed with a vacuum packing machine, and stored at ambient temperature (17°C to 22°C). All bags were opened 150 d after ensiling, the chemical composition and fermentation quality were determined. Then TMR silages from each bag were mixed thoroughly and placed into separate sterile 10 L polyethylene barrel without compaction, which was kept uncovered and stored at ambient temperature (20°C to 30°C) until it had deteriorated. The temperatures of ambience and silages were automatically monitored at 1 h intervals by a thermocouple wire connected to a data logger, and changes of pH and microorganism population of TMR silage were analysed every two days during aerobic exposure. All the ingredients measured in this experiment were according the procedure described by Hu et al. (2015). Results obtained from the experiment were subjected to one way analysis of variance using the general linear model procedure of the SAS (2004) software package, the significance of difference was tested by Tukey's test and was declared at $p<0.05$.

**Results and Discussion** Fermentation characteristics and microbial counts of TMR silage after 150 d of conservation are shown in Table 1. Moisture level affected the fermentation quality significantly ($P<0.001$). The pH of three treatments decreased to below 4.2 and the minimum value was 3.79 in M treatment. L and M treatments obtained higher concentrations of lactic acid and lower ammonia nitrogen which showed better fermentation quality than H treatment. There was a significant difference ($P<0.001$) in the content of residue WSC between different treatments. The microorganism population was inhibited in varying degrees after ensiling, especially yeast counts receded to levels below the detection limit. Changes in microbial counts and pH during aerobic deterioration of TMR silages are shown in Figure 1. Deterioration occurred in the H, M, and L treatments at 3.7d, 18.4d, 39.8d, respectively, significantly affected by the moisture level ($P<0.05$). During the aerobic exposure, aerobic bacteria counts in H treatment showed a large increase before the aerobic deterioration occurred while the yeast counts was still not to be detected. However, a large increase of aerobic bacteria counts in L and M did not results in aerobic deterioration until the yeast counts reached to $10^5$ cfu/g FM, which agree with the study that yeast counts in excess of $10^5$ cfu/g FM are likely to

reduced silage aerobic stability (Borreani and Tabacco 2010). In addition, higher residue WSC concentrations may be one of the reasons for H treatment's rapidly deterioration, because it potentially provided more available substrate for the growth of aerobic microflora when TMR silages are exposed to air. The relationship between aerobic bacteria counts and aerobic stability of high moisture level TMR silage needs further study.

**Conclusion** The data presented here confirm that SPR can be utilized effectively by incorporating it into TMR silages. L and M treatments showed good fermentation quality and aerobic stability. High moisture level results in the decline of fermentation quality and aerobic stability of the TMR silage. Aerobic deterioration occurred of L and M treatments mainly caused by yeast, while H treatment's deterioration mainly caused by aerobic bacteria.

**Table 1**. Fermentation characteristics and microbial counts of TMR silage after 150 d of ensiling.

| Items | L | M | H | SEM | P-value |
|---|---|---|---|---|---|
| pH | $3.86^{B}$ | $3.79^{C}$ | $4.10^{A}$ | 0.007 | <0.001 |
| Lactic acid (g/kg DM) | $36.9^{B}$ | $42.4^{A}$ | $25.0^{C}$ | 0.109 | <0.001 |
| Acetic acid (g/kg DM) | $2.4^{C}$ | $7.0^{B}$ | $13.9^{A}$ | 0.036 | <0.001 |
| Propionic acid (g/kg DM) | ND | 0.4 | 5.5 | - | - |
| Butyric acid (g/kg DM) | ND | ND | ND | - | - |
| Ammonia nitrogen(% TN) | $1.42^{C}$ | $2.48^{B}$ | $6.06^{A}$ | 0.481 | <0.001 |
| DM (g/kg FM) | $507^{A}$ | $356^{B}$ | $216^{C}$ | 1.048 | <0.001 |
| WSC (g/kg DM) | $83.3^{B}$ | $69.8^{C}$ | $114.0^{A}$ | 3.646 | <0.001 |
| CP (g/kg DM) | 168 | 167 | 166 | 1.907 | 0.612 |
| Lactic acid bacteria ($log_{10}$ cfu/g FM) | $5.29^{C}$ | $7.84^{B}$ | $8.08^{A}$ | 0.076 | <0.001 |
| Aerobic bacteria ($log_{10}$ cfu/g FM) | 3.06 | 3.26 | 3.28 | 0.101 | 0.132 |
| Yeasts ($log_{10}$ cfu/g FM) | ND | ND | ND | - | - |

$^{A–C}$ Means in the same row with different superscripts differed (P < 0.05);
L, moisture level-450 g/kg; M, moisture level-600 g/kg; H, moisture level-750 g/kg;
DM, dry matter; FM, fresh matter; WSC, water soluble carbohydrate; CP, crude protein; ND, not detected; cfu, colony-forming units.

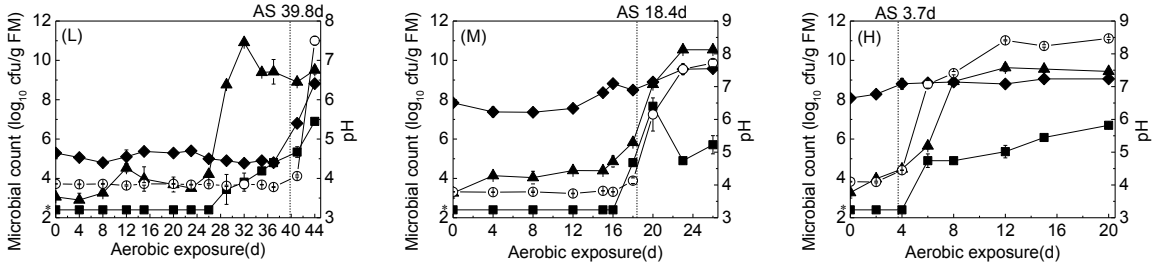


**Figure 1.** Changes in lactic acid bacteria (◆), yeast (■), aerobic bacteria (▲) counts and pH(○) during aerobic deterioration of TMR silages. (L), moisture level-450 g/kg; (M), moisture level-600 g/kg; (H), moisture level-750 g/kg; AS, aerobic stability; *, 2.40, i.e., $log_{10}$ 250 cfu/g, a microbial count below the detection limit was assigned a value corresponding to half of the detection level. Points indicate mean values of triplicate samples, with standard deviations represented by vertical bars. cfu, colony forming units; FM, fresh matter.

**References**

Borreani, G. & Tabacco, E. (2010) The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. Journal of Dairy Science, 93, 2620-2629.

Hu, X.D., Hao, W., Wang, H.L., Ning, T.T., Zheng, M.L. & Xu, C.C. (2015) Fermentation Characteristics and Lactic Acid Bacteria Succession of Total Mixed Ration Silages Formulated with Peach Pomace. Asian-Australasian journal of animal sciences, 28, 502-510.

# Effects of lactic acid bacteria inoculants on fermentation quality and aerobic stability of sweet potato residue TMR silage

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**Keywords:** aerobic stability, fermentation, sweet potato residue, TMR silage

**Introduction** Sweet potato residue, obtained after the production of sweet potato juice, is one of the agricultural by-products that are available in China. Sweet potato residue usually is high in starch, neutral detergent fiber (aNDF) and vitamins, suggesting that sweet potato residue may have potential as a ruminant feed. However its high-moisture content promotes undesirable fermentation and effluent productions. There is an increasing practice to preserve these high-moisture by-products with dry feeds as a total mixed ration (TMR) silage to alleviate the labor prior to feeding rations and may help efficient use of unpalatable or nutritionally unbalanced by-products (Wang, 2018). The objective of this study is to compare the effect of lactic acid bacteria (LAB) inoculants on fermentation quality and aerobic stability of SPR TMR silage.

**Materials and Methods** Sweet potato residue was obtained from a factory and used within 12h of production. The TMR were prepared using alfalfa hay, sweet potato residue, maize meal, soybean meal and molasses in a ratio of 40:30:18:10:2 on a dry matter (DM) basis. Experimental treatments were control (deionized water, CK), inoculated with *Lactobacillus plantarum* (LP) and *Lactobacillus amylovorus* (LA) at a concentration of $10^6$ colony forming units (CFU) per gram of fresh matter (FM). Approximately 900g of pre-ensiled TMR was packed into a plastic film bag silo, degassed and sealed with a vacuum packing machine, then stored in room (20℃ to 25℃). Triplicate silos were opened on days 56 of ensiling for sampling and determining the ensiling characteristics and aerobic stability, according to the methods described by Wang et al. Data were subjected to analysis of variance and statistical significance between means was determined by Duncan multiple comparison. Differences were considered significant when probability was less than 0.05.

**Results and Discussion** TMR silages had DM contents of 54.9%-55.4%. Crude protein (CP), aNDF and acid detergent fiber (ADF) contents had no significantly changes (P>0.05). The LP and LA had lower water soluble carbohydrate contents (P<0.0001) (Table 1).

**Table 1** Chemical composition, fermentation quality and microbial composition of 56-d TMR silages

| Item | Treatments | | | SEM | P-value |
|---|---|---|---|---|---|
| | CK | LP | LA | | |
| Chemical composition(%DM) | | | | | |
| DM(%) | 55.4 | 55.0 | 54.9 | 0.15 | 0.432 |
| CP | 15.7 | 15.8 | 15.8 | 0.02 | 0.178 |
| WSC | 4.8[a] | 4.0[b] | 4.1[b] | 0.13 | <0.0001 |
| aNDF | 29.0 | 27.8 | 27.5 | 0.42 | 0.347 |
| ADF | 21.8 | 21.4 | 21.3 | 0.17 | 0.516 |
| Fermentation quality | | | | | |
| pH | 4.12[a] | 4.08[b] | 4.10[ab] | 0.007 | 0.045 |
| Lactic acid (%DM) | 3.41[b] | 4.53[a] | 4.39[a] | 0.186 | 0.001 |
| Acetic acid (%DM) | 0.90 | 0.87 | 0.99 | 0.054 | 0.724 |
| $NH_3$-N (%TN) | 1.40[a] | 0.74[c] | 0.95[b] | 0.106 | <0.0001 |
| Microbial counts (log cfu /g FM) | | | | | |
| Lactic acid bacteria | 7.4[a] | 6.3[c] | 6.9[b] | 0.16 | <0.0001 |
| Yeasts | ND | ND | ND | - | - |

DM, dry matter; CP, crude protein; WSC, water soluble carbohydrates; aNDF, neutral detergent fiber; ADF, acid detergent fiber; $NH_3$-N, ammonia-N; TN; total nitrogen; ND, not detected SEM, standard error of mean; Value with different superscript letters (a-c).

All the TMR silages were well preserved, as indicated by low pH ($P<0.05$), ammonia nitrogen ($NH_3$-N) contents ($P<0.0001$) and high lactic acid contents ($P<0.01$). Compared with CK, the two LAB inoculated can decrease pH, $NH_3$-N contents and increased lactic acid contents. Propionic acid and butyric acid were not found. The LP had lower LAB populations and the populations of yeasts in TMR silages were all below the detection limit. *L. plantarum* and *L. amylovorus* were both homofermentative LAB, which could encourage rapid pH decline and improve silage conservation by inhibiting some harmful and disadvantage microbiological activities. Figure 1 and Figure 2 showed the aerobic stability and change in temperature of TMR silages during aerobic exposure.

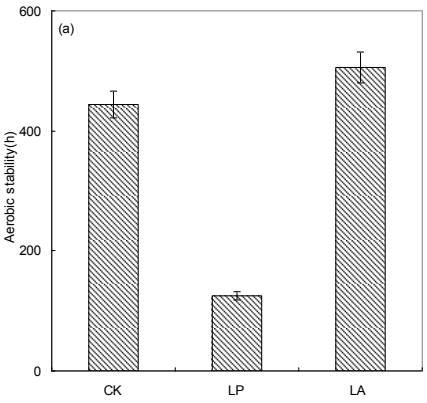


**Figure 1** Aerobic stability of TMR silages treated with *Lactobacillus plantarum*(LP), *Lactobacillus amylovorus*(LA) and the control(CK).

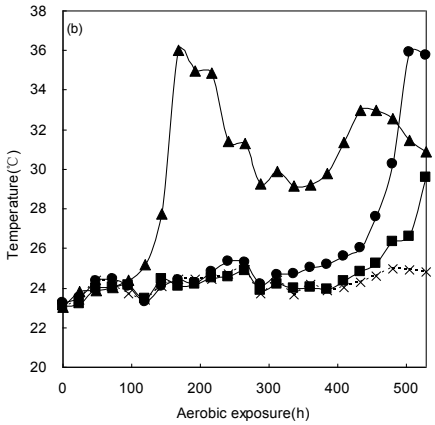


**Figure2** Change in temperature of TMR silages treated with *Lactobacillus plantarum*(LP), *Lactobacillus amylovorus*(LA) and the control(CK) during aerobic exposure. CK(●); LP(▲); LA(■); Room temerature(×).

The aerobic stability of TMR silages were 444 (CK), 125 (LP), and 506 (LA) hours respectively. CK and LA exhibited higher stability and no distinct heating was found within 12 days. LP showed lower stability and heating appeared earlier. Compared with control, silage treated with *L. amylovorus* improved the aerobic stability. Previous studies showed that LAB can improve silage aerobic stability by producing different metabolites, such as acetic acid and 1,2-propanediol, 3-phenyllactic acid and 3-hydroxydecanoic acid, and a phenolic-related antibiotic (Guo 2018). In this study, LA showed better aerobic stability might relate to the higher acetic acid contents after ensiling, because acetic acid could improve aerobic stability of silage by inhibiting spoillage organisms. Further research need to be about the metabolites of *L. amylovorus* which could improve aerobic stability during ensiling.

**Conclusions** Addition of *Lactobacillus plantarum* and *Lactobacillus amylovorus* improved the fermentation quality of TMR silage, and *Lactobacillus amylovorus* showed longer aerobic stability.

**References**

Guo, X.S., Ke, W.C., Ding, W.R., Ding, L.M., Xu, D.M., Wang, W.W., Zhang, P. & Yang, F.Y. (2018) Profiling of metabolome and bacterial community dynamics in ensiled Medicago sativa inoculated without or with *Lactobacillus plantarum* or *Lactobacillus buchneri*. Scientific reports, 8, 357.

Wang, H.L., Hao, W., Ning, T.T., Zheng, M.L. & Xu, C.C. (2018) Characterization of culturable yeast species associating with whole crop corn and total mixed ration silage. Asian-Australasian journal of animal sciences, 31, 198.

# Effects of moisture and *L. buchneri* on the conservation and ruminal degradability of high moisture corn and snaplage

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**Keywords:** aerobic stability, ear corn, fermentation, proteolysis

**Introduction** Ensiling high moisture corn grains (HMC) with cob, husks and shank (= snaplage) is a feasible strategy for harvesting, storing and processing corn grains in beef and dairy farms. At kernel black-layer stage (recommended for harvest HMC), cob and husks have higher content of water than grains, leading to higher moisture in snaplage than in HMC. Hence, higher moisture content in snaplage would stimulate fermentation, breakdown of the protein matrix and, ultimately, increase starch availability for digestion. On the other hand, it has been claimed that snaplage is even more prone to aerobic deterioration than HMC. Therefore, the objective of this work was to compare the conservation (fermentative losses and aerobic stability) and ruminal degradability of snaplage and HMC harvested at two maturities (32% and 37% of moisture) and treated or not with *Lactobacillus buchneri*.

**Materials and Methods** A plot of flint corn (hybrid DKB 390 PRO 2) seeded during the second crop in 2017 at the Experimental Farm of the State University of Maringá was harvested on three dates to obtain HMC and snaplage with 32% or 37% of moisture. The HMC with 37% of moisture was harvested on June 12, HMC with 32% of moisture and snaplage with 37% of moisture on June 19, and snaplage with 32% of moisture on June 27. In the snaplage harvested with 32% of moisture, the grain fraction had 25% of moisture. After harvesting, HMC and snaplage were treated or not with *L. buchneri* at $4 \times 10^5$ cfu/g of fresh matter (LB) and ensiled in mini-bags sealed under vacuum (1 kg per silo, 3 replicates). After 90 d of storage, silages were sampled for measuring fermentative losses, aerobic stability, and ruminal in situ degradability. The aerobic stability was defined as the time elapsed before silage and ambient temperature (24±1.5°C) differed by more than 2°C. For measuring the ruminal degradability, samples were dried at 55°C for 72 h, ground through a 5-mm screen (Wiley mill) and placed (5 g) in nylon bags (10 × 20 cm). Afterwards, bags were incubated in the rumen ventral sac of a cannulated cow for 12 h.

Data were analyzed as a split-plot arrangement using the MIXED procedure of SAS. Grain source (HMC or snaplage) was assigned to main-plot, whereas moisture (32% or 37%), LB (with or without), and interactions were in sub-plot. Means were compared by Tukey's test ($\alpha = 0.05$).

**Results and Discussion** There were two- or three-way interactions for all variables evaluated (Figure 1). Lower moisture content decreased whereas LB increased DM loss during fermentation in both HMC and snaplage. Applying LB increased the pH in HMC, but surprisingly, not in snaplage. Higher pH in silages treated with LB is a common finding (Kung et al. 2007), due to the conversion of lactic to acetic acid and 1,2-propanediol (Oude Elferink et al. 2001). The LB markedly increased the aerobic stability of HMC with 32% or 37% of moisture and snaplage with 37% of moisture. However, the improvement in aerobic stability induced by LB was not significant in snaplage harvested with lower moisture (32%). It has been observed in the field that snaplage is even more prone to aerobic deterioration than HMC. In the current trial, differences in aerobic stability among untreated HMC and snaplage were only numerical, although the snaplage harvested with 32% of moisture was unresponsive to LB inoculation. Snaplage is comprised of three fractions: grains, cob and husks; hence, snaplage has higher fiber content than HMC. In our study, snaplage had 77-80% of grains. Therefore, DM degradability was 18% higher for HMC than snaplage (on average). Harvesting snaplage with lower moisture (32%) decreased DM degradability by 10%. Applying LB led to a numerial increase (3.5%) in DM degradability, in both HMC and snaplage.

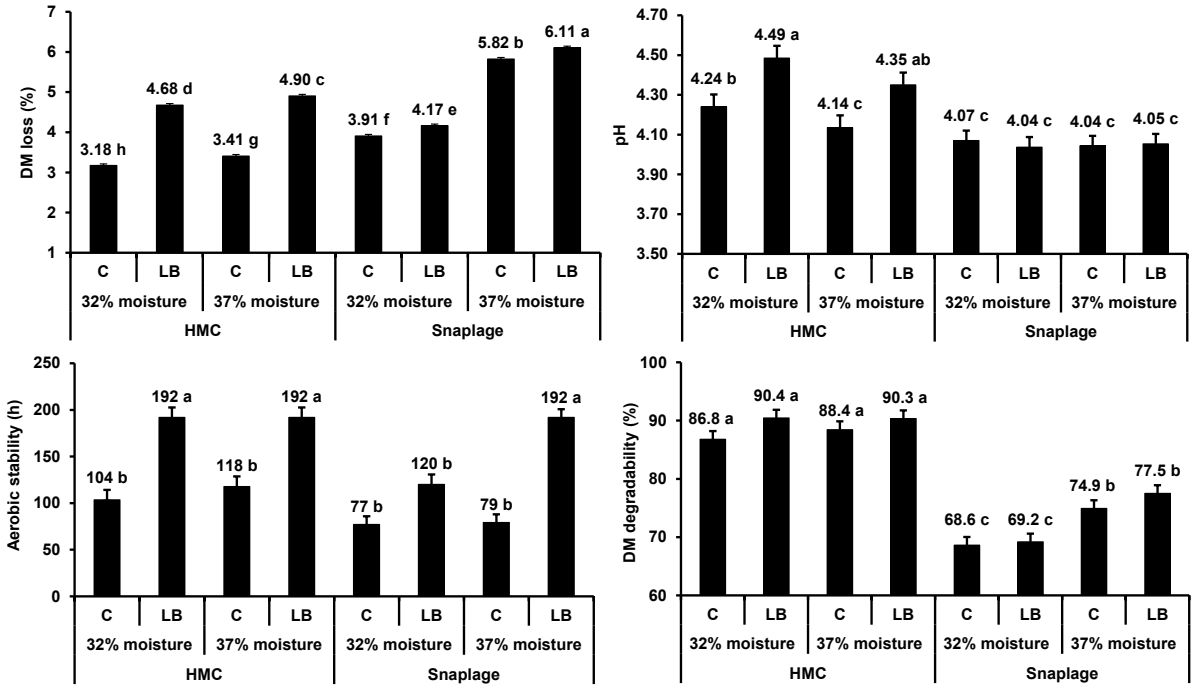


**Figure 1** Dry matter loss, pH, aerobic stability and ruminal in situ dry matter (DM) degradability (12 h) of high moisture corn and snaplage ensiled with 32% or 37% of moisture and treated or not with *L. buchneri* at 4 × $10^5$ cfu/g. $^{a,b,c,d,e,f,g}$Means with different superscripts differ ($P < 0.05$).

**Conclusions** In terms of conservation (fermentation and aerobic stability) and ruminal degradability there was no difference between HMC ensiled with 32% or 37% of moisture, whereas harvesting HMC with 32% of moisture would be more desirable for grain threshing in field. Harvesting snaplage with lower moisture (32%) is not recommended, due to the lower DM degradability and unresponsiveness to inoculate application. Treating HMC (32% and 37% of moisture) and snaplage with 37% of moisture with *L. buchneri* at 4 × $10^5$ cfu/g is a feasible strategy to improve aerobic stability.

## References

Kung, L., Jr., Schmidt, R.J., Ebling, T.E. & Hu, W. (2007) The effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of ground and whole high-moisture corn. Journal of Dairy Science, 90, 2309-2314.

Oude Elferink, S.J., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F. & Driehuis, F. (2001) Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Applied and Environmental Microbiology, 67, 125-132.

# Effects of processing, moisture and length of storage on the fermentative losses and ruminal degradability of reconstituted corn grain silage

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**Keywords:** fermentation, flint corn, proteolysis, starch digestibility

**Introduction** The starch-protein matrix in corn kernels is a physicochemical impairment to starch digestion in ruminants. Ensiling corn grain with original (HMC) or reconstituted (RCS) moisture causes degradation of endosperm hydrophobic proteins during the fermentation process, mainly due to the bacterial activity. The use of baggers with a mounted roller mill to processing and store (at the same time) HMC or RCS is increasing in Brazil. However, rolled corn typically has a higher particle size than corn grounded with a hammer mill. Particle size of corn grain is inversely related to ruminal starch degradability, especially in hybrids with higher proportion of vitreous endosperm. Additionally, particle size affects water holding capacity during the rehydration of RCS. Therefore, the aim of this work was to determine the effects of processing (rolling or grounding), moisture content and length of storage on the fermentative losses and ruminal degradation of RCS. We hypothesized that higher moisture content and longer storage periods would offset, at least partially, the effect of particle size in RCS.

**Material and Methods** Corn kernels with flint endosperm were processed using a hammer mill (5-mm screen) or a roller mill, rehydrated with distilled water to achieve 30%, 35% or 40% of moisture, and stored for 0, 14, 30, 60, 90, 120 and 180 d in vacuum-sealed bags (500 g per silo, four replicates). Treatments were arranged as a 2 × 3 factorial design with repeated measures over time (7 times) (168 bags in total). After each storage period, silos were weighted to compute fermentative losses and samples were oven-dried at 60°C for 72 h. Afterwards, all dried samples (5 g) were placed in nylon bags (10 × 20 cm) and incubated in the rumen ventral sac of a cannulated cow for 12 h, to determine the ruminal degradation. Data were analyzed as repeated measuring using the Mixed procedure of SAS. The model included the fixed effects of processing (rolled or ground), moisture (30, 35 or 40%), length of storage (0 to 180 d), and their interactions. The effect of silo nested within processing × moisture was defined as an error term. Covariance structures were chosen based on the Akaike information criterion.

**Results and Discussion** Rolled corn grains had 2.34 mm whereas ground corn grains had 1.30 mm of mean particle size. Rolled-RCS had lower fermentative losses than ground-RCS, independently of moisture content. On the other hand, ground-RCS with 30% moisture had higher fermentative losses than ground-RCS with 35% or 40% of moisture. The higher particle size of rolled grains might have restricted substrate availability for fermentation, leading to lower losses. On the other hand, the lower moisture content associated with a higher availability of substrate in ground-RCS with 30% of moisture may have favored the development of undesirable microorganisms (e.g. yeasts) and turned the fermentation processes less efficient, with higher DM loss. As expected, ruminal degradability increased with the fermentation progress in all treatments, mainly during the first 60 d of storage. In both, rolled and ground grains, 35% and 40% moisture led to higher ruminal degradation than 30% moisture. Nonetheless, contrary to our hypothesis, rolled corn did not achieve the level of ruminal degradability of ground corn regardless the moisture content and storage period.

**Conclusion** Rolled-RCS did not achieve the ruminal degradability of ground-RCS regardless the moisture content and length of storage. Therefore, ground-RCS might be used more efficiently by animals. On the other hand, grinding rate (ton/h) is likely higher in a roller mill. The decision on rolling or ground before ensiling will depends on the animal physiological status, diet composition (e.g. starch and effective fiber contents), and grinding cost. In both cases, it is recommended storing RCS for at least 2 months before feeding.

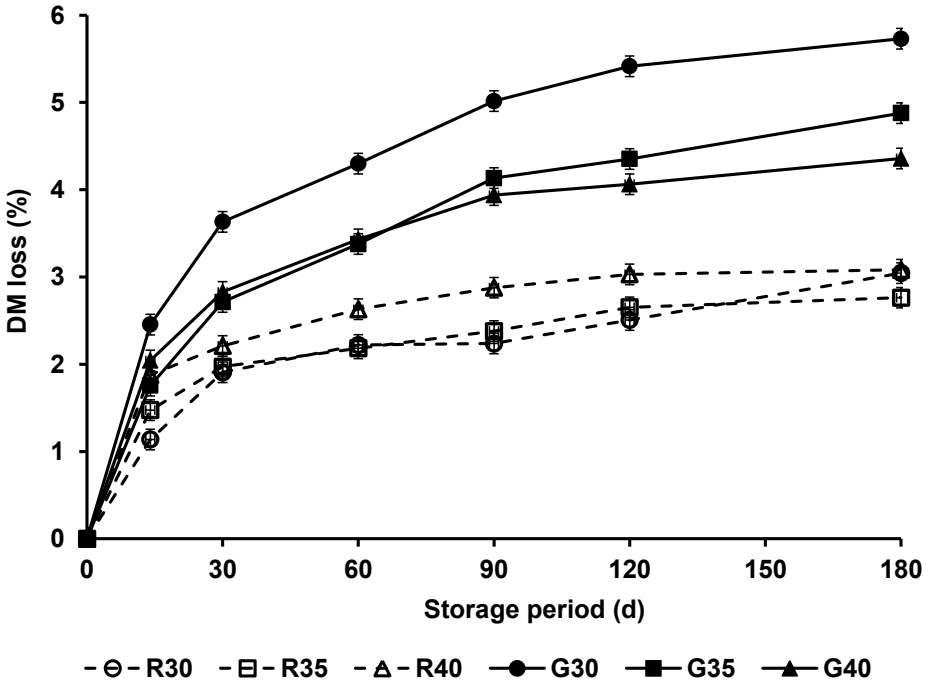


**Figure 1.** Dry matter (DM) loss in reconstituted corn grain silages. R: rolled; G: ground; 30, 35 and 40: moisture percentage; $P < 0.01$ for processing × moisture × storage period interaction effect.

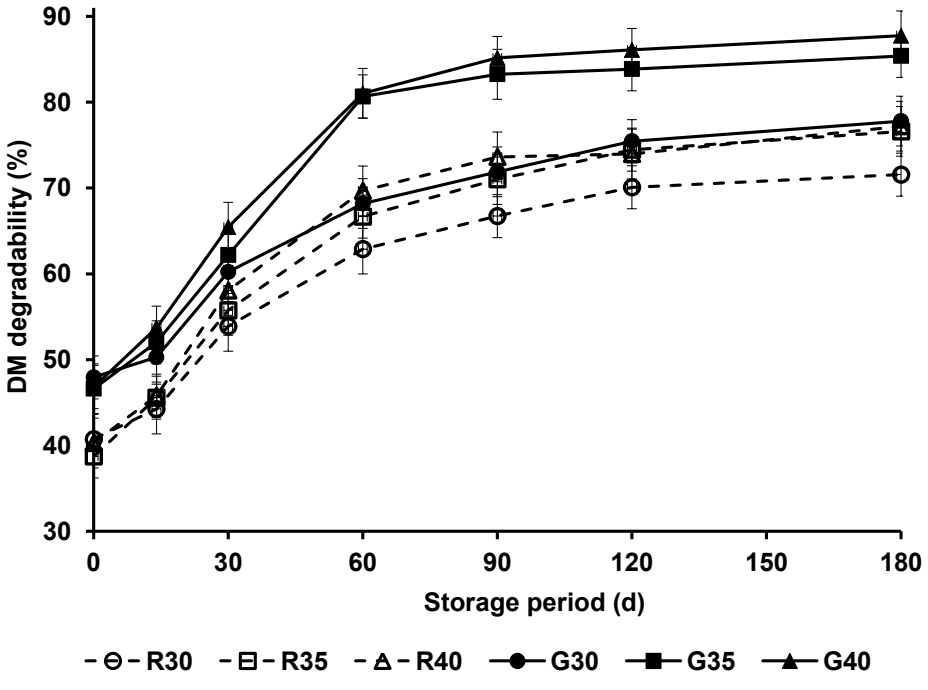


**Figure 2.** Ruminal in situ dry matter (DM) degradability of reconstituted corn grain silages incubated for 12 h. R: rolled; G: ground; 30, 35 and 40: moisture percentage; $P < 0.01$ for processing effect, $P < 0.01$ for moisture effect, $P < 0.01$ for storage period effect, $P \geq 0.17$ for interactions.

# Effects of relocation and microbial inoculants on microbial population and aerobic stability of corn silage

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**Keywords:** aerobic deterioration, dry matter loss, *Lactobacillus buchneri*, *Lactobacillus plantarum,* re-ensiling

**Introduction** Relocation of corn silage is a common practice adopted by the livestock farmers in different regions of the world (Chen and Weinberg 2014). The main reasons are the commercialization of silages or the distance from the silo to the barn. However, corn silage is highly susceptible to aerobic deterioration which would difficult the relocation process. In that case, the exposure time to air in the silage relocation would increase the populations of yeasts and compromise silage quality (Pahllow et al. 2003). Based on that, the objective of this study was to evaluate the effect of exposure time to air in the relocation and the application of microbial inoculants on the lactic acid bacteria, yeasts, and molds counts, and aerobic stability of corn silage.

**Materials and Methods** The experiment was conducted in a completely randomized design, with a 4×6 factorial, with four replications. Studied factors were: microbial inoculant (without inoculant (WI); *Lactobacillus plantarum* + *L. buchneri* (LPLB); *L. plantarum* + *Propionibacterium acidipropionici* (LPPA); and *L. buchneri* (LB)) and time spent with relocation (TR); (non-relocated, 12, 24, 36, 48 and 60 h of exposure before re-ensiling). The hybrid Pioneer 4285 was harvested at the 2/3 milk-line stage (~30% dry matter). Fresh-chopped forage was treated or not with microbial inoculants, at a final application rate of $1\times10^5$ cfu/g fresh forage and packed in 20-L plastic buckets (~550 kg/m$^3$). After 120 d after ensiling, the silages were exposed during the different times previously described (except the 0 h) and re-ensiled in a new bucket. Relocated and non-relocated silages were opened after 90 and 210 days, respectively. We evaluated the pH, temperature and populations of yeasts and molds in the silages after the times spent with relocation. Those same parameters plus the populations of lactic acid bacteria (LAB) and aerobic stability were evaluated in the relocated silages after opening. All data was subjected to ANOVA, including the fixed effects of microbial inoculant (MI), exposure time to air in the silage relocation (TR) and the interaction (MI×TR), and the means were compared by Tukey´s test (α=0.05).

**Results** There was interaction effect on the populations of yeasts and molds in the silages before relocation (P<0.05). Silages treated with LPLB had lower (P<0.05) molds counts (1.0 log cfu/g) than WI-silages (1.7 log cfu/g) when the TR was 24 hours. The molds count decreased when the TR increased. The highest yeasts counts were observed in LPPA-treated silages (5.0 log cfu /g) when the TR was 48 hours. The silages treated with LB consistently decreased (P<0.05) yeasts counts compared with the WI-silages when the TR was 12 h. The highest temperature (P<0.05) was observed when the TR was 60 hours.

The number of LAB (average 4.2 cfu/g) was not different after opening of the relocated silages. Relocated silages treated with LPLB and LB showed lower (P<0.05) molds counts than when the TR was 36 (3.6 and 3.4 log cfu/g, respectively), 48 (3.8 and 3.3 log cfu/g, respectively) and 60 h (3.3 and 3.3 log cfu/g, respectively). The highest (P<0.05) yeasts counts was observed in the relocated silages treated with LPPA, independently of the TR. Silages treated with LB had lower (P<0.05) aerobic stability independently of the TR. Aerobic stability increased (P<0.05) when the TR was 12, 24, 36, 48 and 60 compared with non-relocated silages (Table1).

**Table 1.** Aerobic stability of corn silages treated or untreated with microbial inoculants, relocated or non-relocated

| Inoculation | Exposure time to air in the silage relocation (hours) | | | | | | EPM | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 12 | 24 | 36 | 48 | 60 | Average | 9,29 | <0,05 | <0,05 | 0,08 |
| WI | 122 | 137 | 168 | 168 | 168 | 165 | 155$^{A}$ | | | | |
| LPLB | 105 | 166 | 142 | 141 | 168 | 168 | 148$^{AB}$ | | | | |
| LPPA | 70 | 149 | 143 | 139 | 160 | 157 | 136$^{B}$ | | | | |
| LB | 71 | 94 | 97 | 116 | 103 | 123 | 101$^{C}$ | | | | |
| Average | 92$^{b}$ | 136$^{a}$ | 138$^{a}$ | 141$^{a}$ | 150$^{a}$ | 153$^{a}$ | | | | | |

**Discussion** The results showed the typical silage deterioration where the populations of yeasts start the aerobic degradation of residual water-soluble carbohydrates, starch and lactic acid. Those reactions of aerobic deterioration rapidly increase the temperature, which explain the results of the current study. When the relocated silages were opened, we observed that the populations of yeasts did not differ among the treatments. When compared with the silages before relocation, we observed that relocated silages with 36, 48 and 60 hours of exposure showed significant reduction in the number of yeasts. Based on that, we can affirm that yeasts´ growth was limited when the silages were packed in a new silo because of the low concentration of oxygen to degrade lactic acid and WSC, and possible modifications in the fermentation profile caused by the exposure to air (we could speculate an increase in the acetic acid). On the other hand, the aerobic stability increased when the TR increased, which indicates that all relocated silages had lower amounts of substrates than the non-relocated silage. Relocated silages treated with LB was the lowest among the microbial inoculants. The possible explanation is that the preservation was effective during the first period of fermentation (120 days) and provided more substrates for the yeasts at the final opening because those silages were not previously exposed to oxygen as the relocated ones.

**Conclusions** The microbial inoculants LPLB, LPPA and LB do not improve the aerobic stability of relocated corn silages. Silage relocation appears to decrease the effect of microbial inoculants, specially LB. The time spent with relocation increases aerobic stability of relocated silages because of the aerobic degradation during the silage exposure to air.

**References**

Chen, Y. & Weinberg, Z.G. (2014) The effect of relocation of whole-crop wheat and corn silages on their quality. J. Dairy Sci. 97 :406-410.

Pahlow, G., Muck, R.E., Driehuis, F., Oude Elferink, S.J.W.H. &. Spoelstra, S.F. (2003) Chapter 2: Microbiology. Pages 31–93 in Silage Science and Technology. Agronomy Monograph 42. D.R. Buxton, R.E. Muck, & J.H. Harrison, ed. American Society of Agronomy, Madison, WI.

# Effects of stage of maturity, rollers and chopping length on starch availability, losses and aerobic stability of maize (*Zea mays* L.) silage

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**Keywords:** chopping length, CSPS – Corn Silage Processing Score, forage quality, maize silage

**Introduction** Due to trial results in the USA, a trend towards harvesting maize (*Zea mays* L.) with extended chopping length and alternative kernel processing has reached Europe. However, lack of knowledge exists on the interaction between different kernel processing roller designs and stages of maturity on the starch availability and other items of silage quality. Therefore, the present study evaluated the influence of kernel processor roller design, differential speed, chopping length and dry matter content (DM on the gap clearance, starch availability, losses and aerobic stability) of maize silage.

**Materials and Methods** At research station Futterkamp, (Schleswig Holstein) maize silage was harvested with a self-propelled forage harvester (John Deere 8500i) equipped with a multiknife cutterhead and two different roller designs (reversed sawtooth (HD) and reversed sawtooth with spiral groove (SC)) mounted in the kernel processor housing in 2016. The differential speed of the rolls and the gap clearance were set to 40% and 1mm, respectively. There were 10 different chopping lengths adjusted (3, 5, 8, 11, 14, 17, 20, 23, 26 and 29 mm) at each of four stages of maturity, i.e. whole plant DM content of: A: 27-31%; B: 31-35%; C: 35-39%; D: 39-43%. To analyse the kernel processing, the CSPS-Score was applied (Ferreira and Mertens 2005). Fermentation products, losses and aerobic stability were determined according to DLG guidelines (2018). The statistical evaluation was completed by SPSS (ANOVA and t-test).

**Results** Independent of the kernel processing, the CSPS score on average ranged above 70%. An increased chopping length resulted in a decreased kernel processing (Figure 1), whereas a higher DM content stabilized the kernel processing (not shown). There were no significant differences between the roller designs for kernel processing.

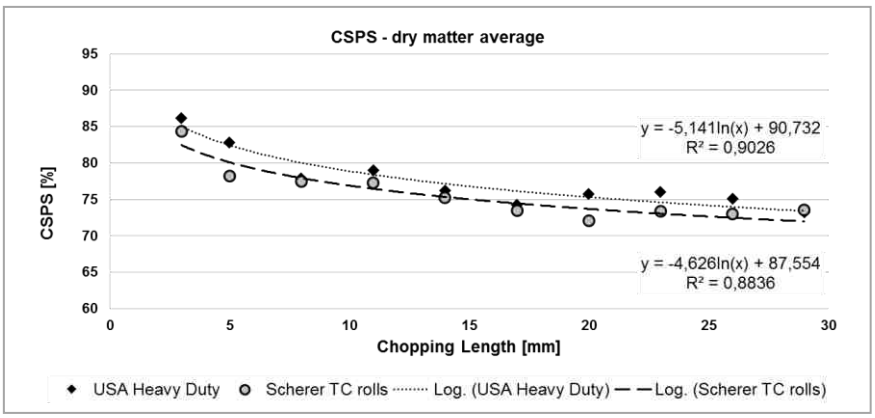


**Figure 1** CSPS % from 3 to 29 mm chopping length by two roller designs (HD,SC)

**Table 1** Silage quality parameters as affected by chopping length and roller designs (HD vs. SC) at four stages of maturity (A-D).

| Chopping length [mm] | | 8 | | 17 | | 23 | | 29 | |
|---|---|---|---|---|---|---|---|---|---|
| Harvest date | Parameter | HD | SC | HD | SC | HD | SC | HD | SC |
| A | CSPS [% DM] | 68.2[a] | 72.8[a] | 69.0[a] | 72.8[a] | 72.1[a] | 72.3[a] | 71.0[a] | 66.3[a] |
| | AA [% DM] | 1.11[a] | 1.44[b] | 1.36[a] | 1.33[a] | 1.42[a] | 1.30[a] | 1.52[a] | 1.52[a] |
| | Eth [% DM] | 1.10[a] | 0.64[b] | 0.85[a] | 0.50[b] | 0.77[a] | 0.67[b] | 0.86[a] | 0.86[a] |
| | Loss [% DM] | 4.78[a] | 4.12[a] | 4.59[a] | 6.11[a] | 4.22[a] | 4.29[a] | 4.33[a] | 4.88[a] |
| | AS [days] | 3.4[a] | 5.0[b] | 4.8[a] | 5.1[a] | 5.2[a] | 5.2[a] | 5.3[a] | 5.2[a] |
| B | CSPS [% DM] | 73.5[a] | 74.8[a] | 71.9[a] | 71.6[a] | 74.4[a] | 77.2[a] | 71.5[a] | 74.1[a] |
| | AA [% DM] | 1.25[a] | 1.33[a] | 1.48[a] | 1.37[a] | 1.36[a] | 1.33[a] | 1.34[a] | 1.38[a] |
| | Eth [% DM] | 0.56[a] | 0.88[b] | 0.92[a] | 1.08[b] | 0.81[a] | 1.20[b] | 0.80[a] | 1.00[b] |
| | Loss [% DM] | 3.43[a] | 3.14[b] | 3.16[a] | 3.30[a] | 3.19[a] | 3.25[a] | 3.36[a] | 3.28[a] |
| | AS [days] | 6.6[a] | 7.0[a] | 6.5[a] | 6.7[a] | 6.8[a] | 6.6[a] | 6.2[a] | 6,8[a] |
| C | CSPS [% DM] | 73.5[a] | 74.8[a] | 71.9[a] | 71.6[a] | 74.4[a] | 77.2[a] | 71.5[a] | 74.1[a] |
| | AA [% DM] | 1.39[a] | 1.29[a] | 1.12[a] | 1.05[a] | 1.03[a] | 1.36[b] | 1.26[a] | 1.32[a] |
| | Eth [% DM] | 0.86[a] | 0.69[b] | 0.61[a] | 0.69[b] | 0.92[a] | 0.56[b] | 0.61[a] | 0.61[a] |
| | Loss [% DM] | 3.47[a] | 3.47[a] | 3.49[a] | 3.62[a] | 3.84[a] | 3.38[b] | 3.64[a] | 3.41[b] |
| | AS [days] | 5.8[a] | 5.9[a] | 5.0[a] | 5.6[a] | 6.0[a] | 5.6[b] | 5.9[a] | 5.3[a] |
| D | CSPS [% DM] | 86.4[a] | 89.1[a] | 76.8[a] | 77.3[a] | 81.7[a] | 81.0[a] | 80.3[a] | 78.0[a] |
| | AA [% DM] | 1.15[a] | 0.89[a] | 0.95[a] | 1.08[a] | 1.05[a] | 0.96[a] | 1.04[a] | 1.03[a] |
| | Eth [% DM] | 1.17[a] | 0.78[b] | 1.13[a] | 1.02[a] | 0.95[a] | 1.00[a] | 1.09[a] | 1.18[a] |
| | Loss [% DM] | 4.19[a] | 4.43[b] | 4.41[a] | 4.31[a] | 4.34[a] | 4.34[a] | 4.31[a] | 4.30[a] |
| | AS [days] | 7.0[a] | 7.0[a] | 6.4[a] | 7.3[a] | 7.2[a] | 6.9[b] | 6.9[a] | 7.5[b] |

CSPS: CornSilageProcessingScore; AA:acetic acid; Eth: Ethanol, Loss: fermentation loss; AS: aerobic stability according Honig 1990; [a,b] symbolize significant differences ($p < 0.05$) within a chopping length and the rollers

Between the four chopping lengths, the four stages of maturity and the two roller designs rather identical results were examined according to the selected silage quality parameters and aerobic stability (Table 1). The aerobic stability of silage samples increased with higher DM content, however, increased chopping length reduced aerobic stability.

**Discussion** The results indicated, that a high starch availability (CSPS) requires a minimum gap of 1mm in combination with a differential speed of the roller of at least 40%, rather than different roller designs. The stage of maturity, the chopping length and the roller design had no effect on silage quality. Moreover, aerobic stability was positively related to the DM content of the forage.

**Conclusions** The standard roller with 40% differential speed and 1mm gap clearance provides excellent kernel conditioning over all ranges of chopping length and maturity. High aerobic stability may be ensured with adapted DM contents of the forage. However, compaction may be an issue with a maturated forage crop. With increased DM content and chopping length, the compaction management is elementary to ensure forage quality including aerobic stability.

**References**

DLG, (2018) DLG Testing Guidelines for the award und use of the DLG Quality Mark for ensiling agents", prepared under the auspices of the DLG commission for ensiling agents, at http://www.dlg.org/siliermittel.html.

Ferreira & Mertens (2005) Chemical and physical characteristics of corn silages and their effects on in vitro disappearance. Journal of Dairy Science Dec. 88 (12): 4414-25.

Honig, H. (1990) Evaluation of aerobic stability. In: Lindgren, S. & Pettersson, K. (eds.), Proceedings of the EUROBAC Conference, Uppsala, Sweden, 1990, special issue, pp. 76-82.

# Estimation of ruminal gas production and utilisable crude protein at the duodenum from native, ensiled and ensiled + toasted peas and field beans

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**Keywords:** *in vitro* gas production, legume grains, protein evaluation, hydro-thermic treatment

**Introduction** Field peas (FP) and field beans (FB) may contribute to reduced inputs of imported soy for animal feed production. Availability of starch and protein from FP and FB is restricted for ruminants, because high percentages are readily fermented in the rumen. We hypothesized that hydro-thermic treatment (toasting) of FP and FB silages improves starch and protein stability against degradation, which was tested *in vitro* estimating gas production (GP), GP kinetics, utilisable crude protein at the duodenum (uCP) and effective uCP (euCP) using an automated system (ANKOM$^{RF}$ GP System).

**Material and Methods** Substrates were maize starch, cellulose (Vitacel® R200), native, ensiled (Rostock Model Silages; Hoedtke and Zeyner 2011) and ensiled + toasted FP and FB. Silages were made from re-moistened grains with ~72% dry matter (DM) and pH 6.6 and 6.4 in FP and FB, respectively. No inoculants were used. Toasting was simulated in a forced air-drying oven (160°C, 30 min). Six consecutive trials were carried out, where ruminal fluid was taken from two cannulated wethers and mixed by 1:2 with a buffer/nutrient solution (VDLUFA 2012, method 25.1; adding $NH_4HCO_3$ by 2 g/L and reducing $NaHCO_3$ by 2 g/L). Ground substrate (0.2 g, Ø 1 mm) and 30 mL inoculum per fermentation bottle were incubated in a shaking water bath (39°C, 80 rpm). Cumulative gas pressures were automatically documented (settings: 1 min recording interval, 1.5 psi threshold for releasing accumulated gases and 150 ms valve open time). Blank-corrected gas pressures were converted to mL by Ideal Gas Law and Avogadro's Law. After 8 and 24 h, samples were taken for $NH_3$-N analysis (FOSS 2300 Kjeltec$^{TM}$). Additional nutrient analyses were carried out using VDLUFA methods (VDLUFA 2012). uCP and euCP were estimated according to Edmunds et al. (2012), the latter for assumed passage rates (*K*p) 2, 4, 6, 8 and 12 %/h. Statistical analysis was performed using SAS 9.4 MIXED with fixed substrate, incubation stage (for GP kinetics), measuring time (for uCP), passage rate (for euCP) and interaction effects (for GP kinetics, uCP and euCP), and random trial effect at $P<0.05$ significance level. Gas production kinetics was modelled using Gompertz function.

**Results** Field pea treatments had 486-564 g starch/kg DM and 180-207 g CP/kg DM; FB treatments had 398-423 g starch/kg DM and 263-276 g CP/kg DM. Gas production (24 h) was highest in starch (46 mL/200 mg DM), followed by FP treatments (41-43 mL/200 mg DM), cellulose (35 mL/200 mg DM) and FB treatments (29-32 mL/200 mg DM). It differed between ($P<0.001$), but not within FP and FB. Throughout incubation, GP was continuously lowest in ensiled + toasted treatments (Table 1).

**Table 1.** Least squares means (LSM) of gas production [mL/200 mg DM] from native, ensiled and ensiled + toasted field peas (FP) and field beans (FB) within stages of the 24 h-incubation.

| | 0-2 h | 2-4 h | 4-6 h | 6-8 h | 8-12 h | 12-24 h |
|---|---|---|---|---|---|---|
| Native FP | $0.4^{eA\mathit{A}}$ | $3.5^{eA\mathit{A}}$ | $7.4^{dA\mathit{A}}$ | $12.5^{cA\mathit{A}}$ | $24.6^{bA\mathit{A}}$ | $39.5^{aA\mathit{A}}$ |
| Ensiled FP | $0.6^{eA\mathit{A}}$ | $3.2^{deA\mathit{A}}$ | $6.6^{dA\mathit{A}}$ | $11.0^{cA\mathit{A}}$ | $21.4^{bA\mathit{AB}}$ | $39.4^{aA\mathit{A}}$ |
| Ensiled + toasted FP | $0.0^{eA\mathit{A}}$ | $2.8^{deA\mathit{A}}$ | $5.7^{cdA\mathit{A}}$ | $9.4^{cA\mathit{A}}$ | $18.7^{bA\mathit{B}}$ | $37.4^{aA\mathit{A}}$ |
| Native FB | $0.1^{eA\mathit{A}}$ | $2.1^{deA\mathit{A}}$ | $4.7^{cdA\mathit{A}}$ | $7.7^{cB\mathit{A}}$ | $14.4^{bB\mathit{A}}$ | $28.7^{aB\mathit{A}}$ |
| Ensiled FB | $0.6^{dA\mathit{A}}$ | $2.1^{dA\mathit{A}}$ | $3.9^{cdA\mathit{A}}$ | $6.0^{cB\mathit{AB}}$ | $10.9^{bB\mathit{B}}$ | $27.1^{aB\mathit{AB}}$ |
| Ensiled + toasted FB | $0.0^{dA\mathit{A}}$ | $0.1^{dA\mathit{A}}$ | $1.7^{cdB\mathit{A}}$ | $3.6^{cB\mathit{B}}$ | $8.2^{bB\mathit{B}}$ | $24.7^{aB\mathit{B}}$ |

DM, dry matter. Standard errors ranged from 1.43 to 1.54 mL/200 mg DM. $^{a,b,c,d,e}$ Within rows, different superscripts mark differences between time stages within species and treatment ($P<0.05$). $^{A,B}$ and $^{\mathit{A,B,C}}$ Within columns, different superscripts mark differences between species within treatment, and between treatments within species ($P<0.05$), respectively.

Utilisable CP at the duodenum was higher after 8 than after 24 h (P<0.01; Table 2). Effective uCP increased with increasing *K*p (Table 2). Native and ensiled FB had more uCP than FP counterparts (P<0.001) and more euCP at *K*p≥4 (P<0.05). Ensiling did not affect uCP and euCP (P>0.05). Among toasted silages, uCP was equal (at 8 h) or lower in FB than in FP (at 24 h, P<0.001); euCP was lower in FB (P<0.05 at *K*p2 and *K*p4). Toasting FP silages increased uCP, and euCP with *K*p≥6 (P<0.05); in FB silages, it decreased uCP at later incubation time (P<0.05), and euCP with *K*p2 and *K*p4 (P<0.05).

**Table 2.** Least squares means (LSM) of utilisable crude protein at the duodenum (uCP) and effective uCP (euCP) of native, ensiled and ensiled + toasted field peas (FP) and field beans (FB).

| | uCP, g/kg DM | | euCP, g/kg DM | | | | |
|---|---|---|---|---|---|---|---|
| | 8 h | 24 h | *K*p2 | *K*p4 | *K*p6 | *K*p8 | *K*p12 |
| Native FP | $278^{aB\beta}$ | $256^{bB\alpha}$ | $247^{aA\alpha}$ | $260^{aB\alpha}$ | $267^{aB\beta}$ | $270^{aB\alpha\beta}$ | $277^{aB\beta}$ |
| Ensiled FP | $290^{aB\beta}$ | $264^{bB\alpha}$ | $225^{bA\alpha\beta}$ | $242^{abB\alpha}$ | $253^{abB\beta}$ | $259^{abB\beta}$ | $269^{aB\beta}$ |
| Ensiled + toasted FP | $402^{aA\alpha}$ | $272^{bA\alpha}$ | $189^{dA\beta}$ | $269^{cA\alpha}$ | $316^{bA\alpha}$ | $348^{bA\alpha}$ | $395^{aA\alpha}$ |
| Native FB | $387^{aA\alpha}$ | $327^{bA\alpha}$ | $287^{cA\alpha}$ | $325^{bbA\alpha}$ | $348^{bA\alpha}$ | $362^{abA\alpha}$ | $385^{aA\alpha}$ |
| Ensiled FB | $391^{aA\alpha}$ | $307^{bA\beta}$ | $251^{dA\alpha}$ | $305^{cA\alpha}$ | $337^{bcA\alpha}$ | $358^{abA\alpha}$ | $390^{aA\alpha}$ |
| Ensiled + toasted FB | $399^{aA\alpha}$ | $226^{bB\gamma}$ | $113^{eB\beta}$ | $221^{dB\beta}$ | $285^{cA\beta}$ | $329^{bA\alpha}$ | $392^{aA\alpha}$ |

DM, dry matter. Standard errors ranged from 5.19 to 8.03 g/kg DM in uCP and from 20.2 to 22.5 g/kg DM in euCP estimates. $^{a,b,c,d,e}$ Within rows, different superscripts mark differences between measuring times (for uCP) or passage rates (*K*p) (for euCP) within species and treatment (P<0.05). $^{A,B}$ and $^{A,B,C}$ Within columns, different superscripts mark differences between species within treatment, and between treatments within species (P<0.05), respectively.

**Discussion** During ensiling, pH values did not decrease (pH 6.5 and 6.4 after 60 d in FP and FB, respectively), contradicting previous findings with natively moist and re-moistened material (~65% DM; Gefrom et al. 2013). Using ANKOM$^{RF}$ GP System, the ranking among substrates for GP and uCP was found to be equal compared to the (modified) Hohenheim Gas Test (Bachmann et al. 2018), which justifies its use for *in vitro* feed evaluation. Hydro-thermic treatment ≥160°C may lead to structural modifications of starch granules and their covering matrices, which might facilitate higher stability of starch against microbial degradation. This would go with lowest GP that we found in toasted silages. Toasting seemed to have contradictory effects on protein degradation in FP and FB silages, which requires further clarification.

**Conclusion** General differences of *in vitro* GP and uCP estimates between FP and FB may result from differing contents of starch and protein. Indications for improved ruminal starch stability after toasting of FP and FB silages were found, but need confirmation *in vivo*. Toasting may increase uCP contents in FP depending on *K*p, whereas in FB, it rather leads to a better protein decomposition. If this can be confirmed, toasting of FB silages might have more potential to be used in monogastrics.

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**References**

Bachmann, M., Kuhnitzsch, C., Martens, S.D., Steinhöfel, O. & Zeyner, A. (2018) Comparison of two *in vitro* systems for the estimation of total gas production and utilisable crude protein at the duodenum from native or ensiled field peas and field beans in ruminants, In: Society of Nutrition Physiology (eds). Proceedings of the 72nd Conference of the Society of Nutrition Physiology, Göttingen, Germany, 2018, p. 137.

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M. & Schwarz, F.J. (2012) Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Animal Feed Science and Technology, 175, 106-113.

Gefrom, A., Ott, E.M., Hoedtke, S. & Zeyner, A. (2013) Effect of ensiling moist field bean (*Vicia faba*), pea (*Pisum sativum*) and lupine (*Lupinus* spp.) grains on the contents of alkaloids, oligo-saccharides and tannins. Journal of Animal Physiology and Animal Nutrition, 97, 1152-1160.

Hoedtke, S. & Zeyner, A. (2011) Comparative evaluation of laboratory-scale silages using standard glass jar silages or vacuum-packed model silages. Journal of the Science of Food and Agriculture, 91, 841-849.

VDLUFA (2012) Die chemische Untersuchung von Futtermitteln. Methodenbuch. 3rd ed. Darmstadt: VDLUFA-Verlag.

# Fermentation and nutritional quality of high moisture alfalfa leaf and stem silage

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**Keywords:** alfalfa, leaf, protein, silage, stem

**Introduction** Alfalfa (*Medicago sativa* L.) is an important perennial forage for dairy production systems in the US. To obtain the most digestible fiber content, alfalfa requires 4 or more cuttings at early bud/late bud per growing season; this increases the cost of production and limits its utilization on farmland. However, there is potential to generate a more nutrient-dense feed from alfalfa, regardless of development stage, by harvesting its protein- and starch-rich leaves separate from its fiber-rich stems. To evaluate the preservation of these fractions under production conditions, high moisture alfalfa leaves and stems were ensiled separately in mini-silos.

**Materials and methods** Alfalfa leaf, stem, and whole plant material was harvested on May 18$^{th}$, May 24$^{th}$, and June 8$^{th}$ of 2016 corresponding to developmental stages early bud, 10-20% bloom, and >50% bloom in Prairie du Sac, Wisconsin. This harvest sequence was repeated on June 16$^{th}$, June 21$^{st}$, and June 29$^{th}$ as a second cutting. Whole plants were wilted to >400 g dry matter (DM) per kg before ensiling (Table 1). Leaves and stems were harvested separately with a prototype leaf stripper and directly-ensiled. All silages were inoculated with 7.63x10$^5$ cfu *Lactobacillus plantarum* (Pioneer 11H50) per g DM. After 0, 1, 3, 21, and 140 days of ensiling, silages were analyzed for nutritive and fermentation characteristics including protein, water-soluble carbohydrates (WSC), fiber, and volatile fatty acids (VFAs). Data were analyzed using a mixed model (MIXED procedures, SAS Inst. Inc., Cary, NC). The effects of plant fraction, development stage, cutting, day of ensiling, and their interactions were analyzed using a split-plot ANOVA and adjusted with Tukey's test for *P*-values.

**Table 1**. DM at ensiling and fraction leaf content for each development stage, cutting, and alfalfa fraction. Leaf content of each fraction resulting from the leaf stripper was measured for silage composition.

| | Cut 1 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Early Bud | | | 10-20% Bloom | | | >50% Bloom | | |
| | Leaf | Stem | Whole | Leaf | Stem | Whole | Leaf | Stem | Whole |
| DM (g/kg) | 272.50 | 241.00 | 449.00 | 240.30 | 255.80 | 459.40 | 305.70 | 371.10 | 545.50 |
| Leaf (g/kg DM) | 911.08 | 378.06 | | 877.01 | 256.31 | | 823.70 | 133.90 | |
| | Cut 2 | | | | | | | | |
| | Early Bud | | | 10-20% Bloom | | | >50% Bloom | | |
| | Leaf | Stem | Whole | Leaf | Stem | Whole | Leaf | Stem | Whole |
| DM (g/kg) | 222.80 | 225.20 | 403.80 | 250.80 | 250.50 | 404.10 | 224.50 | 277.40 | 418.40 |
| Leaf (g/kg DM) | 909.87 | 325.70 | | 900.34 | 271.47 | | 806.15 | 221.95 | |

**Results and discussion** High moisture leaf and stem silages were more heterofermentative than wilted whole plant silages; fermentation shifted from primarily lactic acid (pKa = 3.86) to acetic acid (pKa = 4.75) production after 21 days resulting in a higher pH at 140 days (Figure 1). Butyric acid production did not occur in cut 2 silages. However, leaf fractions at 140 days of ensiling for early bud and 10-20% bloom development stages of cut 1 contained 10.57, and 249.8 mmoles butyric acid per mole VFAs, respectively. WSC declined during the ensiling period but were not completely depleted after 140 days. Independent of ensiling time, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) increased with maturity especially for stem and whole plant silages. Most likely, 10-20% bloom and >50% bloom leaf silages had greater NDF, ADF, and ADL content than early bud leaf silages due to greater stem inclusion at harvest (Table 1). For all plant fractions, protein preservation was about 500 g/kg crude protein (CP) or greater except for early bud leaf fractions

(<400 g/kg CP). A slightly higher proportion of free amino acids (FAA) to peptide amino acids (PAA) was observed in all silages, except in stem silages, which had over twice the amount of FAA to PAA. Nevertheless, leaf silage increased ($P$<0.01) available protein (PAA, soluble and insoluble protein) content by 21-25% compared to wilted whole plant silage after 140 days ensiled (Figure 2).

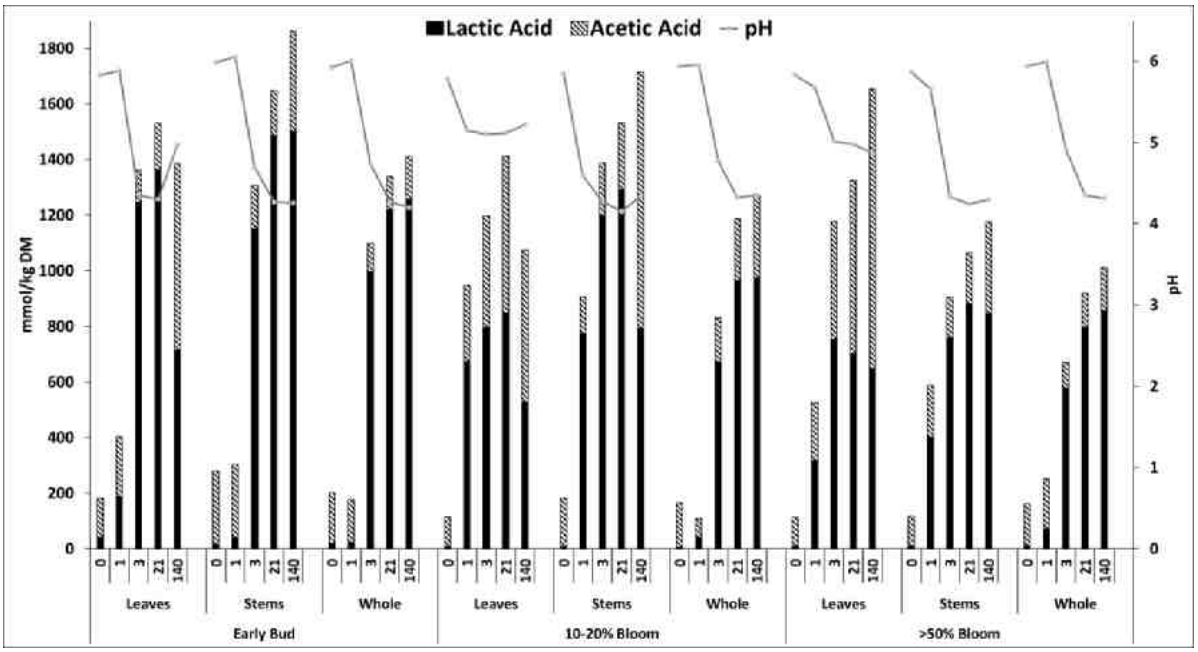


**Figure 1**. pH, and lactic and acetic acid production over the 140 day ensiling period for each development stage and alfalfa fraction of the first cutting.

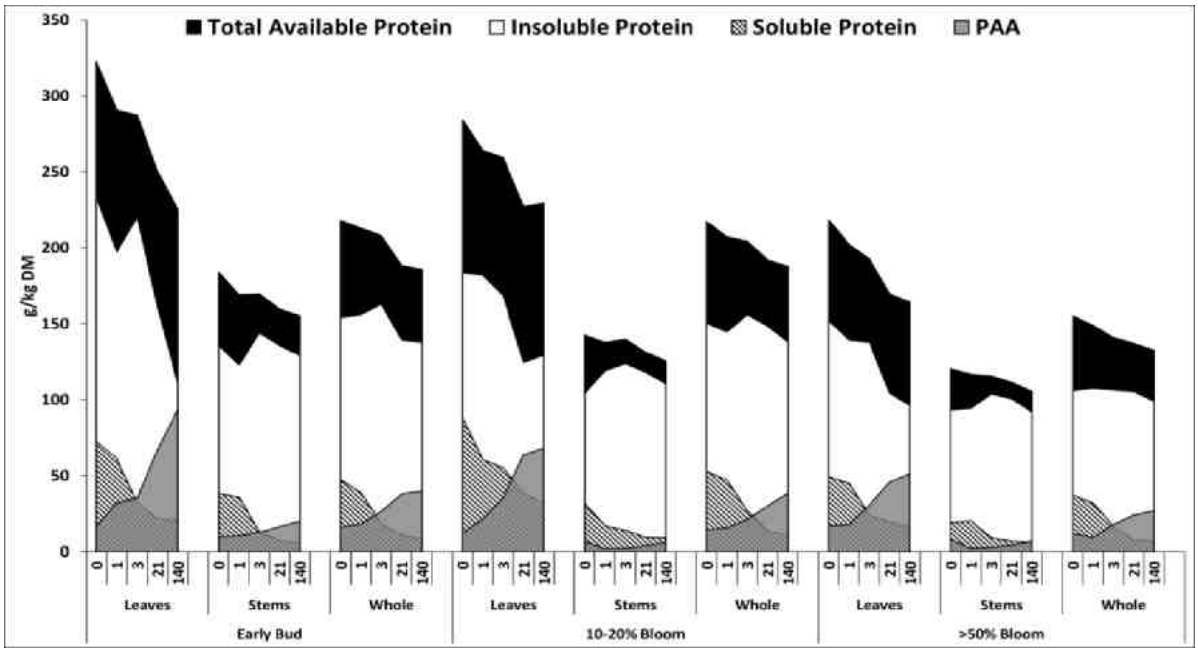


**Figure 2**. Total available protein and its composition over the 140 day ensiling period for the first cutting of each development stage and alfalfa fraction.

**Conclusions** Ensiling alfalfa leaves separate from its stems yielded a protein-rich feed compared to whole plant. The increase in available protein content in leaf silage, regardless of development stage, shows promise that alfalfa may be harvested less frequently during the growing season while yielding a nutrient-rich product. However, a large portion (>500g/kg) of the leaf crude protein degraded during ensiling. Improvements could be made if a more rapid and stable pH decline were attained.

# Fermentation quality of mixed silage of corn stover, broccoli residues and apple pomace

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**Keywords:** apple pomace, broccoli residues, corn stover, fermentation quality, mixed silage

**Introduction** Large amount of broccoli residues are produced during broccoli harvest and sale. The fresh material deteriorates rapidly upon exposure to air because of the high moisture content. This has caused environmental pollution, especialy in broccoli harvested peaks. The crude protein content of broccoli residues is about 20% and has potential as animal feed, but its high moisture content negatively affects its conservation. Ensiling may be a suitable method to preserve high moisture by-products. The aim of this experiment was to evaluate fermentation quality of mixed silage of corn stover, broccoli residues and apple pomace.

**Materials and methods** The broccoli residues (stem and leaves), corn stover and dry apple pomace were ensiled at the ratios of 4:5:1. Approximately 800g of mixed treated material was packed into laboratory silos (polyethylene bottle, 1 L capacity) and stored at the ambient temperature. Three silos were opened on 1, 3, 5, 7, 14 and 30 days after ensiling and the silage fermentation was evaluated. The experimental data were subjected to one-way ANOVA (SAS 9.1).

**Results** Dry matter content did not change significantly ($P > 0.05$) during the initial 5 day of ensiling, and began to decrease significantly ($P < 0.05$) from 7 day, and reaching lowest (179 g $kg^{-1}$DM) at the end of the ensiling. Silage pH value decreased significantly to 3.82 ($P < 0.05$) on day 5, then remained stable until the end of ensiling. The content of lactic acid in mixed silage increased significantly ($P < 0.05$) on day 3, and continued to increase to the highest value (50.4 g / $kg^{-1}$DM) at the end of ensiling. The acetic acid content showed a significant ($P < 0.05$) increase on day 3 then continued to increase until the end of ensiling. Propionic acid and butyric acid were not detected during the full fermentation course. The lactic / acetic acid decreased gradually during the initial 5 day of ensiling, and increased significantly on the 7th day ($P < 0.05$), and then decreased gradually. The ammonia / total nitrogen increased significantly ($P < 0.05$) on day 3, and peaked on day 14( 57.6 $kg^{-1}$TN), and subsequently decreased ($P > 0.05$). The content of water-soluble carbohydrate decreased gradually over the ensiling period.

**Conclusions** The results indicated that mixture of broccoli residues, corn stover and apple pomace at the ration of 4:5:1 was well preserved.

**Table 1** Fermentation quality of mixed silage of corn stover, broccoli residues and apple pomace

| Item | days of ensiling | | | | | | SEM | *P*-value |
|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 14 | 30 | | |
| Dry matter （g·kg$^{-1}$FW） | 232a | 219a | 225a | 198b | 204b | 179c | 4.82 | 0.0009 |
| pH | 4.63a | 4.31b | 3.92c | 3.89c | 3.98c | 3.98c | 0.088 | 0.0489 |
| Lactic acid （g·kg$^{-1}$DM） | 1.12e | 9.86d | 12.0c | 26.9b | 35.5b | 50.4a | 4.31 | <0.0001 |
| Acetic acid （g·kg$^{-1}$DM） | 0.39e | 3.63d | 6.26c | 6.92c | 11.4b | 24.1a | 2.43 | 0.0411 |
| Propionic acid （g·kg$^{-1}$DM） | ND | ND | ND | ND | ND | ND | - | - |
| Butyric acid （g·kg$^{-1}$DM） | ND | ND | ND | ND | ND | ND | - | - |
| Lactic acid / Acetic acid LA/AA | 2.87ab | 2.77ab | 1.93b | 3.99a | 3.13a | 2.09b | 0.472 | 0.6588 |
| Water soluble carbohydrates （g·kg$^{-1}$DM） | 61.6a | 49.9b | 43.2b | 22.3c | 12.7d | 11.7d | 5.31 | 0.0020 |
| Total volatile fatty acids （g·kg$^{-1}$DM） | 0.39e | 3.63d | 6.26c | 6.92c | 11.4b | 24.1a | 2.43 | 0.0411 |
| Ammonia nitrogen / Total nitrogen （g·kg$^{-1}$TN） | 10.7c | 17.2b | 19.6b | 23.6b | 57.6a | 50.3a | 0.099 | <0.0001 |

ND, not detected; values followed by different litter letters in the same row show significant differences ($P<0.05$)

# Field survey on silo dimensions, silage characteristics, and its effect on temperature and density of grass silage in the Netherlands

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**Keywords:** density,grass silage, farm scale, temperature

**Introduction** Applying good silage practices improves preservation and forage quality. Temperature and density are two major issues known for maize silage quality (Andrieu and Demey 2015 a&b). However, little recent data is available about on-farm grass silage characteristics. The aim of this study is to evaluate, in farms, the effect of silo dimensions and silage characteristics on temperature and density of grass silage.

**Materials and Methods** The survey combines the results from 29 farms located in the eastern part of the Netherlands. The grass was ensiled during spring 2016 and the bunker silos were sampled 260 days (±20 days) after ensiling (i.e. winter). Samples were analyzed for nutritional value (dry matter (DM), ash, crude protein, fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), sugar) and microbial counts (moulds with log1 as limit of detection). Density (at top and bottom) (corer sampler attached to a manual hand drill), and temperature (at 10 and 50 cm inside the silo face) of the silage were measured and silo dimension was recorded. Data were subjected to paired T-test, linear regression analysis and univariate ANOVA with mould class (a: <2log and b: ≥2log) as fixed effect (SPSS v.19.0). Significance was declared at $P<0.05$. Outliers were detected with Explore procedure of SPSS (v 19.0). Two farms were removed from all analysis as they were identified as outliers for several of the measured parameters.

**Results and Discussion** Table 1 describes for each of the factors influencing temperature and/or density of the grass silage, the range of values encountered in this field study.

**Table 1**. Description of significant influencing factors of temperature and density of grass silage

| Parameter | Minimum | Mean | Maximum |
|---|---|---|---|
| Silo volume, $m^3$ | 101 | 538 | 3049 |
| Height, m | 1.0 | 1.8 | 2.5 |
| Width, m | 1.8 | 7.2 | 10.4 |
| Speed of feed-out, m/day | 0.7 | 1.4 | 3.0 |
| Dry matter, % | 28.2 | 46.6 | 60.6 |
| Crude fiber, %DM | 20.1 | 23.2 | 28.8 |

Results show that the temperature of the silage at 10 cm was 1.5°C higher than the temperature at 50 cm inside the silo (13.5 vs 12.0°C respectively) ($P<0.05$) (Table 1). Parameters directly influencing temperature are speed of feed-out and moulds content (adjusted $R^2=0.308$, $P<0.05$). Speed of feed out was the most important parameter showing a significant positive correlation with temperature,

r=0.459 (P<0.05). Andrieu and Demey (2015a) also reported that the defacing rate of the bunker seems to influence the temperature of the front face of corn silage. Temperature was 4.9°C higher in silo with moulds >2 log (17.3±4.2°C versus 12.4±4.0 °C respectively). Moulds are known contributors to the spoilage process, resulting in heating of the silage.

Silage density at the bottom was 23.1 kg/$m^3$ higher than density at the top of the silo 212.8 and 235.9 kg DM/$m^3$ respectively (P<0.05) (Table 2). This can be explained due to the self-compaction of the mass. The average density of the investigated silos was 226 kg DM/$m^3$ ± 35 (min. =155 and max. =286 kg DM/$m^3$), which lies within the optimal target of 165 to 240 kg DM/$m^3$ (Idele, 2012). Parameters that directly influence the density are crude fiber, DM content of the silage and volume of the silo (adjusted $R^2$ =0.622, P<0.05). Crude fiber and DM were the most important factors which had a significant correlation with the density. Crude fiber was negatively correlated with density (r=-0.604; P<0.05), which is in line with data on grass silage reported by McGechan (1990) and Messer (1977). DM on the other hand is positively correlated with density (r=0.597, P<0.05). Andrieu and Demey (2015b) reported similar findings on corn, where DM density increased with higher DM content of the forage.

**Conclusions** Results from this field survey suggest that to maintain a low temperature of the silo, the speed of feed-out is an important factor to manage. The presence of moulds in the silage also significantly affects grass silage temperature. Achieving a good density depends on fresh roughage DM and crude fiber content as well as on the volume of the silo.

**References**

Andrieu, B. & Demey V. (2015a) On-farm corn silage investigation: multi-analysis on silage practices, silage quality and its effect on aerobic stability. In: J.L.P. Daniel, G. Morais, D. Junges, L.G. Nussio (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 250-251

Andrieu, B. & Demey V. (2015b) On-farm corn silage investigation: multi-analysis on silage practices, silage quality and its effect on density, In: J.L.P. Daniel, G. Morais, D. Junges, L.G. Nussio (eds). Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 2015, pp. 310-311.

Idele (2012) Densité et rendements des fourrages. http://www.herbe-fourrages-limousin.fr/fileadmin/documents/doc_pascaline/Estimation_de_densite_fourrages_-_novembre_2012.pdf.

Messer, H.M. & Hawkins. J.C. (1977) The influence of the properties of grass silage on bulk density and horizontal pressure. Journal of Agricultural Engineering Research. 22:55-64.

McGechan, M.B. (1990) A review of losses arising during conservation of grass forage: Part 2, storage losses. Journal of Agricultural Engineering Research 45:1-30.

# Field-related quality management system for grass silage production

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**Keywords:** grass harvesting, prediction model, silage quality

**Introduction** The production of high-quality grass silage depends on a wide range of factors, e.g. species composition, plant nutrient supply, morphological stage at mowing, mowing and wilting procedures, weather conditions during wilting and ensiling, dry matter (DM) content of the ensiled grass, use of additives as well as compaction and sealing in the clamp. In general, the evaluation of silage quality and possibly occurring weaknesses within the silage production system is based on laboratory analysis of a core sample taken before or a sample taken at feeding. However, in larger clamps filled with grass from different fields, it is hardly possible to refer the analysis results to the grass swards of the single fields. It was the objective of the work to contribute to a silage quality management system, where the clamp and the single fields are linked. Based on this link, it is possible to discover weaknesses in the whole silage production system, starting at the grass sward, covering the period of mowing, wilting and chopping and ending at the filled, compacted and hermitically covered clamp.

**Material and Methods** The silage quality management system was developed for larger farms in the 1980´s and tested again in 2002 and 2004 (Pickert and Weise 2014). In cooperation with seven farms in Northern Germany in 2015 and 2016, the system was further developed on the base of two prediction models, *WiltExpert* (Pickert et al. 2016) and *SiloExpert* (Pickert et al. 2018). During the studies in 2015 and 2016, it was possible to satisfactorily predict the time of ensiling at an optimal DM content of the grass and to predict the net-energy lactation (NEL) content on different fields with a very good modelling efficiency and a low predictive error (Table 1).

**Table 1.** Mean Absolute Error (MAE) and Modelling Efficiency (EF) of the used models in 2015 and 2016 (Pickert et al. 2016 and 2018).

| model | prediction at | prediction of | n | MAE | EF |
|---|---|---|---|---|---|
| *WiltExpert* | mowing | time of ensiling | 16 | 108 minutes | 0.99 |
| *SiloExpert* | ensiling | silage energy concentration | 16 | 0.14 MJ NEL kg $DM^{-1}$ | 0.93 |

**Table 2.** Evaluation of fodder quality parameters in different phases of the silage production process.

| phase | mowing | ensiling | feeding |
|---|---|---|---|
| material | new mown grass | wilted grass | grass silage |
| scale | field | field | clamp |
| g fresh matter (FM) kg dry matter $(DM)^{-1}$ | + | □ | ■ |
| g crude fibre $DM^{-1}$ | □ | □ | ■ |
| g crude protein kg $DM^{-1}$ | | + | ■ |
| g sugar kg $DM^{-1}$ | | | ■ |
| MJ Net-Energy Lactation kg $DM^{-1}$ | | + | ■ |
| Cation and anion content (e. g. K, Na, Mg, Ca, P, S, Cl) | □ | □ | ■ |

■ silage-related analysis, □ field-related analysis, + field-related model based estimation

The silage quality management system is collecting and evaluating data and information of the grass´ DM and nutrient content, its ensilability and the ensiling conditions instead of conventional silage evaluation of samples at feed-out (Table 2).

In 2017 the silage quality management system was tested in an additional farm of the region.

**Results and Discussion** During the study in 2017, a great variation was found within the grass material from the different single fields contributed to the clamp at ensiling. Only on a limited number of fields, the grass energy content fulfilled the feed requirements of high-yielding milking cows, whereas it was below the need on various other fields. According to the clamp filling flow, six grass bulks with different quality characteristics were identified in different parts of the clamp (Table 3). The minimum size of a clamp part depends on the size and shape of the clamp as well as on the filling and feed-out techniques in the farm. Although the average silage DM content of the whole clamp was in the expected range between 350 and 450 g FM kg $DM^{-1}$, the DM content of the ensiled grass on several single fields was above the optimum. It is necessary to check and perhaps to change the existing harvesting procedure in this farm in order to minimize the risk of exceeding the optimum DM range.

**Table 3.** Silage quality in different parts of a clamp (Organic dairy farm Münchehofe, 1st cut, 2017; clamp 3m high, 12m wide; 40m long; weighted means; bulk density 0.2 t DM $m^{-3}$ ).

| clamp part | 1 | 2 | 3 | 4 | 5 | 6 | total |
|---|---|---|---|---|---|---|---|
| No. of single fields per clamp part | 6 | 4 | 2 | 3 | 6 | 4 | 25 |
| m per clamp part | 10.72 | 4.38 | 4.23 | 4.40 | 6.30 | 10.31 | 40.34 |
| m cumulative | 0.00 | 10.72 | 15.10 | 19.33 | 23.73 | 30.03 | 40.34 |
| m³ | 386 | 158 | 152 | 158 | 227 | 371 | 1452 |
| t DM | 77 | 32 | 30 | 32 | 45 | 74 | 290 |
| mean g FM kg $DM^{-1}$ | 460 | 434 | 413 | 323 | 480 | 488 | 448 |
| min g FM kg $DM^{-1}$ | 383 | 418 | 384 | 298 | 324 | 336 | 298 |
| max g FM kg $DM^{-1}$ | 614 | 447 | 469 | 531 | 676 | 573 | 676 |
| GJ NEL | 476 | 184 | 189 | 182 | 277 | 417 | 1724 |
| mean MJ NEL kg $DM^{-1}$ | 6.16 | 5.85 | 6.19 | 5.75 | 6.10 | 5.62 | 5.94 |
| min MJ NEL kg $DM^{-1}$ | 6.01 | 5.75 | 6.15 | 5.58 | 6.07 | 5.47 | 5.47 |
| max MJ NEL kg $DM^{-1}$ | 6.28 | 5.92 | 6.26 | 5.77 | 6.23 | 6.09 | 6.28 |
| t crude protein | 11 | 5 | 5 | 5 | 7 | 10 | 43 |
| mean g crude protein kg $DM^{-1}$ | 146 | 148 | 171 | 145 | 153 | 134 | 147 |

**Conclusion** On the fields where the grass was harvested at very low energy contents, the farmer has to check and possibly change the sward species composition and/or harvest at an earlier morphological stage. On the fields harvested with too high DM contents, he has to check and possibly change the harvesting procedure. Based on the results of the silage management system, the farmer can ensure the optimal utilization of the produced grass silages depending on the farms´ need and according to the silage quality.

**References**

Pickert, J., Herrmann, A., Hoffmann, T., Mersch, F., Thaysen, J., Weise, G., Weiß, K. & Wellenbrock, K.-H. (2017) Schlag-orientierte Qualitätssicherung in der Grassilageproduktion – ein Paradigmenwechsel? In: Tagungsband 61. Jahrestagung der AGGF, Berlin/Paulinenaue, 105-110.

Pickert, J., Hoffmann, T., Herrmann, A., Thaysen, J., Weise, G. & Wellenbrock, K.-H. (2016) 'WiltExpert' – a model for on-farm prediction of grass wilting time from mowing to ensiling dry matter content. Grassland Science in Europe, 21, 200-202.

Pickert, J., Mersch, F., Herrmann, A., Hoffmann, T., Thaysen, J., Weise, G., Weiß, K., & Wellenbrock, K.-H. (2018) 'SiloExpert' - A model for predicting grass silage quality. Grassland Science in Europe, 23 (in print).

Pickert, J. & Weise, G. (2014) Prediction of energy content of grass silages depending on grass and ensiling conditions. Grassland Science in Europe, 19, 613-615.

# Harvest window: comparison of whole crop rye and whole crop triticale in an early cut system

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**Keywords:** harvest window, rye, triticale, whole crop cereal

**Introduction** Climate change increased (and will further increase in the near future) the number of heat stress days during summer in dry continental regions of Europe, therefore winter whole crop cereals are a major focus to maintain forage supply for dairy farms. However, the short 'harvest window' of the rye and variability of the weather conditions during early cut (boot stage in April) cause difficulties to farmers. Triticale may be an alternative whole crop cereal having a 'wider' harvest window and longer vegetation period. The authors determined green- and dry matter yield, nutrient content, organic matter digestibility (OMd) and $NDFd_{48}$ rumen degradability of a whole crop rye variety (R) and two whole crop triticale varieties ($T_1$ Hungaro, $T_2$ Dimenzio dedicated new variety) according to the different phenological stages in an early cut system in order to compare the different whole crop cereals.

**Materials and Methods** Experimental field was 270 $m^2$ (individual plot size 22,5 $m^2$ per replicate n=4, treatments= 3,). Sowing was carried out on 12 October 2015. Seed quantities were $T_1$ 231 kg/ha; $T_2$ 198 kg/ha R 176 kg/ha. BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale was used to identify the phenological development stages of the triticale. Sampling of two triticale varieties was carried out before heading (3-4 cm head in boot - 21 April 2016, BBCH scale code for cereal: 41; 5-6 cm head in boot – 27 April 2016, BBCH code: 45) and just before heading when the top of the beard could be seen (7 May 2016, BBCH code: 49). Rye harvest was executed at the boot stage (5-6 cm head in boot – 17 April 2016 BBCH code: 45 and 7-9 cm head in boot - 21 April 2016, BBCH code: 49, respectively). Cutting was carried out by hand at 10 cm above ground level (randomized sampling with standard frame: 1 nm, n=4). Green and dry matter yield were measured, crude nutrient content, fibre fractions, organic matter digestibility and $NDFd_{48}$ rumen degradability of R, $T_1$ and $T_2$ were determined by Near-Infra Red Spectroscopy (NIR).

**Results** Triticale varieties had a lower yield (DM yield $T_1$ 5,04±0,52 ton/ha; $T_2$ 4,79±0,56 ton/ha) compared to rye (R DM yield 5,32 ± 0,36 ton/ha) at the date of rye optimal growth stage (7-9 cm head in boot, 21 April 2016, BBCH code: 49) above 65% $NDFd_{48}$. Triticale was at a younger phenological stage on that day (3-4 cm head in boot, BBCH code: 41). 16 days later (7 May 2016), similar $NDFd_{48}$ results ($T_1$ NDF $d_{48}$ 69,6% and $T_2$ 70,0%, respectively) were found in the case of triticale varieties compared to the rye cut earlier (R $NDFd_{48}$ 68,2%, 21 April 2016). Dry matter yield of T1 with BBCH code 49 (7,88±0,56 ton DM/ha, 7 May 2016) and T2 with BBCH code 49 (7,67±0,44 ton DM/ha, 7 May 2016) were significantly ($p \leq 0,05$) higher than R with BBCH code 49 (5,32±0,36 ton DM/ha, 21 April 2016). The T1 variety had a significantly higher protein content, while lower total sugar content compared to T2 in both phenological stages. Dry matter, crude protein, crude fibre, ADF,

$NDFd_{48}$ of R at the stage of BBCH 50 did not differ significantly compared to $T_1$ at the stage of BBCH 50. $T_2$ had a lower crude protein content compared to R and $T_2$ at the similar stage (BBCH 49 and BBCH 50, respectively).

**Table 1.** Dry matter yield of rye var. Ryefood (R), triticale var. Hungaro (T1), triticale var. Dimenzio (T2) according to the different sampling dates

| Variety | BBCH | | Date | Mean |
|---|---|---|---|---|
| R | 45 | Before heading: 5-6 cm head in boot | 17.04.2016. | 3,52aA |
| | 49 | Before heading: 7-9 cm head in boot | 21.04.2016. | 5,32aB |
| $T_1$ | 41 | Before heading: 3-4 cm head in boot | 21.04.2016. | 5,04A |
| | 45 | Before heading: 5-6 cm head in boot | 27.04.2016. | 5,96bB |
| | 50 | Before heading: 6-10 cm head in boot | 06.05.2016. | 7,88bC |
| $T_2$ | 41 | Before heading: 3-4 cm head in boot | 21.04.2016. | 4,79A |
| | 45 | Before heading: 5-6 cm head in boot | 27.04.2016. | 5,94bB |
| | 50 | Before heading: 6-10 cm head in boot | 06.05.2016. | 7,67bC |

a-c Values with different letters within BBCH 45 or 49/50 category differ statistically compared to the rye ($P < 0.05$).
A-C Values with different capital letters within a variety differ statistically – effect of phenological stage ($P < 0.05$).

**Table 1.** Nutrient content and *in vivo* digestibility of rye var. Ryefood (R), triticale var. Hungaro (T1), triticale var. Dimenzio (T2) according to the different sampling dates in fresh forage

| | | **R** | **$T_1$** | | **$T_2$** | |
|---|---|---|---|---|---|---|
| | BBCH code | 49 | 45 | 50 | 45 | 50 |
| | Date | 21.04.2016. | 27.04.2016. | 06.05.2016. | 27.04.2016. | 06.05.2016. |
| Dry matter | g/kg | 157a | 146aA | 147aA | 169bA | 160bA |
| Crude protein | g/kg DM | 193b | 257cA | 196bB | 159aA | 150aA |
| Crude fibre | g/kg DM | 252b | 223aA | 247bB | 231aA | 255bB |
| Total sugar | g/kg DM | 102b | 90aA | 94aA | 165dA | 148cB |
| NDF | g/kg DM | 545a | 540aA | 570c | 532aA | 556bB |
| ADF | g/kg DM | 274b | 243aA | 270bB | 245aA | 271bB |
| ADL | g/kg DM | 20.3a | 21.3aA | 24.7bB | 20.3aA | 23.0bB |
| **$NDFd_{48}$** | **%** | **68.2a** | **76.5b** | **69.6aB** | **76.7bA** | **70.0aB** |
| $dNDF4_8$ | g/kg DM | 372a | 413c | 396bB | 408cA | 389aB |
| OMd | % | 76.4a | 80.5b | 75.3aB | 80.0bA | 75.3aB |

a-c Values with different letters within a row differ statistically compared to the rye ($P < 0.05$).
A-C Values with different capital letters within a variety differ statistically – effect of phenological stage ($P < 0.05$).
NDFd48: in vitro digestibility, 48 hours incubation (NIR), OMD in vitro organic matter digestibility (NIR)

**Conclusions** Results confirmed wider harvest window of two triticale varieties compared to a rye variety. Significantly higher ($p \leq 0,05$) dry matter yield was found with similar $NDFd_{48}$ values for the triticale varieties (BBCH code 50) cut 16 days later cut than the rye variety at stage of BBCH code 49. Nutrient content of R and T1 were similar at the stage of BBCH code 49 and 50, respectively. Therefore triticale varieties can be additional alternative forages in very early cut systems alongside the rye, but mowing can be executed under better weather harvesting conditions (10-16 days later) for wilting.

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# Identifying maize hybrids with optimal traits for snaplage

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**Keywords:** corn varieties, ear corn, starch digestibility

**Introduction** High-moisture shelled corn has been a popular feed among farmers. Another option for harvesting high moisture maize is snaplage, which is composed of kernels, cob, husk, and shank from the ear. In Brazil, there is considerable interest in snaplage to feed ruminants, especially in beef cattle systems. Currently, about 400 maize hybrids are registered for sale on Brazilian market. Thus, we hypothesised that among them there are varieties with more potential to make snaplage. The aim of this study was to evaluate maize hybrids grown across different regions on traits of maize ears.

**Materials and Methods** From January to July 2017, livestock farms were visited in three regions (South, Southeast, and Midwest). Samples were taken from 42 maize fields, totalizing 31 hybrids. As in Brazil there are two growing seasons, hybrids collected from January to March and from May to July were grouped in the first (FS; n = 24) and the second season (SS; n = 18), respectively. All sampling occurred when farmers were harvesting their fields to produce whole-plant silage. On the day of harvest, aerial images were obtained by flying a drone over the maize field. Four random spots (3 linear meter each) were chosen in the area and plants from rows were collected. The plants of each row were weighed and then split into leaves, stem, and ear to determine the proportions of these components. After weighing, the ear was split into grains, cob, and husks. The ear sample and its components were dried at 55°C for 72 h in a forced-air oven and ground to assess dry matter (DM), ash, and neutral detergent fibre (NDF). Ear DM yields were standardised at 65% DM (the standard DM content for snaplage) by using the following equations: $y = 0.309 x + 16.062$ and $y = 0.140 x + 11.293$ for FGS and SGS, respectively, where $x = 65 –$ the obtained DM. The average, maximum, minimum, standard deviation, and 95% confidence interval (CI) of all data were calculated using the PROC MEANS procedure of SAS (2004). Correlation analyses were performed through Pearson's correlation at 5% probability, using the PROC CORR procedure of SAS.

**Results** The mean ear yield for FGS and SGS were 21.2 and 14.7 tons DM/ha, respectively. This parameter showed a CI of 20.1-22.3 tons DM/ha and 13.8-15.6 tons DM/ha for FGS and SGS, respectively. In the FGS, there was a positive trend between ear yield and kernel depth ($r^2 = 0.26$; $P = 0.09$). Whereas, in this season ear yield was negatively correlated with number of grains ($r^2 = –0.68$; $P = 0.01$). In the SGS, ear yield was positively related with kernel depth ($r^2 = 0.51$; $P = 0.03$). In the FGS, the proportions of grains, cob, and husks ranged from 61.2 to 73.0%, from 9.4 to 22.1%, and from 10.1 to 21.5%, respectively. Conversely, In the SGS, the proportions of grains, cob, and husks ranged from 45.7 to 72.5%, from 11.6 to 20.5% and, from 14.6 to 38.5%, respectively. In the FGS, the mean DM and NDF concentrations of grains were 55.2% and 11.9% of DM. In the SGS, the mean DM and NDF concentrations of grains were 51.4% and 13.9% of DM. The mean NDF concentration of cob and husks considering both growing seasons were 81.1 and 81.1% of DM, respectively.

**Discussion** Brazilian producers, especially those who finish beef cattle have had particular interest in snaplage for two reasons: (i) the stalk that remains in the field has been used to protect the soil for no-till system and (ii) the greater concentration of fibre in snaplage than other sources of grain (i.e. silage grain or dry ground shelled corn) may increase chewing activity, since feedlots have used high proportion of concentrates in the diet. Certainly, the greater starch digestibility of ensiled grains when compared to dry ground shelled corn has led feedlots to adopt corn grain silages (Benton et al. 2005). As higher yield reduces harvesting costs per tonne, it is fundamental to choose both the correct hybrid and the suitable season for growing maize. Our results showed that maize hybrids with less and

deeper kernels had greater ear yield. It seems that a larger kernels size compensates a larger number of rows of kernels per ear. Regarding GS, there was an average reduction of 44% in ear yield for maize grown in the SGS than FGS. The growing environment may explain this result, since in the SGS there is a reduction in light incidence, precipitation and temperature (the transition from rainy summer to dry autumn).

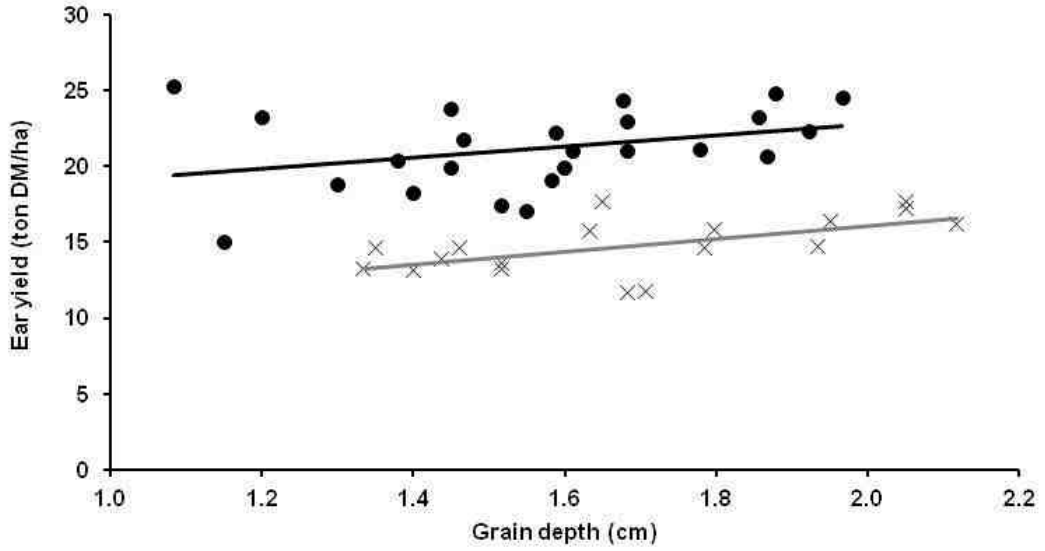


**Figure 1** The relationship between ear yield and kernel depth for the first (n = 24; y = 0.0352x + 0.9789; $r^2$ = 0.26; *P* = 0.09) and second growing season (n = 18; y = 0.089x + 0.6416; $r^2$ = 0.51; *P* = 0.03) in South, Southeast, and Midwest of Brazil. First growing season (●); Second growing season (X).

**Conclusions** Overall, maize hybrids that have less and deeper grains in the ear may have a great potential do produce snaplage, since they show greater ear yield. Farmers should opt for making snaplage in the FGS, since ear yield decreases markedly in the SGS. More data will be collected in 2018 and 2019 for more robust data.

**Acknowledgements** This project was partially funded by Limagrain, Dow AgroSciences, 3r lab, FAPEMIG, and CNPq.

**References**

SAS Institute Inc. (2004) SAS/STAT 9.1 User's Guide. Version 9.1 ed. SAS Inst. Inc., Cary, NC.

Benton, J.R., Klopfenstein, T. & Erickson, G.E. (2005) Effects of corn moisture and length of ensiling on dry matter digestibility and rumen degradable protein. Accessed May 15. https://digitalcommons.unl.edu/cgi/viewcontent.cgi?referer=https://www.google.com.br/&httpsredir=1&article=1150&context=animalscinbcr.

# Impacts of feed-out speed, days after silo opening and surface density on spoilage of silage in the exposed face peripheral area of corn silage piles

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**Keywords:** days after silo opening, feed-out speed, surface density, pile silage, spoilage

**Introduction** Preserving silage in large piles is popular in many areas, including the San Joaquin Valley (SJV) of California. However, pile silos have characteristics, often including a long feed-out phase and large exposed silage area, that make its silage prone to spoilage, especially in the exposed face peripheral area during feed-out. After bacteria initiates spoilage by increasing temperature and pH, yeasts and molds start to proliferate, which is considered to be a sign of an advanced stage of spoilage. Thus, appropriate management of silage piles during feed-out is essential to prevent spoilage and maintain its hygienic quality. Among many management factors, feed-out speed, days after silo opening and surface density are considered to be particularly impactful to the degree of surface silage spoilage. Spoilage of peripheral face silage has been reported to be prevented by high feed-out speed since it decreases the amount of time silage is exposed to oxygen (Borreani and Tabacco 2012, Robinson and Swanepoel, 2016). It has also been reported that high silage density prevents spoilage and helps to preserve nutritive value by limiting ingress of oxygen between silage particles (Ruppel et al. 1995). This study evaluated effects of these characteristics on the degree of peripheral face spoilage in corn silage piles.

**Material and Methods** A total of 9 wedge-type (as in Figure 1) corn silage piles built in late summer/early fall of 2016 on dairy farms in the SJV of California were selected based on defined criteria. Silage samples were collected from 3 'lines' on the surface differentiated by increasing distances from the exposed face (Figure 1); Line 1 was 0.3 m from the face, Line 2 was 3.5 m from the face and Line 3 was 6.5 m from the face. In all cases, Line 1 silage had no plastic cover (it had been removed to facilitate feed-out) whereas Lines 2 and 3 were in the plastic covered area of the pile. Each line had 6 coring locations; locations 1 and 6 were located 1.5 to 1.8 m from the base of the piles (Bottom-side), locations 2 and 5 were ~½ way up the side (Mid-side), and locations 3 and 4 were on the flat top of the pile (Top). From collected samples, temperature, pH, acid and alcohol levels, as well as bacterial community composition were measured in addition to yeast and mold counts in order to investigate the progression of spoilage. Piles were categorized into two groups based on their feed-out speed (high- and low-speed piles), days after pile opening (long and short open piles) and surface density in wet weight (high- and low-surface density piles). Impacts of these three factors on the degree of silage spoilage were separately analysed using the GLM procedure within SAS with each of the three factors described above and core depth (*i.e.*, outer and inner) as fixed factors. Interactions of each factor*depth were also determined. In this study, statistical significance was accepted if $P<0.05$ and a tendency was accepted if $P<0.20$. This rather high $P$ value for a tendency to a difference was selected due to the high inherent variability of most of the response parameters among piles.

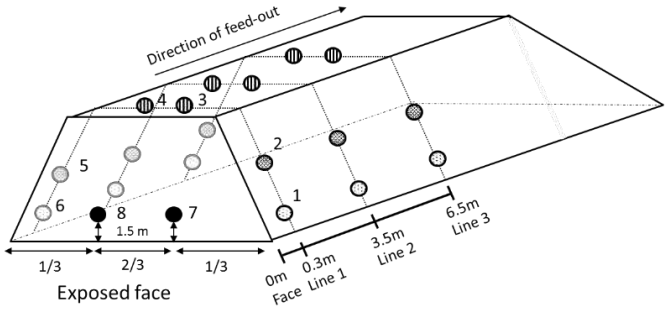


**Figure 1**. Schematic of the sampling locations in the piles. [Dots indicate sample locations from the Bottom-side (1.5 to 1.8 m from the base); Mid-side (middle of the side); Top (flat top of the pile); Deep Mass (lower exposed face)].

**Results and Discussion** Low-speed piles had higher temperature ($P$<0.05) and tended to have higher pH ($P$<0.02) relative to high-speed piles. Lactic acid level and lactic acid bacteria (LAB) abundance were lower ($P$<0.05) in low- *versus* high-speed piles (Table 1). Yeast and mold counts were higher in low-speed piles, however, numerical increase in low-speed piles failed to reach statistical significance. Based on these parameters, low-speed pile silages were judged to be in transition from initiation to mid-stage of spoilage while high-speed piles were judged to be in initiation stage. Feed-out speed of piles was judged to have impacts on spoilage progression in exposed face peripheral area silage. However, since surface spoilage in both low- and high-speed piles were not in an advanced-stage, the average feed-out speed of 0.29 m/day was sufficient to prevent substantive surface silage spoilage in the exposed face periphery.

**Table 1**. Impacts of feed-out speed and depth on degree of surface silage spoilage.

| | Feed-out speed[a] (m/d) | | Depth (cm) | | | *P* | | |
|---|---|---|---|---|---|---|---|---|
| | (Low) 0.29 | (High) 0.62 | (Outer) 0-25 | (Inner) 25-50 | SEM[b] | Spd | Depth | Spd*Depth |
| Temperature (°C) | 38.8 | 30.2 | 35.26 | 33.75 | 2.02 | 0.02 | 0.64 | 0.51 |
| pH | 4.17 | 3.89 | 4.21 | 3.86 | 0.122 | 0.17 | 0.09 | 0.06 |
| Lactic acid (%DM) | 1.98 | 2.67 | 1.51 | 3.14 | 0.359 | 0.03 | <0.01 | 0.38 |
| LAB[b] | 19.7 | 70.3 | 42.2 | 47.8 | 10.8 | <0.01 | 0.69 | 0.37 |
| Yeast (*1000 count/g) | 1089 | 1060 | 1934 | 214 | 822 | 0.97 | 0.02 | 0.90 |
| Mold (*1000 count/g) | 685 | 588 | 1064 | 209 | 462 | 0.80 | 0.04 | 0.62 |

a – The speed that the exposed face moved back due to silage removal; b – % of lactic acid bacteria in total population.

Long-open piles tended ($P$<0.20) to have lower lactic and acetic levels relative to short-open piles. Mold counts also tended ($P$<0.20) to be higher in long- *versus* short-open piles. Based on these parameters, long-open piles were judged to be in transition from the mid- to advanced-stage of spoilage while short-open piles were judged to be between initiation and mid-stage. Therefore, days after opening had impact on spoilage progression at exposed face periphery of silage piles. Since surface spoilage in long-open piles was judged to be in advanced stage, 110 days after silo opening was too long to prevent substantive surface silage spoilage in the exposed face periphery. Low-density piles tended to have higher temperature ($P$<0.20) relative to high-density piles. Yeast and mold counts were higher in low-density piles, but numerical increase in low-density piles failed to reach statistical significance. Low-density piles tended ($P$<0.20) to have lower LAB, and higher acetic acid bacteria (AAB), abundance relative to high-density piles. Surface density had no effect on acid and alcohol concentrations. Based on these parameters, low surface density piles silage was judged to be in transition from the initiation to mid-stage of spoilage, while high density piles were in the initiation stage. However as there were few substantive differences in response parameters between low- and high-density piles, this suggests that an average surface density of 312 kg/m$^3$ was sufficient to prevent substantive surface silage spoilage in the exposed face periphery.

**Conclusion** Faster feed-out speed had positive impacts on suppressing the degree of spoilage and a feed-out speed of 0.29 m/day was sufficient to largely prevent silage spoilage at the periphery of the exposed face. Shorter days after pile opening also had positive impacts on suppressing the degree of spoilage and decreasing the days after opening from 110 to 61 reduced the degree of spoilage at the periphery of the exposed face. Surface density increases in the range of 312 to 360 kg/m$^3$ had little impact on peripheral face spoilage.

**References**

Borreani, G. & Tabacco, E. (2012) Effect of silo management factors on aerobic stability and extent of spoilage in farm maize silages. In: Proceedings of the XVI international silage conference, in July in Hämeenlinna, Finland. p. 71-72.

Robinson, P.H. & Swanepoel, N. (2016) Impacts of a polyethylene silage pile underlay plastic with or without enhanced oxygen barrier (EOB) characteristics on preservation of whole crop maize silage, as well as a short investigation of peripheral deterioration on exposed silage faces. *Animal Feed Science and Technology* 215: 13-24.

Ruppel, K.A., Pitt, R.E., Chase, L.E. & Galton, D.M., (1995) Bunker silo management and its relationship to forage preservation on dairy farms. *Journal of Dairy Science* 78: 141-53.

# Influence of plant population and maturity, microbial inoculation and ensiling time on fermentation profile, nitrogen fractions and starch digestibility in earlage

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**Keywords:** earlage, ensiling time, inoculant, starch digestibility

**Introduction** Earlage (husks, kernels, and cob) is a viable alternative to high-moisture corn. Recently, the use of earlage has received renewed interest by dairy and beef farmers, but literature data evaluating varied harvesting practices is limited. Thus, the objective of this study was to evaluate the effect of plant population, maturity at harvest, microbial inoculation and ensiling time on fermentation profile, N fractions and ruminal in vitro starch digestibility (**ivSD)** in earlage. We hypothesized that early maturity and extended ensiling time would increase ivSD. In addition, earlage treated with a microbial inoculant would have enhanced fermentation profile.

**Material and Methods** Samples from 4 hybrids (used as experimental unit), each planted at 4 different plant populations (64,000 [**64K**], 79,000 [**79K**], 94,000 [**94K**] or 109,000 [**109K**] plants/hectare) and harvested at 2 maturities (1/2 kernel milk line [**ER**] or black layer [**LT**]), were collected at harvest, inoculated without (**CON**) or with $4.0 \times 10^5$ cfu of *Lactobacillus buchneri* 40788 and $1.0 \times 10^5$ cfu of *Pediococcus pentosaceus*/g of earlage (**INOC**; Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI) and ensiled in vacuum-sealed bags for 30, 60, 120 and 240 d. Samples were analyzed for fermentation profile, ammonia-N, soluble CP, starch and ivSD (7 h incubations on dried and 4-mm ground samples) at Dairyland Laboratories Inc. (Arcadia, WI). Data were analyzed as a split-split-split-plot design using Proc Mixed of SAS with the Fixed effects of ensiling time, plant population, maturity, microbial inoculation and their interactions; and the Random effects of hybrid, hybrid × plant population, hybrid × plant population × maturity, and hybrid × plant population × maturity × microbial inoculation interactions.

**Results** No effects of or interactions with plant population were observed ($P > 0.10$). Content of DM was lower ($P = 0.001$; 62.0% vs. 52.5%) for ER than BL. In addition, an inoculation × ensiling time interaction was observed ($P = 0.03$) for DM content; DM content was 1.5%-units greater for CON than INOC on 240 d but not on 30, 60 and 120 d. Maturity × inoculation × ensiling time interactions were observed ($P = 0.001$) for pH, lactate, acetate, and 1,2-propanediol. For ER, pH was greater for INOC than CON throughout the entire ensiling period (4.47 vs. 4.17, on average); this is likely related to lower lactate (0.37 vs. 1.23% of DM, on average) and greater acetate (1.56 vs. 0.93% of DM, on average) and 1,2-propanediol (1.20 vs. 0.45% of DM, on average) concentrations for INOC than CON from 30 to 240 d. However, LT earlage reached a similar pattern only after 60 d for pH, acetate and lactate concentrations or 120 d for 1,2-propanediol concentration. Ethanol concentration was unaffected by inoculation ($P = 0.29$; 0.54% of DM, on average). However, a maturity × ensiling time interaction was observed ($P = 0.001$); ethanol concentration increased for ER but decreased for LT earlage with prolonged fermentation. A maturity × inoculation × ensiling time interaction was observed ($P = 0.001$) for soluble CP. For ER, soluble CP was 3.5%-units greater for INOC than CON on 240 d but not other ensiling times, whereas for LT soluble CP was 3.2% units greater, on average, for INOC than CON on 120 and 240 d but not on 30 and 60 d. A maturity × ensiling time interaction was observed ($P = 0.001$) for ammonia-N; interestingly, despite the increase in ammonia-N for both ER

and LT with prolonged ensiling time, the magnitude of the increase was greater for ER than LT (7.5%- VS. 4.8%-units increase from 30 to 240 d, respectively). Likewise, an inoculation × ensiling time interaction was also observed ($P$ = 0.001) for ammonia-N; this interaction was also related to greater magnitude of the increase for INOC than CON (5.5%- VS. 4.5%-units increase from 30 to 240 d, respectively) throughout the ensiling period. Figure 1 depicts the maturity × ensiling time interaction for ivSD; a gradual increase was observed for LT earlage from 30 to 240 d of ensiling whereas for ER ivSD increased from 30 to 60 d, plateaued from 60 to 120 d and increased from 120 to 240 d. Although an inoculation × ensiling time interaction for ivSD was observed ($P$ = 0.001), it was related to greater and reduced ivSD for CON than INOC on 60 and 120 d, respectively.

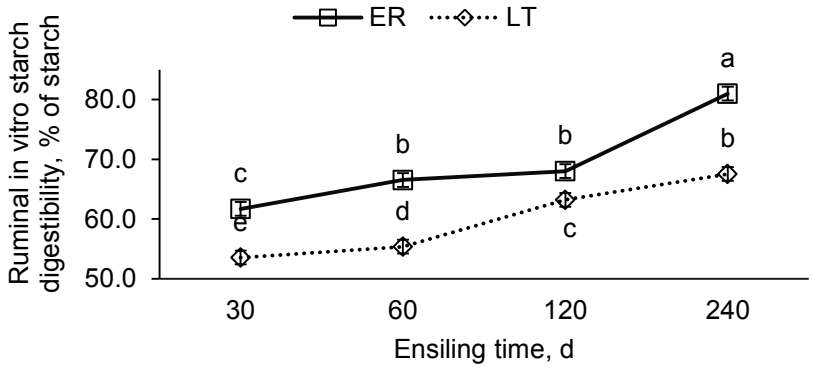


**Figure 1.** Interaction of maturity (ER – early, LT – late) and ensiling time on 7 h ruminal in vitro starch digestibility of earlage. Means with different superscripts differ ($P \leq 0.05$). Effects of maturity ($P$ = 0.001), ensiling time ($P$ = 0.001), and their interaction ($P$ = 0.001); SEM = 1.15.

**Discussion** Fermentation was adequate for all treatments. Moreover, the shift from lactic towards acetic acid observed for INOC is typical of silage inoculation with *Lactobacillus buchneri* (Kung et al. 2007) and underscores that microbial inoculation was effective in the present study. Interestingly, however, this shift occurred at 30 d for ER but only at 60 d for LT. This finding indicates that microbial inoculation with heterofermentative bacteria may require longer storage period to alter fermentation in late compared with early maturity earlage. Gradual increases in ammonia-N, soluble CP and ivSD for all treatments are similar to previous research with whole-plant corn silage and high-moisture corn and highlights the importance of extended storage for optimal starch digestibility (Kung et al. 2018). Furthermore, the interaction between maturity and ensiling time for starch digestibility is in agreement with previous whole-plant corn silage data from our group (Ferraretto et al. 2015) and underscores that ensiling time improves ivSD but does not overcome for the difference caused by greater maturity at harvest. Last, the lack of plant population effect suggest that there is no detrimental effect of greater number of plants in the field on ear quality.

**Conclusion** Fermentation profile, N fractions and ivSD of earlage were influenced to a greater extent by ensiling time, microbial inoculation and maturity than plant populations.

**References**

Ferraretto, L.F., Crump, P.M. & Shaver, R.D. (2015). Effect of ensiling time and exogenous protease addition to whole-plant corn silage of various hybrids, maturities, and chop lengths on nitrogen fractions and ruminal in vitro starch digestibility. Journal of Dairy Science, 98, pp. 8869-8881.

Kung, L., Schmidt, R.J., Ebling, T.E. & Hu, W. (2007). The effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of ground and whole high-moisture corn. Journal of Dairy Science, 90, pp. 2309-2314.

Kung, L., Shaver, R.D., Grant, R.J. & Schmidt, R.J. (2018). *Silage review*: Interpretation of chemical, microbial, and organoleptic components of silage. Journal of Dairy Science, 101, pp. 4020-4033.

# Maize silage in South Africa: Composition, compaction, top layer losses and aerobic stability

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**Keywords:** Evaluation, pH, preservation, silage, starch

## Introduction

Maize silage is an important feed component in dairy and beef cattle diets in South Africa. A system to evaluate silage has been developed and used in South Africa. This system has been used during the Santam/Veeplaas silage competition in 2014, 2015, 2016 and 2017. The evaluation system included nutritional value, fermentation parameters, compaction, top layer losses and aerobic stability of silages. Whole crop maize is easy to ensile as it has high levels of water soluble carbohydrates (WSC) and relatively low protein content (McDonald et al. 1991). Aerobic stability of maize silage is challenging as it contains high levels of residual WSC and lactic acid that can be utilised by yeasts (Wilkins et al. 1999). The aim is to report on the composition, compaction, and aerobic stability of maize silage in South Africa.

**Materials and Methods** A total of 170 maize silage bunkers were sampled from 2014 to 2017. Each bunker was sampled by taking three core samples with a 110 mm PVC silage corer in the middle of the bunker, spaced 1 m apart. Each core sample was taken at three depths: 0-10 cm, 10-20 cm and 20-40 cm. Compaction of the different layers was determined using the weight and volume of each core sample. The three core samples were pooled for each depth and a representative sample was taken, sealed in a plastic bag, kept at 4 ˚C and later frozen pending analysis. The DM, pH and ash content of all silage samples were determined. A representative sample of 300 g from the 20-40 cm pooled sample was loosely placed in a 2 litre plastic container with several holes on its sides to determine aerobic stability. This silage sample was exposed to air for 5 days and then frozen. The DM, ash content and pH was determined to calculate OM losses and monitor aerobic stability. The DM, pH, ash, TDN, CP, starch, NDF, lactic acid, acetic acid, propionic acid and butyric acid of each pooled silage sample taken at 20-40 cm in the bunker was determined at Cumberland Valley Analytical Services (CVAS). The chop length of silage was determined by measuring 10 maize stalks of the composite 20-40 cm sample.

**Results** The composition of 170 maize silage bunkers was: DM 33.6±5.5%, pH 3.81±0.19, TDN 71.1±2.84%, crude protein 8.59±1.15%, starch 28.7±7.15%, NDF 42.4±5.03%, lactic acid 4.09±1.54%, acetic acid 3.52±1.66%, propionic acid 0.27±0.20% and butyric acid 0.02±0.14%. The average compaction in the 0-10cm, 10-20cm and 20-40cm layer was 508±120, 708±128 and 727±105 kg silage/m3 respectively. The OM loss in the top 10 cm layer, 10-20 cm layer and silage exposed to air was 18.7±24.6%, 11.4±13.9% and 8.11±10.1% respectively. The highest OM loss in the top 10 cm was 90.4% while no OM losses were found in the best sealed bunkers. The most stable maize silages had no organic matter losses after 5 days of aerobic exposure, while 58.3% of OM was lost in the least stable silage. The average pH of silage increased from 3.81 before aerobic exposure to 5.24 after 5 days of exposure to air. The pH of the most stable silage did not increase while pH of the least stable silage increased to pH 8.48. The average chop length of maize silage was 12.0±3.34 mm, with the shortest chop length at 5.3 mm and the longest chop length 24.9 mm.

**Discussion** The maize silages were well preserved as indicated by the low average pH of 3.81 at a DM content of 33.6% (McDonald et al. 1991). Some maize silage had a DM content below 25% which then coincided with a low TDN and low starch content, clearly showing the negative impact of ensiling whole crop maize too early. Top layer compaction varied a lot between bunkers. Bunkers with poor top

layer compaction also had higher pH and higher OM losses in the top layer. The silage corer used was very effective to take silage samples and determine compaction of different layers in the silage bunker. Aerobic stability of maize silage is still a challenge and this could be improved by use of hetero-fermentative LAB inoculants (Driehuis et al. 1999).

**Conclusions** It is concluded that whole crop maize silage can still be improved on many farms by ensiling at the correct stage (35% DM). Compaction of the top layer and sealing of the bunker can be improved to reduce top layer losses. Improving aerobic stability of maize silage should get more attention.

**References**

Driehuis, F., Oude Elferink, W.H. & Spoelstra, S.F. (1999) Anaerobic lactic acid degradation during ensilage of whole crop corn inoculated with Lactobacillus buchneri inhibits yeast growth and improves aerobic stability. Journal of Applied Microbiology, v.87, nr4, p.583-594.

McDonald, P., Henderson, A.R. & Heron, S.J.E. (1991) The biochemistry of silage, 2nd Edition, Chalcombe Publications, 13 Highwoods Drive, Marlow Bottom, Marlow, Bucks, SL3 3PU, UK.

Wilkins, R.J., Syrjala-Qvist, L. & Bolsen, K.K. (1999) The future role of silage in sustainable animal production. Proceedings of te 12th International Silage Conference held from 5 July to 7 July at Uppsala, Sweden. Editors: T Pauly and conference scientific committee. Swedish University of Agricultural Sciences, pp. 23-40.

# Microbial inoculant, particle size, and storage time effects on crude protein content and concentration of ammonia nitrogen and soluble protein of reconstituted sorghum grain silage

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**Keywords:** protein solubilisation, rehydration, sorghum grain silage

**Introduction** Sorghum grain has about the same starch content compared to corn grain, however with harder protein matrix surrounding the starch granules, decreasing digestibility. Long storage length seems to increase starch degradation by protein solubilisation, although the interaction with particle size and different microbial inoculants are less evaluated on reconstituted sorghum grain silage. We hypothesized that the protein solubilisation to be positively affected by microbial inoculant comprising heterofermentative species and storage length, and negatively affected by particle size. The aim of this study was to evaluate the effects of microbial inoculant (I), particle size (PS), and storage time (T) on crude protein content and concentration of ammonia nitrogen ($NH_3$-N) and soluble protein of reconstituted sorghum grain silage.

**Material and Methods** Sorghum grains were ground into three PS (8, 2, and 1 mm), rehydrated to reach 650 g DM/kg OM and ensiled in mini silos (300 g). Before ensiling, the mass was inoculated with one of the treatments (1) Control (distilled water); (2) LB, *Lactobacillus buchneri* CNCM I-4323 at $1 \times 10^5$ cfu/g OM (Lalsil® AS – Lallemand Brasil Ltda.); (3) PP, *Pediococus acidilactici* CNCM MA 18/5M at $3 \times 10^4$ cfu/g OM and *Lactobacillus plantarum* CNCM MA 18/5U at $1 \times 10^5$ cfu/g OM (Lalsil® CL – Lallemand Brasil Ltda.); and (4) LBpp, LB + PP. Treatments were evaluated in quintuplicate during 0, 15, 30, 120, and 360 days of storage. Silos were kept inside the lab within controlled temperature (25 °C). Crude protein (CP) was determined by combustion. Soluble protein was analyzed in borate-phosphate buffer. A subsample of 100 g was collected and pressed for $NH_3$-N measurement. Data were analyzed by split-split plot using the Mixed procedure of SAS.

**Results** There was no effect of treatments ($P$ = 0.13) on CP content, where a wide variation was observed throughout the storage time, although there was no difference between the initial and final CP content of the treatments (figure 1). The concentration of soluble protein was affected by PS*I*T interaction ($P$ < 0.01), where the concentration increased linearly with storage time, however the greater protein solubilisation was observed for the treatment with 2 mm of PS inoculated with *L. buchneri* on the d 360 (figure 1). There was PS*I*T interaction ($P$<0.01) for $NH_3$-N concentration, where the peak was observed on d 15 of fermentation with greater concentrations for treatments with 1 and 2 mm of PS inoculated with *L. buchneri*. The concentrations decreased linearly until 120 d and then stabilized with low concentration for all treatments (figure 1).

**Discussion** The results suggest that there was no consumption of CP, but conversion of insoluble protein into soluble form, due the bacteria proteolysis (Junges et al., 2017). The peak of ammonia within the first 15d is likely associated to the degradation of small proteins fragments of the grains, however once it was completely degraded the ammonia concentration dropped due the utilization of this substrate by the bacteria. As the protein matrix (mostly kafirins) has low degradation, the rate of ammonia production turns to be equal to the consumption rate, maintaining the low levels of ammonia throughout the storage time. In the same way, there was a peak of soluble protein within the first 15d followed by a slower increase. The concentration of soluble protein increased for all treatments, indicating that the positive effect of storage time on proteolysis, however this effect was greater when

reconstituted sorghum was ensiled for over 120 d with 2 mm of particle size and inoculated with *L. buchneri*.

**Conclusions** Storing reconstituted sorghum grain for over 120 d with 2 mm of particle size and inoculated with *L. buchneri* increase protein solubilisation without decreasing crude protein content.

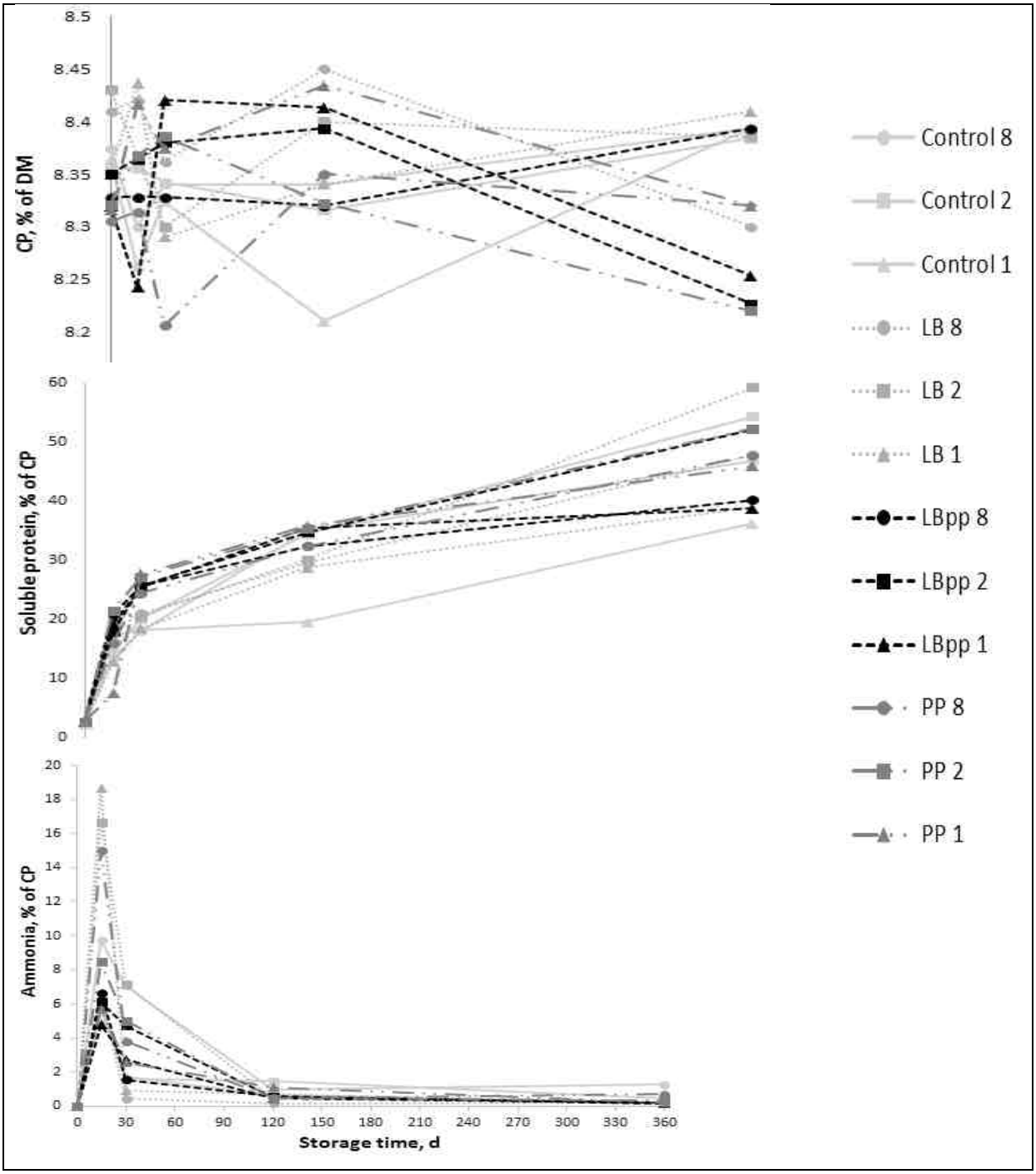


**Figure 1** Microbial inoculant, particle size, and storage time effects on crude protein content and concentration of ammonia nitrogen and soluble protein of reconstituted sorghum grain silage.

**Reference**

Junges, D., Morais, G., Spoto, M.H.F., Santos, P.S., Adesogan, A.T., Nussio, L.G. & Daniel, J.L.P. (2017) *Short communication:* Influence of various proteolytic sources during fermentation of reconstituted corn grain silages. Journal of Dairy Science, 100, 9048-9051.

# Mixed silages of fodder beet and different feedstuffs: quality and nutritive value

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**Keywords**: ensiling, fodder beet, mixed silage

**Introduction** Fodder beet (FB) have by far the highest yield of net energy for lactation (NEL) per ha and are a good catch crop for nitrogen (De Brabander et al. 2012). During the last fifty years, the cultivated area decreased steeply due to labour-intensity and phytopathological threats, as well as preservation difficulties. Nowadays, improved mechanization and availability of disease-resistant cultivars make FB again an attractive crop (ADBFM 2009, Bradshaw 2010). Still, conservation of fresh FB asks a lot of care and attention, and losses can be important (e.g. in case of harvest damage) (Lauwers et al. 2009). To examine the possibilities of ensiling FB, a two-year research project (2015-2017) was performed with main support of the Research Fund of University College Ghent.

**Materials and Methods** FB with high dry matter (DM) content (i.e. 22-23%), 720 g sugar/kg DM, 7.23 MJ/kg DM as NEL, and 81 g protein digestible in the intestine (gDVE) per kg DM were mixed with different feedstuffs and ensiled in order to reduce effluent losses and to improve silage and nutritive quality. Combinations were made with forage-type feeds like maize silage (MS), wilted grass silage (WG), wheat straw (WS), flax chaff (FC) and chopped barley straw (CBS), as well as with concentrates like pressed sugar beet pulp (PBP), wheat gluten feed (WGF), chicory pulp (CP), maize gluten feed (MGF), dried sugar beet pulp (DBP), corn cob mix (CCM), rolled barley (RB), palm kernel meal (PKM) and soybean hulls (SH). The mixtures were ensiled in microsilos (n = 4) with a volume of 2.75 L, equipped with a Bunsen valve and an effluent drain. Additionally, mixtures were ensiled in one-m³ containers to obtain sufficient material for digestibility trials with sheep and rumen incubations with fistulated cows. The FB were mixed with the other feeds in such proportions to reach a global DM content of 330 g/kg fresh matter. After three months of conservation, microsilos were weighed to determine the fermentation losses, and the content was sampled for analysis. The one-m³ containers were weighed and sampled after two and twelve months. Silage quality was evaluated by determining the pH, the ammonia fraction, and the contents of lactic, acetic, propionic and butyric acid and ethanol (De Boever et al 2013). Additionally, samples were analysed for crude protein (CP), crude fibre (CF), crude fat (FAT), crude ash (CA) and sugars. Based on the chemical composition and the digestion coefficients, the NEL was calculated (Van Es 1977). The rumen degradability of CP and organic matter allowed to calculate the gDVE content and rumen degraded protein balance (OEB) according to Tamminga et al. (1994).

**Results and Discussion** Conservation losses and fermentation characteristics are presented in Table 1. Ensiling of low and high DM FB resulted in unacceptable dry matter losses ranging from 37.8 to 46.8 %. CCM and RB were not suitable as mix-partners as they absorbed almost no juice from the FB. Mixtures of FB with MGF, FC, CBS and WS resulted in high DM losses (over 20%), mainly because of fermentation to $CO_2$, water and alcohol. The mixed silages mentioned in Table 1 were characterized by lower DM losses, ranging from 4.8% for WG up to 18.5% for WGF. The most important proximate constituents and nutritive value in terms of energy (NEL) and protein (CP, gDVE and OEB) of the studied mixed silages are summarized in Table 2. The NEL and gDVE of most of the mixed silages were very comparable with the fresh high DM FB, while the sugar content considerably decreased. The lower sugar content can be considered as advantageous for feeding.

**Conclusions** Ensiling FB mixed with MS, WG, PKM and pectin rich feeds (CP,DBP,PBP) in the chosen ratios resulted in acceptable losses of DM and feeding value and a comparable nutritive value

with Fresh high DM FB. This makes it possible to utilize the full yield potential of the FB crop, and to feed FB in mixed silages year-round. However, loss of effluent needs attention in some mixed silages, of course depending on the ratio of FB.

**Table 1**. Conservation losses and fermentation characteristics of some mixed silages.

| Mixed silages | FB ratio | DM (g/kg FM) | | DM loss | Effluent | pH | LA[3] | AA[4] | EtOH[5] | $NH_3$-N / total N |
|---|---|---|---|---|---|---|---|---|---|---|
| | (% DM) | start | end | (%) | (% FM) | | (g/kg DM) | | | (%) |
| Low DM FB[1] | 100 | 153 | 146 | 37.8 | 30.2 | n.d. | n.d. | n.d. | n.d. | n.d. |
| FB[1] | 100 | 220 | 192 | 46.8 | 36.0 | n.d. | n.d. | n.d. | n.d. | n.d. |
| FB-WGF[1] | 53 | 340 | 310 | 18.5 | 2.1 | 3.79 | 53.7 | 47.2 | 30.1 | 5.3 |
| FB-CP[1] | 51 | 347 | 320 | 8.6 | 0.1 | 3.76 | 34.7 | 41.4 | 35.8 | 4.6 |
| FB-DBP[1] | 53 | 342 | 309 | 13.2 | 0.1 | 3.80 | 37.7 | 35.5 | 37.7 | 3.9 |
| FB-PBP[1] | 47 | 236 | 213 | 13.0 | 0.5 | 3.62 | 50.7 | 45.2 | 39.7 | 4.2 |
| FB-MS[1] | 45 | 299 | 284 | 12.8 | 5.0 | 3.75 | 58.5 | 32.6 | 44.9 | 6.1 |
| FB-WG[1] | 44 | 293 | 290 | 4.8 | 0.0 | 4.18 | 37.2 | 33.8 | 98.0 | 5.9 |
| FB-WS[1] | 51 | 348 | 300 | 19.2 | 0.8 | 4.01 | 34.7 | 42.9 | 87.5 | 5.6 |
| FB-PKM[2] | 59 | 342 | 341 | 11.7 | 11.1 | 4.38 | 14.8 | 16.8 | 7.0 | 1.9 |
| FB-SH[2] | 55 | 351 | 343 | 16.4 | 11.9 | 4.33 | 16.2 | 20.2 | 19.6 | 1.7 |

[1] One-m³ containers; [2] Microsilos; [3] LA: lactic acid; [4] AA: acetic acid; [5] EtOH: ethanol.

**Table 2**. Nutritive value of some mixed silages.

| Mixed silages | DM (g/kg FM) | Sugars | CP[1] | CF[1] | CA[1] | NEL (MJ/kg DM) | DVE (g) | OEB |
|---|---|---|---|---|---|---|---|---|
| | | (g/kg DM) | | | | | | |
| FB-WGF | 310 | 57 | 118 | 82 | 65 | 7.27 | 66 | -3 |
| FB-CP | 320 | 39 | 77 | 123 | 68 | 7.55 | 84 | -63 |
| FB-PBP | 213 | 15 | 83 | 137 | 84 | 7.38 | 84 | -57 |
| FB-DBP | 309 | 26 | 85 | 136 | 70 | 7.45 | 89 | -62 |
| FB-MS | 284 | 21 | 73 | 125 | 52 | 7.00 | 60 | -44 |
| FB-PKM | 341 | 145 | 112 | 94 | 51 | 6.90 | 78 | -27 |
| FB-SH | 351 | 52 | 100 | 230 | 60 | 7.05 | 89 | -50 |

[1]Crude Protein, Crude Fibre an Crude Ash

**References**

ADBFM (2009) La Betterave fourragère, Association pour le Développement de la Betterave Fourragère Monogerme, Paris. www.betterave-fourragere.org. In French.

Bradshaw, J.E. (2010) Root and tuber crops - Handbook of plant breeding. Dundee: Scottish Crop Research Institute. 295 p.

De Boever, J., Dupon, E., Wambacq, E. & Latré, J. (2013) The effect of a mixture of *Lactobacillus* strains on silage quality and nutritive value of grass harvested at four growth stages and ensiled for two periods. Agricultural and Food Science, 22, 115-126.

De Brabander, D., De Campeneere, S., Ryckaert, I. & Anthonissen, A. (2012) Dairy cattle feeding. ILVO Announcement 101, 112 p. In Dutch.

Lauwers, T., Vicca, J., Latré, J., Huygens, D. & Lips, D. (2009) Valorization of ensiled fodder beets, Bulletin UASVM Agriculture, 66.

Tamminga, S., Van Straalen, W.M., Subnel, A.P.J., Meijer, R.G.M., Steg, A., Wever, C.J.G. & Blok, M.C. (1994) The Dutch protein evaluation system: the DVE/OEB-system. Livestock Production Science, 40, 139-155.

Van Es, A.J.H. (1978) Feed evaluation for ruminants I - The system in use from May 1977 onwards in The Netherlands. Livestock Production Science, 5, 331-345.

# On-farm evaluation of maize silage: Is it possible to estimate dry matter and methane losses?

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**Keywords:** balance bag, chopping length, density, Hohenheim Biogas Yield Test, input-output-balance

**Introduction** The aim of the study was the comparison of two identical bunker silos, which were filled with maize of two chopping length (12.5 mm, 4.5 mm). The comparison included silage density, dry matter losses, quality and temperature. Furthermore, the objective was to test whether and to what extent the laboratory method to determine the methane losses during ensiling is transferable to on-farm trials.

**Material and Methods** The trial was done at a biogas plant in Northern Germany and started during maize harvest 2014. The first bunker silo was filled with the longer chopped maize (silo 1), the second silo with the shorter maize two weeks later (silo 2). In both cases, 31 weighed balance bags including temperature data-loggers were buried at three different layers (L1: low, L4: middle, L7: top) during the storage process. The target position of the bags is illustrated in figure 1. Additional bags with a distance of only 0.4 m between the layers (L2, L3, L5 and L6) were placed at one vertical line in each silo, highlighted by an arrow. Samples of fresh maize were taken to analyse the content of ingredients in an external laboratory and to determine the specific methane yield (SMY) using the Hohenheim Biogas Yield Test (HBT; Ohl 2011) with the modification, that the samples were used without drying. The removal process started with silo 2 at a mean feed rate of 1.7 m/week, followed by silo 1 at a mean feed rate of 2 m/week. During the removal process balance bags were recovered after silage density was evaluated using a horizontal sampling borer near the bags. Than bags were weighed and silage samples were taken to analyse quality parameters and SMY. Dry matter and methane losses were estimated in accordance with laboratory method described in DLG guidelines of silage additives aim of action 6a (DLG 2016). In addition the results were compared with the input-output-balance of the silos. The Kruskal-Wallis-Test was used for statistical analyses.

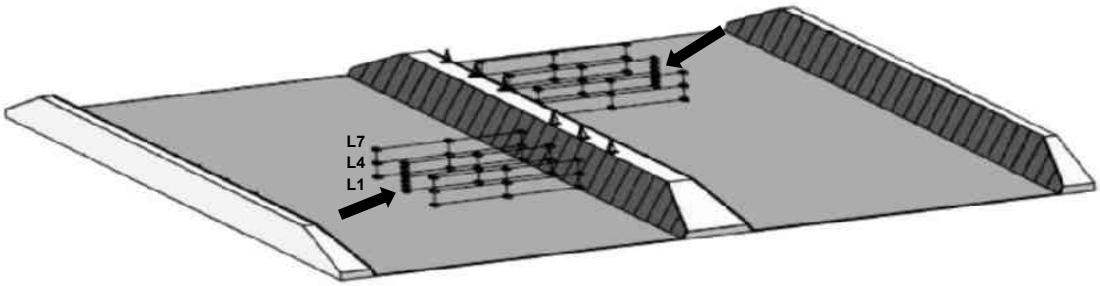


**Figure 1**. Target position of balance bags in silo 1 (left) and silo 2 (right) at different layers (L1: low, L4: middle, L7: top)

**Results and discussion** Silage density in silo 1 was significantly lower (138-300 kg DM/m³, mean 216 kg DM/m³) than density in silo 2 (200-343 kg DM/m³, mean 279 kg DM/m³, figure 2). In silo 1 the lowest density was observed in the top layer. This was connected with reheating and the highest dry matter losses (mean: 8%), whereas dry matter losses in low and middle area were merely up to 5%. In contrast, the lowest density in silo 2 was found at some positions in the middle layer, which was caused probably by high harvested volumes stored in a minimum of time at lower intensity of compaction. The highest dry matter losses up to 22% were found at these positions (mean: 13% at middle layer). Koehler et al. (2013) found a similar range for density and dry matter losses at Bavarian

bunker maize silos using the input-output-balance. For the current study the input-output-balance was too imprecise because of weighing errors during removal due to combined ensiling of different substrates (maize and grass) in silo 2. SMY of fresh maize were in a similar range (silo 1: 395 $L_N/kg_{ODM}$, silo 2: 382 $L_N/kg_{ODM}$), SMY of silages varied (silo 1: 379 $L_N/kg_{ODM}$, silo 2: 414 $L_N/kg_{ODM}$) even though the content of ingredients was similar. Hence mean methane losses of 10% resulted for silo 1 (maximum 18%) and 1% for silo 2 (maximum 12%). The higher SMY of silage in silo 2 partially compensated its higher dry matter losses. However, such a compensation seems rather improbable under practical conditions. Assignment of the samples to different trial runs of HBT rather explains this. All in all the highest methane losses were observed on these measuring points with the highest dry matter loss and the lowest density.

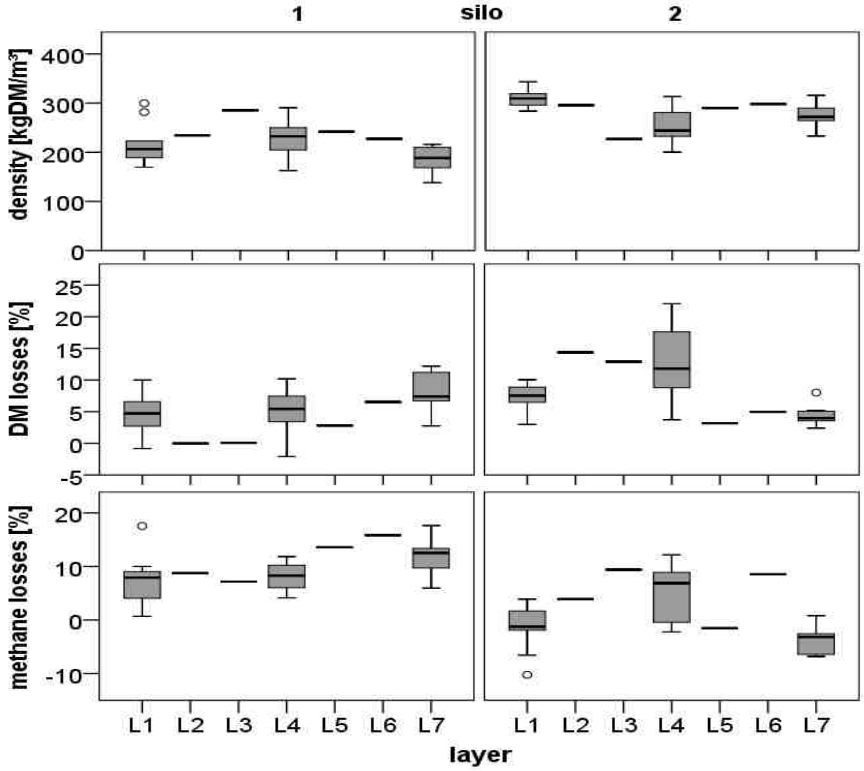


**Figure 2**. Silage density, dry matter and methane losses at different layers (L1: low, L4: middle, L7: top)

**Conclusion** Higher silage density were reached on average in the silo with maize of chopping length 4.5 mm than in the other silo with maize of chopping length 12.5 mm. A lower silage density entailed higher dry matter and methane losses. The use of balance bags in a bunker silo and the investigation of fresh maize and silages in the HBT enable to estimate the dry matter losses and to get an idea about the loss of methane yield. A certain percentage of methane losses is certainly unavoidable during ensiling. It is thus all the more important that dry matter losses and aerobic spoilage has to be reduced to get more methane during biogas production process in order to increase efficiency.

**References**

DLG e.V. (2016) DLG Testing Guidelines for the award und use of the DLG Quality Mark for ensiling agents, prepared under the auspices of the DLG commission for ensiling agents. http://www.dlg.org/siliermittel.html.

Koehler, B., Ostertag, J., Thurner, S. & Spiekers, H. (2013) Dry matter losses of grass, lucerne and maize silages in bunker silos. Agricultural and Food Science, 22, 145-150.

Ohl, S. (2011) Ermittlung der Biogas- und Methanausbeute ausgewählter Nawaro. Forschungsbericht Agrartechnik, 501, PhD Thesis, Kiel. http://eldiss.uni-kiel.de/macau/receive/dissertation_diss_00007295.

# Relationship among economic and nutritional parameters in flint and semi-dent corn silage

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**Keywords:** corn silage, economic parameters, flint corn, nutritional parameters, semi-dent corn

**Introduction** Corn silage is a conserved forage commonly fed to dairy cows; its energy value is affected by the content of neutral detergent fiber (NDF) and starch (STCH). The dry matter (DM) yield (DMY) is a key economic parameter evaluated by dairy farmers. There are differences in nutritional parameters among corn hybrids; e.g. flint corn hybrids have less starch content and more vitreous endosperm than semi-dent corn hybrids (Philippeau et al. 1998). Hybrid selection is done based on weather and soil conditions, silage harvesting practices, and animal requirements. The interaction between hybrid and environment defines the DMY, the nutritional quality, and the milk per ton (MYtonDM). We hypothesize that DMY and MYtonDM of flint (FWCS) and semi-dent (SDWCS) whole-plant corn silage are positively correlated to DM content at harvesting (DMatH) and STCH. Our objective was to analyze the relationship among nutritional and economic variables in FWCS and SDWCS.

**Materials and Methods** During 2017, we sampled a total of 57 silo bags (2.74 m diameter, 75 m length) of whole-plant corn silage from a commercial dairy farm (Grupo Chiavassa; latitude: -32° 02' 60" S, longitude -61° 47' 59" W; Santa Fe, Argentina). The total data set included 39 bags of FWCS nd 18 bags of SDWCS. Before filling each silo bag, each wagon was weight with a scale of 80 tons of capacity and a digital register. Every 2 hours, silage DMatH was determined in a 200-g sample using a forced air oven at 135°C. The DMY (tonDM/ha) was calculated with the average of total DM tons of whole-plant corn chopped divided by the numbers of hectares harvested. After 90 days, samples were collected manually from the side of the silo bag and sent to Rock River Laboratory Inc. for nutritional composition analysis by NIRS. Nutritional variables selected for statistical analysis include ash, STCH, adjusted NDF (aNDF), acid detergent fiber (ADF), and total tract neutral detergent fiber digestibility (TTNDFD). Economic variables selected for statistical analysis included DMY, MYtonDM (kg milk/tonDM; Shaver et al. 2006), milk yield per hectare (MYha, kg/ha), and silage cost per ton (DMC, US$/tonDM; CACF). Days from planting to harvest (DfPtH, days) was calculated as time between plating to harvesting. Growing degree-day (GDD, °C) between planting and harvesting was calculated using the daily average temperature (registered by wireless Vantage Pro 2 weather station in the farm) minus basal temperature (10 °C; Nielsen and Hinkle, 1996). We ran Pearson correlation coefficients (r; InfoStat, Di Rienzo et al. 2017) for FWCS and SDWCS using 12 variables: DfPtH, GDD, DMatH, DMY, STCH, aNDF, ADF, ash, TTNDFD, DMC, MYtonDM, and MYha. Partial correlation coefficients (r) were also run keeping DMY as a constant variable and using 9 independent variables: GDD, DMatH, STCH, aNDF, ADF, ash, TTNDFD, DMC, and MYtonDM.

**Results** For FWCS, DMY (18.14 ± 98 ton/ha) was positively ($P < 0.05$) correlated with DfPtH and GDD, MYha, and negatively ($P < 0.05$) correlated with DMatH, STCH, and DMC. For SDWCS, DMY (14.75 ± 1.64 ton/ha) was positively ($P < 0.05$) correlated with DfPtH and MYha, and negatively ($P < 0.05$) correlated with GDD, DMatH, DMC and STCH (Table 1a). When DMY was kept as a constant variable, significant ($P < 0.05$) partial correlations were found for both hybrids. For FWCS, MYtonDM was positively ($P < 0.05$) correlated with GDD, DMatH and STCH, and negatively ($P < 0.05$)

correlated with aNDF, ADF, ash and TTNDFD. For SDWCS, MYtonDM was positively ($P < 0.05$) correlated with STCH and negatively ($P < 0.05$) correlated with ADF (Table 1b).

**Table 1.** Pearson correlations (a) between DMY and selected variables (*r* values). Partial correlation (b) between MYtonDM and selected variables (*r* values; DMY kept).

| | | (a) DMY, ton/ha | | (b) MYtonDM, kg/ton | |
|---|---|---|---|---|---|
| Variable | | FWCS | SDWCS | FWCS | SDWCS |
| At Harvest | DfPtH, days | 0.31* | 0.59** | | |
| | GDD, °C | 0.42** | - 0.60** | 0.36* | 0.40 |
| | DMatH, % | - 0.63** | - 0.61** | 0.21 | 0.40 |
| Nutritional | ADF, % | 0.18 | 0.29 | - 0.85** | - 0.71* |
| | aNDF, % | 0.04 | 0.21 | - 0.81** | - 0.64 |
| | Ash, % | 0.20 | - 0.05 | - 0.70** | - 0.39 |
| | STCH, % | - 0.31* | - 0.46* | 0.88** | 0.71* |
| | TTNDFD, % | - 0.20 | - 0.15 | - 0.58** | 0.19 |
| Economic | DMC, US$/ton | - 0.64** | - 0.91** | - 0.15 | - 0.32 |
| | MYtonDM, kg/ton | - 0.16 | 0.11 | | |
| | MYha, kg/ha | 0.60** | 0.94** | | |

FWCS: flint corn whole-plant silage; SDWCS: Semi-dent corn whole-plant silage; *$P < 0.01$; **$P < 0.05$

**Discussion** The hybrid and DfPtH affected DMY and MYtonDM because both hybrids differed in growing season length (139 and 152 days to black layer or physiological maturity for SDWCS and FWCS, respectively). Both hybrids shown positive relationship between DMY and DfPtH, but DMY was affected differently by GDD. GDD had a positive correlation with FWCS because its growing season length is longer, while it had a negative correlation with SDWCS because its growing season length is shorter; in agreement with Peyrat et al. (2016). The negative correlation between DMY and DMatH found in our study could be explained by the fact that DMatH was high for both hybrids (40.1 ± 3.1 % and 42.7 ± 5.0 % for FWCS and SDWCS respectively). This probably means that the crop had reached or passed the black layer stage (physiological maturity) at harvest. However, MYha was increased and DMC was reduced significantly with higher DMY. Row et al. (2016) found that DMC was reduced and silage quality was not affected when whole-plant corn was harvested between 35 to 44 %DM. When DMY was kept constant, MYtonDM increased with higher STCH and less ADF for both hybrids, without affecting DMC. Similar results were reported by previous work (Di Marco et al. 2002, Row et al. 2016).

**Conclusions** Our data shows that DMY of both corn hybrids is positively correlated with DfPtH and MYha, and negatively correlated with DMatH and DMC. For the same DMY, our data show for both hydrids that MYtonDM is positively correlated with STCH content and negatively correlated with ADF.

**References**

Di Marco, O. N., Aello, M. S., Nomdedeu, M. & Van Houtte, S. (2002). Effect of maize crop maturity on silage chemical composition and digestibility (in vivo, in situ and in vitro). Animal Feed Science and Technology, 99, 37-43.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2017). InfoStat versión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar.

Nielsen, D.C. & Hinkle, S.E. (1996) Field evaluation of basal crop coefficients for corn based on growing degree days, growth stage, or time. Transactions of the ASAE. American Society of Agricultural Engineers, 39, 97-103.

Peyrat, J., Baumont, R., Le Morvan, A. & Nozière, P. (2016) Effect of maturity and hybrid on ruminal and intestinal digestion of corn silage in dry cows. Journal of Dairy Science, 99(1), 258-268.

Philippeau, C. & Michalet-Doreau, B. (1998) Influence of genotype and ensiling of corn grain on in situ degradation of starch in the rumen. Journal of Dairy Science, 81, 2178-2184.

Row, C.A., Shreck, A L., Bondurant, R.G., Bittner, C.J., Harding, J.L., MacDonald, J.C, & Erickson, G.E. (2016) Effect of Corn Plant Maturity on Yield and Nutrient Quality of Corn Plants, 2- Year Summary. Nebraska Beef Cattle Reports. 906.

Shaver, R.; Hoffman, P.; Lauer, J. & Coors, J. (2006) Corn Silage Evaluation System. Milk 2006 NDFd 30 hours. University of Wisconsin. Madison, 2006. Available: http:// shaverlab.dysci.wisc.edu/spreadsheets/.

# Relationship between economic and nutritional variables in alfalfa silage

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**Keywords:** quality, silage, accumulated degree-days.

**Introduction** To obtain elevated milk yield and composition, dairy cows require high quality forages such as alfalfa silage. Growing degree days (GDD) has been used to predict alfalfa growth (Fischer 1998). We hypothesize that GDD (°C) and milk yield per ton of dry matter (MYtonDM) of alfalfa silage are correlated with amylase-treated NDF modification (aNDF), acid detergent fiber (ADF), and total tract neutral detergent fiber digestibility (TTNDFD) content. Our objective was to analyze the relationship among nutritional and economic variables in alfalfa silage.

**Materials and Methods** From December 2016 (summer) to April 2017 (fall), we sampled a total of 26 silo bags (2.74 m diameter, 75 m length) of alfalfa silage from a commercial dairy farm (Grupo Chiavassa; latitude: -32° 02' 60" S, longitude -61° 47' 59" W, Santa Fe, Argentina). The alfalfa variety was WL 611 (commercial fall dormancy 6). Alfalfa crops was mowed at early bloom and field wilted to reach a minimum of 40% dry matter at harvesting (DMatH). Before filling each silo bag, each wagon was weight with a scale of 80 tons of capacity and digitally registered. Every 2 hours, we measured DMatH on a 200-g sample using a forced air oven at 135°C. The dry matter yield per hectare (DMY, tonDM/ha) was calculated with the average of total DM per ton of alfalfa harvested divided by the numbers of hectares. After 90 days, samples were manually collected from one of the sides of the silo bag and sent to Rock River Laboratory Inc. for nutritional composition analysis by NIRS (model Foss 5000, validated by National Forage Testing Association). Nutritional variables selected for the statistical analysis aNDF, ADF, Crude Protein (CP, %), and TTNDFD. Economic variables selected for the statistical analysis included DMY, MYtonDM (kg milk/tonDM; Shaver et al. 2006), milk yield per hectare (MYha, kg/ha), and silage cost per hectare (DMC, US$/ton; CACF). Between harvesting times, GDD was calculated using the daily average temperature (registered by wireless Vantage Pro 2 weather station in the farm) minus basal temperature (5 °C; Fischer 1998). We ran Pearson correlation coefficients (r; InfoStat, Di Rienzo et al. 2017) using the following variables: DMY, GDD, DMatH, aNDF, ADF, CP, TTNDFD, DMC, MYha, and MYtonDM. Partial correlation coefficients (r) were also run keeping DMY as a constant variable and using 9 independent variables: GDD, DMatH, aNDF, ADF, CP, TTNDFD, DMC, MYha, and MYtonDM.

**Results** DMY (2.8 ± 0.77 ton/ha) was positively ($P < 0.05$) correlated with MYha, and negatively ($P < 0.05$) correlated with DMatH (47.6 ± 7.4 DM%) and DMC. When DMY was kept as a constant variable, significant ($P < 0.05$) partial correlations were found. GDD was positively correlated with aNDF and ADF, and negatively correlated with TTNDFD. MYtonDM was positively correlated with TTNDF and MYha, and negatively correlated with ADF and aNDF.

**Table 1.** Pearson (a) correlations between DMY and selected variables (*r* values). Partial (b) correlations between GDD and MYtonDM and selected variables (*r* values).

| | Variables | (a) DMY | (b) GDD, °C | (b) MYtonDM, kg/ton |
|---|---|---|---|---|
| At harvest | DMatH, % | -0.60* | 0.28 | -0.15 |
| Nutritional | ADF, % | 0.38 | 0.82* | -0.89* |
| | aNDF, % | 0.39 | 0.83* | -0.85* |
| | TTFDND, % | 0.30 | -0.83* | 0.80* |
| | CP, % | -0.35 | -0.76 | 0.61 |
| Economic | DMC, US$/ha | -0.95* | 0.04 | -0.07 |
| | MYtonDM, kg/ton | -0.03 | -0.66 | |
| | MYha, kg/ha | 0.96* | -0.74 | 0.97* |

DMY: dry matter yield; GDD: growing degree days; MYtonDM: milk yield per ton of dry matter; DMatH: dry matter at harvesting; ADF: acid detergent fiber; aNDF: adjusted neutral detergent fiber; TTFDND: total tract neutral detergent fiber digestibility; CP: Crude Protein; DMC: cost per hectare; MYtonDM: milk yield per ton of dry matter; MYha: milk yield per hectare. **P<0.05*

**Discussion** DMY had a relationship negative whit DMatH because the drying rate is faster with less DMY. When DMY was kept constant, GGD was not correlated with DMatH because the alfalfa crop was mowed and wilted in the field before harvest. With higher GDD, ADF (38.3 ± 3.0%) and aNDF (44.1 ± 4.0%) content increased and TTNDFD (34.4 ± 4.8%) decreased, probably because the leaf to stem ratio decreased. Others (Fischer 1998; Don Breazle 1999; Marck et al. 2014) found that NDF was less than 40% when GDD was under 400 GDD. Allen et al. (1992) reported that over 378 GDD, NDF content of alfalfa silage increase 5 percentage units for every 100 GDD over 40% of NDF. This differs with our results probably because the average GDD in our study was 765 ± 142 °C and most of the bags were made during the summer and autumn (n = 16 and n = 4 respectively) in agreement with Coblentz et al. (2008).

**Conclusion** Our data shows that DMY correlates with MYha and DMC, but it does not with MYtonDM.

**References**

Allen, M. Leep, R. & Andresen, J. (1992). Timing Spring Alfalfa Harvest – The Final Word? Departments of Animal Science, Crop and Soil Science, and Geography. Michigan State University. Available: https://forage.msu.edu/extension/timing-spring-alfalfa-harvest-the-final-word/.

Coblentz, W.K., Brink, G.E., Martin, N.P. & Undersander, D.J. (2008) Harvest timing effects on estimates of rumen degradable protein from alfalfa forages. Crop Science, *48*, 778-788.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M. & Robledo C.W. (2017) InfoStatversión 2017. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar.

Don Breazle., Kettle, R. & Munk, G. (1999). Using Growing Degree Days for Alfalfa Production. Coperative Extension, University of Nevada. Available: https://www.unce.unr.edu/publications/files/ag/other/ fs9971.pdf.

Fischer, DB., (1998). Determining Alfalfa Harvest by Growing Degree Days. Dairy Cattle, Illinois Livestock trail. Available: http://livestocktrail.illinois.edu/dairynet/paperDisplay.cfm?ContentID=244.

Mark, R. Kenneth, A. Vance,O. & Jerome, C. (2014)Update on Predicting Harvest Time for Alfalfa. Available: https://fyi.uwex.edu/forage/files/2014/01/alfqualest.pdf.

Shaver, R.; Hoffman, P.; Lauer, J. & Coors, J. (2006) Corn Silage Evaluation System. Milk 2006 NDFd 30 hours. University of Wisconsin. Madison, 2006. Available: http:// shaverlab.dysci.wisc.edu/ spreadsheets/.

# Replacement of polyethylene film with Silostop oxygen barrier film on the nutritive value of corn silage for finishing beef cattle

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**Keywords:** aerobic deterioration, animal performance, corn silage, oxygen barrier film

**Introduction** Aerobic deterioration is the main source of nutrient loss during silage storage and feed-out. Silo covering films with low oxygen permeability restrict the growth of spoilage microorganisms and reduce nutrient loss. To our knowledge, no studies have been carried-out to measure the benefits of covering silage with an oxygen barrier film (Silostop) on the nutritive value of silage for beef cattle. Therefore, the objective of this work was to determine the effect of replacing a conventional polyethylene film (PE) with a PE + ethylene-vinyl alcohol film (OB) on the nutritive value of ensiled whole-crop corn for finishing beef cattle. We hypothesized that OB film would decrease the influx of oxygen and nutrient loss and in turn improve animal performance.

**Material and Methods** The experiment was conducted at the Experimental Farm of the State University of Maringá, Brazil. Whole-plant corn was harvested at ½ milk line stage (31% of DM) with a pull-type forage harvester and packed simultaneously in two 30-t bunker silos, without additive. One silo was covered with a 45-μm PE film and protected with Silostop woven high density polyethylene anti-UV cover. The second silo was covered with a 45-μm OB film and also protected with Silostop anti-UV cover. Nylon bags filled with chopped forage (500 g) were buried during silo filling in the top (15 cm depth from top surface), middle (75 cm depth) and bottom (135 cm depth) layers of the silo to determine DM loss during the storage period. After 93 days of storage, both silages were opened for a feeding trial. Silage feed-out was performed manually once a day (in the morning) using a fork. The proportion of spoiled and visually non-spoiled silage was quantified every day. All the silage under both covering treatments was fed to the animals. Thirty Nellore bulls (352 ± 16.8 kg body weight, BW) were blocked by initial BW and confined in individual pens for 94 d (10 d of adaptation to facilities + 84 d of comparison). The experimental diets contained 40% of total DM as either PE or OB corn silage and 60% of total diet DM as concentrates (based on dry ground corn, soybean meal, urea and mineral-vitamin mix). Dietary ingredients were mixed manually twice daily, before each feeding at 9:00h and 15:00h. Animal assessments included daily feed intake, average daily gain, feed efficiency (gain/feed), carcass dressing, and carcass gain. Additionally, samples of silage were taken from the exposed silo feed-out working face (top, middle and bottom layers, 4 samples from each layer) to measure microbial counts (yeasts, molds and lactic acid bacteria), pH and temperature (at a depth of 20 cm from the front of the face). Statistical analysis was performed using the MIXED procedure of SAS. Animal performance outcomes were analyzed using a model including fixed effects of block and film. Silage data were compared using a model including fixed effects of film, silo layer and film × layer interaction, and a random effect of sampling period. The period × film effect served as the error term for testing the film effect (main plot). Covariance structures were chosen based on the Akaike information criterion. Means were compared by Tukey's test ($\alpha = 0.05$).

**Results** Compared to PE film, OB film decreased yeast counts in the top layer and silage temperature across the feed-out working face ($P < 0.01$). The DM loss measured with buried bags was lower in the top layer of the silo sealed with OB film than for PE film ($P < 0.01$, Table 1). There was no visually spoiled silage in both treatments. Dry matter intake (8.85 kg/d), daily variation in DM intake (8.18%), final body weight (462 kg), average daily live weight gain (1.283 kg/d), carcass dressing percentage

(55.0%), carcass gain (0.910 kg/d) and feed efficiency (0.144 kg live weight gain/kg DM intake) were similar between treatments.

**Table 1**. Characteristics of corn silages under PE and OB films.

| | PE + anti-UV cover | | | OB + anti-UV cover | | | | P-value[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | Top | Middle | Bottom | Top | Middle | Bottom | SEM[2] | F | L | F×L |
| DM, % as fed | 28.7$^{b}$ | 29.7$^{ab}$ | 29.7$^{ab}$ | 28.7$^{b}$ | 29.3$^{ab}$ | 30.0$^{a}$ | 0.77 | 0.99 | 0.03 | 0.67 |
| Temperature, °C | 34.8$^{a}$ | 32.3$^{b}$ | 29.8$^{cd}$ | 32.8$^{ab}$ | 30.9$^{c}$ | 29.4$^{d}$ | 0.35 | <0.01 | <0.01 | 0.31 |
| pH | 3.78$^{ab}$ | 3.64$^{b}$ | 3.43$^{c}$ | 3.79$^{a}$ | 3.67$^{ab}$ | 3.42$^{c}$ | 0.070 | 0.67 | <0.01 | 0.87 |
| Lactic acid bacteria, log cfu/g | 3.70 | 3.35 | 3.60 | 4.57 | 3.73 | 3.53 | 0.479 | 0.14 | 0.12 | 0.33 |
| Yeasts, log cfu/g | 4.93$^{a}$ | 3.42$^{b}$ | 3.49$^{b}$ | 3.42$^{b}$ | 3.09$^{b}$ | 2.68$^{b}$ | 0.211 | <0.01 | <0.01 | 0.05 |
| Molds, log cfu/g | 3.33 | 2.77 | 2.15 | 2.85 | 2.95 | 3.00 | 0.392 | 0.59 | 0.48 | 0.32 |
| DM loss during storage[3], % of DM ensiled | 16.6$^{a}$ | 11.9$^{ab}$ | 5.3$^{c}$ | 9.4$^{bc}$ | 9.3$^{bc}$ | 5.6$^{c}$ | 1.22 | <0.01 | <0.01 | 0.03 |

[1]F = film effect, L = silo layer effect, F×L = interaction effect.
[2]SEM = Standard error of the mean.
[3]Measured with buried bags.
[a,b,c,d]Means within a row with different superscripts differ (Tukey test, α = 0.05).

**Discussion** Oxygen barrier films are recognized by their reduced oxygen permeability compared with standard PE films (Wilkinson and Fenlon 2013). In the current study, the low oxygen permeability of the OB film restricted the growth of yeasts and decreased silage temperature and DM loss during storage, especially in the top 15 cm layer. Contrary to our hypothesis, there was no visually spoiled silage in both treatments, perhaps due to the anti-UV cover and good management during silage feed-out (removal rate > 12 cm/d). Furthermore, the relatively low DM content of the silage might have favored heterofermentation and the formation of antifungal compounds such as acetic acid, decreasing the risk of aerobic deterioration (Wilkinson and Davies, 2012). Animals fed corn silage covered with PE or OB had similar performance reflecting the absence of visually spoiled silage and the relatively low proportion (40%) of silage in the total diet DM.

**Conclusions** The OB film decreased DM loss in the top layer. No significant differences were observed in growing performance of finishing beef cattle fed silages sealed with PE or OB films.

**References**

Wilkinson, J.M. & Davies, D.R. (2012) The aerobic stability of silage: Key findings and recent developments. Grass and Forage Science, 68, 1-19.

Wilkinson, J.M. & Fenlon, J.S. (2013) A meta-analysis comparing standard polyethylene and oxygen barrier film in terms of losses during storage and aerobic stability of silage. Grass and Forage Science, 69, 385-392.

# Response to total mixed ration stabilizers depends on feed quality

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**Keywords:** Additive, Aerobic deterioration, Formic acid, Grass silage, Stability

**Introduction** Total mixed ration (TMR) is a feeding method where forage and concentrate components are mixed into a single feed. TMR can facilitate use of moist feeds, ensure stable rumen fermentation and save labour. Sometimes TMR is prone to spontaneous heating due to activity of aerobic micro-organisms present in the feed ingredients. Warm ambient temperature can intensify the heating problem. Heating can be delayed by chemical preservatives mixed into the TMR together with feed ingredients. Three laboratory scale experiments were conducted where the effect of preservatives on TMR heating were tested. Hygienic quality or moisture concentration of grass silage based TMR was varied. Hypothesis was that the TMR quality modifies the response to preservatives.

**Material and Methods** The TMR in Experiment 1 (Exp. 1) was made of low hygienic quality components while in Experiment 2 (Exp. 2), more stable raw materials were used. Both experiments were made using same 4 preservatives and a control without preservative. The preservatives were calcium propionate (PA); two mixtures of calcium propionate and sodium benzoate (PB1 and PB2) and a blend of formic and propionic acids (FPA). The additives were provided by Eastman Chemical Company (Kingsport, Tennessee, USA). All preservatives were used at a rate of 3 kg/t or 3 l/t. The low and high quality TMRs were prepared on different occasions using different ingredients and recipe. The low quality TMR was prepared from unstable silage, two different batches of crimped barley grain and straw and mixed with farm scale machinery. The high quality TMR was prepared from a stable silage, dry barley and oats grains in laboratory. Both TMRs included rapeseed meal and minerals. The dry matter (DM) concentration of low quality TMR was 475 and that of high quality 400 g/kg.

The third experiment (Exp. 3) was designed to be 3×2 factorial including three DM concentrations of TMR manipulated by water addition and two preservative treatments (control and FPA at a rate of 1.5 l/t). In Exp. 3, the high quality raw materials of Exp. 2 were used but on a different occasion. The DM concentrations of the TMRs were 479 (no water addition), 386 (low water addition) and 330 (high water addition) g/kg. In all TMRs the forage to concentrate ratio was 1:1 on DM basis and three or four replicates per treatment were prepared. The grass silages and crimped barley used were produced without acid based silage additives. All preservatives were diluted into water before mixing to the TMRs (total 10 ml of water-additive mixture per 0.5 kg TMR). Laboratory analyses and aerobic stability measurements were conducted according to Seppälä et al. (2016). Exp. 1 and 2 were combined for statistical analyses. All experiments were analysed using a mixed procedure of SAS. The sum of squares of the Exp. 1+2 was further partitioned into contrasts, where low quality was contrasted against high quality raw material, control against preservative treatments, and interaction between raw material quality and preservative effect. In Exp. 3 sum of squares were partitioned into contrasts to test preservative effect, linear and quadratic effects of water addition and respective interactions.

**Results and Discussion** The microbiological quality of the raw materials is presented in Table 1 showing that aerobic bacteria were rather high for silage and crimped barley 1. Further, crimped barley 1 had high yeast and mould counts. Comparing Exp. 1 and 2, yeast and mould counts of crimped grain were much higher than in components of high hygienic quality, which probably contributed to the faster heating of low quality TMRs (Figure 1A). All preservatives improved the stability of both TMRs in Exp. 1 and 2, but the responses were clearly higher when high quality ingredients were used. Similarly, also Seppälä et al. (2013) detected that response to preservative were reduced by poor quality TMR ingredients. It is a common practice to add water to TMR so that a DM level around 400 g/kg is achieved to prevent diet selection by cattle. Moisture promotes microbial

activity, which is likely to decrease the stability of TMR in response to water inclusion (Figure 1B). The response in stability to water inclusion decreased quadratically. Addition of FPA increased stability significantly and the greatest response to preservative treatment was found with no water addition (P<0.01 for interaction between preservative treatment and quadratic effect of moisture content).

**Table 1**. The microbiological status of TMR ingredients used in the experiments.

| | Aerobic bacteria | | | Yeasts | | | Moulds | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 | Exp.1 | Exp.2 | Exp.3 |
| Silage | $1.4\times10^{8}$ | $1.9\times10^{7}$ | $2.20\times10^{7}$ | $8.2\times10^{2}$ | $1.1\times10^{5}$ | $3.6\times10^{5}$ | $6.4\times10^{2}$ | $<1.0\times10^{3}$ | $<1.0\times10^{3}$ |
| Concentrate 1[1] | $6.3\times10^{7}$ | $3.5\times10^{6}$ | $2.3\times10^{6}$ | $4.0\times10^{6}$ | $3.4\times10^{4}$ | $2.7\times10^{4}$ | $9.0\times10^{6}$ | $8.5\times10^{4}$ | $6.7\times10^{4}$ |
| Concentrate 2[1] | $1.6\times10^{7}$ | $8.9\times10^{6}$ | $6.0\times10^{6}$ | $2.3\times10^{4}$ | $5.1\times10^{4}$ | $6.7\times10^{4}$ | $2.0\times10^{4}$ | $1.1\times10^{5}$ | $8.0\times10^{4}$ |
| Rapeseed meal | $3.7\times10^{4}$ | $3.1\times10^{5}$ | $4.1\times10^{5}$ | $1.8\times10^{3}$ | $7.3\times10^{2}$ | $2.3\times10^{2}$ | $4.5\times10^{3}$ | $6.4\times10^{2}$ | $<1.0\times10^{3}$ |
| Straw | $5.1\times10^{8}$ | - | - | $6.5\times10^{6}$ | - | - | $9.0\times10^{6}$ | - | - |
| Mineral mixture | $4.0\times10^{3}$ | $<1.0\times10^{3}$ | $<1.0\times10^{3}$ | $1.0\times10^{2}$ | $<1.0\times10^{3}$ | $<1.0\times10^{3}$ | $5.5\times10^{2}$ | $<1.0\times10^{3}$ | $<1.0\times10^{3}$ |

[1]Two different batches of crimped barley in Exp. 1; dry barley and oats in Exp. 2.

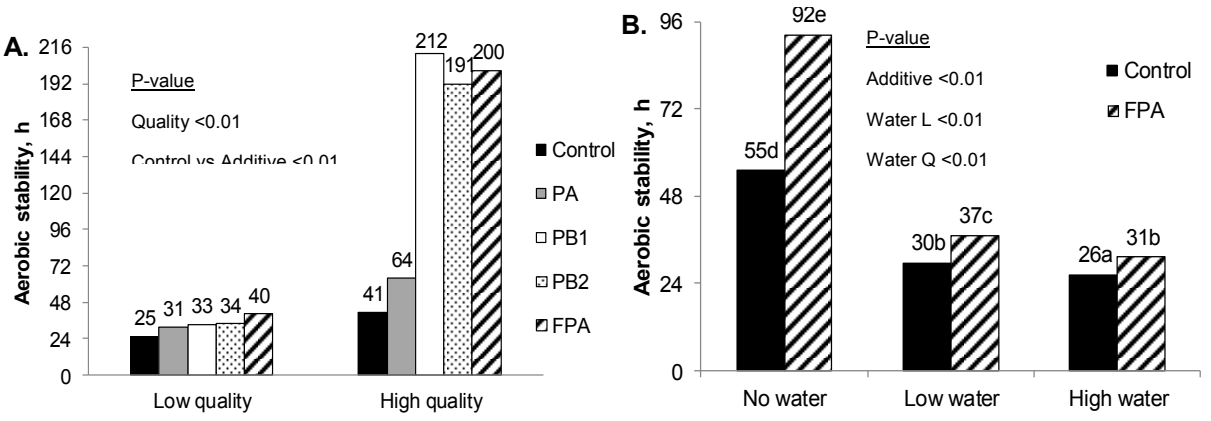


**Figure 1.** Effect of preservatives on aerobic stability of TMRs when quality of TMR ingredients was either low (Exp. 1) or high (Exp. 2) or when dry matter concentration of TMR was varied by different levels of water additions (B, Exp. 3). Preservative treatments in TMR were a control without preservative; calcium propionate (PA); two mixtures of calcium propionate and sodium benzoate (PB1 and PB2) and a blend of formic and propionic acids (FPA).

**Conclusion** Lower microbiological quality of feed ingredients or addition of water into TMR clearly decreased the aerobic stability of TMR as well as response to the use of preservatives. However, responses to preservatives were detected in all materials. Based on the current results, the possibilities to find statistically significant responses to preservative treatments are greatest when high hygienic quality TMR components with high inherent stability are used. Based on Exp. 3, addition of water clearly compromised TMR stability and although it may decrease TMR selection, should be considered carefully if problems in TMR stability are faced.

**References**

Seppälä, A., Heikkilä, T., Mäki, M., Miettinen, H. & Rinne, M. (2013) Controlling aerobic stability of grass silage-based total mixed rations. Animal Feed Science and Technology, 179, 54-60.

Seppälä, A., Heikkilä, T., Mäki, M. & Rinne, M. (2016). Effects of additives on the fermentation and aerobic stability of grass silages and total mixed rations. Grass and Forage Science, 71, 458-471.

# Silage safety - Preventing serious injuries and fatalities

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**Keywords:** accident, entanglement, rollover, silage

**Introduction** Safety is the control of recognized hazards to reach an acceptable level of risk. A hazard does not always affect the person who caused it - the hazard can affect anyone. Accidents are caused by unsafe behavior or conditions due to the actions of people: not cleaning the mud off the ladder of a pack tractor, standing too close the feed-out face of an over-filled bunker silo, or moving a forage harvester without checking all sides and honking three times. A split second of inattention can cause a fatal accident and nothing will ever be the same again. Few farming operations invite as many different opportunities for a serious injury or fatality as a silage program. The silage industry has nothing to lose by practicing safety but it has everything to lose by not practicing it. The objective is to present common hazards encountered in managing silage coupled with case studies involving these hazards.

**Materials and Methods** Three hazards in managing bunker silos and drive-over silage piles are: truck or tractor rollover, entangled in machinery, and run-over by machinery. A detailed account of case studies involving these hazards and ways to avoid each one are presented.

**Results** A 21-year old silage truck driver was killed when a dump-bed rolled over in a bunker silo at a beef cattle feedlot in the state of Texas, USA. According to the Deaf Smith County Sheriff's office, the circumstances of the accident are not uncommon, and silage trucks have been known to rollover if not operated correctly (Hereford Brand 2014). On a small farm in the state of Paraná, Brazil, a 58-year old farmer was killed when the tractor rolled over him when filling a bunker silo. The victim's relatives said that the man was very accustomed to this type of work on the farm, and had operated a tractor since he was 12 years old. According to the local police department, a similar fatal accident happened in this county 1 week before this accident (Intervalo da Notícia 2012).

Guidelines that reduce the risk of tractor and truck rollovers include: 1. use rollover protective structures (ROPS) to create a zone of protection around the tractor operator, 2. never fill a bunker silo higher than the top of the wall, 3. install sight rails on above ground bunker walls, 4. form a progressive wedge of forage, which provides a minimum slope of 1 to 3 when filling bunker silos and building drive-over piles, 5. establish a driving procedure to prevent collisions when two or more pack tractors are used, and 6. only raise the dump bed of a truck when it is on a flat, firm surface. Note: never back onto the forage ramp of a bunker silo or drive-over pile to unload. Trucks are less stable as the bed is raised. A tire rut or depression from a previous truck, low tires on one side, uneven loading of a truck, or a wind gust increase the risk of a truck tipping over during unloading, especially when two or more of these hazards combine at a single time point.

An 18-year-old employee on a dairy farm in the state of New York, USA was fatally injured when he became entangled in a silage defacer. The youth was idling a tractor to allow it to warm up on a cold morning. The defacer was attached on the front of the tractor and was 1 meter above the ground. The energized defacer caught the sleeve of the victim's coat as he walked in front of the unit. The farm owner heard an unusual sound and went to investigate. He immediately shut off the tractor engine, disentangled the victim from the machinery, and began CPR until a local EMT arrived. The youth was pronounced dead at the scene (Bolsen and Bolsen 2017).

A 55-year old man died during the night of March 29, 2016, after having his leg crushed in a forage harvester used for making silage. The accident occurred near the city of São João do Oriente in the State of Minas Gerais, Brazil. According to the local Fire Department, the victim, Lione José de Souza, tried to unclog the forage harvester using his foot. The machine, which was turned on, pulled the man's leg into the chopper, and he was entangled for about 1 hour. He was rescued alive, after the machine was dismantled, and taken to the hospital in Ipatinga. He died that night (Aconteceu no Vale 2016).

Guidelines that reduce the risk of being entangled in machinery include: 1. keep machine guards and shields in place on forage harvesters, wagons, and silage feeding equipment, 2. never attempt to adjust, repair, or unclog any machinery while it is running, 3. shut down the engine and remove and pocket the keys when inspection or service work is needed to prevent accidental starting by another person, 4. before adjusting or unplugging the cutter head of a forage harvester, wait for it to come to a complete stop, 5. never approach the defacer blades while the machine is in operation, 6. all forage harvester, truck, and pack tractor operators should be mature, competent, experienced, and well trained for their task, 7. equipment operators should never take an unnecessary risk, 8. employees should never send or receive text messages while operating equipment, and 9. extra persons, especially children, and bystanders should be kept out of the way of the silage making and feeding activities at all times because they only add distractions to the many responsibilities of the equipment operators.

The 29-year old son of a silage contractor in the state of Nebraska, USA was talking with an employee who was in the cab of a large forage harvester near the edge of a field. After the conversation, the employee drove the machine out of the field. A few minutes later, another employee found the contractor's son lying unconscious in the field, and he died 2 hours later in the hospital. Although the details surrounding the accident are unclear, the son was apparently run-over by a rear tire of the forage harvester (Bolsen and Bolsen 2017).

Guidelines that reduce the risk of being run-over by machinery include: 1. never allow non-working bystanders, especially children, near moving harvest and transport equipment in the field, the traffic area on the farm, or a bunker silo and drive-over pile during filling and feeding, 2. rear view mirrors should be adjusted and back-up warning alarms installed on all trucks and tractors, 3. never walk in front of or behind any truck or tractor that is stopped without first making eye contact with the operator, 4. all employees in a silage program should wear high-visibility safety vests at all times, 5. use reverse alarm devices or a remote video camera on large and oversized machines to increase visibility and warn people in the area that the equipment will be operated in a reverse direction, and 7. use standard agricultural hand signals to communicate with others when operating machinery or when helping with machinery movement.

**Conclusions** The global silage industry has a long way to go to before the senseless fatalities described in this paper are prevented from happening in the future. To that end, every beef and dairy cattle producer and silage operation should have written safety guidelines and procedures for their employees, and they should schedule regular meetings with their employees to discuss safety.

**References**

Aconteceu no Vale (2016) Man dies after leg crushed in machine. Published March 30, 2016. Website accessed February 11, 2018. http://aconteceunovale.com.br/portal/?p=83165#sthash.KSqEtCCY.dpuf

Bolsen, K.K. & Bolsen. R.E. (2017) Silage Safety 101. 1st edition. Austin, Texas. Keith Bolsen Silage Safety Foundation. 33 p.

Hereford Brand (2014) Man killed in truck accident. Website accessed February 11, 2018. http://herefordbrand.com/web/man-killed-in-truck-accident/

Intervalo da Notícia (2012) Tractor rollover kills one person. Published June 16, 2012. http://intervalodanoticias.blogspot.com.br/2012/06/trator-capota-e-mata-uma-pessoa.html

# Technologies applied to the production of corn silage in Brazilian capital of milk during nine years

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**Keywords:** dairy cows, ensiling, forages conserved, nutrition

**Introduction** Brazil is the world's fourth largest producer of milk (FAO 2017). Despite this large volume of production of milk products, there are few surveys of information on the production practices, especially in relation to the production and use of conserved forages (Bernardes 2012). In Brazil, the study region is among the ten cities with the highest volume of milk production and average productivity of approximately 7,000 l/cow/year, much higher than the Brazilian average of 1,709 l/cow/year (IBGE 2017). In addition to the high-tech employment in milk production, the main factor responsible for the high productivity of milk in this region is the animal feed in which corn silage is the feed used in larger quantities (Janssen 2009). Thus, the characterization of silage technologies enables improvements in the production process and is critical to the success of the activity and the entire production chain.

**Material and Methods** In the years 2009-2017 were visited 579 farms, in 33 cities of Campos Gerais region (Centre East of state of Paraná) and south of the state of São Paulo. Silages were evaluated in 1509 silos that were used in animal nutrition. The visited silos, the on-site evaluations were: kind of silo, color polyethylene plastic sheets, coverage of the plastic sheets, method of withdrawal of silage and presence of effluent. A survey was ministered to producers regarding the presence or not of hybrid Bt technology for insect control, harvest of silage, type forage harvester machine, if there was fungicide application in the fields and if inoculant was used during the silage process. We performed a compiled data per year and calculated frequencies for each one of the variables. Regression analysis was performed using SAS 9.4 software using the PROC REG at 5% probability.

**Results and Discussion** The number of samples over the years increased, reflecting the increase of approximately 80% in the area (hectares) of corn silage in the region and by the greater search among the producers to analyse the quality of the silages. In the first year of survey, 100% of the producers used conventional hybrids for silage. Over the years there was a linear increase in the use of technology bt ($r^2$ = 0.7823; $P$ = 0.0015). Between 2009 and 2017, there were no major changes in service delivery to the silage harvest. Most producers contract the harvest (71%), mainly with self-propelled machines (64%). Approximately 46% of farmers apply fungicide in agriculture and only 17% use in ensiling inoculant use that has reduced over the years ($r^2$ = 0.5146; $P$ = 0.0296). 79% of the silos were bunker type (concrete or earth), which provides better compacted silage compared to silages stored in horizontal silos. In 2009, no silo was covered with white-black plastic sheets. Over the years there was an increase in its use ($r^2$ = 0.8989; $P$ = 0.0003) and in 2017, more than half of the silos (61%) were sealed using this kind of material. The opposite occurred with black plastic sheets, which reduced from 61% to 0% of silos ($r^2$ = 0.9605; $P$ = 0.0001). In all the years the ground was the most used silo cover (83%). Presence of ground is considered a positive aspect, as this promotes greater adhesion between the plastic and the mass of silage and protects the plastic sheets contact with oxygen and sunlight. However, the ground may be an impediment to handling (Bernardes 2012). 51% of producers utilizing fork manually withdrawal of the silage and 22% withdrawal with shovel. There was an increase in the use of mechanic cutter between the years 2011 and 2017 ($r^2$ = 0.7486; $P$ = 0.0119).

**Table 1** Characterization of technologies applied by the producers in the ensiling process over the years (%).

| Variable | Technology | Years | | | | | | | | | | Regression | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | $\overline{x}$ | Pr > F | $r^2$ |
| **Hybrid** | *Conventional* | 100 | 98 | 58 | 19 | 6 | 5 | 2 | 3 | 1 | 32 | **0.0015** | **0.7823** |
| | *Bt* | 0 | 2 | 42 | 81 | 94 | 95 | 98 | 97 | 99 | 68 | **0.0015** | **0.7823** |
| **Harvest** | *Own* | 26 | 34 | 36 | 28 | 22 | 33 | 29 | 26 | 25 | 29 | 0.3268 | 0.1370 |
| | *Costume-service* | 74 | 66 | 64 | 73 | 78 | 67 | 71 | 74 | 75 | 71 | 0.3268 | 0.1370 |
| **Machine** | *Self-propelled* | 70 | 55 | 54 | 68 | 74 | 62 | 60 | 69 | 68 | 64 | 0.4742 | 0.0755 |
| | *By tractor* | 30 | 45 | 46 | 32 | 26 | 38 | 40 | 31 | 32 | 36 | 0.4742 | 0.0755 |
| **Fungicide** | *No* | 57 | 62 | 53 | 55 | 52 | 50 | 48 | 45 | 64 | 54 | 0.4864 | 0.0716 |
| | *Yes* | 43 | 38 | 47 | 45 | 48 | 50 | 52 | 55 | 36 | 46 | 0.4864 | 0.0716 |
| **Inoculant** | *No* | 77 | 84 | 81 | 74 | 84 | 83 | 86 | 86 | 90 | 83 | **0.0296** | **0.5146** |
| | *Yes* | 23 | 16 | 19 | 26 | 16 | 17 | 14 | 14 | 10 | 17 | **0.0296** | **0.5146** |
| **Type Silo** | *Bunker* | - | 77 | 75 | 85 | 78 | 84 | 78 | 82 | 76 | 79 | 0.8429 | 0.0071 |
| | *Horizontal* | - | 23 | 25 | 15 | 22 | 16 | 22 | 18 | 24 | 21 | 0.8429 | 0.0071 |
| **Color** | *White-black* | - | 0 | 39 | 52 | 66 | 69 | 83 | 88 | 96 | 61 | **0.0003** | **0.8989** |
| **Plastic** | *Black* | - | 61 | 50 | 40 | 26 | 29 | 14 | 10 | 0 | 29 | **0.0001** | **0.9605** |
| **Sheets** | *White* | - | 39 | 10 | 8 | 8 | 2 | 3 | 2 | 4 | 10 | **0.0371** | **0.5427** |
| **Coverage** | *Without* | - | 8 | 7 | 3 | 7 | 13 | 10 | 14 | 24 | 11 | **0.0222** | **0.6097** |
| | *Ground* | - | 87 | 83 | 92 | 88 | 81 | 87 | 81 | 70 | 83 | 0.0787 | 0.4274 |
| | *Others* | - | 6 | 9 | 5 | 4 | 5 | 4 | 5 | 7 | 6 | 0.5254 | 0.0704 |
| **With-drawal** | *Fork* | - | - | 63 | 63 | 46 | 54 | 47 | 50 | 37 | 51 | **0.0159** | **0.7189** |
| | *Shovel* | - | - | 21 | 18 | 25 | 17 | 24 | 23 | 26 | 22 | 0.2366 | 0.2655 |
| | *Mechanic cutter* | - | - | 14 | 18 | 28 | 28 | 28 | 26 | 37 | 26 | **0.0119** | **0.7486** |
| | *Block* | - | - | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0.1582 | 0.4287 |
| **Effluent** | *Without* | - | 71 | 75 | 69 | 61 | 83 | 74 | 74 | 69 | 72 | **0.0817** | **0.0097** |
| | *Little* | - | 25 | 16 | 30 | 37 | 17 | 25 | 24 | 29 | 25 | 0.7128 | 0.0242 |
| | *Much* | - | 4 | 9 | 1 | 3 | 0 | 1 | 2 | 1 | 3 | 0.1091 | 0.3708 |
| ***n*** | | 53 | 89 | 107 | 120 | 114 | 130 | 192 | 278 | 426 | | | |

**Conclusion** The technological level used in crops and ensiling is high in the studied location, particularly with the increased use of high-tech corn hybrids, costume-service harvest with self-propelled machinery and silos made with quality conservation and withdrawal.

**References**

Bernardes, T.F. (2012) Levantamento das práticas de produção e uso de silagens em fazendas leiteiras no Brasil. Lavras: Universidade Federal de Lavras, 2012. 17p.

Food and Agriculture Organization of the Unites Nations – FAO (2017) Faostat. Available in: < http://www.fao.org/faostat/en/#data> Access in: 07 dez. 2017.

Instituto Brasileiro de Geografia e Estatistica – IBGE (2017) Pesquisa da Pecuária Municipal - PPM 2016. Available in: <https://www.ibge.gov.br/estatisticas-novoportal/economicas/agricultura-e-pecuaria/9107-producao-da-pecuaria-municipal.html?&t=resultados> Access in: 07 dez. 2017.

Janssen, H.P. (2009) Adubação nitrogenada para rendimento de milho silagem em sucessão ao azevém pastejado, pré-secado e cobertura em sistemas integrados de produção. 91f. Dissertation (Master's degree in agronomy) – Universidade Federal do Paraná, Curitiba.

# The effect of processing of Virginia fanpetals (*Sida hermaphrodita* Rusby L.) biomass harvested at different dates on fermentation quality

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**Keywords:** fermentation quality, harvest date, processing, silage, Virginia fanpetals.

**Introduction** Virginia fanpetals (*Sida hermaphrodita* Rusby L.) is a perennial plant of the mallow family (*Malvaceae*) which is grown mainly for energy biomass. Due to its high protein content, exceeding 20% DM, Virginia fanpetals has attracted attention as a potential valuable source of livestock fodder. Previous studies have revealed that silage made from chopped Virginia fanpetals biomass is characterized by low quality (Kwiatkowski et al. 2014). Own research has demonstrated that the stems of Virginia fanpetals are hard and account for approximately 60% DM of plants. However, they contain higher concentrations of water-soluble carbohydrates (WSC) than leaves. It may therefore be assumed that structural damage to stems could facilitate the access of microorganisms to sugars contained in cells, thus accelerating the acidification of the ensiled material and limiting secondary fermentation. The effectiveness of the above treatment should also be evaluated in view of the rapid growth of Sida plants, which leads to considerable changes in the chemical composition of biomass. The aim of this study was to determine the effect of processing Virginia fanpetals biomass harvested at different dates on fermentation quality.

**Materials and Methods** The experimental material was first-cut Virginia fanpetals herbage (*Sida hermaphrodita* Rusby L.) harvested on 5, 12 and 19 June 2016. Herbage was chopped with an electric chopper to a theoretical chopping length of 10 mm. Half of the chopped herbage was ensiled immediately (ChS; n=3), and the other half was crushed using an electric mixing machine (CrS; n=3). The chopped and crushed material (500 g) was ensiled in vacuum bags. The proximate chemical composition (AOAC 2005), the content of water soluble carbohydrates (WSC; Thomas 1977) and NDF, ADF and ADL (Van Soest et al., 1991) were determined in herbage samples using the ANKOM 220 fiber analyzer. After 90 days, the same parameters were analysed in silage; silage samples were also assayed for: pH - with the HI 8314 pH-meter, lactic acid content - using the Shimadzu HPLC system with a VARIAN MetaCarb 67H column, the concentrations of volatile acids - using the **Varian 450-GC** gas chromatograph, and N-$NH_3$ - by direct distillation using the 2100 Kjeltec Distillation unit (Foss Analytical A/S, Hilleröd, Denmark), after increasing the pH of the samples by adding MgO. The results were analyzed by two-way (harvest date and processing) ANOVA.

**Results** Delayed harvest of Virginia fanpetals biomass deteriorated the composition of carbohydrate fractions in herbage. Harvest date had a significant ($p<0.001$) negative effect on the content of protein and structural carbohydrates in herbage, and on the fermentation process. Biomass processing (crushing) significantly ($p<0.001$) improved fermentation parameters at all harvest dates. An increase in lactic acid content, a decrease in the concentrations of butyric acid and acetic acid, and a decrease in N-$NH_3$ contribution to total nitrogen were noted in silage. Significant interactions were also found between the harvest date and the degree of biomass processing with respect to all fermentation parameters.

**Discussion** The considerable differences between herbage and silage could result from the tubular structure of stem and air trapping. A prolonged aerobic phase of fermentation produces more water. DM looses increased with plant age and decreased after crushing. The rate of changes in the content of protein, WSC and structural carbohydrates between cuts was similar to that reported by Tarkowski

et al. (2006). The quality of silage made from biomass that had not been subjected to structural damage was low, which is consistent with previous findings (Kwiatkowski et al. 2014; Purwin et al. 2016). The optimal effects of processing were noted when herbage was harvested on 12 June 2016. The beneficial influence of structural damage to *Sida* biomass prior to fermentation resulted from increased availability of WSC contained in stems. At the latest harvest date, the quality of both silages did not improve despite crushing due to a deficiency of WSC.

**Conclusions** The quality of *Sida* silage is largely determined by the harvest date of herbage. The additional processing of Virginia fanpetals biomass (CrS) improved the fermentation process and, consequently, the quality of silage. The benefits of crushing (CrS) were further enhanced depending on the harvest date.

**Table 1.** Chemical composition of Virginia fanpetals herbage and silage and characteristics of silage fermentation

| Item | Harvest date (HD) | | | | | | | | | SEM | Level of significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5.06 2016 | | | 12.06.2016 | | | 19.06.2016 | | | | | | |
| | Processing (P) | | | | | | | | | | HD | P | HDxP |
| | H | ChS | CrS | H | ChS | CrS | H | ChS | CrS | | | | |
| Chemical composition g·kg$^{-1}$ DM | | | | | | | | | | | | | |
| DM g/kg | 164 | 156 | 158 | 216 | 154 | 186 | 255 | 211 | 221 | 0.66 | *** | *** | *** |
| CP | 205 | 197 | 203 | 160 | 155 | 156 | 127 | 120 | 122 | 0.83 | *** | ns | ns |
| WSC | 119 | 8.60 | 12.6 | 88.7 | 13.9 | 15.9 | 60.0 | 9.80 | 11.1 | 0.08 | ** | * | ns |
| NDF | 373 | 425 | 396 | 456 | 508 | 480 | 507 | 635 | 543 | 2.00 | *** | ** | ns |
| ADF | 299 | 358 | 346 | 354 | 399 | 374 | 392 | 488 | 433 | 1.28 | *** | * | ns |
| ADL | 37.2 | 43.6 | 40.6 | 40.2 | 50.7 | 44.6 | 53.8 | 80.3 | 60.5 | 0.35 | *** | ** | * |
| Fermentation pattern g·kg$^{-1}$ DM | | | | | | | | | | | | | |
| pH | | 4.60 | 4.30 | | 5.49 | 4.26 | | 5.47 | 5.23 | 0.13 | *** | *** | *** |
| LA | | 80.5 | 78.6 | | 19.0 | 71.7 | | 17.7 | 28.4 | 7.17 | *** | ** | ** |
| AA | | 8.13 | 7.09 | | 19.9 | 4.09 | | 11.7 | 2.59 | 1.40 | *** | *** | *** |
| BA | | 0.01 | 0.05 | | 9.49 | 0.02 | | 11.5 | 10.9 | 1.33 | *** | *** | *** |
| N-$NH_3$[1] | | 48.6 | 47.8 | | 116 | 39.5 | | 131 | 69.3 | 0.88 | *** | *** | *** |

[1]g/kg Total N; H – herbage; ChS – chopped silage; CrS – crushed silage; HD – harvest date; P – processing; ns – not significant;

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**References**

AOAC. (2005) Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington. DC. USA.

Kwiatkowski, J., Purwin, C., Sienkiewicz, S., Tworkowski, J., Pysera, B., Wierzbowska, J., Graban, Ł., Lajszner, W., Wyżlic, I., Krzebietke, S. & Żarczynski, P. (2014) The Virginia fanpetals *Sida hermaphrodita* (L.) Rusby used as feedstock for an agricultural biogas plant. In: Wojnowska-Baryła, I. & Gołaszewski J. (eds.). Conversion of waste from agri-food industry to biogas – a system approach. Olsztyn, Poland: UWM Publishing. p. 37-62.

Purwin, C., Fijałkowska, M., Nogalski, Z., Lipiński, K., Antoszkiewicz, Z., & Starczewski, M. (2016) Influence of different additives on the silage quality from Virginia fanpetals (*Sida hermaphrodita* Rusby). In: OKN, Puławy IUNG-PIB, Poland, 2016, pp. 132-134.

Tarkowski, A. (2006) Nutritional and feed value of Sida (Sida hermaphrodita Rusby). Habilitation dissertation, AR Lublin, 308: 1-81.

Thomas, T.A. (1977) An automated procedure for the determination of soluble carbohydrates in herbage. Journal of the Science of Food and Agriculture, 28, 639-642.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991) Methods of dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

# The practical use of a model to predict the wilting time of grass

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**Keywords:** dry matter, grass silage, wilting

**Introduction** Wilting of mown grass up to a recommended dry matter (DM) range (30-40%) improves the fermentation quality and reduces the production of effluent. However, for farmers it is difficult to estimate when the grass has reached this range and thus, when to start ensiling. Therefore, based on field trials in the 1980's, a model named 'WiltExpert' was developed for on-farm prediction of wilting time at the former Institute for Forage Production in Paulinenaue (Germany). In these trials, the wilting process was examined on more than 1.400 plots. The regressions found enabled the description of wilting as a function of weather elements, properties of the grass and technical parameters. Unfortunately, the model fell into oblivion in the wake of the political changes in East Germany in 1989. Implemented in a Microsoft excel-based calculation sheet, the model was tested again on commercial farms (Pickert et al. 2016). There, a mean absolute error of 108 minutes and a modelling efficiency of 0.99 between the observed and the predicted wilting time revealed that the predictive ability of the model is satisfactory. The aim of the present study was to evaluate the use of a further developed version of 'WiltExpert' as on-farm prognosis tool in an EIP (European Innovation Partnerships) project ('Q2GRAS').

**Materials and Methods** The test trials were conducted on six commercial farms in East Germany in May, June and July in 2017. A total of 13 data sets originated from the first and second cut and with or without rain were evaluated. The field name, the time of mowing, the estimated DM at mowing (in some cases, the DM was directly determined and not estimated by the farmer), the target DM (usually within the recommended DM range), the stage of maturity, the estimated yield, the soil conditions, daily evaporation, rain and technical parameters of mowing were used as model input variables and were supplied by the farmer (Figure 1). On the basis of regression equations, the model predicted the time of reaching a target DM desired by the farmer. To evaluate the predictive ability, three samples were each taken at mowing and at the predicted time from swath at the same places after the swath was thoroughly mixed (length of the sampled swath was 6 metres). Of these samples, the DM was determined by oven drying (48 hours at 60°C) in the laboratory. The estimated and the determined DM at mowing as well as the target and the realized DM at the predicted time were compared.

**Input data**

| | | |
|---|---|---|
| Date of mowing: | 07.06.2017 13:30 | |
| Estimated-DM: | 19,0% | |
| Target-DM: | 40,0% | |
| Yield: | 120 | kg/100m² |
| Width of mower: | 9 | m |
| Width of swath | 9 | m |
| Quantity per area: | 1,200 | kg/m² |
| Soil conditions: | 5 | |
| Swath processing: | 2 | |
| Course of evaporation: | 2 | |

| Date | Evaporation values (mm/d) | | |
|---|---|---|---|
| | mm/d | Soil corrections | Swath processing |
| 07.06. | 4,9 | 4,500 | 4,570 |
| 08.06. | 3,4 | 3,120 | 3,170 |
| 09.06. | 3,6 | 3,300 | 3,360 |

Date of harvest without rain: 08.06. 10:54

Current harvest date: 08.06. 14:41

**Rain periods**

| Rain data | | | | Wind strength | Required rain evaporation | | DM before rain | New beginning of wilting | New harvest date |
|---|---|---|---|---|---|---|---|---|---|
| Begin | End | Quantity (mm) | Cumulative (mm) | | current (mm) | cumulative (mm) | | | |
| 07.06. 14:00 | 07.06. 14:10 | 5 | 5,0 | 3 | 1,690 | 1,690 | 21,94% | 07.06. 16:26 | 08.06. 14:41 |

**Figure 2**. Input mask of 'WiltExpert'.

**Results and Discussion** Table 1 shows the results of the use of the model in 2017. The highest realized DM was found at farm 1 (457 g $kg^{-1}$, second cut) and the lowest at farm 5 (225 g $kg^{-1}$, second cut). The mean deviation between the realized and the target DM was 61 g $kg^{-1}$. In the trials with rainfall during the wilting period, the deviation between the realized and the target DM was also high (e.g. farm 4, second cut). In most of the trials, the DM at mowing was poorly estimated by the farmers on the basis of their experiences and the realized DM deviated from the target DM entered (e.g. farm 5, second cut). When the DM at mowing was estimated (or directly determined) by Q-Dry® and was compared with the determined DM by oven drying in the laboratory (reference procedure), the deviation was small and the difference between the realized and the target DM within the first cut was sufficiently.

**Table 8**. Results of the use of 'WiltExpert' as on-farm prognosis tool in 2017 (values in g $kg^{-1}$).

| Farm | Time of mowing* | Estimated DM at mowing ($x_1$) | Determined DM at mowing ($x_2$) | $x_2$-$x_1$ | Target DM ($x_3$) | Realized DM at predicted time ($x_4$) | $x_4$-$x_3$ | Predicted time to reach the target DM |
|---|---|---|---|---|---|---|---|---|
| First cut | | | | | | | | |
| 1 | 11.05.16:00 | 230[e] | 257 | 27 | 400 | 359 | -41 | 12.05.11:51 |
| 1 | 11.05.14:35 | 252[e] | 255 | 3 | 400 | 384 | -16 | 12.05.10:22 |
| 2 | 16.05.13:30 | 210 | 226 | 16 | 350 | 418 | 68 | 17.05.11:00 |
| 4 | 17.05.17:00 | 226[f] | [h)] | - | 350 | 306 | -44 | 18.05.12:05 |
| 5[a] | 19.05.15:20 | 240 | 176 | -64 | 350 | 279 | -71 | 20.05.12:22 |
| 6 | 24.05.09:45 | 197[g] | 249 | 52 | 350 | 410 | 60 | 25.05.13:57 |
| Second cut | | | | | | | | |
| 1[b] | 07.06.13:30 | 192[e] | 223 | 31 | 400 | 457 | 57 | 08.06.14:41 |
| 1 | 07.06.19:30 | 200 | 276 | 76 | 400 | 434 | 34 | 08.06.16:07 |
| 4[c] | 09.06.18:50 | 240 | 306 | 66 | 350 | 406 | 56 | 10.06.13:58 |
| 5 | 12.06.10:30 | 220 | 167 | -53 | 350 | 225 | -125 | 12.06.13:48 |
| 2 | 13.06.15:30 | 220 | 249 | 29 | 350 | 434 | 84 | 14.06.11:55 |
| 3 | 14.06.18:55 | 240 | 212 | -28 | 350 | 306 | -44 | 15.06.11:15 |
| 6[d] | 12.07.11:10 | 200 | 254 | 54 | 350 | 449 | 99 | 14.07.13:41 |
| Mean deviation | | | | | | | 61 | |

a = with 0.3 mm rain; b = with 5.0 mm rain; c = with 2.0 mm rain; d = with 8.5 mm rain; * = indications in day, month and daytime; e = determined by Q-Dry®; f = determined by oven drying (105°C); g = determined by microwave; h) = the sample was discarded after determination; each *n* = 3.

**Conclusion** When using the model, the farmer can receive field-related information when the mown grass will have reached a target DM even under varying sward and weather situations. This information can support the optimization of the harvesting process, particularly if a greater number of various pastures must be involved in the harvest campaign. The main reason for the still occurring deviation between the target and the realized DM was seen in the over- and underestimation of the DM at mowing by the farmer's experiences and in the sampling procedure. Therefore, the future tasks will be both an improvement of the estimation of the DM at mowing and of the sampling to further increase the prediction accuracy and to minimize the deviation. In the next version of the model, time slots will be specified for certain DM ranges.

**References**

Pickert, J., Hoffmann, T., Herrmann, A., Thaysen, J., Weise, G. & Wellenbrock, K.-H. (2016) 'WiltExpert' – a model for on-farm prediction of grass wilting time from mowing to ensiling dry matter content, In: Höglind, M., Bakken, A. K., Hovstad, K. A., Kallioniemi, E., Riley, H., Steinshamn, H., & Østrem, L. (eds). The multiple roles of grassland in the European bioeconomy. Proceedings of the XXVI. General Meeting of the European Grassland Federation, Trondheim, Norway, 2016, pp. 200-202.

# The use of quebracho condensed tannins as additive for rehydrated corn grain silage: effects on fermentation pattern and aerobic stability

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**Keywords:** volatile fatty acids, yeasts, aerobic deterioration

**Introduction** Ensiling is a method used to conserve herbage or grains through acidification by microbial fermentation. However, the development of spoilage microorganisms during storage phase reduces the conservation efficiency. Treating silages with condensed tannins could reduce the risk of undesirable fermentation and spoilage after silo opening. The aim of the current study was to determine the effect of quebracho tannin extract as silage additive on fermentation pattern and aerobic stability of rehydrated corn grain silage.

**Material and Methods** After grinding (5 mm sieve) and rehydration (35% of moisture), rehydrated corn was inoculated with a bacterial inoculant (Bon Silage Brunn/Gebirge, Austria) containing *Lactobacillus buchneri* 1k2075, *Lactobacillus ramnosus* 1k20711, and *Lactobacillus plantarum* 1k2079, in order to reach a theoretical application rate of $2.5 \times 10^5$ colony-forming units (CFU) $g^{-1}$ of silage (as fed). Additionally, a quebracho tannin extract (QTE), derived from *Schinopsis lorentzii*, containing 103 g $kg^{-1}$ DM of condensed tannins (MGM EX, Christian D. Markmann GmbH, Hamburg, Germany), was tested as silage additive. The QTE was added in five dosages: 0 (control), 5, 10, 30 and 50 g $kg^{-1}$ DM of silage. The experimental silos (4 silos per treatment; 3 kg each) were vacuum-sealed bags, remaining stored for 75 days. The buffering capacity was evaluated according Jobim et al. (2007). For the determination of microbial counts, 10 g of silage was diluted with 90 mL of sterile saline solution (9 g NaCl $L^{-1}$). Serial dilutions ($10^{-2}$ to $10^{-8}$) were pour plated (3 repetitions per dilution). Lactic acid bacteria (LAB), was enumerated in the De Man, Rogosa, Sharpe agar (84607.0500; VWR International GmbH, Vienna, Austria) supplemented with natamycin (0.25 g $L^{-1}$). Yeasts and molds were enumerated in potato glucose agar (70139; Sigma-Aldrich, India) acidified to pH 3.5 with lactic acid. The plates were incubated aerobically at 30°C for 2 (LAB), 3 (yeasts) and 4 (molds) days. Colony forming units were transformed to $\log_{10}$ values. For the aerobic stability (AS) test, 2 kg of silage was placed in plastic buckets (4 repetitions per treatment) and allocated in a climate controlled room (20°C) during 336 h. Silage temperature was recorded every 15 min by temperature loggers (Thermochron DS1321G, OnSolution Pty Ltd, Baulkham Hills, Australia) inserted in the geometric center of silage mass. The pH was measured in a silage-aqueous extract (9 g of silage as fed in 60 mL of distilled water). The supernatant was also analyzed for lactic acid (Pryce, 1969), volatile fatty acids (Zhao et al., 2006), ammonia nitrogen (Patton and Crouch, 1977) and ethanol (Ethanol Enzymatic UV-method Kit, Boehringer Mannheim/R-Biopharm, Darmstadt, Germany). The experimental design was totally randomized and data were analyzed by the REG procedure of SAS.

**Results** At ensiling, the corn presented a buffering capacity of 4.31 mEq NaOH $g^{-1}$ DM and a pH of 6.85. The microbial counts were, respectively, 6.79, 5.38 and 4.70 $\log_{10}$ CFU $g^{-1}$, for LAB, molds and yeasts. A quadratic effect was observed for LAB counts in silages, being the minimum point of 6.9 $\log_{10}$ CFU $g^{-1}$ for silage treated with 46.9 g $kg^{-1}$ DM of QTE. On the other hand, the highest pH (4.2) was found at 37.7 g $kg^{-1}$ DM of QTE. No differences were observed in lactic acid concentration (8.35 g $kg^{-1}$ DM). Treating corn silage with QTE reduced the contents of iso-butyric and acetic acids, whereas propionic acid (0.07 g $kg^{-1}$ DM) was not affected. Ammonia nitrogen linearly decreased. For ethanol a quadratic effect was found, being the minimum concentration of 0.3 g $kg^{-1}$ DM at 31 g $kg^{-1}$ DM of QTE. At silo opening, yeasts were observed only in silages treated with 50 g $kg^{-1}$ DM of QTE, however, no molds were observed in this study. The presence of yeasts in the highest QTE dosage reduced the aerobic stability compared to the lower QTE dosages.

**Table 1** Effect of quebracho tannin extract addition (0 – 50 g $kg^{-1}$ DM) on microbial counts, pH, fermentation profile and aerobic stability of rehydrated corn grain silage after 75 days storing.

| Item | Regression equation | $R^2$ | $x_{cr}$[d] | $y_{cr}$[d] | SEM[e] |
|---|---|---|---|---|---|
| LAB[a] | $9.98 - 0.13X + 0.014X^2$ | 0.77 | 46.9 | 6.9 | 0.3 |
| Yeasts[a] | $0.4 - 0.138X + 0.0046X^2$ | 0.96 | 15 | - 0.6 | 0.47 |
| pH | $3.967 + 0.01155X - 0.0001532X^2$ | 0.85 | 37.7 | 4.2 | 0.02 |
| Acetic acid[b] | $0.628 - 0.00968X$ | 0.78 | | | 0.06 |
| Iso-butyric acid[b] | $0.906 - 0.0167X$ | 0.67 | | | 0.09 |
| Ethanol[b] | $1.3 - 0.062X + 0.001X^2$ | 0.62 | 31 | 0.3 | 0.09 |
| Ammonia[b] | $0.0421-0.0005X$ | 0.86 | | | 0.002 |
| Aerobic stability[c] | $312.47 + 7.70X - 0.26X^2$ | 0.95 | 14.8 | 255.5 | 24.86 |

[a] $log_{10}$ CFU $g^{-1}$ silage (as fed); [b] g $kg^{-1}$ DM; [c] hours to silage temperature increase 2°C above environment; [d] Critical point coordinates of *x* and *y*; [e] Standard error of the mean.

**Discussion** In general, condensed tannins reduces microbial activity due to the destabilization of the cytoplasmic membrane, leading to a reduction of the selective permeability, as well as inhibiting enzymes and binding to the protein, negatively affecting the cell development and its metabolism (Scalbert 1991). The lower microbial activity in silages containing QTE decreased the synthesis of fermentation metabolites. The lower content of VFA, mainly acetic acid (Danner et al., 2003), along with the higher pH, enabled the development of yeasts, worsening the aerobic stability, despite the reduction in ethanol concentration. On the other hand, the condensed tannin toxicity to *Clostridium* spp. (Min et al. 2005), as well as the protein binding (meaning less proteolysis during fermentation), led to a reduction in the contents of ammonia and iso-butyric acid.

**Conclusion** The inclusion of quebracho tannin extract up to 30 g $kg^{-1}$ DM did not impair silage fermentation and aerobic stability, but decreased proteolysis.

**References**

Danner, H., Holzer, M., Mayrhuber, E., & Braun, R. (2003) Acetic acid increases stability of silage under aerobic conditions. Applied and Environmental Microbiology, 69, 562-567.

Jobim, C. C., Nussio, L. G., Reis, R. A., & Schmidt, P. (2007). Avanços metodológicos na avaliação da qualidade da forragem conservada. Revista Brasileira de Zootecnia, 36, 101-119.

Min, B. R., Attwood, G. T., McNabb, W. C., Molan, A. L., & Barry, T. N. (2005) The effect of condensed tannins from *Lotus corniculatus* on the proteolytic activities and growth of rumen bacteria. Animal Feed Science and Technology, 121, 45-58.

Patton, C. J., & Crouch, S. R. (1977) Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Analytical Chemistry, 49, 464-469.

Pryce, J. D. (1969) A modification of the Barker-Summerson method for the determination of lactic acid. Analyst, 94, 1151-1152.

Scalbert, A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30, 3875-3883.

Zhao, G., Nyman, M., & Åke Jönsson, J. (2006) Rapid determination of short‐chain fatty acids in colonic contents and faeces of humans and rats by acidified water‐extraction and direct‐injection gas chromatography. Biomedical Chromatography, 20, 674-682.

# Three-dimensional visualization of bulk density and oxygen-induced temperature distributions in silage using a stepwise-profiling penetrometer

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**Keywords:** Density, Penetrometer, Silage, Temperature, Three-dimension

**Introduction** For silage production, high bulk density (BD) is important to minimize aerobic deterioration because it decreases the diffusion rate of oxygen invaded into the silage (Muck and Pitt 1994, Wambacq et al. 2016). Well-compacted silage should not only exhibit a high BD, but a uniform BD distribution as well. For map-based BD measurements, a gamma ray scanner was tested in two studies (Fuerll et al. 2008, Mumme and Katzameryer 2008), where the relative measurement error was about 1 % after calibration. Despite the high accuracy, few producers would be able to effectively use gamma ray due to regulations and the potential danger of exposure to radiation. An improved penetrometer technique for map-based determination of BD in grass bale silage was developed (Sun et al. 2010). Subsequently, a study verified that this novel technique can replace the gamma ray scanner for imaging silage BD distribution (Meng et al. 2012). On the other hand, the invasion of oxygen will result in abnormal rise of temperature which is a key parameter to assess aerobic deterioration (Gastón et al. 2009, Shinners et al. 2009). Previous studies have only provided time-courses of silage temperature based on single-point measurement or the mean value of multiple points (Green et al. 2009, Williams et al. 1997). To precisely assess the packing quality and the deterioration risk of silage, a penetrometer-based mapping system was developed for visualizing bulk density and oxygen-induced temperature patterns of silage in three-dimension (3D).

**Material and Methods** The experiment was conducted in a maize bunker silo and a series of round-bale grass silage. The bunker silo (40 m × 8 m × 3 m), located at a dairy farm in Haus Riswick in Kleve, Germany, was constructed of two concrete side-walls and a back-wall. The silage bales consisted of perennial ryegrass (Lolium perenne) harvested from a grass field at the Frankenforst Experimental Farm, the University of Bonn, Germany. Six bales were used for this test. A penetrometer-based mapping system was developed with a tiny thermocouple sensor integrated into a cone. For 3D-based BD and temperature measurements within the bunker silo and bale silage, a sampling network was constructed. The data processing included filtering of the penetration friction component out of the penetration resistance, transfer of the corrected penetration resistance to BD, incorporation of Kriging interpolation for data expansion and map generation. To visualize temperature patterns within the bales, two of basic functions of ArcGIS 9.2 software, the data post conditioning by ordinary Kriging interpolation and the digital mapping with the interpolated data, were employed.

**Results and Discussion** The result showed that the 3D distributions of density and temperature patterns in silage were informative and clearly interpretable as shown in Figs. 1 and 2. A comparison between the results using the proposed method and core sampling data showed a well agreement between the two measurement approaches (RMSE=6.392 kg $m^{-3}$). Comparing horizontal with vertical arrays, we see that the horizontal BD exhibited smaller variation, but the vertical BD apparently increased with increasing the vertical depth of the bunker silo. The average BD near the top layer was 293 DM kg $m^{-3}$, whereas that of the bottom was 363 DM kg $m^{-3}$. The increasing gradient of BD along with vertical depth was observed in some previous studies. The vertical gradient of BD measured from our core data, indicating that self-compaction occurred in this bunker silo as well. In terms of horizontal BD discrepancy, the samples taken at the center were generally denser than samples taken near the wall. The resulting $R^2$ = 0.9978 between the profiling temperature data and the re-profiling data verified that the contamination due to the invasion of ambient air following the temperature probe penetration could be negligible.

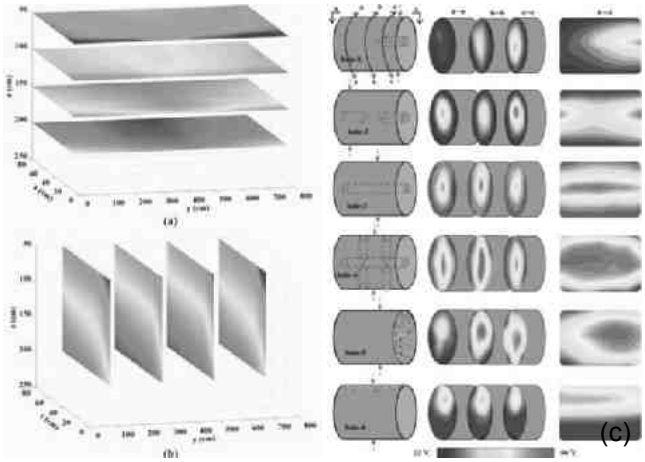


**Figure 1.** The 3D distributions of density and temperature patterns in a maize bunker silo (1a and 1b) and a series of round-bale grass silage (c)

As expected, the proposed stepwise-profiling method and the designed apparatus met the requirement of non-disturbed measurement. Moreover, the high $R^2$ confirmed that the thermocouple sensor remained precise and reliable throughout the experimental period. All 3D maps were generated using the software we developed based on the plot functions of MATLAB 7.0. These temperature patterns of the bales demonstrated the feasibility of using partial differential equation (PDE) models associated with different boundary conditions; the latter depends on the specific damage to the plastic film of bale. In microbiology and food science, it has long been recognized that temperature is a cardinal factor affecting microbial growth rate. Therefore, combining these microbial-growth-rate models with the proposed methods in this study, it is promising to transform the 3D temperature patterns into the relevant maps of microbial responses to the $O_2$ introduction. This is likely to provide more useful information than the model based curves.

**Conclusion** The penetrometer-based bunker silo mapping system yielded digitally imaged silage BD distributions within the outer 1 m of the exposure face. These horizontal and vertical maps are informative and understandable in relation to the bunker silo and packing characteristics. The developed penetrometer-based mapping system can potentially contribute to not only detecting poor compaction management, but also in estimating the risk of aerobic deterioration of feeding materials for farm-scale bunker silos. The 3D maps of temperature presented are informative and clearly interpretable in support of assessing aerobic deterioration for round bale silage at local and global scales. Based on these positive results, the similar map based approach can also extend to square bale silage when the stepwise-profiling apparatus is improved in the future.

**References**

Fuerll, C., Schemel, H. & Koeppen, D. (2008) Principles for measuring density in silages. Landtechnik, 63, 94-95.

Gastón, A., Abalone, R., Bartosik, R.E. & Rodríguez, J.C. (2009) Mathematical modelling of heat and moisture transfer of wheat stored in plastic bags (silobags). Biosystems Engineering, 104, 72-85.

Green, O., Nadimi, E.S., Blanes-Vidal, V., Jørgensen, R.N., Storm, I.M.D. & Sørensen, C.G. (2009) Monitoring and modeling temperature variations inside silage stacks using novel wireless sensor networks. Computers and Electronics in Agriculture, 69, 149-157.

Meng, F., Sun, Y., Buescher, W., Maack, C., Cheng, Q., Berg, A., Lin, J., Ross, F. & Zhang, H. (2012) Extended Penetrometer System for the Map-based Assessment of Bale Density Distribution. Applied engineering in agriculture, 28, 449-455.

Muck, R.E. & Pitt, R.E. (1994) Aerobic deterioration in corn silage relative to the silo face. Transactions of the ASAE, 37(3), 735-743.

Mumme, M. & Katzameryer, J. (2008) Mobile test station for the radiometric measurement of density distribution in bales. Landtechnik, 63, 341-343.

Shinners, K.J., Huenink, B.M., Muck, R.E. & Albrecht, K.A. (2009) Storage characteristics of large round and square alfalfa bales: low-moisture wrapped bales. Transactions of the ASABE, 52, 401-407.

Sun, Y., Buescher, W., Lin, J., Lammers, P.S., Ross, F., Maack, C.,Cheng, Q. & Sun, W. (2010) An improved penetrometer technique for determining bale density. Biosystems engineering, 105, 273-277.

Wambacq, E., Vanhoutte, I., Audenaert, K., De Gelder, L. & Haesaert, G. (2016) Occurrence, prevention and remediation of toxigenic fungi and mycotoxins in silage: A review. Journal of the Science of Food and Agriculture, 96, 2284-2302.

Williams, A.G., Hoxey, R.P. & Lowe, J.F. (1997) Changes in temperature and silo gas composition during ensiling, storage and feeding‐out grass silage. Grass and Forage Science, 52, 176-189.

# Wet ensiling of sugar beets with or without ensiling additive

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**Keywords:** Ethanol, fermentation products, mannitol, silage, sugar beet

**Introduction** Traditionally fodder beets have been fed to dairy cows as fresh beets, which has limited the use to a short period of the year. Conservation as silage could extend to whole year use. Beets can be ensiled in bunker silos, but with substantial effluent losses. Co-ensiling with e.g. maize whole crop can diminish effluents, but is difficult due to coordination of harvest times for beets and maize. Mono ensiling of chopped sugar beets in silos keeping the effluents (wet ensiling) is an attractive alternative; however, the knowledge on fermentation pattern in such silages is scarce. The aim of this study was to examine fermentation characteristics and nutrient changes during the ensiling process when sugar beets were ensiled wet.

**Material and Methods** Two sugar beet silages were made from the same batch of sugar beets, one restricted with a silage additive (SBS+) and one without (SBS-). Beets were washed and mashed and filled into 1000 L intermediate bulk containers using an auger (kg wet weight per container: SBS- 609±25 and SBS+ 603±17). A total of 70 containers were filled, 35 SBS+ and 35 SBS-. The SBS- containers were filled first, thereafter, SBS+ containers; the ensiling additive was pumped simultaneously into the auger (7.6 L per tonne of beet mash). The additive was chemical based and contained 85 % formic/propionic acid buffered with ammonium. After filling, container inlets were closed with a screw cap mounted with a ball valve to allow fermentation gases to escape while keeping effluents in the container. Two SBS- and two SBS+ containers were sampled twice after 26 d and 146 d. Further samples were taken between 154 d - 217 d of storage during a feeding trial (see Hellwing et al. 2017).

Samples of beet silage were prepared according to ICUMSA (1994) as brei and sample solution was clarified with aluminium sulphate (Burba and Puscz 1976). Beet silage samples were analysed for pH, and for glycerol, fructose, mannitol, sucrose, formic acid, acetic acid, citric acid, lactic acid and ethanol using HPLC and gas chromatography. Dry matter was corrected for volatile losses according to Weißbach and Strubelt (2008). Statistical analyses were performed with proc GLM in SAS with sampling time, silage additive and interaction as fixed effects.

**Results** Both silages SBS+ and SBS– ensiled well with pH 4.1 after 26 d and 3.8-3.9 after 146 d (Table 1).

**Table 1.** Effect of days of ensiling (D) and use of ensiling additive (A) on pH, and on nutrients and fermentation products in % of DM. Sugars is sum of fructose, glucose and saccharose. Sum acids is sum of formic acid, acetic acid, citric acid and lactic acid.

| D | A | pH | Fructose | Glucose | Saccharose | Sugars | Mannitol | Sum acids | Ethanol |
|---|---|---|---|---|---|---|---|---|---|
| 26 | - | 4.1 | 18.6 | 1.82 | 3.29 | 23.7 | 12.5 | 5.31 | 0.56 |
| 26 | + | 4.1 | 5.79 | 6.90 | 53.5 | 66.2 | 0.00 | 3.16 | 0.37 |
| 146 | - | 3.8 | 7.06 | 1.11 | 0.0 | 8.18 | 13.4 | 7.73 | 6.60 |
| 146 | + | 3.9 | 20.4 | 21.7 | 24.2 | 66.3 | 0.00 | 4.26 | 0.84 |
| P-values | | | | | | | | | |
| D | | <.01 | 0.3 | <.01 | <.01 | <0.01 | <0.01 | <0.01 | <0.01 |
| A | | <.01 | 0.8 | <.01 | <.01 | <.01 | <.01 | <0.01 | <0.01 |
| DxA | | <.01 | <0.01 | <.01 | <.01 | <0.01 | <0.01 | 0.03 | <0.01 |

Results for nutrients and fermentation products in Table 1 are given as % of dry matter corrected for volatile losses. In the following, components in the silages are listed as 26 d SBS–, 26 d SBS+, 146 d SBS–, 146 d SBS+. Sum of sugars were 24; 66; 8 and 66 %, showing that sugars were preserved in SBS+, whereas sucrose were 3; 54; 0; 24% showing that sucrose fully disappeared in SBS– and over time was changed to other sugars in SBS+. Fructose 19; 6; 7; 20 and glucose 2; 7; 1; 22 concentrations were similar for SBS+, but for SBS- glucose concentrations were much lower than fructose concentrations. Mannitol concentrations were 13; 0; 13; 0 %, high in SBS– but absent in SBS+. Ethanol concentration were 0.6; 0.3; 6.6; 0.8 %, showing a considerable concentration in SBS– after 146 d.

**Discussion** Conservation of beets is essential to allow year around feeding. The present experiment shows that beets can be conserved when ensiled wet either with or without silage additive. The formic and propionic acid based silage additive used in the present experiment generally conserved the sugar, however a large part of the saccharose was hydrolysed to the monosaccharides glucose and fructose. Ensiling without additives resulted in an extensive fermentation, resulting both in fermentation acids and ethanol, especially after 146 days fermentation. Samples taken 154 d - 217 d during the feeding trial indicate that further fermentation takes place and also hydrolysis of saccharose to the monomers continued (Hellwing et al. 2017). Glucose and fructose concentrations in SBS+ were similar, but fructose concentrations in SBS- were considerable higher than glucose concentrations, and SBS- also had a considerable concentration of mannitol. The higher fructose than glucose concentration indicate that glucose is more predisposed to fermentation or to a change to mannitol than fructose. Mannitol is known to have a low energy value in humans (Evrendilek 2012). In ruminants, in vitro studies have shown that mannitol has a low fermentation rate (Ahmed et al. 2013). However, a feeding trial comparing SBS+ and SBS- silages after 154 d - 217 d of storage resulted in highest milk production for the SBS- (Hellwing et al. 2017), indicating that the energy value of SBS- was superior to the SBS+. The positive milk production results for the mannitol and ethanol containing silage (SBS-) indicate that both mannitol and ethanol contribute to milk production. However, further studies are needed to determine the energy value of ethanol and mannitol for ruminants.

**Conclusion** In restricted silages the sugar from the beets was preserved, whereas in unrestricted silages sucrose disappeared, mannitol concentration was high, and ethanol concentration was considerable after 146 d ensiling.

**References**

Ahmed, S., Minuti, A. & Bani, P. (2013) In vitro rumen fermentation characteristics of some naturally occurring and synthetic sugars. Italian Journal of Animal Science, 12, 57, 359-365.

Burba, M. & Puscz, W. (1976) Über die Verwendung von Aluminiumsalzen an Stelle von basischem Bleiacetat zur Klärung wässrigen Breiextrakten der Rüben. Zuckerindustrie, 26, 249-241.

Evrendilek, G.A. (2012) Sugar alcohols (polyols). In: Varzakas, T., Labropoulos, A., Anestis, S. (eds.), Sweeteners. Nutritional aspects, application and production technology. CRC Press, New York. p. 45-78.

Hellwing, A.L.F., Messerschmidt, U., Larsen, M., & Weisbjerg, M. R. (2017) Effects of feeding sugar beets, ensiled with or without an additive, on the performance of dairy cows. Livestock Science, 206, 27-44.

ICUMSA (1994) ICUMSA methods book. International commission for uniform methods of sugar analysis. http://www.icumsa.org.

Weißbach, F. & Strubelt, C. (2008) Die Korrektur des Trockensubstanzgehaltes von Grassilagen als Substrat für Biogasanlagen. Landtechnik, 63, 210-211.

# Statistics and experimental design in silage research: Some comments on design and analysis of comparative silage experiments

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**Keywords:** Sample size, scope of inference, silage experiments, statistical significance, relevance of results

## Introduction

"Reproducible results define the very core of scientific integrity in modern research" (Bello and Renter 2018), but across all scientific disciplines only too a little number of trials generate reproducible results. Misunderstanding and misuse of statistical concepts are contributing factors to the research reproducibility crisis (Nuzzo 2015). Some of these misunderstanding items are *experimental replication* (unclear distinction between experimental and observational unit, role of subsampling), *scope of inference*, *misinterpretation of P-values*, *statistical errors* (multiple comparisons, impact of several traits, and problem of selective reporting). The foundational statistical principles both for experimental design and for data analysis and interpretation should be kept in mind but "there is no rule-of thumb or software automation that can replace careful thought" (Bello and Renter 2018, Udén and Robinson 2015). The critical findings on design, analysis, and interpretation of results will be addressed based on comparative silage experiments in the following.

Initially, a non-comprehensive search in **scientific papers** on silage trials published in *Journal of Dairy Science*, *Grass and Forage Science*, or *Agricultural and Food Science* during the last 8 years yielded the following information: experiments with 1 up to 3 factors (sometimes even more), 3 to 6 replicates per treatment. Statistical analyses were frequently performed by parametric analysis of variance, followed by pairwise comparisons (LSD, Tukey, Bonferroni, Sidak, Duncan, and others), sometimes by non-parametric procedures (Wilcoxon and others).

Furthermore, we decided to conduct a **lab-scale ensiling trial on biostatistical questions:**

**(1)** What can the scope of inference for one ensiling experiment with mini-silos be? Forage material taken from one field location represents exactly this field situation. A composite sample from different locations represents an average field situation, the variability of observations between these sample points cannot be determined. For both situations, the variability of measurements reflects only the different fermentation processes of the replicated mini-silos per treatment from one (perhaps composed) material.
In order to represent arbitrary locations separated replications per location are necessary. In case of one observation per location the variability of measurements is caused by sampling point and fermentation process in the mini-silo, both effects are confounded. To separate the variation, samples with more than one observation from several locations have to be drawn.

Therefore, we sampled several locations of a natural grassland ley as silage material for our trial due to considerable in-field differences in important characteristics that may affect the fermentation process, or aerobic stability. We wanted to know the impact of location and fermentation process on a number of important silage traits.

**(2)** How many replications are necessary for both significance and relevance of results? Do the traits meet the assumptions of normal distribution and variance homogeneity?
Hence, we examined, besides an untreated control, two silage additives each with ten mini-silos as replicates. To restrict the experimental input, we used the composite sample from several sampling points. Thus, the observations represent the average situation in a given field.

From the whole sample subsets of smaller size were drawn and the results of statistical analyses for different sample sizes were contrasted.

**Description of the grassland trial**

A lab-scale ensiling trial was carried out with material taken from three randomly selected sampling points (P1, P2, and P3) of natural grassland. For each sampling point, ten 1.5-L jars were filled with grass material (24, 23, 20% DM) without the addition of silage additives to characterize data distribution pattern. In addition, a composite grassland sample was produced by thoroughly mixing identical quantities of material from each of the three sampling points (MIX). Subsequently, the herbage mixture was ensiled without silage additive (CON), or treated with a chemical additive (CHEM, containing sodium nitrite, hexamethylene tetramine and potassium sorbate) and with a biological additive (LAB, composed of *Lactobacillus buchneri* CNCM-I 4323 and *Pediococcus acidilactici* DSM 11673), respectively. Ten replicates per treatment were prepared. In total, sixty 1.5-L glass jars were stored at 22 °C and opened after 121 days of storage.

The fresh forage was characterised by the following traits, which are known to have an influence on the fermentation process, based on five replicate samples from each location: dry matter (**DM)**, water-soluble carbohydates (**WSC**), nitrate (**NO3**), buffering capacity (**BC**), yeast count **(Yeasts**), and lactic acid bacteria (**Lactobac**).

In the silage, the following parameters were evaluated: **pH**, lactic acid (**LA**), acetic acid (**AA**), 1,2-propanediol (**1,2-PD**), **WSC**, ethanol (**ETOH**), n--propanol (**PROP**), sum of ethyl esters of lactic and acetic acids (**Ester**), aerobic stability (**ASTA**), accumulated temperature during aeration (**ACT**), and anaerobic DM losses (**DML**). As only small quantities of butyric acid were detected and the counts of yeasts and moulds were low (and often below the detection limit), these traits were not further considered. All statistical analyses were performed by SAS, 9.4. The findings reported herein represent only one year and one herbage material, so generalizations cannot be made, yet.

**Results and Discussion**

Table 1 involves simple measures for traits of fresh herbage at ensiling, which can have an effect on the fermentation process. WSC concentrations were always high, nitrate always low (<1 g/kg DM, Kaiser and Weiss, 1997), and all samples had epiphytic lactic acid bacteria in excess of $10^5$ cfu/g fresh herbage. With the exception of nitrate content, variation of other traits were small within sampling locations.

**Table 1.** Mean and variation per sampling point for characteristics of fresh herbage at ensiling (n=5).

| Trait | Mean per field sampling point | | | Variation (s%) per sampling point | | |
|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P1 | P2 | P3 |
| Dry matter[1] | 245 | 227 | 198 | 0.4 | 0.5 | 1.0 |
| WSC[2,3] | 225 | 221 | 214 | 5.0 | 2.0 | 6.0 |
| $NO_3$[3] | 0.20 | 0.13 | 0.41 | 68.5 | 85.9 | 79.2 |
| Buffering capacity[4] | 57.0 | 52.8 | 55.0 | 3.1 | 3.7 | 3.6 |
| Yeasts[5] | $4.54x10^4$ | $2.03x10^4$ | $3.57x10^3$ | | | |
| *$log_{10}$[5]* | *4.66* | *4.31* | *3.55* | *6.2* | *7.6* | *3.5* |
| LAB[5,6] | $5.37x10^7$ | $7.28x10^7$ | $7.09x10^7$ | | | |
| *$log_{10}$[5,6]* | *7.73* | *7.86* | *7.85* | *2.9* | *1.2* | *1.1* |

[1]g/kg, [2]water-soluble carbohydrates, [3]g/kg DM, [4]g lactic acid/kg DM, [5]cfu/g, [6]lactic acid bacteria

The decomposition of the total variation into variations between field sampling points (large-scale variation) and residual variation within sampling point (small-scale variation) are presented in Figure 1. Dry matter showed almost no variation within sampling points, thus the total variation is caused by sample locations, whereas lactic acid bacteria count varied wider within sampling location.

Interestingly, the determined silage fermentation variables (Figure 3) were almost exclusively affected by field sampling point.

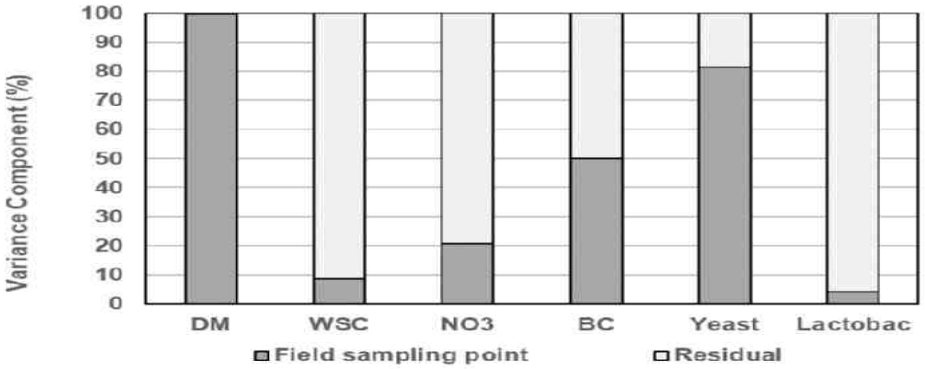


**Figure 1.** Decomposition of total variability of characteristics of fresh herbage at ensiling (3 x n=5) into variance component between field sampling point and variance component within sampling point (residual).

The outcome of the fermentation process was evaluated on the basis of the usually in silage trials measured concentrations of organic acids and alcohols as well as aerobic stability and the parameter DM losses which characterizes the efficiency of fermentation.

**Grassland trial with respect to field sampling locations on untreated silage (CON)**

The scope of inference corresponds to the population to which the results are applicable. Ideally, these population are sampled at random. For the grassland trial, three randomly selected sampling locations were used and the questions were addressed as to how large the variability of observations was between the three sampling locations and whether a composite mixture represented the whole field. Except for ASTA, the observed values are more or less different between sample points, and the values of grassland mixture tended to reflect an average situation for the untreated control (Figure 2).

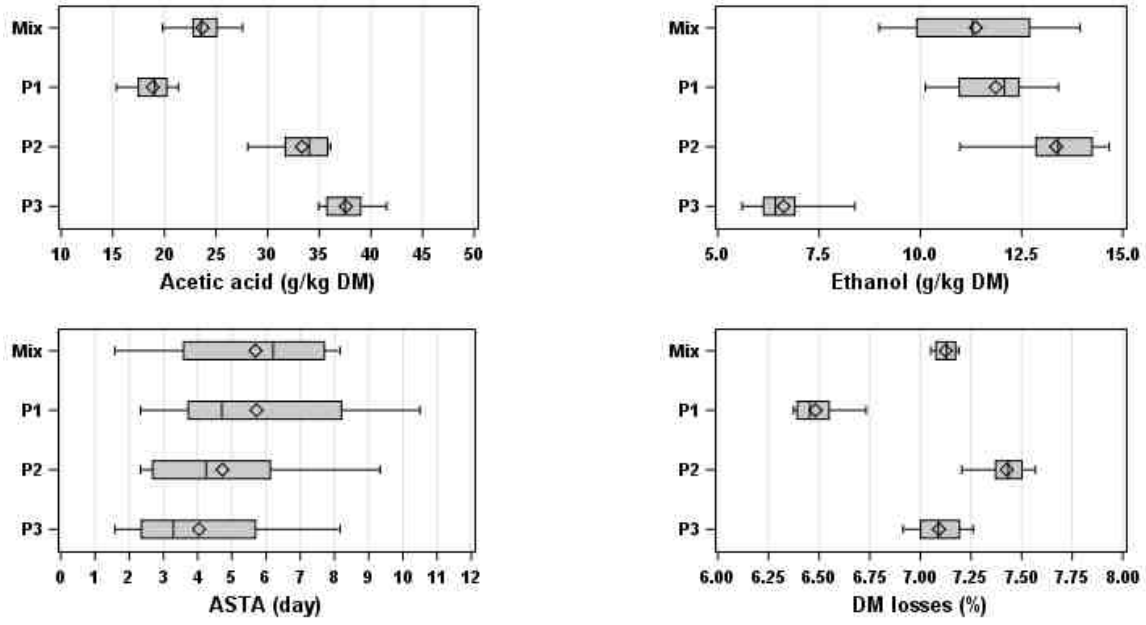


**Figure 2.** Box-Whisker-Plot for three field sampling points (P1-P3) and grass mixture (MIX), n=10.

The decomposition of the total variability showed that, with the exception of ASTA and ACT, the largest fraction of the variation was caused by field sampling point and the remaining residual variation was related to effects of the fermentation process of the ten replicates per sampling point. On contrary, both ASTA and ACT were affected almost completely by the fermentation process (Figure 3).

How far the efficacy of silage additives will be affected by sampling points cannot be shown in our study. In any case, one trial cannot be sufficient for a final evaluation of silage additives. For instance, the *Regulations of the European Union* request at least three laboratory-based studies covering a representative range of materials to which the additive will be applied (EFSA, 2008). However, only one trial per fermentability category is required.

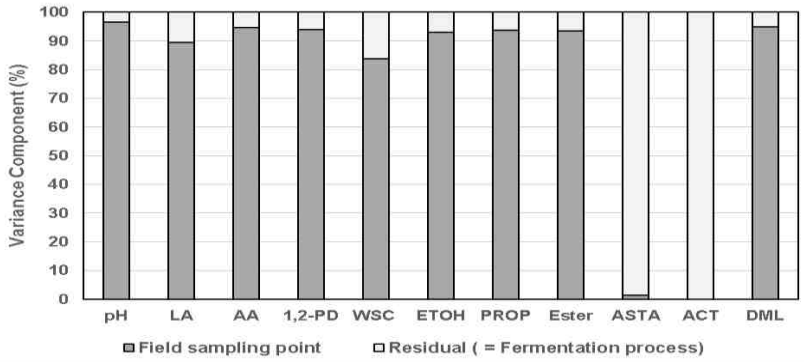


**Figure 3.** Decomposition of total variability of observed values (3 x n=10) into variance component between field sampling point and variance component within sampling point (residual = fermentation process).

**Grassland trial with the grass mixture (CON, LAB, CHEM)**

For the appropriate choice of data analysis comparing the treatments the experimenter should know whether the data observed come from populations with normally distributed data and homogenous variances. The sample data does or does not meet the assumptions. Checking the assumptions by statistical preliminary tests and performing the analysis on the same data are problematic. The experimenter should know the necessary characteristics beforehand. Most statistical software provides, together with the statistical procedures, panels of residual diagnostics for graphical presentation of the underlying assumptions.

For traits as LA, AA and WSC normally distributed data may be assumed. The distributions of traits like counts of yeasts and moulds are typically skewed to the right, and log-transformation is applied. For other traits, including butyric acid, propionic acid and n-propanol, which are frequently below the detection limit (zero values), the assumption of normal distribution cannot be hold. The box-whisker-plots of the experimental data in Figure 4 give an impression about variation and distribution. The four traits of interest will be closer examined: Are homogenous variances between treatments plausible and does a silage additive lead to smaller or larger variation? For data analysis, ANOVA with only one fixed treatment factor was chosen. In Table 2, data are presented for both situations: with one common residual variance (considering variance homogeneity = $VAR_{hom.}$) and with individual treatment variances (considering variance heterogeneity, $VAR_{het}$). For all traits, LAB treatment produced both the highest observations and, except for ETOH, also the highest variation. The use of the chemical additives resulted in particularly low variation for ETOH. Except ASTA, for our trial approaches considering variance heterogeneity are better fitted (smaller AIC values). Here with n=10, the estimation of individual variances is relatively reliable but trials with a small number of replications (n=3) do not allow for reliable variance estimations. Hence, a common residual variance may sometimes be better although the inferences are too strong for treatments with small variation or too weak for treatments with high variation. If the experimenter knows that a treatment does not only have an impact on the magnitude but also on the variation, the number of replicates for reliable variance estimations should be taken into account.

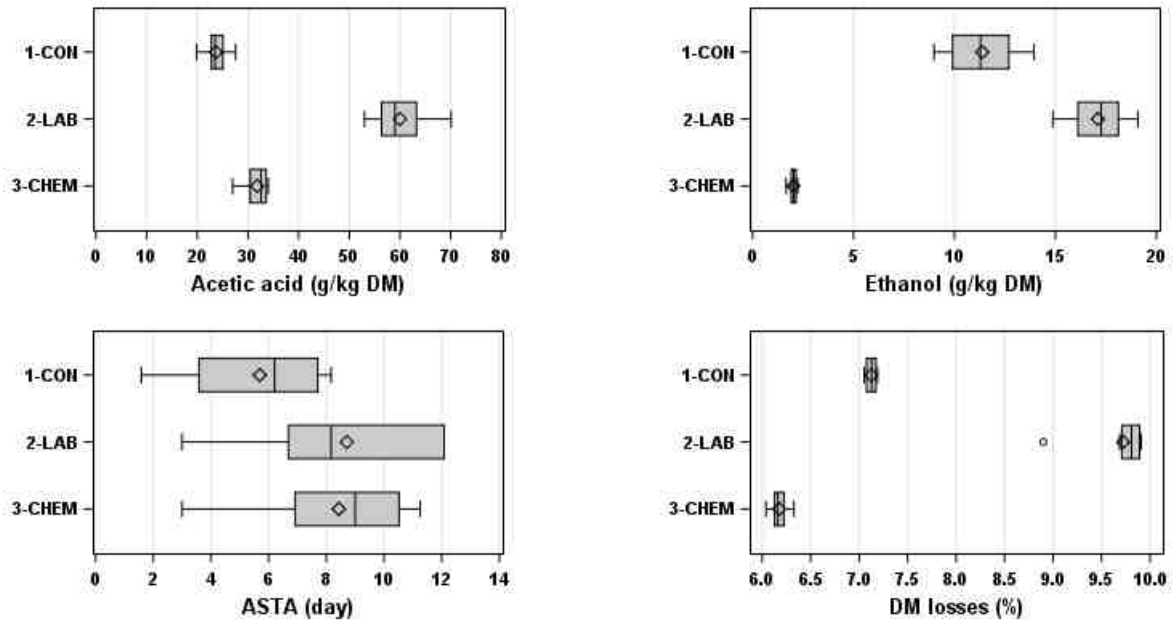


**Figure 4.** Box-Whisker-Plot for grass mixture treated with silage additives (n=10).

**Table 2.** Mean, residual variance and fit criteria for different ANOVA approaches (n=10)

| Trait | Mean | | | Residual variance | | | | AIC[1] (fit criteria) | |
|---|---|---|---|---|---|---|---|---|---|
| | CON | LAB | CHEM | $VAR_{hom}$[2] | CON | LAB | CHEM | $VAR_{hom}$[2] | $VAR_{het}$[3] |
| AA[4] | 23.7 | 60.0 | 31.8 | 13.4 | 4.0 | 31.1 | 5.1 | 155.6 | 147.6 |
| ETOH[5] | 11.4 | 17.1 | 2.0 | 1.54 | 2.61 | 1.98 | 0.03 | 97.2 | 73.1 |
| ASTA[6] | 5.7 | 8.7 | 8.4 | 8.0 | 4.9 | 11.3 | 7.7 | 141.5 | 144.0 |
| DML[7] | 7.1 | 9.7 | 6.2 | 0.033 | 0.002 | 0.090 | 0.007 | -6.3 | -30.3 |

[1] Akaike information criterion (the smaller the value the better), [2]variance homogeneity, [3]variance heterogeneity, [4]acetic acid, g/kg DM, [5]ethanol, g/kg DM, [6]aerobic stability, days, [7]DM losses during fermentation, %.

In our experiment, we used ten replicates compared with the frequently observed situations of n=3 or n=6. So, we extracted from the whole sample n=3 values (→ 120 possible combinations or subsets) or n=6 values (→ 210 possible combinations or subsets) and performed the treatment comparisons for all situations. An overview about selected results for the control treatment and differences to the other treatments treated with silage additive is given in Table 3. Because of smaller sample sizes ANOVA with common residual variance was used although considering variance heterogeneity might be the better approach. Except for ASTA, all pairwise comparisons showed significant differences between CON and additive treatments (Table 3) for the total sample (n=10). These results hold true when considering variance heterogeneity.

The smaller the sample size in the subset the more vary treatment means, treatment differences and Tukey's HSD. Nevertheless, in our study treatment differences for AA, ETOH and DML are large enough to be significant in any case. For ASTA, a few subsets show significant differences, especially for comparisons between CON and LAB.

**Table 3.** Mean for control treatment and differences to LAB and CHEM treatments for total sample (n=10) and all possible subsets with n=6 and n=3.

| Trait | repli- | number of | *Mean* | *Difference* | | HSD |
|---|---|---|---|---|---|---|
| | cates | subsets | CON | CON - LAB | CON - CHEM | α = 5% |
| AA | **10** | | **23.7** | **-36.3** | **-8.1** | **4.06** |
| | 6 | 210 | 22.5 … 24.8 | -40.0 … -32.3 | -10.2 … -6.1 | 3.1 … 7.0 |
| | 3 | 120 | 21.8 … 25.9 | -44.2 … -30.2 | -11.8 … -4.1 | 2.7 … 14.7 |
| ETOH | **10** | | **11.4** | **-5.7** | **9.4** | **1.38** |
| | 6 | 210 | 10.3 … 12.4 | -6.8 … -4.8 | 8.3 … 10.4 | 1.2 … 2.3 |
| | 3 | 120 | 9.6 … 13.2 | -7.3 … -3.5 | 7.5 … 11.1 | 1.0 … 5.0 |
| ASTA | **10** | | **5.7** | **-3.0** | **-2.7** | **3.13** |
| | 6 | 210 | 4.4 … 7.2 | -5.7 … -0.8 | -5.3 … -0.4 | 2.9 … 5.1 |
| | 3 | 120 | 2.9 … 7.9 | -6.9 … 2.1 | -6.8 … 2.1 | 1.7 …10.4 |
| DML | **10** | | **7.1** | **-2.6** | **0.9** | **0.20** |
| | 6 | 210 | 7.1 … 7.2 | -2.7 … -2.5 | 0.9 … 1.0 | 0.07 … 0.35 |
| | 3 | 120 | 7.1 … 7.2 | -2.8 … -2.3 | 0.8 … 1.0 | 0.06 … 0.84 |

How far a significant difference meets practical relevance depends on the effect size the experimenter is interested in. The effect size of interest together with a reliable estimation of the residual variance (random variability of observations) are essentials for sample size calculations. Effect sizes of interest are rarely given. EFSA (2008) gives an effect size only for one trait, ASTA: "…assessment of the aerobic stability of at least seven days after exposure to air, additive shall be stable two days longer than untreated control …". Of course, the design of a trial should be determined not by all but by so-called key traits, which are the most important ones for the evaluation of the treatments tested in the trial. The number of key traits has further consequences: the adjustment of error probability (type I error) due to multiplicity depends not only on the number of treatment comparisons but also on the total number of different response variables. The requirement of "two days longer than control" comes along with the recommendation for one-sided Dunnett test procedure instead of Tukey's test procedure (always two-sided) when the experimenter would only be interested in differences to the untreated control in one direction.

The observations of aerobic stability as one of the most important response are often problematic from the "statistical" point of view. In our study, the duration of air exposure to the silage lasted 12.1 days. Whereas all replications of control and CHEM treatment had shorter ASTA values, four out of ten replicates of LAB were aerobically stable over the entire period of aeration. Frequently, the observed data are more extreme e. g. for untreated control 4.0, 4.8, 2.8 and for treatment with some additive 7.0, 7.0, 7.0 (Weiss et al. 2016) where latter values correspond with the maximal duration of air exposure. Constant (identical) values do not vary and normal distribution can hardly be assumed. For nonparametric rank procedures a sample size of n=3 is rather insufficient. But from the practical point of view the result is totally clear! More replicates of the treated silage would produce further constant ASTA values. A way out could be the calculation of a confidence interval for the expected mean of the untreated control. If the treatment means (regardless of constant values) are not within this confidence interval, then strong evidence for better stability is provided.

**Summary**

Comparative silage experiments are most frequently performed with few replications. The traits of interest for the evaluation of treatments do not meet in each case the assumptions for the chosen statistical analysis procedures. Both, rank procedures in case of non-normality as well as reliable estimations of individual treatment variances in case of variance heterogeneity demand higher sample sizes. Often, the experimenter has information beforehand about treatments which have an impact not only on the magnitude but also on variation. In addition, together with well justified effect sizes of interest, the trials should be designed in such a way that significance and relevance of results come together.

**References**

Bello, N.M. & Renter, D.G. (2017) Invited review: Reproducible research from noisy data: Revisiting key statistical principals for the animal sciences. Journal of Dairy Science, 101, 1-23.

EFSA (2008) Guidance for the preparation of dossiers for technological additives. The EFSA Journal, 774, 121.

Kaiser, E. & Weiss, K. (1997) Zum Gärungsverlauf bei der Silierung von nitratarmem Grünfutter. 2.Mitt.: Gärungsverlauf bei Zusatz von Nitrat, Nitrit, Milchsäurebakterien und Ameisensäure. Archives of Animal Nutrition, 50, 187-200.

Nuzzo, R. (2015) Fooling ourselves. Nature, 526, 182-185.

Udén, P. & Robinson, P. H. (2015) Design and statistical issues in silage experiments. In: Nussio, L. G., de Sousa, D. O., Gritti, V. C., Salvati, G. G., Santos, W. P. & Salvo, P. A. R. (eds.). V International Symposium on Forage Quality and Conservation, Piracicaba, SP, Brazil, 2015, 166-179.

Weiss, K., Kroschewski, B. & Auerbach, H. (2016) Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage. Journal of Dairy Science, 99, 1-17.

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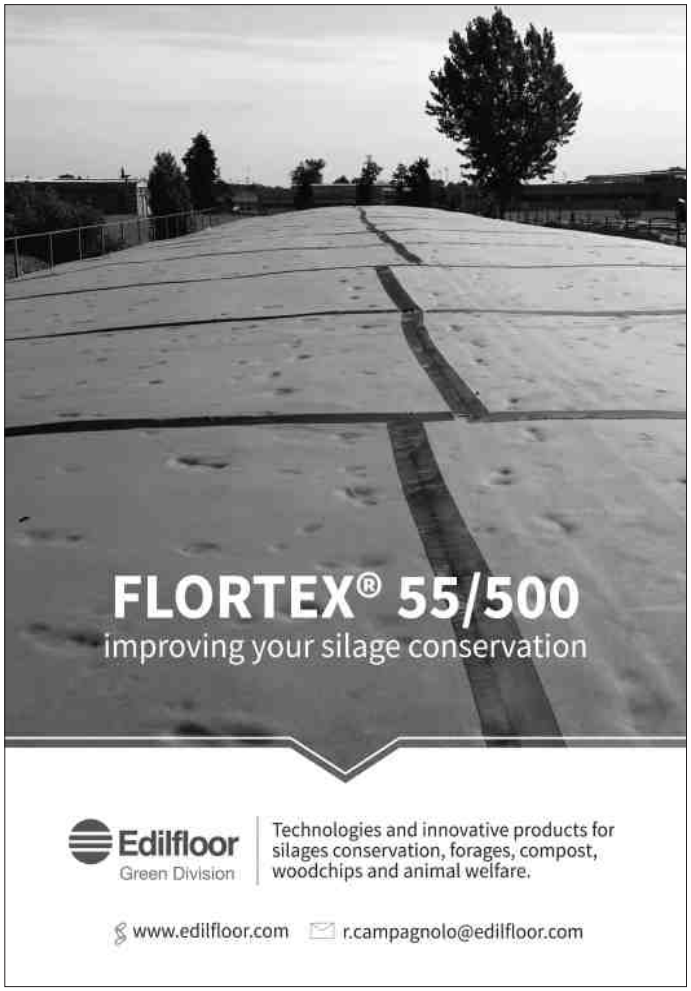


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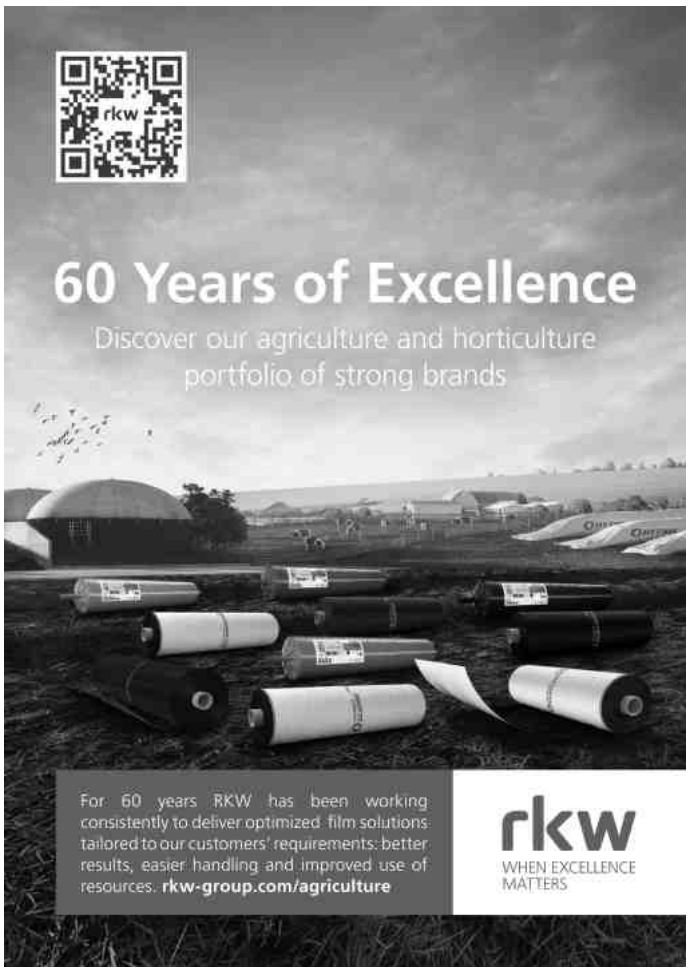

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