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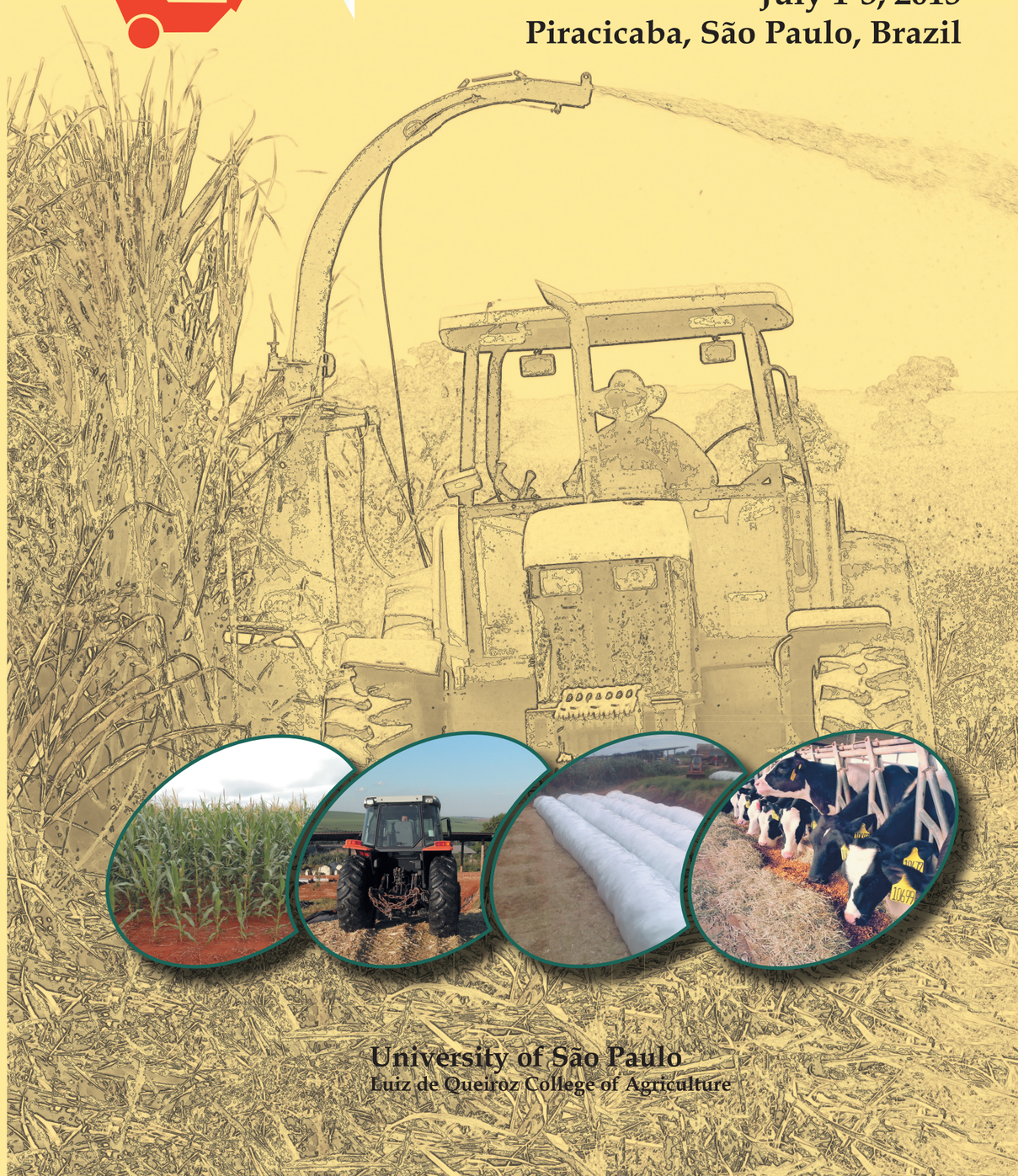
ISSN: 2175-4624
ISBN: 978-85-86481-38-3

XVII International Silage Conference

XVII ISC 2015

XVII International Silage Conference

July 1-3, 2015
Piracicaba, São Paulo, Brazil



University of São Paulo
Luiz de Queiroz College of Agriculture

Proceedings of the
XVII International Silage Conference

IV International Symposium on Forage Quality and Conservation

Printing and Book Design

Editora Gráfica Riopedrense

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Edited by

J. L. P. Daniel, G. Morais, D. Junges and L. G. Nussio

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DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP

International Silage Conference (17.: 2015 : Piracicaba, SP)
Proceedings … / edited by J. L. P. Daniel ... [et al.]. - - Piracicaba : ESALQ, 2015.
623 p. : il.

Publicado com: 4. International Symposium on Forage Quality and Conservation.
Realizado em Piracicaba, SP de 1 a 3 de julho de 2015.
ISBN: 978-85-86481-38-3

1. Forragem - Conservação - Qualidade 2. Silagem I. Daniel, J. L. P., ed. II. Morais, G., ed. III. Junges, D., ed. IV. Nussio, L. G., ed. V. Título

CDD 636.08552
I61p

ISBN 978-85-86481-38-3
ISSN 2175-4624

WELCOME LETTER

Dear Delegate,

We are honored to welcome you to the XVII International Silage Conference, 1-3 July, 2015, in Piracicaba, SP, Brazil. Piracicaba is an indian name which refers to the place where the fish stops at the water fall during the upstream swimming. This meeting was first designed by the steering committee to put together local and international scientific groups to share novel research ideas. Our thoughts were driven towards the integration of knowledge, research partnership, interdisciplinary approach, education and technology transfer in silage science.

The recent scientific progress associated with the tropical zones turned out to be a key tool to improve the animal industry to supply the global market. The increased number of ongoing research projects related with animal science reveals amazing opportunities to integrate the South American scientific communities and the delegates from traditional centers of knowledge, offering mutual benefits. Forage conservation is a key link to accomplish this daring integration initiative.

News from the XVII International Silage Conference was world wide spread and up to date we had almost 10,000 hits on the website from 96 countries, so far resulting in 250 registrations from 32 countries. We had been fortunate enough to receive 209 high quality contributions from around the world. The program will feature in 11 special sessions with 15 outstanding keynote speakers, moreover, 26 oral presentations were selected to strategically fit on addressed subjects and also to give opportunity to some of the new scientists. Four special sessions were dedicated to poster presentations to highlight the importance of personal interaction on the transference of scientific information and networking.

This is the first International Silage Conference in the south hemisphere and is jointed with the IV International Symposium on Forage Quality and Conservation. The organizing committee focused in a meeting with the compromise of coping with some Latin American issues and considered the international agenda in silage science by offering a cosmopolitan meeting.

Thanks for coming and jointing us, wishing you a nice staying in Brazil. On behalf of the Steering Committee,

Luiz Gustavo Nussio

Chair

POSTER SESSION

Agronomic management and forage processing

Silo and silage management

Silage additives

Feed value

Silage microbiology and safety

Grain silage

Major contributions in 45 years of International Silage Conferences

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Keywords silage, fermentation, aerobic deterioration, feeding value, systems

The International Silage Conferences and their predecessors

The early development of this series of Silage Conferences was outlined by Wilkins (1996) and the venues of the meetings are listed in Table 1.

Table 1 Date, venues and titles of meetings

1	1970	Edinburgh, Scotland	Silage Seminar
2	1972	Hurley, England	Silage Seminar
3	1974	Edinburgh, Scotland	Silage Conference
4	1976	Hurley, England	Silage Conference
5	1978	Ayr, Scotland	Silage Conference
6	1981	Edinburgh, Scotland	Silage Conference
7	1984	Belfast, Northern Ireland	Silage Conference
8	1987	Hurley, England	Silage Conference
9	1990	Newcastle, England	Silage Conference
10	1993	Dublin, Republic of Ireland	International Conference on Silage Research
11	1996	Aberystwyth, Wales	International Silage Conference
12	1999	Uppsala, Sweden	International Silage Conference
13	2002	Auchincruive, Scotland	International Silage Conference
14	2005	Belfast, Northern Ireland	International Silage Conference#
15	2009	Madison, USA	International Silage Conference
16	2012	Hameenlinna, Finland	International Silage Conference
17	2015	Piracicaba, Brazil	International Silage Conference##

Also Satellite Workshop of 20th International Grassland Congress

Also 4th International Symposium on Forage Quality and Conservation

The first meeting was held in Edinburgh in 1970 and was entitled 'Silage Seminar'. There were 35 participants, all from the UK and Ireland. There were no papers, but the sessions on particular topics involved a panel of researchers who outlined their recent findings and future intention, prior to a full discussion of the topic. Meetings were then held at two or three year intervals in different centres in the UK until the 10th meeting in Dublin in 1993. The format evolved to that of a conference built around offered papers reporting recent research and development, together with a small number of invited contributions. These include some particularly valuable review papers. Although summaries of most of the papers presented were available at the meetings, a bound collection of papers was not produced until the 5th meeting at Ayr in 1978. From the 2nd meeting, leading researchers from other European countries were invited to the meetings, and attendance increased to around 100.

A major change took place for the meeting in Dublin in 1993. This was entitled the '10th International Conference on Silage Research'. It was an open meeting and the Proceedings were fully published. This pattern has continued to the present, with meetings held at three or four year intervals. Attendance increased from 141 in Dublin to 324 in Hameenlinna, Finland, in 2012. The 2015 17th International Silage Conference is the second meeting to be held outside Europe. Copies of all the Proceedings and reports from previous meetings are held in the Library of Rothamsted Research, North Wyke, Okehampton, EX20 2SB, England (www.rothamsted.ac.uk/northwyke).

A feature of the series has been the presence at the meetings and contributions from researchers from many disciplines. Successful systems of forage conservation and feeding require inputs from agronomists, engineers, chemists, biochemists, microbiologists, physicists, mathematicians, animal nutritionists, animal production specialists, veterinarians and economists. Individuals, or more likely teams, with the capability of integrating information from these different disciplines are needed. The Conferences have provided a forum for people from these disciplines to meet and interact and these contacts have stimulated the development of several successful international inter-disciplinary research projects, including Eurowilt (Zimmer and Wilkins, 1984), Eurobac (Lindgren and Petterson, 1990) and Legsil (Wilkins and Paul, 2001).

Whilst there have been other important national and international conferences on silage, this review concentrates on research reported in the series of meetings listed in Table 1. We will briefly outline the state of knowledge at the time this series of meetings started, discuss the changes in focus that have occurred over the 45 year period, highlight landmark contributions made at the meetings and suggest some important challenges for the future.

State of knowledge in 1970

The Proceedings of the 3rd General Meeting of the European Grassland Federation held in Braunschweig in 1969 on 'Crop Conservation and Grassland' present a good account of knowledge and issues being studied at that time. Significant papers were presented by Zimmer (1969) and Raymond (1969). The major biochemical pathways involved in silage fermentation had been identified and the importance of the contents of dry matter (DM), water-soluble carbohydrates (WSC) and buffering capacity in influencing the course of fermentation was recognised. The magnitude of losses in the field and in the silo had been quantified and it was realised that losses could vary greatly according to method of ensiling and particularly the DM content at ensiling. The main thrust of research on additives was on the use of formic acid and salts, although Gross (1969) reported favourable results with inocula of lactic acid bacteria, particularly when added at high rates. Whilst the digestibility of silages had been shown to generally be similar to that of the crop prior to ensiling, animal outputs from silage-based diets were often disappointing because of low levels of feed intake, but the factors limiting intake had not been elucidated.

Themes at Silage Conferences

There has always been a broad mix of contributions to the Conferences, reflecting silage research activity in progress at the time of the meeting.

Three phases can be identified. The first meetings (1 to 5) were dominated by contributions relating to the silage fermentation, the determinants of feeding value of silage and sources of loss. This work was particularly important to provide a scientific basis for reliable production of silage on farms and for silage to make a major contribution in the feeding of productive livestock at a time when forage conservation in Britain and throughout much of Europe was still dominated by hay. The main thrusts of the next series of meetings (6 to 11) concerned aerobic deterioration, inoculation, the prediction of feeding value and feed complements for silage. More recently (meetings 12 to 16) there has been change and diversification in the contributions, reflecting increased size of meeting and greater global participation. Many papers have dealt with the ensiling and use of silages from a wide range of crops and by-products, including, from the 13th Conference onwards, tropical forages. Research on grasses, legumes and maize included more integral studies concerned with the development and evaluation of whole systems of animal production, including increasing concern with effects on hygiene and health and impact on the environment.

The use of silage for biogas, first featured in the 15th Conference in 2009, opened up a whole new area of research. Previous contributions had been concerned almost exclusively with producing silages for domesticated ruminant animals. However,

Henderson and Whittemore (1976) reported feeding silage to pigs and there was a poster session on feeding silage to horses at Auchincruive in 2002 (Muller, 2002). As a further example of diversification both of ensiled materials and animals used, Orosz *et al.* (2012a) produced tomato pulp silage for deer and wild boar.

Major contributions

Silage fermentation

Contributions highlighted and clarified the importance of a wide range of organisms to the fermentation process. These included yeasts (Weise, 1972), enterobacteria (Lindgren, 1984; Spoelstra, 1984) and acetic acid bacteria (Spoelstra *et al.,*1987). Detailed studies of factors affecting the populations of epiphytic lactic acid bacteria were carried out by Pahlow and Dinter (1987) in Germany and by Muck (1987) in USA.

Additional information was obtained on the effects of plant components on the fermentation. Seyfarth *et al.* (1993) found that fructans are of limited availability to many silage bacteria. Spoelstra (1984) drew attention to the complex effects of nitrate on the fermentation process. These effects were further clarified by Weissbach *et al.* (1993), who concluded that a certain amount of nitrate is required to prevent clostridial fermentation in wilted crops. This led to a new model for assessing the ensiling characteristics in forages which included nitrate content, in addition to DM, WSC and buffering capacity (Kaiser *et al.*, 2002), with this model being successfully tested by Pahlow (2002).

The first model to predict the course of fermentation with time, including the changes in microbial populations and in silage composition, was presented by Neal and Thornley (1981), whilst Chapman and Wilson (1981) reported a multi-variate analysis of inter-relationships between crop composition and silage composition.

Aerobic deterioration

Aerobic deterioration had not emerged as a major issue by 1970 and did not feature in the discussion at the first meeting in Edinburgh that year. There have subsequently been many contributions on the subject, reflecting its importance, complexity of the problem and the need to find practical solutions. Pahlow and Muck (2009) traced the increase in knowledge about the process and how it could be controlled through the papers presented at successive International Silage Conferences. Cook (1972) presented results for the effects of additives on aerobic deterioration and Weise (1972) identified yeasts as contributing to heating when a silo is opened. Through addition of antimycotic and antibacterial chemicals to made silages, Woolford (1976) concluded that bacteria may initiate aerobic deterioration and are followed by yeasts. However, Pahlow and Muck

(2009) concluded that most often lactate-assimilating yeasts utilise lactic acid and raise pH allowing other aerobic organisms such as bacilli to develop.

Weissbach (1996), in a review, highlighted research by Wolthusen *et al.* (1989) that had demonstrated the risk of aerobic instability is reduced with increase in the content of undissociated acetic acid. Driehuis *et al.* (1996), in the first of a number of papers presented to the Silage Conferences, reported that the hetero-fermentative *Lactobacillus buchneri* increased the concentration of acetic acid in silages and resulted in a dramatic increase in aerobic stability compared with silages without additive (Table 2). O'Kiely *et al.* (2005) showed higher aerobic stability in silages made with *L. buchneri* than with inocula of homofermentative lactic acid bacteria.

Table 2 Aerobic stability of silages made from grass and whole crops of maize and wheat without additive and with inoculum of *Lactobacillus buchneri* (from Driehuis *et al.*, 1996)

	No additive	With *L. buchneri*
	Aerobic stability (hours)	
Grass	130	>320
Maize	43	792
Wheat	125	250

Silo management, with effects on silage density and porosity, and the rate of removal of silage determine the extent of air penetration and influence the rate of aerobic deterioration, as discussed by Muck (1993) and Muck and Pitt (1993). Williams *et al.* (1993) reported a model of aerobic deterioration incorporating both physical and biological factors.

Recent work has quantified the effects of aerobic exposure of silage on the animal. Gerlach *et al*. (2012) found an average 57% reduction in DM intake of maize silages differing in DM, chop length and density and exposed to air for 8 days prior to being offered to goats in a preference trial. Dry matter concentration, pH, and counts of yeasts, moulds and aerobic mesophilic bacteria increased during exposure to air, whilst concentrations of fermentation products decreased, with the largest changes occurring between 4 and 8 days exposure. Accumulated increase in silage temperature above ambient during exposure to air was the best predictor of intake. Amaral *et al.* (2012) reported increased digestibility of maize silage and higher milk yield in cows given maize silage made with a surface covering that gave reduced oxygen transmission into the silo.

Voluntary intake

A large research effort was being devoted in the early 1970s to factors that limited silage intake. The general thesis was that intake was lower than that of the unensiled forage and that this was associated both with extensive breakdown of protein during the ensiling process and with high contents of organic acids, particularly lactic acid (Wilkins *et al.,*1971). This was confirmed in an analysis of intake data from 142 silages fed to sheep at Hurley (Wilkins *et al.*, 1978), but the effect of variation in protein breakdown, as indicated by content of ammonia N, was greater than that from total acid content. The precise reason for intake limitation in poorly-preserved silages remains elusive. Barry *et al.* (1976), working with lucerne silages made from one cut, found intake was closely associated with the extent of amino acid decarboxylation that occurred in the silo, but provision of intra-peritoneal methionine did not increase intake. Deswysen *et al.* (1978) found that whilst added acetic acid, often present in large quantities in poorly-preserved silages, influenced the pattern of feed intake, it did not affect total daily intake.

Seminal contributions by Dulphy (1972) and Deswysen and Vanbelle (1976) both demonstrated that chopping before ensiling influenced the pattern of fermentation and increased intake. However, if material ensiled after flail mowing (long particles) was chopped before feeding, intake was increased, but not to the same level as that with silages that had been precision-chopped prior to ensiling. Both physical and chemical factors clearly influenced feed intake.

Lewis (1981) produced the first equations for predicting the intake of grass silages by dairy and beef animals. Intake potential was calculated from DM content, digestibility and ammonia N and this was then modified to take account of animal liveweight, milk yield and concentrate intake. Huhtanen *et al.* (2012) reviewed models for the prediction of silage intake by dairy cows and highlighted the relative silage DM intake index of Huhtanen *et al.* (2002), which included digestibility, total acid concentration and ammonia N.

Efficiency of nitrogen utilisation

In the late 60s it was realised that the efficiency of N utilisation may be low with silages. This was associated with both the extensive breakdown of protein that generally occurs during ensiling and the low level of readily-available carbohydrates in most silages. Many papers presented at the Silage Conferences have progressed our understanding and indicated approaches to improve N efficiency. Generally the approach has been to increase the production of microbial protein in the rumen through either restricting proteolysis in the silo or the rumen or by increasing the supply of readily-available carbohydrates. Waldo and Tyrell (1978) reported that poor supply of protein to the animal with silage feeding resulted in cattle having carcases with increased fat content at the expense of

protein. It was also, realised, as highlighted by Misselbrook *et al.* (2012), that inefficient N utilisation by the animal may result in environmental problems through increased loss of N compounds to water and the environment.

Syrjala (1972) found that N balance in sheep fed grass silage was improved by supplementation with sucrose, but not with starch or cellulose. Large effects of sucrose infusions on microbial protein production were found by Huhtanen and Ala-Seppala (1987) and Chamberlain *et al.* (1993) found that supplementation with sucrose resulted in an increase in microbial protein production that was three times that obtained with starch supplementation. The approach of increasing the supply of readily-available carbohydrates was extended by Jaakkola *et al.* (1993), who found that microbial protein synthesis was more efficient in silages that had undergone restricted fermentation in the silo (and thus had more residual WSC) than in silages with extensive fermentation. Rooke and Armstrong (1987) reported that when animals given grass silage received intra-ruminal infusions of sucrose, there was a further increase in efficiency of microbial N synthesis when casein was infused, but not with urea.

Several approaches have been made to reduce the breakdown of protein in the silo or the rate of breakdown in the rumen. Charmley and Veira (1987) heat-treated lucerne prior to ensiling. This resulted in a large increase in insoluble N in the silage and increase in non-ammonia nitrogen flow into the duodenum, without reducing whole tract N digestibility. Previous attempts to reduce protein breakdown through the use of chemical additives such as formaldehyde had variable success, with N retention being increased in some experiments, but in others over-protection of protein occurred with reduction in whole tract N digestibility (Wilkins, 1972).

Very promising results were reported from the use of legumes with high levels of either polyphenol oxidase or tannins. Jones *et al.* (1993) reported that proteolysis in the silo in red clover was restricted and associated this with polyphenol oxidase in the clover. Subsequent research showed that this resulted in more efficient N utilisation in the animal with red clover than with lucerne silage that did not have polyphenol oxidase (Grabber and Coblentz, 2009). Hymes Fecht *et al.* (2005) compared lucerne silage with birdsfoot trefoil silages made from selections for contrasting contents of condensed tannins. The efficiency of protein utilisation, as indicated by milk urea nitrogen contents, was similar for lucerne silage and the birdsfoot trefoil with low tannin content, but increased with increase in tannin content. Milk production also increased significantly with increase in the tannin content of the birdsfoot trefoil (Table 3).

Table 3 Milk production and milk urea nitrogen with cows fed silages made from lucerne and birdsfoot trefoil with low and normal tannin contents (from Hymes Fecht *et al.*, 2005)

	Lucerne	Birdsfoot trefoil	
		Low tannin	Normal tannin
3.5% fat-corrected milk (kg/day)	31.4[a]	33.8[b]	36.3[c]
Milk protein (kg/day)	0.94[a]	1.04 [b]	1.09[b]
Milk urea N (mg/dl)	10.8[a]	10.8[a]	9.3[b]

Superscripts indicate differences significant at $P<0.05$

Prediction of the energy value of silage

In the early Silage Conferences there was a series of contributions concerned with the prediction of the digestibility or metabolizable energy (ME) of silages. In the first of these papers (Alderman, 1974) the best equation for predicting ME for 45 grass silages involved CP content, digestibility *in vitro* and DM content, but there was still considerable unexplained variation. Some refinements were proposed, including determining the gross energy of the silage and the use of pepsin cellulase or NDF-cellulase, rather than digestibility *in vitro* (eg Givens and Brunnen, 1987). A breakthrough was, however, reported by Kridis *et al.* (1987) who found that organic matter digestibility could be predicted more precisely by near infra red reflectance spectroscopy (NIRS) than from a number of chemical or biological measurements. This set the scene for the extensive use of NIRS, particularly for the routine assessment of farm silages, as outlined by Park *et al.* (2005).

Supplementation of silage

Limitations to the intake and nitrogen utilisation with silages led to considerable research on the composition of supplements to be fed with silage, particularly for dairy cows. Gordon (1976) showed large increases in milk yield of cows fed grass silage of high digestibility with increase in the crude protein (CP) content of the supplement from 9 to 21%, with silage intake tending to be higher with the high CP supplements. Several papers on protein supplementation were presented at the 7th Conference in Belfast in 1984. Small and Gordon (1984) confirmed the response to high CP in the supplement, but with a poorly-preserved grass silage there was no difference between protein supplements which differed in rumen degradability. However, Thomas *et al.* (1984) found greater responses with fish meal of low rumen degradability than with other supplements of higher ruminal protein degradability. The benefits from using protein sources with low degradability in the rumen were confirmed by Girdler *et al.* (1987), using a supplement containing fish meal,

blood meal and bone meal.

Some effects of different carbohydrate sources on microbial protein production were discussed above. Chamberlain and Choung (1993) stressed that with energy supplements it is important to avoid marked depressions in ruminal pH, particularly with silages with restricted fermentation in the silo. They concluded that the ideal sugar supplement should be relatively slowly fermented and would give an acetate/butyrate type of fermentation in the rumen.

Additives

At the beginning of this series of meetings the main research focus was on improving fermentation efficiency and silage intake either through inhibiting growth of undesirable microorganisms, especially clostridia, or by restriction of total microbial activity. Emphasis was on chemical additives, initially formic acid, but then formaldehyde (Wilkins, 1972). Woolford (1972) outlined a procedure for assessing the anti-microbial spectra of potential chemical additives that, with adaptation, became widely used. This approach confirmed that formic acid had specific anti-microbial effects in addition to its effect on crop pH.

The focus changed subsequently to consideration of biological additives, with Woolford (1974) reporting the effects of inoculation with mixtures of lactic acid bacteria. It was not, however, until the 6th Conference in 1981 that substantial attention was given to bacterial additives. Lingvall and Pettersson (1981) reported positive effects of an inoculum of *Pediococcus acidilactici* and *Lactobacillus plantarum* on silage quality and stressed the importance of a high rate of application (10^6 bacteria/ g fresh grass), but Hopkins *et al*. (1981) found that a commercial inoculum had little effect in laboratory and farm-scale silos, probably because insufficient numbers of lactic acid bacteria were added to dominate the fermentation.

Inocula of homolactic acid bacteria such as *Lactobacillus plantarum* had positive effects on fermentation and on feed intake in many papers presented at the Silage Conferences (summarised by Wilkins, 1996). However there was increased risk that such silages were prone to rapid aerobic deterioration. This stimulated interest in the use of the heterofermentative *Lactobacillus buchneri* in an inoculum. As noted earlier, Driehuis *et al.* (1996) found that *L. buchneri* led to increases in silage acetic acid content and increases in aerobic stability. Kalzendorf and Weissbach (1993) produced the first results at a Silage Conference from an alternative approach with the combination of an inoculum of lactic acid bacteria and sodium formate, with the inclusion of the formate leading to marked increases in aerobic stability

Another thrust has been in the use of enzymes, with the main focus on using

enzymes to increase the supply of sugars for fermentation to produce acids or to increase the digestibility of the silage. A session was devoted to the addition of enzymes at the 4th Conference at Hurley in 1976. Wilson (1976) and Henderson and Whittemore (1976) demonstrated that additions of cellulase at ensiling increased lactic acid production and improved preservation of silages made from lucerne and grass respectively. Whilst increasing the supply of substrate for fermentation, the application of cellulase did not increase the digestibility of the silage, as shown by Jacobs and McAllen (1987).

Several papers at the 15th Conference at Madison in 2009 looked at the effects of using a strain of *L. buchneri* (PTA6138) that has ferulate esterase activity. This has the potential of breaking bonds between lignin and cellulose and hemicelluloses and increasing fibre digestion by ruminants, as first reported by Nsereko *et al.* (2008). Bruesermeister *et al.* (2009 a,b) found that an inoculum including this strain of *L. buchneri* improved preservation and also resulted in significant increases in the digestibility of fibre and DM in grass and maize silages. The increases were, however, small at only about 1 percentage point. Similar responses for DM digestibility were found with grasses by Dupon *et al.* (2012). Romero *et al.* (2012) used an *in vitro* technique to evaluate the effects of 18 exogenous fibrolytic enzymes on Bermuda grass haylage. They found large differences in the effects on digestibility, with the most effective treatments increasing NDF digestibility from 31% to 40%. More work needs to be done, but there are clearly possibilities for the development of biological treatments that will result in substantial improvements in the digestibility of silages compared with unensiled forages.

Crops and by-products

Virtually all the research reported at early meetings involved the ensiling of temperate grass or legume species and, from the 3rd meeting, maize. Lucerne was often used to provide a severe test for the efficiency of different management and additive treatments. At the 2nd Meeting no papers focussed on different crops or by-products. Donaldson *et al.* (1978), reported on sunflower silage at the 5th Conference. Subsequently, with participants coming from progressively wider geographic and climatic areas, there was a large increase in contributions involving different forages and feed materials.

A whole session at the 13th Conference at Auchincruive was devoted to silage in tropical, sub-tropical and arid systems with nine papers, including notable contributions on silage in South East Asian systems (Chin, 2002) and in African livestock systems (Titterton *et al.*, 2002).

In each of the three most recent Conferences the focus of more than 30% of the papers has been on contrasts between the forages used or on the ensiling of by-products or tropical forages. In many cases this work has tested the applicability of techniques used

with temperate grasses, legumes and maize to different materials.

The diversity of the materials and applications is illustrated by studies on the ensiling of total mixed rations including many by-products by Nishino *et al.* (2005) and papers at the 16th Conference involving the co-ensiling of waste dates and banana tree by-products (Elahi *et al.*, 2012), and of sugar beet pulp and leguminous shrubs (Sun *et al.*, 2012).

Field management and silos

Several papers reported progress in approaches to increase drying rate after cutting. Particularly influential was the work of Klinner and Hale (1981) on the development of plastic elements for crop conditioning with resultant increases in drying rate.

Two papers presented at the 16th Conference in Hameenlinna opened up the possibility of providing real precision in silage harvesting. Both Suokannas *et al.* (2012) and Thaysen *et al.* (2012) described control systems using NIRS for on-line determination of yield and DM content and for the automatic control of additive application rates.

Knowledge of the physical properties of ensiled grass has much increased and enabled management procedures to be identified to reduce losses during ensiling, storage and feed out. Messer and Neale (1978) described techniques for measuring the porosity of ensiled grass. They concluded that very little, if any, air will flow through grass compacted to 700 and 500 kg/m^3 at DM contents of 16% and 40% respectively. Honig (1987) described the effects of forage type, chop length, DM content and consolidation on gas flow through silage. The data stressed the importance of good consolidation, particularly for crops with high DM content. McGechan (1990a) described a model of losses during ensiling arising from air infiltration.

Although efficient sealing of the silo was well established as a key to reducing losses during ensiling, it is surprising how few contributions to the early meetings were concerned with sealing and sealants. However, Degano (1999) reported beneficial effects on fermentation and surface wastage from covering with co-extruded film of very low permeability to oxygen, compared with using conventional polyethylene. This was followed up by Borreani and Tabacco (2005, 2012ab) and Orosz *et al.* (2012b) who found that a high oxygen barrier film reduced losses and improved the hygienic quality of baled silage and maize silage. Borreani *et al.* (2012) reported promising results with the use of a bio-based biodegradable film.

Animal and human health

There were very few papers on this topic in early Conferences. Fenlon *et al.* (1987) gave the first of a number of reports on the incidence of *Listeria monocytogenes* in big-bale silage. Auerbach and Oldenburg (1993) found that *Penicillium roqueforti* was the

predominant fungal species in moulded silages and 61% of visibly unmoulded silages were also contaminated with *P. roqueforti.*

The importance of silage in relation to animal health was highlighted by Wilkinson (1999), who indicated needs for further research. He discussed the possible importance of undesirable organisms in silage, particularly *Escherichia coli, L. monocytogenes, Clostridium botulinum and P. roqueforti*, and drew attention to effects from undesirable chemicals produced during ensiling, particularly mycotoxins. In the same session Lindgren (1999) discussed microbial hazards in silage in a consideration of the application of Hazard Analysis and Critical Control Points (HACCP) principles to silage. At the 2009 Conference the significance of mycotoxins was highlighted in a session that included a review of challenges in making tropical silage (Adesogan, 2009) and quantification of the role of maize silage as the principal source of mycotoxins in the animal's diet (Driehuis and te Giffel, 2009). In a comprehensive review, Driehuis (2012) discussed potential hazards to human health of contaminating human foods with endospores of heat-resistant bacterial species, Shiga toxin-producing *E. coli* and mycotoxins.

Little work has been reported on metabolic disease or on the reproductive health of the animal in relation to silage quality. Vincente *et al.* (2005) found a higher incidence of sub-clinical ketosis in dairy cows given poorly-preserved silages with elevated concentrations of butyric acid and Davies *et al.* (2012) reported a positive association between inoculant treatment of grass silage and calving to conception interval in dairy cows which they attributed to improved microbial capture of forage nitrogen.

Impact on the environment

In the early meetings the only environmental issue that was featured was silage effluent, which it was realised had an extremely high biochemical oxygen demand and could cause severe pollution to watercourses. The first of a number of papers on the use of absorbents to reduce effluent production was by Woolford and Wilkinson (1978). In later meetings the production of methane and volatile organic compounds emerged as significant issues.

The first paper on reducing ruminal methane emission with silage feeding was by Takahashi *et al.* (1999), who found that nitrate as a dietary supplement could lower methane emission from maize silage. At the following conference he reported that the addition of preparations of *Yucca schidigera* to an *in vitro* system produced a marked reduction in methanogenesis from lucerne and cocksfoot silages (Takahashi *et al.*, 2002). Dietary and animal approaches to reduce methane emissions by dairy cows were outlined by Yan (2009), but further progress is required.

Mitloehner *et al.* (2009) discussed the environmental importance of volatile organic

compounds (VOC), because of possible effects on ozone formation. They demonstrated that open-face silage piles are significant sources of emissions to the atmosphere of VOC on Californian dairies. Whilst the alcohols, that predominated, have only small impacts on ozone formation, alkenes, aldehydes and other compounds could have a greater impact. Montes *et al.* (2009) also concluded that silage was a significant source of VOC and Hafner *et al.* (2009) modelled VOC emissions from silage.

Concern over rising concentrations of greenhouse gases (GHG) in the atmosphere drew attention to the global environmental impact of livestock production (Steinfeld *et al*., 2006; Opio *et al.*, 2013), highlighting the significance of enteric emissions of methane from dairy cattle together with emissions of nitrous oxide (N_2O) from feed production and from N in fertilisers and manure deposited on grazed pastures. Legislation in the USA since 2003 to control the environmental impact of concentrated animal feeding operations (CAFO), where the main emphasis is on control of point-source pollution of watercourses, may therefore be extended to emissions of ammonia and greenhouse gases, and to other regions of the world. Of particular concern is the housing of livestock for extended periods of the year with silage as the predominant feed resource. Research to reduce GHG from silage is likely to assume greater priority as part of a coordinated international effort to reduce the environmental impact of milk and meat production from livestock.

Biogas

The first contributions on silage for biogas production were not made until the 15th Conference at Madison in 2009. Weissbach (2009) showed that biogas yield could be predicted from the content of fermentable organic matter as assessed by *in vivo* digestibility by sheep or predicted from chemical composition. Banemann *et al.* (2009) demonstrated effects of silage additives on biogas yield, with yields reduced in silages that had either undergone poor fermentation in the silo or been subject to aerobic deterioration.

A whole session was devoted to biogas at the 16th Conference in Hameenlinna. Nussbaum (2012) concluded that the highest biogas yields were obtained with the use of heterofermentative inoculants because of improved aerobic stability, whilst Demmig *et al.* (2012) reported that the addition of fibrolytic enzymes at ensiling increased methane yield.

Silage systems

As noted earlier one of the strengths of this series of meetings has been the involvement of contributors from different disciplines, but also a focus on integration and whole systems of silage production and utilisation.

At the 2nd meeting Lingvall (1972) reported systems comparisons between silage, barn-dried hay and artificially dried grass in terms of losses and animal performance by

growing heifers. Economic aspects were introduced by Witney and Beveridge (1974) who calculated the costs of contrasting harvesting systems (direct cut flail harvest; wilting with double chop and wilting with precision chop), including consideration of losses and utilisation by growing beef cattle. The optimal system changed with the number of hectares harvested per year. Information from various sources was used by Adamson and Brooke (1976) to identify optimal cutting dates for silage and McGechan (1990b) described a model of the economics of forge conservation systems in the context of dairy production, including the effects of weather on the growth of grass and losses during conservation and effects on feeding value. Bolsen *et al.* (2012) described a spreadsheet model that calculated the economics of sealing maize silage in bunker silos. They concluded that farmers should pay close attention to efficient sealing and that sealing with oxygen barrier film had a greater economic benefit than sealing with standard plastic.

It is surprising that only few papers in the early meetings were concerned with big bale silage systems as these were being rapidly adopted in Europe from the mid-1970s. However, Lingvall and Nyland (1978) detailed the effects of stretch-film thickness and width on round-bale silage and Morrison *et al.* (1981) compared big bale and precision-chop systems in terms of beef production.

Further research carried out at the systems level was reported by Gordon (1984) who found output of milk per hectare was highest with direct cutting with a fail harvester than with precision-chopped wilted grass. This arose from lower losses with the direct-cut system in the conditions of that experiment. However, other research (Mayne and Gordon, 1984; Zimmer and Wilkins, 1984) has shown that in many conditions system losses are lower with wilted than unwilted silages.

Keady *et al.* (2002) presented a detailed breakdown of the costs of alternative systems of making grass silage in comparison with grazing and other forages. They stressed the need to include all costs and concluded that in Northern Irish conditions the costs per tonne of utilised DM were only some 15% higher than for grazed grass and lower than for most alternative feeds.

Challenges for the future

The application of new technologies to silage making will continue to improve precision of management of silage systems. Further work on in-line sensing of crop composition and epiphytic microflora will assist in determining the need for and type of additive to improve efficiency of nutrient preservation. Multidisciplinary collaborations will continue to improve decision support models for resource use in crop production and silage making.

The use of molecular techniques will lead to the production of bacterial additives

with increased efficacy. The first paper describing the use of such techniques in the context of silage was presented by O'Connell *et al* (1990) and the state of the art was reviewed by Muck (2012), who concluded that more molecular studies are needed on the microbial ecology of contrasting fermentation patterns, so that those species causing problems can be identified and new strategies developed to control their development in silage.

The inclusion of ferulate esterase enzymes in silage additives is a promising development, but more effective enhancement of cell wall digestibility in mature forages with higher lignin concentrations will bring greater nutritional benefits, especially in tropical forage conservation.

The development of an effective material for covering silos that is edible by livestock remains to be achieved. Berger and Bolsen (2006) outlined the criteria for an edible sealant and described experiments with a gelatinised starch/salt matrix. However, the material was expensive to produce and required a protective waxy film to prevent water ingress through cracks in the matrix.

Although progress has been made in establishing that there is polyphenol oxidase activity in cocksfoot and ryegrass and that enzyme activity survives a 12-hour period field-wilting (Hatfield and Marita, 2009), further work is needed to reduce proteolysis in ensiled grasses and lucerne to levels comparable to those in ensiled red clover or birdsfoot trefoil.

Driehuis (2012) distinguished between mycotoxins produced in the field and those produced in the silo. He highlighted the lack of information on the effects of mycotoxins produced during ensilage by *Penicillium* species and *Aspergillus fumigatus*. There is need to quantify the relative importance of crop and field management factors and their influence on undesirable microbial species and their metabolites at harvest, and also those produced in the silo during storage which may be controllable by use of additives. Effects of the hygienic quality of the crop at harvest, silage fermentation quality and aerobic deterioration on mycotoxicosis and other animal diseases, including zoonotic pathogens, remain to be quantified.

With livestock units increasing in size, greater pressure is placed on aged silos that are too small to accommodate the requirement for silage by larger numbers of livestock on the farm. Safety issues were raised by Bolsen and Bolsen (2012) who highlighted the risks associated with complacency while working around silage equipment and silos, avalanches of silage from exposed silo feed faces and injuries from falls from high silos. In the future, greater emphasis on silage safety, coupled with research to understand and increased awareness of safety hazards, will be required for all involved in silage research, development and production.

Conclusions

The most significant contributions to, and benefits from, the silage conferences have been communication of new findings, enhancement of knowledge and collaboration between research groups. The conferences have grown from an informal exchange of recent research between a few workers into the most important gathering concentrating on silage - one of the most important global agricultural crops.

Silage is growing in importance worldwide as livestock units increase in size and as economic development allows the universal application of modern technology to crop conservation. Of fundamental importance is an understanding of the consequences to the animal and its products of getting the silage preservation process right. Of paramount importance is an understanding of the consequences to the animal and human population of getting the process wrong.

The obligation is upon the research community as much as upon farmers, processors and retailers to ensure the highest possible standards of food safety through attention to the biochemical, microbiological, physical and nutritional factors that interact to make silage a high-quality feed for livestock. Success requires sustained investment in silage research as much now as it did 45 years ago in 1970.

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New trends in silage microbiology

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Abstract
In the past decade, PCR-based methods have become more common for profiling microbial communities found in silage and silage-fed animals. Without proper dissemination of studies on clonal library, the use of several fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism has been widespread. These methods have been used to obtain improved snapshots of the silage microbial community, which is more complex than previously thought. In addition, these methods have been employed to evaluate the efficacy of lactic acid bacteria (LAB) inoculants, to gain insight into the heterogeneous distribution of microorganisms in a large-scale silo, and to understand the association of non-conventional and difficult-to-culture microbial species in silage. Moreover, development of quantitative PCR (QPCR) gives us the benefit of a quantifiable assessment, which is a feature lacking with qualitative DGGE. Populations of bacteria and fungi can now be determined by QPCR using universal primers targeting 16S and 26S rRNA genes, respectively. Further advancements will be achieved by incorporating next generation sequencing (NGS) technology. The overwhelming resolution power of NGS will reveal microorganisms that are indeed involved but have never been reported to inhabit silage. If whole genome analysis is performed, vast amounts of unexpected information on metabolism will be introduced. This new information may help select novel LAB inoculants with beneficial activity. Meanwhile, steady efforts to isolate target microorganisms are very important to improve our practice at any stage from farm to table. Using molecular biology-based tools, further advancements can be expected to secure anaerobic fermentation, aerobic stability, and animal health of silage and silage-fed animals.

Introduction

A century has passed since the involvement of bacteria in silage fermentation was first recognized (McDonald et al., 1991). Using large numbers of plate-culture experiments, the importance of the growth of homofermentative lactic acid bacteria (LAB) in the early stages of ensiling has been recognized. Accordingly, the methods, agents, and agricultural technologies aimed at controlling microbial activity have entered common practice. Concentrations of microbial metabolites such as organic acids, alcohols, and

ammonia have been used as criteria to evaluate the quality of preservation. Since the beginning of the 21st century, culture-independent microbial profiling methods have been applied to silage microbiology research. Given that new methods have been developed, why do we need to continue microbiology research after 100 years of history?

One reason is that there are a large number of items to ensile with variable physical and chemical features. Drastic changes often accompany the development of agricultural machinery. Big bale preparation has markedly increased the silage DM content. Heavy wilting, which leads to a loss of 50% moisture content, is now regarded as standard so that acidification by organic acid production cannot be a decisive factor that controls the growth of the microorganisms (Nishino, 2011). In an environment with low water activity, bacterial rather than fungal growth will be suppressed. Thus, concerns should be directed to how fungal activity is controlled under dehydrated conditions. Likewise, large-scale ensiling with sugar-rich material has increased the necessity of microbial control after exposure to air. Lactic acid does not exhibit strong antifungal activity at any pH condition, and many fungal species, such as *Pichia* and *Candida* spp., are capable of assimilating lactic acid under aerobic conditions. The ability to tolerate acidic conditions is also generally greater for fungi than bacteria, indicating that both bacterial and fungal controls are essential to secure large-scale ensiling in modern farming.

Increasing importance of ensiling in tropical and sub-tropical regions has also elevated the necessity of microbiology research (Nussio, 2005). To meet the elevating demand for meat, milk, and other animal products, the production of tropical crop silage is expected to increase further. There is great potential in the fact that the yield of tropical forages is three to four times greater than temperate ones. However, knowledge and information derived from moderate and cool climates does not necessarily work at high ambient temperature with its adapted microorganisms. The fact that acetic acid fermentation often takes place in tropical grass silage has been known since the 1970s (Catchpoole and Henzell, 1971), but measures to control tropical crop ensiling have not been established. In addition, intensive ethanol production may proceed if high sugar crops like sugarcane are ensiled in the tropics (Daniel and Nussio, 2011). Although ethanol fermentation does not indicate energy loss during ensiling, alcohols do not contribute to the acidification process and will evaporate promptly after silo opening. Silage with lactic acid predominance and acceptable aerobic stability is difficult to produce even in temperate regions, thus providing further challenges for secure silage production in the tropics.

Great advancement is expected in the area of probiotics in regards to growth promotion and animal health. Consumers have been attracted to the beneficial claims of LAB and LAB-associated fermented foods. The underlying mechanisms of modulating metabolism and immunity via gut microbiota have been determined, especially in the areas

of human nutrition and physiology. The same concept can be applied to food-producing animals with silage serving as a vehicle to propagate and deliver probiotic LAB species to livestock (Weinberg and Muck, 1996). However, as materials for ensiling are unable to be sterilized before storage, it is difficult to ensure predominance of selected LAB during the entire fermentation. Furthermore, the LAB species commonly found in silage are different from those detected in the ruminant gastrointestinal tract. Yet, as there is opportunity to establish the use of LAB in the production and feeding of probiotic silage, further research on probiosis will continue.

At the previous conference, Muck (2013) emphasized that molecular biology-based profiling methods have opened a new era for silage microbiology. Since then, PCR-based methods have become more widely utilized. In the past, much of the work had been performed using denaturing gradient gel electrophoresis (DGGE), whereas recent work has more often used quantitative PCR (QPCR). Until now, only a few studies using next generation sequencing (NGS) technology have been published, but the numbers will quickly increase in the upcoming two to three years. Application of the high-throughput microbial analyses will boost the development of new practices for better performance, nutrition and health, as well as the hygiene and safety of food-producing animals. In this paper, recent findings in which culture-independent analyses have improved and revised our current body of knowledge will be described.

Culture-independent microbial profiling

In the field of silage microbiology, culture-independent methods, which employed DGGE to examine fungal communities associated with whole crop corn ensiling (May et al., 2001), first appeared in the beginning of the 21st century. Researchers have since been encouraged to use PCR-based techniques in studying the complex silage microbial ecosystems, because the limitation of microbial cultivation, i.e. only a small fraction of all bacteria can be isolated and characterized, was widely recognized. In addition to DGGE, both TRFLP and automated ribosomal intergenic spacer analysis (ARISA) have been used as representative molecular fingerprinting techniques (Muck, 2013). However, fluorescence in situ hybridization (FISH), a culture-independent technique, which enables visualization of microbial cells, has been rarely used in the study of silage. Furthermore, QPCR or real-time PCR has become a popular technique for profiling microbial communities in the past decade. QPCR is often used to help evaluate the effect of LAB inoculants, and its use is expanding to include total and genus-level populations. In the last couple of years, NGS has been introduced to understand silage microbial communities. NGS can offer information related to the occurrence and abundance of microbial genes in a given ecosystem. Therefore, the application of NGS technology will provide vast amounts of information about potential metabolism.

QPCR

Principles of QPCR are the same as conventional PCR-based DNA replication, except the amplification is monitored in real-time using a fluorescent reporter molecule, as opposed to the endpoint observation in conventional PCR (Bokulich and Mills, 2012). A standard curve with known amounts of DNA, or copy numbers of a target gene, is required for the enumeration of a specific microorganism or microbial group in samples. To quantify total bacterial and fungal populations, non-specific primers, which bind to conserved fragments of 16S and 26S rRNA genes, respectively, have typically been employed. To enumerate defined species and groups, specific primers that bind to DNA fragments containing enough variation to distinguish different species and enough similarity to identify microorganisms belonging to particular phylogenetic groups are necessary. Primers specific for individual LAB species have been successfully designed based on 16S rRNA genes. However, several LAB species commonly found in plant material have nearly complete homology in the 16S rRNA genes; hence, further divergent functional genes like the *recA* gene may be selected to secure species-specific QPCR quantification (Stevenson et al. 2006). Detection of functional genes can also present a novel use of QPCR such as discriminating biogenic amine-producing strains of LAB (Nannelli et al., 2008).

DGGE

The first report, which employed DGGE in silage microbiology research, was published by May et al. (2001), who investigated fungal communities of whole crop corn silage with and without LAB inoculation. In DGGE analysis, short fragments of DNA are separated along a chemical gradient as DNA is propelled through a polyacrylamide gel matrix by an electric current until it reaches the point in the chemical gradient at which it becomes destabilized, partially denatured, and immobilized in the gel (Cocolin et al., 2013). Phylogenetic information can be obtained by sequencing the DNA bands excised from the gel. Because of the likelihood of obtaining multiple DNA sequences from a single band due to co-migration, the excised band is often cloned followed by sequencing of each individual clone. Target genes are generally variable regions of 16S and 26S rRNA genes for bacteria and fungi, respectively.

A number of disadvantages have been pointed out about DGGE for community analysis. The DGGE gel is difficult to reproduce, so comparison of the data obtained from separate gels is not sufficiently reliable (Bokulich and Mills, 2012). In addition to band co-migration, rRNA gene multi-copy heterogeneity, as well as chimera and heteroduplex formation may distort interpretation of community analysis. A great shortcoming is that DGGE can provide only a qualitative assessment; however, DGGE is still a useful tool to profile a snapshot of the microbial community of silage.

TRFLP and ARISA

McEniry et al. (2008) used TRFLP to study the microbial ecology of wilted grass silage. This method differentiates microbial populations based on terminal restriction fragment length. Mixed DNA samples are amplified with fluorescence-labeled universal primers, digested with select restriction enzymes, and separated by capillary electrophoresis with molecular standards for estimating fragment size. Only the 5' terminal fragments are detected and compared to a database to determine which microbial populations are represented. TRFLP has several advantages that make it well-suited for microbial community profiling as compared to DGGE, e.g., TRFLP is an automatable process that allows for a large number of samples to be compared.

The sensitivity of ARISA is greater than DGGE or TRFLP (Okubo and Sugiyama, 2009). ARISA involves PCR amplification of the highly variable intergenic regions between 16S and 23S rRNA genes, which display significant heterogeneity in both length and nucleotide sequence. A community-specific complex banding pattern is generated, and each DNA band corresponds to at least one organism. One drawback regarding ARISA is that, although the method has tremendous resolution capacity that can be applied to microbial diversity analysis, information about operational taxonomy units cannot be obtained (Brusetti et al., 2008). Hence, species identification needs to be performed without the advantage of profiling results provided by ARISA.

NGS

Next generation sequencing collectively describes several technologies that achieve massively parallel sequencing of heterogeneous DNA fragments. For the purpose of microbial community analysis, the fragments consist of short segments amplified using universal primers targeting prokaryotic 16S rRNA and fungal ITS genes (Bokulich and Mills, 2012). Two NGS systems, 454 Life Sciences pyrosequencing and Illumina sequencing platforms, are available for use in microbial community profiling. Pyrosequencing can sequence longer reads (up to 700 bp), and thus it theoretically provides greater taxonomic information than Illumina systems (up to 300 bp). However, Illumina systems provide greater sequence coverage per run than pyrosequencing, and this application is growing in the study of microbial ecology. Discontinuation of the pyrosequencing platform was announced in 2013 so the production and support will eventually be terminated.

As for silage microbial community analysis, two published articles used pyrosequencing technology to examine the complete genome sequence of *L. buchneri* (Heinl et al. 2012) and the bacterial community of *L. buchneri*-inoculated grass silage

(Eikmeyer et al. 2013). In addition to genes involved in principal metabolism such as heterofermentative fermentation and lactic acid degradation, large numbers of functional genes were disclosed and their putative functions were determined (Heinl et al. 2012). Phylogenetic classification was also revised by incorporating pyrosequencing results. Community analysis quantitatively revealed that inoculated *L. buchneri* outcompeted other LAB community members (*Lactococcus* spp., *Leconostoc* spp., and *Weissella* spp.), while not eliminating *L. planatrum*, *L. brevis*, and *L. lactis* as dominant species (Eikmeyer et al. 2013). How NGS technology may provide better insight into both taxonomy and activities in silage microbial community studies is exciting to predict.

Monitoring and controlling fermentation

Research has been performed mainly on bacteria involved in naturally fermented and LAB-inoculated silages. Results from untreated silage have demonstrated that bacteria present in pre-ensiled crops are often different from those seen in silage (Li and Nishino, 2011a, b). Hence, fermentation patterns are not well predicted by bacterial profiling of ensiled crops. Use of silage additives, such as LAB inoculants, is recommended to obtain secure fermentation with minimum DM loss and protein degradation. A finding that is difficult to confirm without culture-independent analysis is that many non-LAB species such as *Enterobacter* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Rahnella* spp., *Bacillus* spp. are often detectable in silages, regardless of fermentation patterns (Li and Nishino, 2011c; Eikmeyer et al. 2013; Wang et al., 2014). Likewise, various heterofermentative LAB species can be present even in silages with high lactic acid content, which suggests an exclusively homofermentative LAB community (Eikmeyer et al. 2013). Detection of those bacteria belonging to *Enterobacteriaceae* is not related to the ethanol and 2, 3-butanediol contents. Determination of acids and alcohols provides collective information of microbial activity over the entire fermentation process, whereas alcohol production may take place primarily in a narrow window of time early in the ensiling process. Even though methods for microbial profiling have been improved, sampling times and procedures need to be considered crucial elements.

Use of QPCR has increased the ability to evaluate the LAB inoculation in the ensiling process. Given that species-specific primers are available, QPCR enables quantitative assessment and comparison between inoculants. Representative silage LAB species such as *L. plantarum, L. brevis, L. buchneri, L. lactis, E. faecium, P. pentosaceus,* and *L. casei* can now be enumerated by QPCR (Stevenson et al. 2006). Schmidt et al. (2009) described that slow growth of *L. buchneri*, demonstrated using QPCR, can account for slow response in acetic acid and 1,2-propanediol production for *L. buchneri*-inoculated alfalfa silage. Lynch et al. (2012) used QPCR to determine *L. plantarum* and *L.*

buchneri populations and then discussed why LAB inoculants did not greatly enhance the fermentation or aerobic stability of inoculated silages.

Both DGGE and QPCR have demonstrated that inoculated LAB species may not become predominant in the bacterial community. Parvin et al. (2010) used DGGE to examine the effects of *L. plantarum* and *L. brevis* inoculation. They demonstrated that, although the LAB inoculation can eliminate the indigenous community and alter fermentation of wilted grass silage, the inoculation effect is hard to see in whole crop corn silage because of the robust innate bacterial community. Likewise, Stevenson et al. (2006) examined the effects of five LAB inoculants on alfalfa ensiling. They performed quantitative assessment using QPCR and indicated that no inoculated LAB species exceeded 10% of the total population after 90 days of ensiling. Previously, it was generally thought that inoculated LAB species could predominate over the microbial community. Microbial profiling can refine our understanding and improve the screening process for novel LAB inoculants.

Culture-independent methods have made practical surveys such as monitoring microbial distribution in a large-scale practical silo less complicated. Although plate-culture requires immediate processing after sampling, freezing is acceptable with PCR-based methods and thus a greater number of samples can be examined (Wang and Nishino, 2010; Li and Nishino, 2011c). Wang et al. (2013) and Wu et al. (2014) detected *L. acetotolerans* and *L. pontis* in total mixed ration silage and whole crop corn silage produced in a bunker silo, respectively. These LAB species are regarded as difficult-to-culture, and their presence may not have been disclosed without the application of PCR-based community analysis.

Aerobic stability improvement

The issue of preventing aerobic spoilage has not been fully solved and is thus regarded as a major challenge in silage microbiology research (Wilkinson and Davies, 2012). Yeasts, *Bacillus* spp., *Enterobacter* spp., and *Acetobacter* spp. are regarded as spoilage initiating agents, and no management is considered feasible without inhibitory activity against yeasts. A number of LAB species have been shown to exhibit antifungal activity (Dalié et al., 2010). However, in practice *L. buchneri* has long been commercialized as an exclusive LAB inoculant for this purpose. As *L. buchneri* is a heterofermentative LAB species, there is significant on-going screening to find homofermentative LAB species that can replace *L. buchneri*.

Several naturally fermented silages with innate high aerobic stability, e.g., total mixed ration silage and legume crop silage, could serve as a source from which candidates of novel LAB inoculants could be obtained (Wang and Nishino, 2009). It has been shown

that legume crop silage may remain unheated for more than a week after silo opening. Sugar addition at ensiling has no influence on spoilage after silo opening (O'Kiely and Muck, 1992). Wu et al. (unpublished observation) examined bacterial communities of alfalfa silage with and without molasses addition. They observed that molasses addition enhanced both lactic acid production and aerobic stability of wilted alfalfa silage, for which DGGE analysis suggested that *L. fructivorans* is involved in spoilage inhibition. Because *L. fructivorans* is difficult to grow in MRS medium, the LAB species has never been isolated from any types of silage. Accordingly, Wu et al. changed the medium from MRS to LIS (liver infusion sake), and then successfully isolated *L. fructivorans* as a predominant LAB in molasses-added alfalfa silage. This is an example of how culture-independent analysis can help detect difficult-to-culture LAB species in silage.

Application of culture-independent analysis also has modified our understanding of *A. pasteurianus*. *Acetobacter* spp. are entirely aerobic and known to initiate spoilage when the silo is exposed to air. However, if examined by DGGE, *A. pasteurianus* can frequently be found in whole crop corn silage produced in a bunker, and its presence is not related to heating and significant spoilage (Wang et al., 2014). Furthermore, Minh et al. (2014) found *A. pasteurianus* in elephant grass silage even at the time of silo opening, despite the fact that *A. pasteurianus* has been shown to specifically inhabit whole crop corn and cereal silages (Oude Elferink et al., 2001). It is not certain if the *A. pasteurianus* inhabitation may account for intensive acetic acid production in tropical grass silage. However, these findings would not have been achieved without examining silage microbial communities by culture-independent procedures.

Probiosis, animal health, and food safety

Nearly 20 years have passed since Weinberg and Muck (1996) referred to the potential of silage LAB as probiotics for cattle. LAB inoculation has been shown to occasionally improve animal performance without having any advantageous effect on fermentation. About 30% of the studies in their review demonstrated improvement of feed intake, live-weight gain, energy feed efficiency, and milk production.

One extensively studied silage LAB strain is *L. plantarum* MTD-1, which improved animal performance in those fed silage inoculated with this strain, regardless of fermentation patterns. The strain is shown to survive and stabilize pH conditions in the rumen (Weinberg et al., 2004) and inhibit growth of detrimental microorganisms such as Gram-positive *Micrococcus luteus* and Gram-negative *Pseudomonas aeruginosa* (Gollop et al. 2005). However, even if the *L. plantarum* population, determined by QPCR, was greater in cows fed alfalfa silage inoculated with *L. plantarum* MTD-1 than in the untreated control, no distinctive improvements were seen in feed intake and milk

production (Mohammed et al. 2012). In rumen bacterial communities determined by ARISA, differences due to liquid and solid phases as well as cow-to-cow variations were far greater as compared to the effect of *L. plantarum* inoculation (Mohammed et al. 2012). This suggests difficulty to validate probiotic effects for farm animals, which are kept in a harsh hygienic environment as compared to laboratory animals and humans.

There is debate on how to select LAB species for the purpose of probiosis. The species commonly used in silage inoculants are *L. plantarum*, *L. acidophilus*, *L. casei*, *L. buchneri*, and *E. faecium*. However, the LAB species detected in the ruminant gastrointestinal tract differ from those in silage inoculants; *Streptococcus bovis*, *Lactobacillus vitulinus*, *Lactobacillus ruminis*, *Lactobacillus johnsonii*, and *Lactobacillus murinus* are typically found in cattle (Krause et al., 2003, Hernandez et al., 2008, Nader-Maias et al., 2008). Han et al. (2012) fed non-inoculated, wilted Italian ryegrass silage to goats with and without concentrates, and determined the survival of silage LAB in rumen fluid and feces by DGGE analysis. Although *Enterococcus* sp., *L. plantarum*, *L. brevis*, *L. murinus*, and *Weissella cibaria* were found in silage, only *L. murinus* was detected in rumen fluid and feces regardless of feeding concentrates. Furthermore, Han et al. (2014) evaluated the fate of silage LAB by examining the LAB community in silage, rumen fluid, and fecal material of dairy cows. A total of 14 LAB species were detected in silage samples, of which 5 (*L. acetotolerans*, *L. pontis*, *L. casei*, *L. suebicus*, and *L. plantarum*) were detected in the dairy cow feces. The majority of the DGGE bands for the fecal samples were also detected in the rumen fluid, suggesting that any elimination of silage LAB occurred in the rumen and not in the post-ruminal gut segments. Furthermore, differences are observed between the bacterial communities in the rumen fluid, the solid phase of the rumen, and the rumen wall, while few LAB were found in the three fractions (Cho et al., 2006). Mucosa-associated bacterial communities in the rumen, duodenum, and colon were also found to differ (Collado and Sanz, 2007). Further studies are required to determine how to select potential silage LAB, which may have promise to confer probiotic function. The probiotic ability of silage LAB may also vary according to the strain, the crop, and the ensiling conditions, such as extent of wilting and the degree of anaerobiosis.

For screening and understanding probiotic function of the potential LAB strains, Takahashi et al. (2013) employed a sophisticated in vitro model using a bovine intestinal cell line. The model evaluates interactions between pathogens and epithelial cells, and thus helps clarify the mechanisms involved in the protective activity of probiotic LAB strains against intestinal inflammatory damage. Although immune stimulation is one putative probiotic function in addition to the modulation of fermentation, antimicrobial action, competitive exclusion, and others (McAllister et al. 2011), an improved screening procedure may inspire further probiotic silage LAB research.

Monitoring deleterious and difficult-to-culture microorganisms in the course of ensiling has become less of a burden by utilizing culture-independent analyses. Amado et al. (2012) used plate-culture and DGGE to investigate the fate of *Listeria monocytogenes* in silages. Even if no colonies were grown in the plates, the *iap* gene, which encodes an invasive adhesion protein related to the virulence of *L. monocytogenes,* was detected in the early ensiling period. This suggested that *L. monocytogenes* was present in a viable but non-culturable state. Cook et al. (2013) used QPCR to examine the persistence of *Mycobacterium avium* subsp. *paratuberculosis*, *Escherichia coli*, and *Salmonella typhimurium* in grass/alfalfa silage. *M. paratuberculosis* is extremely slow-growing and may appear in the plates of selective medium after two months. QPCR detection revealed greater survival of *M. paratuberculosis* compared to *E. coli* and *S. typhimurium*. Moreover, DNA from both live and dead *M. paratuberculosis* could be detected by QPCR throughout the 150 days of ensiling. This indicates that the cell wall of *M. paratuberculosis* can resist the ensiling environment and thus support persistence regardless of either the inactive or dormant phase. Elimination of foodborne pathogens is increasingly important because silage is a major source of feed for ruminant livestock. Consequently, solving the challenges of food safety issues related to ensiling will be more attainable by employing culture-independent procedures.

Conclusion

Generally, culture-independent microbial profiling simply confirms previous knowledge obtained through traditional plate-culture experiments. This is because epiphytic LAB are small in number and their growth is not promising, while key practices like high density compaction (lowering redox potential), wilting (reducing water activity), inoculation (fortifying competitive microorganism), and sugar substrates (increasing preservative acids) remain unchanged. One benefit from culture-independent analyses is that there are opportunities to discover non-conventional and difficult-to-culture microorganisms involved in ensiling. This could bring about breakthroughs to overcome unsolved problems and to achieve technical innovation. Further improvements to the field can be expected with the application of NGS technology, encouraging young scientists to participate in the upcoming milestones of silage microbiology research.

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How simulation of fall and winter temperature conditions at ensiling and long-term storage of corn silage influence microbial populations and fermentation during spring thaw

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Keywords lactic acid bacteria, low temperature, corn, microbial diversity, PCR-DGGE

Introduction Many factors affect dynamics of the microbial populations during ensiling and storage of silage. In several countries, fresh temperature at ensiling and cold or even freezing temperatures experienced during storage may affect resident microbial population activities and the fermentation profile. While this situation is common in Nordic countries, very few trials have been conducted to study microbial dynamics in order to define potential strategies to optimise the fermentation process. The objective of this trial was to compare the impact of different temperatures (10°C *vs* 20°C) at ensiling on microbial populations and fermentation profile as well as investigating the effect of low temperature during the storage period.

Materials and methods Forty experimental silos were prepared from whole-plant corn. They were initially incubated at 10°C or 20°C during 60 days (step 1 - 20 silos at each temperature). The silos were then stored at 5 °C for another two months period (step 2). Afterwards, storage temperature was progressively increased from 5°C to 25°C following a weekly increment of 2.5°C (step 3). Targeted sampling temperatures were 5, 10, 15, 20 and 25°C, with 4 repetitions per treatment. Bacterial population was monitored by a culture-independent approach using PCR-DGGE with universal primer set 357F-GC/517R to amplify the V3 region of the 16S rDNA. Fermentation profiles were determined (AGV profiles, NH_3, and pH) and plate counts of microbial population were performed (LAB, enterobacteria, and clostridia).

Results and discussion After 60 days of fermentation, pH and residual water soluble carbohydrates (WSC) differed significantly ($P < 0.01$) between silos incubated at 10°C *vs* 20°C (average pH were 4.04 and 3.74, respectively), (average residual WSC of 95.84 and 35.94 g kg^{-1} DM, respectively). During step 3 of the incubation period, the temperature was slowly increased to simulate spring warmup (Figure 1a). No change in pH and residual WSC was observed for silos initially ensiled at 20°C. However, for silos initially ensiled at 10°C, the pH stayed around 4.00 until the temperature reach 20°C, and dropped to 3.80 during the transition from 20 to 25°C. A similar pattern was observed for residual WSC concentration (Figure 1b).
PCR-DGGE analysis and subsequent amplicon sequencing revealed that LAB species dominated the total bacterial population (51 %), with heterofermentative species such as *Lactobacillus buchneri*, *Lactobacillus brevis*, *Weissella koreensis* and *Leuconostoc citreum*

well represented. Enterobacteria such as *Pantoea agglomerans* and *Enterobacter cowanii* were also detected (Figure 2a). Greater diversity, computed by the Shannon diversity index (H'), was observed in corn silages initially fermented at 10°C compared to 20°C (Figure 2b). H' of silages initially fermented at 20°C was not affected by the rise in temperature during storage ($P > 0.05$). However, in silages which were fermented at 10°C, a significant decrease in H' was observed as storage temperature increased to 25°C ($P < 0.05$). At 25 °C, the H' value of both initial fermentation treatments reached the same value.

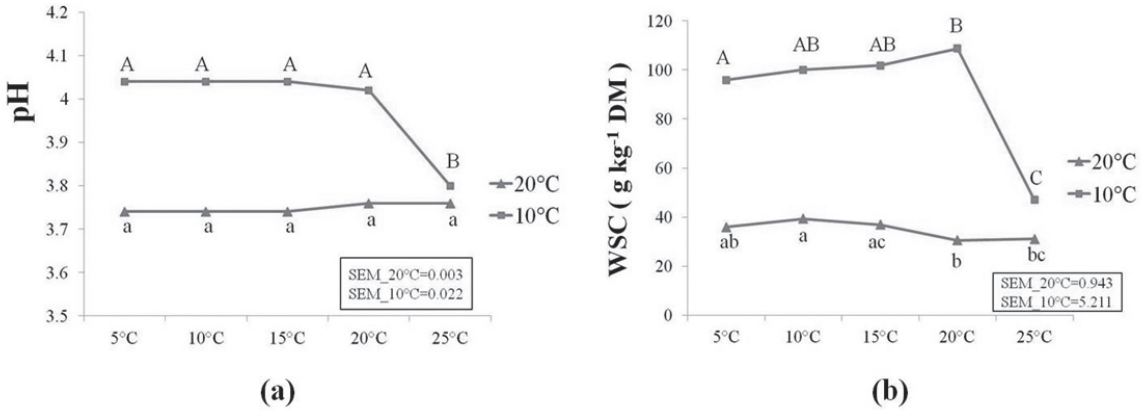

Figure 1 pH (a) and water soluble carbohydrates concentration (b) during increase of storage temperature of corn silage initially incubated at 10 or 20°C for 60 days and subsequently stored at 5°C for another period of 60 days.

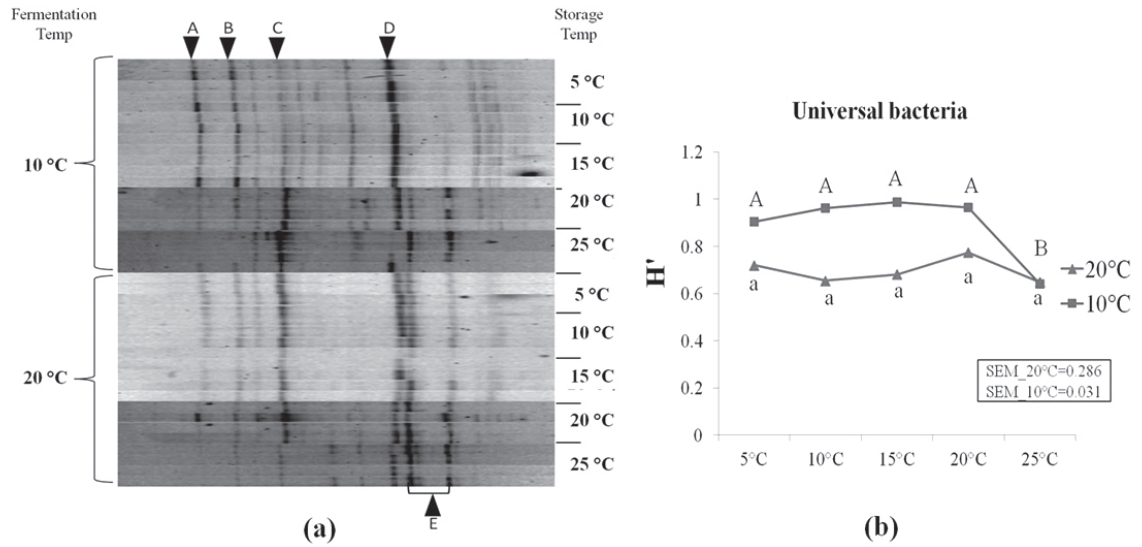

Figure 2 PCR-DGGE profiles (a) using an universal bacteria primer set (357F-GC/517R) of corn silages initially incubated at 10 or 20°C for 60 days and subsequently stored at 5°C for another period of 60 days. Bacterial diversity (b) determined by the Shanon index (H') for the different treatments.

Conclusion Cool temperature at ensiling restricts the fermentation process. However, fermentation will continue and come back to normal parameters following an increase in temperature before opening silo. This is important, as residual WSC are an indicator of aerobic instability.

Metabolomic profiles of alfalfa silage inoculated with or without *Lactobacillus plantarum* and *L. buchneri*. I. Fermentation quality and differentially expressed metabolites

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Keywords silage metabonomics, *Lactobacillus plantarum*, *Lactobacillus buchneri*

Introduction Metabolites in silage, such as organic acids, ethanol and 1, 2-propanediol, are detected conventionally to evaluate the fermentation quality. During fermentation, however, lactic acid bacteria also can produce many other metabolites, *e.g.*, amino acid, fatty acid, oligosaccharide, vitamin, small peptide, flavoring agent and aromatic compound, etc. Little information is available regarding whole picture of metabolites in ensiled forage. Thus, the present study was performed to investigate the metabolomic profiles of alfalfa silage inoculated without or with *Lactobacillus plantarum* or *L. buchneri*.

Materials and methods Alfalfa was harvested and chopped at a DM content of about 400 g/kg. Four silos (vacuum-sealing polyethylene plastic bags packed with 800 g of fresh forage) were individually prepared for each of the following treatments: (a) untreated (control), (b) *Lactobacillus plantarum*, and (c) *L. buchneri*. The application rate of each inoculant to the fresh forage was 1×10^6 cfu/g. The silos were then stored at ambient temperature (18-22 C°) for 90 d. After storage, silage simples were used for conventional chemical and microbial population analysis. Ensiled alfalfa leaves were separated, and 5 g leaves were suspended gently in 50 mL methanol in a shaker at 100 rpm for 2min. Suspensions were subjected to derivatization process and GC-MS (Agilent 7890A/5975C, USA) detection according to Wu et al. (2013). Raw spectra from GC-MS were then processed and subjected to statistical analysis (Wu et al., 2013).

Results and discussion All silages were well preserved with no detectable propionic and butyric acids (Table 1). Inoculation of *L. plantarum* decreased silage pH and increased lactate concentration in ensiled alfalfa. As expected, *L. buchneri* increased acetate concentration in alfalfa silage. As compared to the control, inoculants markedly increased 2,3-butandiol, 4-amino-

Table 1 Chemical composition (g/kg DM), pH, fermentation end products (g/kg DM), and the microbial population (log CFU/g) of alfalfa silages after 90 days of ensiling

Item	DM	NDF	ADF	WSC	pH	Lactate	Acetate	LAB	Molds	Yeasts
Control	403^a	449	280	5.5^b	5.2^b	43.5^a	11.6^a	7.7	2.8	2.3
L. plantarum	400^a	427	266	3.9^a	4.9^a	68.4^c	13.1^a	7.4	2.8	2.0
L. buchneri	414^b	431	282	4.4^a	5.2^b	52.6^b	27.1^b	7.9	<2.0	<2.0
SEM	2.3	12.0	5.4	0.26	0.06	5.08	1.31	0.07	-	-

$^{a, b, c}$ Means in rows with the same superscript do not differ (P<0.05).

butyric acid, adenine, threonine and tyrosine in ensiled alfalfa (Table 2), and L. buchneri had more effect on increasing these substances; L. buchneri increased all the amino acids showed in Table 2 except for glutamic acid, especially for valine and aspartic acid. Inoculation of L. buchneri also markedly increased arabitol, 4-aminobutyric acid and glycerol in ensiled alfalfa. Higher concentrations of polyols such as arabitol, erythritol, glycerol and mannitol were detected in L. buchneri treated silage as compared to L. plantarum inoculation.

Table 2 Relative concentration and fold changes of major metabolites in alfalfa silages with or without inoculation of *L. plantarum* or *L. buchneri* after 90 days of ensiling

Metabolite name	Relative concentration			Fold changes		
	Control	*L. plantarum*	*L. buchneri*	$\log_2^{(P/C)}$	$\log_2^{(B/C)}$	$\log_2^{(P/B)}$
2,3-Butandiol	86.6	171	340	0.981*	1.975*	0.993*
2,3-Butanediol	84.1	28.1	26.9	-1.579*	-1.643*	0.063
2-Aminobutyric acid	61.5	12.5	25.14	-2.294*	-1.291*	1.003*
4-Aminobutyric acid	112	169	272	0.589	1.276*	-0.686
Adenine	2.57	26.2	21.0	3.344*	3.029*	0.315
à-Hydroxyisobutyric acid	3.51	0.77	1.03	-2.188*	-1.762*	-0.423
Aminomalonic acid	5.39	8.81	10.3	0.709	0.938*	-0.228
Arabitol	4.36	6.21	21.4	0.511	2.298*	1.786*
Cadaverine	192	22.1	101	-3.11	-0.924	2.190*
Erythritol	10.4	10.5	39.1	0.006	1.902	1.879*
Glycerol	190	184	301	-0.047	0.663*	0.711*
Inositol	31.7	42.0	40.9	0.406*	0.366	0.038
Ketomalonic acid	0.46	4.05	1.77	3.112*	1.925*	-1.188*
Malonic acid	2.15	10.9	7.68	2.337*	1.832*	-0.505*
Mannitol	0.43	0.47	3.94	0.103	3.184*	3.081*
Phenethylamine	3.62	0.27	1.16	-3.724*	0.762*	2.079*
Threitol	1.51	1.99	3.24	0.400*	1.101*	0.701*
Succinic acid	158	82.9	112	-0.932*	-0.502	0.429*
trans-Ferulic acid	3.91	1.69	5.34	-1.210	0.448	1.659*
Threonine	41.3	64.6	73.9	0.645*	0.841*	-0.195
Tyrosine	3.23	16.7	98.4	4.774*	4.930*	-0.155
Valine	126	172	219	0.089	0.439*	-0.349
Ornithine	28.8	44.4	65.8	0.623	1.192*	-0.568
Lysine	12.1	38.2	50.2	1.654	2.047*	-0.393
beta-Alanine	1.65	2.12	4.04	0.359	1.292*	-0.932
Aspartic acid	140	289	310	1.051	1.153*	-0.101
Glutamic acid	76.9	39.4	28.6	-0.963	-1.426*	0.463

The fold changes were calculated using the formula $\log_2^{(X/Y)}$, where X and Y refer different treatments. * indicate significant ($P<0.05$).

Effects of *Pichia norvegensis* and air exposure on the nutritive value of corn silages for dairy cows

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Keywords corn silage, aerobic exposure, yeast, milk composition, milk fat

Introduction Corn silages are prone to deterioration when exposed to air. The lactate-assimilating yeast species are frequently the first microorganisms to initiate the aerobic deterioration. In a previous study, *Pichia norvegensis*, a lactate-assimilating species, was found as the most frequent yeast specie in corn silages samples. However, in the literature, *P. norvegensis* is not related with corn silage aerobic stability. Some recent field observations reported that aerobically unstable corn silage is associated with reduced feed intake, milk production and milk fat depression. Thus, the objective of this study was to evaluate the performance and milk composition of dairy cows fed corn silages exposed to air and inoculated with *P. norvegensis.*

Materials and methods The trial was conducted at the Department of Animal Science of the College of Agriculture "Luiz de Queiroz". Corn crop was harvested at 31% DM, treated without (C) or with *P. norvergensis* at 1×10^5 cfu/g fresh matter (Y) and packed in large scale bag silos (40 t/silo). After 123 d of storage, silos were opened and fed to lactating dairy cows. Every day, the silages were unloaded and fed immediately (F) or after 48 h of air exposure (E). Twenty Holsteins cows were assigned to five replicated 4×4 Latin squares, with 21 d periods (15 d for adaptation + 6 d for sampling). Diets were formulated to contain 53% corn silage, 8% whole cottonseed, 18% soybean meal, 9.5% citrus pulp, 9% dry corn meal, and 2.5 % vitamin and mineral premix. The four treatments were: control-fresh silage (CF), control-exposed silage (CE), yeast inoculated-fresh silage (YF), and yeast inoculated- exposed silage (YE). Data were analyzed using the MIXED procedure of SAS.

Results and discussion Contrary to our expectation, inoculation with *P. norvegensis* and air exposure did not affect the DM intake (DMI). However, both the inoculation with *P. norvegensis* and exposure of silages to air decreased the yield of 3.5% fat-corrected milk by 1.27 kg/d and 1.38 kg/d, and milk corrected for energy by 1.21 kg/d and 1.30 kg/d, as well as the daily excretion of milk energy (NE_L), 4.6% and 4.9% respectively. In this way, feed efficiency, indicated by NE_L/DMI, was lowered by air exposure (5.7%) and inoculation of silages with *P. norvegensis* (8.5%). Although milk fat content was not affected by treatments, daily yield of milk fat was decreased by both inoculation with *P. norvegensis* (5.8%) and exposure of silages to air (6.2%). Diets containing exposed silages also decreased the yield of milk protein (3.2%) and increased the concentrations of milk urea-N (6.1%) and free fatty acids (19.1%). All animal responses indicate that diets containing silages inoculated with *P. norvegensis* and/or exposure to air had lower content of digestible nutrients.

Conclusion Feeding silages exposed to air for 48 h or inoculated with *Pichia norvergensis* at 1×10^5 cfu/g did not alter feed intake, but decreased the yield of 3.5% fat-corrected milk by 1.38 kg/d and 1.27 kg/d, and feed efficiency by 5.7% and 8.5% respectively.

Table 1 Performance of dairy cows fed corn silage exposed to air and inoculated with *Pichia norvegensis*

	Control		Yeast			P-value[2]		
Item[1]	Fresh	Exposed	Fresh	Exposed	SEM	Y	E	Y×E
Dry matter intake, kg/d	20.67	20.72	21.27	21.17	0.51	0.21	0.95	0.86
Milk yield, kg/d	25.85	25.79	25.82	24.13	1.20	0.12	0.10	0.13
3.5 % Fat-corrected milk , kg/d	27.28	26.45	26.55	24.62	1.17	0.03	0.02	0.34
ECM, kg/d	26.58	25.92	26.00	24.06	1.15	0.03	0.02	0.25
Fat, %	3.82	3.67	3.69	3.68	0.13	0.48	0.36	0.43
Protein, %	3.51	3.55	3.53	3.51	0.07	0.75	0.67	0.38
Urea-N, mg/dL	11.21	11.81	11.12	11.97	0.40	0.92	0.05	0.74
Fat, kg	0.99	0.94	0.95	0.88	0.05	0.03	0.02	0.63
Protein, kg	0.88	0.89	0.89	0.83	0.04	0.13	0.12	0.10
FFA, mmol/10 kg of milk	1.08	1.38	1.12	1.34	0.19	0.99	0.03	0.71
Milk NE_L, Mcal/kg	0.72	0.71	0.71	0.71	0.01	0.42	0.43	0.53
Milk NE_L , Mcal/d	18.61	18.14	18.20	16.85	0.81	0.03	0.02	0.25
Milk NE_L/DMI, Mcal/kg	0.89	0.87	0.84	0.77	0.03	<0.01	0.04	0.39

[1]ECM: energy corrected milk, FFA: free fatty acids, NE_L: net energy for lactation, DMI: dry matter intake.
[2]Effects of silage inoculation with *P. norvegensis* (Y), exposure to air (E) and their interaction (Y×E).

Silage Additives: where are we going?

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Introduction

A wide variety of additives are available to assist in improving various aspects of silage fermentation. In general, additives have been used to improve dry matter (DM) and energy recovery, improve digestibility of nutrients, and improve aerobic stability. This review will briefly discuss the potential future of silage additives relative to the challenges that confront the silage industry.

Additives for improving aerobic stability

In well packed silos, fermentation losses can be relatively low (<6-8% of total DM), but even in such silos, there is still opportunity for large losses of nutrients due to aerobic spoilage during storage and feed out. Both chemical and biological methods have been used to improve aerobic stability and are primarily based on limiting the growth of lactate assimilating yeasts in silage as these organisms are usually the primary initiators of aerobic spoilage.

Chemical additives for improving aerobic stability

Various chemical additives with antifungal properties have been used to enhance the aerobic stability of silages. The most common are short chain organic acids. For example, buffered propionic acid-based products are commonly used (Kung et al., 2000) in the US because of they are less corrosive and safer to handle than the straight acid. It is the undissociated (protonated) form of organic acids that is responsible for their antifungal properties and its prevalence is dependent on pH. Potassium sorbate and sodium benzoate have also been used to improve the aerobic stability of silages. For example, treatment with 0.1% potassium sorbate also improved dry matter recovery and aerobic stability and lowered the final concentration of ethanol in maize silage (Teller et al., 2012). Knicky and Sporndly (2011) reported that an additive containing sodium benzoate, potassium sorbate, and sodium nitrate improved the aerobic stability of a variety of crops with DM > 35%. Synergistic effects of sodium nitrate, potassium sorbate and sodium benzoate against various fungi and bacteria have been reported (Stanojevic et al., 2009). At the time

of writing this review, a factor limiting more wide spread use of chemicals to improve aerobic stability is cost. If production costs of antifungal chemicals can be reduced in the future, use may increase. Identification of other antifungal compounds with low costs and application rates could also prove to be beneficial but would have to overcome regulatory scrutiny to ensure that these compounds were safe.

Inoculants to improve aerobic stability

It is well known that although a homolactic acid fermentation theoretically recovers more overall DM and energy combined compared to a heterolactic fermentation (McDonald et al., 1991), the former type of fermentation minimizes the production of antifungal compounds. As a result, Muck and Kung (1997) reported that homolactic acid inoculation of silages made aerobic stability worse in about a third of the studies they summarized.

The initial commercialization of *Lactobacillus buchneri,* a heterolactic acid bacterium, to improve aerobic stability was controversial because it went against the dogma that only homolactic acid bacteria were suitable as silage inoculants. There was concern that high levels of acetic acid produced by this organism (although antifungal in nature) would lead to a reduction in DM intake and large losses of DM. More than tens years since its introduction by two companies, sales of *L. buchneri* continue to grow and various strains are now sold by numerous companies across the globe. Even with its success, there are still some limitations to using this organism. The area that could use the most improvement is the time that the organism needs to be effective. In general, a robust effect to inoculation with *L. buchneri* is not observed until about 50 to 75 d of ensiling. Schmidt et al. (2008) and Kleinschmit and Kung (2006) showed that this organism is relatively slow growing compared to common homolactic acid bacteria used as inoculants. Identifying a strain of *L. buchneri* that could elicit a response in aerobic stability in less than 1 or 2 weeks would be highly desirable.

Other lactic acid bacteria have also been isolated that produce antifungal compounds. For example, Strom et al. (2002) isolated a strain of *L. plantarum* (MiLAB 393) capable of producing a compound able to inhibit some yeasts and molds. However, to date, specific strains of *L. buchneri* appear to be the most effective in improving aerobic stability. Future research should continue on identifying organisms (including "killer yeasts" [Kitamoto et al., 1993]) that could be beneficial in improving aerobic stability.

Use of Exogenous Enzymes to Improve the Nutritive Value of Silages

Improving fiber digestibility of silages

Fibrolytic enzyme complexes (primarily cellulase and hemicellulase complexes) were originally added to silage to partially degrade fiber to fermentable water-soluble carbohydrates for use by lactic acid bacteria during fermentation. Excessive production of soluble carbohydrates is unwanted because it could lead to issues with poor aerobic stability and excessive effluent. Fibrolytic enzymes also have the potential to improve ruminal fiber digestion. Fiber digestion by these enzymes is complex and also limited by carbohydrate interactions with lignin. In some past studies, use of fibrolytic enzymes has actually resulted in what appeared to be lower fiber digestibility in the resulting silage (Nadeau et al, 2000). In fact, what happened was digestion of the readily digestible fraction of fiber during ensiling leaving the more recalcitrant fraction for feeding. Identification of organisms capable of being competitive in a silage environment and expressing high sufficient fibrolytic activity could have future potential as fibrolytic activity in lactic acid bacteria has been documented (Matthews et al., 2006).

A unique method to improve fiber digestion via the production of ferulic acid esterase by a species of *Lactobacillus buchneri* was introduced commercially several years ago (Nsereko et al. 2008) as a silage inoculant. Rather than digest fiber itself, this application attempted to unlock the link between hemicellulose and lignin resulting in a more readily digestible fiber fraction for the rumen. Although successful in some studies (Kang et al., 2009), results have been nil in others (Lynch et al., 2014, 2015). We theorize that the inoculant may have difficulty expressing sufficient amounts of ferulic acid esterase under varying conditions in the field to yield consistent responses. At the present, direct addition of sufficient amounts of ferulic acid esterase is probably uneconomical, but advances in production of this enzyme for potential use in the biofuels industry (Wong et al., 2011) may lower costs and change this scenario in the future.

Improving ruminal starch availability in maize crops with proteases

Potentially rumen degradable starch increases with time in the silo due to natural proteolytic mechanisms (Hoffman et al. 2011). This process is of particular importance for maize crops. However, high moisture maize (Ferraretto et al., 2014) and whole-plant maize (Der Bedrosian et al., 2012) may require as much as 10 months of storage before reaching peak rumen starch digestibility. Addition of acidic proteases may be a method to lessen the amount of storage time required to achieve peak digestion. Young et al. (2012) were the first to show that addition of acidic proteases at harvest were able to accelerate proteolytic activity during subsequent storage and increase ruminal starch digestion in

less time than it would take under normal conditions. Windle et al. (2014) also reported that addition of an acidic protease was effective in improving ruminal starch digestion in 31% DM and more mature (40% DM) maize silages. Future developments in formulation and production costs are needed before this technology can be applied in the field. The potential of adding bacteria with high proteolytic activity could also move this application forward, but to date, limited research in this area has not been successful (L. Kung, Jr., University of Delaware, unpublished data).

Use of lactic acid bacteria to improve the nutritive value of silages

For more than three decades, animal trials have revealed that some lactic acid bacterial silage inoculants have improved milk production, daily gain and/or feed efficiency (e.g., Weinberg and Muck, 1996). Among these experiments are instances where the inoculant did not affect silage fermentation compared to untreated silage even though animal productivity was increased by inoculation. Such cases have been frequent enough to suggest that the inoculated silage is having some effect on the rumen microbial community.

Recent research has observed differences in the *in vitro* ruminal fermentations of some inoculated vs. untreated silages that may explain improved milk production or gain in livestock fed inoculated silage. A recent review (Muck, 2013) did not find consistent results across studies, but some positive observations relative to animal performance include reduced methane production and increased rumen microbial biomass production. A lactating dairy cow feeding trial to examine inoculant effects on milk production did not observe significant shifts in the populations of the dominant groups within the rumen microbial community due to feeding inoculated silage (Mohammed et al., 2012), but milk urea nitrogen was reduced in the cows fed inoculated silage suggesting improved N efficiency (Muck et al., 2011). Recent analysis of the omasal samples from the study did not show significant increases in microbial N flow from the rumen but did find increased ruminal DM digestibility on the inoculated silage diet (Muck and Broderick, personal communication). It appears that the latter most likely explains the increased milk production on the inoculated silage diet.

Overall, there is accumulating evidence that some lactic acid bacterial strains are positively affecting the rumen environment. Much more research is needed to identify the mechanisms by which silage inoculation is affecting rumen microbial activity. This should lead new criteria to screen for improved inoculant strains that maximize the efficiency by which livestock utilize their diets and thereby increase the milk or meat produced per unit of feed.

Altering amino acid profiles in silage

As indicated earlier, some silage inoculants are affecting ruminal microbial biomass production at least in ruminal *in vitro* studies. Perhaps this is due to differences in the amino acid profiles of the silages. For example, maize silages are known to be low in lysine. Is it possible for inoculant LAB to increase lysine content? Could inoculants be used to shift amino acid profiles to better match the needs of rumen microorganisms and livestock?

Reducing proteolysis in grass and legume silages

High quality grasses and legumes such as perennial ryegrass and alfalfa typically have more than half of the true protein degraded to soluble non-protein nitrogen during ensiling. This may lead to inefficient feed N utilization by ruminant livestock if the diet is not appropriately balanced with other components that contain rapidly degradable carbohydrates and rumen bypass protein. Even so, it is difficult to attain the N efficiency of a diet where the forage source is dry hay.

Reduced proteolysis during ensiling occurs naturally in some forages. Two mechanisms have been found. Some legumes such birdsfoot trefoil, sainfoin and lespedeza contain tannins which bind and precipitate proteins resulting in better protein preservation during ensiling (Albrecht and Muck, 1991). Red clover produces both polyphenol oxidase (PPO) and *o*-diphenol substrates that result in quinones that bind to proteins reducing proteolysis (Jones et al., 1995).

Tannins are polyphenolic compounds that vary widely in composition and protein binding/precipitation ability. Some tannins or tannin concentrations may prevent proteins from being degraded in the rumen or in the lower gastro-intestinal tract. So current research is focused on finding tannins of the right type and concentration to reduce proteolysis in the silo but permit increased N utilization in livestock. This may one day lead to either genetically modified forages that produce the right tannins at the right concentration. It may also lead to tannin-based additives that more precisely control the concentration on the forage at ensiling.

Research on the PPO system in red clover has been focused on understanding that system of protein protection. This has included genetically modifying alfalfa to produce PPO and then adding an *o*-diphenol substrate at ensiling. For forages such as orchard grass that naturally produce PPO, but not the appropriate *o*-diphenols, it is possible inexpensive *o*-diphenols may become silage additives to reduce proteolysis. In non-PPO producing forages such as alfalfa, it is more likely in the distant future that they would be engineered to produce both PPO and an appropriate substrate so that no additive would be needed.

Inhibiting clostridia

Clostridial growth with its production of butyric acid, amines and other compounds is a major ensiling problem because of its effects on intake and health (ketosis) of ruminants. The crux of the problem is an inadequate supply of sugars for LAB to reduce pH sufficiently to inhibit clostridial activity. In drier climates, wilting forages helps to guarantee successful fermentations because the pH to inhibit clostridia increases with DM concentration. In wetter climates (either temperate or tropical), additives may be necessary to stop clostridial activity.

Chemical additives have been the most common approach to preventing clostridial activity. Acids such as formic have been traditionally used in northern Europe to partially reduce crop pH at ensiling, allowing a lactic acid bacteria fermentation to further reduce pH to a level that completely inhibits clostridia. Another approach is the use of nitrite, typically sodium nitrite, for which there is substantial evidence of its inhibitory effect on clostridia. We expect that these approaches will continue for the foreseeable future.

Inoculants to this point cannot consistently prevent clostridial activity. Homofermentative LAB may lower silage pH relative to that in untreated silage, but the degree of reduction may or may not be sufficient to prevent clostridial growth, depending on circumstances. We expect that more direct approaches will come in the future. Bacteriocins, proteinacous toxins produced by bacteria that affect other bacteria, from LAB have been studied in hopes of controlling undesirable organisms such as clostridia and listeria (De Vuyst and Leroy, 2007). Specifically for silages, Flythe and Russell (2004) showed that a bacteriocin (bovicin HC5) from *Streptococcus bovis* HC5 could inhibit the activity of *Clostridium sporogenes* MD1. Marcinakova and Laukova (2004) reported that *Enterococcus faecium* EF9296 produced a bacteriocin like substance against gram positive enterococci and *Listeria monocytogenes*. However, bacteriocins from LAB may also have inhibitory activity against other LAB (Settanni et al., 2005). Consequently inoculants that produce bacteriocins to inhibit clostridia and other undesirable bacteria are possible but do not appear likely in the near future.

Controlling mycotoxins

Under certain conditions, various mold species may produce mycotoxins. *Fusarium*, *Penicillium* and *Aspergillus* species are the most common sources of mycotoxins in silages. These mycotoxins may threaten the health of livestock and farm workers. Mycotoxins may be present on the crop at ensiling, particularly in whole-crop maize and small grain silages. When the silage is again exposed to oxygen during emptying of the silo or through incomplete sealing of the silo, molds may grow and produce mycotoxins while the crop is

in the silo. Mycotoxin development in the silo may be minimized through improved silo management: high density, excellent seal, high feed out rate.

The more difficult issue is how to deactivate mycotoxins that are on the crop at ensiling. Could some of the same binding agents that are fed to livestock be applied at ensiling? Are there LAB or other microbial strains that could reduce mycotoxin concentrations? Are there enzymes that might be effective? This is an area of silage research that merits more attention.

Miscellaneous opportunities

Phages are viruses that can infect bacteria and represent one of the main causes of fermentation failure in the dairy foods industry (Mäyrä-MäKinen and Bigret, 1993). The possibility that epiphytic phages occur in silages has been documented. Tanaka et al. (1995) reported that 25% of 77 samples of silages studied contained phages and many of them were infectious to *L. plantarum*. Kaneshige et al. (1994) showed that phage activity was responsible for failed fermentations in Italian ryegrass. Selection of *L. plantarum* resistant to phage infection (Eguchi et al., 2000) or identifying methods to reduce phage infection of lactobacilli maybe useful in the future.

Quorum sensing is the regulation of gene expression in response to changes in the density of cell populations (Miller and Bassler, 2001). Interactions between microbes in silages via quorum sensing mechanisms has not been studied to our knowledge, and there is obviously potential for quorum sensing to control some aspects of silage fermentation especially because there are major changes in populations of organisms during the fermentation process. Production of bacteriocins is controlled by quorum sensing, and so understanding quorum sensing may be a key to successful development of inoculants that inhibit detrimental bacterial species by bacteriocins.

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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

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Keywords aerobic stability, short fermentation time, LAB, silage additive, oxygen scavenging

Introduction Recently, a novel *Lactococcus lactis* O224 DSM11037 lactic acid bacteria (LAB) strain has been introduced, which is superior in oxygen scavenging and also relatively fast in reducing pH (Hindrichsen *et al.*, 2012). Combining this *L. lactis* O224 DSM11037 strain with *Lactobacillus buchneri* DSM 22501 have shown better results on aerobic stability than previously seen with combinations of *L. buchneri* and high lactate producing strains such as *L. plantarum* at 90 days of fermentation. Recent years, though, many producers have faced feed shortage problems due to season variation. This has forced them to shorten the fermentation time of silage with poor aerobic stability as a negative consequence. The objective of the current study was therefore to evaluate the efficacy of the new *L. lactis* O224 DSM11037/*L. buchneri* DSM 22501 combination on fermentation parameters and aerobic stability at even very short ensiling times. The additive was compared to an untreated reference.

Materials and methods Silages were prepared from whole crop maize containing 34.4 % DM and 3.02 % water soluble carbohydrate (WSC) on fresh matter basis, respectively. Forage was treated the following: no additive (UT) and SiloSolve FC, containing *L. buchneri* DSM22501 and *L. lactis* O224 DSM11037 (TA), Chr. Hansen A/S, Denmark. The target application rate was 150,000 cfu/g forage. The untreated control (UT) received the appropriate amount of sterile water. Whole crop maize was ensiled in 3 l laboratory silos and each treatment was replicated 5 times. Silages were analyzed on day 2, 4, 8, 16, and 32 of storage at 20°C. Aerobic stability (AS) of silages was determined from a 7 day aerobic challenge period after each fermentation time (day 2, 4, 8, 16, and 32 of storage). This was done by monitoring the temperature increase in silages stored aerobically in insulated PVC-tubes at 20 ºC ambient temperature. Aerobic stability is determined by the amount of time it takes the silage temperature to exceed the ambient temperature with more than 3°C. Data were statistically analyzed as a randomized complete block by using the GLM procedure of SAS.

Results and discussion TA resulted in better preserved silage with significantly ($P<0.05$) lower pH and significantly ($P<0.05$) lower yeast and mold counts. When compared with the control (UT) treatment SiloSolve FC treatment reduced ($P<0.05$) dry matter loss at all ensiling times (Table 1). On an average of the different fermentation periods, TA reduced the fermentation losses with 49% ($P<0.05$) compared to the untreated (UT) silages. After 7 days of aerobic exposure pH, yeasts, molds, and DM losses were significantly reduced for the TA treated silages compared to the untreated control silages (UT). TA

treated silages resulted in a significantly enhanced aerobic stability, on average by 49% ($P<0.05$), at all ensiling periods, compared to UT.

Table 1 Silage parameters after different ensiling periods, ensiled with or without additive

Ensiling time	Treatment	DM, %	pH	Yeast, log_{10} cfu/g	Mold, log_{10} cfu/g	Weight loss, %
2d	UT	34.3	4.55	5.08	3.49	0.48
	TA	34.3	4.39*	4.32*	2.52*	0.42*
4d	UT	34.3	4.42	4.72	3.12	0.78
	TA	34.3	4.22*	3.63*	2.41*	0.54*
8d	UT	33.4	4.24	4.63	2.86	1.06
	TA	33.8*	4.05*	2.47*	1.97*	0.61*
16d	UT	32.9	4.11	3.93	2.71	1.18
	TA	33.8*	3.95*	1.34*	1.78*	0.65*
32d	UT	32.7	4.01	3.32	2.67	1.3
	TA	33.7*	3.89*	1.06*	1.45*	0.69*

UT = untreated, TA = *L. buchneri* DSM22501 and *L. lactis* O224 DSM11037, *significantly different from untreated control ($P<0.05$).

Table 2 Silage parameters after 7 days of aerobe challenge, ensiled with or without additive

Ensiling time + aerobic challenge	Treatment	pH	Yeast, log_{10} cfu/g	Mold, log_{10} cfu/g	AS, hours	Weight loss, %
2d + 7d	UT	8.49	7.88	6.69	32.4	4.11
	TA	8.09*	7.50*	6.47*	49.2*	2.83*
4d + 7d	UT	8.30	7.64	7.18	42.0	3.33
	TA	7.83*	7.37*	6.79*	55.2*	2.35*
8d + 7d	UT	7.90	8.00	7.46	69.6	3.78
	TA	6.90*	7.25*	6.25*	98.4*	2.59*
16d + 7d	UT	7.80	8.28	8.69	78.0	3.29
	TA	6.70*	7.39*	7.37*	109.2*	2.24*
32d + 7d	UT	7.58	8.30	8.16	88.0	2.91
	TA	4.77*	7.24*	6.30*	146.4*	1.79*

UT = untreated. TA = *L. buchneri* DSM22501 and *L. lactis* O224 DSM11037. AS = Aerobic stability. *Significantly different from untreated control ($P<0.05$).

Conclusions The novel combination of a hetero- and homofermentative LAB (*L. buchneri* DSM22501 and *L. lactis* O224 DSM11037) was efficient at reducing pH, DM loss, yeast and mold counts at all the investigated ensiling periods in this study. The TA significantly enhanced the aerobic stability of whole crop maize silage compared to UT.

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Nutritive value evaluation of sorghum-soybean ensiled with biological additives in Colombia

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Keywords silage, *Sorghum bicolor*, *Glycine max*, biological additive, nutritive quality, lactic acid bacteria, inoculant

Introduction Forage conservation is the most important element in livestock production, especially for ruminants, when forage availability is not sufficient for animals to prevent low productivity or even mortality of animals during the dry season in tropical countries like Colombia. Tropical grasses and legumes are sometimes difficult to ensile because of their generally low sugar content and high buffering capacity with regard to legumes. Furthermore, the lack of efficient epiphytic lactic acid bacteria (in number and/or species) can be a limitation. The silage additives have been used to improve the fermentation, prevent the production of butyric acid, reduce the loss of dry matter and preserve nutrients. Lactic acid bacteria (LAB) and enzymes are considered fermentation stimulators. Xylanases are fibrolytic enzymes used in silage supplementation to improve the availability of soluble carbohydrates, avoid undesirable microorganisms, reduce the fiber content and improve the digestibility of silage. The rumen fluid may be new option not yet evaluated as a cocktail of enzymes to promote silage fermentation. The objective of our study was to evaluate the nutritional quality of sorghum-soybean when ensiled with different biological additives.

Materials and methods Two plant species were evaluated: sorghum variety Corpoica JJT-18 and soybean forage cv Panorama 29. These plant materials were sown at the International Center for Tropical Agriculture (CIAT) in Palmira, Colombia. Soybean and sorghum whole plants were harvested at about 70 days of growing. The forages were wilted up to ~30 % dry matter (DM) and chopped to a particle size of 2 cm approximately for ensiling. The silages were prepared in the three different mixtures: sorghum 100%/ soybean 0%, sorghum 67%/soybean 33% and sorghum 33%/soybean 67% on fresh matter (FM) base. These three mixtures underwent the following 8 additives: 1) control, 5 different bacterial inoculants 2) lactic acid bacteria (LAB) strain CIAT S66.7, 3) CIAT T735, 4) CIAT S738, 5) CIAT S739, 6) SilAll4x4, 7) fibrolytic enzyme (xylanase from *Orpynomices* sp.) and 8) clarified ruminal liquid. The material was ensiled in vacuum sealer bags (~800 g FM) in triplicate. The silage was stored for 90 days at ~25 °C. Samples were analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), digestible neutral detergent fiber (dNDF), *in vitro* dry matter digestibility (IVDMD). The pH was determined at day 1, 3 and 90. Ammonia-N of total N was determined as indicator for proteolysis. A completely randomized design was used, twenty-four treatments and four replicates per treatment. The dates were analyzed with ANOVA and the means of treatments were compared by Tukey Test (SAS Version 9.3).

Results and discussion The type of forage and the biological additive had a significant influence on pH value ($P<0.0001$). The silage with 100% sorghum resulted in the lowest pH (3.8), followed by mixing sorghum/soybean 67%/33% (4.1).

Table 1 Nutritive value of sorghum-soybean silage

Mixture	Additive	DM (%)	IVDMD (%)	CP (%)	NDF (%)
Sorghum100%/ soybean0%	Control	24.9^{jkl}	69.7^{cdefg}	7.2^{f}	57.2^{bc}
	LAB S66.7	32.0^{defgh}	69.2^{fg}	6.1^{g}	58.6^{ab}
	LAB S738	29.3^{hij}	69.9^{bcdef}	6.1^{g}	56.7^{bc}
	LAB S739	31.3^{fgh}	69.3^{fg}	6.0^{g}	58.5^{ab}
	LAB T735	31.7^{fghi}	69.4^{efg}	5.8^{g}	62.6^{a}
	Silall 4x4	27.3^{ijk}	69.9^{bcdef}	5.3^{g}	58.3^{ab}
	Fibrolytic enzyme	22.2^{l}	69.2^{fg}	5.7^{g}	56.2^{bcd}
	Ruminal fluid	24.1^{l}	70.2^{abcd}	5.7^{g}	55.5^{bcde}
Sorghum67%/ soybean33%	Control	30.6^{fghi}	69.7^{cdefg}	10.5^{e}	51.4^{efgh}
	LAB S66.7	33.1^{cdefg}	70.3^{abc}	11.5^{d}	53.8^{bcdef}
	LAB S738	33.5^{defgh}	70.4^{abc}	10.8^{de}	51.0^{efghi}
	LAB S739	35.7^{bcde}	69.3^{fg}	10.8^{de}	53.2^{cdefg}
	LAB T735	35.1^{bcdef}	70.1^{abcde}	11.2^{de}	49.7^{fghijk}
	Silall 4x4	32.2^{efgh}	70.2^{abcd}	10.6^{e}	52.4^{cdefg}
	Fibrolytic enzyme	30.5^{fghi}	70.2^{abcd}	11.0^{de}	50.9^{efghi}
	Ruminal fluid	30.2^{ghi}	70.4^{abc}	10.7^{de}	53.1^{cdefg}
Sorghum33%/ soybean67%	Control	30.4^{fghi}	70.4^{abc}	14.8^{ab}	44.5 ^{l}
	LAB S66.7	37.9^{abc}	70.1^{abcde}	13.9^{ab}	46.7 ^{hijkl}
	LAB S738	41.1^{a}	70.8^{a}	14.9^{a}	45.1^{kl}
	LAB S739	38.4^{ab}	60.1^{g}	12.9^{c}	50.4^{fghi}
	LAB T735	37.2^{abcd}	70.5^{abc}	14.2^{ab}	46.4^{ijkl}
	Silall 4x4	31.3^{fghi}	69.8^{bcdef}	14.6^{ab}	48.4^{ghijkl}
	Fibrolytic enzyme	32.7^{efgh}	69.5^{defg}	13.2 c	49.1 ^{fghijkl}
	Ruminal fluid	32.9^{efgh}	70.6^{ab}	14.3^{ab}	45.9 ^{jkl}
mixture*additive Probability		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$

A higher level of inclusion of soybean improved DM, CP, ADF and dNDF ($P<0.001$). The mixtures had a better IVDMD ($P < 0.0001$) than sorghum only (70.1%). There was no difference for NDF among additive but there was interaction between mixture and additive. It is interesting that the LAB employed, S66.7 and S738 strains were able to limit proteolysis (38.6 and 36.6 g NH3-N/kg total N), whereas the control treatment (67.7 g NH3-N/kg total N) has the highest protein degradation by additive effect.

Conclusions The mixture sorghum/soybean 67/33 is a feasible option for good anaerobic fermentation and a high nutritional quality when ensiled with the CIAT LAB S738 and CIAT S66.7 respectively.

Effects of silage additives in whole-crop maize and grain silages to enhance methane yield

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Keywords additives, methane yield, test scheme, aerobic stability, losses, silage

Introduction Biogas production based on energy crops is very common in Germany. In practice silage additives are offered to improve the biogas yield (Banemann et al. 2010). Nussbaum et al. (2012a) developed a reasonable test scheme that can verify the effects of silage additives on possible improvements of methane yield. The test scheme focusses on all processes - from harvesting energy crops, through silage fermentation, including all losses with or without air stress, up to the processes in the biogas reactor - and works with small quantities of silage on a laboratory scale level. Different additives (homo-, heterofermentative and their combination as well as chemical additives in combination with homofermentative inoculants) have been tested with whole crop silages in 2012 and with maize silages from 2012 and 2013. The present research work focusses on results of 2 selected commercial products with maize silages in 2012.

Materials and methods The applied testing scheme (Figure 1) allows testing of quite different silage additives under different ensiling conditions (6a airtight, 6b with air stress), which were completed by determining aerobic instability. Silages were prepared routinely on laboratory scale. Losses were recorded by weighing. Stability tests were operated by periodic temperature measurements. The correction of the dry matter content for volatile substances was conducted according Weissbach and Strubelt (2008). Methane yields were recorded by Hohenheim biogas yield test (HBT). The accounting for the evaluation of silage additives included losses as well as specific gas yields. The benchmark was the methane yield of the original material (OM) prior to ensiling.

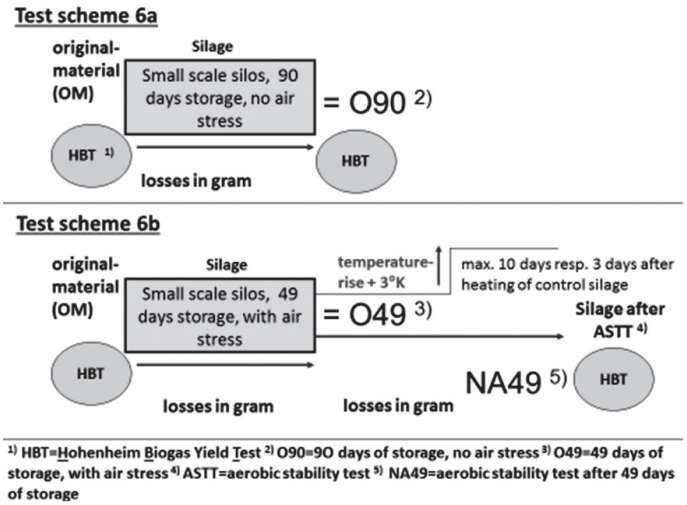

Figure 1 Test schemes for silage additive assessment on laboratory scale.
The additive used in maize silages 2012 are both combination inoculants of homo- and heterofermentative strains (table 1). Two grams of both inoculants were diluted in 1.0 L water (concentration 200.000 cfu/g), 1.0 L of these solutions were applied on 1.0 t original material.

Table 1 Composition of the inoculants used in maize silage of 2012

Additive name	SILASIL ENERGY (SE)	SILASIL ENERGY XD (SE XD)
ingredients	*Lactobacillus buchneri* (NCIMB 30141)	*Lactobacillus buchneri* (NCIMB 30141)
	Lactobacillus plantarum (NCIMB 30142	*Lactobacillus diolivorans* (LAC 1129)
	Lactobacillus rhamnosus (SBE 10070)	*Lactobacillus rhamnosus* (SBE 10070)

Results and discussion Except the silage of SE XD (O90) the methane yields of all other tested silages (Table 2) were lower than of the original material. None of the 2 products enhanced the methane yield after 49 days of air stress storage, but both indicated significant effects at silo-unlading conditions after the aerobic stability test. In case of 90 days storage under airtight conditions only SE XD indicated significant effects of improvement of the methane yield. The difference in performance of the 2 inoculants might be due to bacterial strain composition. Comparable results were reported by Nussbaum (2012b).

Table 2 Effects of 2 inoculants on methane yield of maize silages 2012

		Methane yield on base of 1 kg DM [NL/kg DM]			
			49 days (6b)		90 days (6a)
		OM	O49	NA49	O90
Control	$\bar{x}$	375[1)]	350	324	365
	SD		9	17	6
SE	$\bar{x}$		365	348	365
	SD		12	4	13
	p(<0.05)		n.s.	*	n.s.
SE XD	$\bar{x}$		358	369	378
	SD		15	11	2
	p (<0.05)		n.s.	*	*
Difference Control-SE [%]			4.3	7.3	-0.2
Difference Control-SE XD [%]			2.1	13.8	3.5

[1)] methane yield of original material as benchmark

Conclusions If aerobic instability is expected, it is recommend to use homo-/ heterofermentative inoculants to enhance methane yield up to 13 % compared to untreated control. Compared to control and SE, the product SE XD achieved 3 % more methane yield under airtight conditions.

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Impact of silage additives on aerobic stability and methane production from sorghum

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Keywords aerobic stability, anaerobic digestion, methane yield, silage additive, sorghum silage

Introduction Due to its high yield potential and its drought tolerance, sorghum is recently integrated as an alternative to maize in energy crop rotations and used as feedstock for biogas production. Sorghum biomass usually reveals adequate levels of water-soluble carbohydrates (WSC) and a low buffering capacity for successful preservation by ensiling. However, favourable silage fermentation characteristics can make sorghum silages prone to aerobic deterioration during silage feed-out. So far, only few studies on the effects of aerobic deterioration on methane production exist. The objective of the present study is to investigate the effects of a range of silage additives on silage fermentation, aerobic stability and methane production of sorghum silage, with focus on the impact of aerobic deterioration on methane yield.

Materials and methods Sorghum hybrid (*Sorghum sudanense* × *bicolor*) variety Lussi was harvested at the end of flowering stage of maturity, chopped to a nominal particle size of 8 mm and ensiled in 1.5 L glass silos after inoculation with silage additives. Silos were stored for 49 days at 25°C under anaerobic conditions and air stress conditions, respectively. Air stress involved opening of two 1 cm holes placed in the top and bottom of the silos for 24 hours at day 28 and day 42. All ensiling experiments were performed in triplicate. Aerobic stability was measured for 7 days applying the temperature method according to Honig (1990). Silages were analysed for dry matter (DM) and organic dry matter (ODM) content (equates to DM content exclusive of ash), pH, products of silage fermentation, and methane yield as described previously (Herrmann et al., 2011). The DM content was corrected for loss of volatile compounds during oven drying (Weissbach and Strubelt, 2008). Methane yields were measured in batch anaerobic digestion tests which were conducted in 2 L reactors at 35°C over 30 days. Methane yields are based on the original organic dry matter available in freshly harvested biomass prior to ensiling (ODM_{orig}), thus silage losses are taken into account. Data were analysed applying the GLM procedure in SAS 9.3 for analyses of variance, and the test-procedure SIMULATE for pairwise comparisons.

Results and discussion Sorghum biomass was characterised by low DM content (25 %) at harvest which resulted in high concentration of fermentation products and DM losses of 4.5 to 8.9 % (Table 1). Only traces of butyric acid were found, implying absence of clostridial fermentation. Silage additives significantly affected silage fermentation. Application of sodium benzoate and sodium propionate (BP) resulted in lowest DM losses. Inoculation with homofermentative lactic acid bacteria (LAB) *L. plantarum* (LP) did not increase lactic acid fermentation compared to the control, probably due to high counts of LAB (8×10^5 cfu/g) present before additive application. Inoculation with additives containing *L. buchneri* significantly increased the acetic acid and alcohol content and reduced the content of lactic acid, but also increased DM losses (Table 1). Despite differences in fermentation products between additive treatments, no significant differences in methane yield

considering storage losses were found. The control silage (CON) was aerobically stable for 1.8 days which was further reduced by air stress during storage. Chemical additive and inoculants with heterofermentative LAB successfully improved the aerobic stability (> 7 days). Instability during exposure to air led to a rapid degradation of organic acids which are valuable substrates for anaerobic digestion. Thus, aerobic deterioration of sorghum silage resulted in a decline in methane yield of 16-19 % within 7 days of exposure to air (CON, LP).

Table 1 Effects of silage additives and storage method on fermentation parameters, aerobic stability and methane yield of sorghum silages

	After silage fermentation (49 days)							After exposure to air (7 days)				
Silage additive	DM loss %	pH	LA g/kg DM	AA g/kg DM	ALC g/kg DM	MY L_N/kg ODM_{orig}	ASTA days	pH	LA g/kg DM	AA g/kg DM	ALC g/kg DM	MY L_N/kg ODM_{orig}
Anaerobic storage												
CON	5.2^{cd}	3.7^{f}	84.4^{a}	14.7^{e}	8.5^{c}	302.1	1.84	7.9^{b}	1.2^{d}	6.2^{d}	4.4^{d}	246.0^{b}
BP	4.5^{d}	3.8^{c}	87.7^{a}	17.5^{d}	10.2^{c}	286.4	> 7	3.8^{d}	82.4^{a}	13.8^{c}	4.3^{d}	275.9^{a}
LP	7.3^{b}	3.7^{e}	75.2^{b}	12.6^{f}	21.9^{b}	293.6	1.88	8.2^{a}	1.6^{d}	3.6^{d}	0.0^{e}	243.5^{b}
LB+E	5.9^{c}	3.8^{d}	85.2^{a}	22.7^{c}	17.1^{b}	300.8	> 7	3.7^{d}	80.9^{a}	17.3^{b}	7.7^{c}	276.0^{a}
LB-FE	8.6^{a}	4.2^{a}	14.9^{d}	69.7^{a}	36.2^{a}	284.2	> 7	4.3^{c}	8.4^{c}	54.1^{a}	17.6^{b}	278.7^{a}
LP+LB	7.6^{ab}	4.1^{b}	34.1^{c}	51.3^{b}	36.8^{a}	288.3	> 7	4.1^{c}	25.4^{b}	51.5^{a}	20.9^{a}	287.5^{a}
Sign.	***	***	***	***	***	ns	np	***	***	***	***	***
Storage with air stress												
CON	6.8^{d}	3.8^{c}	72.5^{b}	13.0^{d}	15.4^{c}	290.6	0.58	8.9^{a}	0.0^{d}	3.8^{d}	0.1^{c}	240.7^{d}
BP	5.6^{e}	3.8^{c}	84.2^{ab}	17.5^{d}	10.0^{d}	299.1	> 7	3.8^{c}	80.5^{a}	12.8^{c}	4.6^{b}	286.7^{ab}
LP	7.5^{c}	3.8^{c}	72.3^{b}	11.6^{d}	23.9^{b}	291.8	1.04	8.8^{a}	0.7^{d}	3.6^{d}	0.9^{c}	245.8^{cd}
LB+E	6.8^{d}	3.8^{c}	87.7^{a}	24.4^{c}	15.1^{c}	282.0	2.00	7.6^{b}	2.4^{cd}	2.9^{d}	0.6^{c}	254.4^{c}
LB-FE	8.9^{a}	4.1^{a}	20.7^{d}	62.6^{a}	21.4^{b}	294.9	> 7	4.2^{c}	9.8^{c}	64.4^{a}	19.0^{a}	280.4^{b}
LP+LB	8.0^{b}	4.0^{b}	42.1^{c}	55.0^{b}	31.0^{a}	287.2	> 7	4.0^{c}	46.9^{b}	43.6^{b}	19.6^{a}	292.8^{a}
Sign.	***	***	***	***	***	ns	np	***	***	***	***	***

DM: dry matter; LA: lactic acid; AA: sum of acetic and propionic acid; ALC: sum of ethanol, 1-propanol, 1,2-propanediol, 2,3-butanediol; ASTA: aerobic stability; MY: methane yield; CON = control without additive; BP = sodium benzoate/sodium propionate (3.5 g/kg); LP = *L. plantarum* (3×10^5 cfu/g); LB+E = *L. buchneri* + enzymes (β-glucanase, α-amylase, xylanase, 10^5 cfu/g); LB-FE = *L. buchneri*, ferulate esterase producing strain (1.1×10^5 cfu/g); LP+LB = *L. plantarum*/*L. buchneri* (2×10^5 cfu/g).
[a-f]Means within columns with no letter in common differ significantly ($P < 0.05$, Adjust = Simulate), ns: not significant, np: not performed.

Conclusions Aerobic spoilage of biogas silages considerably reduces the methane production potential. Chemical additives and heterofermentative LAB are effective to enhance the aerobic stability, and can prevent losses and assist in preserving energy-rich feedstock for biogas production.

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Gases and VOC in silages: occurrence, environmental and animal issues[1]

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[1] *The present proceedings paper contains excerpts from: Malkina I.L., A. Kumar, P. G. Green, and F. M. Mitloehner. 2011. Identification and quantitation of volatile organic compounds emitted from dairy silages and other feedstuffs. J. Environ. Qual. 40:1–9.*

Abstract

Ground-level ozone formation continues to be a critical problem in the United States. The problem is especially severe in California, generally, and Central California's San Joaquin Valley (SJV), specifically. Dairies are one of the major sources of volatile organic compounds (VOCs) in SJV and have recently attracted considerable attention from the regulatory agencies. A number of recently conducted studies have reported actual emissions data from different dairy sources. However, there is currently limited data available for feed storage and silage piles, which are potentially significant contributors to ozone formation. The impact of different VOCs on ozone formation varies significantly from one species to another. Comprehensive measurements of VOC emissions are required to fully characterize and include all the important contributors to atmospheric reactivity. Therefore, the identification of emitted VOCs is needed to properly assess the wide spectrum of chemicals involved in ozone formation. This research study aims to identify and quantify the VOCs emitted from various silages and other feed sources. We have conducted experiments in an environmental chamber using large representative samples under controlled conditions. Over eighty VOCs were identified and quantified from corn, alfalfa, and cereal silages, total mixed rations, almond shells, and almond hulls using gas chromatography-mass spectrometry (GC/MS). Emissions of aldehyde compounds and acetone were measured using high performance liquid chromatography. The results revealed high fluxes of alcohols and other oxygenated species. Lower, but perhaps comparably significant, concentrations of highly reactive alkenes and aldehydes were also detected. Additional quantitation and monitoring of these emissions are essential for assessment of and response to the specific needs of the regional air quality in the SJV.

Introduction

Tropospheric ozone is one of the most important pollutants throughout the United States. Currently, higher ozone levels are found not only in densely populated areas and

areas with intense agricultural operations, but also in remote areas. The United States Climate Change Science Program and the Subcommittee on Global Change Research have recently reported that over the past 50 years the ozone at the land surface has risen in rural areas of the United States, and is forecast to continue to increase during the next 50 years (U.S. Climate Change Science Program, 2008).

The Central California's San Joaquin Valley (SJV) has long suffered from some of the worst air pollution in United States, in general, and high ozone levels in particular.

Ground level ozone formation is caused by the gas-phase reaction of emitted VOCs and oxides of nitrogen (NOx) in the presence of sunlight. The United States Environmental Protection Agency (EPA) has identified the SJV as a "severe non-attainment" area based on the federal 8-hour ozone standard. In March of 2008 the EPA adopted a new 8-hour ozone standard of 0.075 ppm (US EPA, 2008). In order to attain this new standard for agriculturally intensive regions, the reduction of agricultural emissions of VOCs and NOx is essential.

Considerable effort at ozone reduction has been attempted in the past few decades by reducing the total mass of VOC emissions (US EPA, 2008). However, impacts of various VOCs on ozone formation differ significantly from one species to another. This makes the determination of individual VOCs crucial for the assessment of ozone reduction strategies. In particular, non-traditional VOC control strategies take into account the pronounced differences in "reactivities" of VOCs (Carter et al., 1995), and therefore further provide the means for additional ozone reduction, which could supplement mass-based control approaches. Additionally, VOC reductions have been more effective in reducing ozone in dense urban areas where, due to higher NOx levels, VOCs are the limiting factor in ozone formation. Away from dense urban areas, NOx are limiting, and the natural background of VOCs (from soils, grasses and trees) can make anthropogenic VOCs less dominant. Based on current models of the SJV air basin, even complete elimination of all sources of all types of anthropogenic VOCs would not achieve attainment of the ozone standard; in fact, it would only produce modest improvement. NOx reductions are paramount but, nevertheless, an increase in VOCs, especially the more reactive ones, would necessitate even greater NOx reductions.

Although the vast majority of ozone precursors' sources are well characterized, and their control has proven effective at reducing urban ozone (ARB, 2005, Kumar and Viden, 2007, EPA, 2008), data on dairy emissions remain sparse. Dairies are believed to be one of the largest sources of VOCs and their high concentration in the SJV is of particular concern (CARB, 2006). To make matters worse, the combination of extensive and intensive agriculture, stagnant air and low wind speeds coupled with high summer air temperatures, high summer levels of solar irradiation and cloudless skies provide the optimal conditions

for ozone formation in the SJV. Therefore, evaluation and understanding of emission sources, speciation of a wide range of dairy- and agricultural-related compounds and assessment of their reactivities are critical.

Several research efforts have been undertaken in the past few years to better quantify emissions from dairies and agricultural sources.

A total of 113 compounds were identified at the Washington State University Knott Dairy Farm (Filipy et al, 2006) using GC/MS, sorbent tubes, and cryogenic traps techniques. The wide range of VOCs included alcohols in which ethanol was dominant, aldehydes, ketones, esters, ethers, sulfides, carbonyls, aromatics, and other hydrocarbons. VOC emissions from dairy cows and their waste at various stages of the lactation cycle were measured with a proton-transfer-reaction mass spectrometer (PTR-MS) using a facility at the University of California, Davis (Shaw et al., 2007). The measurements of alcohols, VFAs, phenols, and methane (CH4) emitted from non-lactating and lactating dairy cows and their manure under controlled conditions were reported by Sun et al. (2008).

Ngwabie et al. (2008) reported chemical ionization mass spectrometry and photo-acoustic spectroscopy measurements of mixing ratios of VOCs over a two week measurement period in a large cowshed in Mariensee, Germany. Numerous VOCs were detected with alcohols (ethanol, methanol, C3–C8 alcohols) being dominant, followed by acetic acid and acetaldehyde, and included ketones, amines, sulfides, aromatic compounds, and VFAs. These results indicated that animal husbandry VOC emissions are dominated by oxygenated compounds.

Alanis et al (2008) quantified emissions of six VFAs from non-enteric sources at a small dairy located on the campus of California State University Fresno. Both animal feed and animal waste were found to be major sources of VFAs, with acetic acid contributing 70–90% of emissions from the sources tested. Measured total acid fluxes during the spring (with an average temperature of 20o C) were 1.8± 0.01, 1.06 ± 0.08, (1.3 ± 0.5) × 10-2, (1.7± 0.2) × 10-2 and (1.2 ± 0.5) × 10-2 gm-2 h-1 from silage, total mixed rations, flushing lane, open lot and lagoon sources, respectively with silage being the highest contributor. These data indicated high fluxes of VFAs from dairy facilities.

Recently reported studies provided improved information regarding VOC emissions from dairy facilities in general and animal waste in particular. However, while fermented cattle feed (silage) could arguably be one of the largest, and perhaps the largest, sources of dairy-related VOCs, currently there is no experimental data available on the identification and characterization of VOC emissions from silage and other feed sources. We have utilized a combination of GC/MS and high performance liquid chromatography (HPLC) with specific objectives to: (1) identify gaseous compounds emitted from different types

of silage and other feed sources in order to better understand their contribution to ozone formation; (2) quantify emitted VOCs concentration and compare different silage types across the dairy; (3) measure concentration of aldehydes and ketones emitted from silages.

Reported experiments were conducted under controlled conditions, which further allow comparison of different types of typical dairy silage, and other feed sources and eliminate the influence of ambient conditions.

Materials and methods

Silage and other feed samples were collected from commercial dairy located approximately 20 miles northwest from campus of University of California at Davis. This is a typical large size Californian dairy, representative of most western dairy operations. In this relatively new and modern facility, approximately 3000 cows are housed in freestall naturally ventilated barns with open walls. Silage piles are used as forage in dairy rations; placed aside and near other feed storage structures. The layout of these structures allows forming a feeding center.

The feed (total mixed ration-TMR) is a mixture of various components formulated to provide the optimum amount of energy and nutrition to the animals at the dairy. Silage is the largest component of the TMR. Typically, there are few different forage piles located at the dairy. Except for the vertical open-face, silage piles are covered with black plastic sheet and sealed along the sides. Tires are used for holding plastic tightly against the top surface of the pile. This helps to prevent silage spoilage, due to air exposure, and reduces emissions.

Typically, 6-12 inches of forage are removed from the face of the pile daily leaving this open part of the pile exposed to ambient air. All forage samples (corn, alfalfa, and cereal silages) within the dairy were collected early in the morning, right after a new portion of silage was removed. High moisture ground corn pile was not covered and samples were also collected immediately after new portion was removed for the TMR preparation. Other piles of feedstuff (almond hulls and almond shells) were covered for sun and rain protection (roof only, no walls structure) and their samples were collected in a similar manner.

Various feed components are loaded into a large truck where they are mechanically mixed and delivered to the animals. This operation normally takes place twice each day. The TMR samples were collected as soon as it was delivered to the animals. Large plastic bags (doubled to avoid emissions leakage) were tightly closed and immediately transported to campus.

Experiments were conducted in an Environmental Chamber (4.4m x 2.8m x 10.5m) at the Department of Animal Sciences, University of California at Davis. Background and

inlet air samples were collected throughout all experiments.

Approximately 40-70 kg of silage or other feed sample were placed in large round shape bin (diameter 1.92 m) located in the center of the chamber and spread to a depth of approximately 30 cm. Chamber door was closed and sealed. All major experiments were conducted in duplicates.

Multiple air samples from the chamber outlet port were collected using 6 L SUMMA ® passivated stainless steel canisters from two manufactures: TO-Cans from Restek (110 Benner Circle, Ballefonte, PA) and Model S6L-G AeroSphere sampling canisters from LabCommerce Inc. (San Jose, CA). Canister sampling could be performed in two modes: either grab or time integrated sampling (up to 24 hours). Sampling procedures, canisters cleaning and preparation were performed according recommendations of EPA method TO-15 for the determination of toxic organic compounds through analysis of ambient air samples collected in specially-prepared canisters which are further analyzed by GC/MS (US EPA, TO-15 method) and the Laboratory Standard Operating procedures for ambient air analysis used by the California Air Resources Board (CARB, SOP MLD 059).

Results and discussion

A total of 24 compounds were identified and quantified from silage and TMR emissions. These included 6 alcohols, 5 VFAs, and 13 carboxylic acids esters. Alcohol emissions from all silages and TMR were the dominant VOCs, with ethanol concentrations being the highest among all emitted alcohol compounds. The highest concentrations of ethanol and propanol were detected from corn silage. Significant concentration of 2-butanol was also detected from corn silage. In addition, low concentration of isopentyl alcohol was measured from corn and cereal silages. Emission fluxes of hexanol were detected from all silage samples at relatively small concentrations and not quantified. Corn silage was found to emit the highest concentration of alcohols.

Volatile fatty acids were identified as the second most abundant group of compounds emitted from silages and TMR, with acetic acid having the highest concentration within VFA emissions. High concentration of acetic acid observed in our experiments could be correlated to its presence (up to several percent by mass) in silage (Danner et al., 2003, Kung and Shaver, 2001). Propionic, isobutyric, butyric, and isovaleric acid emissions were also detected and quantified from the alfalfa silage and TMR. These findings are consistent with recently reported data on the evaluation of non-enteric emission fluxes of VFAs from five different locations including silage and TMR (Alanis et al., 2008). Similar to our results, the emissions of acetic acid were found to be higher (1-2 orders of magnitude depending on the source) from all selected sources among all measured VFAs (Alanis et al. (2008). Further, in our chamber experiments, the VFA emissions from alfalfa silage

and TMR were also measured using sorbent tubes method described in details by Sun et al. (2008). In these experiments we have detected fluxes of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acids. It is important to underline that despite the relatively high concentrations of emitted VFAs from dairy silages found in this report and study conducted by Alanis et al. (2008), these compounds are known to have insignificant effect on ozone formation (Carter, 1994).

A wide variety of carboxylic acids esters have been identified and quantified in addition to alcohols and VFAs emitted from silages and TMR. The emitted propyl acetate, propyl propionate, as well as ethyl, propyl, and butyl esters of butyric acid had the highest concentrations for corn silage. The highest concentration of ethyl acetate was detected from cereal silage. The composition and concentrations of identified emitted esters varied significantly among tested silage and TMR samples. Corn silage was found to emit the widest range and highest concentrations (except for ethyl acetate) of carboxylic acids esters.

Emission of only several VFAs and propyl propionate was detected from dry food components (almond hulls and almond shells), with their concentrations being below the quantification limit.

The results show that the majority of VOCs identified in the environmental chamber experiments were oxygenated compounds with alcohols being the major contributors. Total concentration of alcohols was found to vary in the range of 500-600 ppb from the TMR, alfalfa silage, and high moisture ground corn to approximately 1.7 and 2 ppm from corn and cereal silages, respectively. Among alcohols, ethanol was the most abundant throughout measurements of all silages and TMR. Besides ethanol, significant concentrations of propanol and other isomers of C3-C4 alcohols were also detected with the highest concentration emitted from corn silage. Ethanol is expected to be a dominant VOC compound since it is produced by yeast fermentation of the plant material as part of ensiling process. The combined alcohols (excluding methanol) accounted for over 80% of the total VOCs emissions measured by the canisters analyses, with the ethanol concentration alone exceeding 70% and 90% of total alcohols emissions for silages, TMR and high moisture ground corn, respectively. The ethanol emissions from cereal silage were determined to be the highest, followed by emissions from corn and alfalfa silages. However, the variability in emissions of alcohols in general and ethanol emissions in particular could vary significantly due to number of factors. In general, silages made from grass and winter grown cereals with lower carbohydrate content are expected to produce less ethanol than corn and grain silages. Furthermore, silage preparation methods, different additives, management style, climate, and ambient conditions could contribute to the variability in emissions.

In addition, the density of silage piles could also play an important role. The plant material during silage production is compressed to the point where no oxygen is present and anaerobic conditions are established that promote the growth of autochthonous lactic acid bacteria (Neureiter et al., 2005). The microbial conversion of free soluble carbohydrates into lactic acid and the resulting decrease in pH prevents the growth of undesirable microorganisms. In case of incomplete compression, the amount of oxygen could be sufficient for yeast and ferment carbohydrates to ethanol. This could be an indication of poor quality of silage.

Therefore, our experiments have demonstrated that levels of alcohol emissions from different silage types vary significantly and determine total VOC emissions.

Since silage is typically the largest component of TMR, significant but lower ethanol fluxes were detected from TMR. Alcohols are very volatile and rapidly vaporize during preparation and distribution of TMR. While the alcohols and other VOC emissions are lower from the TMR compared with silage, the feed (TMR) typically spread over a much larger area than the silage pile face. Thus, because of the larger surface area the potential for emissions from TMR could be greater than from silage.

Conclusions

This research has demonstrated the diversity of VOCs emitted from various types of silages and other feed sources. The measurements indicated that open-face silage piles are likely a significant source of VOCs in California dairies. The bulk of emitted compounds identified here are oxygenated VOCs in which alcohols are dominant, and known to have a small impact on ozone formation. However, emissions of alkenes, alkynes, diene compounds, and aldehydes from silage, which were identified and quantified here, could make a significant contribution to ozone formation. The atmospheric implications of these findings may include effects on the local air quality in agricultural areas. Comprehensive measurements of fluxes of a suite of oxygenated VOC emitted from assorted dairy feed sources are needed to assess their importance in regional chemistry.

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References are available from the corresponding author upon request.

Volatile organic compounds (VOC) in maize silages at German dairy farms

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Keywords ethanol, ethyl ester, maize silage, volatile organic compound, farm silage

Introduction Volatile organic compounds (VOC), e.g. alcohols, organic acids and esters thereof, are frequently found in silages (Weiss et al., 2009; Weiss and Auerbach, 2012), and empirical evidence shows negative effects on feed intake by dairy cows. The aim of this survey was to investigate the incidence of VOC in maize silages from German dairy farms and to monitor the concentrations of ethanol, n-propanol and the corresponding esters ethyl acetate, ethyl lactate and propyl acetate, depending on the sampling site in the silo and the compaction of silages.

Materials and methods The survey was carried out in the northern German State of Schleswig- Holstein. It included a detailed examination of silages stored in bunker silos on 52 dairy farms. Most silages were produced without silage additives (n=43), whereas 9 farms had used biological additives. A total of 155 samples were taken from the cutting face during feed-out from the silage core (middle and bottom cores) and from the top edge areas. Silages were analyzed for fermentation characteristics (pH, acids, alcohols, esters, water-soluble carbohydrates, ammonia) using methods described by Weiss and Auerbach (2013). The dry matter (DM) content, yeast counts and aerobic stability (AS) were measured by standard methods. Before coring, silage density was evaluated by means of a horizontal sample borer. Data were subjected to statistical analysis by employing PROC MIXED of SAS.

Results and discussion The highest contents of fermentation acids (acetic, lactic and propionic acids) and alcohols (methanol, ethanol, n-propanol) in maize silages were found in the bottom, highly compacted core and to some extent in middle core samples taken from bunker silos (Table 1), which supports empirical observations by Weiss et al. (2009). Ethanol was detected at up to 17.8 g/kg DM and the highest n-propanol level was 20.2 g/kg DM. In agreement with data by Weiss et al. (2009), ethyl lactate (EL) concentrations in maize silages were higher than the levels of ethyl acetate (EA) and propyl acetate (PA). The contents of total esters (up to 925 mg/kg DM) were higher than in silages from laboratory ensiling trials (Weiss et al., 2009). With increasing compaction, the concentrations of n-propanol and ethanol as well as those of the ethyl esters EA and EL (Figure 1) and aerobic stability ($r^2 = 0.920$, $P<0.001$) increased (data not shown). This may be explained by the usually lower pH in the bottom, more compacted and less air-affected zones in farm silos. Esterification processes were shown to be stimulated by low pH (Weiss and Auerbach, 2013).

Conclusions The results demonstrate the occurrence of VOC in maize silages on dairy farms. Sampling site affects the concentrations of alcohols, acids and esters. Elevated levels of VOC, especially alcohols and esters, occur in well-compacted silages stored in bunkers.

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Table 1 Fermentation characteristics of maize silages on 52 German dairy farms in different sections of bunker silos (mean ± SEM, g/kg DM unless otherwise stated)

Parameter	BC[4]	MC[5]	TE[6]	*P-Value*
DM (%)	34.1 ± 0.5	33.4 ± 0.4	34.0 ± 0.5	*0.950*
pH	$3.85^{a,b}$ ± 0.18	3.83^{a} ± 0.02	3.89^{b} ± 0.03	*0.036*
Lactic acid	49.3^{b} ± 2.6	51.4^{b} ± 1.9	41.8^{a} ± 2.1	*0.001*
Acetic acid	23.0^{b} ± 1.2	19.5^{a} ± 0.9	19.6^{a} ± 1.0	*0.009*
Prop. acid[1]	0.8^{b} ± 0.2	0.4^{a} ± 0.1	$0.6^{a,b}$ ± 0.1	*0.028*
Methanol	0.3^{b} ± 0.0	0.2^{a} ± 0.0	0.3^{b} ± 0.0	*0.008*
Ethanol	6.9^{b} ± 0.5	$5.9^{a,b}$ ± 0.4	5.1^{a} ± 0.4	*0.001*
2-Butanol	0.2^{b} ± 0.1	$0.2^{a,b}$ ± 0.1	0.1^{a} ± 0.0	*0.015*
n-Propanol	4.4^{b} ± 0.7	2.7^{a} ± 0.5	2.1^{a} ± 0.4	*0.001*
Ethyl acetate[2]	$51^{a,b}$ ± 4	40^{a} ± 3	59^{b} ± 5	*0.007*
Ethyl lactate[2]	210^{b} ± 17	$176^{a,b}$ ± 15	150^{a} ± 14	*0.003*
Propyl acetate[2]	44 ± 17	30 ± 7	46 ± 16	*0.626*
Total esters[2]	305 ± 24	246 ± 18	255 ± 24	*0.080*
Ammonia	1.3^{b} ± 0.0	1.1^{a} ± 0	1.1^{a} ± 0.0	*<0.001*
WSC[3]	8.2^{a} ± 0.7	10.5^{b} ± 1.0	9.9^{a} ± 0.7	*0.001*
AS (d)	7.2 ± 4.8	6.6 ± 4.1	6.3 ± 4.2	*0.2613*
Yeasts (log cfu g^{-1} FM)	4.7^{a} ± 4.6	6.2^{b} ± 5.9	6.1^{b} ± 5.8	*<0.001*
Compaction (kg/m^3)	256 ± 5.6	226 ± 5.8	217 ± 5.9	*<0.001*

[1]Propionic acid; [2]mg/kg DM, [3]water-soluble carbohydrates; [4]Bottom core; [5] Middle core; [6]Top edge; means in rows with unlike superscripts differ at $P<0.05$ (Tukey's test).

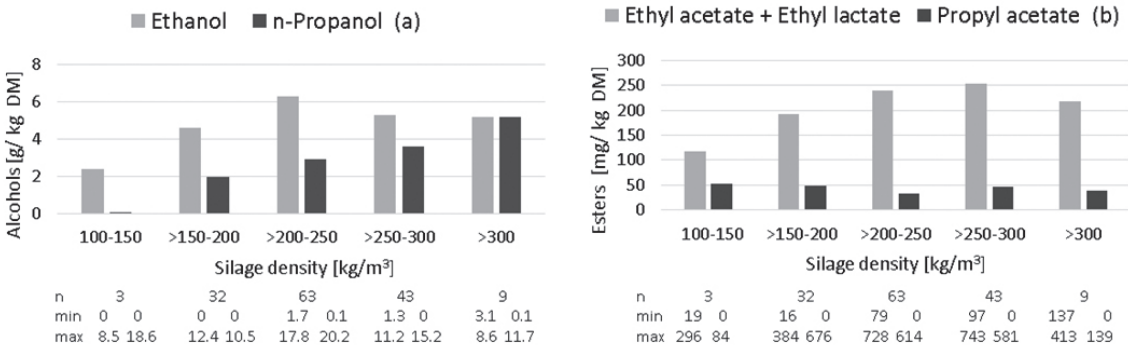

Figure 1 Average concentrations of ethanol and n-propanol (a) and the esters ethyl acetate + ethyl lactate and propyl acetate (b) as affected by silage density.

Ensiled whole sugar beets and their influence on preference and feed intake by goats

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Keywords chemical additive, ethanol, fermentation quality, ruminant, sugar

Introduction Sugar beets (*Beta vulgaris ssp. vulgaris*) are characterized by both high yields per hectare and high sugar content. Although primarily grown for sugar production, sugar beets and their by-products can also be used for biogas production and feeding ruminants. Using sugar beets continuously during the whole year as an energy-rich diet ingredient for ruminants is only possible when a safe preservation is achieved. The ensilage of fresh, whole sugar beets seems to be a promising method in terms of preserving most of the nutrients and having a conserved feed with consistent quality throughout the year. However, because of the fermentation of sugar by yeasts, high contents of ethanol can be produced during anaerobic storage. Formation of ethanol in silages is accompanied by high dry matter (DM) losses and should therefore be avoided. This study compares two different methods of conserving sugar beets and evaluates the effect of ensiled beets on DM intake (DMI) and preference when used as diet ingredient for goats.

Materials and methods Whole sugar beets were harvested and then ensiled in 120-L barrels equipped with a perforated second bottom to separate beets from effluent and a one-way valve for exit of gases. Two types of sugar beet silage were examined, one being ensiled without additive (CON, control beet silage) and one with a chemical additive (TBS, treated beet silage; 6 L/t of an additive containing 85% formic and propionic acids). After ensilage for six months, sugar beets were chopped and mixed in a total mixed ration (TMR) in different proportions. The TMR had a DM content of 439 g/kg and contained maize and grass silage, straw and soybean meal. In the first trial inclusion levels (DM basis) of 0, 5, 10, 15 and 20% TBS were examined; and 0, 6.25, 12.5, 18.75 and 25.0% CON DM were mixed in the TMR in the second trial. After mixing the different diets, they were stored anaerobically in vacuum-sealed polyethylene bags in a cooling chamber for 2-15 days (d) until fed. Ensiled sugar beets and components of the TMR were analysed for proximate constituents, fibre fractions and fermentation products. Furthermore, the microbial status of the beet silages at opening of the silos was determined. For both types of beets, one preference trial (Burns et al., 2001) was done with Saanen-type wethers (n = 10, body weight 93 ± 10.9 kg), each one lasting 15 d. During the experimental phase, each possible combination of two silages (n = 10) was offered to the goats as free choice for 3 h. Data were analyzed using the SAS procedure multidimensional scaling (MDS) and analysis of variance after averaging DMI of each diet (averaged across each combination, n = 5). Within the treatments, means were separated using the minimum significant difference (MSD) from the Waller-Duncan k-ratio t-test.

Results and discussion Both types of sugar beet silage showed a high sensory quality and a low pH of 3.5 (CON) and 3.9 (TBS). Microbial status was also good with numbers of yeasts, moulds and aerobic mesophilic bacteria being below orientation values for (maize) silages. Concentrations of lactic, acetic and butyric acids were 29, 31 and 0.3 g/kg DM for CON and 4, 9 and 0 g/kg DM for TSB. By using the chemical additive, considerable

amounts of sugar were preserved and the production of ethanol was prevented. The TSB contained 700 g sugar and 30 g ethanol per kg DM whereas CON contained 203 g sugar and 220 g ethanol per kg DM. The conversion of sugar into ethanol did not result in loss of gross energy, so both sugar beet silages had a metabolizable energy (ME) content of 14 MJ/kg DM. In the short-term preference experiment (Table 1) increasing amounts of sugar beet silage in the diet increased DMI ($P<0.05$) with a strong preference for those diets containing high amounts of sugar beets ($P<0.05$). Initial DMI after 30 minutes was 4-5 fold increased when comparing diets without (CON/TBS-0) and with the highest beet concentration. Both silages contained high concentrations of sugar (especially TBS) and ethanol (especially CON). It is assumed that DMI was positively influenced by sugar – sweet feeds are generally preferred by ruminants (Forbes, 2007) – or the associated higher concentration of ME. Ethanol had either no effect or a positive influence on feed intake, likely due to its energy content, which was also shown previously (Daniel et al., 2013). Nevertheless, as formation of ethanol is associated with excessive DM losses during storage and feed-out (volatilization), yeast activity should be impaired, for instance by applying chemical additives.

Table 1 Short-term dry matter (DM) intake of diets containing different proportions (% of DM) of control beet silage (CON) and treated beet silage (TBS) shown by ten goats (n = 40)

	CON-0	CON-6.25	CON-12.5	CON-18.75	CON-25	Mean	MSD
g/30 min	83^{c}	217^{b}	296^{b}	459^{a}	443^{a}	303	86
g/3 h	322^{d}	511^{c}	588^{b,c}	709^{a}	692^{a,b}	567	112
	TSB-0	TSB-5	TSB-10	TSB-15	TSB-20		
g/30 min	133^{c}	228^{c}	395^{b}	547^{a}	527^{a,b}	366	129
g/3 h	355^{c}	487^{c}	680^{b}	887^{a}	810^{a,b}	644	133

MSD = Minimum significant difference (Waller-Duncan k-ratio t-test)
$^{a, b}$ means within a row having different superscripts differ significantly ($P<0.05$)

Conclusions Ensiling whole sugar beets was a successful method of conservation, especially when a chemical additive was applied which impaired extensive conversion of sugar to ethanol. Both types of beet silage significantly increased preference and short-time DMI when used as ingredient in TMR for goats. Feeding ensiled sugar beets as energy-rich, highly-palatable compound to ruminants seems therefore to be promising.

Acknowledgement We thank KWS Saat AG, Einbeck, Germany for provision of ensiled beets.

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Effect of partial replacement of grass silage with faba bean whole crop silage on the rumen fermentation characteristics and plasma metabolites of dairy cows

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Keywords *Vicia faba*, *Triticum aestivum*, whole crop silage, dairy cow

Introduction Grain legumes are an interesting alternative to grass as cattle forage owing to their nitrogen fixing ability and high biomass production. The aim of this study was to investigate the effects of partial replacement of grass silage with faba bean-wheat whole crop silage on feed intake, nutrient utilization and milk production of dairy cows. Further, the effects of protein supplementation level of the silages were studied.

Materials and methods Eight multiparous Finnish Ayrshire cows, of which four were rumen-cannulated, were used in a replicated 4 × 4 Latin square study with 2 × 2 factorial arrangement of treatments. The cows were in average 100 d in milk. Experimental treatments consisted of first cut timothy-meadow fescue (*Phleum pratense* - *Festuca pratensis*) grass silage (GS) and a mixture of GS and faba bean-wheat (*Vicia faba* - *Triticum aestivum*) whole crop silage (FB) (1:1 on a dry matter (DM) basis). Both forages were fed *ad libitum* and supplemented with 13 kg/d of concentrate containing 2.0 or 3.5 kg (on air-dry basis) rape seed meal (RSM). The crude protein concentration of the concentrate was 175 or 200 g/kg DM, respectively. Rumen fermentation characteristics and plasma metabolite data were subjected to analysis of variance using the MIXED procedure of SAS® (version 9.3).

Results and discussion Forage type had no effect on DM intake, milk production or concentrations of milk constituents (Table 1) despite the lower concentration of *in vitro* digestible organic matter (678 vs 611 g/kg DM) of FB than GS. Milk fat concentration decreased with the higher RMS amount ($P < 0.05$) in both forages. Replacing GS partially with FB increased NH_3-nitrogen concentration in rumen ($P = 0.01$). Ruminal butyric acid concentrations decreased with increasing amount of RSM in GS but not in FB-GS (interaction, $P < 0.01$) (Table 1). The FB decreased plasma NEFA concentrations and increased plasma histidine, leucine and valine concentrations when compared to GS ($P < 0.05$).

Conclusions Partial replacement of grass silage with faba bean-wheat silage had no adverse effects on dry matter intake and milk production. Silage type and amount of RSM had only minor effects on rumen fermentation characteristics. Results of plasma amino acid and rumen ammonium nitrogen concentrations suggest that there might be differences in nitrogen utilization between silage types.

Table 1 Dry matter intake, milk production and rumen fermentation characteristics

	Treatments[1]					*P*-value		
Silage	GS		FB-GS					
Concentrate CP, g/kg DM	175	200	175	200	SEM	Silage	CP	Interaction
n	8	7	6	8				
Dry matter intake, kg/d	25.0	25.2	25.8	25.0	0.48	0.459	0.550	0.272
Milk, kg/d	35.5	35.4	36.1	35.8	1.02	0.226	0.565	0.760
Fat, g/kg	46.0	44.6	46.5	43.6	1.45	0.795	0.032	0.444
Protein, g/kg	35.4	35.5	35.8	35.3	0.84	0.726	0.511	0.377
Lactose, g/kg	44.3	44.1	44.4	44.7	0.30	0.138	0.816	0.322
Rumen fermentation								
pH	6.31	6.28	6.29	6.33	0.094	0.672	0.856	0.382
NH_3-N, mmol/L	6.89	6.43	9.03	9.19	1.180	0.010	0.857	0.742
Total VFA, mmol/L	103	104	105	103	3.7	0.478	0.729	0.366
Molar proportions, mmol/mol								
Acetic acid (AA)	646	647	643	645	4.1	0.421	0.633	0.853
Propionic acid (PA)	181	186	184	180	2.3	0.229	0.659	0.004
Butyric acid (BA)	130	124	131	131	3.4	<0.001	0.002	0.003
Isobutyric acid	13.2	13.2	13.5	13.8	1.00	0.647	0.829	0.85
Valeric acid	18.2	18.4	18.2	18.8	0.96	0.859	0.668	0.809
Caproic acid	6.81	6.03	6.50	5.97	0.270	0.448	0.014	0.617
(AA+BA) /PA –ratio	4.31	4.16	4.24	4.35	0.071	0.197	0.642	0.016
Protozoa, $\times 10^5$ U/mL	11.5	7.69	9.35	10.6	1.606	0.817	0.466	0.316

[1] Treatments: GS = grass silage; FB = faba bean-wheat silage; CP = concentrate crude protein concentration.

Table 2 Arterial concentration of plasma metabolites and amino acids

	Treatments[1]					*P*-value		
Silage	GS		FB-GS					
Concentrate CP, g/kg DM	175	200	175	200	SEM	Silage	CP	Interaction
n	8	7	6	8				
Glucose, mmol/L	3.84	3.87	3.76	3.81	0.071	0.208	0.443	0.754
Insuline, mmol/L	11.9	16.1	12.5	15.3	1.73	0.943	0.021	0.633
NEFA, mmol/L	0.095	0.098	0.078	0.086	0.0076	0.022	0.311	0.712
BHBA, mmol/L	0.833	0.859	0.820	0.830	0.0551	0.657	0.678	0.858
Acetic acid, mmol/L	0.095	0.102	0.097	0.093	0.0081	0.633	0.860	0.466
Arginine, µmol/L	88.3	95.8	101	98.4	6.52	0.134	0.560	0.318
Histidine, µmol/L	50.3	57.6	66.3	61.6	3.82	0.016	0.689	0.108
Isoleucine, µmol/L	121	129	130	131	5.2	0.179	0.223	0.304
Leucine, µmol/L	122	132	138	138	6.4	0.039	0.250	0.279
Lycine, µmol/L	106	109	122	113	7.1	0.099	0.660	0.297
Methionine, µmol/L	22.7	22.7	23.0	21.5	0.78	0.570	0.274	0.284
Phenylalanine, µmol/L	44.1	45.6	45.4	43.7	1.76	0.825	0.967	0.278
Threonine, µmol/L	128	122	127	123	6.6	0.998	0.185	0.844
Tryptophan, µmol/L	46.1	47.0	44.2	43.9	1.61	0.114	0.830	0.692
Valine, µmol/L	236	252	261	267	10.5	0.008	0.073	0.441
ΣBCAA	479	514	529	536	21.0	0.024	0.135	0.338
ΣEAA	964	1031	1057	1037	36.0	0.149	0.455	0.200
ΣNEAA	1269	1204	1346	1263	52.1	0.098	0.064	0.812

[1] Treatments: GS = grass silage; FB = faba bean-wheat silage; CP = concentrate crude protein concentration. NEFA = non-esterified fatty acids, BHBA = β-hydroxybutyric acid, ΣBCAA = Sum of branched chain amino acids (Ile, Leu, and Val). ΣEAA = Sum of essential AA (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, and Val). ΣNEAA = Sum of non-essential AA (Ala, Asp, Cys, Glu, Gly, Pro, Ser, and Tyr). ΣTAA = Total AA

A new *in vitro* ensiling technique for silage research

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Keywords silage, methodology, sterilization, microflora

Introduction Normal ensiling techniques do not allow separating effects of pre-ensiled microbial and chemical composition on ensiling results. Crop sterilization or artificial substrates and inoculation with specified floras could alleviate this problem. However, sterilization by gamma-irradiation (Heron et al., 1986) or using simulated silages (Woolford and Wilkins, 1975) are either expensive or have not been validated. This abstract summarizes work done at our laboratory in developing a new inexpensive *in vitro* ensiling methodology to facilitate field floral research and inoculant evaluation (Mogodiniyai Kasmaei et al., 2014). The methodology included dry-heat sterilization of dried forage, reconstitution and inoculation with original microfloras and ensiling. Validation was made by comparing ensiling results with untreated controls.

Materials and methods Samples of mixed grass species, mixed grass-clover species, white clover and maize were used in four identical experiments. The samples were collected in fall 2013, chopped and frozen until needed. Samples were thawed and inoculated with spores of *Clostridium tyrobutyricum* at a level of 10^3 cfu/g fresh matter. Samples had dry matter (DM) contents between 250 and 390 g/kg. One and half kg of each inoculated sample was then divided in two. From each forage replicate, 20 g and 50 g were sampled for microbial analysis and microbial isolation, respectively. An additional 70-80 g was compacted into glass tubes (20 cm length, 3 cm inner diameter) and sealed with water-lock. Remainders were dried at 60°C overnight to DM >900 g/kg before being milled on a hammer mill to pass a 1-mm screen. An amount of 35 g of ground samples was sterilized by heating at 103°C for 15 h in a forced-drought oven. The 50-g forage samples were macerated with 300 mL of 0.25-strength Ringer solution fortified with Tween®80 (0.5 mL/L). Forage microflora was obtained from the solution (on average 74% of added Ringer solution and sample water) by means of centrifugation (15500 × *g* for 40 min) and sterile filtration (first 0.45 and then 0.22 µm pore size filters) of the supernatant and subsequent homogenization of the pellet and filters. When reconstituting, it was attempted to maintain the ratio of microbes:forage fresh weight similar to untreated samples. The fresh weight corresponding to the isolated microflora was estimated from the volume of microbial solution. It was assumed that forage microflora was entirely removed from samples and evenly distributed in the solution after maceration. Calculated amounts of sterilized DM, distilled water and inocula were then mixed to reach to DM contents similar to untreated samples. Ensiling of 32-42g of reconstituted forages was done in sealed Falcon tubes (45 mL) fitted with water locks. Silos were opened after 60-63 d and sampled for microbial analyses, and juice was also extracted by squeezing. Microbial analyses, which were based on culture-dependent techniques, were conducted on thawed fresh,

sterilized-reconstituted and ensiled samples. Viable lactic acid bacteria, enterobacteria, yeasts and moulds and spores of clostridia were counted. The ground and the sterilized samples were analyzed for water soluble carbohydrates (WSC), buffer soluble N (BSN), acid detergent insoluble N (ADIN). Silage samples were analyzed for short chain fatty acids and alcohols by HPLC and ammonia-N by flow injection technique. Statistical analyses of data were performed on SAS (v. 9.2; SAS Institute Inc., Cary, NC, USA) using the MIXED procedure with forage as random factor.

Results and discussion The sterilization procedure reduced the counts of microbes below detection limits but also reduced WSC and BSN by 49% and 22%, respectively and increased the ADIN by 53%. It appears that the drying step before the dry-heat sterilization was not sufficient to hinder Maillard reactions and protein denaturation. Despite the chemical alteration, fermentation profiles of reconstituted silages were comparable to untreated silages. Concentrations of volatile fatty acids, 2,3-butanediol and total organic acids plus alcohols were similar between the two treatments but concentrations of lactic acid, ethanol and ammonia-N were reduced in reconstituted silages by 18%, 20% and 37%, respectively. Microbial profiles of silages were not affected by the reconstitution treatment. It is concluded that the methodology could be useful for evaluating relative effects of microfloras on fermentation quality and for inoculant research. A further improvement on the sterilization procedure is however needed to reduce the heat damage to chemical composition.

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Impact of ensiling on the traceability and authentication of foods of animal origin

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Introduction

Consumers of animal-derived foods are increasingly interested in the background origin of such foods and, for this reason, publications on food traceability and authentication are now common both in the popular and scientific literature. Traceability has been defined as "the ability to follow the movement of a food through specified stages of production, processing and distribution" (WHO/FAO, 2007). It requires a record of the various steps in the journey of a food from its site of production to consumption and "each link requires keeping records of preceding and succeeding links" (TRACE, 2010). Traceability systems depend on the maintenance of accurate records, paper- or computer-based, but they can be open to error or fraud. Authentication, defined as "the process by which a food is verified as complying with its label description" (Dennis, 1998), is therefore necessary to support traceability systems and to prove beyond doubt that a particular food product is as is stated on the product label. Thus the concept of an "authenticity based traceability system" has emerged (TRACE, 2010).

Foods of animal origin, such as dairy and meat products, can have a particular value associated with the production system, e.g. grass or pasture-based, from which they derive (Enser et al., 1998; McCluskey et al., 2005). Producers increasingly seek to maximise the market potential of such products, based on the nutritional profile of the products or the sustainability of the production system, for example. The same principle could be applied to food products derived from animals consuming predominantly ensiled feedstuffs. Worldwide, approximately 250 million tonnes of silage dry matter are produced annually from a range of temperate and tropical forage crops (e.g. grass, legume), whole-crop cereals (e.g. maize, wheat), other agricultural products (e.g. moist grains/cobs, beet leaves, beet roots, damp straw) and industrial by-products (e.g. beet pulp, brewers grains, distillers grains). The silages are used mainly as a feed for ruminants producing meat/milk for human consumption. Is there a unique and valuable compositional 'signature' associated with such dairy and meat products? If so, is there potential to authenticate these products? This paper sets out to answer these questions as they pertain to dairy and meat products from animals fed ensiled feedstuffs.

The tools for authentication

Authentication of meat and dairy products from different animal production systems has, not surprisingly, focussed on the measurement of components in these products that derive directly or indirectly from animal feedstuffs (Vasta and Priolo, 2006; Prache, 2009). One approach is to measure components that directly reflect the diets consumed by the animals. These include the measurement of components at a molecular or elemental level, including fatty acids (Alfaia et al, 2009) volatile organic compounds such as terpenes and phenolics (Priolo et al., 2004), carotenoids (Prache et al., 2003), vitamin E (Röhrle et al., 2011a), stable isotope ratios (Heaton et al., 2008) and trace elements (Franke et al., 2008). A second, 'fingerprint', approach can be taken whereby non-invasive spectroscopic

techniques are used to determine differences in the optical properties of foods derived from different production systems (Prache, 2009). Furthermore, molecular genetics techniques can be used to study the impact of production systems on gene expression (Hocquette et al., 2009). Comprehensive reviews of these methodologies as they apply to authentication of foods are available (Lees, 2003; Luykx and van Ruth, 2008; Sun, 2008; Primrose et al., 2010). The methods involve chromatography (GC, HPLC), isotope ratio mass spectrometry (IRMS), spectroscopy (IR, NMR, UV, fluorescence, Raman), molecular genetics (DNA and PCR-based) and enzymatic techniques. In addition to the measurement techniques themselves, appropriate statistical analysis of the data collected is critical in establishing whether a particular product is authentic or not.

Fatty acids

The influence of dietary forage on the fatty acid composition has been recently reviewed for beef (Daley et al., 2010, Shingfield et al., 2013, Scollan et al., 2014) and for milk (e.g. Dewhurst et al., 2006; Lourenco et al., 2008). The findings of the large number of studies now available are generally consistent. Thus, feeding fresh grass compared to concentrates, results in higher concentrations of n-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid in muscle and milk lipids. Despite some loss of alpha linolenic acid (18:3 n-3) during the ensiling process, feeding grass silage compared to concentrates still results in higher concentrations of n-3 PUFA and CLA (French et al., 2000; Scollan et al., 2006). The concentrations of these fatty acids therefore have potential as biomarkers of grazed/ensiled pasture or concentrate feeding of ruminants.

With respect to the type of silage, wilting of grass prior to ensiling did not negatively impact on the n-6: n-3 PUFA ratio, but increased the CLA concentration of beef (Noci et al., 2007) while restricted and extensive patterns of fermentation during ensilage of pasture did not influence the fatty acid composition of beef from cattle fed the resulting silages (Moloney et al., 2002). Feeding mixtures of grass and white clover silage compared to grass silage alone increased the deposition of both n-6 and n-3 PUFA in bovine (Scollan et al., 2002). In contrast, animals fed red clover silage, grass silage, or a 50:50 mix of the two, had an increasing content of PUFA in muscle with increasing red clover silage content (Richardson et al., 2005). Substitution of grass silage with maize silage or whole-crop wheat silage led to a decrease in the proportion of n-3 PUFA in beef reflecting the lower concentrations 18:3 n-3 in maize silage (Moloney et al., 2013).

Dewhurst et al. (2006) reviewed the effects of clover silages on fatty acids in milk. In comparison with milk from cows fed grass silages, both red clover and white clover silages led increases in the proportion of 18:3 n-3. Van Dorland et al. (2008) included red clover silage or white clover silage at 40% of forage dry matter and increased the 18:3 n-3 proportion from 0.9% of milk fatty acids (grass silage control) to 1.04% (red clover silage) and 1.14% (white clover silage). In a meta-analysis of eight published studies, Steinshamn (2010) statistically confirmed the above findings and reported an average increase in milk 18:3 n-3 proportion from 0.53 to 0.91% due to feeding red clover silage compared to grass clover silage. They found no statistical difference between white clover and red clover silage. Vetter and Schroeder (2010) attributed higher levels of phytanic acid and its degradation product pristanic acid, in organic dairy products compared to conventionally-produced dairy products, to the predominant use of grass-based feedstuffs in organic production. These authors set a target value of at least 200 mg phytanic acid/100 g lipid for the verification of grass-fed, organic dairy products. However, this assumes that all conventional production is ‘less’ grass-based and uses diets that are sufficiently different

from the organic diets.

In general, the differences in the concentrations of individual fatty acids in meat and milk due to ensiling *per se* or to different types of silage, while often statistically significant are rather small compared to the differences between pasture and concentrate-based rations and are unlikely to be better biomarkers than others considered in this paper. An additional potential limitation to the use of specific fatty acids as biomarkers is that consumption of fatty acids from different sources may give similar concentrations in food e.g. for food products of grassland production, inclusion of non-grass sources of fatty acids could give 18:3 n-3, 18:2 n-6 or CLA contents in meat and milk similar to those derived from grass (Shingfield et al., 2013). Use of the full fatty acid profile to develop a specific "fingerprint" of the diet might overcome this limitation. Using this approach (a stepwise forward discriminant analysis to select the most relevant variables for classification followed by a canonical discriminant analysis), Alfaia et al. (2009) achieved a 100% correct classification of beef from bulls finished on concentrate, on pasture or on pasture followed by concentrate. In a recent study (Roehrle et al., 2015), a discrimination model of muscle fatty acid data permitted differentiation of beef from animals raised on grass, a barley-based concentrate or on grass/concentrate combinations over a 12 month period with a correct classification of 92.9 %. The miss-classified samples related to beef from animals raised on pasture for 12 months prior to slaughter being classified as beef from animals fed grass silage for six months followed by grass at pasture for six months. This is probably the most challenging scenario i.e. can beef be distinguishing between cattle fed grass silage or pasture earlier in life? Effectively, however, both groups could be considered grass-fed, since the silage is ensiled grass; therefore the miss-classification is not of major significance from the perspective of grass-fed beef authentication and pooling these groups together gave 100 % correct classification of beef according to diet. When beef from cattle finished on concentrates, or grass silage, maize silage or wheat silage (alkalage) each supplemented with three kg of concentrates, 83% of wheat silage beef samples were correctly classified but 17% were classified as maize silage-fed while 14% of grass silage-fed beef samples were classified as concentrate-fed (Raes et al., 2006). Similarly, using the fatty acid profile yielded a correct classification of 80% when maize silage replaced cereal in the finishing ration of bulls (Martinez Martin et al., 2013). Either different or additional biomarkers would be required to achieve 100% correct classification for these silage-based rations.

The use of the full fatty acid profile has also been used to distinguish fully retail organic bovine milk from conventional milk (Molkentin and Giesemann, 2007; Capuano et al., 2015). However in the latter study, organic milk could not be clearly distinguished from milk that was labelled as "pasture" milk. Using the full fatty acid profile, Engel et al. (2007) were able to distinguish between milk from cows fed <25% corn silage and those fed >30% corn silage. Similarly, Hurtaud et al. (2014) compared the fatty acid profiles of milk from different farming systems i.e. a feeding system based on "herbage" (fresh and preserved), a feeding system based on corn silage and a feeding system based on corn silage supplemented with flaxseed (a source of linolenic acid). All milk samples from "herbage" and corn silage plus flaxseed were correctly classified and 98% of milk samples from the corn silage were correctly classified. Povola et al. (2012) using a similar approach showed promising separation of cheese made from milk of cows that grazed *Trifolium alpinum* or *Festuca nigrescens*.

Volatile organic compounds

Among the volatile components in meat influenced by the diet of animals are branched chain fatty acids, lactones, aldehydes, phenolic compounds, indoles, 2,3-octanedione, terpenes and sulphur compounds (Vasta and Priolo, 2006). Of these compounds some are directly incorporated into tissues from the diet while others, for example certain sulphur compounds and lipid oxidation products, are indirect markers of dietary background because they are generated by reactions, involving the absorbed dietary compounds, that occur during cooking of meat fat. For example, Vasta et al. (2007), using dynamic headspace GC-MS, identified 33 significant compounds (from a total of 114) which contributed to discrimination of concentrate *vs* pasture-fed lambs. In a study of beef from animals raised at pasture, on concentrates or on grass silage/pasture/concentrate combinations, skatole, 3-undecanone, cuminic alcohol and 2 methyl-1-butanol were identified as compounds that allowed discrimination between beef from animals fed grass at pasture or on grass silage/pasture/concentrate combinations (Vasta et al., 2011) (Figure 1). The results suggest that the "chemical memory" of a silage-based diet persisted over a long period (six months) during which the animals were fed either pasture or pasture plus concentrate.

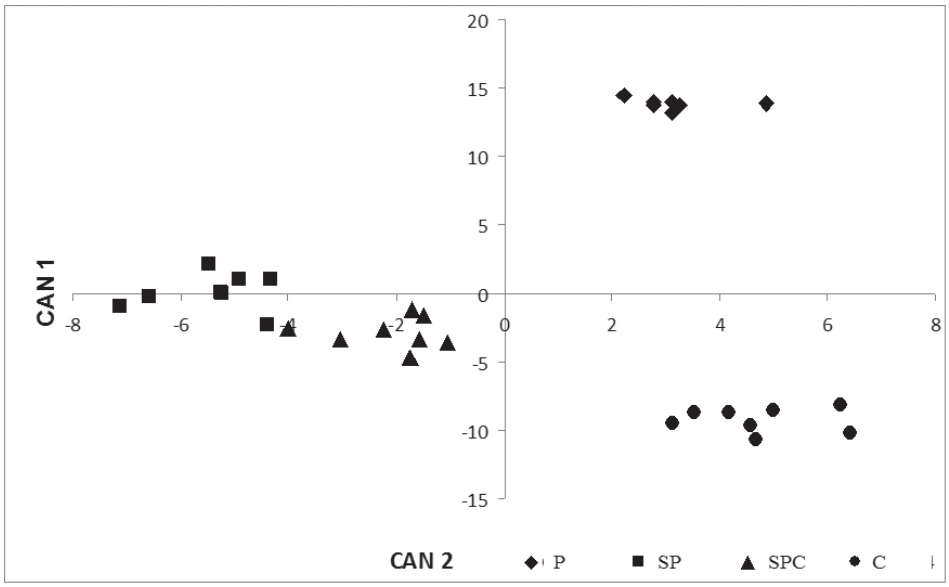

Figure 1 Discrimination of dietary background on the basis of canonical discriminant analysis using 16 volatile organic compounds identified in beef from animals fed grass at pasture for 12 months (P); grass silage for 6 months (November-April) followed by grass at pasture for 6 months (SP); grass silage for 6 months (November-April) followed by grass at pasture supplemented with a barley-based concentrate (0.5 of total dry matter) for 6 months (SPC); or a barley-based concentrate for 12 months (C) (from Vasta et al., 2011).

Stable isotopes

In studies attempting to relate foods of animal origin to the diet consumed by the animal, or to its geographical origin, stable isotope ratio analysis (SIRA) has found widespread usage and been shown to be of immense value. SIRA involves the measurement, usually using IRMS, of ratios of naturally occurring stable isotopes of bioelements, mainly carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), hydrogen (D/H or $^{2}H/^{1}H$), oxygen ($^{18}O/^{16}O$), and sulphur ($^{34}S/^{32}S$) and expression of results as delta (δ) values in per mil

(‰) units as $d^{13}C$, $d^{15}N$, $d^{2}H$ (δD), $d^{18}O$ and $d^{34}S$, respectively (Kelly, 2003). It is now well established that the composition of the diet of animals influences the stable isotope composition of these bioelements in animal tissues (De Niro & Epstein, 1978); therefore SIRA of animal tissues, or foods such as milk or meat derived from animals, can provide information about the diet consumed by animals. For instance, in pecorino cheese, Manca et al. (2001) used $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in cheese from three regions of Italy for regional designation, with differences being attributed to differences in farm production practices between regions. In cow's milk, Renou et al. (2004) demonstrated differences in $^{18}O/^{16}O$ between milk of animals raised in two regions of France (Brittany *vs* the Massif Central). They also showed differences between milk in the Massif Central produced in spring from pasture, in winter from grass silage and in winter from hay, but in Brittany there was no difference between milk produced in winter from maize silage and in winter from hay.

In meat, SIRA has also been shown to be particularly useful in the assignment of dietary background (Piasentier et al., 2003; Bahar et al., 2008). Piasentier et al. (2003) reported $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in lamb samples from six European countries, encompassing three broad production systems. The potential for multi-element stable isotope analysis (C, N, H, S) to discriminate between lamb sourced in different parts of Europe has been demonstrated (Camin et al., 2007). The influence of production system was clearly evident, with lamb samples from island pasture-based sources on the western seaboard of Europe (Ireland and the Orkney islands) clustering separately, mainly on the basis of their grass-based production systems and lower $^{13}C/^{12}C$ ratios, from samples originating in mainland Europe, where supplemental cereal or maize-based feed inputs are more common (Figure 2).

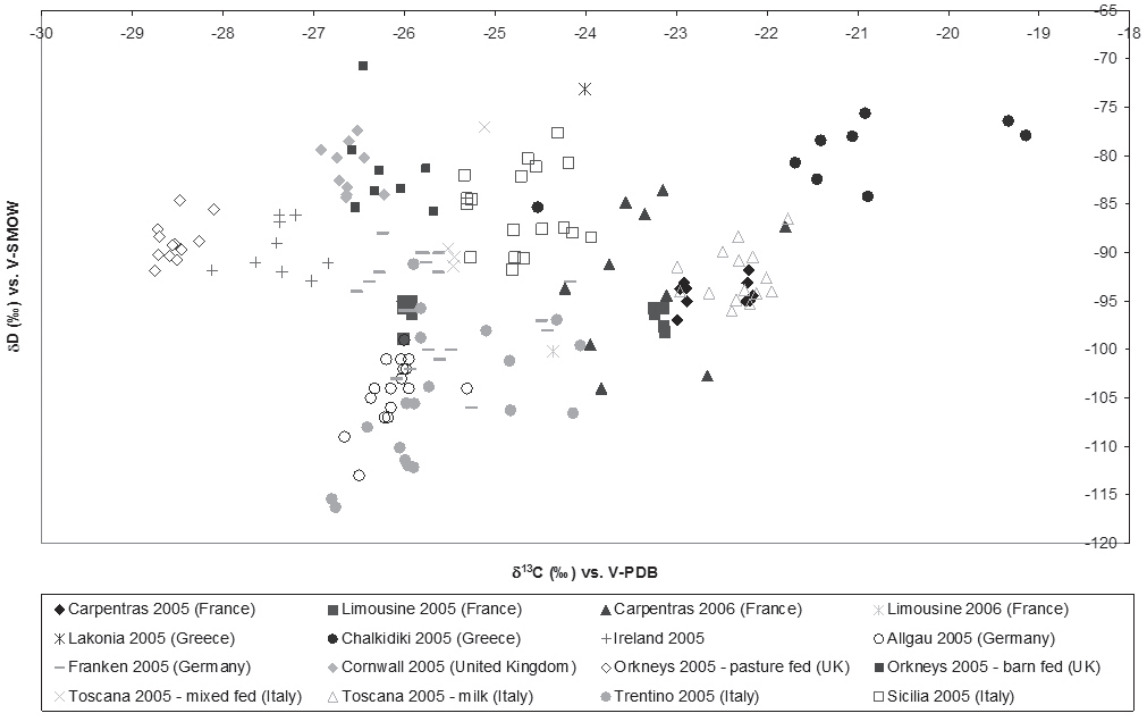

Figure 2 Carbon and hydrogen isotopic ratios of lamb from animals reared in different regions of Europe in 2005 and 2006 (from Camin et al., 2007).

The underlying reason for large differences in $d^{13}C$ values in meat from different production systems is often the different proportions of C3 and C4 plant species in the diets of animals (Smith and Epstein, 1971). For example, beef from cattle consuming a predominantly C3 (grass silage) diet ($d^{13}C = –29.6$) was clearly distinguishable from that of animals consuming a predominantly C4 (maize silage) diet ($d^{13}C = –11.8‰$) (Figure 3) (Bahar et al., 2005). The $d^{15}N$ values of the diets (8.1‰ and 3.3‰ in the grass and maize silages, respectively) were also clearly reflected in those of the beef.

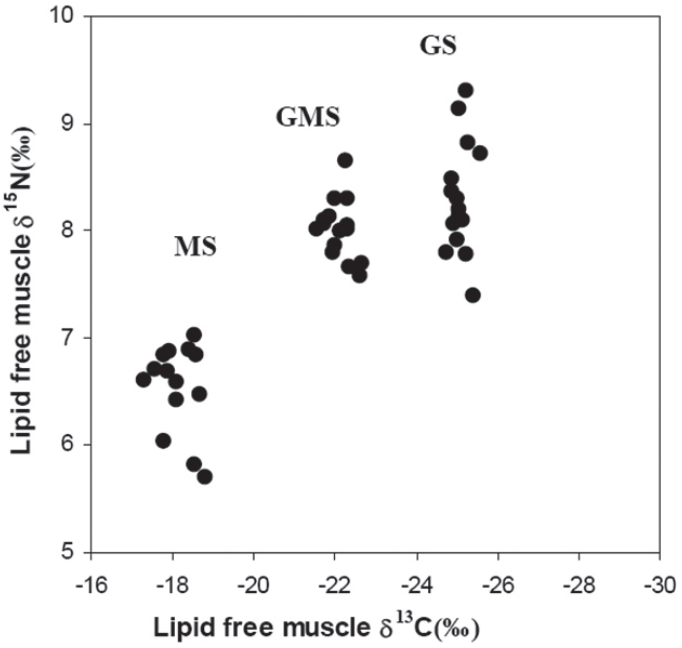

Figure 3 $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of bovine muscle of animals fed *ad libitum* as follows: grass silage (GS), maize silage (MS) or an equal mixture [dry matter (DM) basis] of grass silage and maize silage (GMS). In addition, all animals received 3 kg concentrates (composition per kg: 310 g citrus pulp, 460 g barley, 160 g soybean, 50 g molasses and 20 g mineral/vitamin mixture) daily (from Bahar et al., 2005).

Distinguishing meat or dairy products from animals receiving different diets, but with similar stable isotope ratios (e.g grazed grass vs grass silage), is more challenging. Nevertheless, even if the dietary isotopic spacing is small, for example barley *vs* grass-based diets (both C3 plant species) in which a dietary spacing of 2 to 3‰ in $d^{13}C$ values exists, it is possible to discriminate between beef from animals raised on the these different diets (Monahan et al., 2006; Moreno-Rojas et al., 2008; Osorio et al., 2011) (Figure 4).

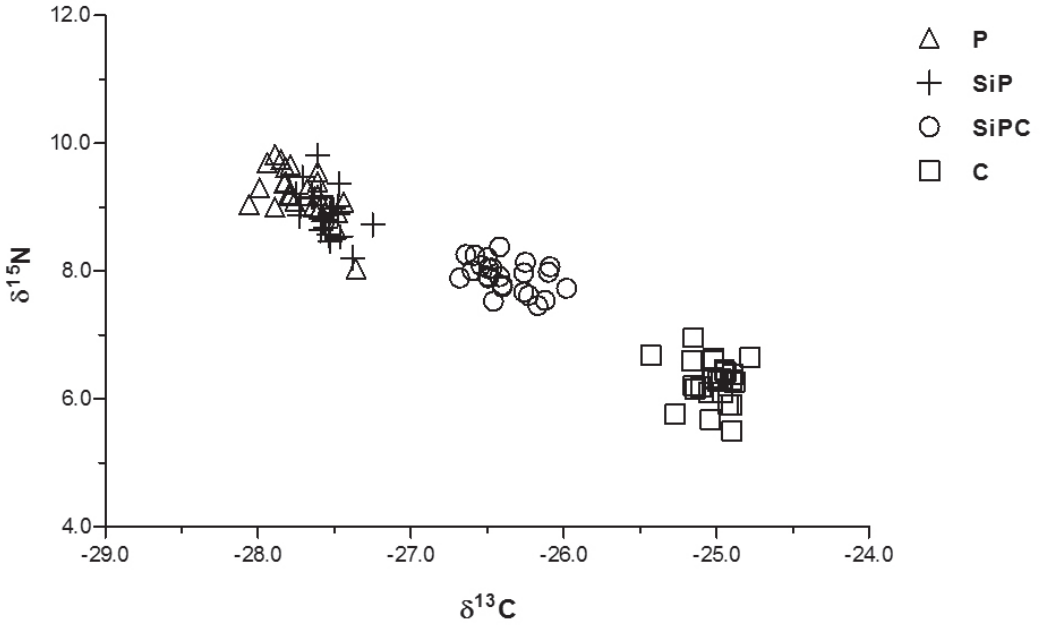

Figure 4 $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of muscle of beef cattle fed grass at pasture for 12 months (P); grass silage for 6 months (November-April) followed by grass at pasture for 6 months (SiP); grass silage for 6 months (November-April) followed by grass at pasture with a barley-based concentrate (0.5 of total dry matter) for 6 months (SiPC); or a barley-based concentrate for 12 months (C). The $d^{13}C$ values of feedstuffs were –30.9, –29.2, and –27.9‰ for the pasture, silage and concentrate, respectively. The $d^{15}N$ values of feedstuffs were 6.4, 5.0 and 3.4‰ for the pasture, silage and concentrate, respectively (from Osorio et al, 2011).

While beef from animals fed either grass at pasture (P) or ensiled grass (SiP) for six months was analysed in the study of Osorio et al (2011) (Figure 4) both groups were fed grass at pasture for a subsequent six month period prior to slaughter thus 'diluting' the impact of the silage feeding on the stable isotope profile of the beef. When canonical discrimminant analysis was applied to the stable isotope data from this study, 87% of beef samples from the four dietary treatments (P, SiP, SiPC and C) classified correctly, with the miss-classified samples all falling between the P and SiP treatments. Had the SiP group instead received grass silage for the entire duration of the study to slaughter, the likelihood is that the miss-classification would not have occurred, i.e. the comparison would have been between beef from animals fed entirely grass at pasture or entirely grass silage. The likelihood of obtaining a different isotopic signature in beef from the grass *vs* grass silage fed animals is supported by the clearly different stable isotope profiles of the grass at pasture and the grass silage (Table 1). However, it is important to note that in comparing the stable isotope composition of grass and grass silage seasonal differences in the stable isotope profile of grass, grazed or ensiled, need to be considered (Osorio et al., 2011). Thus differences in stable isotope ratios between grass and grass silage may reflect differences in the time of sampling, stage of grass growth or duration of ensiling. In addition, comparisons of the water fractions of both the feedstuffs (e.g. grass silage) and the animal derived foods (milk and meat products) may be more useful; Sun et al. (2014), in a comparison of the water fraction of grass at pasture and silage demonstrated a ~5‰ and ~60‰ difference in $d^{18}O$ and $d^{2}H$ values, respectively. The authors also showed that

the atmospheric conditions during exposure (relative humidity, exposure time, and isotopic composition of the air vapour) in the feed vessel strongly affect the isotopic composition of silage water ingested.

Table 1 Isotopic composition of the grass and grass silage dry matter (mean ± SD) (from Osorio et al., 2011)

Dietary components	δ^{13} C (‰)	δ^{15} N (‰)	δ^{2} H (‰)	δ^{34} S (‰)
Grass	–30.9 ± 0.8	6.4 ± 1.8	–128.8 ± 7.8	4.9 ± 3.0
Grass silage	–29.2 ± 0.3	5.0 ± 1.0	–142.2 ± 7.6	3.9 ± 0.5

Optical properties

Visible reflectance spectroscopy has been used to distinguish between meats produced under different animal production systems, including pasture *vs* cereal-based systems. Prache and co-workers used reflectance spectroscopy in the visible region (450-510 nm) to discriminate between lamb production systems (Prache and Theriez, 1999; Prache et al., 2009) and advocate measurements on both adipose tissue reflectance and carotenoids in blood to lower the likelihood of miss-classification (Prache et al., 2003). With regard to the potential to discriminate between food products from animals consuming grass at pasture *vs* grass silage, differences in the carotenoid composition of these feedstuffs may be useful. For example, the carotenoid content in cut (zero-grazed) grass was found to be higher than in the grazed grass (Serrano et al., 2006) and this has been attributed to the fact that cut grass may consist of herbage taken at a stage of growth when it is particularly rich in carotenoids. In our laboratory we found a 1.8 fold higher mean β-carotene level in grass silage compared to the mean for pasture sampled monthly over a one year period (Röhrle et al., 2011b).

In agreement with the work of Prache and co-workers, findings in beef showed contrasting reflectance spectra (400-700 nm) for subcutaneous adipose tissue from animals fed pasture (P) *vs* a barley-based concentrate (C) for a 12 month period (Röhrle et al., 2011b). Furthermore, and of particular interest in the context of the current paper, subcutaneous adipose tissue from a group fed grass silage for six months followed by grass pasture for six months (SiP) was distinguishable at slaughter from that of the group fed grass at pasture for 12 months, indicating an effect of a diet consumed six months earlier on adipose tissue reflectance at slaughter (Figure 5) (Röhrle et al., 2011b).

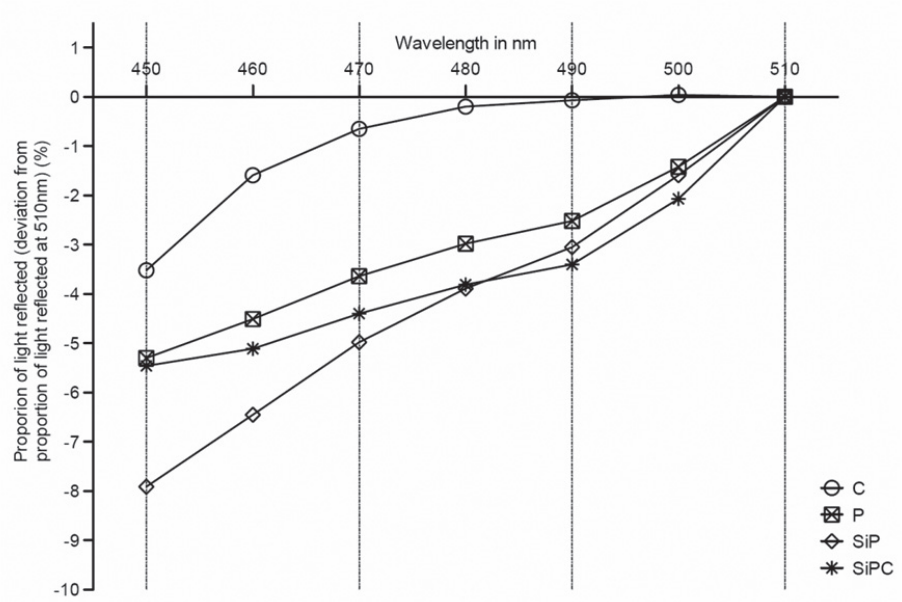

Figure 5 Mean reflectance spectra of subcutaneous adipose tissue of beef cattle fed grass at pasture for 12 months (P), grass silage for 6 months (November-April) followed by grass at pasture for 6 months (SiP), grass silage for 6 months (November-April) followed by grass at pasture with a barley-based concentrate (0.5 of total dry matter) for 6 months (SiPC) or a barley-based concentrate for 12 months (C) (from Röhrle et al., 2011b).

The reasons for these differences in reflectance spectra require further investigation. However, the β-carotene concentration in adipose tissue from the concentrate-fed animals (C) was significantly lower ($p<0.05$) than that in the adipose tissue of animals fed grass or grass silage. In addition, and particularly relevant in the context of an effect of ensiling on authenticity, the lutein concentrations differed significantly ($p< 0.05$) between all treatments, in the order P > SiP > SiPC > C (Figure 6).

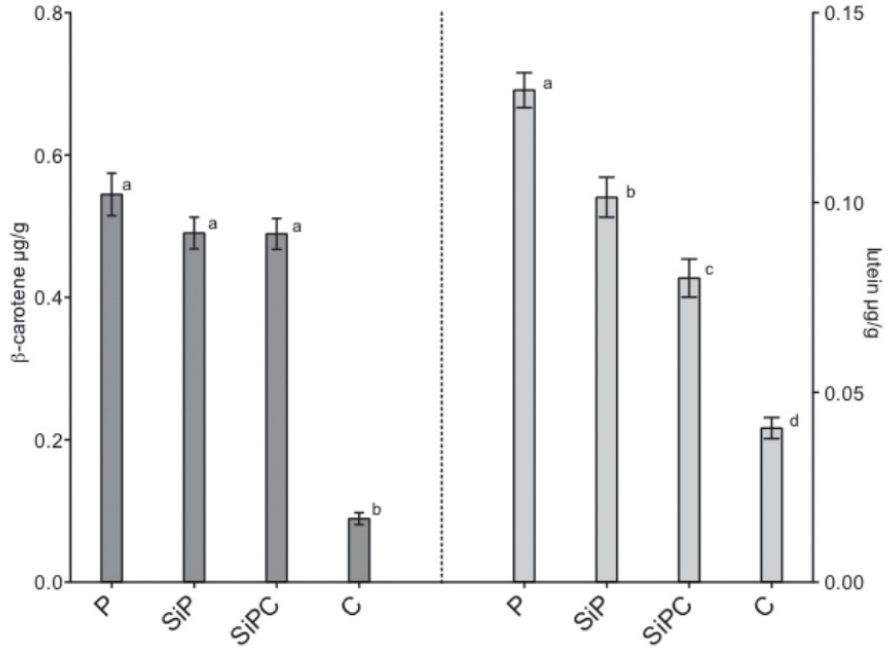

Figure 6 Mean (± sdev) β-carotene (µg g^{-1}) and lutein (µg g^{-1}) concentration in subcutaneous adipose tissue of beef cattle fed grass at pasture for 12 months (P); grass silage for 6 months (November-April) followed by grass at pasture for 6 months (SiP); grass silage for 6 months (November-April) followed by grass at pasture with a barley-based concentrate (0.5 of total dry matter) for 6 months (SiPC); or a barley-based concentrate for 12 months (C). For each treatment, bars with different letters (a, b, c, d) are significantly different ($P < 0.05$) (from Röhrle et al., 2011b).

Practical factors such as the pooling of milk and its bulk storage prior to processing pose a significant challenge in terms of authentication of milk or the processed dairy products derived from it. With regard to dairy products, Noziere et al. (2006a,b) attempted to relate the carotenoid content of milk to production system. The nature of the forage is considered the main variable influencing milk fat β-carotene (Noziere et al., 2006b) with seasonal variation, affected by the stage of growth, also a contributory factor. Reflectance measurements have been applied to milk in an attempt to distinguish between grass *vs* hay and concentrate feeding (in individual cows) – this was possible provided there was at least a 36 day interval between time of diet switch from the low carotenoid (concentrate, hay) to the high concentrate (pasture) diet (Noziere et al., 2006a).

Near infra-red spectroscopy (NIRS) has shown promise as a tool for authenticating meat or milk from grassland. Thus, Cozzolino et al. (2002) showed that using NIRS, 98% of beef samples from cattle fed exclusively on pasture were correctly classified while 86% of samples from cattle fed mainly on corn silage were correctly classified. Dian et al. (2008) examined the ability of NIRS measurement of lamb perirenal fat between 400 and 2500 nm and reported correct classification of 97.5% and 97.8%, respectively, for samples from pasture-fed and concentrate-fed lambs. In a survey of 486 bulk milk samples from 172 farms in France and Italy, Coppa et al. (2012) reported that using NIRS, 95.5%, 91.5% and 93.3% of samples were correctly classified when pasture was compared with maize silage, hay of grass silage/haylage, respectively. Valenti et al. (2013) concluded, however, that mid infra-red spectroscopy (MIRS) was better than NIRS when discriminating between milk from cows offered hay or pasture-based diets and between milk from cows

offered maize silage or pasture-based diets (97% correct classification, approximately). Osorio et al. (2009) applied Fourier transform MIRS to perirenal and omental fat from early weaned/milk replacer-fed lambs and lambs suckling until slaughter and reported 100% correct classification for perirenal fat and a 9% error rate for omental fat but this approach has not been applied to ration composition *per se*. A similar approach (Fourier transform Infrared spectroscopy) has been applied to milk from cows pasture grazing, having grass *per se* in the ration or from organically managed cows (Capuano et al., 2014) while Gori et al. (2012) further showed that Fourier transform infra-red spectroscopy coupled with artificial neural networks could distinguish the dietary regime of butters produced in the Parmigiano Reggiano cheese region in Italy.

Hyperspectral imaging provides spatial information, as regular imaging systems, along with spectral information for each point in the image, as single-point spectroscopic techniques (Kamruzzaman et al., 2015).The main advantage of hyperspectral imaging over spectroscopic techniques is that by combining simultaneous measurements of spectral and spatial information, it allows for a more detailed investigation of the sample. Hence, hyperspectral imaging offers the possibility to display hidden image information that can qualitatively or quantitatively describe the properties of tested samples usually not detected by imaging methods. Image processing techniques enable the visualization of the attributes detected by spectral analysis on a sample, making hyperspectral imaging an attractive method for food authentication (Kamruzzaman et al., 2012).

Application of multiple measurements for authentication

From among a large array of individual measurements, scientists have sought to determine, using appropriate chemometric techniques, an optimum combination of biomarkers for confirming the dietary background of animals and of the meat and dairy products derived from them. In the case of both meat and dairy products multivariate statistical approaches have been applied to data sets incorporating a number of different variables with a view to discriminating between different production systems (Osorio et al., 2013; Martin et al., 2005). For instance, a classification technique (partial least squares discriminant analysis) was used to build models which would allow confirmation of production claims for four different dietary treatments (Osorio et al., 2013). The treatments chosen reflected different beef production systems in Ireland and elsewhere, ranging from grazed grass at pasture to cereal concentrate fed indoors and combinations of grass silage, pasture and concentrate feeding. Chemometric models were created for each dietary treatment using a set of variable derived using a number of different methodological approaches (Table 2). Meat from each dietary treatment was identified with a high level of accuracy (correct classification between 90.8% and 100%) using a discriminant approach and after elimination of insignificant variables, accuracy was maintained or marginally improved (Osorio et al., 2013).

Table 2 List of 83 variables measured in the study (from Osorio et al., 2013).
* branched-chain fatty acids (i, *iso* series; a, *anteiso* series)

Carotenoids and α-tocopherol	β-carotene, lutein, α-tocopherol
Tri-stimulus colour parameters	L (lightness); a (redness); b (yellowness); C (saturation); H (Hue angle)
Stable isotope ratios	$\delta^{13}C$; $\delta^{15}N$; $\delta^{2}H$; $\delta^{34}S$
Fatty acids	C14:0; C15:0i*; C15:0a*; C14:1; C15:0; C16:0i; C15:1; C16:0; C17:0i + C16:1t9; C16:1t11; C16:1t12; C16:1c9 + C17:0a; C17:0; C17:1c9; C16:2c9,c12; C18:0; C18:1t9; C18:1t10; C18:1t11; C18:1t13; C18:1c9; C18:1c11; C18:1c13; C18:1t16; C18:1c15 + C18:2 10,14 + C19:0; C18:2 10,13 + C18:2 11,14; C18:2c11,t15; C18:2t10,c15; C18:2n-6; C18:3n-3; C20:1t9; CLA(C18:2c9,t11); C22:0; C20:3n-6; C20:4n-6; C23:0; C22:2n-6; C20:5n-3; C22:5n-3; SFA; MUFA; PUFA; PUFA:SFA ratio; n-6 fatty acids; n-3 fatty acids; n-6:n-3 fatty acid ratio
Volatile compounds	Pentanal; heptane; pentanol; n-octane; 1-octene; (E)-4-octene; hexanol; heptanal; n-heptanol; n-decane; n-octanal; 2, 3, 5-trimethyl-pyrazine; p-cymene; 2-butyl-thiophene; 2-nonanone; n-nonanal; 2-undecyne
Reflectance spectral measurements	450 nm; 460 nm; 470 nm; 480 nm; 490 nm; 500 nm; 510 nm; the integral values of the reflectance spectrum at wavelengths between 450 and 510 nm

* branched-chain fatty acids (i, *iso* series; a, *anteiso* series)

Functional genomics

Among the recent approaches with potential for use in discriminating between production systems is functional genomics, in particular transcriptome (Hocquette et al., 2009; Prache, 2009) and proteome (Shibata et al., 2009) profiling. Cassar-Malek et al. (2009), in a comparison of outdoor pasture *vs* indoor concentrate feeding of Charolais cattle, found Selenoprotein W to be under-expressed in pasture-fed animals and proposed it as a putative gene marker of the grassland system. Duckett et al. (2009) studied expression of genes involved in lipogenesis in muscle and found up-regulation of stearoyl-CoA desaturase, fatty acid synthase and Spot-14 and down regulation of signal transducer and activator of transcription-5 (STAT5) in the subcutaneous fat of grazing steers finished on a high-concentrate diet compared with a pasture only diet.

Differences in the energy density of the diets, with subsequent effects on animal growth rate and fat deposition, may contribute to differences in gene expression, especially for genes associated with lipogenesis. Using 2-dimensional electrophoresis with mass spectrometry and Western blot analysis, Shibata et al. (2009) showed that differential expression of muscle proteins, attributed to a change in muscle fibre type and changes in metabolic enzymes, occurred during the fattening period in concentrate-fed *vs* grazed cattle. To our knowledge no studies to date have investigated the effect of consumption of ensiled forages on gene expression.

Conclusions

To date little research has focussed specifically in the traceability or authenticity of food products derived from animals consuming ensiled feedstuffs. In this paper, using the limited research that has been conducted, we have sought to demonstrate that there is potential to identify unique characteristics or "signatures" associated with food from animals consuming ensiled feedstuffs. The focus of the work to-date has been on ensiled grass from temperate regions but the principles demonstrated could be applied to other crops. As stated in the introduction, ensiling applies to a broad range of crops and products produced in both temperate and tropical regions and herein lies both challenges and opportunities. The range in the content and quality of both energy and protein provided by these silages is vast, reflecting the huge array in crop production and harvesting (and processing), and silage storage and feedout conditions that prevail. In addition, they contribute a small or large proportion of the animal's dietary intake for part or most of the year. In terms of opportunities, animal production systems incorporating defined species and levels of ensiled feedstuffs are likely to yield food products for human consumption with a distinctive compositional signature that can be of value in tracing the origin of and authenticating such products.

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An overview of silage production and utilization in Brazil

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Introduction

In Brazil, most cattle production is based on grazing systems. However, forage conservation has been an important agricultural activity since the commercial production of livestock became important at the beginning of the last century (Cotrim, 1913; Alves and Silva, 1936). In tropical environments, hay production presents certain obstacles due to high humidity and frequent rainfall. Artificial dryers are expensive for farmers, and buildings are often not available. Consequently, ensilage is the main method of forage preservation in countries with hot and humid climates (Adesogan, 2009).

Since the implementation of silage as part of cattle dietary plan over a hundred years ago, no data have been published describing practices of silage production and utilization. Unlike countries in which the livestock industry is well developed, Brazil has neither an annual census of farmers nor official statistics about farming practices. The only Brazilian data on the production of silages are found in the book World Silage (Wilkinson and Toivonen, 2003); however, these data are limited to some characteristics of the state of Minas Gerais. Recently, aspects of silage making on Brazilian farms have been an important research subject (Bernardes et al., 2012a; Bernardes and Do Rêgo, 2014); these studies evaluated silage management practices, particularly on Brazilian dairy farms, and they are the basis for this article.

Crops for silage making

In Brazil, whole-plant corn (*Zea mays* L.) silage is the major forage source throughout the year in intensive systems (i.e., confinement) and during part of the year in grazing-based systems. This finding is likely due to the desirable characteristics of this species, including high ensilability, productivity, and nutritive value (Allen et al., 2003). Additionally, corn crop has been traditionally grown since the beginning of the last century (Cotrim, 1913) and is well adapted to the environmental conditions of Brazil.

Sorghum (*S. bicolor* (L.) Moench) is the second most important crop for silage production. In the last years, it has been increasingly used in Brazil and in other parts of the world (Bolsen et al., 2003; Berenji and Dahlberg, 2004; Lima-Orozco et al., 2013). The increased popularity of sorghum for dairy and beef cattle can be attributed to its desirable

nutritive value, ensilability, and agronomic traits in comparison with those of corn (Bolsen et al., 2003). Sorghum has a higher drought resistance, which is an advantage it has over corn.

Tropical grasses, especially elephant grass and cultivars of the genus *Panicum sp.,* are also considered to be important. Native to the Africa and now widespread in the tropics, the forage potential of these species was recognized in Brazil about 50 years ago, mainly because they can be used under grazing, have high dry matter (DM) yields, show excellent response to fertilizers, and are persistent. However, tropical grasses typically have low contents of DM and water-soluble carbohydrates, which result in poor fermentation and high effluent production (Catchpoole and Henzell, 1971). Wilting has been an alternative method of raising DM content and minimizing losses, but in Brazil, equipment is often unaffordable for most producers, and tropical grasses have a slow drying rate; hence, additives that have high water-holding capacity have been used in tropical-grass silages.

While ethanol and sugar production have been expanding in Brazil, the use of sugarcane (*Saccharum officinarum* L.) as cattle feedstuff has also steeply increased. Its advantages as a forage crop include: 1) a high DM yield, 2) a high sucrose accumulating capacity, 3) the harvest window occurs during the dry season of the year, and 4) the unique ability to maintain consistent quality for several months as a standing crop in the field. Both fresh-chopped and ensiled sugarcane have been used to feed livestock. Sugarcane silage, however, is susceptible to a high degree of ethanol fermentation by yeasts, leading to high losses of DM (Kung and Stanley, 1982) and net energy (Daniel and Nussio, 2011). Thus, Brazilian researchers have conducted several studies to improve sugarcane silage fermentation so that farmers may use it on a large scale.

Other species have been used as silage crops, such as oats, rye, barley, and wheat. They are grown specifically in the southern region of the country due to the favorable climatic conditions (autumn and winter with milder temperatures and rain, between 22°32’S and 33°41’S).

Silo types

At the beginning of the last century, tower and pit silos were predominant on Brazilian farms (Alves and Silva, 1936), especially because plastic sheets were not available at that time for sealing horizontal silos (Wilkinson, 2014), and the herds were much smaller than they are currently. In recent decades, the majority of farmers have bunker silo on their farms. As a major advantage, bunker silos allow for the handling of large amounts of feed very rapidly (Savoie and Jofriet, 2003). Thus, they are the preferred storage system in most large feedlot operations. Another popular method of making silage is the stack silo. In most tropical regions, farmers choose strategies that do not require

major expenses, as in the case of stack silos, where the only structural investment is the plastic film.

More recently, pressed-bag and wrapped-bale technologies have been used for storage of forage in countries where the livestock industry is well developed. Wrapped-bale is gaining popularity because it enables selling, shipping, and flexible use of silage according to specific needs (Weinberg et al., 2011). However, in Brazil, these preservation methods are much less common. The lack of appropriate equipment has been the main barrier for most farmers. Additionally, bale silage is a technology for making pasture plant silage, which is used less often on Brazilian farms, as described above.

Custom services for forage conservation

Although forage outsourcing is common in North America and Europe, in Brazil, the majority of farmers use their own equipment for growing and harvesting the silage crop. However, some producers, especially large ones, are opting to outsource rather than produce their own forages. This could be explained by the fact that ruminants, such as dairy cows, require substantial amounts of forage. Additionally, in recent years, the number of outsourcing companies in Brazil has increased. The other part of producers has borrowed equipment from neighbors, the municipal government, or cooperatives because of the lack the economic power in outsourcing services or purchasing equipment (Bernardes and Do Rêgo, 2014).

Forage harvesters

In Brazil, the majority of harvesters are pulled by a tractor. The use of pull-type forage harvesters is still very common, mainly due to the high cost of self-propelled harvesters. This scenario is different from that observed in other countries with traditions in forage conservation. According to Muck and Shinners (2001), worldwide sales of traditional ensilage machines decreased from 7,000 units in 1990 to 3,500 in 1995, whereas the sales of self-propelled harvesters increased from 1,800 to 2,500 units within the same period.

A survey carried out in Brazil (Bernardes et al., 2012a) observed that the use of traditional harvesters often results in long particles with poor uniformity; hence, this cutting pattern increases the risk of silage deterioration because the rougher particles hinder the compaction and increase oxygen penetration due to the higher porosity of the mass (Muck et al., 2003). This fact is verified by the appearance of mold on the silo working face. Furthermore, very long particles may compromise the precision of the nutritional management in dairy herds with medium to high production potential. The selection against long particles in the feedbunk by the animals (Leonardi and Armentano, 2003)

is a risk for the occurrence of ruminal disturbances (Heinrichs et al., 1999), in addition to consequent nutrient imbalance. Furthermore, poor chopping by traditional machines results in less grain being cracked or damaged. Intact kernels typically have low starch digestibility (Owens et al., 1986). Mechanical processing of grains by the forage harvester is an important method of breaking down the pericarp and exposing the starch granules to the digestion process (Roberge et al., 1998). Bal et al. (2000) showed that processing corn silage improved dry matter intake, starch digestion, and lactation performance. To minimize such problems when utilizing pull-type harvesters, special care should be taken with machine setup and maintenance, especially the sharpening of knives and clearance between the cutter-head and shear bar.

In 2013, two hundred sixty Brazilian dairy farmers answered a survey asking how often they sharpen the harvester knives (Bernardes and Do Rêgo, 2014). Only 54.6% of the respondents indicated that they sharpen the knives of the harvesters daily. Half of the farmers reported sharpening only at the beginning of the harvest (26.9%), every two days (11.2%), or less frequently than every two days (7.3%). As discussed before, the two main concerns that must be addressed in the maintenance of forage harvesters are keeping the knives sharp and adjusting the knives and shear bar (O'Dogherty, 1982; Shinners, 2003). The use of dull knives and the poor adjustment of the equipment results in torn, rather than cut, material. In some cases, the machine may halt due to the presence of particles stuck between the knives and shear bar, resulting in wasted fuel and poor cut quality (Wild et al., 2011). Tearing of the material results in long particles, low cut uniformity, and therefore problems in the compaction of the mass (Shinners, 2003).

Filling and packing

Of the stages of the production process, filling and packing procedures have had prominence among Brazilian farmers. They recognize that these steps are important for the exclusion of air from the silo to ensure an anaerobic environment and decrease nutrient loss. However, the lack of equipment has been an obstacle for small farmers. Although large operations have made high-quality silages, they over-fill the bunker silos. This was the main problem highlighted by a study involving 32 top dairy farms in five Brazilian States (De Oliveira, 2015). According with that survey, over-filled bunker silos had poorer fermentation and higher yeast counts in the upper layers of the silo compared with farms where corn silages were maintained below the containing walls of the bunker.

Silage cover

Double-sided polyethylene film (white-on-black) has been the main tool used for sealing horizontal silos in Brazil. However, nearly one-fifth of the producers still use

black plastic for sealing (Bernardes and Do Rêgo, 2014), which is considered to be a poor practice. Snell et al. (2003) reported the effects of color on the temperature of the film surfaces. They determined that during the morning hours, temperature peaks were up to 16°C higher for black film compared to white film. The quality of the film used in sealing horizontal silos is a key factor in limiting silage losses, especially in the peripheral area of the mass (Bernardes et al., 2012b).

Besides the plastic sheet, most Brazilian farmers use some material to cover the film after sealing the silo. Soil has been the preferred material of producers, followed by tires (Bernardes et al., 2012a; Bernardes and Do Rêgo, 2014). This strategy protects the plastic film from solar radiation and climatic conditions, allowing for less degradation of the coverage, particularly in tropical environments. Covering the plastic film decreases the air infiltration, delays the onset of aerobic deterioration in the upper layer of the silo, preserves the nutritional quality of the silage, and even increases the performance of lactating dairy cows (Amaral et al., 2010; Amaral et al., 2014). Although it is effective, protecting the plastic film is sometimes laborious, depending on the size of the silo and the amount of silage produced (Muck et al., 2003). In cases where covering the plastic sheet is not possible, the quality of the film is indispensable.

Unloading and face management

In Brazil, the majority of farmers remove silage manually (i.e., without the use of machinery). Other than during harvesting, a lack of equipment is also observed during silage unloading and feeding. Adesogan (2009) reported that in tropical countries, the lower nutritive value of forages, the attack of pests and diseases, and the climatic conditions affect farmers of both low and high economic power; however, economic and infrastructure barriers mainly affect those of low economic power.

Bernardes and Do Rêgo (2014) reported that on small-scale dairy farms producers removed silage from the silo manually. Conversely, farmers of high economic power have used machines. One advantage of mechanical unloading is that silage removed from the silo face is deposited into a feed wagon. The forage may then be mixed with other ingredients and delivered in the feedbunk. Among silage unloaders, the scrapers leave a smooth face, whereas frontload buckets result in an uneven face with cracks through which air may penetrate; hence, they must be avoided (Holmes and Bolsen, 2009).

With respect to the width (on a percentage basis) of the face that is removed from the silo daily, a small number of farmers (approximately 30%) reported that they remove silage from the entire face of the silo (Bernardes and Do Rêgo, 2014). Heterogeneously removing the silage is a reflex brought on by poor silo design (oversized) in relation to the number of animals to be fed. One method to reducing silage DM losses at the removal step

is to maintain an adequate feedout rate (> 40 cm/d) and remove silage from the entire face of the silo (Mahanna and Chase, 2003). Bernardes and Do Rêgo (2014) stated that small farmers had greater difficulty in scaling their silos. Several studies (Ashbell et al., 2001; Reiber et al., 2009) showed that the silage bag is an interesting tool for the storage of feeds on small properties located in tropical areas because of its low cost and ease of use. Thus, this technology can be transferred to small farmers in order to improve the silage-making process in Brazil.

The majority of the Brazilian farmers do not discard silage with deteriorated appearance before unloading and feeding the animals. Studies with cattle (Wichert et al., 1998; Whitlock et al., 2000) and goats (Gerlach et al., 2013) demonstrated that feeding deteriorated silage, even in small quantities, has a strong negative effect on feed intake and on the nutritive value of the ration, resulting in a significantly lower intake of digestible DM. Moreover, the intake of spoiled silage can have negative health consequences (Lindgren et al., 2002) and can contaminate animal products, such as milk and cheese (Vissers et al., 2007; Tabacco et al., 2009).

Silage additives

A Brazilian silage survey showed that approximately one-third of dairy producers, especially larger ones, applied an additive when ensiling the crops (Bernardes and Do Rêgo, 2014). Another study conducted in southern Brazil, including 120 dairy farms, determined that 18% of all farmers applied additives to corn silage and that all of these additives were bacterial inoculants (Bernardes et al., 2012a). Wilkinson and Toivonen (2003) reported that, in many countries, the practice of using additives is insignificant, possibly reflecting doubts about their cost-effectiveness. These authors also commented that biological additives are preferred by farmers, as new products are being launched to improve the aerobic stability of cereal silages. In other countries, it seems that additives are more commonly used by farmers. For example, in Israel, where wheat silages are widely used in the diet of dairy cows, a study indicated that approximately 47% of the silages were treated with additives, most of which were chemicals or bacterial inoculants (Weinberg et al., 2009).

High-quality silage can be made without the use of additives, assuming that producers have control over many management aspects. Nevertheless, silage additives can be useful in different circumstances. For example, they have been used to prevent the production of butyric acid in wet silages. In addition, additives are used to reduce dry-matter loss and preserve nutrients during fermentation and the feed-out phase (Kung et al., 2003).

Microbiology and mycotoxins

Microbial analyses of silage produced on Brazilian farms are uncommon, whereas silage microbiology has been assessed in research trials. Although some Brazilian labs are beginning DNA-based analysis to identify microbial populations (Ávila et al., 2010; Santos et al., 2013; Carvalho, 2014), most of the published papers presented microbial counts, mainly of lactic acid bacteria (LAB), yeasts, and molds, based on plate techniques and their derivations.

Microbial counts of fresh and ensiled corn (Assis et al., 2014), C4 grass (Coan et al., 2007; Santos et al., 2011) and C3 grass (Souza, 2015) were assessed in many trials to evaluate the effects of additives on silage conservation. The reported counts are highly variable and in most cases are similar to those reported in the international literature. A diversity of factors such as type of crop, weather conditions, forage processing, sampling and analytical methods are related to those variations on microbial populations and must be considered when different trials are compared.

Other than these findings, the most important contributions of Brazilian research on microbial analysis are related to sugarcane silages. In Brazil, since the beginning of the last decade, this forage has been highly investigated, and a large data set is available to provide understanding and to estimate the very high fermentative losses that are often found. Microbial profiles, especially of yeast populations, are key factors for these losses.

Ávila et al. (2010) identified the yeast populations in sugarcane silages by PCR, beyond the characterization of LAB and filamentous fungi. The authors found nine different species of yeast from five different silages, where four species were predominant: *Torulaspora delbrueckii, Pichia anomala, Saccharomyces cerevisiae,* and *Candida glabrata*. As expected, the population size and the number of species were variable among samples of different silages. Most of the isolated yeast species were able to metabolize lactate in addition to glucose and sucrose. This explains the increase of dry matter loss when facultative heterofermentative (homolactic) bacteria-based additives are used for sugarcane ensiling. In a meta-analysis, Schmidt (2009) verified that the dry matter losses were increased in 11 of 12 studies where *Lactobacillus plantarum* was used as an additive for sugarcane ensiling. The author highlights that the lactic acid content of the silages was increased in just one of six trials evaluating these bacteria, probably due to the metabolism of lactic acid by yeasts.

The identification and isolation of *Lactobacillus buchneri* strains from sugarcane silages were described by Ávila et al. (2009). These authors compared an indigenous strain with a commercial strain and verified that both were effective for decreasing yeast population and improving the aerobic stability of the silages. Similarly, Carvalho et al. (2014) evaluated the microbial profile of sugarcane silage inoculated with different strains

of indigenous LAB that were isolated from sugarcane silages in Brazil. Fourteen strains were tested by the authors, who found that inoculation of obligatory heterofermentative strains (*L. brevis* and *L. hilgardii*) led to a higher content of acetic acid, whereas decreased the ethanol concentration and DM losses. The effect of applying wild or commercial stains of the same bacteria is still not conclusive. However, searching for new strains that occur naturally in silages seems to be an effective way to isolate and replicate microorganisms that can alter and improve fermentation.

Another recent subject of silage studies in Brazil is the occurrence of mycotoxins. These secondary metabolites produced by toxigenic fungi can cause several undesirable effects in humans and animals. Silage can be an important mycotoxin source, and little is known about the potential synergistic or antagonistic effects of mycotoxins that lead to chronic health problems (Driehuis et al., 2008). Mycotoxins can be derived from the field or introduced during ensilage. Contamination during ensilage is directly related to air infiltration across the storage and silo feedout, mainly in silos with high-porosity or a low rate of silage removal (Gonzales-Pereyra et al., 2008).

Brazilian dairy farmers frequently associate reproductive or metabolic disorders with the intake of fungi-spoiled silages. However, farm silages are rarely analyzed for mycotoxins because of the high analytical costs and the lack of reference values for forages. Recently, a field survey was performed by Schmidt et al. (in press) to identify the profile of mycotoxins in maize silages. The authors tested 327 samples from 109 farms and found a highly variable mycotoxin profile, with a high incidence of zearalenone and fumonisin B1 (72.8% and 48.6% of the samples, respectively). The concentration of mycotoxins was not correlated with the temperature of the silo face or the management of silage feedout. This survey concluded that most mycotoxins are probably field-derived and that the average concentrations in maize silages are relatively low.

Nutritive value of silages

Nutritive value is the potential that a feedstuff has to provide nutrients to animals. Therefore, to achieve a high level of nutrient intake, animals must be able to eat and digest the feed properly. Under practical feeding conditions, feed nutritive value is synonymous of the amount of digestible DM (= DM intake × DM digestibility) that animals are able to eat under a given physiological status (e.g., growing, lactating). Although more than 60% of the variation in energy intake is related to differences in DM intake (DMI) (Mertens, 1994), diet digestibility has a large influence on DMI and animal performance (Friggens et al., 1998). In Brazilian conditions, dairy diets typically contain concentrate ingredients. Because the concentrates have high DM digestibility, most of the variation in DM digestibility is dependent on the digestibility and proportion of silage in the diet

(Nousiainen et al., 2009).

Several studies have reported that the concentration of neutral detergent fiber (NDF) and its digestibility (NDFD) are by far the most important variables related to forage nutritive value. However, in ensiled forages, the nutritive value is further affected by the fermentation pattern (extent and profile), mainly by influencing the DMI (Huhtanen et al., 2007).

Corn silage

Corn silage is the most energy dense forage source (high starch content) used in Brazilian animal husbandry systems. However, compared with corn silage produced in temperate climates, tropical corn silage tends to present lower starch and higher fiber concentrations (Adesogan, 2010). The NDFD is also lower in corn plants grown in warm climates (Cone and Engels, 1990; Adesogan, 2010).

Besides the lower starch content, corn grow in Brazil is predominantly flint, with a higher proportion of vitreous endosperm (Correa et al., 2002). Vitreousness is inversely related to starch digestibility (Phillipeau and Michalet-Doreau, 1997; Correa et al., 2002; Lopes et al., 2009; Nellis et al., 2013). Whereas forage processing is a feasible strategy to improve starch availability (Bal et al., 2000; Weiss and Wyatt, 2000; Ferraretto and Shaver, 2012), most Brazilian farmers use pull-type harvesters without a processing device (Bernardes and Rêgo, 2014).

However, when corn is ensiled, differences in starch digestibility seem to be attenuated during the storage period. Fernandes (2014) ensiled flint and dent corn grains harvested at different maturity stages. Although vitreousness and maturity impacted starch digestibility at ensiling (nonfermented grains), no differences were observed in starch digestibility after 60 days of silage storage due to the degradation of prolamins in the starch-protein matrix by proteolytic activity. In fact, Correa et al. (2003) did not observe any differences in starch digestibility (*in vivo*), DMI, or milk yield in dairy cows fed diets based on flint or dent corn silages. As starch and NDF are the major sources of nutrients in corn plants and starch digestibility typically increases across the fermentation, NDFD became an important trait for the selection of hybrids for silage production (Lopes et al., 2009; Zopollatto and Sarturi, 2009). Other than having improved starch digestibility, corn silages stored for long periods are even more nutritive because of their higher aerobic stability, as a consequence of higher acetic acid content and lower yeast counts (Sá Neto et al., 2013).

In comparison with temperate conditions, corn silages fermented in warm climates present a more heterolactic fermentation (Kim and Adesogan, 2006). Although heterolactic fermentation leads to higher DM losses (McDonald et al., 1991), this type of fermentation

is still advantageous in terms of aerobic stability (Driehuis et al., 1999). However, tropical climates (more humid and hot) leave silages more prone to spoiling (Ashbell et al., 2002; Adesogan, 2009). Therefore, even with more heterolactic (spontaneous) fermentation, tropical corn silages still respond to additives capable to improve the aerobic stability, such as weak acids (e.g., benzoate and sorbate; Bernardes et al., 2014) and heterolactic inoculants (e.g., *L. buchneri*; Sá Neto et al., 2013). Because naturally fermented silages have higher concentrations of acetic acid (Kim and Adesogan, 2006; Adesogan, 2010; Wand and Nishino, 2013), the inoculation of corn silages with heterofermentative strains (e.g., *L. buchneri*) would depress the DMI (Kleinshmitt et al., 2013).

Sugarcane silage

Sugarcane is another competitive tropical forage source, due to its high DM yield (e.g., 30 to 50 t DM/ha) and suitable nutritive value at maturity. Although its harvesting window coincides with the period of pasture shortage, sugarcane may be ensiled to prevent crop lodging and loss by accidental fire, to avoid daily harvesting, chopping and hauling, and to facilitate the agronomic management. As sugarcane is a semi-perennial tropical grass, its field lifespan may be prolonged by uniform harvesting and post-harvesting management. However, due to its high content of soluble carbohydrates and large yeast population (Alli et al., 1982; Ávila et al., 2010), ensiling sugarcane results in the conversion of part of the soluble sugars into fermentation end-products, which are characterized by high levels of volatile organic compounds, mainly ethanol (Kung Jr. and Stanley, 1982; Daniel et al., 2013c).

For many years, farmers and technicians have claimed that ethanol would depress DMI. To date, there is sufficient evidence indicating that ethanol does not impair the feed intake (Daniel et al., 2013a; Raun and Kristensen, 2011; Randby et al., 1999; Ham et al., 1994) and is actually used as an energy source. However, because the ethanol is partially metabolized to acetate in the rumen (Durix et al., 1991; Yoshii et al., 2005), part of the ethanol gross energy is lost as methane. Therefore, the net energy of ethanol is similar to that of carbohydrates (Ham et al., 1994; Daniel et al., 2013a). Hence, alcoholic fermentation is still undesirable due to the partial volatilization of ethanol and the loss of net energy (Daniel and Nussio, 2011). When effective additives are applied in sugarcane ensiling (e.g., sodium benzoate), most of soluble carbohydrates are recovered and silage nutritive value become similar to those of fresh-chopped sugarcane (Queiroz et al., 2008; Mari, 2008).

Sugarcane silage is composed of three main fractions: soluble carbohydrates (mainly sucrose), fermentation end-products, and NDF; whereas crude protein, ether extract and ash comprise less than 7% of sugarcane silage DM. The true digestibility of the soluble

fraction (soluble carbohydrates + fermentation end-products) is almost complete (Van Soest, 1967); therefore, the nutritive value is primarily a function of the concentration and digestibility of NDF.

The concentration of sugarcane silage NDF is mainly dependent on crop maturity, genotype, soil fertility and the use of effective silage additives, which ultimately affect the recovery of soluble sugars and, consequently, the NDF concentration in the silo. However, sugarcane NDFD is typically low (< 35%; Andrade and Pereira, 1999; 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) when compared with temperate grass and corn silages (Huhtanen and Krizsan, 2011). Low sugarcane NDFD has been associated with low DMI (Andrade and Pereira, 1999; Correa et al., 2003). Thus, two strategies have been highlighted for thriving with the low NDFD in sugarcane based systems. Primarily, chopping sugarcane into fine particles is not only beneficial for silage packing and preservation, but it also decreases the sorting of dietary ingredients, avoiding nutrient imbalance and digestive disturbances. Moreover, sugarcane NDF retains its high physical effectiveness, even when it has a short particle length (Goulart et al., 2009; Campos, 2015). Santos (2010) reported a linear increase in milk yield without alteration in milk fat content of crossbred Holstein-Zebu cows fed sugarcane with lower particle size. Hypothetically, finely chopped particles increased the ruminal turnover and lowered the digesta load (Gherardi et al., 1992).

Another strategy for improving the nutritive value of sugarcane silage-based rations is to increase the dietary proportion of concentrates (Biondi et al., 1978; Costa et al., 2005). Sá Neto et al. (2014) demonstrated that formulating dairy rations with an equal, physically effective NDF allows for similar performance when corn and sugarcane silages are exchanged. The physical effectiveness factor of sugarcane silage NDF is approximately 20% higher than that of corn silage (Goulart et al., 2009; Sá Neto et al., 2014). However, this strategy is currently valid for mid- to late-lactation cows and should be further evaluated before it can be recommended for early-lactation cows. Additionally, it is inadvisable to feed high-yielding dairy cows with sugarcane (fresh or ensiled) as an exclusive forage source during the pre-partum transition period. The high content of potassium (> 1% of DM) is likely to predispose the occurrence of metabolic diseases such as hypocalcemia (Goff, 2006).

Considering its high physical effectiveness, sugarcane silage is a very attractive forage source for beef feedlots because a low dietary inclusion is enough to achieve the fiber requirements. Additionally, silage moisture assists in bunk management, reducing dust and fines, and improving the ration texture compared with dry roughages (e.g., hay). Hence, a very high stocking rate is feasible in feed yards operating with sugarcane silage-

based diet.

Tropical grass silage

Like temperate grasses, tropical grasses present a high level of moisture at harvest. Accordingly, the addition of absorbent substrates or wilting are feasible pre-ensiling strategies for reducing water activity and the risk of effluent formation (Ribeiro et al., 2009), resulting in lower nutrient losses and well-preserved silages (Nussio, 2005). However, because the main grass species utilized for silage production in Brazil are tall and have thick stems (e.g., elephant grass, guinea grass), the former strategy (absorbents) seems more appropriate. Additionally, ensiling tropical grasses with absorbents may lead to silages with high intake potential and improved DM digestibility, depending on the additives selected (Table 1).

Table 1 Influence of wilting and absorbent additive on the nutritive value of tropical grass silage (*Brachiaria brizanthia*, cv. Marandu) (adapted from Bergamaschini et al., 2006)

Item	Control	10% Dried citrus pulp	Wilting
DM, %	24.1	31.1	47.9
NDF, %	73.9	62.1	75.2
pH	4.94	4.17	4.58
N-NH_3, % N	34.8	6.8	8.0
DM intake, kg/d	4.71	5.33	5.83
DM digestibility, %	65.7	69.3	66.6
Intake of digestible DM, kg/d	3.09	3.70	3.88
Relative nutritive value[1]	79	95	100

[1]Potential of digestible DM intake, relative to the wilted silage.

In Brazilian conditions, dry by-products (e.g., soybean hulls, citrus pulp, wheat bran, rice bran, corn gluten feed), energetic meals (e.g., corn meal, sorghum meal, pearl millet meal), or even protein meals (e.g., cottonseed meal, peanut meal, sunflower meal) have been recommended as absorbents for grass ensilage. Coffee hulls, rice hulls and cotton hulls were previously used in specific situations; nevertheless, the poor digestibility and the presence of anti-nutritional factors render the nutritive value of the final silage many times unsatisfactory (Carvalho et al., 2007). Shorting the particle size has benefits on silage preservation (Crestana et al., 2000); whereas its effects on animal performance are small under practical feeding conditions (Paziani et al., 2006). The same trend has been reported for temperate grass silages (Rinne and Seppälä, 2011).

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Essential oils as additives for sugarcane ensiling

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Keywords effluent, gases, losses, microbiology, soluble compounds, yeast

Introduction Sugarcane silage has been used as a feedstuff for ruminants. However, the high concentration of soluble carbohydrate and the high population of epiphytic yeasts in the fresh forage may lead to silages with reduced quality and low DM recovery. Thus, the use of additives at sugarcane ensiling is required. Essential oils are already widely used in animal nutrition, but there are few studies on its antimicrobial effects on silages, mainly in terms of yeast and mold control. This work aimed to evaluate two essential oils (thymol and carvacrol) as additives at sugarcane ensiling to decrease fermentative losses.

Materials and methods The trial was performed at the Centro de Pesquisa em Forragicultura (CPFOR), Federal University of Paraná, in Pinhais – Paraná – Brazil. At harvesting sugarcane variety clone RB03-6066 crop had 24.2°Brix and 31.1% DM. The forage was chopped in a stationary machine. The treatments were: **control** (no additives), **thymol** (600 $mg.kg^{-1}$ fresh basis), **carvacrol** (400 $mg.kg^{-1}$ fresh basis) and **combined** (250 $mg.kg^{-1}$ of thymol and 250 $mg.kg^{-1}$ of **carvacrol,** fresh basis**)**, with five replications. The experimental silos (20L) were equipped to allow determination of fermentative losses, according to Jobim et al. (2007). Silos were filled to achieve bulk density of 650 $kg.m^{-3}$ (fresh basis). After 90 days of storage, the silos were opened, the mass was homogenized and sampled to determine DM content in order to calculate total DM losses, gas production, and effluent production. The microbial population was evaluated according to Kung (1996). Data were statistically evaluated by ANOVA and treatments means were compared by Tukey test at 95% confidence level using the software SAS 9.3.

Results and discussion Treatments did not affect ($P > 0.05$) yeast and mold counts. Neither carvacrol nor combined (thymol plus carvacrol) silages had the development of LAB altered, but thymol decreased LAB population (3.7 log $cfu.g^{-1}$ fresh basis) However, carvacrol silages had the lowest pH value ($P < 0.05$). These effects may be due to the lower production of lactic acid and consequently lower nutrient availability to yeast growth. The isolated essential oils increased ($P < 0.05$) DM content in effluent relative to control and combined treatments. These data suggest that the oils can carry the soluble compounds of the silage, especially sugar, since that the effluent °Brix of silages added with oil was bigger than control silage ($P < 0.05$). However, there were no differences ($P > 0.05$) for DM losses, gas losses and effluent losses among treatments.

Conclusions The essential oils did not show efficiency in reducing total DM losses at sugarcane ensiling, and thymol even reduced the LAB population in treated silages. Both essential oils were not able to control the growth of spoilage microorganisms.

Table 1 Fermentative losses of sugarcane silages

Variables	Treatments[1]				SEM[3]
	Control	Thymol	Carvacrol	Combined	
pH	3.67[b]	3.71[b]	3.62[c]	3.77[a]	0.02
DM losses, % DM	21.79	18.67	16.10	18.11	5.88
Gas losses, % DM	18.58	15.21	13.04	14.43	6.16
Effluent losses, kg.t^{-1} fresh basis	60.1	61.1	54.7	62.8	0.11
Effluent DM, %	13.9[b]	15.6[a]	15.2[a]	14.6[ab]	0.7
Effluent Brix, °	15.0[b]	16.0[a]	16.3[a]	15.9[a]	0.45
Efluent pH	3.3	3.3	3.3	3.4	0.01
LAB[2], log cfu.g^{-1}, fresh basis	6.2[a]	3.7[b]	5.4[ab]	5.9[a]	1.14
Yeast, log cfu.g^{-1}, fresh basis	6.0	5.7	5.6	5.5	6.2
Molds, log cfu.g^{-1}, fresh basis	1.2	1.6	3.0	2.2	23.8

Means within a row with different superscript letters are different ($P < 0.05$)

[1] Control – without essential oils; Thymol – 600 mg.kg^{-1} fresh basis; Carvacrol 400mg.kg^{-1} fresh basis; Combined - 250 mg.kg^{-1} of thymol + 250 mg.kg^{-1} of carvacrol.

[2]LAB: lactic acid bacteria; [3]SEM: standard error mean

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Effect of a chemical additive containing sodium benzoate, potassium sorbate, and sodium nitrite on the microbial populations and aerobic stability of sugarcane silage

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Keywords aerobic deterioration, chemical additives, dry matter recovery, ethanol, yeasts

Introduction Sugarcane is a difficult crop to ensile because it typically has a high water soluble carbohydrates content, its fermentation is dominated by yeasts that produce high concentrations of ethanol resulting in low DM recover, and its aerobic stability is poor. Various chemical and biological additives have been used to control the yeasts and improve aerobic stability (Pedroso et al., 2008; Siqueira et al., 2011) but the results from using these additives have been inconsistent. Thus, the objective of this experiment was to evaluate effect of a chemical additive containing a mixture of sodium nitrite, potassium sorbate, and sodium benzoate (**SFE**) on the microbial populations and aerobic stability of sugarcane silage.

Materials and methods Whole plant sugarcane (32% average initial DM) was harvested from the dairy farm of the "Universidade Federal de Viçosa" and was chopped using a stationary chopper (2 mm theoretical chop length). The chopped forage was further divided into five piles and treated with 1) nothing (**Ctrl**), 2) *Lactobacillus buchneri* 40788 (**LB**; Lallemand, Brazil; theoretical application rate of 1 ´ 10^5 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**). 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 in five 10-L laboratory silos at a packing density of approximately 170 kg of DM/m^3 and sealed with plastic lids. Weights of empty and full buckets were recorded at packing and again at opening. Silos were opened after 21 and 100 d of storage (22 ± 2°C). Apparent DM recovery was determined. Representative samples were collected and analyzed as previously described with the addition of aerobic stability (number of h before a rise in silage temperature of 2 °C in relation to the ambient temperature (22 ± 2°C)). Data were analyzed considering the effects of treatment (T), day of ensiling (D) and their interaction (T × D) by using the software SAS ($P < 0.05$).

Results and discussion All variables were affected by the interaction T × D ($P < 0.05$; Table 1). The DM content was lower in the Ctrl and LB than the other treatments for the both periods of ensiling (21 and 100 d). The number of yeasts in the S3 and S5 was the lowest after 21 d of ensiling. After 100 d of ensiling treatment with CaO and S2 improved DM recovery to a greater extent when compared to Ctrl and LB. However, addition of S3 and S5 further improved DM recovery which was greater than all other treatments. The aerobic stability was highest in the SFE-treated silages than the other treatments.

Conclusions Addition of Safesil (2 L/t) markedly improved the fermentation by

controlling yeasts which, resulted in more DM recovery and better aerobic stability of sugarcane silages.

Acknowledgments This research project was supported by the CNPq and Salinity Agro.

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Table 1 The DM content, pH, microbial composition (fresh weight basis), aerobic stability and DM recovery of sugarcane silage

	Treatment[1]						SEM	*P*-value[2]		
	Ctrl	LB	CaO	S2	S3	S5		T	D	T×D
Day	DM, %						0.43	*	*	*
21	24.84^{Ac}	25.11^{Ac}	30.95^{Ab}	31.26^{Aab}	31.84^{Aab}	32.3^{Aa}				
100	24.64^{Ac}	24.74^{Ac}	31.07^{Aa}	28.02^{Bb}	31.81^{Aa}	32.74^{Aa}				
	pH						0.04	*	*	*
21	3.22^{Bc}	3.20^{Bc}	4.11^{Aa}	3.20^{Bc}	3.22^{Bc}	3.34^{Bb}				
100	3.65^{Ab}	3.62^{Ab}	4.10^{Aa}	3.54^{Ac}	3.55^{Ac}	3.65^{Ab}				
	Lactic acid bacteria, log cfu/g						0.18	*	*	*
21	7.98^{Ab}	8.02^{Aab}	8.56^{Aa}	7.28^{Ac}	6.97^{Ac}	6.97^{Ac}				
100	6.93^{Babc}	7.88^{Aa}	7.79^{Aab}	6.40^{Babc}	5.39^{Bcd}	4.00^{Bd}				
	Molds, log cfu/g						0.08	*	*	*
21	2.00^{Ba}	2.00^{Aa}	2.00^{Ba}	2.00^{Aa}	2.00^{Aa}	2.00^{Aa}				
100	2.70^{Aab}	2.00^{Ab}	3.71^{Aa}	2.00^{Ab}	2.42^{Ab}	2.00^{Ab}				
	Yeasts, log cfu/g						0.18	*	*	*
21	5.92^{Aa}	5.16^{Aa}	4.76^{Aa}	4.67^{Aa}	2.56^{Bb}	2.56^{Ab}				
100	2.12^{Bb}	3.29^{Bab}	2.84^{Bab}	3.87^{Aa}	3.85^{Aa}	2.24^{Ab}				
	DM recovery, %						1.42	*	*	*
21	75.42^{Ac}	76.38^{Ac}	90.22^{Ab}	96.56^{Aa}	98.10^{Aa}	99.20^{Aa}				
100	74.55^{Ac}	74.97^{Ac}	89.60^{Ab}	84.99^{Bb}	97.67^{Aa}	99.72^{Aa}				
	Aerobic stability, h						8.60	*	*	*
21	51.21^{Ab}	58.13^{Ab}	63.43^{Bb}	123.58^{Ba}	164.83^{Aa}	164.58^{Aa}				
100	37.93^{Ac}	38.97^{Ac}	137.57^{Ab}	191.83^{Aa}	178.72^{Aab}	179.03^{Aab}				
	Maximum temperature, °C						1.03	*	*	*
21	40.63^{Ab}	39.50^{Ab}	35.10^{Ab}	25.50^{Aa}	21.80^{Aa}	22.38^{Aa}				
100	36.6^{Aa}	35.60^{Aa}	24.70^{Ba}	23.00^{Aa}	24.50^{Aa}	23.59^{Aa}				

[1]Ctrl = control; LB = *Lactobacillus buchneri* 40788 (application rate of 1 × 10^5 CFU/g); CaO = calcium oxide (10 g/kg); S2 = 2 L Safesil/t fresh weight; S3 = 3 L Safesil/t; S3 = 4 L Safesil/t.

[2]T = effect of treatment, D = effect of day of ensiling; T × D = interaction between treatment and day.

[a-c]Means followed by the same uppercase letter in the columns and lowercase letter in the rows are not significantly different based on Tukey's test ($P < 0.05$).

Corn Shredlage: Equipment, storage and animal perspectives

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Introduction

High quality whole-plant corn silage (**WPCS**) contributes greatly to supplying the energy, starch and forage neutral detergent fiber needs of high-producing dairy cows, reducing purchased feed costs from expensive grain and byproduct supplements, and generating milk revenues for dairy producers throughout the world. Figure 1 is an overview of the factors that influence the nutritive value of corn silage. Kernel processing WPCS to improve starch digestibility was reported to be effective across a wide range of dry matter (**DM**) contents and theoretical length of cut (**TLOC**) settings, but did not overcome the adverse effects of very high DM content on total tract starch digestibility and was ineffective at very long TLOC in a recent meta-analysis (Ferraretto and Shaver, 2012b).

Garnering much recent interest by dairy producers and their nutritionists has been a new method of harvesting WPCS. The resultant feedstuff has been called corn shredlage (**SHRD**) by the developer of the shredlage processor (Shredlage™, LLC; http://www.shredlage.com/). This new method allows WPCS to be harvested at a longer TLOC while still maintaining or improving the degree of kernel processing. Although a recent development with limited information available, we conducted two feeding trials with SHRD fed to lactating dairy cows and an on-farm survey of dairy farms about their corn silage harvest, processing and feeding practices, and collected WPCS samples during feed-out for determination of processing score and particle length. The purpose of this article is to provide an assessment of SHRD based on the results from the feeding experiments and the on-farm survey.

Equipment and storage

The SHRD is harvested with a commercially available self-propelled forage harvester (**SPFH**) fitted with after-market cross-grooved crop-processing rolls set for 2- to 3-mm roll gap and greater roll speed differential than has typically been used (32% versus 21%). Also, the developer of this processor recommends that the SPFH be set for

a longer theoretical length of cut (**TLOC;** 26 to 30 mm) than has typically been used in the past (19-mm TLOC). For harvest of the SHRD in our first feeding trial (Ferraretto and Shaver, 2012a), a SPFH equipped with the new shredlage processing rolls was set for a 30-mm TLOC by removing half of the knives and the processor roll gap set at 2.5 mm. Most SPFH manufacturers recommend against removing knives when harvesting WPCS, because of added stress and wear on SPFH components like the cutter-head, processor and blower. Therefore, in practice a 26-mm TLOC setting is most common for SHRD so that knife removal is not required. During our second feeding trial harvest of the SHRD was done with the SHRD processor set at a 2-mm roll gap and with the SPFH set for a 26-mm TLOC. Thus far this processor unit has been adapted only for Claas SPFH, although shredder roll kits have been made available for the other makes of SPFH. During the 2014 harvest approximately 600 shredlage processors and shredder roll kits were in operation according to the developer of the shredlage processor. Most of these were shredlage processor units on Claas SPFH.

Recently, we performed a dairy farm survey of WPCS harvest, processing and feeding practices, and equipment-related information. Samples of WPCS during silo feed-out were also collected from these farms for determination of processing score and particle length. Only equipment-related information and pack density will be discussed in the present article. The rest of the survey information is presented in a companion abstract submitted to this conference. The survey included farmers that had been feeding SHRD in their herds for only 4 months to as long as 3 years. The following statistics were calculated from farmers using SHRD harvested with either a shredlage processor (n=46) or shredder roll kits (n=5) who had previously been using conventional processors. Processing rolls were relatively new with only 23% of respondents reporting usage on more than 30,000 as-fed tons. Most respondents reported either no change or being unsure about tons per hour throughput (63%) and roll wear (82%). Approximately 67% of the respondents reported no change or were unsure about fuel usage, whereas 30% reported an increase. Further research evaluating equipment-related aspects of corn shredlage is warranted.

Although increased particle size of SHRD may cause concerns about packing density, only 4% of the respondents reported decreased packing density with SHRD. Furthermore, an increase in packing density was reported by a majority (51%) of the surveyed farmers. This perception of an increase in packing density may be related to SHRD particles being intermeshed within the silo similarly to alfalfa or grass silages. However, quantitative silo bag and bunker silo packing density measurements at the UW-Madison dairy facility have revealed no differences between SHRD and conventional-processed corn silage. Further research is warranted to evaluate packing density of SHRD in silos of varying types.

Animal perspectives

Feeding trial 1

We conducted a feeding trial (Ferraretto and Shaver, 2012a) with SHRD fed to lactating dairy cows at the UW Blaine Arlington Dairy during Oct. – Dec., 2011 following a September, 2011 harvest at UW Arlington Agricultural Research Station (**AARS**). Compared to conventional-processed WPCS (**KPCS**) the most obvious visual difference for SHRD was a greater proportion of long stover particles in SHRD. When fed in rations for lactating dairy cows, this can increase the physically-effective fiber (**peNDF**) content of the ration which is important for proper rumen function, cow health and milk fat content. The cross-grooved rolls used for producing SHRD may cause greater damage to the coarse stover particles and allow for greater digestibility of the NDF, but this has yet to be confirmed with controlled research data. An 8 ha field at UW AARS planted with a dual-purpose corn hybrid was used for the study. One day apart in early September, 2011 half the field was harvested as SHRD while the other half was harvested as KPCS. The SHRD and KPCS were stored in separate side by side 2.5-m diameter by 61-m long silo bags and allowed to ferment for approximately 6 weeks before commencing the dairy feeding trial. For harvest of the SHRD, a SPFH equipped with the new shredlage processing rolls was set for a 30-mm TLOC by removing half of the knives and the processor roll gap set at 2.5 mm. The SHRD harvest was done by a custom operator (Kutz Farms, Jefferson, WI) and SPFH was set up by Shredlage™ LLC and Scherer representatives. Harvest of the KPCS was done using the UW AARS SPFH set for a 19-mm TLOC and equipped with conventional processing rolls set at 3-mm roll gap. For samples collected at harvest, the corn silage processing score (**CSPS**; % of starch passing through a 4.75 mm screen) was 60.3 ± 3.9 for KPCS and 75.0 ± 3.3 for SHRD.

The SHRD and KPCS were similar in DM (35.0% ± 1.9 versus 34.7% ± 1.4) and starch (37.6% ± 5.2 versus 38.7% ± 4.9) concentrations, pH (3.59 ± 0.05 versus 3.61 ± 0.03), and silo bag packing density (272 kg DM/m^3, on average). The proportion of coarse particles was greater for SHRD than KPCS for samples collected during feed-out from the silo bags throughout the feeding trial (31.5% versus 5.6% retained on the 19 mm screen of the Penn State Separator Box). For TMR fed throughout the trial, the proportion of coarse particles was greater for TMR prepared with SHRD than KPCS (15.6% versus 3.5% retained on the 19 mm screen of the Penn State Separator Box). Our measurements of weigh-backs during the trial did not reveal feed sorting for either treatment.

Fourteen 8-cow pens, balanced by breed, parity and days in milk (**DIM**), were randomly assigned to either SHRD or KPCS treatment TMR (7 pens and 56 cows per treatment). At the start of the feeding, SHRD and KPCS cows were 114 ± 35 and 117 ± 36

DIM. All pens were fed a 50:50 mixture (DM basis) in the TMR for a 2-week covariate adjustment, followed by an 8-week treatment period pens received their respective treatment TMR containing 50% (DM basis) from either SHRD or KPCS. Both TMR treatments contained 10% alfalfa silage and 40% (DM basis) of the same concentrate mix comprised of dry ground shelled corn, corn gluten feed, solvent and expeller soybean meal, rumen-inert fat, minerals, vitamins and monensin. Statistical analysis of the data was done using pen as the experimental unit.

The DMI tended ($P < 0.08$) to be 0.7 kg/day per cow greater for SHRD than KPCS, while milk yield (43.7 vs. 42.8 kg/day per cow for SHRD vs. KPCS) and feed efficiency (1.72 vs. 1.73 kg Milk/kg DMI for SHRD vs. KPCS) were similar ($P > 0.10$). Yield of 3.5% FCM tended ($P < 0.08$) to be greater for SHRD than KPCS (45.5 vs. 44.5 kg/day per cow for SHRD vs. KPCS). A week by treatment interaction was detected ($P < 0.03$); there was no difference between the treatments at week 2, FCM yield tended ($P < 0.10$) to be greater for SHRD compared to KPCS at weeks 4 and 6, and FCM yield was ($P < 0.01$) 2.0 kg/day per cow greater for SHRD than KPCS at week 8. Milk fat, protein and urea-nitrogen contents were unaffected ($P > 0.10$) by treatment and averaged 3.72%, 3.20% and 13.8 mg/dL, respectively. Body weight (710 kg on average) and condition score (3.04 on average) and body-weight change (0.30 kg/day per cow) were similar ($P > 0.10$) for the two treatments.

Ruminal in situ starch digestibility was greater for SHRD than KPCS (80.8 vs. 64.2%, respectively). Likewise, total-tract starch digestibility was 1.5%-units greater for SHRD than KP. This response is related to greater kernel breakage during passage through rollers and corresponding increased surface area allowing for enhanced bacterial attachment and digestion.

Feeding trial 2

In the second feeding trial, we evaluated: 1) the response to corn shredlage in a brown midrib (**BMR**) WPCS hybrid, and 2) whether the greater TLOC setting on the SPFH for the harvest of SHRD increased the peNDF content of the WPCS (Vanderwerff et al., 2014).

A BMR WPCS hybrid (F2F627; Mycogen Seeds) was harvested in September 2013 with a Claas 940 SPFH equipped with either a Claas conventional processor or a SHRD processor on the same day at 50% kernel milk line stage of maturity. The conventional processor was set for a 2-mm roll gap and 40% roll speed differential with the SPFH set for a 19-mm TLOC for harvest of the KPCS. Harvest of the SHRD was done with the SHRD processor set at a 2-mm roll gap and 32% roll speed differential with the SPFH set for a 26-mm TLOC. The KP and SHRD were stored in separate silo bags until the bags were

opened to begin the feeding trial in January, 2014.

Mid-lactation Holstein cows were used in a 16-week continuous-lactation experiment in our university dairy herd with 15 replicated pens of 8 cows each. The respective treatment TMR contained 45% (DM basis) from either SHRD or KPCS. Both TMR treatments (SHRD and KP) contained 10% alfalfa silage and 45% (DM basis) of the same concentrate mix comprised of dry ground shelled corn, corn gluten feed, solvent and expeller soybean meal, rumen-inert fat, minerals, vitamins, and monensin. Additionally, a third treatment TMR (**KPH**) was included in the experiment to focus on the peNDF question. This ration was formulated with 35% KPCS, 10% alfalfa silage, 10% chopped hay, and 45% (DM basis) of the same concentrate ingredients adjusted in proportions in the mix to balance dietary crude protein and starch concentrations across the three treatments.

The SHRD and KPCS were similar in average DM (39%) content and pH (3.9). Corn silage processing scores on feed-out samples averaged 72% for SHRD and 68% for KPCS with less variation observed for SHRD over the duration of the experiment. The sample range (difference between maximum and minimum samples) was 10%-units for SHRD and 21%-units for KPCS. For SHRD, all processing scores were above 65%. However, for KP 43% of the samples obtained on a weekly basis throughout the feeding trial were at or below a processing score of 65% (refer to Figure 2).

The proportion of coarse stover particles was greater for SHRD than KPCS for samples collected during feed-out from the silo bags throughout the feeding trial (18% versus 7% as-fed particles retained on the top screen of the shaker box). For the TMR fed throughout the trial, the proportion of as-fed particles on the top screen of the shaker box was greater for SHRD than KP or KPH. Our measurements of weigh-backs during the trial indicated minimal sorting and no differences in sorting among the three treatments.

Averaged over the treatment period, milk yield was 1.5 kg/day per cow greater for SHRD than KP with the SHRD cows averaging 51.3 kg/d; feed efficiency was similar for the two treatments. Milk yield was 3.4 kg/d per cow lower and feed efficiency was reduced for KPH compared to KP. Milk yield by week on treatment is summarized in Figure 3.

Milk fat content was greater for KPH (3.7%) than KP or SHRD (3.3%). Rumination activity measured using the SCR rumination collars averaged 8.4 hours per day and was not different among the treatments. Using milk fat content and rumination activity data to assess peNDF suggests that the peNDF content of SHRD was not improved despite its longer TLOC and increased percentage of as-fed particles on the top screen of the shaker box compared to KPCS. Milk fat yield was not statistically different among the treatments, but was numerically greatest for KPH and lowest for KPCS. Similar to the milk yield differences, milk protein and lactose yields were greatest for SHRD and lowest for KPH. Body condition score (3.1 on average) and body-weight change (0.6 kg/d per cow on

average) were similar among the three treatments.

Total-tract DM and organic matter (**OM**) digestibility were greater for cows fed KP and SHRD than for cows fed KPH. Total-tract NDF digestibility (**TTNDFD**) tended to be greatest for KPH and lowest for SHRD. Lower TTNDFD for SHRD may be related to increased dietary starch content for SHRD compared to KPH and increased kernel processing and ruminal starch digestibility for SHRD compared to KP and KPH. The ruminal in situ starch digestibility was greater for SHRD than KPCS (88.3 vs. 76.0%, respectively). Total-tract starch digestibility was greater for SHRD than KP. Differences in total-tract starch digestibility between SHRD and KP were, however, biologically small (0.5%-units) and starch digestibility was near 100% for all treatments. Small differences in total-tract starch digestibility along with much larger differences ruminally may be explained by post-ruminal compensatory digestion of starch. Nearly complete digestion of starch in the total-tract may be explained by the nearly 6 month lag between ensiling and the midpoint of the feeding trial, since length of the ensiling period has been shown to increase starch digestibility in WPCS (Ferraretto et al., 2015).

Summary & Conclusions

In summary, greater lactation performance was observed for corn shredlage compared with conventional-processed corn silage either when using a conventional or a BMR corn hybrid. Furthermore, feeding corn shredlage increased total tract starch digestibility in both trials and may be a potential tool for dairy producers and their nutritionists desiring to feed higher corn silage diets without compromising kernel breakage and energy availability for whole-plant corn silage chopped at a greater length of cut. However, despite a longer length of cut setting on the SPFH and increased particle size for corn shredlage relative to conventional-processed corn silage, milk fat content and rumination activity were not increased. Further research is warranted to evaluate ruminal fermentation patterns and in vivo digestion kinetics to better understand the impact of adding corn shredlage in diets for high-producing dairy cows. In addition, more data is needed regarding NDF digestibility for corn shredlage and the relative peNDF for corn shredlage compared to hay-crop silage, whole cottonseed, and chopped hay or straw, to allow for better decisions on how best to utilize corn shredlage in dairy cattle diets. Assessment of particle size of corn shredlage as an indicator of peNDF and CSPS as an indicator of starch digestibility is essential to determine the best ration formulation strategies.

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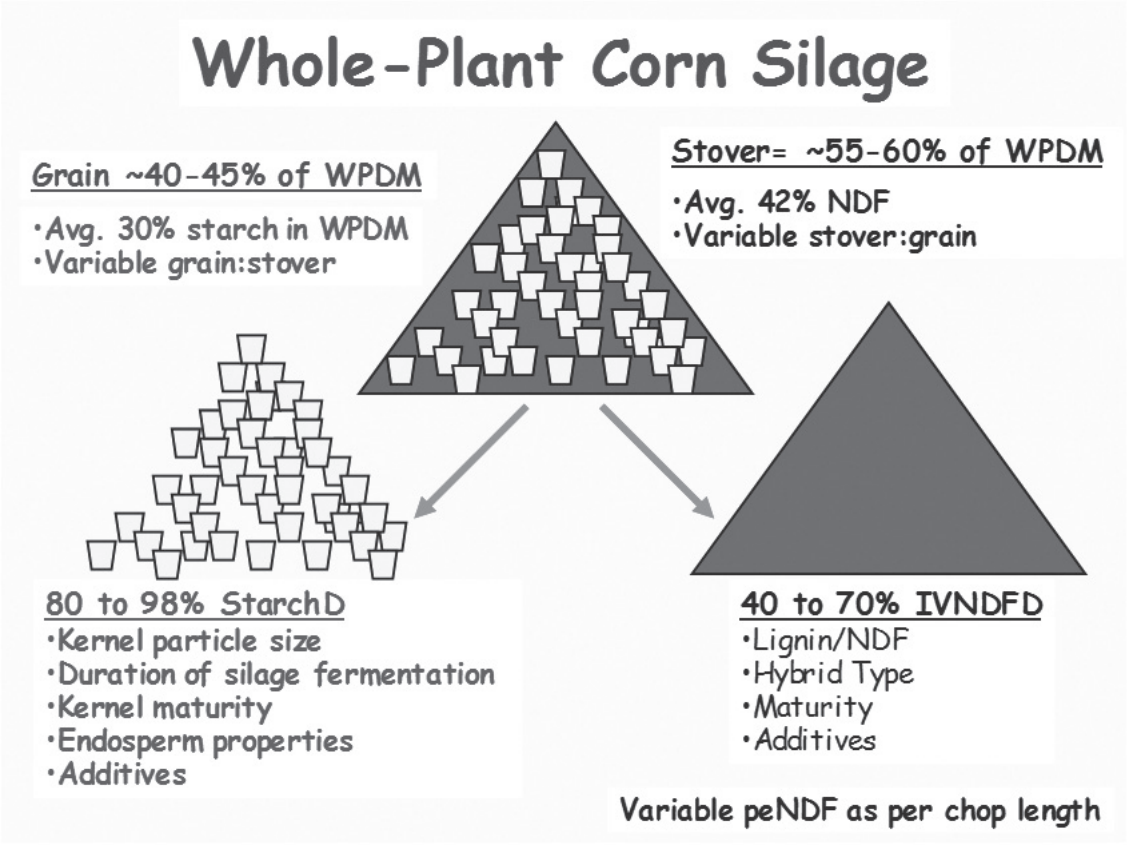

Figure 1 Overview of the factors that influence the nutritive value of corn silage (Adapted from J.G. Lauer, University of Wisconsin-Madison).

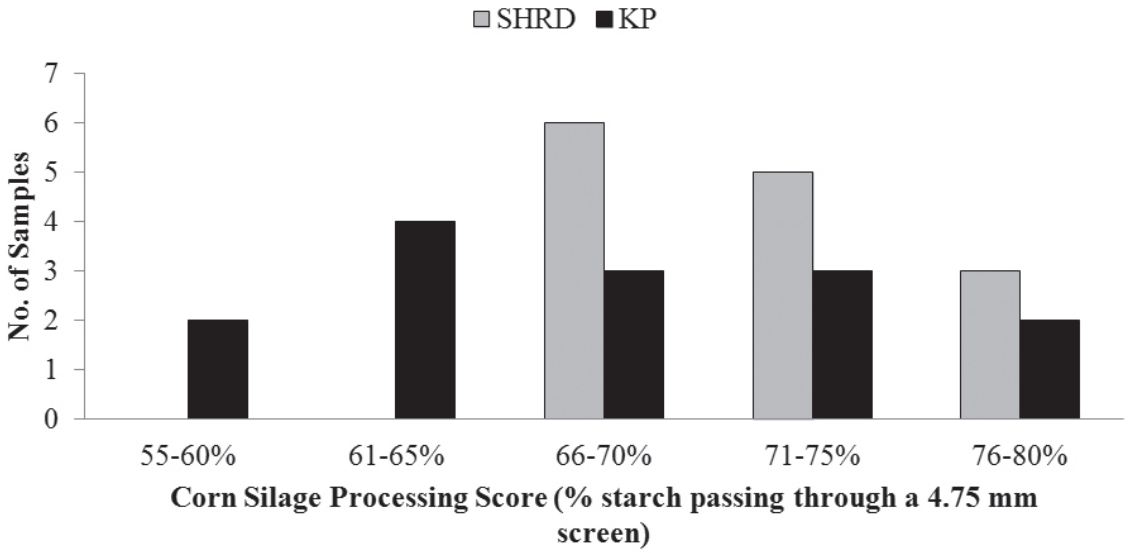

Figure 2 Frequency distribution for corn silage processing score on samples of brown midrib corn shredlage (SHRD) and conventional-processed corn silage (KP). Sample were obtained weekly during feed-out from the silo bags of feeding trial 2 (Vanderwerff et al., 2014).

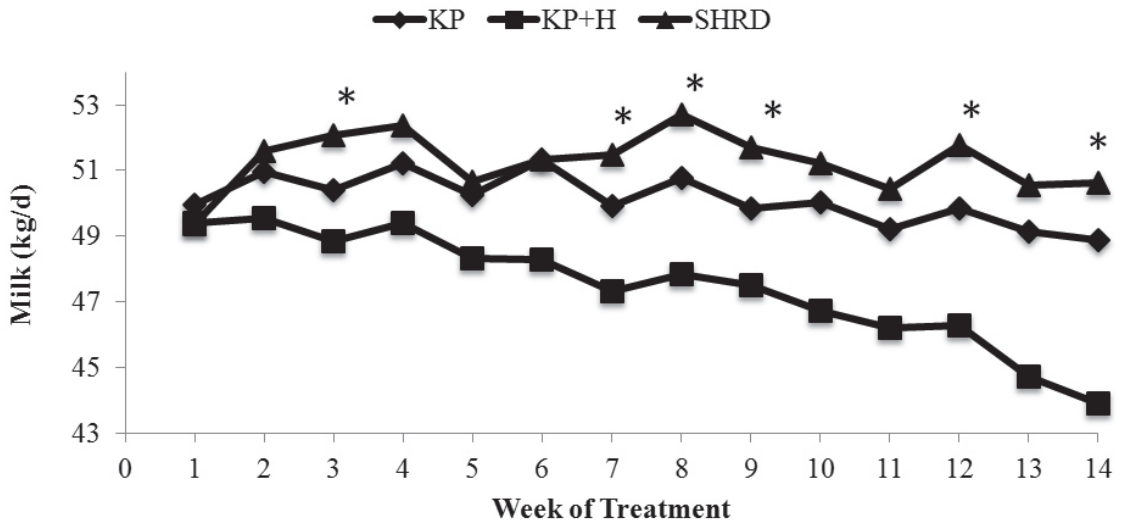

Figure 3 Milk yield by week on treatment for total mixed rations containing brown midrib corn shredlage (SHRD), brown midrib conventional-processed corn silage (KP), and brown midrib conventional-processed corn silage plus hay (KP+H). Week x Treatment interaction effects ($P < 0.0001$). Means within the same week with * differ ($P < 0.05$). Data from corn shredlage feeding trial 2 (Vanderwerff et al., 2014).

Relationship between theoretical length of cut and mean particle length in whole-plant corn silage

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Keywords theoretical length of cut, mean particle length, hydrodynamic separation

Introduction Dairy farmers increase theoretical length of cut (**TLOC**) with the aim of increasing mean particle length (**MPL**) of whole plant corn silage (**WPCS**) to provide greater physically effective fiber. However, others factors can influence the MPL in WPCS, such as kernel processing, dry matter content and the ratio of grain:stover. Thus, the objective of this study was to evaluate the relationship between verbal TLOC and MPL measured through two methodologies in both as fed whole-samples or stover fractions.

Materials and methods Eighty WPCS samples from 2 field trials and a field survey were selected to represent varied verbal TLOC settings and processor types and settings. Particle size distribution and MPL were measured on as-fed samples using either the Penn State Particle Size Separator (**PSPS**) or the Wisconsin Oscillating Particle Separator (**WI-OS**). The PSPS procedure was conducted manually using 3 sieves (19-mm, 8-mm, and 1.18-mm) and a pan according to Kononoff et al. (2003). Samples measured with WI-OS were sieved mechanically using 5 sieves (26.9-mm, 18-mm, 8.98-mm, 5.61-mm, and 1.65-mm) and a pan (ANSI, 2001). A sub-sample of 1 kg as fed of each sample was used to separate grain and stover fractions through the hydrodynamic separation procedure (Savoie et al., 2004). All samples were dried at 60°C for 48 h in a forced-air oven prior to immersion in water. After stover and grain fractions were separated, the stover fraction was re-dried at 60°C for 48 h in a forced-air oven. The dried stover fraction samples were used to determine MPL using the PSPS as described above. Sequentially, stover samples were recombined and then sieved mechanically using the WI-OS as described above. Regressions to determine linear and quadratic relationships were performed using Proc Reg of SAS (SAS Institute, 2004). Best-fit regression (linear or quadratic) was chosen using the highest coefficient of determination ($\mathbf{R^2}$) and lowest root mean square error (**RMSE**) as indicators. Statistical significance and trends were declared at $P < 0.05$ and $P > 0.05$ to $P < 0.10$, respectively.

Results and discussion Verbal TLOC was not related to MPL of as fed samples measured by either PSPS ($P = 0.23$) or WI-OS ($P = 0.58$). However, a relationship between MPL measured by PSPS with MPL by WI-OS was observed ($R^2 = 0.62$, $P = 0.001$, Figure 1). This relationship was also observed when MPL of stover was evaluated ($R^2 = 0.60$, $P = 0.001$, Figure 2). Even after the hydrodynamic separation procedure the MPL of stover fractions measured by both methods were not related to TLOC (PSPS, $P = 0.17$; WI-OS, $P = 0.68$). Although our hypothesis was that MPL measured on the stover fraction would be related to TLOC due the elimination of the grain fraction, the drying after hydrodynamic separation may have resulted in shattering of particles and further particle size reduction

during the sieving process. Alternatively, effects of DM content at harvest and type and setting of processing rolls may also have affected the MPL results.

Conclusions Verbal TLOC was not related to MPL of WPCS. In addition, the elimination of grain through hydrodynamic separation did not improve this relationship. Perhaps specific TLOC guidelines are warranted for specific DM contents and kernel processor types and settings to achieve greater physically effective fiber. A strong relationship between the two different set of sieves on estimation of MPL in WPCS and in the stover fraction was observed suggesting that MPL may be measured adequately on farm.

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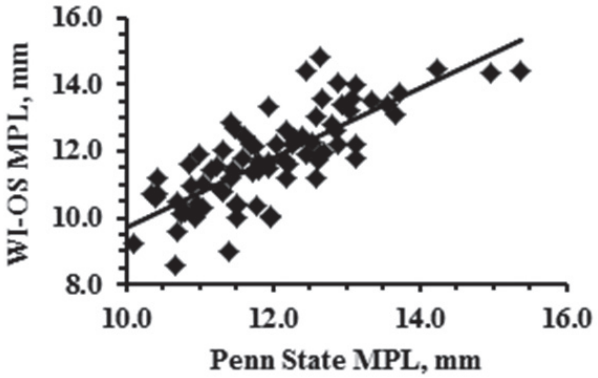

Figure 1 Relationship between whole-plant corn silage samples MPL (mm) measured by PSPS and measured by WI-OS. Prediction equation: y = -0.73 (±1.13) + 1.05x (±0.09); n = 79, RMSE = 0.89, R^2 = 0.62, *P* = 0.001.

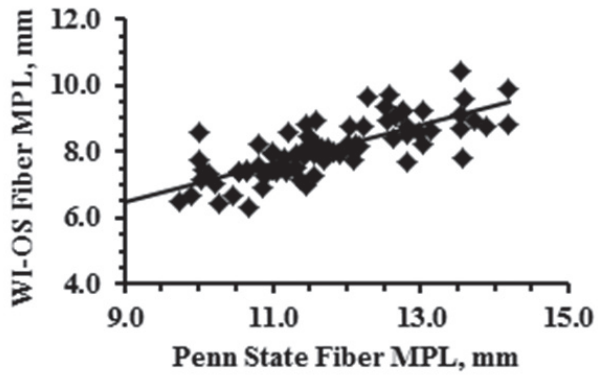

Figure 2 Relationship between stover fractions MPL (mm) measured by PSPS and measured by WI-OS. Prediction equation: y = 1.13 (±0.65) + 0.59x (±0.06); n = 79, RMSE = 0.57, R^2 = 0.60, *P* = 0.001.

Effect of corn silage kernel processing score on dairy cow starch digestibility

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Keywords corn silage, kernel processing, dairy fecal starch, starch digestibility

Introduction With the increased cost of grain and forages in the USA, dairy producers have turned their attention to the feed efficiency of milk production. Low on-farm starch digestibility contributes to reduced feed efficiency. Several researchers have demonstrated that measuring fecal starch (**FS**) is highly correlated to total tract starch digestibility in dairy cows. One factor affecting the digestibility of starch in lactating dairy cows is the extent of the processing of the corn kernel during the harvesting of corn silage. Kernel processors are employed on the forage harvesters to break the corn kernel into smaller fractions. The extent of kernel processing varies based upon the management of the kernel processing equipment, kernel maturity, hardness of the corn, and extent of corn silage fermentation. A kernel processing score (**KPS**) was developed by Mertens (2005) which involves submitting a corn silage sample to a laboratory where it is dried and sifted through variable sized mesh sieves. A starch analysis is performed and the portion of the starch that passes through a 4.75 millimeter sieve is determined more digestible by the lactating dairy cow. Guidelines for KPS are >70%, excellent; 50 to 70%, adequate; and <50% poor.

Materials and methods From December, 2012 to July, 2013, 35 dairy operations ranging in size from approximately 1,200 to 6,800 cows participated in a study to evaluate the effect of KPS of ensiled corn silage on the content of starch in the feces of cows fed the corresponding corn silage. These dairy operations which represented a total of approximately 58,000 cows were located in the states of Wisconsin, Iowa, Minnesota, and Illinois. A minimum of at least 6 core samples from the face of corn silage bunkers or drive-over piles was consolidated and a sub-sample sent to a commercial lab (Rock River Laboratories, Watertown, Wisconsin) for KPS determination using the method of Mertens (2005). On the same farms and same day, fresh floor fecal samples were collected from at least 20 cows per pen from pens with cows less than 120 days-in-milk with a composite sample analysed for FS by Rock River Laboratories (Watertown, WI). Some dairies were sampled more than once as they changed sources of corn silage.

Results and discussion Figure 1 plots the KPS score compared to the respective % FS (DM basis) of each dairy arranged from high to low KPS. Linear lines were plotted for both KPS and % fecal starch. A negative relationship between % FS and KPS % ($R^2 = 0.58$, $P = 0.001$, Figure 2) was observed. Ferguson (2003) established a positive relationship ($R^2 = 0.782$) between FS and total tract starch digestibility (TTSD) in lactating dairy cows. Also, Ferraretto and Shaver (2012) demonstrated a negative relationship between FS and total tract starch digestibility ($R^2 = 0.94$). Ferguson (2003) estimated that for each one percentage unit increase in FS above 5% (DM basis), a corresponding decrease of 0.35 kg of milk yield per cow per day can be expected. This excess of starch in manure decreases feed efficiency of milk production, adds to the manure load on a dairy

farm, and represents wasted money on feed that is not digested.

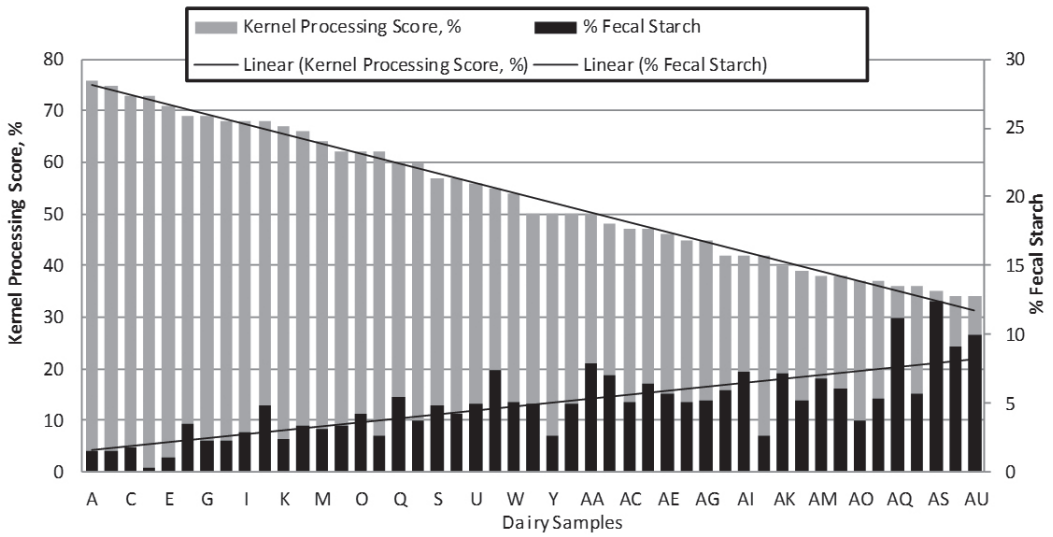

Figure 1 Percent fecal starch plotted against respective corn silage kernel processing score (%) for each dairy sample (n=47).

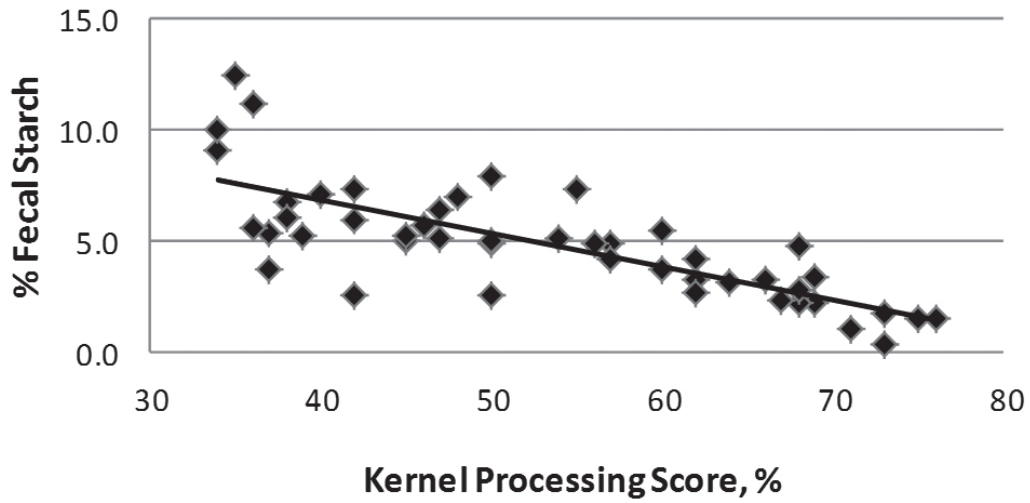

Figure 2 Relationship between kernel processing score and % fecal starch (DM basis). % fecal starch prediction equation y = 12.90 (±1.04) – 0.15x; R^2 = 0.58; *P*=0.001.

Conclusions There was a wide variation in kernel processing scores (34-76%) on the Midwest USA farms that participated in this research. A high negative correlation between corn kernel processing score and fecal starch in high producing lactating dairy cows suggests that improving corn kernel processing during corn forage harvest is a management tool that can increase total tract starch digestibility and increase milk production efficiency.

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The effect of an exogenous protease on the fermentation and nutritive value of corn silage stored at two temperatures

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Keywords corn silage, protease, starch digestion

Introduction Silage fermentation can be affected by the temperature during storage. For example, cool temperatures may retard microbial activity whereas warm temperatures (>40°C) may cause silages to undergo an altered or restricted fermentation (Weinberg et al., 2001). Recent research (Windle et al., 2013) has shown that application of an exogenous protease to corn silage at ensiling improved the digestibility of starch by degrading the prolamin matrix surrounding the starch granules within the endosperm of the corn kernel. In those studies, silages were stored at ~22°C. However, in some cases, silages are harvested under warm conditions and often reach and maintain relatively high temperatures in the deep core of the silo for prolonged periods of time, which could also affect enzyme activity and performance. The objective of this experiment was to determine the effect of moderate and high storage temperatures on corn silage that was ensiled with or without an exogenous protease.

Materials and methods Corn plants were harvested from 5 locations in a single field. Two piles of forage were prepared from each location, and were treated with either a phosphate buffer (pH 5.5) or a phosphate buffer with dissolved acid-protease (Novozymes, Bagsvaerd, Denmark), resulting in an application rate of 2000 mg of protease formulation per kg of wet forage weight. After application, approximately 500 g of chopped forage from each pile was packed into nylon-polyethylene pouches, vacuumed to remove air, and heat sealed. Bags were stored between 22 ± 1°C (**LW**) or at 40 ± 1°C (**HI**) for 2, 7, 45 and 90 d. Water extracts, prepared from representative samples of fresh forages and silages were analyzed for NH_3-N, water-soluble carbohydrates, lactic acid, acetic acid, ethanol, and yeasts and molds. Fresh forages and silages were analyzed for DM, NDF, ADF, total N, starch, 7-h in vitro ruminal starch digestibility (**IVRSD**), and soluble protein. Data were analyzed as a 2 × 2 × 4 factorial arrangement of treatments with the main effects of storage temperature, protease, time of ensiling and their interactions.

Results and discussion Treatment with protease did not affect the concentrations of lactic acid or ethanol in silages. Silages that were ensiled for 7 and 45 d had lower concentrations of acetic acid, and those stored for 90 d had lower concentrations of lactic acid if they were stored at HI compared to LW conditions, indicating a more restricted fermentation in silages that were stored at 40°C. The concentration of NH_3-N increased over time of storage and was higher in silages that were treated with protease than in untreated silages. Soluble CP increased with time of storage and after 45 d it was highest in silages that were treated with protease and stored at HI (55.6%), followed by silages that were treated with protease and stored at LW (48.7%), then untreated silages that were stored at HI (45.5%), and was lowest in untreated silages that were stored at LW (40.7%). There was an interaction between time of storage and treatment with protease on IVRSD.

There was no effect of the experimental protease on the IVRSD in silages that were ensiled for 2 and 7 d, but after 45 and 90 d, the IVRSD was higher in silages that were treated with protease compared to untreated silages. After 45 d of ensiling, silage treated with protease and stored at HI had numerically greater IVRSD than silages treated with protease but stored at LW. Silages that were treated with protease and stored for 45 d had IVRSD similar to silages that were untreated and stored for 90 d.

Conclusions An exogenous protease added to whole plant corn at ensiling did not improve IVRSD after 2 and 7 d of fermentation regardless of storage temperature. However, after 45 d of fermentation, treatment with protease improved IVRSD at moderate and high temperatures of storage and the effect was numerically greater when the silage was stored the higher temperature, presumably due to greater enzyme activity.

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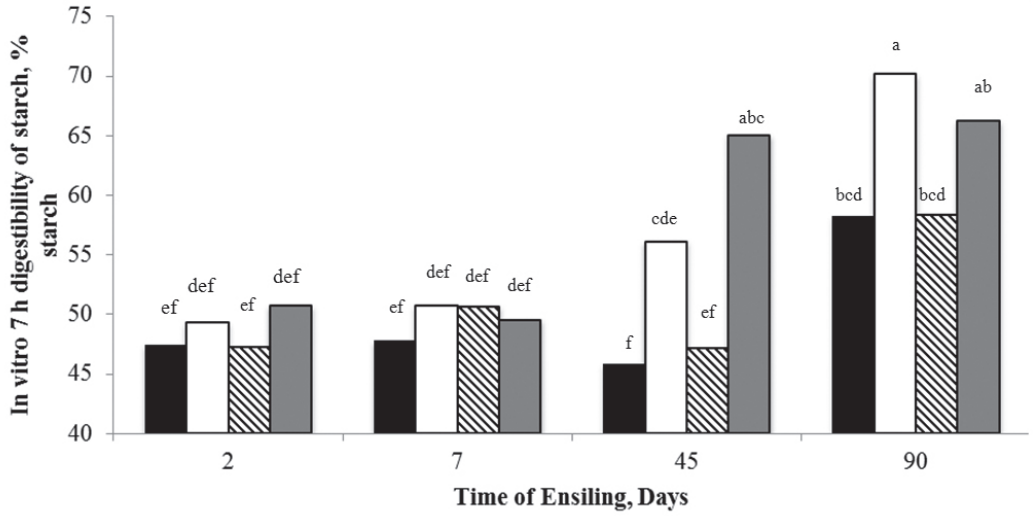

Figure 1 The IVRSD (% of starch) of corn silages that had been untreated and stored at 22°C (black bars), treated with a protease enzyme and stored at 22°C (white bars), untreated and stored at 40°C (striped bars), or treated with a protease enzyme and stored at 40°C (gray bars). Bars with unlike letters (a-f) differ ($P < 0.05$). SEM = 1.95. Effect of protease, $P < 0.01$; effect of temperature of storage, $P = 0.23$; effect of time of storage, $P < 0.01$; protease × temperature interaction, $P = 0.88$; temperature × time of storage interaction, $P = 0.09$; protease × time of storage interaction, $P < 0.01$; protease × temperature × time of storage interaction: $P = 0.06$.

Sorghum-legumes mixtures silage: aerobic stability and organic matter loss

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Keywords aerobic spoilage, organic matter loss, pH, soybean, temperature

Introduction Aerobic spoilage of silage is associated with the penetration of oxygen into the silage during storage or feeding. In aerobic environment, at feed-out phase, the spoilage microorganisms such as filamentous fungi, yeast and aerobic bacteria multiply rapidly, promoting intense metabolic activity, and causing an increase in the temperature and pH of the silage, as well as elevating the loss of quality silages. Resistance to these changes in the standard silage is what characterizes the aerobic stability of silage. Silage of legumes generally has lower aerobic stability that grass silage; however, greater amounts of protein and minerals, thus, the intercropping can be an alternative to overcome this problem. In this sense, the objective was to evaluate sorghum-legumes silages and its aerobic stability and organic matter loss.

Materials and methods It was evaluated two silages produced from associated crops, being the dwarf grain sorghum (*Sorghum bicolor* (L.) cv. Surgho) with soybean (*Glycine max* (L.) var. Mitzuko NT), and giant grain sorghum (*Sorghum bicolor* (L.) cv. Sweet virginia BMR) with common beans (*Phaseolus coccineus* (L.) var. Neckargold). During feed-out phase, five samples were collected (5 kg each) at different points for each silo (with capacity of 216 m^3), placed in plastic buckets and maintained in a closed place at room without control of environmental variables. Aerobic stability was evaluated for a week, by measurement of silages and ambient temperature, in addition to the pH values. The temperatures were measured twice daily (9h00 and 17h00), using a digital thermometer (Model Checktemp® 1; Hanna, Ann Arbor, Michigan, USA) and the pH checked once a day (17h00) by the potentiometer method (Kung Jr. et al., 1984). Daily, sub-samples were collected from silage to determine the levels of ash and estimation of the organic matter loss in aerobiosis, according to equation described by Ashbell and Weinberg (1992). Data were subjected to analysis of variance and regression and the means compared by F test ($P<0.05$) using the statistical program SAS (Statistical Analysis System - 2009).

Results and discussion There was a positive linear behavior for pH values as a function of air exposure time with greater depth in giant sorghum+bean silages (Figure 1). This could be due to its lower density compared to dwarf sorghum+soybean silages (145.37 *vs.* 158.68 kg of DM/m^3) favoring the penetration of oxygen in the silo panel and favoring the activity of microorganisms spoilers. At the beginning of aerobic exposure, there were no differences ($P>0.05$) between the pH of dwarf sorghum+soybean silages (4.21) and giant sorghum+bean silages (4.12). However, from the third day, giant sorghum+bean

silages showed rapid increase in pH, continuing to a lesser extent, the following days, until reaching the maximum value of 8.53. For dwarf sorghum+soybean silages, there was an increase in pH (P<0.05) when compared to first days, only on the sixth day of aerobic exposure.

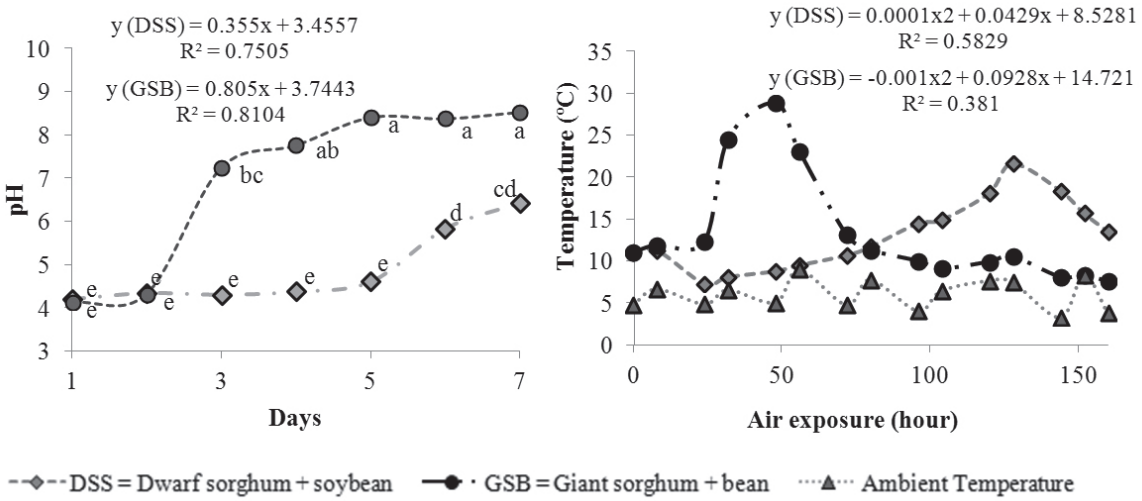

Figure 1 Regression equations for pH and temperature of the silage as a function of time of air exposure.

The higher pH increases occurred simultaneously to higher temperatures. There was a quadratic effect of temperature on both silages, with a tendency to stabilize close to the value of the ambient temperature, after reaching the point of maximum warming, that occurred in the third and sixth day to the giant sorghum+bean silages (25.95°C) and dwarf sorghum+soybean silages (19.85°C), as verified in Figure 1. The amounts of organic matter losses were higher (P<0.05) in the association of giant sorghum+bean silages compared to dwarf sorghum+soybean silages (1.6 vs. 0.34%). These values can be explained by the high temperatures and pH observed in the giant sorghum+bean silages, suggesting a high activity of microorganisms spoilers and low aerobic stability of these silages.

Conclusions Dwarf sorghum+soybean silage has higher aerobic stability and smaller losses of organic compounds that giant sorghum+bean silages, constituting an important alternative for the production of silages with good quality.

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Design and statistical issues in silage experiments

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Keywords silage, experimental design, sampling, statistics

Abstract
The relevance of silage research objectives, designing experiments to meet objectives and silage sampling problems are discussed, as well as comments on statistical analyses of silage experiments. Study objectives must always be clear, achievable and relevant, with the latter judged relative to the targeted forum, such as farmers/advisors, scientists or relative to geographic regions. In designing silage studies, crop(s) which are potentially responsive to the treatment should be selected, but factors influencing plant composition and silo conditions which are likely to influence treatment outcome must be considered. Although analytical and statistical replicates are needed in silage studies, they are often confused. Analytical replicates are necessary to reduce overall experimental variability, but observations from, for example, a single plant source and treatment should be averaged before statistical analysis. In contrast, true replicates are units on which the statistical analysis is based. They may be multiple sources of a specific plant, different plant species, different batches of inoculum or from different harvest dates, and are fundamental to identification of treatment impacts. Judging from the literature, the failure to consider the existence of interactions is the biggest flaw in design of silage experiments. Mini-silos allow an increase in the number of analytical and statistical replicates, and to complete experiments under controlled conditions thereby enabling the study of interactions. While mini-silos may or may not be model silos, they can be a useful starting point to identify promising treatments in experiments with commercially sized structures, where the number of analytical and statistical replicates will be much lower. Sampling silage to obtain representative samples is challenging and appropriate methods vary dependant on objective. Sampling to obtain information on average silage composition, spatial differences in the silo or of the silage actually fed to the animals differ. While a few analytes can be obtained from the fresh chop crop, products of fermentation can only be obtained from samples collected before or after silo opening. The statistical model must always be consistent with the objectives and should be described exactly, as well as describe which observations were included. Scrutiny of 10 years of published silage papers

indicates that statistical models are seldom described in detail, software (of limited use to a reader) is almost always reported and most models incorrectly use analytical replicates as statistical replicates. In studies based on dose response treatments, polynomial contrasts, or use of dose as a covariate, are often the most powerful statistically. A very few reviews have been published on analysis of multiple silage data sets. Such reviews have merits, but are not free from problems with respect to data quality and interpretation of results. It seems that simple treatment comparisons are often more suitable than studies to predict ensiling results, due to incomplete information of potentially important variables.

Introduction

Silage has been an important preservation technique of seasonally available forages for a very long time in most dairy areas of the northern hemisphere. Initially silos were simply holes in the ground, and sometimes they still are, but the last 50 yrs has seen a plethora of new and modified silo structures and techniques ranging from tower silos (with and without oxygen barriers), to concrete bunkers, to large compacted piles, to use of plastic covers (with or without weights) and, more recently, use of inner plastic coverage films with and without enhanced oxygen barriers. As the size of dairy farms has increased in many dairy areas so has the need for silage, and sizes of silo structures have increased dramatically in some areas. Bunkers and piles of 15,000 tonnes fresh weight silage are now common in some dairy areas of the United States.

Diets of high-producing dairy cows often contain silage at a level of about 50% of diet dry matter (DM). This makes silage hygienic quality, and nutritional composition (which can vary substantially among day), critically important to animal performance. Sources of variation in silage characteristics are many; vary among silo structures, among crops and with how the silo masses were constructed. However they can all impact diet composition and quality, particularly in the mixed rations which are fed to the cattle, unless rigorous quality control is exercised.

Corporate and university/government research efforts have devoted much effort to finding ways to create silages with maximum nutritive value and minimum mould/yeast counts. Thousands of papers on forage species, varieties, maturities and moisture levels, as well as silage additives, silo types, cut lengths, pack densities and silo sealing techniques have been published over the last century. This research has changed direction over time as the nature of the silages, silo structures and issues relative to silages have changed. However, often only one or two variables have been investigated simultaneously and, generally, the experiments cannot be repeated in any meaningful fashion due to the unique nature of the crops used. This has meant that general statements addressing the fundamentals of silage making have changed only modestly over the years, and that

specific recommendations are generally of regional interest, meaning that they are highly specific to the actual silo structures, crops, soils and climates. Nevertheless, valuable information and recommendations have come out of this research and they have been successfully adopted in many areas. To enable research to predict and manipulate the outcome of ensiling, more effort must be devoted to design and statistical evaluation of silage experiments to resolve questions related to the whens, whys and hows of ensiling techniques and technologies.

Our objectives are to discuss the relevance of silage research objectives, how to design experiments to meet objectives, how to obtain sufficient information on silage composition by sampling, and to provide some advice on statistical analyses of silage experiments.

About formulating objectives in silage research papers

Clear objectives and hypotheses (if any), are absolutely essential in all scientific studies. They may be (and often are) refined during the course of completing and analysing the experimental data, but they should always be present in the final manuscript, preferably as the final paragraph of the introduction section (Robinson et al., 2007), and the stated objectives should always relate directly to the conclusions section of the study.

The importance of clear and achievable objectives was discussed by Udén et al. (2012). It is essential in determining the type of statistical analysis that should be completed and the most appropriate statistical model. Three key issues are of importance in formulating objectives. First, are they clear, second, can they be met and, third, are they scientifically relevant? These criterion are often more complex with silage studies due the generally unique combination of conditions which lead from a sown crop to a silage. For example, an objective such as:

> "To investigate effects of inoculation of alfalfa with *Lactobacillus plantarum* on silage fermentation quality"

is clear, but the objective cannot be met if the author ensiled a single crop source harvested at their research site. In this case, use of a single site means that the specific conclusions of the study are highly specific to that site, and so only general implications can be made to other areas. Thus, if the objectives statement is modified to a more specific objective, such as:

> "To investigate effects of inoculation of alfalfa FSG229CR with *L. plantarum* on silage fermentation quality harvested at 55% DM in Dade County (WI, USA) in 2010 and 2011 on silage fermentation quality"

is now clear and achievable, thereby meeting criterion 1 and 2 above, but its specificity to a single geographical area (and to its soils and climate) means that its findings

are only locally relevant thereby questioning its scientific relevance. If the objectives of the study are further modified to create scientific relevance to read:

"To investigate effects of inoculation of alfalfa FSG229CR with *L. plantarum* on silage fermentation quality harvested at 55% DM in Dade County (WI, USA) and in Kings County (CA, USA) in 2010 and 2011 on silage fermentation quality."

is now clear, achievable and scientifically relevant, thereby meeting all the criterion listed above, but complexity and cost may make it functionally impossible to complete. Thus, the silage scientists' dilemma is to identify the appropriate (i.e., achievable, scientifically relevant) level of specificity and complexity for the study. This may best be decided by choice of forum. Does it consist of dairy farmers in Dade County growing mainly alfalfa FSG229CR, or is it the scientific community? If the former, then a study limited to Dade County is appropriate, but may be unpublishable in a peer reviewed scientific journal. If the latter, then it is critical that more than one geographic area be in the study in order to draw scientifically relevant conclusions. Phrased differently, while it is always possible to draw specific and general conclusions from a study, if those specific conclusions are regionally specific, then results will be of interest to individuals in the region but, scientifically, it may be of little or no relevance. That general conclusions can be drawn does not necessarily make the results scientifically relevant since general conclusions are, by their nature broad, and so stated objective may make the study scientifically unimportant in general. The question of generality of objectives and conclusions impacts choice of statistical replicates, which will be further dealt with in the section on experimental design.

About designing silage experiments to examine impacts of treatments

Experiments dealing with treatments in the form of plant sources, often linked to physical, chemical or microbiological treatments, are the most common in silage research. A search of the literature revealed 296 papers published with at an English abstract between 2004 and 2014 wherein the specifications used were:

Title: silage/ensil*,

Topic: statist*,

Type: non-agronomic, non-feeding, non-conference, non-modelling, non-prevalence studies, non-animal production.

Only 13 of these papers consisted of single experiments and so they were scrutinized in detail. An additional 3 papers were meta-analyses, which were evaluated separately. Only some of these papers having, in our view, clear merits will be cited.

A general problem in a majority of these silage papers was that variability due to treatment and variability due to measurement were confused. When using a single forage

source, the evaluation method itself creates variability and, if animals are involved in the evaluation, the variability will be higher than when only chemical analyses are employed. Another serious problem is that many authors ignored the likely existence of treatment interactions.

The choice of silo type for research purposes plays an important role. Because ensiling studies with commercially sized silos are a logistical challenge, leading to limited observations and statistical limitations, use of mini-silos facilitate ensiling of multiple plant sources at different maturities and times of the year prepared in different ways with several treatments and/or levels. The issue, never addressed by mini-silo users, is whether a 10 kg mini-silo is actually representative of, for example, a 10,000 tonne silage pile. The likelihood that mini-silos are indeed mini-silos rather than model-silos is a serious criticism of the usefulness of using mini-silos as a model for commercially sized silos.

Choosing a crop for ensiling requires specific considerations. Which type of crop is likely to be responsive to a specific treatment and how important are characteristics such as DM content and water activity, growing conditions, plant maturity, time of year of harvest and climatic conditions during growth and harvest? Some of these variables affect plant composition, silo environment (i.e., osmotic pressure and water activity) and probably also field microfloral composition, which are often accepted to be the main crop variables influencing silage outcome. In addition, there are variables related to silo management. If silage effluent is ignored, then they mainly relate to oxygen availability during filling and ensiling. Management variables can be influential to detecting treatment effects amongst background variation (i.e., from functionally unknown sources).

The influence of as many of the variables noted above should be known before initiating an experiment. Knowledge of other variables, such as field flora and oxygen availability, require an experimental design that takes them into account. For example, when examining an additive to improve aerobic stability of silage, the experimentalist may know that farmers have stability problems with a certain crop in the 2nd cut when wilted to DM of 50 to 60%. What may not be known are interacting effects of plant composition, field microflora and O_2 availability during storage. Thus a logical design for an experiment should include field replicates to introduce micro-floral variability, if it exists, and several harvest dates to create compositional variability, either as a treatment or as replicates, and some treatments with different levels of O_2 ingress. Indeed, the *Deutsche Landwirtschafts-Gesselschaft* (DLG; www.dlg.org) require air stress during the ensiling phase, as well as at least 5 ensiling experiments, in order to grant quality labels of additives aimed at improving aerobic stability. Whether this protocol achieves realistic variation in O_2 ingress is uncertain, but may warrant research effort as little has been done in the area.

Those treatments which have high variability should be replicated to enable

generalizations. For example, it is well known that crop composition varies considerably. However additive addition and chopping under field conditions introduce other sources of variation. Thus, if chop length and additive levels are possibly influential to outcome, and interact, not dealing with them in the experimental design will limit interpretations, possibly even creating false conclusions, thereby substantially reducing the value of the experiment.

An issue of controversy relates to the concept of analytical *versus* statistical replicates. Both are needed, but for different reasons. To be clear, analytical replicates as those used to obtain as correct an answer as possible about a sample (Udén et al., 2012). This means that, in the example above, silos with the same plant source and treatment are analytical replicates in the same way as replicated chemical analyses are analytical replicates since the only variability in both is that introduced by the process. While such replicates have value by reducing overall variability, they should be averaged before statistical analysis. In contrast to analytical replicates, true replicates are the statistical units which are fundamental to identification of treatment interactions. Ignoring interactions is likely the biggest error in silage experiment design, at least based on our examination of silage papers published since 2004. The most important interactions are likely to be treatments (e.g., additives) which interact with crop composition, O_2 availability and field flora. An excellent example of examining interactions is the study of Lima et al. (2011) in which 2 sorghum and 1 soybean variety were used in a 72 treatment mini-silo study. Treatments included the pure forages with 3 proportions of sorghum inclusion, all with 4 water soluble carbohydrate addition levels with or without a bacterial inoculant. Treatment interactions and orthogonal contrasts were effectively evaluated by this design. However, the issue of whether the findings of this study, completed with mini-silos, are relevant to practical commercially sized silos makes the findings of uncertain value outside of the conditions of the laboratory study. Nevertheless, the great advantage of mini-silos is that, because they allow an increase in the number of observations, the ability to complete experiments under controlled conditions with respect to crop composition, aerobic conditions and temperature, for example, is possible. This may be a useful starting point in identifying promising treatments to use in further experiments with commercially sized structures where the number of analytical and statistical replicates will be lower. Interestingly, no efforts were found in the literature to use mini-silos to simulate other than anaerobic conditions, which likely prevails in the inner mass part of a commercial silo, but is unlikely to exist in the surface 'skin' of a silo.

Conducting silage research at commercial silo scale often makes statistical evaluation so difficult as to be functionally impossible. In the study of Borreani et al. (2007), two types of plastic underlay cover films were compared on two farms with different

managements. Each horizontal farm silo was divided lengthwise with each half covered with different films. Net bags of fresh chop crop (i.e., 'buried bags') were incubated in different silage layers in 4 replicates and analysed after recovery. The methodology of dividing the silo lengthwise, thereby introducing the possibility of an untestable side by treatment interaction (which could lead to a false conclusion of the impact of the films), and the assumption that buried bags are a model of the surrounding silage mass, are unverified. However an experimental design without information from the 'buried bags' prevents a meaningful statistical analysis of losses because each pile has both covers. In this case, the experimentalist has a 2 (silo) x 2 (treatment) design with only one degree of freedom (df) in the error term. If the data from the 'buried bags' is accepted and the 4 replicates averaged, the model would have a 2x2x2 design with 4 df for error. Such a design remains limited by the number of true statistical replicates (i.e., silos) but may make it a more useful general concept for on-farm research with local (i.e., geographical or conceptual) importance. Then again, an experimentalist cannot possibly examine a plastic underlay film in a mini-silo since it is obvious that the mini-silo is not a model-silo relative to this treatment. Thus, the scientific value of such study may only be fully mined in a subsequent strategic or meta-analysis of a number of similar studies, as is examined below.

Sampling silage

Silage sampling objectives vary. Particularly for large horizontal structures, it is critical that the purpose of the sampling be defined. If the purpose of sampling is to document differences in composition relative to location on/in the face then a different sampling protocol will be required *versus* an objective to document differences in composition of the silage being fed to the cows within and/or among days, or to obtain an average value of the entire silage mass. The sampling protocol will also be influenced by prior knowledge of variability of similar type silages.

There were only a few publications found in a second literature search covering 1980-2014 which included sampling approaches and statistics of analytical data from commercial silos (Haslemore and Holland, 1981; Stone, 2003; O'Brien et al., 2006; Krnjaja et al., 2012; Schenk et al., 2013; Weiss et al., 2014).

Sampling silo structures prior to opening

Identification of appropriate schedules/methods to sample silages from silos prior to opening can be relatively simple (i.e., in mini-silos which can be emptied and sampled), not so difficult (i.e., wrapped bales and silage tubes where every bale, or location in the silage tube, can be accessed), difficult (i.e., in large horizontal commercial structures where the bulk of the structure is functionally unavailable), and impossible (i.e., vertical silo

structures).

The entire mass of silage in individually wrapped bales and silage tubes are relatively accessible for sampling prior to feedout, for example over time of fermentation, using appropriate sampling apparatus. For example, O'Brien et al. (2006) core-sampled 10 undamaged 1.2 x 1.2 m round bales for microbial analysis from 4 horizontal angles at 2 distances from the top of the upright bales to a depth of 20 cm. This perennial grass silage had an average DM content of 416 g/kg and a pH of 5.2, and among bale SD for yeast was 3.1 log cfu/g as opposed 1.3 within bale (based upon analysis of original data supplied by the first author). It is likely that if damaged bales had been included, that the SD's would have been higher. Due to a skewed distribution for moulds, which is often the case in silage samples, mould data was not analysed for its variance profile. Non-normal distributions of organisms in silage, as well as calculation of meaningful averages are discussed below.

Vertical silo structures cannot be sampled at the sides, or at the bottom, while sampling from the top will likely create unacceptable health and safety risks, and have little interpretive value since the limited distance that can be penetrated into the mass from its top will mean that only a small fraction of the silage is actually sampled.

Large walled and unwalled horizontal silos can likely only be sampled prior to opening to a limited depth, meaning that data to represent the deep mass is functionally unavailable. However, this sampling procedure may be suitable if the objective is to determine the composition of outer silage layers, perhaps to determine surface spoilage prior to opening.

Sampling silo structures after opening

Wrapped silage bales are normally rapidly fed our after opening. Thus sampling can theoretically occur in the mixer wagon, but this requires rapid in line analysis to be useful, and breaking up the silage, which in large wrapped bales is generally not chopped, will likely defy effective sampling. A better alternative is generally to collect core samples directly from the intact bales at, or immediately before, feedout.

Vertical silo structures can be sampled at unloading from the silage being removed (i.e., top or bottom of the silo mass dependent upon the loadout mechanism). Such samples could be used to assess the change in characteristics of silage with time of unloading relative to an applied treatment, or it could be used to create samples to represent changes within or among day in silage characteristics which are of relevance to animal performance.

Considerable variation exists among the layers of horizontal silos and it was considerably larger for most components in haylage *versus* maize silage in the US study of Stone (2003). If the purpose of sampling an exposed face of a horizontal structure is

to document differences in composition from top to bottom, side to side and due to depth into the exposed face, then there will be a need for a face sampling protocol in terms of grid and depth. This can be dangerous if the exposed faces are tall and/or have overhangs. Nevertheless, such a protocol will be required if, for example, the stated objective involves use of methods to create higher silage pack density and its impact on silage quality at the exposed face (which is, after all, the silage taht is fed to the cows). To document differences in composition of the silage being fed to the cows within and among days, then the sampling protocol needs to sample what is actually going into the feed mixer or bunk. In this case, a face sampling protocol will not be required, as this documents the characteristics of the silage on (and in) the face, in favour of a sampling schedule to document the characteristics of the silage used to feed the cows.

Studies reporting variation in microbial and chemical composition within silos are scarce. An example of a study on variability within a horizontal silo is that of Krnjaja et al. (2012). The ensiled maize grain had water contents, total mould counts and mycotoxin concentrations which depended on depth in the silo. Water content decreased from the top (720 g/kg) to the bottom layer (676 g/kg). Mould counts and mycotoxin levels were highest in the middle layer, except for deoxynivalenol. The presence of zearalenone was 60% in the middle layer samples, whereas samples at the bottom layer had non-detectable levels. The study of Green et al. (2012) is an example of large spatial variation in O_2 and temperature of two horizontal silos (with and without intact covering). They noted the highest heterogeneity towards the centre of the silos. Schenck et al. (2013) compared techniques to monitor prevalence of fungi in 150 round bale haylages in Sweden and Norway. When fungal species were plated after core sampling, as opposed to after sampling visible fungal colonies on the silage surface, prevalence differed substantially. These three reports emphasize the problems of the heterogeneous nature of silage which will have a direct impact on research objectives and experimental design.

Weiss et al. (2014) evaluated variability in silage composition due to sampling, day-to-day and analytical variation. They collected corn silage (CS) and hay crop silage (HCS) samples from 16 farms and took duplicate daily samples over 14 days and analysed each sample in duplicate in a single laboratory, resulting in 448 samples per silage type. The data was analysed for within-farm variation (Table 1).

Table 1 Standard deviations of samples taken from farms representing analytical, sampling and day-to-day variation (% of dry matter)

Dry matter		NDF[a]		Starch	CP
CS[b]	HCS	CS	HCS	CS	HCS
1.78	3.31	1.94	2.14	1.79	1.08

[a]Neutral detergent fibre; [b]CS=corn silage; HCS=hay crop silage (Weiss et al. 2014).

Analytical variation was the smallest contributor (<10%) whereas, variation due to sampling ranged from 30 to 70% of the total, and was the major source of variation for neutral detergent fibre (NDF) and starch in corn silage and crude protein (CP) in hay-crop silage. Day-to-day variation ranged from 20 to 65% of total variation, particularly for DM concentrations and NDF of hay crop silage. These high SD values likely relate to farm staff collecting samples on each farm using different (undefined) methods, rather than by individuals trained to sample by a common method, something which was recommend by the authors for on-farm use. The authors discuss the implications of the findings relative to diet formulation.

Sampling before ensiling

Under many conditions, silage composition and nutritive value can be predicted to some extent from that of the crop at ensiling (Huhtanen et al., 2005), with the exception of fermentation end-products (Mogodiniyai Kasmaei et al., 2013) and hygienic quality variables. Thus sampling at the time of ensiling should increase predictability of the composition of the resultant silage, although repeated sampling of incoming truckloads of fresh chop crop will require a very high number of samples to adequately represent the fresh chop crop put into the silage structure, especially for horizontal structures where layering of the incoming crop is at an ~45 degree angle. Thus, such samples at silo building may be useful relative to loadout time samples for vertical silos, silo tubes and wrapped bales. They will be of much less use for horizontal silos where face removal is vertical but building was at an ~45 degree angle. Although no data was found in the literature on sampling errors at time of ensiling, we surmise that differences in concentrations of some analytes between crop and silage will be mainly determined by the balance of losses due to effluents and fermentation *versus* accumulation due to losses of other constituents, mainly in the form of effluents and CO_2.

About analysing silage experiments statistically

Today, almost all data is analysed by statistical packages requiring only a model statement and a data set to obtain probabilities for treatment differences. There are fundamental problems with this approach, as pointed out by Robinson et al. (2007), is that the analysis can be done without any actual understanding of the statistical model used. It also may encourage uncritical 'shopping' for statistical procedures to find one which yields a desired statistical outcome.

Statistical Models

It is imperative that the statistical model(s) are consistent with the experimental design and objectives. A description of a statistical model should include the model in words as well as presenting it as an equation, unless the model is very simple. An unambiguous and complete description of how all available observations were used in the model should always be reported (see Udén et al., 2012). The general impression from our scrutiny of 10 yrs of published silage papers are that statistical models are often not described in sufficient detail, whereas the software (of limited use to readers) is almost always reported. Most studies used some form of 'analytical' replicates but, in several studies, the absolute number statistical units could not be deduced.

A discussion of the virtues of fixed and random effects, and associated models, is beyond our objectives. It should be noted, however, that a standard error of the mean (SEM) from a 'mixed model' procedure is always larger than from a model with only fixed effects (Littell et al., 2000). Thus it is less meaningful than a standard error of the difference (SED) because it also contains covariance and can therefore be larger than the least significant difference (LSD).

A common design of silage studies is to examine incremental level of silage additives, often with the objective of identifying an optimal level. In such cases, multiple range tests are inappropriate and create problems in preparing readily interpretable tabular presentations. Thus, analysing dose level responses by polynomial contrasts, or including dose as a covariate, is generally more effective (Robinson et al., 2007). Such models also make the statistical test stronger by increasing the degrees of freedom in the error term.

Non-normal distributions

When analysing data statistically with parametric methods based on probability distributions (e.g., analysis of variance), it is advisable to first test errors for normal distribution. If they are not, transformation may be necessary, often as log-transformation. Particle size, hydrogen ion concentrations and microorganism levels in silages are candidates for non-normal distributions. However, a problem arises with interpretation of means. The correct arithmetic mean number of organisms, for example, estimated from replicated sampling, can only be calculated on untransformed values and log-transforms will yield the geometric mean. For pH, this may be the only option as the arithmetic mean of the hydrogen ion concentration at many silo points is unlikely to be the same as the concentration of a perfectly mixed silo due to buffering.

The issue of how to deal with response variables with extreme variation is not simple. For example, as microorganisms in a silo can proliferate extensively in pockets while existing at negligible levels elsewhere, it is possible that data cannot be normalized

and meaningful averages cannot be calculated. Even collecting multiple samples may be of little use if the range is very high in the response parameter. For example, if 20 silage samples are collected from two treatments, and 39 have undetectable mould counts but one has a mould count of 30,000,000 cfu/g (presumably because it is in a 'hot spot'), then averaging all samples within treatment will result in a mean of 1,500,000 cfu/g for one treatment but 0 cfu/g for the other, which is obviously not representative of the treatment impact. In the case of such response parameters, it may be necessary to create biologically meaningful *a priori* 'cut points' which represent a biological impact, and then report results as a distribution profile.

About using multiple studies to test treatments

Papers with shortcomings in terms of design, in the sense that few general conclusions can be made, may have use if a sufficient number of similar studies are available to enable strategic or meta-analyses. This can be the case for data from commercial and mini-silo experiments. The statistical approaches used in the three published silage meta-analyses are worthy of comment.

Wilkinson and Fenlon (2013) collected data from 51 paired comparisons where two types of plastic underlay films had been used to 'seal' the silage of horizontal farm-type silos, mini-silos and bales. Loss during storage was one of the variables compared and a simple one-tailed paired t-test was successfully used to test differences.

In the well-planned meta-analysis of Kleinschmit and Kung (2006), effects of adding *L. buchneri* at ensiling of whole-crop maize, small grains and grasses in unspecified silos were analysed in a set of 43 experiments from 23 sources with standard errors (SE) reported. Data was divided into high and low level bacterial inclusion in the statistical analysis where study was used as a random effect in a mixed model. Orthogonal contrasts were used to test differences among treatments. A regression was also used to investigate effects of the numbers of yeasts on the acetic acid level of the silages. As maize silage fermentation differed from whole-crop small grain and grass silages, data was further divided into silage type prior to analysis.

A meta-analysis of mini-silo experiments was completed by Mogodiniyai Kasmaei et al. (2013) with the aim of obtaining prediction models for fermentation products. They assumed that crop composition and field flora could explain concentrations under similar, and strictly anaerobic, conditions. A total of 118 observations of silages from a single laboratory made from crops (i.e., 30 grasses, 7 legumes, 15 grass and legume mixtures, 66 whole-crop maize) harvested nearby over 7 yrs were available. Multiple regression models were fitted 'stepwise' and, if residuals were not normally distributed, response variables were logarithmically transformed.

Analysis of multiple silage data sets has merits, but it is not free from problems with respect to data quality and interpretation of results. It seems likely that simple treatment comparisons, such as those of Wilkinson and Fenlon (2013) and Kleinschmit and Kung (2006), are more suitable for analysis because the number of known differences among treatments within study are lower, and the number of unknown differences may also be lower. However, identification of the underlying reasons for ensiling results (Mogodiniyai Kasmaei et al., 2013) is probably seriously affected by the lack of information on plant, micro-floral and other interactions which is considered to be important, and/or reported in the papers which comprise the data set.

Conclusions

Relevance of, particularly, silage studies published for the benefit of science, are often limited as a result of not considering treatment interactions. Use of mini-silos makes it possible to study ensiling under controlled conditions, and to increase the number of observations, thereby allowing more powerful study designs which also enable analysis of interactions. However, mini-silo experiments can only be starting points to identification of promising treatments to explore in experiments with commercially size silos. To make it possible for mini-silos to become relevant model silos of these commercially sized silos, further improvements are needed in technique as well as a better understanding of important variables. Statistical models published in the literature are generally poorly described and include analytical replicates to allow acquisition of a sufficient number of observations to make a statistical analysis apparently possible, even though it is incorrect. Sampling of silage poses considerable challenges and the method chosen must be relevant to the stated objective of the sampling.

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Influence of different slurry application methods on grass silage quality

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Keywords slurry application, clostridia, grass silage, silage quality

Introduction The silage quality is influenced by many factors. The composition of the forage at ensiling as well as the technique of silage preparation are important. With the application of slurry many clostridia spores are spread to the field and to the forage. According to Lorenz and Steffens (1996) the forage which had been fertilized with a broadcast system had higher butyric acid contents in comparison to a band-spread system. On the other hand, Beck (2011) did not find differences between the two systems. The objective of this study was to compare the effect of different slurry application methods as well as a mineral N fertilizer treatment on the number of clostridia spores and the grass silage quality.

Materials and methods The experiment at Agroscope in Tänikon (535 m a.s.l.) included three different slurry application techniques (broadcast, band-spread and trailing-shoe), as well as a mineral N fertilizer treatment in a small-plot scale (18 m^2, three repetitions per treatment). Slurry (4-5% dry matter (DM) content, 30 kg NH_4-N per ha and cut) and mineral fertilizer (30 kg N/ha and cut) were applied at two times (early: 1-3 days after the preceding cutting; late: 7-10 days afterwards). The forage which contained only grasses was cut five times a year. Samples of the first, third and fourth cut were ensiled in 1.5 l laboratory silos and analysed 90 days after ensiling for silage quality (butyric acid). In addition, DM and nutrient contents, as well as clostridia spores, were determined in the fresh forage.

Results and discussion At the time of ensiling the forage samples had an average DM content of 22, 30 und 33% for the three different cuts. The fermentability coefficients were 42, 45 and 49. The ash and crude protein content amounted to 72, 80 and 77 and 120, 116 and 143 g/kg DM, respectively, and the crude fibre level was 254, 224 and 207 g/kg DM for the three cuts. The number of clostridia spores was relatively low (Fig. 1). The highest values were detected for broadcasted and band-spread, late applied slurry. A reason for the lower number of spores in the late slurry application treatments is that there was less rain between the slurry application and the harvest. Despite the low number of clostridia spores butyric acid was produced during the fermentation process (Fig. 2). Particularly, the silages of the first cut showed in all treatments high butyric acid contents. This is partially explainable with the low DM content. For the third and fourth cut, the highest butyric acid contents were detected for broadcast and late application. These observations confirm the results reported by Lorenz and Steffens (1996).

Conclusions The different slurry application methods influenced the number of clostridia spores on the forage as well as the silage quality. Additionally, the time of slurry

application had an effect on both clostridia values and silage quality.

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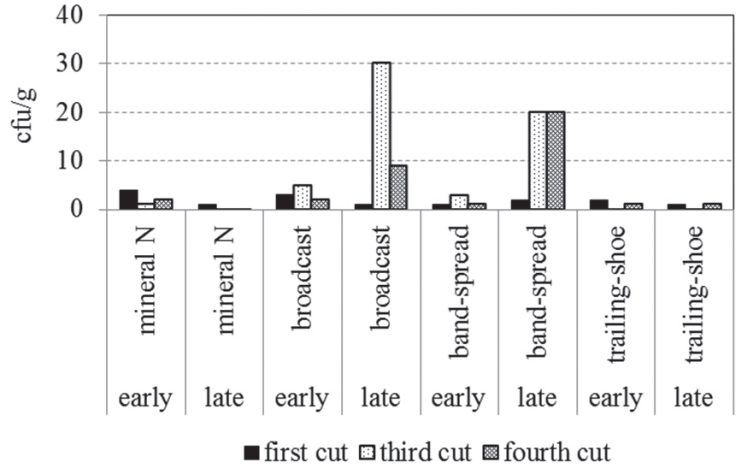

Figure 1 Clostridia spores of fresh forage samples at different application techniques and application timings (cfu: colony format units).

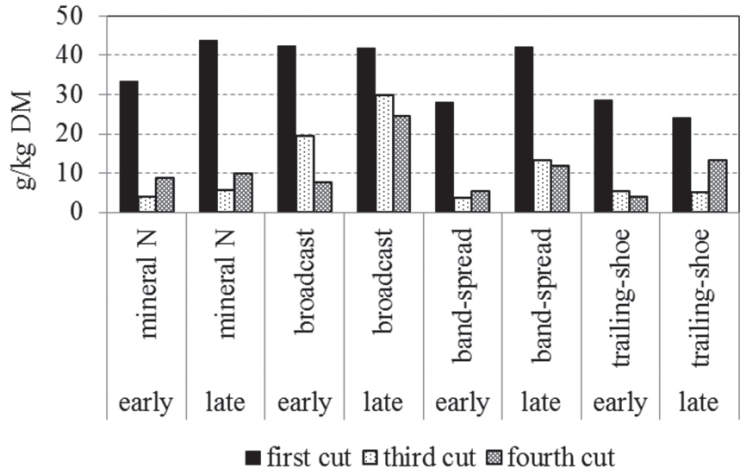

Figure 2 Butyric acid content of the silages at different application techniques and application timings.

Effect of aerobic exposure before and after ensiling on maize silage quality

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Keywords air exposure, density, sealing time, aerobic stability, maize silage quality

Introduction When silages are exposed to air, deterioration may occur as a result of microbial activity. This process provokes loss of dry matter (DM) and nutritive value (Tabacco et al., 2011). Air can exert its detrimental effects during the silo filling phase until sealing and during the feed-out phase. The aim of this study was to determine the effects of aerobic exposure before and after ensiling and DM density on DM losses, aerobic stability and microbial composition of maize silage.

Materials and methods Whole-crop maize (28% DM) was harvested in September 2013 on the research station Frankenforst (University of Bonn, Germany), chopped, mixed and thereafter ensiled in 120-L plastic barrels (six per treatment). Treatments evaluated were: **Lo0** - low density (196 kg DM/m^3), sealing: prompt; **Lo2** – low density (194 kg DM/m^3), delayed sealing after 2 days (d); **Lo4** - low density (191 kg DM/m^3), delayed sealing after 4 d; **Hi0** – high density (232 kg DM/m^3), sealing: prompt; **Hi2** - high density (235 kg DM/m^3), delayed sealing after 2 d; **Hi4** - high density (234 kg DM/m^3), delayed sealing after 4 d. Barrels were stored anaerobically for 175 d (Lo0/Hi0), 217 d (Lo2/Hi2) and 259 d (Lo4/Hi4) under comparable ambient temperatures. After opening the barrels silages were taken out, mixed and aerobically exposed indoors for 6 d (mean ambient temperature across all treatments: 20 ± 1.5 °C) as a quadratic heap with a constant layer height of 12 cm. Ambient temperature as well as the temperature of each silage treatment was recorded every 4 h by data logger inserted in the center of the material to determine aerobic stability over the time of aerobic exposure. On each day of aerobic exposure a composite sample per treatment was taken for microbiological analysis, sealed anaerobically and kept cool until analysis. The DM losses were determined as the difference between the weight of the plant material placed in each barrel at ensiling and the weight of the silage at the end of conservation and were calculated according to Weissbach (2005). Data were analyzed as a completely randomized design by employing PROC MIXED of SAS 9.2.

Results and discussion Sealing time affected the DM losses ($P<0.001$) with the highest losses found in Lo4 and Hi4. This can possibly be explained by the longer time of air exposure before sealing the silos. Delayed sealing by more than 2 d led to DM losses of about 11% (Lo4). Prompt sealing as well as delayed sealing for 2 d resulted in moderate DM losses (Table 1). Sealing time also influenced the aerobic stability. Silages sealed after 4 d showed the lowest aerobic stability (47 h for Lo4, 52 h for Hi4), whereas promptly sealed silages were stable for 64 h (Lo0) and 65 h (Hi0), respectively. Silage density had no influence on DM losses and on aerobic stability after 6 d of aerobic exposure. Data on the effects of aerobic exposure on microbial populations after silo opening are shown in Table 2. When silages were exposed to air, the initial yeast count of $\log_{10}$ 5.15 cfu/g increased rapidly to $\log_{10}$ 7.26 cfu/g after 2 d of exposure, and a further increase was observed until d 4 and 6 to $\log_{10}$ >8.00 cfu/g. Mould numbers did not change until d 4 of aerobic exposure but a marked increase was seen on d 6 of exposure to air. Bacterial counts measured on d 4

and 6 of air exposure were higher than those determined on the day of opening and after 2 d.

Conclusions Sealing time influenced DM losses and aerobic stability. Delaying sealing by 4 d caused high DM losses and low aerobic stability. The longer the aerobic exposure after opening, the more pronounced was the microbial development. Yeast counts responded faster to aerobic exposure than the numbers of moulds and bacteria. On the contrary to moulds, prolonged aerobic exposure for ≥4 d resulted in no further increases in yeast and bacterial counts.

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Table 1 Effect of density and sealing time on dry matter (DM) losses during ensiling and aerobic stability of maize silage (mean ± SEM[1])

	Density						Effects[2]		
	Low			High					
Sealing time (d)	0	2	4	0	2	4	D	S	D x S
DM losses (%)	3.7[a] ±0.7	5.7[a] ±0.7	10.7[b] ±0.7	5.5[a] ±0.3	5.8[a] ±0.4	9.0[b] ±0.4	*NS*	*<.001*	*0.018*
Aerobic stability[3] (h)	64[bc] ±4.4	48[ab] ±3.4	47[a] ±3.1	65[c] ±3.1	57[ac] ±3.8	52[ac] ±3.4	*NS*	*0.016*	*NS*

[1]standard error of the mean, [2]D = density, S = sealing time, D x S = density x sealing time interaction, NS = non-significant ($P > 0.05$), [a-c] means in rows bearing unlike superscripts differ ($P \leq 0.05$, according to Tukey-Kramer), [3]defined as the number of hours the silage remained stable before a temperature rise by ≥ 2 °C above ambient temperature over 6 d of aerobic exposure.

Table 2 Effect of aerobic exposure after opening on microbial counts of maize silage

	Days of aerobic exposure					
Microbial population[1]	0	2	4	6	SEM	*P-value*
Yeasts	5.16[a]	7.26[b]	8.26[bc]	8.20[c]	0.19	*<.001*
Moulds	3.13[a]	2.70[a]	2.70[a]	4.84[b]	0.37	*0.001*
Bacteria[2]	5.84[a]	5.63[a]	8.46[b]	8.79[b]	0.40	*<.001*

[1]$\log_{10}$ cfu/g, [2]total aerobic mesophilic bacteria, [a-c] means in rows bearing unlike superscripts differ ($P \leq 0.05$, according to Tukey-Kramer).

Silage safety issues for large-scale bunker silos and drive-over piles re-visited: avalanches

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Keywords silage, avalanche, fatality, bunker silo, drive-over pile

Introduction Few farming operations invite as many different opportunities for injury or fatality as a silage program. One of these is an avalanche or collapsing silage. It only takes a fraction of a second for part of a silage face to silently break off and fall, and the result can be deadly for anyone located beneath it. There have been numerous avalanche fatalities in the United States the past few years, including an 11-year old boy in New Hampshire, a 30-year old truck driver in Idaho, and a 63-year old employee in Pennsylvania (Bolsen and Bolsen 2012; Bolsen and Bolsen 2013). Although rarely reported, the authors have heard many stories about someone having a near miss or suffering a serious injury in a silage avalanche. This paper documents silage avalanche tragedies and looks at ways they can be avoided in the future.

Materials and methods The most common hazard in managing bunker silos and drive-over piles, avalanche or collapsing silage, is presented and discussed.

Results and discussion On January 13, 2014, Jason E. Leadingham was working in a bunker silo near Dexter, New Mexico when a massive amount (10 to 15 tonnes) of maize silage collapsed onto him and the floor of the silo (Tucker 2014). Jason's body was not recovered from the silage until about 2 and 1/2 hours later, and it was determined that he died of mechanical asphyxia. There was a sample bag near Jason's left hip. He was clutching silage in his hands and had silage in his mouth, which suggest that Jason struggled to survive in the final moments of his life. This tragedy should never have happen.

A Nebraska newspaper reported the following fatal accident in October 2013 (Bolsen and Bolsen 2014). A 53-year old Norfolk man died Monday, October 21, 2013 in a feedlot accident. Stanton County Sheriff Mike Unger said Matthew Winkelbauer died after he was buried by a large silage pile that fell in an open silage pit at Four-Quarters Feedlot east of Norfolk. Winkelbauer, who was the owner and operator of Four-Quarters, was pronounced dead at the scene. A co-worker was seriously injured in the accident. The victim was standing in front of the feedout face, which was about 4 m height, and the avalanche pushed the falling silage more than twice that distance away from the face.

A New York newspaper reported the following tragedy on a farm in Ontario County (Sherwood 2010). Sheriff deputies said David Mark Crouch was shoveling silage into a feeder for cows on his family's Fort Hill Road farm when part of the silage pile collapsed on top of him. The pile was estimated at about 3 m high. He was buried under about 1.2 m of the wet, heavy silage and suffocated. He was found unconscious by a family member and taken by ambulance to Geneva General Hospital where he was pronounced dead.

Similar tragedies happen all over the world (Bolsen and Bolsen 2014). In a July 2013 email, Professor Ali Assadi-Alimouti, University of Tehran, Tehran, Iran, described the serious injuries he received in a silage avalanche. "It was March 15, 2010 and I went to

see a large dairy farm client. Two of the herdsmen and I went to the large bunker silo (8,000-tonne capacity). The height of the feedout face was about 6 meters. After visual appraisal of the silage, we were walking out of the bunker and a large silage avalanche fell on us. Observers testified later that it was around 10 tonnes of silage. One of the herdsmen remained outside of the silage from his head, and thank God, he could call to others to save us. The worst injuries happened to me, including multiple fractures to my tibia and femur, and I was in a coma for 30 hours in a hospital. The other herdsman suffered a broken leg and had respiratory problems due to inadequate oxygen for 10 minutes. I was the last one rescued, being trapped under the silage for about 20 minutes. It is by the grace of God that I am alive. God gave me another chance for life."

Far too many bunkers and piles are just too large to ever be safe for the crew filling them and the one feeding it out. Higher crop yields and/or growing herd sizes mean more silage needs to be stored. But unless new bunkers are added, the footprint for drive-over piles is enlarged, or packing density is increased significantly, there is nowhere for additional silage to go but up. As it does, so does the risk of an avalanche tragedy. It is not uncommon for cattle feedlots and large dairies to have bunkers and piles with silage faces that are 5.5 to 7.5 m tall. Common sense tells us that a 6 to 7 m tall silage face is far more dangerous than one that is only 3 to 3.5 m tall.

Here are guidelines that can decrease the chance of having a fatality or serious accident caused by a silage avalanche: 1) Never allow people to stand near the feedout face, 2) A rule-of-thumb is never stand closer to the feeding face than three times its height, 3) Suffocation is a primary concern and a likely cause of death in any silage avalanche, so follow the '*buddy rule*' and never work alone in a bunker or pile, 4) Use caution when removing plastic or oxygen-barrier film, tires, tire sidewalls or gravel bags near the edge of the feedout face, 5) Never ride in a front-end loader bucket, 6) Never park vehicles or equipment near the feedout face, 7) Post warning signs, '*Danger! Silage Face Might Collapse'*, around the perimeter of bunkers and piles, and 8) Avoid being complacent and never think that an avalanche cannot happen to you.

Conclusions Unfortunately, the global silage industry has a long way to go to eliminate these senseless fatalities and serious injuries. Keep in mind, an avalanche can occur anywhere, anytime, in any bunker or pile, without warning, and in any ensiled forage. We cannot stop avalanches from happening, and they are impossible to predict, but we can prevent people from being under them. Every farm, feedlot, and dairy should have safety policies and procedures for their silage program, and they should schedule regular meetings with all their employees to discuss safety. If a silage program is not safe, then nothing else about it really matters.

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Mycotoxins in high moisture grain silages and ensiled grain by-products

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Introduction

Grains that are stored and fed as high moisture grain silage include maize, wheat and barley. The advantage of high moisture storage is that it saves the energy costs for drying. In addition, it allows earlier harvesting which increases the flexibility of the operator/ farmer and sometimes increases yield. The lactic acid fermentation process that occurs in high moisture grain silage is essentially similar to that occurring in other types of silages: development of a population of lactic acid bacteria that convert mono- and disaccharides to lactic acid as the primary fermentation product (and usually some acetic acid), resulting in a decline of the pH. The moisture content (or more correctly: the water activity) is a key factor for the ensilability of high moisture grain silage. The moisture content needs to be high enough to allow growth and lactic acid fermentation by lactic acid bacteria. For optimal ensiling conditions of high moisture grain silage, the ideal moisture content is between 300 and 350 g kg^{-1}, depending on the type of crop. However, in practice the moisture content of high moisture grain silage is usually within the range of 200 to 350 g kg^{-1} (1). This means that micro-organisms that are less susceptible to low moisture conditions, such as moulds, may become the predominant flora in silages with a moisture content in the lower part of this range. The role of the moisture content in mycotoxin formation in high moisture grain silages is described in more detail later in this paper. The second key factor determining the quality of high moisture grain silages is oxygen. The oxygen level needs to be low enough to prevent growth of moulds, yeasts and other undesirable aerobic micro-organisms. Therefore, it is important to quickly establish and maintain anaerobic storage conditions. High moisture grain silages are usually stored in sealed horizontal bunkers or vertical silos. Sealing and packing are important to achieve maximal air exclusion. Grinding or cracking the grains prior to storage improves packing and reduces the risk of occurrence of air pockets. Apart from the mycotoxins that may be formed during storage of high moisture grain silage, grains may already be contaminated by mycotoxins during growth of the crop in the field. These so-called field mycotoxins also occur in other grain-based feeds, such as dry-stored grains and whole crop silages. Since levels of field mycotoxins increase in time, early harvesting of grains for high moisture storage leads to lower levels of these mycotoxins in comparison with dry-stored grains.

By-products of the milling or processing of grains for food, feed or biofuel purposes, such as distiller's grains, brewer's spent grains and maize gluten feed, are common

ingredients of the diet of dairy cattle. These by-products are mostly fed in dry form but sometime in wet form. In a number of countries, including the Netherlands, certain wet by-products are stored as silage to extend the shelf life.

This paper summarizes scientific knowledge about the major mycotoxins occurring in high moisture grain silages and by-products, conditions under which they are formed and how they can be prevented. Toxic effects in cattle, the metabolism of mycotoxins in ruminants and their carry-over into milk are briefly described. Toxic effects of mycotoxins in humans, analytical methods for detection of mycotoxins and legislative aspects are not described in this paper. Information about these topics can be found elsewhere (2-8).

Major classes of toxinogenic moulds and mycotoxins

Moulds and mycotoxins that are of relevance for the major crops for high moisture grain silage are listed in Table 1. A distinction can be made between mycotoxins that are formed before harvesting, i.e. during growth of the plant in the field, and mycotoxins that are formed after harvesting, i.e. during processing, storage or feeding. In this paper, these groups are referred to as field mycotoxins and storage mycotoxins, respectively. It is important to make this distinction because different types of moulds, different types of mycotoxins and different types of agricultural factors influencing mycotoxin levels are involved.

Table 1 Major mycotoxigenic moulds and mycotoxins in maize and small grain cereals (wheat, triticale, rye, barley)

Mycotoxin group	Major toxin(s)	Mould species	Field- or storage-derived
Aflatoxins	Aflatoxin B_1 (M_1), B_2, G_1, G_2	*Aspergillus flavus, A. parasiticus*	Field and storage
Trichothecenes	Type A: T_2, diacetoxyscirpenol	*Fusarium langsethiae, F. poae, F. sporotrichioides*	Field
	Type B: DON, nivalenol	*F. graminearum, F. culmorum*	Field
Fumonisins	Fumonisin B_1, B_2	*F. verticillioides, F. proliferatum*	Field
Resorcylic acid lactones	Zearalenone	*F. graminearum, F. culmorum*	Field
Cyclic hexadepsipeptides	Enniatin A, A1, B, B1, beauvericin	*F. oxysporum, F. subglutinans* and many other *Fusarium* species	Field
Ochratoxins	Ochratoxin A	*A. ochraceus, Penicillium verrucosum*	Field and storage
Alkaloids	Clavines, lysergic acid amide, ergotamine	*Claviceps purpurea*	Field
P. roqueforti toxins	Roquefortin C, mycophenolic acid	*P. roqueforti, P. paneum*	Storage (silage)
A. fumigatus toxins	Gliotoxin, fumigaclavines	*A. fumigatus*	Storage (silage)

Field mycotoxins

The major toxinogenic moulds capable of producing field-derived mycotoxins are different *Fusarium* species, *Aspergillus flavus* and *A. parasiticus* and *Claviceps* species. Important factors influencing mould growth are moisture (water activity (a_w)), temperature and availability of nutrients and oxygen. Moulds are generally tolerant to low a_w. Mechanical damage or insect attack of plants or grain kernels plays an important role in mould infestation and subsequent mycotoxin contamination because these events cause disruption of the protective plant cell wall. This creates entry points for infective moulds. In addition, it causes release of nutrients from the plant endosperm that can be used by moulds for growth. *Fusarium* moulds produce a wide variety of mycotoxins. The most frequently occurring *Fusarium* mycotoxins are deoxynivalenol (DON), nivalenol, T2-toxin and HT2-toxin (all belonging to the trichothecene group of mycotoxins), zearalenone, fumonisins, enniatins and beauvericin. *Fusarium graminearum* and *F. culmorum* are important species associated with the occurrence of trichothecene mycotoxins and zearalenone in maize/corn and wheat. These species cause common plant diseases, such as ear rot and stalk rot in maize and ear blight in wheat. Fumonisins are found exclusively in maize/corn and are formed by *F. verticillioides* (syn., *F. moniliforme*) and *F. proliferatum*. Enniatins and beauvericin are structurally related mycotoxins, belonging to the so-called cyclic hexadepsipeptides. These mycotoxins are produced by a large number of *Fusarium* species. The toxic mechanism of this group of mycotoxins is not yet fully understood. They probably act as ionophores and are believed to have antimicrobial activity, which may affect rumen fermentation.

The *Aspergillus* species *A. flavus* and *A. parasiticus* are associated with the production of aflatoxins. Aflatoxins are the mycotoxins of highest concern from the perspective of food safety and human health because of their high toxicity and carcinogenicity. Aflatoxin B_1 is the most prevalent and most toxic form. Aflatoxin B_1 is transformed into aflatoxin M_1 in the liver of cattle, the form in which it is (partially) excreted into milk. The carry-over of aflatoxin from feed to milk of, on average, approximately 2.5% is much higher than that of other the feed-associated mycotoxins, for which the carry-over is less than 0.01% (Table 2). Though *Aspergillus* is often classified as a mould associated with mycotoxin production during storage of commodities, it can infect crops in the field under favourable conditions, especially in subtropical and warm temperate climates. *A. flavus* and *A. parasiticus* are associated with aflatoxin production in a number of crops, including maize. Maize plants can become infected by *Aspergillus* conidia from the environment, usually soil or insects. A high level of insect damage increases the risk of infection. Aflatoxin development in the kernels occurs within narrow ranges of moisture content and temperature. Drought stress generally increases *Aspergillus*

infection and aflatoxin development in maize.

Moulds of the genus *Claviceps,* in particular *Claviceps purpurea*, are associated with the occurrence of ergot alkaloid mycotoxins in grain crops, of which rye and triticale are the crops with highest susceptibility. Plants are infected by *Claviceps* when flowering. The moulds produces a resting structure, called sclerotia or ergots, that is comparable in size to grain kernels and allows the mould to survive adverse conditions. The ergots contain high concentrations of ergot alkaloids. Ergot alkaloids represent a diverse group of toxic substances, including clavines, ergopeptines and lysergic acids. Ergotism caused by high levels of ergot alkaloids in grains is one of the oldest known forms of mycotoxicoses: severe epidemics occurring in the Middle Ages in Europe, known as St Anthony's fire, were caused by the consumption of bread and flour from ergot-contaminated grains. Detailed information about ergot alkaloid mycotoxins can be found elsewhere (2, 9).

Storage mycotoxins

Moulds associated with spoilage and formation of mycotoxins during storage of grains belong to the genera *Aspergillus* and *Penicillium*. The most relevant species are *A. flavus*, associated with aflatoxins (discussed in the previous chapter), *A. ochraceus* and *P. verrucosum*, associated with ochratoxin A, and *P. roqueforti* and *P. paneum*, associated with roquefortin C, mycophenolic acid, PR-toxin and a number of other mycotoxins. The most important abiotic factors that influence growth and mycotoxin production by these moulds in stored grains are water activity, availability of oxygen, temperature, pH and the presence of antifungal substances, for instance organic acids such as lactic acid, acetic acid and propionic acid. With respect to the ochratoxin A-producing moulds, *P. verrucosum* tends to be more prevalent in cooler climates and *A. ochraceus* in warmer climates. *P. roqueforti* and *P. paneum* are the species that are the most tolerant to acidic conditions and require low oxygen levels for growth (as low as 0.1% v/v). *P. roqueforti* is also the predominant spoilage mould in ensiled forages, for instance grass and whole crop maize silage, and by-products.

Toxic effects of mycotoxins in cattle, metabolism in the rumen and carry-over into milk

The toxic effects of feed-associated mycotoxins in cattle vary widely, ranging from immunosuppression and reduced reproductive ability to reduced productivity and non specific signs of illness. Diagnosis of mycotoxin intoxication is difficult. Factors such as the level and time of exposure, occurrence of multiple toxins, nutritional status, environmental conditions and breed may influence the degree of intoxication. Reduced feed intake or feed refusal and reduced milk production is a symptom occurring for

almost every mycotoxin. High levels of mycotoxins in feed can cause acute health problems in cattle. Cases of acute mycotoxicosis in dairy cattle are relatively rare in developed countries. However, the impact of the frequent exposure of animals to low doses of multiple mycotoxins on chronic health problems is probably much greater than that of acute health problems. The only effective treatments of animals suffering from mycotoxicoses is to cease feeding the contaminated feedstuff or to apply detoxification methods, for instance the addition to the diet of agents that adsorb mycotoxins or degrade them to less toxic or non-toxic metabolites in the gastrointestinal tract.

The significance of a mycotoxin occurring in feed with respect to animal health and the safety of animal food products for consumers is dependent on its metabolism in the animal, its toxicological effects in man and animals, and its carry-over from feed into milk, meat or organs. After intake via silage or another feed, mycotoxins, like other xenobiotics, follow the typical pharmacokinetic cascade of uptake from the gastro-intestinal tract to the blood, internal distribution, metabolism, storage/remobilization and excretion.

The rumen has an important function in the metabolism of mycotoxins in ruminants. It contains a complex and dense microflora with a high biodegradative power. Some mycotoxins are rapidly metabolized in the rumen into less toxic metabolites, some are transformed into equally toxic or more toxic metabolites and some are not transformed at all (5, 10, 11). Table 2 gives an overview of the knowledge about detoxification of mycotoxins in the rumen and their carry-over rate into milk. DON and ochratoxin A are examples of mycotoxins that are transformed into less toxic metabolites in the rumen. For that reason cattle are less sensitive to these mycotoxins than non-ruminant animals such as pigs. Zearalenone is transformed in the rumen into different metabolites, with varying toxic activities. Fumonisins and aflatoxin B1 are not metabolized in the rumen. Aflatoxin B1 is transformed into aflatoxin M1 in the liver of ruminants. Aflatoxin M1 is less mutagenic and genotoxic than aflatoxin B1, but the cytotoxicity of aflatoxin M1 and B1 is similar. Information concerning the metabolism of *Claviceps* alkaloid mycotoxins and *A. fumigatus* mycotoxins is lacking. Aflatoxin B1 is the only mycotoxin with significant carry-over into milk: between 1 and 6 percent is excreted in milk as aflatoxin M1. Carry-over rates of DON, zearalenone, fumonisin B1, ochratoxin A, alkaloids, roquefortin C and mycophenolic acid appear to be at least about 100-fold lower (5, 10, 11).

Table 2 Available information about detoxification of mycotoxins in the rumen and carry-over rates into milk (5, 10, 11)

Mycotoxin	Detoxification in rumen	Carry-over rate
Aflatoxin B_1	No	1 to 6% (average 2.5%)
Ochratoxin A	Yes	≤ 0.03% [1]
DON	Yes	≤ 0.02%
T2-toxin	Yes	≤ 0.02%
Zearalenone	No	≤ 0.01%
Fumonisin B_1	No	< 0.01%
Claviceps alkaloids	No	< 0.01%
Roquefortin C	No	< 0.005%
Mycophenolic acid	No	< 0.005%
Enniatins, beauvericin	Unknown	Unknown

[1] Based on study with lactating goats

Stability of mycotoxins in high moisture grain silage

The general view is that most field mycotoxins occurring in harvested grains remain stable during storage in high moisture grain silage. However, the information in literature is not fully conclusive. No studies investigating the stability of mycotoxins in high moisture grain silage in the course of storage time were available in literature (or at least, were not found by the author) and studies investigating the stability of mycotoxins in silages are sometimes contradictory. The available data indicate that zearalenone and fumonisins acid are stable during ensilage and therefore probably also during storage in high moisture grain silage. The available information concerning the stability of DON in silages indicates that DON is stable or may be degraded to only a limited extent. For aflatoxin B_1 and ochratoxin A and ergot alkaloids partial degradation has been reported to occur in the course ensilage of maize, barley and grass, respectively (12-17). However, it is unknown whether these mycotoxins are degraded in high moisture grain silage and, if so, to what extent.

Development of mycotoxins in high moisture grain silage

Almost no experimental data are available in the literature about the development of mycotoxins in high moisture grain silage. But despite the lack of experimental data, some general statements can be made based on existing knowledge about growth and mycotoxin

formation in moist grains and knowledge about the physiology and ecology of lactic acid bacteria in silage processes. The chance of development of mycotoxins in high moisture grain silage will depend mainly on factors that control mould growth, i.e. water activity (a_w), availability of oxygen, temperature, pH and the presence of antifungal substances. A number of studies investigated the influence of moisture content, temperature and modified atmospheres on the growth and mycotoxin production by moulds during storage of grains. Table 3 summarizes the relation between moisture content and water activity of maize and wheat and growth and ochratoxin A production by *P. verrucosum* and growth and aflatoxin B1 production by *A. flavus*. It also includes data for growth of lactic acid bacteria.

Table 3 Water activity (a_w) and moisture content of maize and wheat grain and growth of lactic acid bacteria and growth and aflatoxin B1 (AF1) production by *A. flavus* and growth and ochratoxin A (OTA) production by *P. verrucosum* at different a_w values (18-20)

a_w	Moisture content (%)		LAB	*A. flavus*		*P. verrucosum*	
	Maize	Wheat	growth	growth	AF1	growth	OTA
0.98	30-32	30-34	+	+	++	++	+
0.95	26-27	26-28	-	++	+	+	+
0.92	24-25	23-24	-	+	+	+	++
0.90	23-24	21-22	-	+	+	+	+
0.80	16-17	16-17	-	~	-	-	-
0.70	15-16	14-14,5	-	-	-	-	-

Lactic acid bacteria are unable to grow at less than 0.98 a_w. In contrast, *A. flavus* and *P. verrucosum* are able to grow and produce mycotoxin up to 0.85 to 0.90 a_w. Aflatoxin B1 production by *A. flavus* is optimal at 0.99 a_w, whereas ochratoxin A production by *P. verrucosum* is optimal between 0.90 and 0.95 a_w (18, 19). The a_w range where growth and mycotoxin production by moulds is possible and growth of lactic acid bacteria is not is the critical range with respect to mycotoxin development in high moisture grain silage. When under these conditions sufficient oxygen is available moulds will have the opportunity to grow without having to compete with lactic acid bacteria for the available substrates and without the hurdle of a low pH and the inhibitory effect of lactic acid, acetic acid and possibly other antifungal substances produced by lactic acid bacteria. Carbon dioxide in the gas phase has been shown to have an inhibitory effect on growth and ochratoxin A production by *P. verrucosum* and *A. ochraceus* during storage of moist grain (21). However, it remains to be investigated how these findings relate to the development of ochratoxin A by these moulds in high moisture grain silage.

About 30 years ago, there were unexpected cases in Sweden with aflatoxin-contaminated moist grain, predominantly barley and rye, causing incidents of aflatoxin M1 contamination of milk. Treatment of moist grain with a formic acid-based preservative was found to be the cause of growth and aflatoxin production by *A. flavus*/*A. parasiticus* (22, 23). Due to inaccurate dosing of the acid, the treatment inhibited lactic acid fermentation and the corresponding pH drop and created environmental conditions which allowed growth and aflatoxin production by the *Aspergillus* moulds. This case in Sweden illustrates the importance of lactic acid fermentation in the control of mould growth and mycotoxin production in high moisture grain silages.

It seems probable that mycotoxins formed by the silage-associated mould species *P. roqueforti*, such as roquefortine C and mycophenolic acid, may occur in well-fermented high moisture grain silages that are insufficiently sealed from air. Experimental data confirming this assumption are, however, lacking.

Occurrence data of mycotoxins in grains

Data about mycotoxins in high-moisture grain silages are scarce. In contrast, there is a vast amount of data about the occurrence of mycotoxins in feed commodities. Also the amount of data about mycotoxins in grass en maize silages is increasing. A number of recent reviews summarized the occurrence of the major field mycotoxins in cereal grains, in particular maize and wheat. Recently the results of a survey programme which monitored the occurrence of mycotoxins in animal feed and feed commodities worldwide between 2005 and 2014 were published (24-26). Overall, 72% of the samples contained detectable levels of aflatoxins, DON, zearalenone, ochratoxin A and fumonisins. Detection of two of more mycotoxins was observed in 38% of the samples. Part of the data from this study are summarized in Table 4. The highest incidence and highest average concentrations were detected for DON and fumonisins. The data in Table 4 also show that maize is a far more important source of aflatoxins and fumonisins than wheat, whereas these commodities appear to be of equal importance for DON and ochratoxin A.

Table 4 Occurrence of DON, zearalenone (ZEA), fumonisins B1 and B2 (FUM), alfatoxins B1, B2, G1 and G2 (AFLA) and ochratoxin A (OTA) in maize and wheat for feed, sourced worldwide in 2012, 2013 and 2014 (adapted from 25, 26). LOQ: limit of quantification

	DON	ZEA	FUM	AFLA	OTA
LOQ (µg/kg)	250	40	250	0.6	2
Maize					
No. of samples tested	3705	3302	2478	2647	2186
% positive samples	69%	49%	76%	25%	9%
Average concentration positive samples (µg/kg)	1817	364	2353	48	5
Wheat					
No. of samples tested	1527	1131	658	663	675
% positive samples	65%	26%	12%	5%	12%
Average concentration positive samples (µg/kg)	1037	101	514	6	3

As expected, differences were observed with regard to the incidence and concentrations of the mycotoxins in different regions of the world (26). For instance, the highest incidence and concentrations of aflatoxins were detected in samples originating from South and South-east Asia, whereas ochratoxin A was highest in Europe and fumonisins was highest in South America. A high incidence of DON was detected in almost all regions of the world, but the highest average concentrations were observed in North America, North Asia and North and Central Europe. Highest contamination levels of zearalenone were recorded in North Asia en South America. Climate and weather conditions have a large impact on mould proliferation and mycotoxin contamination of field mycotoxins. Contamination of maize by DON and zearalenone has been shown to be favoured by high humidity conditions and is associated with high rainfall amounts late in the growing season (27, 28). These findings have been confirmed repeatedly, for instance by data concerning DON and zearalenone concentrations in maize silage in the Netherlands (29) and data concerning DON and zearalenone in maize grain in the United States and Australia reported in a worldwide survey study (24).

Mycotoxins in by-products

By-products of the processing of grains for food, feed and bio-ethanol production are valuable feedstuffs for dairy cattle. In particular the supply of by-products from

bio-ethanol production has greatly increased with the worldwide expansion of biofuel production during the last decade. Maize, sugar cane, wheat and sorghum are commonly used crops for bio-ethanol production, of which maize has become the major one. The major by-product from bio-ethanol production from maize and wheat is distillers grains with solubles (DGS). Other popular by-products include maize gluten, brewers grains and pressed sugar beet pulp. The majority of by-products are preserved by drying but wet by-product feeds are increasing in popularity. The use of wet by-products is popular in regions where the distance between grain processing plants and dairy farms is relatively small. Wet by-products are sometimes used without any form of preservation, but the shelf life of those feeds is generally short. Ensiling can successfully be used to extend to shelf life of wet by-products to 6 to 12 months. Ensiling and storage is generally at the farm. Examples of wet by-products are brewers grains, pressed sugar beet pulp, maize gluten feed and pressed potato pulp. In the Netherlands, ensiled by-products represent on average approximately 5% of the total diet of dairy cows and about half of the dairy farms feed at least ensiled by-product (30).

During the last five years a lot of data have been published about the occurrence and concentrations of mycotoxins in maize DGS from bio-ethanol production. Unfortunately, studies that investigated mycotoxins in ensiled by-products are scarce. However, since the stability of most mycotoxins during storage as silage is high (see above), the data concerning DGS and other dried by-products are valuable also for ensiled by-products.

Studies that investigated the fate of aflatoxins and the major *Fusarium* mycotoxins occurring in maize during the ethanol fermentation process and their distribution in different by-product fractions have been reviewed recently (31). Concentrations of aflatoxins and DON in DGS were approximately three times the concentration in the original grain (on dry matter basis). Since approximately two-third of the original grain mass is transformed into ethanol and all non-soluble matter is collected in DGS, it was concluded that there is no or very little degradation of aflatoxins and DON during the entire process (31, 32). For zearalenone and fumonisins concentration factors in DGS between 1.5 and 2 have been reported (31), suggesting partial degradation of these mycotoxins during ethanol fermentation. Comparison of results of the worldwide survey of mycotoxins in different maize-based feeds indicate that levels in maize gluten are equal or higher than in maize grains (26). Data about mycotoxin levels in ensiled DGS and ensiled maize gluten are lacking, but given the high stability of most mycotoxins in silage similar levels as in the dry feeds can be expected.

The development of aflatoxins during storage of wet brewers grain (not ensiled) at dairy farms was recently demonstrated in two studies from South America (33, 34). As part of a survey of the occurrence of mycotoxins in the different ingredients of the diet of dairy

cows at Dutch dairy farms, 15 samples of ensiled brewers grains and 12 samples of ensiled pressed sugar beet pulp were analysed. No aflatoxins, DON, zearalenone, ochratoxin A or fumonisins were detected in these samples. Five samples (one sample of brewers grains and four samples of pressed sugar beet pulp) contained low concentrations of roquefortin C, mycophenolic acid or both. These mycotoxins are associated with *P. roqueforti*, the predominant mould species in ensiled feeds, and can be expected to occur in all types of ensiled by-products that show signs of aerobic spoilage.

Conclusions

High moisture grain silage can be contaminated with a wide range of mycotoxins, originating from infection of the crop in the field or from growth of moulds during storage. Prevention of contamination of high moisture grain silage by mycotoxins requires different strategies. Field-derived mycotoxins can be reduced by application of adequate agricultural practices in crop production. Storage-derived mycotoxins can be reduced by application of adequate storage management, with emphasis on moisture content, compaction and prevention of infiltration of air. Little information is available about concentrations of mycotoxins in high moisture grain silage. Knowledge (or hypotheses) about mycotoxins in high moisture grain silage is mainly derived from the occurrence of mycotoxins in unprocessed grains and from mould spoilage of moist grains.

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Short and long term storage of wet by-products fed by ruminants

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Abstract
The authors reviewed the current situation with respect to a wide range of wet by-products that are available and applicable in ruminant nutrition. The review shows the importance of the wet by-products as feedstuffs and volume produced worldwide. The authors give a general description of the industrial processes having an influence on nutritive value and fermentability of the different wet by-products, their role in feeding of ruminants and nutrient content. The main aim of the review was to show the short- and long term storage background, details, opportunities, difficulties and potential future of the wet by-products worldwide.

Keywords by-products, sugar beat pulp, sugar cane top, sugar cane bagasse, wet distillers' grain, wet brewers' grain, wet corn gluten feed, wet citrus pulp, wet tomato pomace, wet cassava pomace

1. Introduction

This paper reviews the current situation with respect to a wide range of wet by-products that are available for ruminant nutrition with some details of the short- and long term storage opportunities and difficulties. The global annual yield of the major by-products can be estimated to be in the range of 1000 million tonnes/annum (Section 2). Whilst many of these by-products are of low dry matter content (range 15-25% DM) they offer a massive feed resource particularly with the current global demands on land and water for the production of food, feed and fuel. Therefore it would be immoral if we, as 'silage scientists' did not make considerable efforts in maximising the use of these nutrients by optimal storage and feeding. There are some other factors that have made this topic of increasing importance over the past decade.

1. The dramatic increased use of corn and cereals for the production of bio-ethanol as a liquid fuel has increased the supply of various types of wet grain by-products.
2. The increase in biogas plants that need a continuous supply of energy rich feeds for the production of biogas.
3. There is an opportunity to save arable lands for food-, bio-ethanol and biogas industry using the wet by-product in animal nutrition.

4. Disposal of by-products for example burning of sugar cane top on the field has environmental concerns.
5. In many countries the costs of landfill has increased significantly making their re-use for other purposes an economic proposition.
6. Most of these wet by-products (as the 'traditional forages'), are available only at a certain period of the year making long-term storage a real need.
7. Wet by-products have a very short shelf life if appropriate storage conditions are not used, due to the deterioration instigated by the growth of undesirable microorganisms.
8. Fermentation, as a conservation method, has better economic prospects compared to energy demanding drying.
9. Nutritive value of some wet by-products are higher than conventional forages, so as part of a balanced diet, they can make a significant contribution to ruminant diets and therefore production efficiency.

The initial part of this paper describes the processes whereby the different by-products are produced in the factory. It is worthy of note that some of these processes result in specific challenges for both the storage and feeding of some of these by-products. For example, many of the processes for brewers' and distillers' grains involve a heating process that will eliminate microflora but not *Clostridia* spores. Therefore a short description of each production methodology has been included as a background of each by-product (Section 3). Section 4 briefly describes the nutritional ranges of the different by-products. Short and long term storage methods are shown in Sections 5 and 6, respectively. The final section (Section 7) gives a brief look forward and attempts to identify research needs in the future.

2. Quantity of wet by-products available globally

Estimated total volume of different by-products produced worldwide are shown in Table 1.

Table 1 Estimated volume of different by-products produced worldwide

Types of by-products	Volume (yield ton/year)	Main producers in the world	Reference
Sugar factory			*http 1*
Sugar beet pulp	2013: 246.5 million t/ year	Russian Federation, France, USA, Germany, Turkey, Ukraine, Poland, Egypt	*http 2*
Sorghum by-products: bagasse, top, press mud, leaves	2013: 62.3 million t/ year	USA, Nigeria, Mexico, India, Sudan, Ethiopia, Argentina, China, Australia, Brazil, Burkina Faso, Niger, Cameroon	*http 3, 4*
Sugar cane bagasse, top and press mud	2010: 252-421 million t DM /year top 2010: 37-63 million t DM/year top for feeding	Brazil, China, India representing 75 % of world production	*Naseeven, 1988*
Brewers' by-product			
Wet brewers grain	35-40 million t/year 8 million t/year in EU	Brazil, China, USA,	*Mussatto, 2006*
Distillers' by-products			
Wheat distillers grain	2010: 1.9-5.5 million t/year	Europe: France, Canada, Australia	*Bonnardeaux,, 2007*
Corn distillers grain	USA 1992: 2 million tons/year USA 2010: 34 million tons/year (biofuel 97%)	USA (exported 4.5 million tons -81 %of by-products traded worldwide). China, Canada, Germany and Poland.	*Moreau et al., 2011* *Hoffman, and Baker, 2010.*
Starch or ethanol production (wet-milling)			
Corn gluten meal and corn gluten feed	2011: 14.9 million t/year	USA (5.6 million t), the European Union (3 million t), South Korea (1 million t), Japan (0.94 million t) and other Asian countries (1.6 million t). Main importers for 2010-2011 were the EU, South Korea, Turkey, China, Japan, Israel, Egypt and Indonesia.	*RFA, 2008*
Sorghum by-products (DDGS and brewers' grain)	2006: 0.4-0.8 million t/year	USA (ethanol production), Africa (clear or opaque beer): South Africa, Nigeria, Zimbabwe.	*Taylor et al, 2006,* *Jessen, 2010*
Fruit and vegetable by-products: temperate and mediterrean region			
Tomato pomace, tomato skins and tomato seeds	2007: 11 million t/year, 2007: 4 million t/year of tomato pomace	China, India, USA, Turkey, Egypt, Italy, Iran, Spain, Brazil and Mexico	*Ventura et al, 2009,* *Knoblich et al, 2005* *Cotte, 2000.*
Olive oil cake and by-products	2013: 2,8 million t/year	Spain, Italy, Greece, Tunisia, Turkey, Syrian Arab Republic, Morocco	*http 5*
Fruit and vegetable by-products: tropical and subtropical region			
Citrus by-products (pulp)	2013: 135 million t/year	USA, China, India, Mexico, Spain, Egypt, Turkey, Italy, South Africa, Pakistan, Indonesia, Iran, Argentina, Brazil, Philippines, Ecuador, Thailand, Nigeria, Costa Rica,	*Göhl, 1978,* *http 6-9*
orange pulp, fresh and silage	2013: 71,4 million t/year	Brazil, USA, China, India, Mexico, Spain, Egypt, Turkey, Italy, South Africa, Pakistan, Indonesia, Iran.	*http 6*
mandarin pulp, fresh and silage	2013: 28.7 million t/year	China, Spain	*http 7*
lemon pulp, fresh and silage lime pulp, fresh and silage	2013: 15.2 million t/year	India, Mexico, China, Argentina, Brazil.	*http 8*
grapefruit pulp, fresh and silage	2013: 8.5 million t/year	China, USA	*http 9*
Banana by-products (leaves and stems, dehydrated or fresh green and ripe peels, rejected fruits)	12–15 million t/year fruit 7–9 million t /year of plantains	India, China, Philippines, Brazil, Ecuador, Indonesia	*Babatunde, 1992*
Pineapple by-products and leaves (skins, crowns, bud ends, cores, the pomace, leaves)	2013: 7-9 million t/year	Costa Rica, Brazil, Philippines, Thailand, Indonesia, India, Nigeria, China	*Sruamsiri, 2007, Hepton and Hodgson 2003*
Mango by-products	150,000 and 400,000 t/year	India, China, Thailand, Indonesia, Mexico, Pakistan, Brazil	*Cruz Medina, and Garcia, 2002, Jedele,2003*
Coconut by-products (copra meal)	2013: 175,000 t/year (oil meal)-	Indonesia, Philippines, India	*http 10*
Other by-products			
Cassava by-products (cassava peels and pomace, leaves)	85.000 t/year (cassava leaves)	Nigeria, Thailand, Indonesia, Brazil, Democratic Republic of the Congo, Angola, Ghana, Mozambique	*http 11*

3. General description of wet by-products fed by ruminants

3.1. Wet by-products derived from the sugar industry

3.1.1.Wet sugar beet pulp

The wet sugar beet pulp has a long history among the other wet by-products as feed for ruminants and significant potential importance in the future as a substrate for biogas production having high dry matter yield per hectare and gas production per kg dry matter (Wagner et al., 2009). The sugar beets are first washed and shredded then sliced into V-shaped strips (2 mm wide) passed into a rotating diffuser drum immersed in water at 72°C (Kelly, 1983). The extracted pulp has 5-7% dry matter content. It is screw-pressed and the wet sugar beet pulp finally has 10-15% dry matter content. This can be further pressed raising the dry matter content to 20-25%. Some pulp is sold as pressed pulp at this stage, but most is molassed and dried. The quantity of molasses added is generally 18-22% of the dry matter (Kelly, 1983). The wet sugar beet pulp is a palatable but slightly laxative feed, containing approximately 5% sugar in the dry matter. It is rich in rumen-degradable NDF (20-25 %DM hemicellulose) for cattle (Kelly, 1983).

3.1.2 Sugarcane by-products

3.1.2.1. Sugarcane bagasse

Bagasse is the residual fibre resulting from the extraction of sugar cane juice and is relatively common in the tropics and subtropics. There are two main types of bagasse (Heuzé et al, 2012). 'Factory bagasse' from industrial processes involving repeated extraction steps resulting in a fibrous by-product „Farm bagasse" is obtained from on-farm or small factory cane fractionation that use only 2 or 3 crushers. Due to the reduced efficiency of the extraction process (50% compared to 70% efficiency), it has a higher sugar content and so is much more valuable for ruminants (Preston, 1995). Bagasse is extremely unhomogeneous comprising *circa* 30-40% „pith" fibre,from the core of the plant and stem fibre *circa* 60-70%. Raw bagasse is a poor, fibrous roughage with a low nutrient density and digestibility, mostly used for ruminants (Preston, 2009) and needs considerable supplementation for high animal production.

3.1.2.2. Sugarcane tops

Sugarcane tops are one of the main by-products of sugarcane milling (Heuzé et al., 2015a) they are discarded from extraction because they contain components that prevent optimal crystallization and cause an undesirable color in sugar. The tops represent 15 to 25% of the aerial part of the plant consisting of green leaves, bundle sheath and variable amounts of immature cane (Naseeven, 1988). The tops are generally left in the field, burned and remain as fertilizer (McKenzie et al., 2007). Sugarcane tops are used fresh, dried or ensiled for feeding livestock. Their nutritive value is highly variable and depends

on preharvesting methods, stalk cutting point, plant maturity and the quantity of dry leaves (McKenzie et al., 2007; Ortiz-Rubio et al., 2007). As they are a seasonal crop, ensilage can be useful to increase all year round supply (Naseeven, 1988). Sugarcane tops ferment readily and can be used for ethanol or lactic acid production (Serna Cock et al., 2007). The estimated animal consumption has risen from 37-63 million tons DM in 1986 to 252-421 million ton dry matter in 2010.

3.2. Wet by-products derived from brewers: wet brewers' grains

Wet brewers grains are the solid residue left after the processing of germinated grains (malt) for the production of beer, malt extracts and vinegar. Barley is the main grain used for brewing, but beers are also made from wheat, maize, rice and sorghum. During the brewing process, grains are soaked in water, germinated, dried to produce the malted grain then milled and steeped in hot water to allow the enzymatic conversion of starch into sugars. The sugar-rich liquid is boiled, filtered and fermented to produce beer/bioethanol. Brewers grains are collected, once all sugars are removed from the grain (Heuzé et al., 2014a). The remaining product is a concentrate of proteins and fibre that is suitable for animal feeding, particularly for ruminants (Crawshaw, 2004). There is seasonality of supply of brewers' grains (more produced during summertime), which generally doesn't have a good fit to balanced ruminant feeding. They can be supplied wet or dried and can be ensiled (Blezinger, 2003). Wet brewers grains (20-25% DM) are a highly perishable and bulky product that is costly to transport. The growth of bacteria, yeasts and fungi even during short term storage is a challenge (Allen et al., 1975a; Allen et al., 1975b) and palatability of brewers grains decreases with storage time. Brewers grains has a protein content of 27-33% DM. The protein degradability is generally lower than other plant derived protein, resulting in a higher level of by-pass protein. Intestinal digestibility of nitrogen ranges from 74% to 84% (Sauvant et al., 2004). Organic matter digestibility are quite variable and range from 55 to 75%. The ADF content is 17-26% DM, which makes them suitable for ruminants. Wet brewers grains are a bulky feed with a low energy content, which can limit their use (Table 2).

3.3. Distillers' wet by-products

3.3.1.Barley distillers' wet by-products

3.3.1.1.Whisky (barley) distillers' by-products

The major source of barley distillery by-products is the production of malt whisky, which is usually based on malted barley (Heuzé and Tran, 2014; Russell et al., 2014). The increase in drying costs, as with other wet by-products, has caused a renewed interest in marketing wet distillers grains (McKendrick et al., 2003). Excess malt distillers (not

consumed by livestock) is ensiled, often alongside grass (during the summer) for the following winter (Miller, 1969). Fresh malt distillers produced in tropical countries have a short shelf life (3-7 days). Treatment with 2% propionic acid was effective for short-term (7 days) preservation (Geetha et al., 2009). Malt distillers product has a high crude protein content (17-23% DM). It is poor in starch and sugars (Crawshaw, 2004) and OM digestibility of about 50-54% due to its large concentration of fibre (NDF > 60% DM). Its energy value is disputed, with reported ME values in the 9-12 MJ/kg DM range (Crawshaw, 2004).

3.3.2.Wheat distillers' by-products

3.3.2.1.Wheat distillers processing (ethanol production by-products)

Wheat distillers grain is the main by-product of the distillation of alcohol from wheat grain. Barley is still a minor feedstock for ethanol production, used mostly in Europe and in the USA, and it is always blended with other cereals (Kalscheur et al., 2012). So wheat distilleries produce alcoholic beverages, industrial ethanol and ethanol biofuel with the following by-products: spent grains, wet grains, wet distillers grain (WDG), wet distillers grain with solubles (WDGS), distillers wet grains with solubles (DWGS), dried distillers grain (DDG), dried distillers grain with solubles (DDGS), condensed distillers solubles (CDS), dried distillers solubles (DDS) (Heuzé et al, 2013a). While the USA mainly fulfil their needs with maize-based ethanol, wheat is the primary feedstock for biofuel in Europe (France), Canada and Australia (Moreau et al., 2011).

The *dry-milling* (or *dry-grind*) process is the dominant methodology for producing ethanol (Heuzé et al, 2013a). The grain is ground to produce a bran-free flour, mixed with water in order to make a mash, then cooked to kill the undesirable bacteria. After that, enzymes are added to the mash to release sugar from the starch. Finally, yeast is added to start the fermentation process, which produces a „beer" and $CO_{2,}$ (distilled). The product that remains at the bottom (whole stillage) is centrifuged and provides the wet grains (also called spent grains) and thin stillage (Heuzé et al, 2013a). Condensed distillers solubles and distillers grain are often blended together to prepare wet or dried distillers grain and solubles (WDGS or DDGS). If the bran has been removed from the grain prior to fermentation, it may be added to the wet grains.

3.3.2.2.Whisky (wheat) distillers by-products

Wheat is the main substrate in whisky distilleries in some regions (Crawshaw, 2004). The process is similar to that described for barley distillers. The draff is dried or pressed before being fed to animals. The alcohol-free effluent that remains at the bottom of the distillation column is called spent wash or spent lees. This product, which contains

enzymes and yeast, can be dried to yield dried distillers solubles. Wheat distillers solubles contains xylans and arabinoxylans that are not broken during the process and that result in a high viscosity level in the solubles. Wheat solubles are therefore more difficult to dry than maize solubles (Crawshaw, 2004). Like in ethanol production, the spent grains are often mixed with the solubles, resulting in wheat distillers dark grains (Crawshaw, 2004). Wheat DDGS has a protein content ranging from 30 to 40% DM, which is slightly higher than for maize DDGS. However, wheat DDGS has a low starch (4.5% DM) and sugar content. When the bran is removed before the fermentation and added back at the end of the process, the starch content can be increased to 10-25% DM. Wheat distillers are a good source of energy and by-pass protein for ruminants. The digestibility of dried distillers grain and solubles (DDGS) from wheat and wheat/barley mixtures was of 76%, 50% and 87% for organic matter, resulting in ME of 12.1 MJ/kg DM and NE for lactation of 7.3 MJ/kg DM (Losand et al., 2009).

3.3.3.Corn distillers' by-products

3.3.3.1.Corn distillers grain (ethanol by-products)

Corn distillers grain is the main by-product of the distillation of alcohol from maize grain. The DDGS (maize-based, dry-milling ethanol production) is now the dominant distillery by-product (Heuzé et al., 2015b). Distilleries produce alcoholic beverages, industrial ethanol and ethanol biofuel from maize with the by-products described above (WDG, WDGS, DWGS, DDG, DDGS, CDS also called syrup, DDS). Condensed distillers solubles and distillers grain are often blended together to prepare wet or dried distillers grain and solubles (WDGS or DDGS). There are two main distillery processes, dry-milling distillery and wet-milling distillery. The wet-milling process yields numerous other by-products including maize gluten meal, maize gluten feed and maize germ meal, which will be described in Section 3.4.

Corn distillers grains are a valuable feed ingredient. High protein DDG contains more than 40 % DM of crude protein but the lysine content is particularly low (Fastinger et al., 2006). Corn distillers grain is low in cell wall content (lignin: 3.9 % DM), which explains the high digestibility of NDF in ruminants. Residual starch is low (< 8 % DM) for ethanol DDGS. The average OM digestibility is 73.5 ± 6.2 %, which corresponds to a mean ME content of 12.6 MJ/kg DM (Woods et al., 2003). The proteins in maize DDGS contains a higher by-pass fraction than that of the original grain. Values for ruminal undegradability of protein (RUP) variable, with mean value of 55 % (Woods et al., 2003; Kleinschmit et al., 2006).

3.4.Wet by-products derived from the starch or ethanol production (wet-milling)

3.4.1.Corn gluten feed

Corn gluten feed (CGF) is the by-product of the wet-milling of maize grain for starch (or ethanol) production (Hoffman et al., 2010). The wet-milling process of maize yields 5 main products: maize starch, maize germ oil meal, corn gluten meal, corn gluten feed and maize steep liquor (Heuzé et al, 2014c). Corn gluten feed consists mainly of maize bran and maize steep liquor (liquid separated after steeping), but may also contain distillers solubles, germ meal, cracked maize screenings, as well as minor quantities of end-products from other microbial fermentations (Stock et al., 1999). *Wet-milling process* (Heuzé et al., 2014c): after cleaning and removal of foreign material, the maize grain is usually steeped in water with sulfur dioxide (SO_2) for 24-40 hours at a temperature of 48-52°C. The role of sulfur dioxide is to weaken the glutelin matrix by breaking inter- and intramolecular disulfide bonds. Steeping at 45-55°C favours the development of lactic acid bacteria that produces lactic acid, lowering the pH of the medium and thereby restricting growth of most other undesirable organisms. At the end of steeping phase, the maize kernels (contain about 45% water) become sufficiently softened (BeMiller et al., 2009). After steeping, the maize kernels are coarsely ground so that the germs are separated from the endosperm and used for oil extraction (which yields maize germ oil meal). The remaining steeping water is condensed into a steep liquor. The endosperm undergoes further screenings that separate the fibre from gluten (protein fraction) and starch slurry. Fibre (bran) can be mixed with steep liquor and maize germ oil meal to create corn gluten feed. The fibre-free endosperm is centrifuged in order to separate the starch fraction and the gluten, which have different densities, resulting in almost pure starch (99% starch), and corn gluten meal (CRA, 2006).

Corn gluten feed is a feed ingredient mostly used in cattle diets as a source of energy and protein (Ash, 1992). Corn gluten feed is usually sold in the dried form, but maize processors may save on the drying cost by selling wet corn gluten feed. Wet corn gluten feed contains 40 to 60% DM (Stock et al., 1999). *In vivo* organic matter digestibility values found for dried corn gluten feed vary from 70 to more than 80%. Published ME values vary from 11.4 to 12.2 MJ/kg DM (NRC, 2001), while NE for lactation values range from 6.9 (NRC, 2001). Corn gluten feed contains *circa* 20-25% DM CP with about 24-30% by-pass protein (NRC, 2001) and low in lysine. It is important to note that corn gluten feed should not be confused with corn gluten meal, which contains about 65% crude protein instead of 20-25%. The starch content is highly variable ranging between 11% to greater than 30% DM. Wet corn gluten feed is rather acidic (pH approx. 4.0-4.5). Wet corn gluten feed spoils very quickly and must either be fed within 6-10 days or stored in anaerobic conditions in a sealed structure (RFA, 2011).

3.5.Sorghum grain by-products

Sorghum grain is used by various food industries, including milling, starch production, brewery and distillery, resulting in numerous by-products (Heuzé et al., 2013b). Sorghum brewer's grains (sorghum brewers' grain, sorghum brewers' spent grain, sorghum spent grain, draff, sorghum beer residues) are the by-product of brewery based on sorghum grains (Heuzé et al, 2013b). It can be used fresh or dried (artificially or sun-dried). Sorghum has been the basis of traditional African beers (dolo and pito) and the opaque beers of southern Africa. Like other spent grains from the brewing process, sorghum brewers' grains are relatively rich in protein (26% DM) and contain a good amount of fibre (ADF 25% DM). Malted sorghum sprouts are another brewery by-product, similar to barley culms (sprouts and rootlets). Sorghum gluten feed is a by-product of the manufacture of sorghum starch or syrup by wet-milling. It has a bitter taste and should be mixed with a more palatable ingredient such as molasses (Göhl, 1982).Sorghum gluten meal is another by-product of sorghum starch extraction. Sorghum gluten meal is mainly a protein source but with a much lower protein content than corn gluten meal (48 *vs.* 67% DM). Sorghum and its by-products may contain cyanogenic glucosides (Adewusi et al., 1994).

3.6.Citrus by-products: citrus pulp

Citrus is one of the most important fruits crop type worldwide (Heuzé et al., 2014b). Oranges, mandarins, lemons, limes (several species) and grapefruits are the main cultivated species.Oranges accounted for 61% of the world citrus production (USDA-FAS, 2010). The main producer of citrus fruit for processing is Brazil (47% of the world production), followed by the USA (29%) (USDA-FAS, 2010). Citrus pulp is the solid residue that remains after fresh fruits are squeezed into juice. It amounts to 50-70% of the fresh weight of the original fruit and contains the peel (60-65%), internal tissues (30-35%) and seeds (0-10%) (Crawshaw, 2004; Göhl, 1978). Citrus pulp is usually made from oranges but may also contain by-products of other citrus fruits, notably grapefruits and lemons (Crawshaw, 2004). Large amounts of fresh citrus pulp are available in the harvest season, which in many countries coincides with the dry season when grass is scarce (Göhl, 1978). Fresh citrus pulp has a natural acidity, but is still quite perishable due to its high content of water and soluble sugars. It ferments and sours quickly when it is in contact with the air (Fuller, 2004). It can be treated by ensiling (as it stores well in the absence of air), or alkali treatment for longer storage (Wing, 2003).

Citrus pulp can be fed fresh or as silage to ruminants. Fresh citrus pulp typically contains up to 40% DM soluble fibre (pectins) and carbohydrates 5-10% DM. Dry matter is usually about 20% (Crawshaw, 2004). Citrus pulp is used as a cereal substitute in ruminant

feeds, due to its high energy content and good digestibility in ruminant species. Fresh citrus pulp is palatable to cattle but may require some adaptation (Crawshaw, 2004). High levels of citrus pulp in ruminant diets can result in rumen parakeratosis (Arthington et al., 2002). Intakes of 11 kg/day citrus press cake silage have been reported for mature cows in Florida (Becker et al., 1946). Fresh or ensiled citrus pulp, as all citrus by-products, have an unbalanced Ca: P ratio that may cause milk fever in cattle at, or soon after, parturition.

3.7.Tomato by-products

Tomato processing yields the following by-products, which represent 5-13% of the whole tomato (Heuzé et al, 2013c): tomato pomace is the mixture of tomato peels, crushed seeds and small amounts of pulp that remain after the processing of tomato for juice, paste and ketchup (Ventura et al., 2009). Skins (peels) are a by-product of the peeling of tomatoes used for canning (Knoblich et al., 2005). Tomato seeds are a by-product of tomato cannery, notably from the production of de-seeded canned tomatoes (Cotte, 2000).

Tomato by-products are highly heterogenous products. Tomato pomace is relatively rich in protein (17-22 % DM) and fat (10-15 % DM). The NDF (50-72 % DM) consists largely of ADF (39-60 %) with high lignin content (ADL 20-30 % DM; Feedipedia, 2011). But some tomato pomaces contain less than 7 % DM of ADL. Tomato by-products are usually fed to ruminants due to their high fibre content. An important fraction of the crude protein is acid-detergent insoluble nitrogen (Weiss et al., 1997; Ventura et al., 2009). Therefore the pomace is not an excellent feed ingredient, being less digestible than most other protein sources. *In sacco* protein degradability of dried tomato pomace in the rumen ranged between 65-78 % (Chumpawadee, 2009; Abbeddou et al., 2011; Ben Salem et al., 2008; Valizadeh et al., 2009). Dairy cows fed with tomato-maize (12% DM) silage had the same DM intake (3.74 % BW) compared to cows fed with maize silage alone and had the same milk yield (35 kg/d) and milk composition (Weiss et al., 1997).

Tomato pomace and skins are low in DM betwen 2 and 20% (NRC, 1983) and spoil very quickly, in less than 2 days in some cases (Caluya, 2000). Unless they can be fed immediately to livestock, they must be preserved either by drying or by ensiling.

3.8.Cassava by-products

The processing of cassava tubers yields the following by-products that can be valuable livestock feeds when properly processed (Heuzé et al., 2015c; Aro et al., 2010). Cassava peels can represent 5 to 15% of the root (Aro et al., 2010; Nwokoro et al., 2005a). They are the result of tubers that have been washed and peeled (Aro et al., 2010). They may contain high amounts of cyanogenic glycosides and have a higher protein content than other tuber parts (Tewe, 2004). Cassave pomace (also called cassava fibre, cassava

bran, cassava bagasse, cassava starch residue and cassava pulp): all these terms refer to the solid fibrous residue (up to 17% of the tuber) that remain after the flour or starch content has been extracted (Aro et al., 2010). The quality and appearance of these residues vary with plant age, time after harvest and industrial equipment and method used (Cereda et al., 1996).

Fresh cassava peels have 3 main problems: they spoil very quickly, they contain phytates and large amounts of cyanogenic glycosides. Sweet varieties roots contain less than 0.01% DM hydrogen cyanide and leaves 0.1% DM hydrogen cyanide (Murugesrawi et al., 2006). The bitter type cassava peels should be processed in order to reduce cyanogenic potential and phytate content and to preserve their nutritive quality (Murugesrawi et al., 2006; Oboh, 2006; Salami et al., 2003; Tewe, 1992). Cassava peels have a low protein content (< 6% DM) with high and variable fibre content (crude fibre: 10-30%DM). Cassava peels are highly digestible products, with reported values of 78% and 81% for DM and OM total tract digestibility, respectively (Baah et al., 1999).

4. Nutritive value of wet and some dry by-products fed by ruminants

Crude nutrient content and digestibility of the most important by-products fed by ruminants worldwide are shown in Table 2.

Table 2/1. Crude nutrient content and digestibility of the most important by-products fed by ruminants worldwide (cited by Heuzé 2012, 2013a,b,c, 2014a,b,c,d, 2015a,b,c, Heuzé and Tran, 2014)

		Dry matter	Crude protein	Crude fiber	NDF	ADF	lignin	EE	Ash	Total sugars	OMd	ME	N dig. ruminants
		%				%DM					%	MJ/kg DM	%
Sugar factory by-products													
sugar beet pulp, fresh	mean	24.1	8.6	20.8	49.3	24.8	1.8	0.5	6.8	5.1	83	11.2	69
	min.	19.2	7.6	18.6	48.4	22.4	1.7	0.2	4.7	1.2			
	max.	27.8	10.2	23.9	55.7	25.7	2	0.8	10.4	9.5			
sugar beet pulp, silage	mean	23.9	9.5	21.4	50	25.2	1.6	1.2	8.4		82.4	11	70.2
	min.	15.7	8.7	17.5	39.9	22.3	1.2	1	5.5				
	max.	27.8	10.5	28	54.6	26.1	2	1.3	12.2				
sugar cane bagasse, fresh	mean	46.00	1.8	45.9	86.9	58.4	12.5	0.6	5.9		49.7	7	
	min.	31.3	1.4	35.8	72.6	55.1	11	0.4	2.7				
	max.	62.9	2.4	50.3	91.9	62.2	13.6	0.7	10.6				
sugar cane top, fresh	mean	26.8	4.9	34	67.7	39.2	5.6	1.5	7.7			57	8.0
	min.	18.1	2.5	29.5	64.8	34.4	3.9	1.1	5.3			48.4	
	max.	35.6	6.3	37.8	79.9	54.6	8.9	2.3	9.9			65.4	
sugar cane top, silage	mean	30.9	6.7	35	69.6	40.3	5.8	1.6	8			55.1	7.8
	min.	29.6	6.3	34.1				1.3	7.2				
	max.	32.1	7.2	35.8				1.9	8.7				
Citrus by-products													
orange pulp, fresh	mean	23.0	6.0	10.4	25.6	16.9	1.4	4.6	3.6		92.4	13.6	72.2
	min.	21.0	4.8	8.2	24	13.0	1.0	3.4	3				
	max.	24.9	8.0	14.0	27.1	20	1.6	5.6	4.9				
grapefruit pulp, fresh	mean	20.3	6.4	10.5	18.2	12.8	0.4	4.1			92.2	13.4	70.2
clementine pulp, fresh	mean		5.9	7.4	15.3	10.1	1.2	4	3.9		96.5	14.1	74.1
mandarine pulp, fresh	mean		8.0	10.0	21.4	17.4	2.1	6.1	4		92.9	14	75.4
lemon pulp, fresh	mean	21.5	6.1	10.9	33.7	16.8	1.8	5.7	3.8		91.7	13.5	71.1
	min.	18.5	4.8		29.6								
	max.	23.8	7.1		36.4								
orange peel ,fresh	mean	16.1	6.8	6.2	14.1	9.1		1.9	3.7		98.1	14.6	76.8
orange peel ,silage	mean	19.6	7.7	14.3	21.8	16.1		2.6	5.1		87	12.6	67.7
grapefruit peels, fresh	mean	17.9	6.7	10.6	18.3	12.9	1.7	3.9			92.1	13.5	70.6
grapefruit peels, silage	mean	19.2	7.3	13.0	20.6	15.0	2.0	4.2			88.8	13	68.5
lime peels, fresh	mean	18.3	7.8	16.9	24.3	18.3	5.0	3.6			83.4	12.5	64.5
lime peels, silage	mean	23.0	10.6	21.0	28.2	21.9	6.4	9.5			77.8	10.7	66.7
Cassava by-products													
cassava peels, fresh	mean	28,2	4,8	21	19,6	17,1	7,2	1,3	5,7				59,7
	min.	17,9	3,7	10,3	15,8	15,6	4	0	3,4				
	max.	38	5,9	31,8	23,5	18,6	12,1	3,3	8				
cassava pomace, fresh	mean	13,1	1,7	17,7				1,3	3,7				
	min.	10,4	1,1	16,1				0,3	2,8				
	max.	15,8	2,4	19,3				2,4	4,6				
Tomato by-products													
tomato pomace, dehydrated	mean	93.5	21	39	54.9	44.3	25.4	11.9	5.2	21.8	55.1	9.3	65.4
	min.	90.4	17.3	27.6	43.2	36.6	20.6	8.9	3.4		52.4		61.1
	max.	95.2	35	56.9	71.6	60.4	40.5	22	8.4		57.9		71.1
tomato skins, dehydrated	mean	92.7	19.8	37.8					3.3		53.7		61.1

Table 2/2 Crude nutrient content and digestibility of the most important by-products fed by ruminants worldwide (cited by Heuzé 2012, 2013a,b,c, 2014a,b,c,d, 2015a,b,c, Heuzé and Tran, 2014)

		Dry matter	Crude protein	Crude fiber	NDF	ADF	lignin	EE	Ash	Starch*	Total sugars	OMd	ME	N dig. ruminants
		%					%DM					%	MJ/kg DM	%
Distillery by-products														
malt distillers grains (draff), fresh (derived from malted barley)	mean	24.1	20.3	17.6	65.1	26.4	5.9	8.2	3.3	1.8	0.5	51.7	8.9	73.9
	min.	22.0	17.8	16.3	61.8	24.7	5.1	7.6	2.4	1.4	0.4	49.8	8.9	73
	max.	26.8	23.5	20.7	68.9	27.4	6.8	8.9	3.8	2.1	0.6	53.6	10.8	74.8
malt distillers dark grains, dried (derived from malted barley)	mean	90.7	27.8	11.6	39.7	15.5	3.8	8.5	5.8	3.2	4.3	71.2	12	
	min.	88.1	24.3	8	23.2	11.6	2.4	5.5	4.4	2.4	1.7	64.8	12	
	max.	92.9	31.1	13.7	49	18.8	6.3	13	6.8	3.8	11	75.1	15.3	
barley distillers grains (ethanol) (derived from non-malted barley	mean	91.3	28.2	13.8	60.1	23.8	5.1	7.5	5.4	0.9		66.9	11.3	
	min.	87.5	21	10.1	40.1	12.9	0.9	4.2	3.9					
	max.	94.3	34.3	16.1	73.7	33.9	9.3	10.2	8.4					
wheat distillers grain with solubles	mean	90.6	37.3	7.7	34	14.5	4.6	5	5.9	4.2	2.9	78.5	12.5	77.1
	min.	88.1	33.5	6.5	25.5	10.1	2.7	3.5	3.6	2.1	0			
	max.	94.7	40.5	11.2	49.6	21.4	8	6.7	7.3	6.4	6.9			
wheat distillers grain with solubles, starch min.7	mean	92.4	31.4	5.7	27	7.1	2.5	4.9	5.0	16.1	6	82.3	13.1	76.3
	min.	90.7	28.4	4.5	16.4	5.2	1.4	2.7	4.2	9.4	2.3			
	max.	94.5	36	6.9	35.1	8.9	6.8	7.1	6.1	25.1	8.5			
maize distillers dried grains and soluble	mean	89	29.5	7.9	34.2	13.6	4.3	11.1	5.4	9.3	1.7	83.3	14.2	77
	min.	86.6	25.2	6	18.3	7.9	1	7.1	3.4	3.9	0.2	71.6		
	max.	91.9	33.5	9.9	47.4	25.1	8.4	15.7	7.5	15.2	4.8	83.3		
maize distillers dried grains and solubles, high protein	mean	92.2	44	7.5	28.8	12.7	3.1	5.1	2.6	8.2	1.6	84	13.7	78.7
	min.	89.5	40.8	7.4	22.5	6.6	1.2	3.2	1.3	2.7	0.9			
	max.	94.9	48.4	7.6	36.6	22.9	4.6	12.8	6.1	11.4	2.3			
maize distillers dried grains and solubles, fat max. 6 %	mean	88.3	27.9	8.3	39.8	14	2.3	4.2	6.8	12.3	0.6	82.4	12.4	76.6
	min.	85.6	23.4	7.0	27.6	10.1	0.4	2.8	5.8	8.7	0.2			
	max.	91.1	30.8	9.9	47.3	19.8	5.4	6.5	7.9	17.5	1.7			
maize distillers wet grains and solubles	mean	92.2	44	7.5	28.8	12.7	3.1	5.1	2.6	8.2	1.6	84	13.7	78.7
	min.	89.5	40.8	7.4	22.5	6.6	1.2	3.2	1.3	2.7	0.9			
	max.	94.9	48.4	7.6	36.6	22.9	4.6	12.8	6.1	11.4	2.3			
sorghum distillers' grains (with or without sol.), fresh	mean	31.6	35.5		35.6	25.6		11.7				80.8		78.3
	min.	23.5	31.2		20	22.4		11						
	max.	36	41.9		45.4	28.5		13.3						
Starch and ethanol production by-products (wet mill)														
corn gluten meal, dried	mean	90	67.2	1.2	4.1	1.6	0.3	2.9	2.1	17.6	0.5	96.1	16.6	81
	min.	87.3	56.9	0.4	1.1	0.3	0.2	1	1.1	9.1	0.2	94.2	16.6	81
	max.	96.2	76.2	2.7	8.6	3.7	0.6	6.5	4.6	26	1.2	99.9	18.2	97.4
corn gluten feed, dried	mean	88.3	21.7	8.3	39.6	10.6	1.2	3.4	6.9	21.5	1.8	82.4	12.2	74.4
	min.	84.3	17.3	5.3	31.0	8.4	0.6	1.5	4	11	0.7	72.4		67.6
	max.	94.5	27.2	11.4	49.1	13.3	2.7	6.9	10.3	33.8	4.4	82.4		80
Brewers by-products														
wet brewers grain, fresh	mean	24.9	25.9	16.4	49.6	20.8	5.7	7	4.1	5.7	1	61.9	10	76.5
	min.	21.7	20.3	7.8	34.3	17.2	3.5	5.8	2.7	3.3	0.7	55.3	10	73
	max.	28.9	30.6	21.2	62.5	24.8	8	9.3	4.9	9.6	1.3	75.4	12.5	81
wet brewers grain, ensiled	mean	25.6	29.2	16	57.5	21	5	7.4	4.2			62.6	10.2	78.3
	min.	24.6	23.9	10.1	42.6	13.3	3.1	5.4	2.4					
	max.	27.5	33.3	19.2	66.3	24.5	7.7	8.5	6.4					

5. Short term storage of wet by-products

Short-term storage (2-14 days) of wet by-products (brewers' grains, wet corn gluten feed) is associated with the periodic delivery of fresh material from breweries to farms. Storage facilities are usually concrete or plywood boxes, but unprotected piles are not uncommon as a method of keeping wet brewers'grains or wet corn gluten feed during the feeding period between delivery dates. In some cases, storage during this interval has resulted in mold and yeast growth as well as dry matter breakdown in surface layers of the grains. Losses appear to vary with the length of storage, the type of storage facility and

the ambient temperature during the storage period (Allen et al., 1975a, 1975b). There is an inverse relationship between dry matter losses associated with the storage of high-moisture material and the degree of air-tightness achieved (Allen et al, 1975a).

5.1.Aerobic spoilage on the surface and subsurface processes of wet by-products during short term storage

Wet grain by-products are generally stored in horizontal structures. Covering of the silos with plastic film of at least 150 µm or oxygen barrier film sealing immediately following filling is essential for maximal recovery of nutrients during extended storage periods (>10 days), but is also recommended even for short term storage.

5.1.1.Deterioration processes in aerobic top layer of untreated wet by-products during short term storage

Deterioration is more pronounced in the upper, aerobic layer (soft material on the top: 0-10 cm depth) compared to the lower, subsurface layer in wet brewery grain. It was confirmed by an elevated ammonia-N content in the upper layer at 20°C (ammonia-N in total N: aerobic layer 2.31 % *vs.* subsurface layer 0.85 %) and the poor microbial status of the untreated wet brewery by-product (molds: aerobic layer 4.88 log10 CFU/g *vs.* subsurface layer 2.72 log10 CFU/g) (Orosz et al., 2010).

Extensive mold growth, discoloration and dry matter deterioration were noted in fresh wet brewers' grains at 23% dry matter and 4.7% DM total nitrogen in uncovered piles (placed outside on polyethylene sheeting) during a 14-day storage period for treatments of 85% formic acid at 0.20% and 0.40%; propionic acid and formic-propionic mixture (1:1) at 0.20, 0.30, and 0.40%; molasses at 2% and the untreated control, respectively (Allen et al., 1975a).

The wet corn gluten feed is another fairly perishable material (dry matter: 400-420 g/kg, crude protein: 200-220 g/kg DM), but in many cases short term storage is the only option due to periodic delivery. In a trial (Orosz, 2007 unpublished), the aerobic surface of wet corn gluten feed (at 10 cm depth) and the subsurface layer (50 cm depth) were stable during 6 days according to the measured pH and temperature (open container with a capacity of 1 m^3 placed in a barn protected against sunshine and rain, filled with non-packed fresh corn gluten feed). On the 14th sampling day, mold growth, discoloration were found on the top, while the wet CGF in the subsurface layer (50 cm depth) visually was similar to the initial stage .The temperature and pH results on the 14th day showed accelerated spoilage in the top 10 cm (initial temperature: 20 °C *vs.* day 14: 47 °C) and pH (initial pH: 4.2 *vs.* day 14: 5.5), while the subsurface layer had not changed significantly compared to the start (initial temperature: 20 °C *vs.* day 14: 22.1 °C) and pH (initial pH:

4,2 *vs.* day 14: 4,1). It can be concluded that the inner wet CGF was significanty more stable due to limited access of oxygen compared to the top, aerobic layer (Day 14 pH 10 cm: 5.5 *vs.* pH 50 cm: 4.1, p£0.05; temperature 10 cm: 47 °C *vs.* temperature 50 cm: 22.1 °C, p£0.05)

5.1.2. Undesirable processes in the subsurface layer of wet by-products during short term storage

The subsurface layers in wet brewers' grains were more stable in a trial (Orosz et al, 2010) shown by lower ammonia-N content and better microbial status (aerobic bacteria count: aerobic layer 6.48 $\log_{10}$ CFU/g *vs.* semi-anaerobic layer 5.03 $\log_{10}$ CFU/g, p£0.05, molds: aerobic layer 4.88 $\log_{10}$ CFU/g *vs.* semi-anaerobic layer 2.72 $\log_{10}$ CFU/g, p£0.05,). Otherwise, a considerable acetic acid fermentation and ethanol production were found in the subsurface layer compared to the upper (aerobic), untreated material (acetic acid: aerobic layer 1,37 g/kg DM *vs*. anaerobic layer 6,66 g/kg DM p£0.05, ethanol: aerobic layer 0 g/kg DM *vs.* anaerobic layer 1.84/kg DM, p£0.05, fermentation products: aerobic layer 3.03 g/kg DM *vs.* anaerobic layer 10.56 g/kg DM, p£0.05) (Orosz et al., 2010). Similar results were found in another trial, where a rapid increase in acetic acid concentration in the subsurface samples (75 cm depth) was detected for brewers' grains in the untreated control (Allen et al., 1975). The process can be described as non-controlled fermentation by yeasts and undesirable facultative anaerobic bacteria. As aerobic deterioration in the semi-anaerobic subsurface and inner layers (on 20°C for 10 days) were not so intensive as in the aerobic top layer (significantly less aerobic bacteria and mold count in the subsurface layers), thus causing a lower animal health risk during short term storage.

In a trial (Orosz, 2007 unpublished) the wet CGF in the subsurface layer (50 cm depth) was quite stable, but significant microbial activity was found showing deterioration processes had already started (initial stage aerobic bacteria count: 2.9 $\log_{10}$ CFU/g, mould count: 2.0 $\log_{10}$ CFU/g, day 14th aerobic bacteria count: 4.6 $\log_{10}$ CFU/g, mold count: 5.2 $\log_{10}$ CFU/g, yeast count 5.8 $\log_{10}$ CFU/g, p£0.05, respectively). A lactic acid concentration of 7 g/kg DM was observed in the subsurface layer (50 cm depth) in wet CGF on the 14th day of storage.

5.2. Inhibitory effect of different treatments on deterioration of wet by-products during short term storage 5.2.1.Effect of different treatments on aerobic spoilage in the top layer of wet by-products during short term storage

Some chemicals are effective at preventing growth of undesirable microorganisms, heating and spoilage of wet by-products. Propionic acid and its salts, formic acid and

its salts, ammonia, urea, sulfur dioxide, sodium benzoate and potassium sorbate can be options as additives applied just to the top (aerobic) layer, or to the entire bulk of wet by-products during short term storage (maximum 10 days) before feeding (Buchanan-Smith et al., 2003). Toxigenic fungi (molds) generally grow under humid, warm, aerobic conditions. Therefore wet by-products are particularly at risk
(i) in the field (the grain can be infected by molds and contaminated by mycotoxins before harvest),
(ii) during the industrial process (mycotoxin concentration will be increased in the by-product due to starch and protein extraction),
(iii) on farm, during the short term storage in open-air (often non-covered) horizontal piles before feeding (especially in humid and warm tropical-subtropical climates and continental regions in the summer time when the temperature is above 20°C).

Propionic acid is an active fungicide that prevents the mold proliferation and mycotoxin contamination of wet by-products. Propionic acid levels required to preserve high moisture corn are 4.5-17.5 g/kg wet material for the moisture content in the range of 180-400 g/kg. The propionate solution at pH 4.86 was as effective as pure propionic acid (pH = 1.70), it is likely that the propionate ion does contain considerable antimicrobial activity (Raeker et al., 1992). Levels of propionate, rather than propionic acid, and propionate-acetate mixtures that are required for effective preservation of wet by-products are likely to be slightly greater than the pure acid alone. Lower application rates may be used for shorter (anaerobic) storage times for grain by-products compared to acid application for long term anaerobic storage. Schneider et al. (1995) found that 5 g/kg dose propionic acid applied to wet brewers' grain (DM: 185-300 g/kg) preserved the material for periods of 90 days. Solubilization of nitrogen and formation of NH_3 can be decreased by propionic acid application relative to untreated wet by-products during long and short term storage (Schneider et al., 1995; Sebastian et al., 1996). The propionic acid reduces the lactic acid production in the subsurface layers, but does not eliminate it (Schneider et al, 1995; Sebastian et al, 1996). Formic acid is also an important component of mixtures to preserve wet brewers' grain, since acidification, mold and yeast inhibition are important aspects of preserving these by-products (Allen et al., 1975). Mixture of 2.5 g/kg propionic acid and 2.5 g/kg formic acid added to wet corn gluten feed (438 g/kg DM) preserved it at 26.5 °C for periods up to 21 days.

Orosz et al. (2010) found that a dose of 0.3% propionic acid-formic acid (1:1) treatment in the upper soft and aerated layer of wet brewers' grain (top 0-10 cm depth) did not inhibit the deterioration compared to the control (pH 6.36 *vs*. control pH 5.97, respectively, p£0.05). Moreover, the added propionic acid (0.15%) concentration decreased by 96% in the aerobic layer over 10 days compared to the initial concentration and was

lower than the propionic acid concentration in the anaerobic layer (aerobic layer: 5.9 g/kg DM, anaerobic layer: 10.1 g/kg DM, p£0.05). Presumably, undesirable aerobic bacteria and molds were able to use the propionic acid as substrate in the dose of 0.15% in aerobic circumstances. Therefore application of 0.3% dose of propionic and formic acid mixture (1:1) is not recommended as an application for the upper surface treatment, because it presumably supplies substrate for aerobic undesirable micro-organisms causing accelerated deterioration compared to the untreated wet by-product in the aerobic surface.

Two rates (0.20 and O.40%) of formic acid and the high rate (0.40%) of propionic acid were unable to reduce the amount of surface spoilage (Allen et al., 1975).

Acid treatments were effective in inhibiting the deterioration process in the aerobic layer (10 cm depth) of wet CGF stored in open tanks for 14 days (Orosz, 2007 unpublished). The temperature in the top layer of wet CGF was significantly lower when applying propionic acid treatment (dose: 0.25%) and ammonium-propionate (control: 47.4 C, propionic acid 0.25%: 34.5ºC, ammonium-propionate 0.1%: 41.3, ammonium-propionate 0.2%: 36.5; p£0.05). Propionic acid treatment (P dose: 0.25%) and ammonium-propionate (AP dose: 0.2%) were effective at reducing the aerobic spoilage of the top in wet CGF (stored in open tanks without packing) causing significant differences in pH compared to the untreated control (control pH: 5.5, PA treatment pH: 4.3; AP treatment pH 4.2; p£0.05). The lower dose ammonium-propionate treatment (AP dose: 0.1%) was not effective in spoilage inhibiton.

The acids and acid mixture applications are irritants and corrosive, therefore ammonia-buffered products can be used, as less harmful additives. The efficiency of these ammonia-buffered acid mixtures were investigated (Orosz et al., 2010) in anarobic and semi-anaerobic conditions in wet brewers' grain during short term storage (10 days). The buffered acid mixture (dose: 0.5%, composition: 50.4 % formic acid, 29.7% ammmonium-formate, 18.1% propionic acid, 1% mono-propylene-glycol and 0,8 % water) inhibited the spoilage compared to the untreated wet brewery grain in the upper (top 10cm) aerobic layer (aerobic bacteria count of control: 6.48 $\log_{10}$ CFU/g; 0.3% PA:FA 1:1 treatment: 6.48 $\log_{10}$ CFU/g; 0.5% buffered mixture treatment: 4.12 $\log_{10}$ CFU/g; p≤0,05). Otherwise, it was less effective than the treatment of 0.5% un-buffered acid (PA:FA 1:1) mixture (aerobic bacteria count at dose 0.5% PA:FA 1:1 treatment: 3.00 $\log_{10}$ CFU/g, and 0,5% buffered mixture treatment: 4.12 $\log_{10}$ CFU/g, p≤0.05). The aerobic mesophil bacteria- and mold count was significantly higher in the top 10 cm layer treated with the buffered acid mixture compared to the dose of 0.5% of acid mixture (PA: FA 1:1) in wet brewers' grains.

Since inner layers are more stable microbiologically than the top, and whole treatment of the wet by-products on the farm has a potential health hazard for the farm-worker and is expensive, it is recommended to apply a surface acid or acid mixture

treatment by spraying keeping the rules of health and safety. Treatment of the wet by-product pile surface with 0.5% acid mixture (PA:FA 1:1) can be effective to inhibit undesirable aerobic microorganism growth during short storage (for a maximum 10 days) (Orosz et al., 2010; Orosz, 2007 unpublished). Lower doses can be effective, but it is recommended to consider that significant amounts of acids will volatilize from the surface. For this reason, covering the piles is recommended even for short term storage of wet by-products.

5.2.2.Effect of different treatments on spoilage in the subsurface layer of wet by-products during short term storage

A rapid increase in acetic acid in the subsurface samples (75 cm depth) was detected for brewers' grains in untreated control and treated with the low and medium rates (0.20 and 0.30%) of both propionic and formic-propionic acid, 2.0% molasses. The two rates (0.20 and O.40%) of formic acid and the high rate (0.40%) of propionic acid were effective in reducing subsurface deterioration of wet brewers' grains (Allen et al., 1975a).

The 0.3% dose of acid mixtures (PA:FA 1:1) had a beneficial effect on deterioration in the semi-anaerobic layer compared to the control. The dose of 0.5% was significantly more effective in mold inhibition than the dose of 0,3% acid mixtures (PA:FA 1:1) in the subsurface layer (0.3%: AEMB 4.53 $\log_{10}$ CFU/g *vs.* 0.5%: AEMB 3.0 $\log_{10}$ CFU/g, p£0.05). The effect of buffered acid mixture (0.5%, PA:FA 1:2.5) on wet brewers' grains profile was similar to the dose of 0.5% of acid mixture (PA: FA 1:1) in the subsurface layer (Orosz et al., 2010).

It was found that 0.5% of acid mixture (PA: FA 1:1) could inhibit effectively (at 20°C and 20% DM content of wet brewers' grains) the deterioration process compared to the control in both aerobic and subsurface layers (Orosz et al., 2010). There were none significant differences between the aerobic and subsurface layer characteristics in the case of 0.5% acid (PA: FA 1:1) treatment.

6. Long term storage of wet by-products

Before discussing the long storage it is worth briefly reminding ourselves of a few management factors affecting the storage of wet by-products, be they 'forages' or 'concentrates'. Woolford (1986) stated that the presence of air is the 'Achilles heel' of the ensiling process. This is probably even more true of wet by-products than any other ensiled feed. Carbon-dioxide is heavier than air and it has a tendency to gravitate to the bottom of the silo from where it escapes, thus leaving a void, filled by air generally at the top of the silo (Honig, 1991; Williams et al., 1997). The oxygen drawn into the silo is rapidly used producing more CO_2, therefore a continuous gaseous exchange process can be detected

between O_2 and CO_2. The extent depends on a number of factors such as silo type (Jiang et al., 1989), compaction density, dry matter and particle size (Pitt et al., 1991; Pitt 1986; Williams et al.,1997). So, if we now consider the case of ensiling wet by-products there are a number of important additional risk factors worthy of note.

1. The by-product has lost most, if not all, of the biological activity with respect to respiration, such that oxygen entering the store will be entirely available for the growth of aerobic microorganisms increasing the population size of these undesirable organisms.
2. The quantity ensiled/stored is generally limited, giving it a larger surface area:volume ratio making it more vulnerable to CO_2 egress and O_2 ingress. Therefore the plastic tube method would be the more efficient silo type for many wet by-products than other silo types with higher capacity and slower filling rates.
3. As the wet by-products have a low dry matter content (15-50 % DM range), and the structure is variable their consolidation on farm is a challenge. For this reason again the plastic tube system is a potential solution as the prefered silo type in order to obtain anaerobic conditions as quickly as possible, with little human effort required. There is little or no data on effluent production on ensiling of the different by-products. Grass, at 15% dry matter can produce an effluent volume of 200 l/ton diminishing to 0%, when the dry matter content reaches 30% (Bastiman, 1976). Most of the wet by-products fit this dry matter range. Obviously it is not correct to extrapolate data derived from whole plant silages (such as grass) to processed by-products. There are many different factors (particle size and the hygroscopic properties), that will exert their effects on effluent production. Another unknown aspect is the composition of the effluent and its effect on nutritive value of the wet by-product silage.
4. The procedures for sealing the bunker silo is a challenge, allowing a greater chance of carbon dioxide egress and oxygen ingress and overall gaseous exchanges with these type of wet by-products.
5. The hygenic status of the wet by-products recieved on farm is variable. Even in the vicinity of the factory (1-200 km), signs of aerobic deterioration can be found in many cases. The temperature measurement can be a monitoring tool on the farm. The fermentation efficiency will be affected in an aerobically deteriorated wet by-product.
6. Soil contamination is also a high risk in the case of leafy by-products derived from the arable (eg. sugar cane top) and when the factory or the farm store the by-product temporarly in open-air.
7. There may be remnants of different chemicals used during the industrial processes to extract starch, sugar etc, which may have an effect on the fermentation process and aerobic stability.
8. Some of the by-products are treated with acids or salts (eg. 3 l/ton propionic acid

treatment of wet CGF (Orosz, 2015 unpublished) by the factory in order to prevent aerobic deterioration during preliminary storage and transport. This is likely to have a positive effect on the long term storage processes and aerobic stability.

The following section serves as a 'taster' of the information available on some of the more common by-products.

6.1. Long term storage of wet by-products derived from the sugar industry

6.1.1. Sugar beet pulp and molassed sugar beet pulp

6.1.1.1. Fermentation characteristics of wet sugar beet pulp

The ensilage of sugar beet pulp (SBP) and molassed sugar beet pulp (MSBP) has been carried out for over 70 years. Olsen described the fermentation process as early as 1951, the author compared pulp ensiled alone or with the addition of 4% molasses. The addition of molasses (Olsen, 1951) resulted in an almost pure lactic fermentation with *Streptobacterium plantarum* and *S. casei* dominating the initial process. However, after 2-3 months only *Betabacterium breve* could be found. This fermentation resulted in losses of 8 %, as a result of CO_2 production. In the case of wet sugar beet pulp without molasses, the butyric acid fermentation dominated as a result of Clostridia activity and dry matter losses of 12-15% were measured due both to CO_2 and effluent production.

Kilic and Saricecek (2011) investigated the use of 8 different ensiling treatments (1.5 l glass laboratory silos, 60 day) and the effect on nutrient content, pH, physical factors and *in vitro* digestibility of SBP. The treatments were the following: control; AIV system; urea at 1% FW of SBP; formic acid at 2.5 kg/ton FW, Maize-all inoculant applied at 1 x 10^5 CF/g FM (*L.plantarum, L. salivarius, P. acidilactici* + Amylase), Sil-All inoculant applied at 1 x 10^5 CF/g FM (*L. plantarum, L. salivarius, E. faecium, P. acidilactici* + cellulase, hemicellulase, pentonase and amylase); 0.5 kg/ton FM dry sodium formate; 5-7 kg/ton mix of sodium formate and formic acid. The ash content was significantly higher in the AIV treatment at 167 g/kg DM (all other treatments 54.8 to 77.5 g/kg DM), while the urea treatment was intermediate at 107 g/kg DM. The pH was the lowest in the AIV treatment at 1.45, all other treatments ranged from pH 3.17 to 3.55. The urea treatment significantly increased crude protein levels to 212 g/kg DM from *circa* 115 – 120 g/kg (inoculated and untreated SBP), while the formic acid and AIV treatments were significantly lower at 88 and 99 g/kg, respectively. The energy content was the greatest in the inoculated silages and lowest in the AIV treatment. The authors concluded (Kilic and Saricecek, 2011) that the AIV treatment was not advisable, but the other treatments all had merits. Including the urea treatment, which did not affect fermentation adversely and had the benefit of increasing crude protein content. In a study examining a wide range of additives Li and Yu (2009) concluded that SBP silage made without additives had a poorer fermentation quality than those ensiled with 7 different silage inoculants. Sodium sulphite and sodium sulphate

treatment also showed an improvement compared to the contol SBP.

Ensiled SBP was shown to increase bio-ethanol production by 50% compared to fresh SB (Zheng et al., 2012). In this study they showed no benefit of inoculating the SBP before ensiling with *L. fermentum* compared to the untreated control, as this inoculum is hetero-fermentative this is probably not a surprising result. This study also indicated that the ensiling process not only offers a storage solution, but can significantly increase production efficiency of some processes.

6.1.1.2. Technical aspects of wet sugar beet pulp ensiling

The wet sugar beet pulp can be stored in a heap if covered to exclude air, and stored for up to two weeks during October-January. For longer storage it should be ensiled. Ensiling of pressed sugar beet pulp has shown that the conservation in plastic tube silos can be excellent even for long storage periods of time (up to 18 months) and this technique offers a cost effective storage method (Wagner et al., 2009). Due to the rapid filling the fermentation can begin quickly and due to the rapid feed-out rates the risk of aerobic spoilage is low. Since its introduction in 1993/94 more than 1 million tonnes of pressed sugar beet pulp across Europe have been stored in plastic tube silos according to Budissa Bag Technologies (Engelhard et al., 1994).

6.1.2. Wet sugar cane top, as by product of sugar cane harvest for the sugar industry

6.1.2.1. Fermentation of wet sugar cane tops

Sugar cane tops were traditionally removed and left in the field as this improved the economics of the sugar industry. These tops provide a valuable feed resource compared to other forages with a higher energetic potential. Nussio et al. (2009) ensiled manually separated and chopped (10mm length) sugar cane top using 4 treatments. Namely untreated control, CaO (1.5% on a FM basis), dried citrus pulp (10% on a FM basis) and *L. buchneri* (at an inoculation rate of 4 x 10^5 CFU/g FM). The silos consisted of 20 l plastic buckets, fitted with gas and effluent release and measurement mechanisms, with 4 replicates per treatment and filled with 13 kg FM. At ensiling the sugar cane tops had a dry matter of 34%. After 90 days chemical analyses and aerobic stability was measured. Results are shown in Table 3.

Table 3 Effect of different additives on the ensiling of sugar cane tops (Nussio *et al.*, 2009)

	Untreated	CaO	Dried citrus pulp	L. buchneri
Dry matter%	26.3bc	27.8ab	29.6a	25.1c
Crude protein (%DM)	4.30b	4.05c	4.12c	4.51a
NDF (% DM)	71.2c	87.8a	64.5d	77.8b
ADF (%DM)	51.2b	58.2a	48.6c	43.8d
In vitro digestibility (% DM)	45.0c	49.0a	47.9b	43.8d
pH	3.76c	6.68a	3.81c	4.16b
Total losses (% DM)	12.0b	21.8a	10.1b	9.6b
Total Gas loss (% DM)	10.9b	21.6a	9.8b	8.6b
Effluent (kg/T FM)	12.75a	2.03b	3.37b	1.70c
Aerobic stability (h)	78b	216a	30b	62b

Means within rows with different superscripts differ significanctly, $p \leq 0.05$

Results showed that the calcium oxide treatment had a large negative effect on fermentation losses at 21.8% DM. No significant differences were observed between the other treaments in terms of total losses. However, the untreated silage had significantly more effluent at 12.75 kg/ton FM than the other treatments. The untreated and dried citrus pulp had the lowest pHs both below 4, where as the CaO treatment was very high at pH 6.2. This paper indicates that the sugar cane tops can be successfully ensiled.

6.1.2.2. Aerobic stability of sugar cane tops

Aerobic deterioration could be challenging with the exception of the CaO at 216 h compared to 78h for the untreated silages (Nussio et al., 2009).

6.1.2.3. Technial aspects of ensiling sugar cane tops

The silage can be done in small plastic bags in above ground low-cost silos, as well as in small or large concrete silos. The tops can be ensiled without other materials, thereby providing a low protein silage. Ensiling leaves with the tops tends to decrease silage digestibility (Naseeven, 1988). Sugarcane tops can also be ensiled with materials that will increase the nitrogen content, such as urea and molasses, ammonium sulphate and molasses, or poultry litter (Mthiyane et al., 2001; Naseeven, 1988). It is generally a bulky (DM < 30%), low protein (protein < 6 % DM) and fibrous (crude fibre > 30% DM) material (McKenzie et al., 2007).

6.1.3. Sugar cane bagasse

6.1.3.1. Fermentation characteristics of sugarcane bagasse silage

Sugarcane bagasse, is the major by-product of the sugar industry, it is a complex by-product containing about 50% cellulose, 25% hemicellulose and 25% lignin and low

organic matter digestibility. Periera et al. (2009) ensiled sugar cane bagasse with or without chopping. The fresh material had a good sugar concentration of 16.4% and a dry matter of 51%. The dry matter loss of the unchopped bagasse silage was not significant, however the unchopped material loss was 7.5 %. These differences were accounted for by difficulty of compacting the unchopped material leading to a much slower fermentation. There was no difference in the pH (chopped 3.7; unchopped 4.0). However the top part of the whole bagasse treatment had a pH of 7 and was completely degraded indicating an extensive aerobic deterioration process.

6.2. Long term storage of wet brewers' grains

6.2.1. Fermentation characteristics of wet brewers' grains

Wet brewers grains (WBG) are very perishable and the fermentation can be a problem due to the low dry matter- and high crude protein content. Ensiling results in a low organic acid concentration (LA: 1.69 %DM, AA: 2,69%DM) in untreated WBG after 90 days of ensiilng according to Schneider et al. (1995). Despite the WBG having a rather low pH (pH 4,1). Schneider et al. (1995) conducted a number of studies examining different preservation methodologies with wet brewers'grian (WBG). The studies examined the use of inoculants (1 x 10^5 CFU/g compared to 1 x 10^6 CFU/g), acids (propionic acid 0.5% w/w) and absorbent materials with sugar beet pulp pellets (inclusion rate: 15% FM), on the preservation of WBG across 5 different trials. The DM of the WBG ranged between the experiments from 18.5% to 30 % indicating high variability of the WBG. Schneider et al. (1995) showed that the use of an inoculant containing a mix of Homofermentative Lactic acid bacteria improved fermentation quality over untreated silage as measured by increased levels of lactic acid and reduced levels of both acetic and butyric acids. The level of inoculation of either 1 x 10^5 CFU/g compared to 1 x 10^6 CFU/g also had a significant effect, with the higher inoculation rate being significantly better. The higher inoculation rate (1 x 10^6 CFU/g) significantly reduced butyrate concentrations after 57 days of ensilage compared to the untreated control and 1 x 10^5 CFU/g inoculation. In their further studies (Schneider et al., 1995), propionic acid applied at 0.5% w/w (on a FM basis) did not indicate an inhibition of the fermentation. Propionic acid application (0.5% w/w) had a positive effect on the ratio of lactic to acetic of 1:8 compared to 1:2.6 in the untreated WBG. They also found significant differences in total acid content (PA 0.5%: 50.5 g/kg DM vs. untreated: 39.5 g/kg DM). The use of beet pulp (BP) pellets (Schneider et al., 1995) at 15% inclusion rate (FM) increased the dry matter content from 29.5% (untreated control) to 40% in the BP treated WBG. The inclusion of the BP reduced the crude protein content from 31.9% to 22.9%. The BP had a debatable effect on fermentation quality. When BP (15% FM) treatment was combined with an inoculant (homofermentative

bacteria, 10^5 CFU/g), the lactic acid fermentation was improved (LA:AA ratio BP 15% and inoculant:1.5, BP 15% alone: 0.95).

Orosz et al. (2008, un-published) mixed WBG with wilted chopped lucerne (331 g/kg DM, 174 g/kg DM crude protein, 431 g/kg DM NDF, chop size: 50% - 8-19 mm) in a ratio of 40 WBG:60 L and 60 WBG:40 L (based on fresh weight) and baled with a special baler (Göweil LT Master, high density bales: 1,1 ton/bale, density: 846 kg FM/m^3). The lucerne applied as a structural fiber for bale formation allowed a long storage period of 6 months to be achieved. Fermentation quality was very poor (Table 4): pH 6.0, low lactic acid-, very high acetic- and rather high butyric acid content were found with extremely low LA:AA ratio after 90 d of ensilage. Ammonia-N content was elevated indicating intensive protein breakdown. The mold counts were low probably due to the combination of the high levels of undesirable acids and the rapid baling ensiling technology that quickly reduced the oxygen concentration.

Table 4 Fermentation parameters of wet brewers' grain ensiled with fresh chopped lucerne in high density bales (Orosz et al., 2008a, un-published)

(n=3)	pH		Lactic acid		Acetic acid		Propionic acid		Butyric acid		Total acid	
			g/kg DM		g/kg DM		g/kg DM		g/kg DM		g/kg DM	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
40%WBG: 60% L	6.0a	0.2	11.4a	4.1	47.7a	2.9	10.7a	0.6	22.2a	1.8	95.7a	3.6
60%WBG:40% L	6.0a	0.1	16.8b	0.9	42.9b	1.5	10.7a	0.7	26.9b	0.3	106.7b	1.7

	Ethanol		LA:AA		NH_3-N/total N		Aerobic bacteria		Mould	
	g/kg DM		g/g		%		lg_{10} CFU/g		lg_{10} CFU/g	
	mean	std	mean	std	mean	std	mean	std	mean	std
40%WBG: 60% L	5.6a	0.3	0.2a	0.1	22.8a	0.77	5.85a	0.77	0.61a	0.12
60%WBG:40% L	4.5b	0.2	0.4a	0.0	31.6b	0.48	5.03b	0.51	0.20b	0.35

Different letters indicate significant difference $p \leq 0.05$

6.2.2. Technical aspects of wet brewers' grain ensiling

The brewers' grain can easily be consolidated to a high density due to its particle size and low dry matter content. Well preserved brewers' grains in bunker silos can be found on farms. The silo should have proper drainage to collect runoff. Brewers grains silage fermentation is complete by 3 weeks and the ensiled material will keep for 6 months, and even more if a silage additive is used (Boessinger et al., 2005). However, the plastic tube system is more common worldwide as a proposition to store WBG. Advantages of this technology are the flexible capacity, the quick filling and long term maintainance of the anaerobic conditions. Although, gas and effluent production can be hazards during the first 1-3 days of fermentation. The propionic acid treatment (1-3 l/ton) with or without ground cereal or maize silage addition can also be found on farms. Orosz et al. (2008, un-published) recommended the special (high density) baling (as flexible volume silo type) for the long term storage of WBG mixed with structural fiber sources. Packing and ensiling

characteristics can be improved by blending the wet brewers grains prior to ensiling with dry materials such as dry forage, bran or hulls, or with a source of fermentable carbohydrates such as molasses or cereal grains (Blezinger, 2003; Göhl, 1982).

6.3. Long term storage of wet by-products derived from the starch or ethanol production

6.3.1. Wet corn gluten feed

The wet corn gluten feed (WCGF) is a rather perishable by-product due to its 60% moisture content, therefore short-term storage (7-10 days) as fresh wet CGF may cause an animal health risk on the farm. Fermentation of wet CGF is a solution for long term storage, but there is limited information about the fermentation quality and aerobic stability of the ensiled wet CGF after the silo is opened.

6.3.1.1. Fermentation characteristics of wet corn gluten feed

Orosz and Kapas (2010) examined the ensiling characteristics of WCGF. The WCGF had a low organic acid content on the 30th day of fermentation (Exp 1: 12.1 g/kg DM, Exp 2: 8.5 g/kg DM), accompanied by pH of 4.7- 4.8). However, LA:AA ratio was relatively good (Exp 1 LA:AA: 9.7, Exp 2 LA:AA: 4.0). Ethanol production was considerable in the WCGF (Exp 1 ET: 19.0 g/kg DM, Exp 2 ET: 31.3 g/kg DM). Summarizing, the WCGF had a low fermentation intensity with a very high ethanol concentration (Orosz and Kapas, 2010). In this trial Orosz and Kapas (2010) examined the ensiling characteristics of WCGF in combination with 10% dry ground corn grain or 20% maize silage. Maize silage improved fermentation intensity of WCGF (organic acid 12.1 g/kg DM *vs* 18.2 g/kg DM), however significantly increased the acetic acid production and reduced the LA:AA ratio compared to the WCGF control (9,7 *vs* 2.2). The dry corn increased the lactic acid concentration (6.6 g/kg DM *vs.* 9.3 g/kg DM) and significantly reduced the ethanol conent of the WCGF (31.3 g/kg DM *vs* 23.4 g/kg DM) between the 14-30 day. However, acetic acid production was more intensive compared to the control (1.7 g/kg DM *vs.* 2.9 g/kg DM).

Orosz et al. (2008b, unpublished) investigated the use of silage additives in the preservation of WCGF. This study involved the ensilage of WCGF for 30 days either alone or after treatment with one of the following additives: a chemical mixture (59% formic acid, 20% propionic acid, 4.3% ammonium formate and 2.5% potassium sorbate) applied at 3 rates either 4, 5 or 6 l/t (FPA4, FPA 5, FPA 6) or a silage inoculant (INOC: containing *L.plantarum, P. acidilactici, L. salivarius, E. faecium* plus enzymes: cellulase, hemicellulase, amylase applied at 2 x 105 CFU/g) or the above inoculant with K sorbate and Na benzoate (INOC + PRES: K-sorbate, Na-benzoate, applied dose: 250g/ton AF, 3 litre water/ton). The authors concluded that chemical treatments had a negative effect on lactic acid production, the acetic acid concentration did not change considerably, while there was a significant decrease in the ethanol concentration (Table 5).The residual sugar content was significantly higher in the WCGF treated with acid mixtures showing a less intensive fermentation processes. The authors did not find significant differences between

the dose rates applied. They concluded that silage additive containing mixtures of formic- and propionic acids (59% formic acid, 20% propionic acid, 4,3% ammonium-formate, 2,5% K-sorbate), at a rate of 4 litre/ton was sufficient to inhibit yeast growth and ethanol production.

Table 5 Fermentation parameters of wet corn gluten feed treated with different acid mixtures and inoculants (Orosz et al., 2008b, unpublished)

n=3		pH	Lactic acid g/kg DM	Acetic acid g/kg DM	Total acid g/kg DM	T/E	Ethanol g/kg DM	NH3-N % of total N	Total sugar g/kg DM
WCGF	mean	4.81a	9.1a	1.3a	10.4a	7.3a	25.6a	4.2a	15.1a
	std.	0.01	0.4	0.1	0.4	0.8	2.1	0.3	1.7
FPA4	mean	4.17b	7.2b	1.3a	8.5b	5.7b	0.9c	4.4a	52.4b
	std.	0.03	1.0	0.0	1.0	0.9	0.1	0.1	2.0
FPA5	mean	4.24b	8.2b	1.3a	9.5b	6.1b	2.0d	4.6a	49.3b
	std.	0.02	0.6	0.0	0.6	0.4	0.6	1.1	2.0
FPA6	mean	4.16b	6.8b	1.3a	8.0b	5.4b	0.7c	4.9b	49.6b
	std.	0.01	1.5	0.1	1.5	1.0	0.1	0.2	3.2
INOC.	mean	4.83a	7.4b	1.2a	8.6b	5.9b	19.3b	4.8b	12.3a
	std.	0.02	1.1	0.1	1.2	0.7	1.9	0.1	2.6
INOC +	mean	4.83a	7.8b	1.5a	9.3a	5.5b	15.7b	4.5a	15.2a
PRES	std.	0.02	1.1	0.4	1.1	1.4	8.9	0.1	4.0

Different letters indicate significant difference $p \leq 0.05$

FPA4, FPA 5, FPA 6: 59% formic acid, 20% propionic acid, 4.3% ammonium formate and 2.5% potassium sorbate applied at 3 rates either 4, 5 or 6 l/t, INOC: containing *L.plantarum, P. acidilactici, L. salivarius, E. faecium* plus enzymes: cellulase, hemicellulase, amylase applied at 2 x 105 CFU/g) or the above inoculant with K sorbate and Na benzoate, INOC + PRES: (*Enterococcus faecium, Pediococcus acidilactici, Lactobacillus salivarius, Lactobacillus plantarum,* K-sorbate, Na-benzoate, applied dose: 250g/ton AF, 3 litre water/ton).

Orosz et al. (2008a, un-published) mixed WCGF with wilted chopped lucerne (331 g/kg DM, 174 g/kg DM crude protein, 431 g/kg DM NDF, chop size: 50% - 8-19 mm) at a ratio of 70% WCGF:30% L and 60% WCGF:40% L (based on fresh weight) and baled with a special baler (Göweil LT Master, high density bales: 1,05 ton/bale, density: 846 kg FM/m^3). Fermentation quality of the mixture 70% WCGF:30% L was intensive (Table 6): pH was 4.3 associated with 84 g/kg DM total acid and 62.7g/kg DM lactic acid after 90 d of ensilage. The fermentation quality was not so good in the case of the 60% WCGF:40% L ratio (pH 4.4, total acid: 95.7 g/kg DM, acetic acid: 32.3 g/kg DM, ammoniaN in total N: 13.2%). Butyric acid was not found in any case. Microbial composition was advantagous and showed anaerobic stability in the high density bales.

Table 6 Fermentation parameters of wet corn gluten feed ensiled with fresh chopped lucerne in high density bales (Orosz et al., 2008a, un-published)

n=3	pH		Lactic acid g/kg DM		Acetic acid g/kg DM		Propionic acid g/kg DM		Butyric acid g/kg DM		Total acid g/kg DM	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
70%WCGF: 30% L	4.3a	0.0	62.7a	10	20.8a	1.1	2.2a	0.1	0a	0	84,1a	11,1
60%WCGF :40% L	4.4a	0.0	60.9a	11.3	32.3b	3.2	2.6b	0.1	0a	0	95,7a	8,7

	Ethanol g/kg DM		LA:AA g/g		NH3-N/total N %		Aerobic bacteria lg_{10} CFU/g		Mould lg_{10} CFU/g	
	mean	std	mean	std	mean	std	mean	std	mean	std
70%WCGF: 30% L	6.5a	0.4	3.0a	0.4	10.8a	0.45	4.55a	0.07	0.75a	0.26
60%WCGF :40% L	7.2a	0.6	1.9b	0.5	13.2b	0.34	3.88b	0.48	0.69a	0.12

Different letters indicate significant difference $p \leq 0.05$

6.3.1.2. Aerobic stability of wet corn gluten feed

Orosz and Kapas (2010) found that the WCGF aerobic stability was rather poor (49 hours/1°C and 39 hours/1°C) during aerobic deterioration studies (after 30 days of fermentation). Maize silage (20%) increased the mold proliferation compared to the control WCGF, while dry corn decreased the proteolysis (ammonia N%: 7.0 *vs* 5.8) during the aerobic deterioration stage. Maize silage had an undesirable harmful effect on the aerobic stability (49 hours/1°C, *vs* 28 hours/1°C), while dry ground corn significantly improved the aerobic stability of WCGF (39 hours/1°C *vs* 84 hours/1°C).

In a latter trial Orosz et al. (2013) found, the mixture of formic- and propionic acids (application rate of 4 l/ton, 5 l/ton, 6 l/ton dose, respectively) were effective in maintaining the low pH and low ethanol concentration during the aerobic deterioration phase compared to the control. Homofermentative bacteria inoculation at ensiling of WCGF did not have a positive effect on aerobic parameters (pH, acetic acid, ammonia-N, aerobic bacteria, molds), moreover it increased significantly the ethanol concentration compared to the control during the aerobic deterioration phase. Combination of homofermentative bacteria and preservative salts (Na-bensoate and K-sorbate) did not have any significant effect on aerobic parameters (pH, acetic acid, ethanol, ammonia-N, aerobic bacteria, moulds). The aerobic stability of the ensiled WCGF was investigated by monitoring the time taken for the temperature to rise to 1°C above ambient temperature (Orosz et al., 2013) Chemical treatment at 5 and 6 l/t gave the longest aerobic stability of 157 and 135 h respectively. Formic- and propionic acid treatment at an application rate of 6 litre/ton did not have any additional effect on aerobic stability compared to 5 litre/ton. This was followed by the chemical at 4l/ton (90 h) and the combination of inoculant and chemical preservatives (76 h). The untreated (36h) and homofermentative inoculant (36h) were unstable after exposure to air.

Summarizing, the silage additive containing the mix of formic- and propionic

acids (59% formic acid, 20% propionic acid, 4,3% NH4-formiate, 2,5% K-sorbate), at an application rate of 5 litre/ton is recommended to apply for fermentation of WCGF in order to improve aerobic stability after silo-opening.

6.3.1.3. Technical aspects of wet corn gluten feed ensiling

The WCGF could be consolidated easily to a high density, but a density of 443 and 477 kg DM/m^3 caused high weight losses (8,8% and 8,4%, respectively) due to intensive gas and effluent production (Orosz and Kapas, 2010). Maize silage increased the weight losses compared to the control WCGF (+1,1%). Dry ground corn (10%) reduced the weight losses (-1,8%) compared to the control (8,4% vs 6,6%, p£ 0,05). The plastic tube system is sensitive to gas production, therefore it is recommended to reduce the density to 370-390 kg DM/m^3. Orosz et al. (2008, un-published) recommended the special (high density) baling (as a flexible volume silo type) for the long term storage of WCF mixed with structural fiber sources providing almost instantaneous and excellent anaerobic conditions. Wet corn gluten feed stored in a tube silo for one year maintained its composition. In cold climates, freezing temperatures actually extend the storage life of wet corn gluten feed and it was even possible to store unprotected wet corn gluten feed on the ground in winter (North Dakota) with little spoilage for up to three to four weeks. However, high summer temperatures reduce freshness to only three to four days, causing palatability problems (Schroeder, 2010).

6.3.2. Whole plant sorghum by products of the ethanol industry

6.3.2.1. Fermentation characteristics of whole sorghum plant by products

Sorghum is a drought-tolerant plant and the sugar-type sorghum plant has a potential green yield of 50-90 ton/ha with 4-9 ton/ha total sugar yield. These facts make it a very important feed-stock for the bio-ethanol industry. Thus there are a number of different by-products derived from the sugar-type sorghum whole plant after sugar extraction of the stem for bio-ethanol production. In this case the stem would be used for the sugar extraction leaving behind the leaf and seed head. A study was conducted (Bellus et al., 2013) to assess the nutritive value and ensiling characteristics of whole crop sorghum, leaves and 50:50 FM mix of leaves and seed heads on the 40th day of the fermentation. Sorghum leaf and seed head manually separated, chopped (5 mm length) and ensiled in model silos. Nutritive value of the leaves and seed head-leaf mixture was limited (Table 7), but acceptable for extensive animal performance. The study showed that the by-products significantly increased the dry matter content of the silage from 29% in the whole crop to 40% and 45% in the leaf and seed head - leaf mixed silages, respectively. These changes in the dry matter content significantly reduced the total acid content produced during the

40 days ensiling period and resulted in higher pHs compared to the whole crop silage (pH of whole crop silage 3.7 compared to 5.0 and 5.6 in the leaf and seed head-leaf mixed silage). The whole crop silage was also found to have a much lower mold and yeast population at 2.3 log CFU/g FM compared to 4.4 and 5.1, respectively for leaf and seed leaf mixed silages. The authors concluded that the by-products of the sorghum had a poorer fermentation than the whole crop.

Table 7 Nutrient content of the whole crop sorghum silage (WCS), the sorghum seedhead-leaf mixed silage (SSL) and the sorghum leaf silage (SL) on the 40th day of fermentation (Bellus et al., 2013,)

n=5		**Whole crop sorghum**	**Seed head and leaf**	**Sorghum leaf**
Dry matter	g/kg	292a	401b	453b
Crude protein	g/ kg DM	52a	54a	45b
Crude fat	g/ kg DM	15a	18a	25b
Crude fiber	g/ kg DM	304a	330b	340c
Crude ash	g/ kg DM	52a	57a	57a
NDF	g/ kg DM	610a	745b	740b
ADF	g/ kg DM	342a	416b	409b
ADL	g/ kg DM	53a	57a	41b

Means within rows with different superscripts differ significanctly, $p \leq 0.05$

In another trial (Orosz and Bellus, 2012 unpublished), inoculant (*Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus salivarius,* a-amylase, inoculation rate of 1 x 10^5 CFU/g fresh material) was applied to the seed head-leaf mixture (dry matter: 351 g/kg, crude protein 67 g/kg DM, NDF 666 g/kg DM, sugar: 18.5 g/kg DM) in model silos (density: 193 kg DM/m^3). The seed head and leaf ratio was 1:3.6 followed the natural occurence on the field (plant parts: seed head 4.9%, leaf 16.6%, stem: 78.5% based on fresh matter). Fermentation of the untreated seed head-leaf mixture was not intensive, with a poor LA:AA ratio. Inoculation was effective and significantly reduced the pH and the ethanol content, while improved the lactic acid concentration found after 7, 21 and 35 days of fermentation.

Table 8 Fermentation parameters of wet sorghum seed head and leaf mixture silages (Orosz and Bellus, 2012 unpublished)

n=5		Day 7		Day 21		Day 35	
		Control	Inoc.	Control	Inoc.	Control	Inoc.
pH		4.9a	4.4b	4.7a	4.3b	4.9a	4.4b
Lactic acid	g/kg DM	11.4a	23.0b	15.0a	25.3b	16.9a	25.5b
Acetic acid	g/kg DM	7.5a	6.1a	11.3b	8.1b	9.2b	7.7a
Ethanol	g/kg DM	5.2a	3.0b	4.1b	3.7b	6.3c	4.0b
Total acid	g/kg DM	19.3a	29.7b	27.0b	33.8c	29.3b	31.7c
LA:AA ratio	g/g	1.5a	3.8b	1.3a	3.4b	1.8a	4.1b
AmmoniaN	%total N	12.0a	10.7a	10.3a	11.1a	13.7a	12.2a

Means within rows with different superscripts differ significanctly, p≤ 0.05

6.3.2.2. Aerobic stability of whole sorghum plant by products

The homofermentative bacterial inoculation did not have a significant effect on seed head-leaf mixture parameters on the 7th day of exposure to air (Orosz and Bellus, 2012 unpublished), except the pH and yeast count (Table 9). Although, aerobic stability (hours needed for +1 ºC above ambient temperature) of the untreated control silage was 84 hours, while the inoculated was 122 hours.

Table 9 Parameters on the 7th day after exposure to air of sorghum seed head and leaf mixture silage (Orosz and Bellus, 2012 unpublished)

n=5		Control	Inoc.
pH		5.3a	4.3b
Total sugar	g/kg DM	5.0a	5.3a
Acetic acid	g/kg DM	26.2a	29.0a
Ethanol	g/kg DM	6.7a	8.1a
Total acid	g/kg DM	26.2a	29.0a
AmmoniaN	%total N	31.0a	30.0a
Aerobic bacteria	log10 CFU/g	8.5a	7.8a
Mould	log10 CFU/g	1.8a	5.1b
Yeast	log10 CFU/g	8.1a	3.0b

Means within rows with different superscripts differ significanctly, p≤ 0.05

6.3.2.3. Technical aspects of ensiling whole sorghum plant by-products

Compaction was a problem making the mixture and the leaves much more prone to yeast and mold growth (Bellus *et al.,* 2013). Difficulties were also observed in the case of seed head leaf mixtures in the other trial (Orosz and Bellus, 2012 unpublished).

6.4. Long term storage of wet citrus pulp

6.4.1. Fermentation characteristics of wet citrus pulp

As with many of the by products, low dry matter content in wet citrus by products (WCP) is a major challenge. Megıas et al. (1993) concluded that a pre-ensiling, drying or moisture reduction was necessary with orange peel silage in order to obtain an adequate storage. Fermentation acids and pH indicated that the ensiled orange peel resulted in a lactic acid fermentation (Megıas et al., 1993), which was not found during ensiling of orange pulp (Cervera et al., 1985). Other research has shown that WCP may be ensiled alone or mixed with cereal crop residues, such as wheat straw and poultry litter to increase the DM. Migwi *et al.* (2001) concluded that by ensiling of wet citrus pulp (in proportions 0, 150, 300, and 450 g/kg DM of silage) with wheat straw and poultry litter, a good and safe preservation process could be achieved. In these studies (Migwi *et al.* 2001) increasing the level of wet citrus pulp in the silage resulted in a linear decrease in pH and a linear increase in the total acid content of the silage after 60 days of fermentation.

Scerra et al. (1999) used 10 strains of *Penicillium* spp. as inoculant for colonization of bergamot fruit peel which resulted in an increase in CP, crude fat and structural carbohydrates *versus* untreated bergamot fruit peel. Such an approach would be questionable by many silage microbiologists, but non mycotoxigenic strains of *Penicillium* are used in many cheese making processes so why not consider using similar non mycotoxigenic strains to scavenge oxgen in the silo and possibly improve nutrient availability.

Taking a more conventional approach Sarturi et al. (2009) examined the effect of sodium benzoate (0.18% o a FM basis) either applied on the surface of the silo or mixed with WCP at the point of ensiling on fermentation quality and aerobic stability. The totally mixed treatment showed significantly lower gross energy losses after 10 days of 7.2 % compared to the surface treated and untreated, respectively (12.1% and 12.8%). The mixed benzoate treatment also appeared to control extent of fermentation with a significantly higher pH.

6.4.2. Aerobic stability of wet citrus pulp

After 120 days of ensilage the WCP in the study by Sarturi et al. (2009) was subjected to an aerobic stability assessment and the sodium benzoate mixed treatment was significantly more unstable taking 74h to reach its maximum temperature, compared to 154h and 175h, respectively for untreated and surface treated WCP.

6.4.3. Technical aspects of ensiling citrus pulp

Citrus pulp silage has a much higher weight per volume than that of grass or maize

silage, therefore silos in which it is to be made should be adequately reinforced. This problem does not apply to trench silos (Göhl, 1978).

6.5. Long term storage of wet tomato pomace

6.5.1. Fermentation characteristics of wet tomato pomace

Weiss et al. (1997) conducted a study where tomato pomace (WTP) was added (at 0, 6 or 12% on a DM basis) with whole crop corn silage prior to ensiling in laboratory silos. The whole crop maize had a dry matter of 36.6% (WTP 24.7% DM, CP 20% DM). After 58 days there was a significant linear decrease in lactic acid and non protein nitrogen concentrations with increasing level of WTP. The data showed that even at *ca.* 25% DM, the WTP preserved relatively well compared to the corn silage with a pH of 3.92 (corn silage: 3.92); lactic 1.04 % DM (corn silage: 3.11 % DM); acetic 0.92 %DM (corn silage: 1.20 %DM); butyric 0.13 % DM (corn silage: 0.01 %DM), ammonia-N 3.1 %TN (corn silage: 10.3 % TN) and NPN 6.91 % TN(corn silage: 19.78 %TN). The latter finding suggests that maybe there are components in the WTP that protect the protein from breakdown, this does warrant further investigation.

Gallo et al. (2013 a,b) conducted a number of experiments both at small scale in 150-180 kg barrel silos and at farm scale in special, high density bales. Under aerobic conditions storage was poor but under anaerobic conditions storage was relatively good with a good microbial status (pH 4.35; total acid content 55.9 g/kg DM; lactic acid: 36.0 g/k DM, acetic acid: 19 g/kg DM, butyric acid: 0.9 g/kg DM, aerobic bacteria: 4.03 log CFU/g, mold: 3.81 log CFU/g). Within these studies the authors examined a range of treatments including mixing 20% whole grain wheat, 20% ground corn, mixed with NaCl, sealed with NaCl 1kg/barrel) and treated with or without mixed species of homofermentative inoculants.Their studies showed that even alone the TP produced an adequate silage with 55.9 g/kg DM total acid content, lactic:acetic ratio of 1.89 low butyric acid of 0.64 g/kg DM in the barrel silos. The use of non-ground wheat reduced the acetic acid fermentation in the barrel silos. However, there was an increase in losses in the aerated and soft surface layer, so these authors did not recommend this approach. Use of 20% ground maize in high density bales improved the preservation process. Therefore the authors recommended to use dried ground cereal (corn) as an additive (20%) to increase dry matter and energy content, moreover to improve volatile fatty acid composition of the wet tomato pulp silage. The calculated lactation net energy content was similar to a maize silage harvested with approx. 25-30% starch content. The addition of salt did not have a benefit on the fermentation. Whereas the use of the inoculant improved the preservation process in the high density bales when added alongside the 20% inclusion of dry ground corn.The calculated lactation net energy content was similar to a maize silage harvested

with 25-30% starch content.

An approach of using wheat straw at 10, 15 and 20% (FM basis) incorporation with WTP was used by Denek and Can (2006). These authors also compared this approach to ground wheat grain inclusion at 0, 2, 4 and 6% on a FM basis. The primary aim of the research was investigation of the nutritive value and not detailed assessment of silage preservation. The WTP at ensiling had 14.2% DM and 19.2 % CP content. The pH of all silages ranged between 4.00 and 4.07. The inclusion of wheat straw (as expected) reduced *in vitro* digestibility from 57.5 to 54.7% (at 20% straw inclusion), whereas the addition of ground wheat (6%) increased digestibility from 53.8% to 58.5%.

6.5.2. Technical aspects of wet tomato pomace ensiling

Gallo et al. (2013 b) used the special (high density) baling system and confirmed that it was able to form well-shaped and stable bales even with wet by-product such as fresh tomato pulp + ground corn with a small particle size (initial dry matter range of the mixture was 362.6-375.7 g/kg). Extreme bale weight (1120 ± 12.6 kg/bale, n=6), high density (355 ± 4.0 DM kg/m^3 , n=6) and low density-deviation were achieved with the new technology due to high pressurization (130 bar) and small particle size. High density, quick wrapping (within 120 sec after bale-forming) had a beneficial effect on fermentation quality. The authors recommended the special (high density) baling for the long term storage of wet tomato pulp mixed with ground cereal.

In the Phillipines, Caluya et al. (2000) recommended mixing the fresh tomato pomace with chopped wilted grasses or roughage such as rice straw or maize stover in plastic containers, or, alternatively, to pack the materials layer by layer (pomace then chopped grasses aternately). They suggest the mixture should be approximately 70% pomace and 30% dry grass and that the silage is ready for use after 14 days.

6.6. By-products, as silage additives

By-products partially or completely dried, as additives to conventional forages can be applied as absorbents. There is some research showing their use as absorbents to reduce effluent flows (Jones and Jones, 1996; Wilkinson, 1988). O'Kiely (2002) showed that molassed sugar beet pulp and citrus pulp, both dried, had a positive effect on the ensiling process of a 15% DM grass. Inclusion rates of 25, 50 and 75 g/kg were used. The author concluded that both materials promoted a lactic fermentation with citrus pulp being superior to molassed sugar beet pulp at the same inclusion level. Bernandes et al. (2005) showed a similar effect as above using citrus pulp pellets (at 50 and 100 g/kg inclusion rate), when ensiling Marandu grass, with a more rapid pH decline and reduced ammonia-N as the level of citrus pulp increased from 0 to 100 g/kg inclusion.

7. Conclusion and future research needs

This paper has attempted to collect disparate information on numerous wet by-products produced globally. There is a huge quantity of these wet by-products available, as seen in Table 1. Additionally, Table 2 has shown, that as part of balanced diet, they can make a significant contribution to ruminant diets and therefore production efficiency. However, when comparing the published work on grass, legumes, whole crop cereal, maize and sorghum and the wet by-product literature, the research published on effective storage is somewhat 'ad hoc'. Much of the cited work has been conducted for feeding experiments and has not examined the details of the storage and fermentation processes that can have such a big influence on the feed intake and utilization of the product in the animal. This paper will hopefully serve as a conduit for researchers and governments to examine these processes in deeper detail and the research to be conducted in a structured and methodical way.

There are a number of key challenges for the future of wet by-product preservation:

1. Low dry matter content and short shelf life of many of the wet by-products.
2. The effect of the factory processing on the feed value/storage efficiency.
3. Proximity of the supplier and end-user (duration of shipment and the deterioration processes).
4. Effect of intermediate storage (on the field or at the factory) before transportation to the end-user.
5. Problems such as microbial and chemical contamination (soil, heavy metals, mycotoxins, acids and salts) need to be investigated.
6. Methodologies for both short and long term storage, including additives and silo technology developments may increase the value of such products.

Efficient use of wet by-products on farm has to be the end goal. Short term storage of these by products is adding to some problems on farm, for example a highly aerobically spoiled wet brewers' grain product mixed with the TMR will cause large heating losses of the whole diet and can increase the risk of mycotoxins. The plastic tube silo has huge potential to improve many of these aspects in the practical farm situation. However, there are some other significant gains that need further investigation. For example, Nishino et al. (2003, 2005) reported that spoilage could be avoided when wet brewers' grain was stored as part of a TMR (mixed with lucerne hay, dried beet pulp, cracked maize, wheat bran and molasses) in plastic bags with high aerobic stability in the presence of air after opening. Additionally, ensiled and dried grape marc (the solid residue after wine making) reduced methane emissions when fed to cows 5 kg /day. The data on tomato pulp (Weiss, 1997) suggests that the wet pomace has a protective effect on protein degradation in the

silo. This needs more research, as maybe there is a possibility of using tomato pulp by-product as an additive to the conventional high protein crops to reduce proteolysis in the silo and improve protein utilization in the animal. The ensiling fermentation has been shown to reduce phytate content (down to 0.7%) in wet cassava by-products (Oboh, 2006), further investigations are warranted to examine the effect of the ensiling process on other anti-nutritional factors in other by-products. Mycotoxin contamination of distillers' and brewers' grain is another challenge.

So, in final conclusion, there is much more research to be done worldwide, for a better and deeper understanding of the wet by-product preservation, including safe and efficient utilization in ruminant nutrition.

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Can lactic acid bacteria bind aflatoxin B_1 in silage contaminated with the toxin?

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Keywords aflatoxin B_1, binding, lactic acid bacteria, silage

Introduction We recently demonstrated that certain silage lactic acid bacteria (LAB) can bind aflatoxin B_1 (AFB_1) in vitro but the binding extent varied with the bacterial strain, population, and viability. The greatest proportions of AFB_1 were bound when nonviable cells of *Lactobacillus buchneri* and *L. plantarum* or viable cells of *Pediococcus acidilactici* were acidified at pH 2.5 (60.5, 66.5 and 56.9%, respectively). The objective of this study was to determine if adding viable and acid or heat-treated forms of certain silage bacteria could reduce the concentration of AFB_1 in artificially contaminated silage.

Materials and methods A corn hybrid was harvested at 35% DM, chopped to achieve a theoretical length of 1.9 cm and treated in quadruplicate with deionized water (Con) or 30 µg/kg of AFB_1, or with a mixture of AFB_1 and viable, heated or acid-treated forms (1×10^9 cfu/mL) of *L. plantarum* R2014 (Lp), *L. buchneri* R1102 (Lb), or *P. acidilactici* EQ01 (Pa). Each of the inoculants was incubated for 1 h in 30 mL of phosphate-buffered saline (pH 6) at 37°C (viable) or 85°C (heated) or in 30 mL of 0.003 M HCl (pH 2.5) at 37°C (acid-treated) before application. Silages (3 kg) were stored for 21 d in polythene bags. Concentrations of AFB_1 were measured after 0, 24, 42, and 72 h of ensiling and silage samples from d 21 were analyzed for chemical composition, pH, volatile fatty acids and lactate. Data were analyzed using the GLIMMIX procedure of SAS (Version 9.2 SAS Institute Inc., Cary, NC) and a statistical model that included bacteria, processing method, silage sampling time (for AFB_1) and all interactions of these terms.

Results and discussion Despite addition of 30 µg/kg of AFB1 at ensiling (0 h), concentrations of AFB_1 were less than 2 µg/kg within 2 h of ensiling, suggesting immediate binding of AFB_1. Silage AFB_1 concentrations decreased linearly during ensiling to a safe level (< 0.5 µg/kg; US Food and Drug Administration) within 3 d (Figure 1). Inoculation with bacteria or altering bacteria viability did not increase AFB_1 removal. Bacterial viability had no effect on the AFB_1 concentration of Lb and Lp silages ($P > 0.10$) but acid-treated or heated Pa silages had less AFB_1 than the viable Pa silage ($P = 0.01$). Treatment with AFB_1 reduced the CP concentration and increased the butyric acid concentration but inoculation largely prevented these effects.

Conclusion The AFB_1 concentration of contaminated corn forage decreased linearly during ensiling across treatments. Despite contamination with 30 µg/kg at ensiling, safe levels (<0.5 µg/kg) of AFB_1 were achieved within 72 h. Inoculation with bacteria did not increase AFB_1 removal but prevented the increase in butyric acid and decrease in CP caused by the toxin.

Acknowledgement We are grateful to Lallemand Animal Nutrition for funding this study.

Table 1 Effect of applying bacteria (10^9 cfu/mL) subjected to different viability-altering processes to corn forage spiked with aflatoxin B_1 on silage chemical components (% of DM)

Treatment Additive	Process[1]	CP	NDF	Lactic	Acetic	Propionic	Butyric	Total VFA
Control (Con)		8.83	55.2	1.52	2.97	0.52	0.37	6.52
Toxin		8.25	58.9	1.17	2.97	0.47	0.62	8.44
L. plantarum	Viable	8.48	50.4	3.97	3.50	0.33	0.09	50.4
	Heat	8.43	50.4	2.77	3.34	0.36	0.09	50.4
	Acid	8.30	46.7	2.55	4.11	0.53	0.10	46.7
L. buchneri	Viable	8.20	50.7	0.8	2.90	0.48	0.12	50.7
	Heat	8.30	54.8	2.38	2.38	0.42	0.14	54.8
	Acid	8.43	50.9	0.78	3.04	0.39	0.09	50.9
P. acidilactici	Viable	8.28	53.5	2.37	3.34	0.35	0.13	53.5
	Heat	8.78	52.5	2.09	2.28	0.32	0.08	52.5
	Acid	8.03	50.2	0.52	2.47	0.50	0.18	50.2
SEM		0.18	1.92	0.38	0.44	0.11	0.05	1.29
		Contrast *P*-values						
Con vs others		0.02	0.11	0.31	0.89	0.39	0.001	0.20
Con vs Toxin		0.03	0.18	0.56	0.99	0.78	0.001	0.27
Toxin vs Lb		0.78	0.01	0.77	0.68	0.74	0.001	0.52
Toxin vs Lp		0.48	0.01	0.01	0.14	0.61	0.001	0.12
Toxin vs Pa		0.61	0.01	0.34	0.55	0.53	0.001	0.18
Lb viability		0.47	0.38	0.10	0.73	0.56	0.89	0.62
Lp viability		0.62	0.44	0.01	0.67	0.42	0.90	0.26
Pa viability		0.58	0.36	0.03	0.05	0.65	0.94	0.06

[1] Viable, heated and acid-treated cells incubated in phosphate-buffered saline at 37 or 85°C or with 0.003 M HCl at 37°C, respectively.

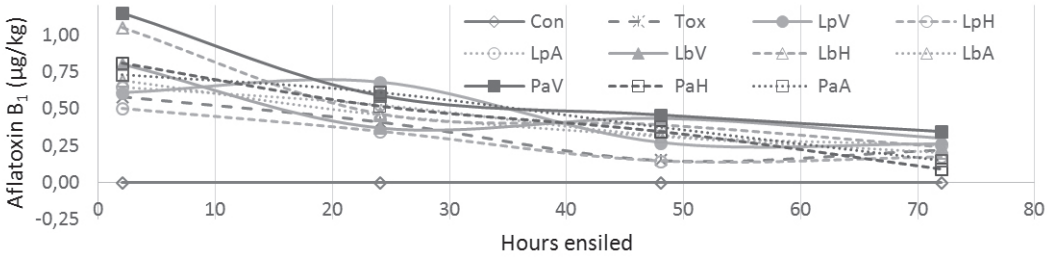

Figure 1 Effects of treatment with nothing (Con), 30 µg/kg of aflatoxin B_1 (AFB_1; Tox) or with Tox and viable (V), acid (2 M HCl)-treated (A) or heated (85°C) forms of L. *plantarum* (Lp)*, L. buchneri,* or *P. acidilactici* (Pa) on silage AFB_1 concentration. Exactly 30 µg/kg of Tox were added at 0 h to all treatments except Con.

Mycotoxin characteristics of farm silages: A two year Irish national survey

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Keywords mycotoxins, silage, fungi, grass, baled, pit, survey

Introduction Over one million hectares, which is one quarter of the total area farmed in Ireland, is used for silage production. Silage fed to ruminant livestock accounts for 24% of the total dry matter intake, but this overhead accounts for 32% of the total feed cost. In Ireland, 90% of farms produce silage and, after grazed grass, silage is the most important crop produced. A previous study of baled silage identified visible moulds, including *Fusarium* and *Penicillium,* in over 90% of the bales surveyed, indicating that a strict anaerobic environment was not maintained within these bales. *Fusarium* and *Penicillium* are toxicogenic and can produce a range of secondary metabolites, namely mycotoxins. Mycotoxins can induce a range of detrimental effects in livestock which include reduced feed intake, vomiting, lameness, immuno-supression and abortions. Some mycotoxins can travel through the feed chain into food via meat (ochratoxin A) and milk (aflatoxin B_1/ M_1). This study was developed to include all mycotoxins that are regulated in Commission Directive (EC) No. 32/2002 and recommendation (EC) No. 576/2006 and emerging mycotoxins of concern as identified by the E.U Commission. The objectives of this study were to identify and quantify the challenge posed to livestock from mycotoxins in Irish grass and maize silages and determine whether conventional chemical characteristics could be used as indicators of mycotoxin incidence.

Materials and methods One silage sample from each of 150 farms was collected between Dec. and March in both 2012-2013 and 2013-2014. Of the 300 silages sampled, 290 were grass and 10 were maize. Samples were assayed for conventional chemical characteristics (dry matter (DM); dry matter digestibility; ash; crude protein (N * 6.25); water soluble carbohydrates; pH; ammonia-N; lactic acid; volatile fatty acids; ethanol) and 20 mycotoxins (deoxynivalenol, aflatoxins B_1, B_2, G_1 and G_2, HT-2 toxin, fumonisins B_1 and B_2, mycophenolic acid, roquefortines C and E, ochratoxin A, T-2 toxin, zearalenone, andrastin A, enniatins A_1, A, B_1 and B and beauvericin). Mycotoxin analysis was performed by validated LCMS/MS. Mycotoxins concentration data (Table 1) were analysed using a Kruskal Wallis test accounting for silage type (within year) and year, and contains positive value samples only. Chemical characteristics identified as significantly associated with the likelihood of mycotoxin detection were modelled using binary logistical regression.

Results and discussion Mean chemical characteristics of baled (b) and precision chop (pc) silage in Year 1 were: DM (b) 291, (pc) 239 g/kg; pH (b) 4.5, (pc) 4.0; lactic acid (b) 58, (pc) 75 g/kg DM and NH_3-N (b) 145, (pc) 41 g/kg N, and the corresponding values in Year 2 were DM (b) 324, (pc) 254 g/kg; pH (b) 4.5, (pc) 3.9; lactic acid (b) 89, (pc) 158 g/kg DM and NH_3-N (b) 96, (pc) 90 g/kg N. Silage DM concentration values indicate that baled and precision chop silages underwent wilting, with herbage used for baled silage experiencing a more extensive wilt. The higher ($P<0.001$) DM values in baled compared

to precision chop silages lead to a more restricted fermentation as evidenced by their lower (P<0.001) fermentation product contents and higher (P<0.001) pH and WSC values compared with precision chop silages. There was a higher incidence (Table 2) of four enniatins in Year 2 compared to Year one and a higher incidence of mycophenolic acid in Year 1.

Table 1 Mycotoxin concentrations for baled and precision chop silages collected during the winter period in Ireland (2012-2014) – values relate only to samples testing positive for mycotoxins

	Year 1						Year 2						
	Baled (n=56)		PC* (n=94)			Silage type	Baled (n=59)		PC* (n=91)			Silage type	Year
Mycotoxin (µg/kg DM)	Mean	s.d.	Mean	s.d.	s.e.d	sig	Mean	s.d.	Mean	s.d.	s.e.d	sig	Sig
Andrastin A	816.0	373.0	404.0	-	-	NS	506	305	< LOQ	-	-	-	NS
Beauvericin	55.0	51.0	26.8	9.41	25.3	NS	30.5	34.5	21.6	8.27	14.5	NS	NS
Enniatin A	10.5	-	17.2	3.72	-	NS	29.4	25.2	20.1	16.3	9.81	NS	NS
Enniatin A1	31.8	10.95	37.5	4.01	6.6	NS	41.7	44.7	50	28.5	14	NS	NS
Enniatin B	364.0	429.0	240.0	357	127.7	*	308	383	276	350	83.1	NS	NS
Enniatin B1	121	74.9	63	94.5	42.88	NS	142	181	112	101	44.1	NS	NS
Mycophenolic acid	1366.0	1756.0	239.0	9.4	1314.3	NS	349	-	< LOQ	-	-	-	NS
Roquefortine C	653.0	732.6	837.0	603	631.0	NS	3358.00	-	< LOQ	-	-	-	NS
Zearalenone	< LOQ	-	< LOQ	-	-	-	33.10	-	73.00	42.70	-	NS	-

*PC: Precision chop; < LOQ: Below limit of detection.

Table 2 Incidence of mycotoxin occurrence detected on baled and precision chop silages collected during the winter period in Ireland (2012-2014)

		Year 1					Year 2					
		Silage type					Silage type					
	Detection	Baled (n=56)		PC* (n=94)		Silage type	Baled (n=56)		PC* (n=94)		Silage type	Year
Mycotoxin (µg/kg DM)	limit	n pos	% of total	n pos	% of total	sig	n pos	% of total	n pos	% of total	sig	Sig
Andrastin A	100	2	3.6	1	1.1	NS	2	3.6	0	0.0	NS	NS
Beauvericin	10	3	5.4	4	4.3	NS	7	12.5	6	6.4	NS	NS
Enniatin A	10	1	1.8	2	2.1	NS	17	30.4	8	8.5	**	***
Enniatin A1	20	10	17.9	3	3.2	**	19	33.9	13	13.8	**	**
Enniatin B	50	16	28.6	22	23.4	NS	35	62.5	43	45.7	NS	***
Enniatin B1	50	12	21.4	5	5.3	**	27	48.2	21	22.3	**	***
Mycophenolic acid	80	5	8.9	2	2.1	NS	1	1.8	0	0.0	NS	*
Roquefortine C	80	3	5.4	2	2.1	NS	1	1.8	0	0.0	NS	NS
Zearalenone	20	0	0.0	0	0.0	-	1	1.8	2	2.1	NS	NS

*PC: Precision chop. n pos = number of samples testing positive for the mycotoxin.

Several chemical characteristic categories (high ash, low and medium DMD, low fermentation products, high propionic acid, low lactic acid and high crude protein were identified using binary logistical regression, as covariates that increase the likelihood of the incidence of enniatin or beauvericin in baled and pit farm silages in this study. Mycotoxin profiles in baled and precision chop silages were similar and contained pre- and post-harvest mycotoxins. Only one E.U regulated mycotoxin (zearalenone) was detected, in Year 1 of the study, but this was well below the E.U. threshold of 3000 µg/kg DM for complete feeding stuffs.

Conclusions Despite significant differences in chemical compositional traits between and within baled and precision chop silages, and between both years, the concentrations of mycotoxins were generally similar across silage types and years. The mycotoxin concentrations found did not exceed E.U directives or guidelines.

Ensiling wet distillers grains with solubles: A review

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Keywords corn silage, corn stover, ensiling, soybean hulls, wet beet pulp, wet distillers grains with solubles

Introduction Corn distillers grains with solubles have become readily available to livestock producers through expansion of the dry milling industry in the United States over the past decade. Corn distillers grains have been shown to be an excellent feed for ruminants, providing a source of protein, energy, and minerals (Schingoethe et al. 2009). Wet distillers grains with solubles (WDGS) has been demonstrated to be a high quality, economical, and readily available feedstuff, but long-term storage can be an issue. Many farms are unable to consume large amounts of WDGS in a short period of time; therefore the development of preservation methods for WDGS is important for livestock producers. Blending and ensiling WDGS with other feedstuffs with a complementary nutrient profile with lower protein, energy, and minerals is a strategy to develop ensiled feeds that can be fed directly to cattle as part of a total mixed ration. Consequently, a series of experiments were conducted to evaluate fermentation characteristics of WDGS ensiled in combination with several different types of feedstuffs including soybean hulls, beet pulp, corn silage, and corn stover.

Materials and methods Four studies were conducted evaluating ensiling characteristics of WDGS with different feedstuffs at various levels of inclusion. Experiment 1 evaluated fermentation characteristics of WDG blended with soybean hulls (Anderson et al. 2009). Experiment 2 evaluated fermentation characteristics of WDGS blended with wet beet pulp (Kalscheur et al. 2004). Experiment 3 evaluated fermentation characteristics of WDGS blended with whole plant corn (Mjoun et al. 2011). Experiment 4 evaluated fermentation characteristics of WDGS blended with corn stover (Anderson et al., 2010). For each respective experiment, samples were taken from the mini-silos (Exp. 1) or silobags (Exp. 2, 3, and 4) and analyzed for nutrient composition and fermentation characteristics including pH, ethanol, volatile fatty acids, and ammonia. Experiment 3 also evaluated the aerobic stability of WDGS and its blends with whole plant corn. Data were analyzed using a mixed model (MIXED procedure, SAS Inst. Inc., Cary, NC). The effects of treatment and day of ensiling were analyzed using a split-plot ANOVA, with treatment as the main factor and day as the split-plot factor. Treatment means were compared using least squares means and adjusted with Tukey's test for P-value significance.

Results and discussion Distillers grains with solubles often contain about 30% CP, 25 to 40% NDF, and anywhere from 4 to 12% crude fat (Schingoethe et al. 2009). New fractionation processes continue to alter the nutrient profile of WDGS, so it's important to analyze the feedstuff to know how to formulate it into diets. In 3 of these studies (Kalscheur et al. 2004, Anderson et al. 2009, Mjoun et al. 2011), WDGS was ensiled

alone and then in combination with the test feedstuff, whereas the 4th study (Anderson et al. 2010), corn stover was combined with WDGS and evaluated with and without an ensiling additive. One of the advantages of freshly produced WDGS is that it has a low pH (<4.0) which helps in its preservation whether it is alone or blended with other feeds. When blended with other feeds, pH of these blends drop quickly aiding in its preservation. Blending WDGS with other feeds resulted in fermentation patterns that differ from the "traditional" lactic acid fermentation towards more production of acetic acid. Ensiling WDGS with whole plant corn resulted in a shift from lactic acid fermentation to an increase in acetic acid production and improved aerobic stability compared to whole plant corn silage. Fermentation characteristics for the blends of corn stover and WDGS were similar for both treated and untreated treatments. Combinations of WDGS with soybean hulls or wet beet pulp preserve very well when bagged individually or blended together. Ultimately, the goal of the blends is to extend storage capabilities of the feeds, but also to improve palatability of these feeds to cattle which result in potentially improved productivity.

Conclusions Feeding WDGS presents challenges to smaller livestock producers as it often spoils quickly. Combining WDGS with other feedstuffs prior to ensiling is an approach to increase their use in diet formulation and improve their long-term storage capability. Blending WDGS with alternative feedstuffs is an innovative approach to extend feed supplies and reduce feed costs.

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On-farm corn silage investigation: multi-analysis on silage practices, silage quality and its effect on aerobic stability

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Keyword corn silage, silage practices, aerobic stability, silage inoculants

Introduction Poor silage quality has a major impact on production cost, especially when it represents more than 50% of the daily intake for ruminants (Paragon et al., 2004). Silage practices from harvest management to bunker management and the use of an appropriated silage inoculant greatly influences the forage quality and thus the profitability of the farms. Spoiled silage represents not only dry matter (DM) losses, but it also depresses feed intake and performances (Hoffman and Ocker, 1997; Whitlock et al., 2000). A silage investigation kit and its accompanying software [Corn Silage Investigation (CSI)] was designed to assess silage quality and aerobic stability on the one hand and silage practices on the other. This information identifies areas for improvement (Andrieu et al., 2012). This article combines the results of different surveys, made in Europe using the CSI, in one multi-analysis.

Materials and methods During the 2012 and 2013 springs and summer, corn silage on 149 dairy farms located in France, Italy and Greece have been investigated according to the standardized "CSI" method (Andrieu et al., 2012). One part of the survey collected information from harvest practices (cutting height, use and type of inoculant, feed-out rate) whilst the other part investigated the silage quality based on measurements of the temperature at 20 cm depth from 6 points on the silo face. One sample of each silo was also drilled and sent to the lab for analysis of the fermentation profile (NIR determination of DM, Ash, pH, lactic acid, VFA, 1,2-propanediol). The three surveys assessed in total 76 untreated silos (Control), 27 treated silos with competitor's products (Competitors) and 46 silos inoculated with *L. buchneri* 40788 (LB 40788) (300,000 cfu/g of forage, Lalsil Fresh, Lallemand Animal Nutrition, Blagnac, France).

Results and discussion Results of this survey show a correlation between some of the investigated parameters and the aerobic stability of the silage. Silos with a higher density have lower average temperatures on the silo face (209±47 kg DM/m^3 for 25±4.7°C vs. 238±48 kg DM/m^3 for 22±3.0°C, $P<0.05$). These findings are in line with Johnson et al. (2002) who states that silage temperature was negatively correlated with the packing density of the silos. The silo design also plays a role on the temperature of the silos. This is mainly a result of the variation of compaction linked to the design. Bunker silos are more likely to have better compaction and were significant cooler than drive over pile silos (24±3°C vs. 25±4°C, $P<0.05$). Cutting height of corn silage has a significant influence on the ash content of the silages. Results show a negative correlation between ash content and the cutting height ($P<0.05$, $r^2 = -0.32$). Higher ash content in the forage is indicative of soil contamination and can lead to increased numbers of clostridia (Knický, 2005), which can lead to aerobically unstable silage (Tabacco et al., 2010). The use of an appropriate inoculant shows a significant and consistent effect over the silo temperature. The LB 40788 treated silos were significantly cooler than both the control and competitors silos (22±2.7°C vs. 26±3.4°C vs. 24±3.6°C, $P<0.05$). There was no significant difference between the control and competitors silos. A meta-analysis conducted by Kleinschmidt

and Kung (2006) showed a consequent improvement of aerobic stability when silages were inoculated with *L.buchneri* 40788. This can be explained by the differences of the fermentation profiles during the ensiling process as observed also in this multi-analysis: LB 40788 silos showed significantly more acetic acid than control ($P<0.05$), and numerically more than competitor products (Table 1). Weinberg *et al.* (2001) observed that the more stable corn silages had higher acetic acid concentrations.

Table 1 Fermentation characteristics of silages from 149 farms (± SEM)

	pH	Lactic acid	Acetic acid
Control	3.85 ± 0.02	30.4 ± 2.62	11.1 ± 0.7 [a]
Competitors	3.83 ± 0.01	28.6 ± 4.70	18.7 ± 2.8 [ab]
LB 40788	3.80 ± 0.01	34.1 ± 3.47	21.2 ± 3.5 [b]

[a,b] differ when $P<0.05$

The defacing rate of the bunker also seems to influence the temperature of the front face. There is a trend for lower temperatures on the front face with higher feeding out rates (>20 cm/day), confirming the results from Pitt and Muck (1993).

Conclusions The temperature of the silo face is one of the signs of aerobic stability and this can be easily assessed on farm. This temperature is correlated with several parameters. CSI allows an assement of the parameters that can be improved. Among the "cooling" practices, the use of *L.buchneri* 40788 based inoculants shows a consistent effect in controlling the silos temperature,

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Effect of *Lactobacillus buchneri* on fatty acids profile of corn silage

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Keywords lactic acid bacteria, PUFA, silage inoculant

Introduction In silages the majority of the fatty acids (FA) occur as free fatty acids (FFA) due to lipolysis (Steele and Noble, 1984). Since lipolysis is a prerequisite of rumen biohydrogenation, a high amount of FFA in the forage could result in a high biohydrogenation rate (Van Ranst et al., 2009). Conversely, a lower lipolysis inside of silo could result in a lower rumen biohydrogenation (Van Ranst et al., 2009). Although one of the major causes of variation of FA in silages is the dry matter (DM) content (Khan et al., 2012), the knowledge about the effect of silage inoculants on the FA profile is unclear and needs to be better studied. Thus, our objective was to investigate the effect of the application of *Lactobacillus buchneri* on fatty acids profile of corn silage.

Materials and methods A corn hybrid (cv. 2B688Hx) was harvested at 312 g/kg of dry matter (DM) and chopped to achieve a length of 10 mm. Corn plants were treated with water (0.7 L/t; control) or 1×10^5 cfu/g of fresh forage of *L. buchneri* NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI, USA; inoculated). The inoculant was dissolved in water (0.7 L/t) and then sprayed on piles of fresh forage under constant mixing. Two bunker silos were filled on the same day with 60 t of corn forage each at a packing density of 530 kg/m^3. The silos were stored outside during the Brazilian summer and autumn seasons with an average temperature of 23.2ºC for 70 d. The fatty material was extracted using a mixture of chloroform-methanol (Bligh and Dyer, 1959), and the fatty acid methyl esters (FAME) were obtained by the ISO 5509 method (ISO, 1978) using the method n-hexane, methanol and KOH and a mixed standard (C4 to C24). The qualitative and quantitative measurements of the fatty acid content were performed by gas chromatography using a chromatograph (Shimadzu, Kyoto, Japan - Model GC-14B with a Communication Bus Module - CBM 102) with a flame ionization detector (FID) and fused silica capillary column (Omegawax 250), which was 30 m in length and 0.25 mm in diameter and had a film thickness of 0.25 μm (Supelco SP-24136). Helium was used as a carrier gas at a flow of 1 mL/min. A 1-µL aliquot of the fatty acids dissolved in ester was injected into a "split" at a division ratio of 1/100 and temperature of 250°C. The temperature of the oven was programmed to remain at 100°C for 2 min and then increase to 220°C at an interval of 4°C/min for 25 min, whereas the detector was at 280°C. The identification and quantification of the methyl esters of the fatty acids were achieved by a comparison with the retention times and concentrations of the methyl esters of the standard fatty acids. The data were analyzed with a mixed model using the PROC mixed of SAS (v 9.2), and differences between means were determined using the DIFF procedure. Differences were declared significant at $P \leq 0.05$.

Results and discussion The majority of FA evaluated was not changed by inoculant (Table 1). However, inoculated silage had lesser concentration of palmitic (16:0), margaric (17:0) and lignoceric (24:0) acids, which resulted in lower SFA concentration; whereas the majority of the FFA were not affected by inoculation. Conversely, MUFA and PUFA were no changed by inoculant. These results suggests that total FA profile in a well-sealed silage is, at least to some extent, independent of the type and extent of fermentation (Van Ranst et al., 2009). In general, the silage inoculant has few effects on the FA profile.

Table 1 Fatty acid profile (%) of corn silages

Item	Control	Inoculated	SEM	*P*-value
12:0[1]	0.280	0.225	0.013	0.092
14:0	0.345	0.280	0.024	0.199
15:0	0.050	0.050	0.007	1.000
16:0	17.895	16.210	0.185	0.023
16:1	0.135	0.175	0.011	0.127
17:0	0.230	0.170	0.007	0.026
17:1	0.045	0.040	0.008	0.698
18:0	3.105	3.005	0.056	0.333
18:1 *n*-9	29.635	30.540	0.557	0.369
18:1 *n*-7	0.750	0.620	0.114	0.504
18:2 *n*-6	38.735	40.910	0.787	0.190
18:3 *n*-6	4.570	3.940	0.452	0.428
18:3 *n*-3	0.050	0.080	0.021	0.422
20:0	0.895	1.115	0.230	0.568
20:1 *n*-9	0.545	0.585	0.205	0.902
22:0	0.475	0.445	0.011	0.198
23:0	0.105	0.095	0.005	0.292
24:0	2.075	1.475	0.090	0.042
SFA	25.47	22.98	0.278	0.001
MUFA	31.03	32.03	0.469	0.182
PUFA	43.47	44.95	0.738	0.205

[1]12:0 = lauric, 14:0 = myristic, 15:0 = pentatonic, 16:0 = palmitic, 16:1 = palmitoleic, 17:0 = margaric, 17:1 = cis-10-heptadecanoic, 18:0 = stearic, 18:1 *n*-9 = oleic, 18:1 *n*-7 = vaccenic, 18:2 *n*-6 = linoleic, 18:3 *n*-6 = □-linolenic, 18:3 *n*-3 = linolenic, 20:0 = arachidic, 20:1 *n*-9 = 5-eicosanoic, 22:0 = docosanoic, 23:0 = tricosanoic, 24:0 = lignoceric, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids.

Conclusions The *Lactobacillus buchneri* no markedly changes the fatty acid profile of corn silage.

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Solid-state fermentation of lupin (*Lupinus albus*) meal bio-processed with *Lactobacillus plantarum* and supplemented in aqua-diets

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Keywords solid state fermentation, *Lupinus albus*, *Lactobacillus plantarum*, aqua-diets, proximal composition, organic acids enrichment

Introduction Solid-state fermentation (SSF) is based on microbial (bacteria, yeast and fungi) transformation of solid substrates (Pandey, 2003) in the absence of free water (Raimbault, 1998). Although filamentous fungi species have been widely used in SSF, several bacteria have been utilized in this bio-process (Raimbault, 1998; Manpreet et al., 2005). *Lactobacillus plantarum* is a gram-positive, with probiotic properties and aero tolerant Lactic acid bacteria (LAB) commonly used in silage (Ohashi et al., 2009). During the anaerobic conditions of ensilage, *L. plantarum* quickly dominate the microbial population producing lactic and acetic acids (Contreras-Govea et al., 2013). Traditionally fish meal has been used as main dietary protein source in aqua-diets. Shortage and high cost have made fish meal little profitable (Pandey, 2003). Plant protein meals have been widely used as fish feed ingredient. The main drawback of plant meals is the unbalanced nutritional profile and anti-nutrient content (Torstensen et al., 2008). Protein content of lupin (*Lupinus albus*) grains is higher in comparison with other crops (Flengmark et al., 1984). The SSF using lupin meal has been well demonstrated (Lone et al., 2003). It has been well studied that fermentation of plant substrates with LAB improves its nutritional profile (e.g. protein and amino acids), anti-nutrient reduction (Contreras-Govea et al., 2013) and enrichment with secondary metabolites (organic acids) (Torstensen et al., 2008). Present study is aimed to assess effects of solid state fermentation in proximal composition and organic acid enrichment of lupin meal alone and after partially being supplemented in aqua-diets.

Materials and methods *Lactobacillus plantarum* 229v was used. Bacteria solution was inoculated (until reach 60% of humidity) in previously sterilized and chopped lupin grains. During fermentation process (37ºC, pH = 4.48 h), CFU of *L. plantarum* were assessed at 0 h, 36 h and after drying. Afterwards, resulted substrate was minced and submitted to proximal composition, and organic acid analysis. Using fermented and non-fermented lupin meal, five diets were designed and manufacture as follows: C (control diet), L15% (diet partially supplemented with 15% of non-fermented lupin), FL15% (diet partially supplemented with 15% of fermented lupin), L30% (diet partially supplemented with 30% of non-fermented lupin) and FL30% (diet partially supplemented with 30% of fermented lupin). In order to assess effects of partial supplementation of fermented lupin meal in aqua-diet for salmonid, experimental diets were submitted to a proximal composition and organic acid analysis.

Results and discussion Protein (40.67%), ether extract (8.51%) and gross energy (21.10 MJ/kg) were slightly increased in fermented lupin meal compared to non-fermented

(protein: 38.37%; ether extract: 7.90%; gross energy: 20.40 MJ/kg). Dry matter (93.87%), fiber (3.02%) and non-nitrogenous extract (47.47%) remained slightly higher in non-fermented lupin meal compared to fermented one (dry matter: 91.09%; fiber: 2.81%; non-nitrogenous extract: 44.77%). Lactic acid content of fermented lupine meal (62 mg/g DM) was higher than that of non-fermented lupin meal (0.0 g/g DM). Citric acid content of fermented lupine meal (6 mg/g DM) was lower than that of non-fermented lupine meal (13 mg/g DM). Acetic acid content of fermented lupine meal (14 mg/g DM) was higher than that of non-fermented lupine meal (4 mg/g DM). The CFU counts were 7×10^3/g at 0 h (70% humidity) of fermentation process. At 36 h of fermentation, CFU was 10×10^7/g (65% humidity). After drying fermented lupin, CFU was 15×10^3/g. Dry matter, protein, ether extract, ash and gross energy did not recorded significant ($P>0.05$) differences among experimental diets. Fiber showed significantly ($P<0.05$) lower values in experimental diets compared to C. Nitrogen-free extract showed significantly ($P<0.05$) lower value in C compared to experimental diets. Lactic acid was not detected in C. L15% recorded a higher value (17.4 mg/g DM) compared to FL15% (10.1 mg/g DM). Lactic acid showed a higher value in FL30% (75.6 mg/g DM) compared to L30% (12.9 mg/g DM). Citric acid was not detected in diets C, L15% and FL15% whereas L30% showed a higher value (4.9 mg/g DM) compared to that shown in FL30% (2.2 mg/g DM). Acetic acid showed similar values between L15% and FL15% (4.5 mg/g DM and 4.0 mg/g DM, respectively) whereas FL30% showed a higher value (10.4 mg/g DM) compared to L30% (4.1 mg/g DM).

Conclusions The SSF with *L. plantarum* produced minimum or meaningful differences in proximal composition of fermented lupin meal and aqua-diets partially supplemented (15% or 30%) with this fermented substrate. SSF with *L. plantarum* notoriously enrich lupin meal with lactic acid and acetic acid. Partial supplementation level (30%) of fermented lupine meal considerably enriched aqua-diets with lactic and acetic acid.

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Influence of plant population on the quality of corn silage

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Keywords corn silage, planting density, dairy cow

Introduction Nowadays, corn silage is the major energy and fiber source in diets for dairy cattle (Gonzalo Ferreira et al., 2014). Plant population is one of the factors which influence the nutritive value of corn silage. The present experiment compared different plant populations on the nutritional quality of whole-plant corn for silage.

Materials and methods The corn variety Jindan 42 was seeded with four densities: 52500, 63000, 73500 and 84000 plants per hectare (three plots per treatment) in the spring of 2013. Plants were harvested at the dough stage and ensiled in the silage bags. After 90 days of storage, silages and fresh forage were analyzed for dry matter (DM) , crude protein (CP), NDF, ADF, ash, starch and water soluble carbohydrate (WSC) by wet chemistry. Silages were also analyzed for lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) by high performance liquid chromatography (HPLC).

Results and discussion The yield of fresh matter and DM of 83000 and 73500 plant densities were significantly ($P<0.01$) higher than that of 63000 and 52500 treatments. In addition, different populations had not significant ($P>0.05$) effect on nutrient contents of fresh plants (Table 1) and silages (Table 2). In this way, the four planting density had no significant ($P>0.05$) influence on silage pH, AA, AN, LA, PA and BA contents and DM recovery (Table 3).

Conclusions Different plant population had no obvious effect on the quality of corn silage. Higher DM yields were achieved with higher plant populations.

Acknowledgements The authors wish to thank all the staves of the Institute of Grassland Science, China Agricultural University, China, for their help. This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (Contract No. 201303061) and the National Key Technologies R&D Program (Contract No. 2011BAD17B02-02).

Table 1 Characteristics of corn fresh forage in different plant populations

Population, Plants/ha	Fresh weight, t/ha	Dry weight, t/ha	DM, %	CP, %	NDF, %	ADF, %	Ash, %	Starch, %	WSC, %
52500	54.0± 1.25[c]	16.71± 0.36[b]	30.91± 2.49	8.88± 0.26	56.40± 2.70	26.35± 2.39	4.24± 0.80	31.24± 2.29	12.49± 1.08
63000	61.11± 1.11[b]	17.80± 0.40[b]	29.12± 2.91	8.67± 0.31	54.54± 4.39	25.34± 1.99	3.75± 0.65	31.96± 3.15	11.42± 2.50
73500	69.09± 0.81[a]	20.00± 0.26[a]	28.94± 2.48	8.12± 0.28	54.48± 3.37	25.40± 1.33	3.98± 0.58	31.82± 2.48	13.08± 1.54
83000	69.72± 0.95[a]	20.55± 0.33[a]	29.48± 2.55	8.09± 0.93	56.29± 2.36	27.10± 1.19	4.26± 0.50	30.23± 3.03	12.68± 1.40

Means with different letters in the same column differ (P<0.01).

Table 2 Nutrient composition of corn silages from different plant populations

Population, Plants/ha	Dry matter recovery, %	DM,%	CP,%	NDF,%	ADF,%	Ash,%
52500	96.44±3.24	31.61±2.68	9.24±1.39	57.13±2.20	27.26±1.54	3.99±0.60
63000	97.07±2.55	31.03±1.67	9.63±1.37	56.14±1.49	25.14±3.03	4.13±0.85
73500	97.20±3.65	29.68±2.37	8.75±1.36	55.44±2.54	26.11±2.10	4.17±0.14
83000	96.49±1.76	30.69±1.20	8.05±1.32	56.07±2.36	27.43±1.29	4.01±0.38

Table 3 Fermentation profile of corn silage according to plant population

Population, Plants/ha	pH	AN,%	LA,%	AA,%	PA,%	BA,%
52500	3.87±0.08	1.76±0.19	4.78±0.18	0.31±0.06	0.36±0.02	nd
63000	3.50±0.04	2.07±0.07	5.21±0.10	0.39±0.07	0.33 ±0.01	nd
73500	3.71±0.02	2.22±0.02	4.48±0.56	0.30±0.02	0.34±0.03	nd
83000	3.47±0.03	1.77±0.07	4.12±0.71	0.33±0.10	0.29±0.19	nd

nd: not detected.

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Harvesting corn silage at different maturity stages

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Keywords corn silage, harvest, nutrients, fermentation quality

Introduction Corn is the most important feed in the word. In recent years, due to the rapid development of animal husbandry, corn silage as a forage has becoming an inevitable trend. Silage has advantages of good palatability, high nutritive value and storable. However the maturity at harvest can affect the fermentation quality and nutrient composition.

Materials and methods The trial have compared four the harvest and ensiling of corn plants at four maturity stages: 80 days after seeding is forming stage group, 100 days after seeding is milk stage group, 115 days after seeding is dough stage group and 130 days after seeding is ripe stage group. After 90 days of storage, silos were opened and samples of the silages were were taken to be analysed. The dry matter (DM) was measured by oven method, crude protein (CP) with Kjeldahl method, neutral detergent fiber (NDF) and acid detergent fiber (ADF) with Van Soest method. Lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) were determined by HPLC method.

Results and discussion The results showed that productivity (fresh weight) of milk stage, dough stage and ripe corn was significantly (P <0.01) higher than forming stage (Table 1). The NDF content of ripe stage were significantly (P <0.05) higher than that of the dough stage. The ADF content of forming stage were significantly (P <0.01) higher than that of the dough stage. The pH of corn silage of the forming stage were significantly (P <0.01) higher than that of the milk stage and the dough stage (Table 2). The NH_3-N content of the forming stage were significantly (P <0.05) higher than that of the milk stage and the dough stage. The DM content of corn silage were significantly (P <0.01) different at the four stages (Table 3). The NDF content at ripe stage were significantly (P <0.01) higher than that of the dough stage. Silage plays a major role in the maintenance of good palatability, promoting health of cow, improving milk quality (Zhu Yu et al., 2011). The different maturity at harvest for ensiling did not affect AA, PA, Ash content and dry matter recovery (DMR) (P> 0.05). However, a lower pH, fewer PA content, higher LA and AA content were found in the dough stage.

Conclusions With the growth process of corn, the nutrients of corn changes, which is suitable harvested for silage at dough stage.

Acknowledgements The authors wish to thank all the staves of the Institute of Grassland Science, China Agricultural University, China, for their help. This research

was supported by the Special Fund for Agro-scientific Research in the Public Interest (Contract no. 201303061) and the National Key Technologies R&D Program (Contract no. 2011BAD17B02-02).

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Table 1 Forage composition of corn harvested at different periods

Harvest time	Fresh weight t/hm^2	Dry weight t/hm^2	DM，%	CP，%	NDF，%	ADF，%	Ash，%	Starch，%	WSC，%
Forming stage	38.4±2.12^{Bc}	5.91±0.99^{Da}	18.39±2.57^{Dd}	9.67±0.15^{Aa}	57.89±2.05^{ab}	31.20±0.11^{Aa}	4.49±0.18	5.10±5.48^{Bb}	8.32±1.22^{Bb}
Milk stage	58.2±1.99^{Ab}	13.1±0.85^{Cc}	24.67±2.17^{Cc}	8.51±0.65^{ABab}	55.72±3.92^{ab}	28.30±6.03^{ABab}	4.24±0.46	15.55±6.41^{Bb}	11.40±2.22^{ABab}
Dough stage	68.4±2.11^{Aa}	21.7±0.85^{Bb}	31.82±2.22^{Bb}	8.46±0.61^{ABab}	54.84±2.33^{b}	25.57±2.50^{Bb}	3.95±1.09	30.34±8.55^{Aa}	13.14±1.08^{Aa}
Ripe stage	61.8±2.03^{Ab}	25.5±0.89^{Aa}	41.35±3.21^{Aa}	7.38±0.55^{Bb}	59.42±3.12^{a}	28.28±5.06^{ABab}	4.41±1.43	31.44±6.04^{Aa}	11.80±2.10^{ABab}

Note: Different capital letters in the same column means most significant difference at 0.01 level (P<0.01), lower case letters means significant difference at 0.05 level (P<0.05).

Table 2 Fermentative profile of corn silages harvested at different periods

Harvest time	pH	NH_3-N,%	LA,%	AA,%	PA,%	BA,%
Forming stage	4.19±0.09^{Aa}	2.08±0.14^{a}	3.24±0.18^{b}	0.23±0.03	0.18±0.01	0.25
Milk stage	3.51±0.10^{Bc}	1.34±0.16^{b}	4.91±0.00^{a}	0.19±0.03	0.14±0.04	0.05
Dough stage	3.55±0.11^{Bc}	1.37±0.17^{b}	4.96±0.09^{a}	0.23±0.02	0.13±0.04	-
Ripe stage	3.89±0.26^{ABb}	1.74±0.30^{ab}	4.19±0.51^{ab}	0.25±0.05	0.23±0.13	-

Note: Different capital letters in the same column means most significant difference at 0.01 level (P<0.01), lower case letters means significant difference at 0.05 level (P<0.05),"-"means not detected.

Table 3 Nutrient composition of silages from corn harvested at different period

Harvest time	Dry matter recovery	DM，%	CP，%	NDF，%	ADF，%	Ash，%
Forming stage	96.38±2.27	19.28±2.87^{Dd}	9.82±1.00^{Aa}	58.22±2.19^{ABab}	31.23±1.35^{Aa}	4.85±0.60
Milk stage	97.31±2.26	25.39±3.99^{Cc}	9.17±0.93^{ABab}	56.83±2.15^{ABab}	29.22±2.27^{ABab}	4.37±0.36
Dough stage	97.23±2.29	30.37±2.13^{Bb}	9.01±0.21^{ABab}	54.63±2.98^{Bb}	26.21±1.31^{Bb}	4.38±0.38
Ripe stage	97.31±2.29	39.74±2.87^{Aa}	8.65±0.31^{Bb}	61.68±2.86^{Aa}	30.27±2.42^{ABab}	4.64±0.19

Note: Different capital letters in the same column means most significant difference at 0.01 level (P<0.01), lower case letters means significant difference at 0.05 level (P<0.05).

Effect of five varieties on nutritional quality of summer planting corn for silage in China

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Keywords corn silage, summer planting, nutritional quality

Introduction Ensiled whole-plant corn is one of the most important feed sources for ruminants in many parts of the world, constituting 50-75% of the dry matter intake for dairy cows (Dunière et al., 2013). The objective of this study was to determine the effects of genotype on the nutrient quality of whole-plant corn for silage.

Materials and methods This study was performed with Jin ping guo 608, Hua yu 14, Qiang sheng 30, Jun shi 9 and Zhong liao xiang tian 23 varieties corn. Five varieties were planted in experimental plots within a cornfield. The planting density was 63,000 plants per hectare. The planting date was June 13, 2013. At dough stage (about 30% DM), the fresh material of corn were sampled and ensiled. After 90 days of storage, the fresh material and silage were analyzed. The concentrations of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), water-soluble carbohydrate (WSC), ash, lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA) were measured by wet chemistry.

Results and discussion Among the five corn varieties planted in China summer, fresh and dry weight of Zhong liao xiang tian 23 was significantly (P <0.01) lower than other four varieties (Table 1). The AA content of silage Jin ping guo 608 was significantly (P <0.01) higher than that of the Jun shi 9 and Zhong liao xiang tian 23 (Table 2). The DM content of silage Zhong liao xiang tian 23 was significantly (P <0.01) lower than that of the other four varieties (Table 3). Five varieties corn also showed differences in dry weight, fresh weight, DM and NDF. However, there were some differences in the ADF content (Ruhua Yu et al., 2011). This may be due to the different periods of planting and different places caused.

Conclusion The Zhong liao xiang tian 23 variety was inferior than the other four varieties.

Acknowledgements The authors wish to thank all the staves of the Institute of Grassland Science, China Agricultural University, China, for their help. This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (Contract no. 201303061) and the National Key Technologies R&D Program (Contract no. 2011BAD17B02-02).

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Table 1 The fresh material from five corn varieties planting in China summer

Varieties	Fresh weight, t/ha	Dry weight, t/ha	DM,%	CP,%	NDF,%	ADF,%	Ash,%	Starch,%	WSC,%
Jin ping guo 608	57.21± 5.64[a]	19.28± 1.90[a]	33.71± 5.58[a]	8.92± 0.44	57.62± 4.02[a]	31.27± 4.06	4.37± 0.17	28.38± 8.98	7.83± 1.81
Hua yu 14	70.86± 7.55[a]	22.18± 2.36[a]	31.30± 6.10[a]	8.43± 0.22	52.88± 4.02[a]	29.36± 4.11	4.38± 0.90	27.62± 6.01	9.30± 1.85
Qiang sheng 30	57.06± 9.08[a]	18.32± 2.91[a]	32.09± 4.16[a]	8.10± 0.45	51.51± 5.77[a]	30.65± 1.38	3.63± 0.58	26.06± 7.01	9.47± 1.36
Jun shi 9	60.84± 8.57[a]	18.56± 2.61[a]	30.49± 8.25[a]	7.42± 1.15	53.07± 3.38[b]	31.40± 1.94	3.87± 0.84	25.60± 7.96	8.38± 1.90
Zhong liao xiang tian 23	18.84± 4.24[b]	5.37± 1.21[b]	28.51± 4.09[b]	9.68± 1.81	55.29± 2.48[b]	29.68± 2.61	4.35± 0.36	27.83± 3.32	10.04± 1.52

Different lowercase letters means significant difference at 0.05 level (P<0.05). The same symbol is used for other tables.

Table 2 The fermentation quality of silage from five corn varieties planting in China summer

Varieties	pH	AN,%	LA,%	AA,%	PA,%	BA,%
Jin ping guo 608	3.70±0.06	3.51±0.37	4.24±0.64	0.33±0.04[a]	0.29±0.13	n.d.
Hua yu 14	3.54±0.06	2.64±0.34	4.46±0.61	0.28±0.00[a]	0.25±0.16	n.d.
Qiang sheng 30	3.65±0.03	3.03±0.34	4.55±0.21	0.28±0.09[a]	0.26±0.02	n.d.
Jun shi 9	3.51±0.04	3.60±0.27	4.80±0.74	0.15±0.02[b]	0.23±0.13	n.d.
Zhong liao xiang tian 23	3.61±0.04	3.14±0.26	5.17±0.43	0.17±0.04[b]	0.25±0.10	n.d.

n.d.: not detected.

Table 3 Dry matter recovery and nutrient composition of silage from five corn varieties planting in China summer

Varieties	DM recovery,%	DM,%	CP,%	NDF,%	ADF,%	Ash,%
Jin ping guo 608	97.31±2.25	31.34±2.22[a]	9.08±0.83	55.23±1.61	30.38±2.26	4.49±0.26
Hua yu 14	97.31±1.23	32.89±2.65[a]	8.67±0.86	53.12±3.53	28.23±2.73	4.37±0.02
Qiang sheng 30	98.24±2.23	30.28±4.69[a]	8.98±1.05	53.24±3.60	30.86±3.77	4.38±0.11
Jun shi 9	97.28±1.31	31.83±3.95[a]	8.12±0.95	54.23±4.61	30.19±2.78	4.64±0.14
Zhong liao xiang tian 23	97.27±2.35	27.00±5.50[b]	10.25±0.74	53.19±3.17	30.09±4.38	4.33±0.28

Effects of different spring maize varieties on the quality of fresh forage and silage

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Keywords maize variety, spring planting, silage quality

Introduction Maize (*Zea mays* L.) as cereal crop plays an important role not only in agriculture, but also in animal husbandry. According to official statistics, there are more than 70 percent of the maize used for feed production in China. At the end of 2007, China had 106 million of cattle and 286 million of sheep heads. In order to improve the efficiency and quality of grazing animal production, we must supply high-quality corn silage. Then, the objective of this study was to compare maize varieties for silage production during the spring season in China.

Materials and methods Five maize varieties Jindan 42, Qiangsheng 30, Jindan 56, Shengyu 366 and Jinnuo 8 widely planting in Shanxi Province were selected as the test varieties and tested with the randomized block arrangement method. Each treatment was replicate three times. At the dough stage, three plants from each test plot were chopped and packed into plastic bag, then stored for 90 days in a shading place after vacuum sealing using a vacuum heat-sealer. Dry matter (DM) was determined by oven drying. Crude protein was determined by Kjeldahl method. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by Van Soest method. The content of water-soluble carbohydrates (WSC) was determined by anthrone-sulfuric acid colorimetry. Ash was determined by burning. Starch determined by polarimetry. In silages, the pH was determined with a pH meter, NH_3-N determined by phenol-sodium hypochlorite colorimetric method and organic acids determined by liquid chromatography.

Results and discussion Both fresh weight and DM weight of Jinnuo 8 was significant (P<0.01) lower than other four varieties (Table1). Dry matter content of Jinnuo 8 was markedly (P<0.01) lower than the other four varieties. The content of NDF was lower in Shengyu 366 and Jinnuo 8 than in the other three varieties (P<0.05). The pH, N-NH_3, lactic acid (LA), acetic acid (AA), and propionic acid (PA) of the five spring maize varieties were no significantly different (Table2). Considering the yield of both fresh and dry matter among the five maize silage varieties, Jinnuo 8 was significantly lower than the rest of the four varieties.

Conclusion The Jinnuo 8 variety was inferior than the other four varieties.

Acknowledgements The authors thank all the staves of the Institute of Grassland Science, China Agricultural University, China, for their helps. This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (Contract no. 201303061) and the National Key Technologies R&D Program (Contract no. 2011BAD17B02-02).

Table 1 Fresh forage yield and composition of five spring maize varieties in China

Varieties	FMY (t/ha)	DMY (t/ha)	DM (%)	CP (%)	NDF (%)	ADF (%)	Ash (%)	Starch (%)	WSC (%)
Jindan 42	68.34±11.79^{a}	21.26±3.12^{a}	32.31±5.90^{a}	8.46±0.61	54.31±7.62^{a}	25.52±7.58	4.48±0.83	31.15±6.81	13.77±1.06
Qiangsheng 30	65.77±7.48^{a}	21.20±2.37^{a}	31.79±6.68^{a}	7.99±1.18	52.14±5.87^{a}	28.70±8.52	4.26±0.56	31.20±7.37	13.56±0.90
Jindan 56	63.57±9.13^{a}	20.53±3.14^{a}	32.21±6.56^{a}	8.65±0.64	51.27±6.61^{a}	26.74±8.53	5.06±0.74	30.79±7.07	11.84±0.65
Shengyu 366	60.90±8.62^{a}	20.73±3.14^{a}	34.09±5.93^{a}	7.92±0.33	46.10±8.77^{b}	27.20±5.39	3.76±0.27	30.96±5.32	13.14±0.73
Jinnuo 8	49.39±7.42^{b}	12.84±2.05^{b}	26.19±7.94^{b}	9.51±0.61	44.50±7.53^{b}	25.18±4.52	4.51±1.00	33.73±5.32	12.53±1.14

FMY: fresh weight yield, DMY: dry matter yield, DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, WSC: water soluble carbohydrates. Means within a column with different superscripts differ (P<0.05).

Table 2 Fermentation profile of maize silage from five spring varieties in China

Varieties	pH	N-NH_3 (%)	LA (%)	AA (%)	PA (%)	BA (%)
Jindan 42	3.65±0.17	2.16±0.29	5.09±0.86	0.20±0.01	0.35±0.23	n.d.
Qiang sheng 30	3.65±0.08	3.07±0.41	4.38±0.45	0.18±0.11	0.26±0.17	n.d.
Jin dan 56	3.58±0.08	2.21±0.5	4.48±0.13	0.33±0.12	0.27±0.17	n.d.
Sheng yu 366	3.63±0.04	2.16±0.25	4.23±0.41	0.16±0.04	0.25±0.14	n.d.
Jin nuo 8	3.67±0.09	3.67±0.10	5.04±0.14	0.22±0.08	0.22±0.14	n.d.

LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid, n.d.: not detected.

Table 3 Chemical composition of maize silage from five spring varieties in China

Varieties	DM recovery(%)	DM (%)	CP (%)	NDF (%)	ADF (%)	Ash (%)
Jindan 42	98.25±2.41	31.29±2.97	8.62±0.02	53.78±3.77	26.23±4.04	4.68±0.25
Qiang sheng 30	97.32±2.95	30.91±5.12	8.31±0.34	54.28±4.20	29.21±1.89	4.45±0.95
Jin dan 56	97.15±5.06	33.48±2.32	8.74±0.34	53.96±4.35	27.23±2.16	4.87±0.93
Sheng yu 366	97.22±5.08	32.87±4.87	8.87±0.23	48.25±4.08	28.61±2.22	4.17±0.68
Jin nuo 8	96.49±4.95	28.31±3.65	9.84±0.3	46.29±3.54	27.72±6.65	4.84±0.41

Effect of plant population on the morphology and yield of corn plants and the chemical composition of corn silage

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Keywords corn silage, plant density, corn morphology

Introduction Planting density can have a direct effect on plant yield and morphology, which interferes with the quality of the fresh material used to make silage. The central region of Argentina, a traditional dairy area, is highly dependent on corn silage to feed cows (Ferreira et al., 2014). Few studies have been done to define the optimal corn plating density on this area. This project aimed to determine the effect of planting density on the morphology and yield of corn plants and the chemical composition of corn silage.

Materials and methods Corn hybrid Decalb 7210 was sown on five densities: 56,000 (T1), 57,000 (T2), 63,500 (T3), 73,000 (T4) and 74,000 (T5) plants/ha on December 15th and harvested on April 24th at San Basilio, Argentina. Thirty plants per treatment were collected to evaluate plant weight and height, number of nodes, DM yield and relation ear/plant weight. Corn forage was harvested at approximately 34% DM (R5 growth stage), and chopped at 15 mm theoretical particle size. Fresh material was packed into each of three laboratory replicated silos at density of 225 kg/DM. Silos were opened after 60 days of fermentation and samples were collected to evaluate chemical composition and fermentation profile. Silage extract was prepared by mixing 25 g of corn silage with 225 mL of water in a stomacher for 3 min and an electrode was used to measure the pH. The concentration of volatile fatty acids was evaluated by the use of high performance liquid chromatography. Corn silage samples (300g) were used to determine the concentrations of dry matter, crude protein (N*6.25), ash, fat, NDF, ADF and starch. The experiment had a completely randomized design with five treatments with four replicates. Data were analyzed using the MIXED procedure of SAS (version 9.2). Differences between means were determined using the DIFF procedure. Significant differences were declared at $P < 0.05$.

Results and discussion Chemical concentration of the components were unaffected by treatment except that T2 had the lowest DM (34.26%, $P = 0.018$) and T1 the highest pH value (3.84, $P = 0.007$) (Table 1). Despite of differences on pH values, all silages had pH bellow 4.0. Size of plants and number nodes were similar between treatments. The lowest plant weight was observed for the T2 treatment (0.64 kg, $P < 0.001$) and T4 had the highest DM yield (19.33ton/ha, $P = 0.01$). Despite of the different values observed for the ratio of ear to plant weight that was not enough to promote higher starch levels for the treatment T3, T4 and T5. Probably the participation of grain and cob within the corn ear are not the same between treatments. Further data will be evaluated to confirm that hypothesis.

Conclusion The second highest plant population was the most promising treatment for corn silage production on the central region of Argentina. Corn sown at 73,000 plants/ha has the highest yield of DM with high contribution of the ear in relation to the total plant weight.

Table 1 Effect of population density on the chemical composition and fermentation profile of corn silage

	T1[1]	T2[2]	T3[3]	T4[4]	T5[5]	SEM[6]	P-value
DM, %	36.53[ab]	34.26[b]	37.00[a]	36.00[a]	35.36[ab]	0.49	0.018
CP, % DM	7.56	6.87	7.20	6.97	7.23	0.17	0.128
NDF,% DM	33.60	37.26	35.90	35.96	35.32	1.21	0.37
ADF,% DM	21.30	23.26	21.97	22.16	21.71	0.74	0.46
Ash,% DM	3.65	3.41	3.19	3.20	3.40	0.11	0.10
Ether extract,% DM	3.80	3.36	3.69	3.72	3.82	0.06	0.29
Starch, % DM	40.93	37.73	39.43	39.37	39.66	1.29	0.57
pH	3.84	3.79	3.78	3.80	3.82	0.01	0.007
Lactate, % DM	5.06	5.07	5.60	5.03	4.83	0.32	0.55
Acetate, % DM	1.58	1.79	2.90	1.66	1.69	0.46	0.31
Propionate,% DM	0.25	0.18	0.74	0.16	0.24	0.36	0.51

1)T1 =56,000 plants/ha; 2) T2 =57,000 plants/ha; 3)T3 =63,500 plants/ha; 4) T4 =73,000 plants/ha; 5) T5 =74,000 plants/ha; 6) SEM =standard error of mean. Means within rows with unlike superscripts ($P < 0.05$).

Table 2 Effect of population density on the chemical composition and fermentation profile of corn silage

	T1[1]	T2[2]	T3[3]	T4[4]	T5[5]	SEM[6]	P-value
Nodes[7]	8.5	8.5	7.5	7.5	8	0.44	0.41
Height, m	2.18	2.16	2.14	2.11	2.05	0.08	0.79
Weight, kg	0.69[b]	0.64[c]	0.72[ab]	0.73[ab]	0.74[a]	0.005	<0.001
DM yield, ton/ha	14.51[c]	16.57[b]	14.18[c]	19.33[a]	13.17[c]	0.24	0.01
Ear/Plant[8]	48.74[ab]	47.47[b]	51.03[a]	50.51[a]	49.85[a]	0.36	0.01

1)T1 =56,000 plants/ha; 2) T2 =57,000 plants/ha; 3)T3 =63,500 plants/ha; 4) T4 =73,000 plants/ha; 5) T5 =74,000 plants/ha; 6) SEM =standard error of mean; 7) Nodes = number of nodes/plant; 8) ratio Ear/ plant weight. Means within rows with unlike superscripts ($P < 0.05$).

Reference

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Morphological composition of three hybrids of corn harvested at different maturity stages

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Keywords agronomic composition, preserved feed, ruminant nutrition

Introduction Obtaining high quality silage goes through several processes, where the choice of a suitable and adapted hybrid to the region, the planting season and the crops are keys to the final quality of the product. Many corn hybrids are presented annually by companies to upgrade feeding to high production ruminants. Such indications are based normally on the basis of the structural characteristics of the plant, the productive potential of dry mass per area and chemical characteristics. In this context, the aim of this study was to evaluate different corn hybrids (*Zea mays L.*) in different maturity stages.

Materials and methods The experiment was conducted in the Núcleo de Produção Animal (NUPRAN) of the Center for Agricultural and Environmental Sciences, University of the Midwest (UNICENTRO) in Guarapuava - PR. The experimental design was in blocks with factorial arrangement 3×4, comprised of twelve treatments with eight replications, being three hybrids (P2530, P30B39H, P30R50H) and four maturity stages (milk grain stage, R2; pasty grain, R3; dough grain, R4; and hard grain, R5). The proportion of structural components was evaluated for each treatment combination, considering: stalk, leaves, husk plus cob and grains. Data were compared by statistical analysis using ANOVA procedure and the means were compared by Tukey test at 5% significance level. Data were also submitted to polynomial regression analysis, considering the variable evaluation period (days after emergence), through the PROC REG of SAS.

Results and discussion With the advancement of physiological maturity of corn plants, the percentages of stalk, leaves and husk plus cob reduced numerically, while the participation of grains increased in the plant structure. According to the literature, to evaluate the quality of a corn hybrid destined for the production of silage, one must observing these anatomical components because they have influence on plant digestibility by ruminants. One important aspect refers that a good corn hybrid should contain high percentage of grains in its dry basis providing nutrients for a good fermentation in the silo and so, keeping the nutritional quality of silage as close to the harvest time. In counterpoint, the higher the proportion of straw and plant cob, there is the tendency to increase the fibrous fraction and so decrease the final quality of silage. However, some reports demonstrate that not always the largest proportion of grains in forage confer quality to the silage, indicating that the nutritional value of silage is also associated with the quality of the vegetative fraction (stalk and leaves).

Table 1 Percentage of stalk, leaves, husk plus cob and grains (DM basis) of corn hybrids for silage at different maturity stages

Hybrid	Stages (days after emergence)				Regression[1]
	R2 (93 d)	R3 (100 d)	R4 (107 d)	R5 (114 d)	
			Percentage of stalk		
P 2530	25.5	21.3	20.5	17.0	Y = 60.2162 – 0.3781D; CV: 8.9; R²: 0.7432; P=0.0003
P 30B39 H	25.0	21.1	23.7	21.6	Y = 34.3995 – 0.1114D CV: 11.4; R²: 0.1180; P=0.2743
P 30R50 H	24.5	23.0	21.7	20.7	Y = 41.3681 – 0.1824D CV: 11.9; R²: 0.2517; P=0.0965
Average	25.0	21.8	22.0	19.8	Y = 45.3279 – 0.2240D CV: 11.6; R²: 0.3287; P=0.0003
			Percentage of leaves		
P 2530	23.4	19.8	19.1	16.8	Y = 49.7490 – 0.2895D CV: 6.7; R²: 0.7756; P=0.0002
P 30B39 H	26.0	21.1	20.8	19.6	Y = 50.9452 – 0.2809D CV: 10.8; R²: 0.5083; P=0.0093
P 30R50 H	26.7	21.4	20.6	19.1	Y = 56.7047- 0.3357D CV: 11.6; R²: 0.5595; P=0.0052
Average	25.4	20.8	20.2	18.5	Y = 52.4663 – 0.3021D CV: 10.7; R²: 0.5323; P=0.0001
			Percentage of husk and Cob		
P 2530	22.7	16.8	16.4	14.8	Y = 53.1114 – 0.3424D CV: 9.9; R²: 0.7384; P=0.0003
P 30B39 H	22.8	19.4	14.8	15.2	Y = 58.8752 – 0.3943D CV: 9.7; R²: 0.7885; P=0.0001
P 30R50 H	22.9	19.3	16.9	17.0	Y = 48.7276 – 0.2871D CV: 8.7; R²: 0.6902; P=0.0008
Average	22.8	18.5	16.0	15.7	Y = 53.5714 – 0.3413D CV: 9.6; R²: 0.7120; P=0.0001
			Percentage of grains		
P 2530	28.4	42.1	43.9	51.4	Y = -62.9371 + 1.0086D CV: 10.5; R²: 0.7972; P=0.0001
P 30B39 H	26.1	38.4	40.6	43.7	Y = -44.0471 + 0.7852D CV: 14.1; R²: 0.6213; P=0.0023
P 30R50 H	25.9	36.2	40.9	43.1	Y = -46.9567 + 0.8067D CV: 10.6; R²: 0.7594; P=0.0002
Average	26.8	38.9	41.8	46.1	Y = -51.3136 + 0.8668D CV: 12.7; R²: 0.6716; P=0.0001

[1]D: days after plant emergence, ranging from 93 to 114.

Conclusion The hybrid P2530 was superior to other hybrids tested by presenting a balance between their vegetative fractions and superiority in grain production.

Comparison of maize silage from varieties cultivated in northern regions

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Keywords maize silage, chemical composition, fermentation quality

Introduction Maize (*Zea mays* L.) is a widely cultivated crop. Possibilities for a warming climate and recent experience of warmer weather have increased interest in maize in cooler climatic regions. Recently, cultivation of maize for silage production has increased in more northern latitudes. Improvements in agronomic practices and effective plant breeding have made this possible, having provided relatively cold temperature tolerant and earlier maturing varieties. The aim of this study was to investigate the chemical composition and fermentation parameters of maize silage made from different varieties.

Material and methods Seven maize varieties: 'DKC3014' (FAO 220), 'ES Bodyguard' (FAO 200), 'ES Ardent' (FAO 200), 'ES Regain' (FAO 200), 'Crescendo' (FAO 180), 'Drim' (FAO 200), 'Avenir' (FAO 200) were used. Maize seeds were sown at the rate of nine germinated seeds per $1m^2$ on the same field, in sample plots of varieties, on 23.05.2013. Fertilizing was identical and the sum of effective temperatures (≥5 °C) in the growing period was 1,344 °C. Whole-crop maize were harvested and silages were made on 16.09.13. Silages were packed into double vacuum plastic bags, in triplicates for each variety, and stored for 90 days in a dark room under constant temperature conditions (20 °C) until opening. Silages were analysed for chemical composition and fermentation parameters and statistical differences between varieties were analysed with the t-test. Results were corrected for multiple testing with the Bonferroni method.

Results and discussion The most important criterion when choosing maize varieties is early harvest. In the context of northern latitudes, varieties should reach a silage DM content of 310-330 g/kg before mid-October or before the first frost (Mikkelsen and Halling, 2014). In this experiment three varieties achieved that DM level (table 1) by mid-September, the time of harvest. Varieties were seeded at the same time on the same field, thus neither the soil nor growing period could have affected the maize chemical composition. However, silage made from 'ES Ardent' had higher crude fat content and lower crude protein content compared with the other varieties. For fermentation quality, all silages had low pH levels and ammonia contents. Silage from the variety 'Crescendo' contained significantly more ethanol. The butyric acid content was statistically higher in the 'DKC3014' silage, and absent in the silage 'Avenir'. Contents of 1.2-propanediol and 2.3-butanediol were also higher in the 'DKC3014' silage. Lactic acid contents did not significantly differ between the varieties.

Conclusions Varieties achieving a dry matter content of 300 g/kg for harvested whole-crop maize were suitable for silage production in northern latitudes.

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Table 1 Mean values (SD in parentheses) of chemical composition of maize varieties

Item	DKC3014	ES Bodyguard	ES Ardent	ES Regain	Crescendo	Drim	Avenir
g/kg							
Dry matter	287.5 (9.3)	295.1 (14.1)	313.3 (10.1)	305.9 (11.5)	327.7 (6.9)	279.5 (17.9)	332.1 (2.5)
g/kg in DM							
Crude protein	78.0 (1.6)	79.5 (2.7)	70.5^{a} (0.6)	76.8 (2.8)	78.0^{a} (0.8)	72.7 (5.2)	76.6 (2.4)
Crude ash	34.5 (1.1)	33.8 (1.4)	34.6 (2.1)	33.1 (1.1)	34.1 (1.7)	34.6 (2.0)	35.8 (1.8)
Crude fat	31.8^{a} (1.5)	34.9^{b} (1.4)	43.0^{abcd} (1.2)	34.1^{c} (1.5)	38.2^{f} (1.5)	28.6^{df} (1.6)	34.9 (2.0)
NDF	403.2 (35.5)	463.4 (23.0)	443.2 (80.5)	425.0 (28.8)	411.5 (22.9)	421.0 (28.2)	397.3 (36.2)
ADF	224.5 (14.9)	230.0 (17.4)	209.4 (7.9)	206.8 (19.1)	231.0 (32.7)	232.5 (24.7)	197.7 (15.7)
Metabolizable energy, MJ/kg	10.4 (0.1)	10.4 (0.0)	10.4 (0.0)	10.4 (0.0)	10.5 (0.1)	10.3 (0.0)	10.3 (0.1)
Metabolizable protein	77 (0.0)	77 (1.0)	75 (1.0)	77 (1.0)	77 (1.0)	76 (1.0)	77 (0.0)
Ethanol	4.5^{a} (0.9)	8.0^{b} (0.8)	6.7^{c} (0.5)	14.1 (4.3)	15.6^{abcde} (0.3)	6.4^{d} (0.2)	6.9^{e} (0.9)
Acetic acid	12.4 (1.3)	13.0 (1.7)	10.4 (1.3)	14.1 (2.3)	11.9 (1.8)	15.8 (2.1)	10.3 (1.3)
Propionic acid	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.1)	0.1 (0.1)	0.1 (0.0)	0.1 (0.1)
Butyric acid	0.2^{abcd} (0.0)	0.2 (0.1)	0.1^{ae} (0.0)	0.1 (0.1)	0.1^{bf} (0.0)	0.1^{cg} (0.0)	0.0^{defg} (0.0)
Lactic acid	54.7 (7.6)	40.4 (5.2)	38.7 (2.8)	42.5 (3.5)	40.4 (1.5)	45.3 (3.2)	35.2 (3.3)
1.2-propanediol	0.7^{ab} (0.1)	0.2^{a} (0.1)	0.2 (0.2)	0.1 (0.0)	0.2 (0.2)	0.1^{b} (0.1)	0.2 (0.2)
2.3-butanediol	3.8^{abc} (0.3)	2.0^{a} (0.2)	0.8^{b} (0.1)	2.1 (0.6)	2.7 (0.3)	2.7 (0.3)	0.5^{c} (0.0)
pH	4.0 (0.1)	4.1 (0.0)	4.1 (0.0)	4.1 (0.0)	4.2 (0.0)	4.0 (0.0)	4.2 (0.0)
NH_3-N/total N	27.0 (1.7)	22.7 (2.3)	22.7 (1.5)	20.3 (1.5)	24.3 (0.6)	21.3 (0.6)	19.7 (1.2)

$^{a, b, c, d, e, f, g}$ - Least square means within a row with same superscript letters differ statistically significantly after Bonferroni correction

Fermentative profile and losses in *Sorghum bicolor* silages from different cultivars

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Keywords dry matter, ensilage, forage conservation, pH

Introduction *Sorghum bicolor* is a type of tall sorghum, higher than two meters, mainly characterized by having sweet and juicy stalk. For silage production, it has an easy cultivation, high yield, lower moisture demand and particularly good quality fermentation of the produced silage, achieved through adequate concentration of soluble carbohydrates, which are essential for lactic fermentation, without additives to stimulate fermentation, and nutritional value similar to corn silage (Gonçalves and Borges, 1997). Inside this context, we aimed to evaluate the fermentative profile and losses in *Sorghum bicolor* silages from different cultivars and two different periods of cultivation.

Materials and methods The experiment was conducted in University Federal of Mato Grosso in partnership with Embrapa Agrosylvipastoral. Sorghum was grown on a useful area of 600m², with due soil preparation and fertilization. The plots had 0.75 m spacing between planting rows with10 m length, resulting in 120.000 plants per hectare populations, with 10 rows per plot and a population of 9 plants per meter. The sorghum crop cycle lasted 90 days for the cultivars seeded on 28/02/2012 and 100 days for the cultivars seededon18/02/2012. The silages were prepared in 20 PVC mini-silos, with a volume of 2.75 liters, provided with Bunsen valves. Two sorghum cultivars of Embrapa, varieties CMSX 647 and BRS 506 early and late, were evaluated with two periods of cultivation (90 and 100 days), resulting in four treatments and five replicates per treatment: T1 – Early variety (90) CMSX 647, T2 – Late variety (100) CMSX 647, T3 - Early variety (90) BRS 506 and T4 - Late variety (100) BRS 506. After opening the silos, we evaluated titratable acidity and pH, according to methodologies from Silva and Queiroz (2002). Evaluations of the produced effluent were quantified as the difference in the weight of the silo and sandbag set before and after ensiling, compared with the fresh mass of the ensiled sample. The loss in dry matter (DM), which results from gas production, was determined by the difference of gross weight of DM at the ensiling and at the opening, relating with the ensiled DM, discounting the total weight of the set at the ensiling and opening. Total dry matter loss (TDM_L) was determined by the difference between the gross weight of DM at the ensiling and at the opening, compared with the ensiled DM. The experiment followed a completely randomized design, with five replicates per treatment. The analyzed characteristics were compared by partition of square sum of treatment in orthogonal contrasts, assessing: Contrast1=CMSXvsBRS; Contrast2 =CMSX 90vs CMSX100 and Contrast3 =BRS90vsBRS100, at 5% probability for type I error.

Results and discussion Values of dry matter (DM), pH, titratable acidity (TAC), effluent losses (EFF_L), gas losses (GAS_L) and total dry matter losses (TDM_L) are presented in

Table 1. Dry matter (DM) contents were significant (P<0.05) when CMSX 90 and CMSX 100were compared, as well as BRS 90 vs BRS 100.The DM content varies with the age of cut, type of stem and the percentage of grains. The pH range is in the optimal pattern of an ideal silage, between 3.8 and 4.2 (McDonald et al., 1991), ranging from 3.62 (CMSX 90 andBRS100) to 3.72 (BRS 90), with significant difference (P<0.05) only between BRS 90 and BRS100). Values of TAC ranged from 20.70 in BRS 90 to 25.01 in CMSX 90. Significance was found (P<0.05) in comparison with CMSX and BRS and comparing CMSX 90 and BRS100.

Table 1 Dry matter, fermentation profile, effluent losses, gas losses and dry matter loss of *Sorghum bicolor* silages

	Treatments				SEM[3]	Contrast *P*-value*		
	CMSX90	CMSX100	BRS90	BRS100		1	2	3
DM[1]	24.51	23.83	22.50	26.17	0.35	0.2239	0.0014	<0.0001
pH	3.62	3.63	3.72	3.62	0.16	0.1055	0.7460	0.0456
TAC[2]	25.01	23.51	20.70	21.97	0.64	<0.0001	0.0212	0.0779
EFF_L[3]	4.64	5.67	4.67	3.14	0.28	<0.0001	0.0021	<0.0001
GAS_L[3]	16.90	18.29	13.47	18.30	1.08	0.3540	0.7203	0.1174
TDM_L[3]	20.88	23.10	15.80	20.96	1.04	0.0973	0.5528	0.0849

[1] %; [2]Expressed in mL of 0.1N NaOH until pH reached 7.0; [3]% DM; [4]Error of the means; *Contrast 1 = CMSX vs BRS; Contrast 2 = CMSX 90 vs CMSX 100 and Contrast 3 = BRS 90 vs BRS 100

Effluent losses were higher for CMSX100 and lower for BRS100, being significant (P<0.05) for all contrasts. According to McDonald et al. (1991), these values are within the suitable considered limit for silage, ranging around 5-7% of total energy losses in the process, though not desirable during ensiling. Gas losses did not differ (P<0.05) in the evaluated contrasts, but were relatively high. Total DM losses were considered high, even if they have not been significant between treatments, with an average of 20.19%. According to Gourley and Lusk (1977), low DM losses are expected in silages with high content of soluble carbohydrates and DM greater than 20%. These losses are mainly from the formation of effluent, since high moisture content at the time of ensiling leads to greater losses of DM.

Conclusion The evaluated cultivars of *Sorghum bicolor* showed potential of ensilage, with adequate fermentative profile and low losses, with potential use in animal feed.

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Fermentation characteristics of different sorghum cultivars for ensiling at second crop

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Keywords dry matter, fermentability coefficient, silage, water soluble carbohydrates

Introduction Corn is the standard culture for silage since it presents ideal characteristics for ensiling and high nutritional value. However, in Midwest region of Brazil, corn use is more common as second crop, after summer cultivation, with production losses in case of late seeding. Sorghum crop is an interesting alternative for silage production as it presents similar nutritional value to corn and also ideal characteristics for ensiling, but additionally it has higher tolerance to drought and greater seeding range (Machado et al., 2012). Among sorghum types there are: grain; forage and sweet sorghum, which differ in plant height and nutritional value. There are new cultivars of grain and sweet sorghum that can be used for silage production, but there is little information about their characteristics for ensiling. Thus, this work aimed to evaluate the fermentation characteristics of different purpose sorghum for silage production at second crop.

Materials and methods Trial was conducted at the Plant Production Department of the Federal Institute of Education, Science and Technology of Rondônia, Colorado do Oeste Campus, and chemical analyses were performed at the Laboratory of Animal Nutrition, at Federal University of Mato Grosso, Cuiabá Campus. Experimental design was a randomized block with six replications. Treatments consisted of six sorghum cultivars of different purpose (BRS 308 and BRS 310, grain sorghum; BR 655 and BRS 610, silage sorghum; BRS 506 and CMSXS 647, sweet sorghum). Seeding was done on March 22, 2012, while crop was harvested when plants presented grains at hard dough stage. Ten plants per plot were collected and chopped. The mass was homogenized and a sample was freeze-dried at -70° C until it reached constant weight. Samples were ground to pass a 1 mm screen with a Wiley mill. With pre-dried samples, it was performed the water soluble carbohydrate content analysis (WSC) according to Deriaz (1961), and dry matter content (DMc) analysis, in a 105°C oven. The fermentability coefficient was determined according to Weissbach (1996). After harvesting, forage was chopped into approximately 1 to 2 cm lengths immediately before ensiling. Experimental units consisted of experimental silos (glass jars), with 2.5 L volume. Silos remained closed for 30 days. At silos opening, samples were collected at the geometric centre. Subsequently, samples were freeze-dried, at -70º C, ground to pass a 1 mm screen with a Wiley mill, and analysed for WSC determination. Difference between the WSC content of the forage and silage was obtained by subtracting the content before and after ensiling. Data were subjected to analysis of variance (PROC GLM of SAS) and means compared by LSD test, adopting the probability level of 5%.

Results and discussion Effect of cultivars was observed on all evaluated variables ($P<0.01$). Grain sorghum cultivars presented higher dry matter content (DMc), while sweet sorghum cultivar CMSXS 647 had the lowest DMc (Table 1). According to McDonald et al. (1991), the ideal forage DMc for ensiling is 30-35%, which was only observed in grain sorghum cultivars. Higher forage water soluble carbohydrates (WSCf) was found in sweet

sorghum cultivars, while the lowest content was observed in grain sorghum cultivars. Similarly, silage of sweet sorghum cultivars presented higher water soluble carbohydrates content (WSCs), while grain sorghum cultivars and forage sorghum cultivar BRS 610 presented the lowest values (Table 1). According to Johnson et al. (1971), the minimum WSCf content required for good fermentation is 15%, which was not obtained by grain sorghum cultivar BRS 310. The difference between forage and silage WSC content (dWSC) was higher on sweet sorghum cultivars, while grain sorghum cultivars presented lower differences. This is probably due to the higher DMc in grain sorghum cultivars, which may minimize effluent losses inside the silo, with lower WSCf losses. Another possible explanation could be the ethanol production at sweet sorghum cultivars, due to its high WSCf content, which promotes rapid proliferation of yeast and ethanol production, with soluble nutrients loss. Although some cultivars did not present ideal minimum levels of DMc and WSCf, all treatments presented pH lower than 4.2, which indicates good fermentation. According to Weissbach et al. (1996), forage with fermentability coefficient (FC) greater than 45, as observed in all treatments, probably had adequate fermentation.

Table 1 Fermentation characteristics of different purpose sorghum forage and silage, in 2012

Cultivar	BRS 308	BRS 310	BRS 605	BRS 610	BRS 506	CMSXS 647	SEM
DMc	302.0^{a}	314.0^{a}	282.1^{b}	256.1^{c}	254.1^{c}	219.9^{d}	7.10
WSCf	152.2^{c}	127.5^{c}	211.6^{b}	199.5^{b}	345.4^{a}	313.6^{a}	17.07
WSCs	45.4^{c}	52.4^{c}	80.7^{b}	45.5^{c}	128.1^{a}	110.1^{a}	7.21
dWSC	106.8^{cd}	75.1^{d}	130.9^{bc}	154.0^{b}	217.3^{a}	203.5^{a}	11.20
FC	49.5^{de}	46.5^{e}	61.4^{bc}	55.5^{cd}	72.5^{a}	64.0^{b}	20.78

SEM = Standard error of mean. Means followed by the same letter in the line do not differ by LSD-Test ($P<0.05$). DMc: dry matter content (g kg^{-1}), WSCf: forage water soluble carbohydrate content (g kg^{-1}); WSCs: silage water soluble carbohydrate content (g kg^{-1}); dWSC: difference between WSCf and WSCs (g kg^{-1}); FC: fermentability coefficient = DMc + (8 × WSCf / buffering capacity).

Conclusion Based on the fermentation characteristics, all evaluated sorghum cultivars can be recommended for ensiling at second crop.

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Field yield and fermentative losses of ryegrass silages according to seeding rate and method for increasing dry matter content

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Keywords absorbent, Barjumbo, soybean hulls, wilting

Introduction The annual ryegrass (*Lolium Multiflorum Lam)* is largely used in grazing based systems. Ryegrass can also produce high quality conserved feed for ruminants. In establishment of annual ryegrass fields, seeding rate is an important aspect and can lead to differences in morphology of the plant, affecting DM yield, chemical composition and wilting time for ensiling. In southern Brazil ryegrass has been conserved only as wilted silage. An alternative to conserve this grass with lower field losses is the use of water absorbents as silage additive. Soybean hulls is an easily found by-product that have great capacity to absorb water. It is commonly used by farmers for dairy cattle nutrition. This trial aimed to evaluate the DM yield of a tetraploid ryegrass at different seeding rates and two ensiling methods.

Materials and methods The trial was performed in the Centro de Pesquisas em Forragicultura (CPFOR). The annual ryegrass (cv. Barjumbo) was seeded in May 31, 2014, in 16 plots (5×5 m), allocated in four blocks. The seeding rates were: 20, 30, 40 or 50 kg ha^{-1}. At harvests, a 0.5 m border was discarded. The first cut was made in August 5, 2014 and second cut in October 8, 2014. Dry matter yield was assessed in both cuts by weight all forage from each plot. Silages were made in the second cut, according to two ensiling methods for each plot: 1) field wilting or 2) absorbent additive. Silages added with absorbent (15% of soybean hulls, as fed basis) were made immediately after the harvest. Ryegrass (3.95 kg) was chopped (~8 cm) and the absorbent was homogenized, being immediately ensiled. The remaining ryegrass was kept in the same plots for wilting during 6 hours. After that it was chopped and ensiled. Plastic buckets (7.6 L) were used as lab silos. Each silo had 2 cm of sand in the bottom, to measure effluent production (EP). Silo weights were recorded before and after fermentation to assess gravimetric DM losses (DML) and gas losses (GL) according to Jobim et al. (2007). After a 90-day storage period, silos were opened and samples were taken to assess DM content (forced-air oven at 55°C for 72 h) and pH (25 g of silage in 225 mL distilled water). Data were analyzed as randomized block design with factorial arrangement 4 × 2 (four seeding rates × two ensiling methods). Data were submitted to ANOVA, and significantly data ($P < 0.05$) had their means compared by Tukey test (α=5%). All analyses were performed using R software.

Results and discussion Means of DM yield were different in first and second cut to all the seeding rates (Table 1). This is a common finding for annual ryegrass, as recently reported by Tonato et al. (2014). Differences among seeding rates on DM yield were observed only in the first cut. The total DM yields were low, and this variable was probably affected by the weather conditions. Silage DM content was higher for wilted silages when compared

to silage with soybean hulls (Table 2). However, wilted silages presented higher variation on DM content among plots. Both processes were effective in avoiding effluents (EP), and the values of this variable were negligible for all treatments. The addition of soybean hulls decreased silage pH, probably due to its soluble carbohydrate contribution. Likewise, immediately ensiled forage has higher concentration of soluble carbohydrates than wilted ones. The seeding rate presented low influence on silage variables, mainly when compared to the effect of the processes of ensilage. The gas losses and the total DM losses were similar among treatments and ensiling methods. This result shows the effectiveness of using soybean hulls to increase the DM content of ryegrass and avoid fermentative losses. This technology is ready to be applied by farmers, mainly when climate conditions do not allow wilting the forage on the field.

Conclusions Seeding rates of annual ryegrass had no significant effect on the DM yield. The DM content and pH of the silages demonstrates the potential of soybean hulls as an additive for unwilted ryegrass silages.

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Table 1 Dry matter yield (kg ha-1) of a tetraploid annual ryegrass at different seeding rates

	Seeding rate (kg ha^{-1})					
	20	30	40	50	Mean	SEM[1]
First cut	1328^{Bb}	1748^{Ba}	1603^{Ba}	1750^{Ba}	1607	52.9
Second cut	2922^{A}	2770^{A}	2826^{A}	2631^{A}	2787	113.5
Total	4250	4518	4429	4381	4394	-

Means followed by different letter, uppercase in column or lowercase in row, are different by Tukey test (P<0.05).
[1] Standard error of the mean

Table 2 Dry matter content, pH and fermentative losses of ryegrass silages wilted or added with soybean hulls, at different seeding rates

	Ryegrass silage with soybean hulls				Wilted ryegrass silage				
	Seeding rate (kg ha^{-1})				Seeding rate (kg ha^{-1})				
Variables[1]	20	30	40	50	20	30	40	50	SEM[2]
DM, %	26.3^{b}	26.6^{b}	26.8^{b}	26.5^{b}	32.6^{ab}	35.3^{a}	33.6^{ab}	33.3^{ab}	0.93
pH	4.21^{b}	4.21^{b}	4.29^{ab}	4.40^{ab}	4.66^{ab}	4.66^{ab}	4.68^{a}	4.69^{a}	0.04
EP, kg t^{-1}	3.74	4.01	2.75	2.89	3.91	3.01	3.12	3.39	0.22
GL, % DM	6.55	6.62	7.85	8.43	5.01	4.68	6.02	8.11	0.45
TDML, % DM	6.90	7.00	8.10	8.70	5.39	4.97	6.32	8.43	0.44

Means followed by different letters in the same row are different by Tukey test (P <0.05).
[1] DM: dry matter content, EP: effluent production, GL: gas losses, TDML: total dry matter losses.
[2] SEM: standard error of the mean.

Effect of glyphosate as desiccant on the aerobic stability of oat haylage

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Keywords losses evaluation, preserved forage, glyphosate, haylage

Introduction The wilting restricts the fermentation extension during the preservation process of silage, reduces the spoilage microorganisms development and the effluent production. The use of desiccants to haylage production can quickly improve the dry matter content and reduces the operational costs. However, researches involving this technology are uncommon. This study aimed to evaluate the effect of glyphosate as desiccant in black oat haylage production and its implications in aerobic stability.

Materials and methods The research was conducted in the State University of Maringá (UEM). The experiment was in a randomized design, using four repetitions per treatment. The forage evaluated was the black oat *(Avena strigosa* cv. Agrocoxilha). The application of product Roundup Transorb® over the culture was when it reached the milk grain stage. The doses applied were zero (control treatment), 500, 750, 1000 and 1250 mL/ha. The silos were stored in a climate-controlled room, adjusted to 25ºC during the test. To evaluate the aerobic stability, the temperature was taken directly in the silos, using a digital long stem thermometer model *Gulterm 100,* which one was inserted in the center of forage mass. The pH was determined using a digital pH meter. Samples were collected every day for determination of ash concentration. The organic matter loss was determined using an equation described by Paredes et al (2000). The statistical procedures for all data analysis were performed using Bayesian Inference as described by Rossi (2011).

Results and discussion The regressions obtained for the aerobic stability, mean temperature, maximum pH and mean pH were non-significant. However, the use of glyphosate increased the maximum temperature of silages upon air exposition. The temperature rising in silages after the silo opening is a result of microbial activity, particularly yeasts. This effect may be related to the kind of fermentation occurred in this silages, because, the use of glyphosate caused a reduction in acetic and iso-valeric acids concentrations (data not showed), two molecules capable to reduce the aerobic spoilage. The regression equations to the variables sum of temperatures and maximum pH (hours) showed negative linear effect. The means and the regression equation that describes the effect of the desiccant in organic matter losses was significant ($P > 0.05$). The use of glyphosate can enhance organic matter losses after the silage exposure to the environment.

Conclusions The use of desiccant during the ensiling of black oat increased the aerobic deterioration after silage feedout.

Table 1 Bayesians estimative (means, standard deviation and regression equation) of the parameters evaluated during the aerobic stability test

Variable		Control Silage	Regression Equation			$^1X_{cr}$	$^1Y_{cr}$
			b0	b1 X	b2 X^2		
Organic matter losses (%)	Mean	12.29	3.33	0.010			
	sd	4.84	4.48	0.005			
Aerobic stability (hours)	Mean	37.32	18.10	0.013			
	sd	17.46	12.75	0.014			
Maximum temperature (ºC)	Mean	29.27	48.10	-0.039	0.0001	556.79	32.31*
	sd	0.17	5.18	0.013	0.0001		0.71
Sum of temperatures (ºC)	Mean	241.50	265.92	-0.023			
	sd	3.93	6.14	0.007			
Temperature mean (ºC)	Mean	26.83	25.15	0.002			
	sd	0.44	2.90	0.003			
Maximum pH	Mean	7.87	9.15	-0.002			
	sd	1.06	1.24	0.001			
Maximum pH (hours)	Mean	74.00	78.72	-0.009			
	sd	0.02	3.70	0.004			
pH mean	Mean	6.00	6.19	-0.001			
	sd	1.07	1.18	0.001			

*Significant difference (p>0,05) between the treatment and control, by Bayesian contrast; sd – standard deviation; [1]Coordinate of critical point of quadratic regression (X_{cr} in mL/ha).

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Effect of glyphosate as desiccant on the nutritional composition and dry matter recovery of oat haylage

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Keywords preserved forage, dry matter losses, haylage

Introduction Ensiling crops with excessive moisture can lead to effluent production and undesirable fermentations, increasing the dry matter (DM) losses. In this way, the use of desiccants to haylage production can quickly improve the DM content and reduces the operational costs compared with wilting. However, researches involving this technology are scarce. This study aimed to evaluate the effect of glyphosate as desiccant in oat haylage production and its implications on the chemical composition and DM recovery.

Materials and methods This study was developed in the State University of Maringá (UEM). The experiment was in a randomized design, using four repetitions per treatment. The crop evaluated was the white oat *(Avena sativa* cv. Corona). The application of product Roundup Transorb® over the culture was when it reached the milk grain stage. The doses applied were zero (control), 500, 750, 1000 and 1250 mL/ha. After harvesting, the forage was ensiled in PVC silos (10 kg), remaining sealed by 150 days. The chemical analysis performed were: neutral detergent fiber (NDF) and acid detergent fiber (ADF), according to Van Soest et al. (1991); lignin (LIG) using 72% sulfuric acid solution, after determination of FDA, as described by Detmann (2012). Hemicellulose was calculated as: HEM = NDF - ADF. Cellulose was computed as: CEL = FDA - LIG. Crude protein (CP), DM and mineral matter (MM) analyses were performed as described by AOAC (1997). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were measured in samples after determination of NDF and ADF. Organic matter was calculated as: OM = 100 - MM. The pH was determined using a digital pH meter. Dry matter recovery (DMR) was calculated according to Jobim et al. (2007). The statistical procedures for all data analysis were performed using Bayesian Inference as described by Rossi (2011).

Results and discussion The regression analysis performed for OM (921 g/kg of DM), ADF (367 g/kg of DM), LIG (39.9 g/kg of DM) and ADIN (2.7 g/kg of DM) were non-significant. The DM, HEM, CP and pH were linearly effect by the treatments. The use of glyphosate might cause alterations in the stomatal conductance and modify the selective permeability of membrane cells, since the use of desiccant changes the functionality of aquaporins, reducing the water absorption by the plant. Harvesting the crop with low DM stimulates the development of acid lactic bacteria, due to the high water content and soluble plant constituents' consumption. The application of glyphosate can decrease the CP content, due to inhibition of essential aromatic amino acid synthesis. The DMR, NDF and NDIN were quadratic effect by the treatments. The use of glyphosate reduced the DM losses. The effect observed to NDF is to a dilution effect. Probably the use of glyphosate decreased the consumption of soluble constituents by the microorganisms during the silage

fermentation.

Table 1 Bayesians estimative (means, standard deviation and regression equation) of chemical composition, recovery rate of dry matter (DMR) and pH of oat silage

Variable		Control Silage	Regression Equation			$^1X_{cr}$	$^1Y_{cr}$
			b0	*b1* X	*b2* X^2		
DM[2]	*Mean*	270.7	368.3	-0.07			
	sd	0.71	0.81	0.001			
DMR (%)	*Mean*	81.11	31.41	0.144	-0.0001	864.2	93.44*
	sd	3.17	14.65	0.036	0.0001		2.12
NDF[3]	*Mean*	665.3	809.5	-0.3	0.001	1.141.32	642.8*
	sd	1.46	2.41	0.006	0.0001		0.33
HEM[3]	*Mean*	309.5	329.5	-0.05			
	sd	1.21	1.6	0.002			
NDIN[3]	*Mean*	2.4	5	-0.01	0.001	829.14	2.3
	sd	0.02	0.07	0.001	0.0001		0.01
CP[3]	*Mean*	103.8	71.7	0.04			
	sd	0.4	0.64	0.001			
pH	*Mean*	3.81	4.02	-0.001			
	sd	0.04	0.04	0.001			

*Significant difference (P<0,05) between the treatment and control, by Bayesian contrast; *sd* – standard deviation; [1]Coordinate of critical point of quadratic regression (X_{cr} in mL/ha); [2]g/kg of natural matter; [3]g/kg of dry matter.

Conclusions The use of desiccant enhances the dry matter recovery, without great alterations in nutritional composition of white oat silage.

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Fermentation profile of black oat silage treated with chemical desiccant

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Keywords *Avena strigose*, glyphosate, haylage, fermentation end-products

Introduction Ensiling crops with a high moisture content might led to increased dry matter (DM) losses, including the formation of effluent and the development of undesirable microorganisms during the fermentation. In this way, the use of chemical desiccants is a strategy to speed crop dry down. However, studies with the application of desiccants previous to the crop ensiling are very scarce. Therefore, the objective of this study was to evaluate the effects of applying glyphosate on black oat crop and the consequent alterations in silage fermentation.

Materials and methods A plot of black oat (*Avena strigosa* cv. Agrocoxilha) was seeded in 15/05/2013 at The State University of Maringá, PR, Brazil. When the crop matches the grain pasty maturity stage (19/08/2013), the desiccant was manually sprayed to the crop, resulting in five treatments: 0 (control), 500, 750, 1000 and 1250 mL/ha of glyphosate (Roundup Transorb®). Approximately 72 hours after the desiccant application, the crop was mechanically harvested and ensiled in lab scale silos (15 L buckets). The experimental design was completely randomized with four repetitions. After 150 days of storage, the silos were opened and the silages were sampled. Then, 25 g of silage was diluted in 225 mL of distilled water and homogenized in a blender for 1 min. After filtering, the water extract was used for measuring the pH and concentrations of fermentation end-products (lactic acid by colorimetry, volatile fatty acids and alcohols by gas chromatography, and ammonia by Kjeldahl method).The DM content was determined in an oven at 60°C e corrected for volatile compounds (DM_{corr}). All data were subjected to the statistical analysis by Bayesian inference (Rossi, 2011).

Results and discussion All silages had a reasonable conservation. As expected, the DM_{corr} content increased with the application of glyphosate (24% in controle vs. 32% in glyphosate treatments), whereas there is no treatment effects on lactic acid (7.16% DM_{corr}), butyric acid (0.26% DM_{corr}), ethanol (0.08% DM_{corr}), propionic acid (0.05% DM_{corr}), formic acid (0.05% DM_{corr}), iso-butyric acid (90 mg/kg DM_{corr}), 1-propanol (42 mg/kg DM_{corr}), and N-NH_3 (4.2% TN) concentrations. The contents of 2-butanol increased linearly with the doses of glyphosate, whereas the increase in 2,3-butanediol followed a quadratic trend. On the other hand, the concentrations of acetic and iso-valeric acids were decreased in a quadratic way (Table 1). Even though lactic acid was the major fermentation end-product, other compounds has been formed in the black oat silages. The lower concentration of acetic acid observed in the treated silages is probable an effect of higher DM concentration, which may have restricted the activity of enterobacteria. On the other hand, the application of glyphosate may have impaired the metabolism of some proteolytic microorganisms (i.e., lower iso-valeric acid) whereas favored others (i.e., higher 2-butanol). Thus, no difference was observed in the ammonia nitrogen.

Table1 Bayesian estimates for significant alterations in silage fermentation

Item		Control silage	Regression			$^1X_{cr}$	$^1Y_{cr}$
			b0	*b1*X	*b2*X^2		
			%DM$_{corr}$				
Acetic acid	*Mean*	2.42	1.21	-0.002	0.0000089	947.6	0.56*
	SD	0.79	0.40	0.001	0.0000018		0.05
2,3-Butanediol	*Mean*	0.16	-0.35	0.0002	-0.0000009	869.3	0.35*
	SD	0.03	0.30	0.001	0.0000014		0.04
			mg/kgDM$_{corr}$				
2-Butanol	*Mean*	38	-3.86	0.012			
	SD	20.9	4.58	0.005			
Iso-valeric acid	*Mean*	258	41.2	-0.010			
	SD	20.9	4.58	0.005			

[1]Coordenates of the critical point in the quadratic regression (X_{cr} = mL/ha).
SD: standard deviation.
*Bayesian contrast: Treatment vs. Control, significant at α = 0.05.

Conclusion Desiccation with glyphosate prevents the occurrence of undesirable fermentation in black oat silages. A dose of 500 mL/ha is recommended.

Reference

Rossi, R.M. 2011. Introdução aos métodos Bayesianos na análise de dados zootécnicos com uso do WinBUGS e R. ed. EDUEM, Maringá-PR. 191p.

Fermentation profile of white oat silage treated with chemical desiccant

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Keywords *Avena sativa*, glyphosate, haylage, fermentation end-products

Introduction Wilting is widely used to increases the dry matter (DM) concentration of highly moist forages, preventing the occurrence of undesirable fermentations and effluent losses during silage make. In this way, applying chemical desiccants, such as glyphosate, some days before harvesting, would decreases moisture in excess and reduce labor demand and field losses associated with tedding, raking, and windrow collecting. However, studies with the application of desiccants previous to the crop ensiling are very scarce. Therefore, the objective of this study was to evaluate the effects of applying glyphosate on white oat crop and the consequent alterations in silage fermentation.

Materials and methods A plot of white oat (*Avena sativa* L. cv. Corona) was seeded in 15/05/2013 at The State University of Maringá, PR, Brazil. When the crop matches the grain pasty maturity stage (19/08/2013), the desiccant was manually sprayed to the crop, resulting in five treatments: 0 (control), 500, 750, 1000 and 1250 mL/ha of glyphosate (Roundup Transorb®). Approximately 72 hours after the desiccant application, the crop was mechanically harvested and ensiled in lab scale silos (15 L buckets). The experimental design was completely randomized with four repetitions. After 150 days of storage, the silos were opened and the silages were sampled. Then, 25 g of silage was diluted in 225 mL of distilled water and homogenized in a blender for 1 min. After filtering, the water extract was used for measuring the pH and concentrations of fermentation end-products (lactic acid by colorimetry, volatile fatty acids, esters and alcohols by gas chromatography, and ammonia by Kjeldahl method).The DM content was determined in an oven at 60°C e corrected for volatile compounds (DM_{corr}). All data were subjected to the statistical analysis by Bayesian inference (Rossi,2011).

Results and discussion The treatments had no effect on the concentrations of lactic acid (8.8% DM_{corr}), acetic acid (0.95% DM_{corr}), ethanol (0.35% DM_{corr}), and 1-propanol (31 mg/kg DM_{corr}). Glyphosate increased the formation of 2,3-butanediol and formic acid compared with the control. Although the concentrations of propionic and iso-butyric acids varied across the treated silages, there is no difference among the control and desiccated silages. On the other hand, the desiccated forages led to silages with lower contents of 2-butanol, ethyl lactate, and butyric acid. Due to the desiccation with glyphosate increased the silage DM_{corr} (27.9% in control vs. 31.7% in glyphosate treatments) it is likely that the activity of clostridia has been restricted. Even tough, the levels of $N\text{-}NH_3$ were higher in treated silages. The application of glyphosate may have increased the concentration of free amino acids in the fresh forage, which are prone to proteolysis during the silage fermentation.

Table 1 Bayesian estimates for significant alterations in silage fermentation

Item		Control silage	Regression			$^1X_{cr}$	$^1Y_{cr}$
			b0	*b1* X	*b2* X^2		
			% DM_{corr}				
Butyric acid	*Mean*	0.45	-1.06	0.003	-0.0000017	900.8	0.34*
	SD	0.33	0.34	0.001	0.0000005		0.05
2,3-Butanediol	*Mean*	0.57	-1.28	0.005	-0.0000027	861.7	0.73*
	SD	0.05	0.43	0.001	0.0000006		0.06
Formic acid	*Mean*	0.11	-0.42	0.002	-0.0000008	874.3	0.18*
	SD	0.05	0.21	0.001	0.0000003		0.03
			mg/kg DM_{corr}				
Ethyl lactate	*Mean*	323	604	-1.112	0.0006765	808.6	147*
	SD	70	165	0.408	0.0002322		23.7
2-Butanol	*Mean*	59	-60.6	0.191	-0.0001074	888.5	24*
	SD	19	24.3	0.060	0.0000546		3.4
			% Total nitrogen				
$N\text{-}NH_3$	*Mean*	3.80	0.70	0.010	-0.0000056	916.5	5.00*
	SD	0.42	1.44	0.004	0.0000020		0.20

[1]Coordenates of the critical point in the quadratic regression (X_{cr} = mL/ha).
SD: standard deviation.
*Bayesian contrast: Treatment vs. Control, significant at $\alpha = 0.05$.

Conclusion Treating white oat crop with glyphosate 3 d before harvesting prevents the occurrence of undesirable fermentation and improved the conservation process. The dose of 500 mL/ha was most cost-effective.

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Rossi, R. M. 2011. Introdução aos métodos Bayesianos na análise de dados zootécnicos com uso do WinBUGS e R. ed. EDUEM, Maringá PR. 191p.

Effects of N fertilization and phenological stage on the quality of wheat hay

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Keywords conserved cereal, nutritional value, temperate grass

Introduction The efficiency of conserved feed production is crucial to the profitability of any production system. Therefore, one should seek alternatives to the greater forage production with higher quality and reduction of nutrient losses. In this context, the use of higher doses of nitrogen could expand the productive potential of wheat for conservation purposes. Productivity and nutritional quality of hay are also determined by the phenological stage at harvesting. The DM production increases with plant maturity, however, gradual reduction may occur in non-structural carbohydrate and protein concentrations, whereas structural carbohydrates increase steeply. The aim of this study was to evaluate the composition of wheat hay, harvested on two phenological stages and with two levels of nitrogen fertilization.

Materials and methods The experiment was conducted in the Núcleo de Produção Animal (NUPRAN) of the Center for Agricultural and Environmental Sciences, University of the Midwest (UNICENTRO) in Guarapuava - PR. The experimental design was in blocks with factorial arrangement 2×2, comprised of four treatments with four replications, being two growth stages (pre-flowering and grain dough) and two levels of nitrogen fertilization post-emergence (88 and 148 kg ha^{-1}). The levels of neutral detergent fiber (NDF) were determined according to Van Soest et al. (1991), acid detergent fiber (ADF) according to Goering and Van Soest (1970) and hemicellulose content was computed as NDF minus FDA. The statistical analysis was conducted using ANOVA procedure and the means were compared by F test at 5% significance level.

Results and discussion As expressed in Table 1, phenological stage affected the DM (83.33 vs. 77.32%), NDF (65.03 vs. 54.12%), CP (11.53 vs. 5.33%) and hemicellulose (26.04 vs. 17.53%) contents. However, no difference ($p>0.05$) was observed for ADF concentration (38.98 vs. 36.58%). According to the literature, the dehydration rate post-cutting in pre-flowering stage is higher than in grain dough stage and this fact could be explained by the increased participation of leaves in the pre-flowering hay. the contents of NDF, hemicellulose and CP decreased with the maturity advance. This is due to the dilution effect. When comparing levels of nitrogen fertilization for wheat hay production, there was no significant difference ($p>0$ .05) for chemical composition.

Table 1 Dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP) and hemicellulose (HEMI) in wheat hay harvested at different phenological stages and cultivated with two levels of nitrogen fertilization

Levels of nitrogen fertilization	Phenological stages		Average
	Pre-flowering	Grain dough	
	DM, %		
88 kg ha^{-1}	81,66	77,66	79,66 a
148 kg ha^{-1}	84,99	76,99	80,99 a
Average	83,33 A	77,32 B	
	NDF, % da MS		
88 kg ha^{-1}	64,57	53,12	58,84 a
148 kg ha^{-1}	65,49	55,11	60,30 a
Average	65,03 A	54,12 B	
	ADF, % da MS		
88 kg ha^{-1}	38,55	36,94	37,74 a
148 kg ha^{-1}	39,42	36,21	37,81 a
Average	38,98 A	36,58 A	
	CP, % da MS		
88 kg ha^{-1}	11,13	5,24	8,18 a
148 kg ha^{-1}	11,93	5,42	8,67 a
Average	11,53 A	5,33 B	
	HEMI, % da MS		
88 kg ha^{-1}	26,01	16,17	21,09 a
148 kg ha^{-1}	26,07	18,90	22,48 a
Average	26,04 A	17,53 B	

Means followed by different letters in lines and columns were statistically different ($P<0.05$) by F test.

Conclusion Level of nitrogen fertilization post-emergence did not affect the chemical composition, whereas the nutritional quality was improved with the late harvesting (grain dough stage).

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Digestibility of whole crop legume-cereal silages harvested at three different dates

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Introduction Producing whole crop small grain cereal silages provides an opportunity to improve the efficiency of forage production for ruminants under Northern European conditions. Inclusion of grain legumes (faba bean, pea) or vetches may improve the nutritional quality of the feed. *In vitro* digestibility has been shown to be high for pea and faba bean whole crops and inclusion of them increases the crude protein (CP) concentration of grain legume and cereal mixed crops (Kuoppala et al. 2014). We investigated the effect of growing time on fermentation quality and *in vivo* digestibility of two legume-cereal whole crop silages.

Materials and methods Two legume-cereal mixtures, pea (*Pisum sativum*, cv. Florida) + wheat (*Triticum aestivum*, cv. Anniina) (PW) and faba bean (*Vicia faba*, cv. Fuego) + wheat (cv. Anniina) (FBW) were grown at Luke (former MTT Agrifood Research Finland) research farm in Ruukki, Finland (64°N, 25°E). The fields were fertilized with manure compost or cattle slurry (mean 44 kg N/ha). The amount of seeds was 138 kg pea with 75 kg wheat and 174 kg faba bean with 75 kg wheat per hectare. Both whole-crop mixtures were harvested three times, at 10, 12 and 14 weeks after sowing. The stands were cut by mower conditioner (Elho 280 Hydro Balance) and harvested with an integrated round baler wrapper (McHale Fusion 2) 2-3 hours after cutting. Silages were preserved with formic acid based additive applied at a rate of 6 L/t. The bales were opened 90 days after ensiling, chopped with a TMR mixer wagon and kept in a freezer at -21 °C until used in a 6 × 4 incomplete Latin square digestibility experiment with six wether lambs (average live weight 53±3.6 kg) using total collection of feces.

Results and discussion Both forages contained high proportion of legumes (mean 0.911 and 0.824 g/g DM for PW and FBW, respectively) and low concentration of DM (Table 1). The unintentionally low DM concentration was mainly caused by the high proportion of legumes; the weather was good before and during the harvests. Exceptionally warm early summer may have improved the competitiveness of legumes in the mixed stands. The average DM yield increased 68 kg/d/ha for PW and 151 kg/d/ha for FBW. Legume-cereal silages contained more DM than the original herbages indicating remarkable effluent losses. The fermentation quality was typical for very wet silages: low pH and abundantly lactic acid. The high proportion of ammonium N indicates excessive degradation of protein and poor quality especially for PW silages. Concentration of indigestible NDF was very high particularly for PW. Digestibility of organic matter (OM, Table 2) was higher for FBW than for PW and for FBW it increased with the growing time but decreased for PW. Digestibility of CP was higher for PW silages than for FBW silages which reflected the higher average CP concentration of PW to FBW (172 vs. 157 g/kg DM) but the growing time did not affect it. The average digestibility of NDF did not differ between the two legumes but it decreased for PW and increased for FBW when the crops were harvested

later. The digestibility of potentially digestible NDF (pdNDF) was higher for PW and it decreased with postponed harvest. Metabolic fecal OM output was typical but showed an increase with postponed harvest.

Conclusions The low DM concentration of legume cereal whole crop silages should be avoided. It is connected to the proportion of plant species in a mixed crop, method of harvest and weather conditions. Digestibility of OM was low for all silages compared to grass silages commonly recommended for dairy cow feeding. High yields of DM and CP per hectare can be obtained by using mixtures of grain legumes and cereals for whole crop silages.

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Table 1 Description of pea wheat (PW) and faba bean wheat (FBW) silages harvested at different harvesting dates (units g/kg dry matter unless otherwise stated)

	PW			FBW		
Weeks from sowing	10	12	14	10	12	14
Dry matter, g/kg	200	218	270	215	230	251
Dry matter yield, kg/ha	5038	6839	6954	4750	7009	8970
pH	3.96	4.03	4.21	4.00	4.00	4.21
Ash	71	74	82	86	64	63
Crude protein	164	172	181	144	161	166
Neutral detergent fiber (NDF)	504	482	479	513	516	457
Indigestible NDF	220	224	224	203	199	170
Acetic acid	9.1	13.7	13.0	10.7	10.1	7.7
Propionic acid	3.6	3.4	3.1	3.3	4.2	3.2
Butyric acid	0.6	0.6	0.4	0.5	0.5	0.5
Lactic acid	72	92	74	66	43	26
Water soluble carbohydrates	67	53	54	53	71	90
Ethanol	2.3	5.8	4.2	3.2	1.9	4.8
Ammonium N, g/kg N	72	92	87	53	51	49

Table 2 The effect of growing time on the *in vivo* digestibility of pea wheat (PW) and faba bean wheat (FBW) silages

	PW			FBW				P values[2]		
Weeks from sowing	10	12	14	10	12	14	SEM[1]	Leg	Harv	Leg × Harv
Organic matter	0.651	0.630	0.605	0.630	0.629	0.660	0.0048	0.02	0.20	<0.01
Crude protein	0.756	0.738	0.720	0.690	0.695	0.689	0.0117	<0.01	0.17	0.17
NDF[3]	0.568	0.527	0.503	0.541	0.536	0.552	0.0105	0.28	0.04	0.01
pdNDF[4]	1.014	0.984	0.952	0.897	0.873	0.879	0.0148	<0.001	0.025	0.18
NDS[5]	0.746	0.739	0.711	0.744	0.742	0.761	0.0071	0.017	0.27	0.01
MFOM[6]	107	114	125	101	106	112	2.6	0.010	<0.01	0.20

[1]SEM = standard error of the mean; [2]Data was analyzed by ANOVA with orthogonal contrasts, probability of the effects due to legume species (Leg), growing time (Harv) and their interaction; [3]NDF=neutral detergent fiber; [4]pdNDF=potentially digestible NDF (NDF-indigestible NDF); [5]NDS=neutral detergent soluble (organic matter -NDF); [6]MFOM = fecal metabolic OM output, g/kg DM intake

Comparative analysis of nutritive value of some summer annual green fodder mixtures (oat, pea and vetch) and effect of different seed number and seed ratio at sowing

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Keywords green fodder, oat, nutritive value, vetch

Introduction The authors investigated the effect of different seed number (per ha) and seed-ratios on yield, nutrient content and digestibility of summer annual mixtures (six oat-pea and six oat-vetch combinations, respectively). Optimal seed ratio is needed to achieve good protein and fermentable carbohydrate concentration in order to produce high quality silage.

Materials and methods Six different oat-pea and oat-vetch mixtures were sown on 28th March 2013. Nitrogen fertilization was carried out prior to sowing (200 kg/ha NPK and 54 kg N/ha). Experimental plot size was 15 m^2 per repetition (n = 4). Treatments (6 seed number combinations) were trialled (Tables 1-2). Sampling was carried out 19th June (oat and the legume in flowering stage) by hand (randomized sampling with standard frame: 1 m^2, n = 4). Crude nutrient content, fiber fractions, protein- and organic matter (OM) digestibility, NDF degradability were determined by NIR method (BLGG AgroXpertus database, Wageningen, spectra: NEN-EN-ISO 12099). Wet chemical reference methods can be provided by the authors. Applied statistical models were the following: Levene test for homogeneity and UNIVARIATE MODELLING, ANOVA (comparison of the different mixtures).

Results and discussion Results are shown in Tables 1 and 2. Mixtures of oat (2.0 x 10^6 seed/ha) and pea (0.65 x 10^6 seed/ha) had the highest fresh and DM yield (4.9 ton/ha) compared to other combinations, with the lowest digestible protein-content, low OM-digestibility, and the highest NDF-, ADF- and dNDF content. Mixtures of oat (1.5 x 10^6 seed/ha) and vetch (2.0 x 10^6 seed/ha) had the highest fresh and DM yield (6.3 ton/ha) compared to the other oat-vetch combinations, with the highest ADF-content and lowest OM digestibility, digestible OM- and net energy content among the other combinations. The authors concluded that the oat-pea and oat-vetch combinations with high yield had generally worse protein content and/or nutritive values than mixtures having lower DM yield. It was found that the increasing seed number of vetch (per ha) in combination with the same oat ratio improved the crude protein content and the organic matter digestibility. Therefore combinations of 3.000.000 vetch seed/ha with 2.000.000 oat seed/ha or 2.500.000 oat seed/ha are recommended for additional investigations and farm practice. The oat-pea and oat-vetch whole crop mixtures (each seed rate) had considerable fibre content (NDF: 488-532 g/kg DM) with low level of ADL (ADL: 25-33 g/kg DM) harvested in flowering stage. These silages made from the investigated whole crop

mixtures can be excellent sources of degradable NDF (dNDF) in the cattle diet. Increase of oat in the oat-pea mixtures resulted in lower ash content, presumably due to lower soil contamination. The vetch increased the ash content compared to the oat-pea mixtures, due to higher soil contamination. The high ash content is a risk from animal health and fermentation point of view, therefore the potentially lower ash content is recommended to consider in farm practice. It was found that the phenological stage applied in this trial at harvest could provide a limited concentration of crude protein (similar to a poor quality lucerne silage) and limited net energy content (10% lower than a normal maize silage), but the protein:energy ratio was balanced compared to lucerne- or maize silage. Authors summarized, the oat-pea and oat-vetch mixtures (harvested in flowering stage of the oat) are recommended to feed of growing heifers at high performance farms, based on limited protein and energy content, balanced protein:energy ratio and high dNDF value.

Table 1 Fresh matter yield, dry matter (DM) yield and nutrient content of whole crop oat-pea and oat-vetch mixtures

Seed/ha		FM yield	DM yield	DM	Crude protein	Crude ash	Total sugar	Starch	Digest. protein
		t/ha	t/ha	g/kg	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM
Oat	Pea								
1 000 000	500 000	22.2a	4.2a	192a	153a	92bc	67.8bc	45.8a	58.0bc
3 000 000	500 000	18.9c	3.8c	201a	143a	90b	69.0bc	53.8a	57.3ab
1 500 000	650 000	24.0a	4.2a	176a	166a	107a	64.5a	35.0a	61.5ab
2 000 000	650 000	27.5b	4.9b	180a	149a	100ac	56.8b	37.5a	54.5a
2 500 000	650 000	24.4a	4.3a	174a	164a	97ab	61.0ac	51.0a	63.0bc
3 500 000	850 000	20.6ca	3.9c	191a	164a	94ab	73.3ab	44.0a	63.3c
SE		*1.32*	*0.17*	*1.62*	*1.80*	*1.18*	*0.28*	*2.96*	0.67
Oat	Vetch								
1 500 000	2 000 000	34.9a	6.3a	182a	157a	105a	57.8a	29.0a	54.3a
3 000 000	2 000 000	23.2bc	4.1b	176a	170bc	103a	50.8ab	28.0a	57.8a
2 000 000	2 500 000	24.9b	4.6b	185a	159ab	110a	45.5a	46.5a	56.8a
2 500 000	2 500 000	22.2c	3.9b	177a	157a	105a	56.0ab	39.0a	57.3a
2 000 000	3 000 000	22.0c	3.9b	176a	178c	103a	44.3b	36.3a	63.3b
2 500 000	3 000 000	24.3bc	4.2b	174a	176c	103a	46.3b	42.8a	64.0b
SE		*1.29*	*0.27*	*2.13*	*2.34*	*1.51*	*0.37*	*3.70*	*0.98*

^{abc}Means in the same column with different letters differ compared the impact of different seed /ha within the same type of mixture ($p \leq 0.05$).

Table 2 Nutrients, digestibility and energy content of whole crop oat-pea and oat-vetch mixtures

		NDF	ADF	ADL	NDFd	dNDF	OMd	NEl	DOM
		g/kg DM	g/kg DM	g/kg DM	%	g/kg DM	%	MJ/kg DM	g/kg DM
Oat	Pea								
1 000 000	500 000	517a	304a	27a	63abc	324ac	68.5bc	5.7bc	622b
3 000 000	500 000	523a	310a	30a	63abc	327ab	68.0bc	5.6ac	619ab
1 500 000	650 000	512a	302a	31a	65a	333ab	70.4a	5.8ab	629ab
2 000 000	650 000	532a	313a	29a	64ab	338a	67.8b	5.6a	610a
2 500 000	650 000	492a	299a	32a	63bc	308bc	69.6ac	5.8ab	628ab
3 500 000	850 000	488a	311a	33a	61c	297c	68.9ab	5.8b	624b
Std error		3.45	1.90	0.47	0.39	4.77	0.82	*0.027*	*2.36*
Oat	Vetch								
1 500 000	2 000 000	528a	310a	28ab	64ab	340ab	67.5a	5.5a	605a
3 000 000	2 000 000	532a	301a	26b	65ab	344a	68.8ab	5.7ab	617ab
2 000 000	2 500 000	515ab	302a	29a	63a	326ab	68.5a	5.6a	611ab
2 500 000	2 500 000	511ab	293a	25b	65ab	330ab	69.0ab	5.7ab	617ab
2 000 000	3 000 000	522ab	296a	27b	66b	342ab	70.9b	5.9b	635b
2 500 000	3 000 000	495b	294a	26b	64ab	319b	70.8b	5.9b	635b
Std. error		4.56	2.94	0.51	0.25	3.56	1.04	*0.043*	*4.01*

^{abc}Means in the same column with different letters differ compared the impact of different seed /ha within the same type of mixture ($p \leq 0.05$), OMd -organic matter digestibility, NDFd -NDF rumen degradability, dNDF -rumen degradable NDF, DOM digestible organic matter.

Corn silage processing score in farm samples collected in Hungary

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Keyword corn processing, corn silage, CSPS

Introduction The Corn Silage Processing Score (CSPS) was developed (USDA Forage Research Center) as a tool to define adequacy of kernel processing by forage harvesters. The CSPS is the percentage of starch passing through the coarse 4.75 mm screen (Ferreira and Mertens, 2005). Processing of corn silage may improve dry matter intake, starch digestion, and lactation performance (Bal et al., 2000 a,b). Dry matter intake (25.9 vs. 25.3 kg/d) and milk (46.0 vs. 44.8 kg/ d) and fat (1.42 vs. 1.35 kg/d) yields were higher for the processed corn silage treatments (at a 1 mm roll clearance) compared with the control corn silage (Bal et al., 2000a). Kozakai et al. (2007) have found more rapid and greater colonization of the processed compared to unprocessed silage, by the rumen bacteria which facilitates ruminal digestion and fermentation. Authors investigated Corn Silage Processing Score (CSPS) of 211 corn silage samples collected from Hungary in 2013 and 2014.

Materials and methods Samples from different areas of Hungary collected by farmers during 2013 (n.147) and 2014 (n. 74) were assessed. Samples were dried (70 °C, 8 hours) according to method EN ISO 6496:1993. Starch content was determined by Near-InfraRed Spectroscopy. Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser). Reference starch method: NEN-EN-ISO 15914. Corn silage processing score (CSPS) was determined on dried samples with Ro-Tap Sieve shaker according to Ferreira and Mertens (2005). Modified starch digestibility, modified digestible starch and modified net energy content according to CSPS value were calculated based on Schwab et al. (2003).

Results and discussion Results and distribution of the quality are given in Table 1. The ratio of inadequately processed corn silages were rather high (2013: 28% vs 2014: 23%). Although, 65% of the corn silage samples were adequate from processing point of view (2013 and 214). According to our results, 7% (2013) and 12% (2014) of the analysed corn silages were in the optimal quality range. Detailed results of starch digestibility based on the actual CSPS value and calculated losses can be found in Table 2. The CSPS mean values of corn silages derived from 2013 were adequate, but not optimal causing losses of 41 g/kg DM starch and 0.29 MJ/kg DM net energy compared to the original laboratory results (without CSPC) due to undigested starch in inadequately processed corn silages (seed size: >4.75 mm). The losses (based on undigestible starch content) were equivalent to 14 ha corn grain, as average (2013). The CSPS value of 58% (2014) were equivalent to 17.3 ha corn grain (based on undigested starch content). Inadequate corn processing can cause serious losses due to the lower real starch digestibility. It is recommended to introduce *corn silage processing quality control* on the dairy farms during the corn harvest period, to improve the management and technical background in order to reduce the financial losses caused by inadequate CSPS values.

Table 1 Distribution and evaluation of Corn Silage Processing Score of fresh whole corn crop (chopped) and corn silage

Quality distribution according to CSPS value		Harvest 2013		Harvest 2014	
		No. sample	%	No. sample	%
<50	inadequate	41	28	17	23
50-70	adequate	96	65	49	65
>70	optimal	10	7	9	12

Table 2 Corn Silage Processing Score, starch digestibility and net energy content of corn silages

		Fresh forage and corn silage			Corn silage	
	CSPS	Original starch	Modified starch digestibility	Modified digestible starch	Original NEl	Modified NEl
	%	g/kg DM	%	g/kg DM	MJ/kg DM	MJ/kg DM
Harvest 2013, n= 147						
Average	55	286	86	245	6.32	6.03
St. deviation	11	55	9	50	0.23	0.26
Starch losses					41 g/kg DM starch	
NEl losses					0.29 MJ/kg DM	
Milk losses (energy equvivalent, 7kg DMI silage, 3 MJ NEl /kg milk)					0.69 kg milk/day/cow	
Losses (starch equvivalent 1 year, 500 dairy cow, 7 kg DMI silage)					14.0 ha corn seed	
Harvest 2014, n= 75						
Average	58	357	87	306	6.51	6.13
St. deviation	10	62	9	46	0.21	0.25
Starch losses					51 g/kg DM starch	
NEl losses					0.38 MJ/kg DM	
Losses (energy equvivalent, 7kg DMI silage, 3 MJ NEl /kg milk)					0.93 kg milk/day/cow	
Losses (starch equvivalent 1 year, 500 dairy cow, 7 kg DMI silage)					17.3 ha corn seed	

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Screening of nutritional quality and particle size of corn silage samples in the Valley of Lerma, Argentina

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Keywords silage, corn, quality, particle size, dairy farm

Introduction Most of the dairy farms in northwestern Argentina are changing towards intensive production systems. In this way, the dietary inclusion of whole corn silage (WCS) is an essential tool to achieve this goal. However, the lack of effective fiber (eNDF) in this feedstuff can be a serious limitation for its use in dairy rations. The eNDF can be calculated by measuring the size and homogeneity of the feed particles using the Penn State Particle Separator (PSPS), according to the methodology proposed by Heinrichs and Kononoff (2002).The aim of this study was to evaluate the nutritional quality and describe the particle size distribution from WCS samples collected in dairy farms located in the Valley of Lerma region, Argentina.

Materials and methods Feed samples were analyzed. In total, 29 WCS samples were considered for both quality and particle size determinations. This 29 WCS samples were collected from 12 farms selected at random. Because these farms are using both types of silos, 48.3% of the samples came from bunker silos and 51.7% of them were from silos bag. The chemical composition parameters as follows: dry matter (DM, conventional dry oven at 60 ° C for 72 hours), crude protein (CP, by Kjeldahl method) according to Thiex et al. (2002), neutral detergent fiber (NDF) and acid detergent fiber (ADF) by ANKOM method according to Mertens and Fahey (1994), and silage pH. The particle size distribution was determined by PSPS system according methodology proposed by Heinrichs (2013). Data were analyzed using Infostat software (Di Rienzo et al., 2001).

Results and discussion The results of corn silage quality are shown in Table 1. The value of 37% of DM is above the optimal value and 6.3% of CP is below the national average value in WCS, according as indicated in Gallardo (2013). The value of the mode on DM data is close to 50%, this indicates chopped material after its optimum time with low moisture content, along with a value of CP mode a point lower than optimal, which is a consequence of water deficit during the crop growth which increases stem lignification and impairs the absorption and translocation of nitrogen. The mode values of ADF and NDF are above the national average value in WCS, according as indicated in Gallardo (2013). Regarding fermentation quality, the pH value indicated a good silage making process. The results show a low percentage of particles in the lower sieve (Table 2), which decreases the density achieved during storage in WCS made after the optimum date (Bragachini et al., 2008). In the evaluated dairy farms, the difference between average values of larger particles that are retained in the upper sieve and what is considered eNDF (Palladino et al., 2014), is 5.98 percentage points. Considering this value, a bag silo with 210 t of fresh matter (DM 37.3% on average) would lose 5.98% of DM in the trough, about 4.6 tons of DM per bag.

Table 1 Nutritional quality of corn silage samples of 12 dairy farms located in the Valley of Lerma (Salta province, Argentina) (n=29)

Parameter	Mean	Minimum	Maximum	Mode*
DM, %	37.3	28	54.2	49.7
CP, % DM	6.3	4.5	8.2	6.9
ADF, % DM	24.9	20.7	31.7	22.5
NDF, % DM *	46.5	38.8	60	45
pH	3.6	3.1	4	3.6

* The mode is the value that appears most often in a set of data (Di Rienzo et al., 2008)

Table 2 Particle size distribution (%) of corn silage samples collected from 12 dairy farms located in the Valley of Lerma (Salta province, Argentina) (n= 29)

Particle size	Mean	Minimum	Maximum	SD
Upper , %	11.48	2.77	28.48	6.95
eNDF, %	5.50	0.62	17.98	4.24
Middle , %	73.45	59.11	83.97	7.41
Lower , %	11.30	5.97	22.57	3.80
Bottom , %	3.77	1.13	7.20	1.36

Conclusions Based on the results obtained in this study about quality of corn silage in the Valley of Lerma (Salta province) should be considered for the next chopping season regulate the size of particles in the forage harvester, along with an selection of the chopping schedule, to improve the quality of corn silage in this region.

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Aerobic stability and pH of corn silage rehydrated and total mixed rations with different particle sizes

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Keywords nutrient losses, feed quality, high moisture grain, maize

Introduction Although the use of corn in ruminant diets is broad, there are inconsistencies on animal performance, usually limited by faults in the storage, processing or variations in the chemical composition. In this sense the use of corn grain silage in rehydrated form appears as a good alternative, but this technique requires the correct use of the foundations of conservation in order to obtain a final product with quality and can in fact provide improvements on animal performance. Assessment of stability and pH are important steps in this context, because the aerobic spoilage can result in increased temperature of the silo, loss of nutritional value and dry matter losses according to the breakthrough in the use of the silo, thus triggering a negative impact on the production and animal performance (Whiter and Kung, 2007). The purpose of this study is to evaluate the aerobic stability of corn grain silages rehydrated with different particle sizes and with or without inclusion of protein concentrate.

Materials and methods The CGR (Corn grain rehydrated) was processed at approximately 140 g/kg of dry matter (DM) and submitted a rehydration process for highest moisture in composition, the CGR was a three types of processes in a hammer mill, with inclusion or not of protein concentrate (38% CP) aiming at the analysis of total mixed rations with high proportion of energy. The following treatments are applied to the corn grain: Corn grain rehydrated 1 mm (CGR1), Corn grain rehydrated 1 mm with commercial concentrate (CGR1+C) Corn grain rehydrated 3 mm (CGR3), Corn grain rehydrated 3 mm with commercial concentrate (CGR3+C), Corn grain rehydrated 7 mm (CGR7), Corn grain rehydrated 7 mm with protein concentrate (CGR7+C). After processing the samples were ensiled in vacuum (1000g material / sill) in plastic bags to remove the air and sealing the experimental sills in order to obtain an average density of 600kg/m^3 of silage. The silos were packed in a box and stored at room temperature and opened at 120 days. To determine the aerobic stability, 0.5 kg of material were placed in plastic buckets and maintained in a closed space at room temperature (average 25ºC). The temperature of the silage and the ambient temperature were measured every 12 hours during the aerobic exposure and the silages were sampled to determine the levels of pH by 6 days. The experimental design was a randomized block design with six treatments and three repetitions. The collected data for each variable were subjected to variance analysis to compare means at the 5% significance through the SAS (2004).

Results and discussion The data concerning the aerobic stability and pH of corn grains rehydrate and corn grains rehydrate with protein concentrate are shown in Table 1. The processing and protein concentrate inclusion did not affect the dry matter at the opening of

the silo (p>0.05) among silages. Regarding the pH was no significant difference (p<0.05) between treatments, and it was observed that the treatments without the inclusion of protein concentrate, had pH values lower and more stable when compared to treatment with inclusion. The GMR1 treatment was demonstrated that during the six days of evaluation as stable pH, compared to the other.

Table 1 Aerobic stability during aerobic exposure of silage of corn grain rehydrates and corn grain rehydrates added protein concentrate

Time (days)	Treatmensts						
	GMR1	GMR1+C	GMR3	GMR3+C	GMR7	GMR7+C	SEM
			Dry matter (g/kg)				
0	630A	615A	635A	620[a]	635A	620A	-
			pH				
0	4.13C	4.41ABC	4.20BC	4.45AB	4.24ABC	4.53A	0.005
1	4.13C	4.36B	4.13C	4.43AB	4.32B	4.48	0.0008
2	4.18	4.35	4.18	4.44	4.35	4.46	0.003
3	4.26A	4.46A	4.40A	4.54[a]	4.55A	4.54A	0.007
4	4.27C	4.42BC	4.44BC	4.56AB	4.68A	4.74A	0.001
5	4.49BC	4.50BC	4.44C	4.62ABC	4.69A	4.64AB	0.001
6	4.19A	4.41A	4.29A	4.48[a]	4.48A	4.96	0.07
			Aerobic estability (ºC)				
0	22.25A	23.35A	23.15A	23.45A	21.45A	21.55A	0.30
1	23.25B	23.30AB	23.30AB	23.55AB	23.55AB	23.60A	0.005
2	22.80C	22.95BC	22.90BC	23.00BC	23.15AB	23.45A	0.005
3	23.30A	23.30A	23.35A	23.50A	23.40A	23.5A	0.003
4	23.60A	23.70A	23.85A	23.90A	23.90A	24.35A	0.06
5	22.90A	23.20A	23.10A	23.00A	23.10A	23.80A	0.33
6	22.40A	22.50A	22.40A	22.60A	22.70A	23.55A	0.07

[1] CGR1 - Corn grain rehydrated 1 mm; CGR3 - Corn grain rehydrated 3 mm; CGR7 - Corn grain rehydrated 7 mm; CGR1+C - Corn grain rehydrated 1 mm with commercial concentrate; CGR3+C - Corn grain rehydrated 3 mm with commercial concentrate; CGR7+C - Corn grain rehydrated 7 mm with commercial concentrate.
Means followed by differences letters, capital letters in the lines differ (P<0.05) by Tukey test; SEM = standard error of the mean

On the aerobic stability, also found statistically significant differences (*P*<0.05), but was not observed the same pH behavior, only on days 1 and 2, these differences were observed in the remaining days the values were similar. The differences observed on pH can be explained by the greater availability of starch in the treatments without addition of concentrate, but the grain size factor seems to exert a strong influence on this characteristic, a fact observed when comparing the treatments with and without inclusion during the days of exposing the material to aerobic environment. Jobim et al. (2007) confirming the data, also found stable pH for corn grain silages rehydrated, but the materials with inclusion of protein sources had unstable pH behavior. The temperature of the studied silage can be considered stable, according to the methodology recommended because it did not exceed 2°C to room temperature (25°C) for six days of aeration.

Conclusions The particle size and the inclusion of concentrate protein affect the pH of silages, indicating that the smaller the particle sizes the better pH stability. The temperature during exposure did not affect aerobic stability, showing that all the silages, even with an increase in the evaluated days were stable during the days of evaluation.

Chemical composition of corn silage rehydrated and total mixed rations with different particle sizes

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Keywords chemical composition, grain processing, maize, reconstituted grain

Introduction In Brazil, corn is one of the main cereals produced and used in diets of ruminants, has as its main features, high in carbohydrates, especially starch, which makes it an energy food. However, to improve efficiency in the use of corn processing has shown advantages such techniques aim to increase digestibility and availability of starch in the rumen and this results in a positive change in the nutritional quality of food leading to better performance animals (Owes et al., 1997). The grain milling process is more simple and practical to obtain particles of different sizes by varying the proportions degradation in the rumen, as well as the reconstitution of corn, i.e., the recovery of the humidity around 35 - 40%, which results in better digestibility of dry matter and protein degradability (Benton et al, 2009;. Hale, 1973). The objectives of this study to assess the chemical composition of maize grain rehydrated with or without the inclusion of protein concentrate.

Materials and methods The CGR (Corn grain rehydrated) was processed at approximately 140 g/kg of dry matter (DM) and submitted to a rehydration process for higher moisture in composition, the CGR was a three types of processes in a hammer mill, with inclusion or not of protein concentrate (38% CP) aiming the analysis of total diets mixed with high proportion of energy. The following treatments were applied to the corn grain: Corn grain rehydrated 1 mm (CGR1), Corn grain rehydrated 1 mm with commercial concentrate (CGR1+C) Corn grain rehydrated 3 mm (CGR3), Corn grain rehydrated 3 mm with commercial concentrate (CGR3+C), Corn grain rehydrated 7 mm (CGR7), Corn grain rehydrated 7 mm with protein concentrate (CGR7+C). After processing, the samples were ensiled in vacuum (1000g material / sill) in plastic bags to remove the air and sealing the experimental sills in order to obtain an average density of 600kg / m^3 of silage. The silos were packed in a box and stored at room temperature and opened in 120 days for chemical analysis. In the pre-dried samples, were determined the total dry matter (DM) in a greenhouse at 105 ° C, crude protein (CP), mineral matter (MM) by incineration at 550 ºC for a period of 4 hours. We also determined the fiber content in neutral detergent fiber (NDF), using α amylase heat-stable and acid detergent fiber (ADF). The total digestible nutrients content (TDN%) were obtained via equation [TDN,% = 87.84 - (0.70 x ADF)] (Queiroz and Silva, 2002). The experimental design was a randomized block design with six treatments and three repetitions. The collected data for each variable were subjected to variance analysis to compare means at the 5% significance through the SAS (2004).

Results and discussion The data of chemical composition and TDN are disposed in the Table 1. For the analyzed parameters: DM, NDF, ADF, CP, ASH, TDN statistical differences were observed among treatments ($p < 0.05$). It is observed that the differences in the composition of the silage, that is, corn grains rehydrated x total diet were observed

for fractions NDF, ADF, ASH and CP, being those parameters of interest during fermentation stages and during the storage and use phases, being able to influence the conservation. According to Kung and Whiter (2001) and Bernardes et al. (2007), materials rich in nitrogen change the production of organic acids, consequently the stability of the material and the composition, by exerting a buffering action, as well as the presence of some micro and macro minerals. About NDF and ADF, the values are similar to those found by Moura et al. (2014) and Pereira et al. (2012), for the treatments with CGR. For the treatments with inclusion of protein concentrate it can be noticed that the values are higher ($p<0.05$) than the treatments with CGR and yet differed among grain particle sizes. There was no statistical difference between the CGR treatments on protein composition and on the treatments with inclusion of protein concentrate. Jobim et al. (2007) evaluating the addition of protein sources (urea, sunflower and soybean) in the ensiling of CGR, observed differences in CP composition and also on the degradability when compared to corn grain silage with the addition of protein sources.

Table 1 Chemical composition and total digestible nutrients of corn grains and the total diet submitted to three processes, rehydration and silage

Parameter[2]	Corn grain rehydrated ensiled[1]			Total diet rehydrated ensiled			EPM
	CGR1	CGR3	GMR7	CGR1+C	CGR3+C	CGR7+C	
DM	62.0 AB	61.33 B	63.5 A	62.0 AB	63.5 A	62 AB	0.35
NDF	8.28 D	12.1C	9.88CD	19.74 B	22.23 B	26.56 A	1.31
ADF	2.18 C	2.29 C	2.42 C	5.11 B	6.20 A	6.07 AB	0.13
CP	5.70 B	6.09 B	5.67 B	10.86 A	11.30 A	11.83 A	0.07
ASH	0.94 D	0.99 D	0.94 D	3.66 B	3.03 C	4.19 A	0.01
TDN	86.3A	86.2A	86.14A	83.50 C	84.26 B	83.59 BC	0.06

[1]CGR1 - Corn grain rehydrated 1 mm; CGR3 - Corn grain rehydrated 3 mm; CGR7 - Corn grain rehydrated 7 mm; CGR1+C - Corn grain rehydrated 1 mm with commercial concentrate; CGR3+C - Corn grain rehydrated 3 mm with commercial concentrate; CGR7+C - Corn grain rehydrated 7 mm with commercial concentrate.
[2]DM – Dry matter; NDF – Neutral detergent fiber; ADF – Acid detergent fiber; CP – Crude protein; TDN – total digestible nutrients.
Means followed by differences letters, capital letters in the lines differ ($P<0.05$) by Tukey test; SEM = standard error of the mean.

Conclusions Total mixed rations ensiling affects the final composition of silage and these parameters may cause changes on fermentation processes of silage, even with changes and noticeable losses, this technique may enable practical benefits, related to storage, logistics, practicality and economy.

Impact of corn shredlage on ruminal NDF and starch digestion kinetics evaluated through varied in situ methods

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Keywords in situ, NDF digestibility, starch digestibility, corn shredlage

Introduction A novel method of harvesting whole-plant corn silage, corn shredlage (**SHRD**), allows corn silage to provide both physically effective fiber and greater degree of kernel processing. Recently, SHRD was reported to improve lactation performance and total tract NDF and starch digestibility by dairy cows compared with conventional-processed corn silage (**CPCS**). However, research evaluating ruminal digestion kinetics is still warranted. Therefore, the objective of this study was to evaluate ruminal in situ NDF and starch digestion kinetics of SHRD compared with CPCS through varied in situ methods. Our secondary objective was to determine relationships between ruminal in situ digestibility of DM and NDF or starch.

Materials and methods Samples from SHRD and CPCS were obtained from bunker silos located at the University of Wisconsin – Madison Emmons Blaine Dairy Research Center (Arlington, WI) after 4 months of fermentation. Both treatments were harvested using a self-propelled forage harvester fitted either with novel cross-grooved rolls (2.5 mm gap) and chopper set at 30 mm theoretical length of cut (**LOC**) or conventional rolls (3 mm gap) and 19 mm of LOC for SHRD and CPCS, respectively. Ruminal in situ NDF (6, 12, 24, 30, 48, 72 and 96 h) and starch (6, 12 and 24 h) digestibilities using Dacron polyester cloth bags (9 × 18 cm) containing 5 g DM of samples, incubated in duplicate in 3 cows. Three methods of samples preparation were used: 1) whole-sample not dried and unground, 2) grain or stover dried and unground, and 3) grain or stover dried and 6-mm ground. Grain and stover components were obtained through hydrodynamic separation. The in situ bags for the respective treatments for each time-point were placed in a nylon laundry bag (30 × 40 cm) and then positioned in the ventral rumen. Each laundry bag contained a blank bag to allow correction for any infiltration of DM into sample bags. Two bags for each treatment and sample preparation (0 h bags) were soaked for 30 min in warm water and washed with the rest of the sample bags. Data was fitted to a first-order exponential model with discrete lag using the PROC NLIN of SAS to calculate the soluble (**A**), potentially degradable (**B**) and undegradable (**C**) fractions, and rate of digestion ($\mathbf{k_d}$). Extent of digestion was calculated using the effective ruminal degradability equation (**ERD**) assuming a passage rate of 0.034/h and 0.146/h for NDF and starch, respectively. Data were analyzed for each method of sample preparation separately using PROC MIXED of SAS and the model included treatment as fixed effect and cow as a random effect. Ruminal in situ NDF and starch digestibility data were analyzed using PROC MIXED of SAS and the model included treatment, hour and treatment by hour interaction as fixed effects and cow as a random effect. Relationships between ruminal DM digestibility and ruminal NDF or starch digestibility were determined using Proc REG of SAS.

Results and discussion Particle size (26.3% vs. 4.5% of particles retained on a 19 mm screen of Penn State Shaker Box Separator) and corn silage processing score (71.6% vs. 65.0% of starch passing through a 4.75 mm screen) were greater for SHRD than CPCS. Ruminal NDF digestibility was greater ($P = 0.001$) for SHRD than CPCS at 12, 24, 30, 48 and 96 h when whole-samples not dried and unground were used, but not for both methods with dried samples ($P > 0.10$). Fraction A and k_d of NDF did not differ ($P > 0.10$) independently of sample method used. However, fraction B was greater ($P < 0.05$; 4.2-% units on average) and C lower ($P < 0.05$; 4.0-% units on average) for SHRD than CPCS when whole-samples not dried and unground or stover samples dried and 6-mm ground were used, but did not differ ($P > 0.10$) for stover dried and unground. The NDF ERD was greater ($P = 0.001$; 61.3% vs. 57.5%) for SHRD compared with CPCS when whole-samples were used, but not ($P > 0.10$) with the other methods. A strong positive relationship was observed ($P = 0.001$; $R^2 = 0.99$) between ruminal DM and NDF digestibilities for both methods of stover samples. Ruminal starch digestibility was greater ($P < 0.05$) for SHRD than CPCS independently of sample method but at different time-points. Starch digestibility was greater ($P = 0.001$) at 6 and 24 h but lower ($P = 0.001$) at 12 h when using whole-samples; greater ($P = 0.01$) at 6, 12 and 24 h when using grain samples dried and unground; and greater ($P = 0.02$) at 12 and 24 h for grain samples dried and 6-mm ground. Fraction A was greater ($P = 0.01$; 0.49% vs. 0.0%) for SHRD compared with CPCS when whole-samples were used, however, no differences were observed ($P > 0.10$) on fractions B and C, k_d, or ERD (89.7%, 10.1%, 0.19/h, 51.4% on average, respectively). Fraction A was unaffected by treatment ($P > 0.10$) for neither grain sample methods. However, fraction B was greater for SHRD than CPCS when grain unground ($P = 0.08$; 93.5% vs. 89.4%, respectively) and grain 6-mm ground samples were used ($P = 0.03$; 97.4% vs. 85.9%, respectively). In contrast, fraction C was 4.1%- and 2.0%-units lower for SHRD than CPCS for both grain unground ($P = 0.08$) and 6-mm ground ($P = 0.03$) samples. Starch k_d tended ($P = 0.07$) to be greater for SHRD compared with CPCS when grain dried and 6-mm ground samples were used, but not when using grain dried and unground samples. Starch ERD, however, was greater ($P = 0.05$; 62.5% vs. 59.4%, respectively) for SHRD compared with CPCS only when using grain samples dried and unground. Ruminal digestibilities of DM and starch were positively related ($P = 0.001$; $R^2 = 0.95$) for both grain sample methods.

Conclusions Digestibility of NDF was greater for corn shredlage compared with conventionally-processed corn silage only when whole-samples not dried and unground were used. Despite the strong relationship between DM and NDF ruminal digestibilities, the use of stover fractions for evaluation of NDF digestibility merits further studies. Starch digestibility, however, was greater for SHRD independently of in situ method. These data suggest that degree of kernel processing provided by shredlage rolls is advantageous for dairy farmers. In addition, similarity of results between in situ methods combined with the strong relationship between ruminal DM and starch digestibilities suggest that the use of grain samples dried and unground are a feasible alternative to determine ruminal in situ starch digestibility of corn silage.

Corn silage processing: Dairy farm survey

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Keywords kernel processing, harvesting practices, shredlage

Introduction Digestibility of starch in whole plant corn silage (**WPCS**) is influenced primarily by kernel processing and length of storage. Starch digestibility is important because about half of digestible energy of WPCS comes from the starch provided by the grain fraction. Varied types of kernel processors have been implemented on self-propelled forage harvesters (**SPFH**) being used on farms and thus may affect degree of kernel breakage and corresponding starch digestibility. Additionally, there has been a lot of recent interest, especially with the feeding of higher WPCS diets, about setting the SPFH for a longer theoretical length of cut (**TLOC**) with aim of increasing physically effective fiber of WPCS. The use of shredlage processors may allow for both increased degree of kernel processing and increased TLOC and thus its use has been increasing. Therefore, the objective of this survey was to evaluate WPCS samples harvested using a Shredlage® processor and to summarize the harvesting practices adopted by the farmers.

Material and methods Forty-six WPCS samples were obtained from 41 dairy farms during farm visits April to August 2014. Farms were located in Minnesota (n = 2), Wisconsin (n = 38), and Illinois (n=1). One sample was collected from each farm except for 5 farms that were feeding from two silos at the time of our visit. The samples were collected from the pile that had been shaved from the exposed face for feeding. Dry matter (**DM**) content was determined on WPCS samples by drying at 60°C for 48 h in a forced-air oven. Dried samples were then ground thru a 1-mm Wiley Mill screen for determination of starch content by near-infrared spectroscopy. As-fed samples were used to determine particle size distribution using the Penn State manual shaker box (**PSU-SB**) with 3 sieves and a pan (Kononoff et al., 2003). Also, as-fed samples were sieved mechanically using the Wisconsin Oscillating Particle Separator (**WI-OS**) to determine mean particle length (**MPL**; ANSI, 1998). Corn silage processing score (**CSPS;** Ferreira and Mertens, 2005) was determined on dried samples. During each farm visit a survey questionnaire was completed to assess harvesting practices.

Results and discussion Bunkers (93%) and inoculants (90%) were used by most farms. Corn hybrids were solely dual-purpose type for 46.3% of the farms. Most farmers reported a 22-26 mm TLOC (90%) and a 1.5-2.5 mm roll gap (69%). There was no attempt to verify the reported settings on the equipment. The average DM and starch contents were indicative of high-quality Midwest-USA WPCS, the range among farms was wide for both at 21.5%-units and 25.5%-units for DM and starch, respectively. This likely reflects the challenges of weather conditions and harvest scheduling. The average percentage retained on the top PSU-SB sieve for shredlage was substantially lower than feeding trial (20% versus 32%; Ferraretto and Shaver, 2012a). The ranges for PSU-SB top sieve, PSU-SB top 2 sieves, and WI-OS MPL in the samples were 32%-units, 21%-units, 6 mm, respectively. Thirteen samples (28%) were classified as excellent and 33 samples (72%) as adequate for kernel processing based on CSPS measurements (Excellent: >70%, Adequate: 50%-70%, Poor <50%). A survey of commercial testing labs over 2005 to 2012 (Ferraretto and Shaver, 2013) showed a high percentage of WPCS samples categorized with poor

processing (up to 42%) and a low percentage of samples categorized with excellent processing (7 to 17%).

Conclusions The physical form (PSU-SB, MPL, and CSPS) and DM results indicate considerable opportunity to improve WPCS quality by reducing variation through better process control during harvest for the shredlage processor.

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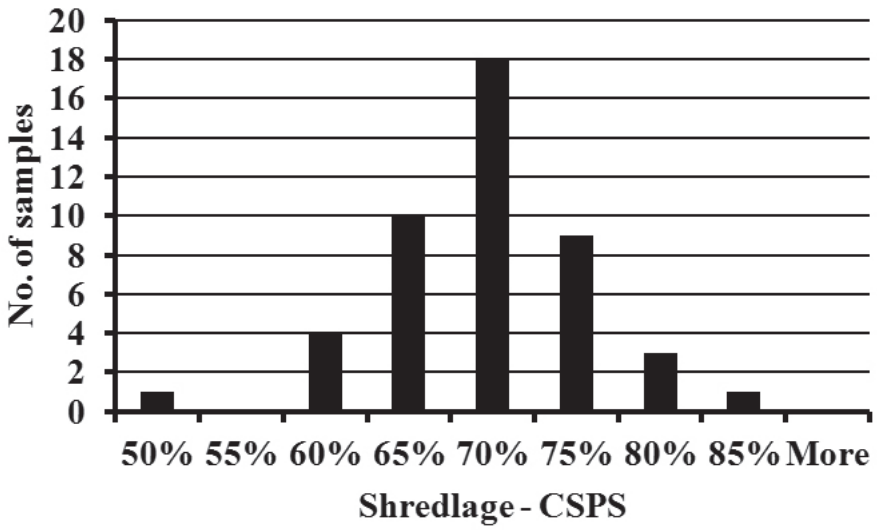

Figure 1 Frequency distribution for CSPS on all samples.

Table 1 Survey descriptive statistics for WPCS dry matter % (DM; as-fed basis) and starch % (DM basis), as-fed % retained on top (19 mm) and top 2 (8 and 19 mm) sieves of PSU-SB, MPL (mm) on as-fed samples with the WI-OS, and CSPS on dried samples using a Ro-Tap shaker (% starch passing thru a 4.75 mm sieve)

Item	DM %	Starch %	% Top sieve	% Top 2 sieves	MPL (mm)	CSPS %
Mean	34.5%	33.6%	19.6%	75.7%	11.9	67.2%
Stdev	4.0%	6.2%	7.8%	5.4%	1.4	5.9%
Min	25.6%	17.1%	7.2%	65.1%	9.0	49.5%
Max	47.1%	42.6%	39.9%	85.9%	14.8	82.7%

Prolonged storage period increases the starch degradability of flint corn silage

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Keywords corn silage, length of storage, prolamin, proteolysis, starch degradability

Introduction Although silage pH typically drops and stabilize within a week, periods between three weeks and one month have been recognized as being suitable for stabilizing the fermentation. Nonetheless, several studies reported that the fermentation proceeds for prolonged periods, resulting in increased levels of soluble protein (SP) and ammonia. This proteolytic activity has been associated with higher availability of starch granules and, consequently, higher starch digestibility. Thus, the objective of this study was to evaluate the effects of microbial additives on the nutritional quality of flint corn silage stored for almost two years.

Materials and Methods Whole corn plants (hybrid Pioneer 30F90 Bt) were harvested at 40.3% dry matter (DM), chopped (without kernel processing), and packed in lab-scale silos (20 L buckets). Microbial inoculants were applied as follows: Control – no additives, *Lactobacillus buchneri* DSM 13573 (applied at 1×10^5 cfu/g fresh forage), and *Lactobacillus plantarum* + *Enterococcus faecium* + *Pediococcus acidilactici* + cellulolytic and hemicellulolytic enzymes (applied at 1×10^5 cfu/g of fresh forage). Silos were stored for 3, 7, 15, 30, 60, 210, 390, 480, and 570 days. In each scheduled date, 9 silos were opened. Samples were oven dried and analyzed for chemical entities and another one was frozen at −20°C for the *in situ* assay. The content of prolamin was measured according to Nellis et al. (2013), whereas the content of SP was determined in borate-phosphate buffer (Krishnamoorthy et al., 1982). The starch content was evaluated according to Hall (2009). At the end of the storage period, frozen samples (70 g fresh matter) were thawed, allocated in macro-bags (20 cm × 40 cm) and positioned into the rumen ventral sac for 24 h, in two cannulated non-lactating cows fed a diet containing maize silage (60 % DM) and concentrates (40% DM). After incubation, bags were placed into cold water to stop the fermentation and washed in a machine by five cycles. Then, bags were dried in an oven at 60°C for 72 h and starch degradability was calculated as: DEG (%) = 100 × [Initial strach (g) − Residual starch (g)]/ Initial starch (g). Data were analyzed using the Mixed procedure of SAS.

Results and Discussion Due to the lack of responses to additives, only storage length will be issued on this report. The concentration of SP increased ($P < 0.01$) continuously throughout the storage period ranging from 22.7 to 52.3% (crude protein basis) for the silage stored for 3 and 570 d, respectively. Contrary, the content of prolamin decreased ($P < 0.01$) across the storage from 2.3 to 1.1% (DM basis) (Figure 1), and the ruminal degradability of starch steeply increased ($P < 0.01$) across the storage period (Figure 2). In addition, a positive correlation ($R^2 = 0.83$; $P < 0.01$) was found between SP and DEG24-starch. The increasing levels of SP reflected increases in starch degradability, probably due to the proteolysis of the protein matrix that surrounds the starch granules. The DEG24-starch was negatively correlated to the prolamin concentration ($R^2 = 0.42$; $P < 0.01$). Therefore, prolonged length of storage is a strategy to increase the starch available to be degraded in the rumen. Kung et al. (2014) noted that in silages with high dry matter concentrations, the fermentation process is slower compared to that of materials with a lower dry matter concentration, reflecting the need for a greater fermentation time to allow proteolysis in the starch-protein matrix and consequently better animal performance.

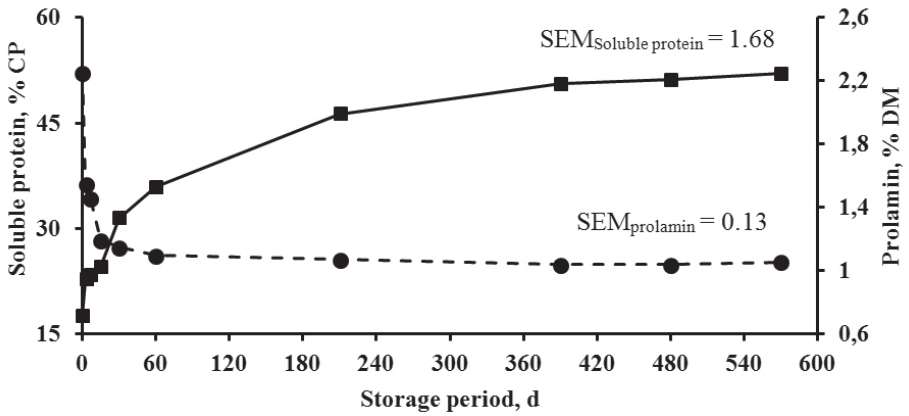

Figure 1 Content of soluble protein (■) and prolamin (●) in corn silages across the storage period.

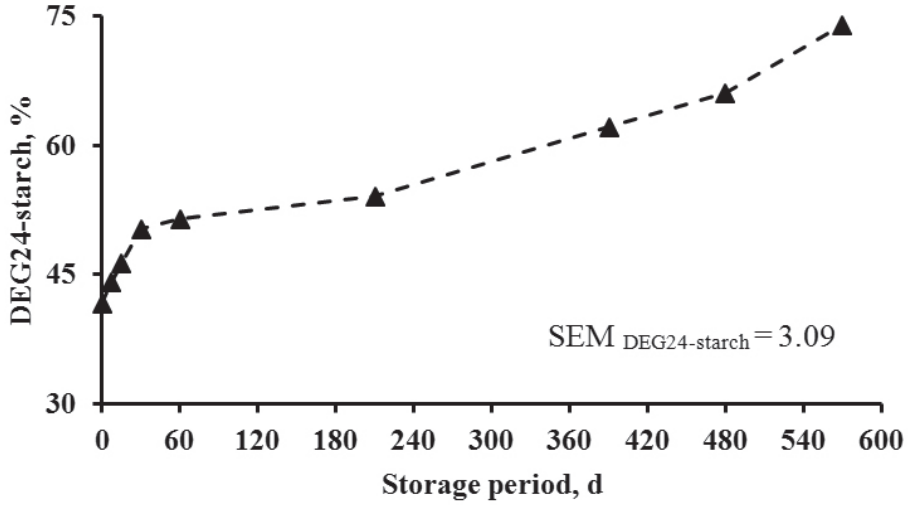

Figure 2 *In situ* ruminal degradability of starch during 24 h (DEG24-starch) in corn silage across the storage period.

Conclusion The ruminal degradability of starch steadily increased throughout the storage period of flint corn silage.

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Effects of length of ensiling and variety on chemical composition and *in vitro* ruminal degradation of whole-crop maize

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Keywords fermentation quality, *in vitro*, maize silage, protein quality, starch degradation

Introduction Maize silages as a major part of ruminant rations are usually stored anaerobically for a period of 1 to 12 months before being fed. Proximate constituents do not change in concentration markedly once the main fermentation process is finished yet inconsistency in literature exists about potential continual changes in ruminal starch degradation and protein quality. Especially for silages with high dry matter (DM) concentrations (> 400 g/kg), starch degradability was reported to increase with time of storage (Dorenbroos and Van Laar, 2014), whereas other studies observed no influence of storage length (Cone et al., 2008). Decreases in protein quality as a result of prolonged storage are known from grass silages but have not been studied in detail in maize silages. Both changes in starch degradation as well as in protein quality may require changes of diet composition throughout the feeding period. The aim of this study was to evaluate the effect of storage length of different silage maize varieties on chemical composition, protein quality and *in vitro* ruminal degradation of nutrients.

Materials and methods Nine varieties (three maturity groups, DM 343 ± 5.6 g/kg) of whole-crop maize were harvested at two dates in September 2013, chopped and ensiled octuple in laboratory-scale silos. Each variety was sampled at harvest and after 30, 60, 90 and 120 days (d) of ensiling. Silages were then freeze-dried and analyzed for chemical composition (proximate constituents, fermentation products and pH), starch, non-protein N (NPN) and NH_3-N. Each sample and its neutral detergent fibre (NDF) fraction were incubated in the Hohenheim gas test system. *In vitro* gas production was measured after 0, 2, 4, 8, 12, 24, 36, 48, 72 and 96 h of incubation. Gas production of the neutral detergent solubles (NDS) fraction (mainly starch) was calculated using a curve subtraction method (Schofield and Pell (1995). Data was analyzed using SAS 9.3. Gas production dynamics over time were estimated using a non-linear regression equation. For these parameters and chemical constituents, a 2-factorial analysis of variance (storage length, maturity group and their interaction) using the general linear models procedure was conducted. Separation of treatment means was accomplished using the Tukey-Kramer procedure ($P \leq 0.05$).

Results and discussion After 30 d, all silages were well fermented with a low pH and a lactic acid dominated fermentation profile. Butyric acid was not detected. Most fermentation products and proximate constituents only changed until 30 or maximum 60 d of ensiling (Table 1). Concentrations of ethanol showed a strong correlation to total ethyl ester concentration (r = 0.9, $P<0.001$), and both did not change with length of storage.

The rate of *in vitro* gas production from NDS increased as a result of the ensiling process from 0.070/h (unensiled) to 0.087/h at d 30, but after 60 d no further increase occurred. In general, only few changes concerning the *in vitro* nutrient degradation were detected after the first 30 d of ensiling. Ensiling per se increased the ruminal degradability of the NDS fraction, but there was no further increase caused by a prolonged duration of storage. However, extensive changes in protein quality occurred continually with an increase in NPN and NH_3-N compounds from 0 to 120 d of storage, indicating continual protein and amino acid degradation.

Table 1 Influence of ensiling period on chemical composition, protein quality and ruminal degradation of neutral detergent solubles (NDS) in nine varieties of silage maize [g/kg dry matter (DM) unless stated otherwise]

	Length of storage (days)					
	0	30	60	90	120	SEM
Dry matter (g/kg)	342	346	346	342	344	3.6
Crude protein	73.4^{a}	73.6^{a}	72.9^{a}	72.0^{a}	65.8^{b}	1.12
Starch	355	358	358	355	368	7.5
pH	4.85^{a}	3.94^{b}	3.92^{b}	3.96^{b}	3.88^{b}	0.25
Lactic acid	0.0^{c}	58.7^{b}	62.6^{b}	66.3^{a,b}	73.4^{a}	1.81
Acetic acid	2.59^{b}	10.2^{a}	10.1^{a}	11.3^{a}	11.4^{a}	0.65
Ethanol	0.31^{b}	3.70^{a}	4.81^{a}	5.11^{a}	4.89^{a}	0.37
Ethyl lactate (mg/kg DM)	0^{c}	130^{b}	158^{a,b}	161^{a,b}	176^{a}	8.9
Ethyl acetate (mg/kg DM)	0^{b}	229^{a}	236^{a}	204^{a}	159^{a}	22.8
Water-soluble carbohydrates	79.8^{a}	6.9^{b}	10.4^{b}	12.8^{b}	13.5^{b}	2.24
Non-protein N (NPN, g/kg N)	209^{d}	474^{c}	521^{b}	648^{a}	676^{a}	83.0
Ammonia-N (NH_3-N, g/kg N)	18.3^{d}	78.1^{c}	91.9^{c}	117.2^{b}	145^{a}	2.1
Rate of gas production from NDS (/h)	0.070^{c}	0.087^{b}	0.091^{ab}	0.092^{a}	0.091^{ab}	0.0013
Metabolizable energy (MJ/kg DM)	10.6^{b}	10.8^{a,b}	10.9^{a}	11.0^{a}	10.9^{a}	0.07

^{a,b} means within a row having different superscripts differ significantly ($P<0.05$)

Conclusions When ensiling whole-crop maize with recommended DM contents (280-350 g/kg) and observance of a minimum length of ensiling of 60 d, little to no further changes in nutrient degradation occur with increased length of storage. On the other hand, a continuous increase in NPN and NH_3-N fractions was observed which decreases silage protein quality for ruminants and should therefore be considered in formulation of diets.

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A meta-analysis of the effects of length of storage on starch digestibility and aerobic stability of corn silages

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Keywords aerobic deterioration, ensiling, fermentation, protein matrix

Introduction Corn silage is the main forage source in Brazilian dairy farms (Bernardes and do Rêgo, 2014). Although silage pH stabilizes within a week, several studies have reported increased starch digestibility across prolonged storage periods (Newbold et al., 2006; Kung, 2013). Furthermore, enhanced aerobic stability would be expected, since a higher concentration of acetic acid and lower count of yeasts have been observed in silages stored for long periods (Kung, 2013). However, there is no practical recommendation for a minimal storage period that would results in silages with improved nutritive value. Thus, the aim of this meta-analysis was to recognize a 'minimal' period of storage that would enables gains in both starch digestibility and aerobic stability.

Materials and methods A meta-analysis based on published experiments with corn silage was conducted to study the effects of length of storage on the starch digestibility and aerobic stability. For the starch digestibility measured as in vitro-7h, in vitro-12h, in situ-3h or in situ-12h, the data set included a total of 95 treatment means from 8 studies; whereas for the aerobic stability (time until silage temperature reached 2°C above ambient temperature) a data set comprising 96 treatment means from 12 studies was collected. Data were analyzed using the NLMIXED procedure of SAS with a random study effect (St-Pierre, 2001). Studentized residuals with absolute values exceeding 3 were considered outliers and deleted. Additionally, adjusted data for random study effect were modeled with segmented (broken-line) regressions (Robbins et al., 2006). Those approaches have practical claims because a field recommendation may be obtained (break-point). The fitness of the models was assessed by the Akaike's information criterion (AIC), root mean square error (RMSE) and R^2-adjusted.

Results and discussion Although the number of studies comprising the current meta-analysis was not abundant, the data set was broad and displayed a large variation in corn genotypes and storage periods (0 to 570 d). Among the tested models for starch digestibility, a broken-line regression with the first segment linear followed by a second segment linear had the best fitness. Thus, the starch digestibility increased 0.28 percentage units per day, during the first 30 d of storage. Afterward, the starch digestibility still enhancing over time, but the rate of increase lowered approximately 8 times in comparison with the first segment (e.g., 0.033 percentage units per day) (Figure 1). For the aerobic stability, the best model was a broken-line regression with the first segment linear followed by a plateau. Thus, the aerobic stability increased 0.4 h per day until 113 d of storage, when reached a plateau (Figure 2).

Conclusion The starch digestibility and aerobic stability of corn silage increase along the storage period. Based on the current meta-analysis, corn silage should be stored for at least 1 month to obtain improvements in starch digestibility. However, if the aim is to maximize

aerobic stability and enhance starch digestibility, the corn silages should be fermented for at least 4 months.

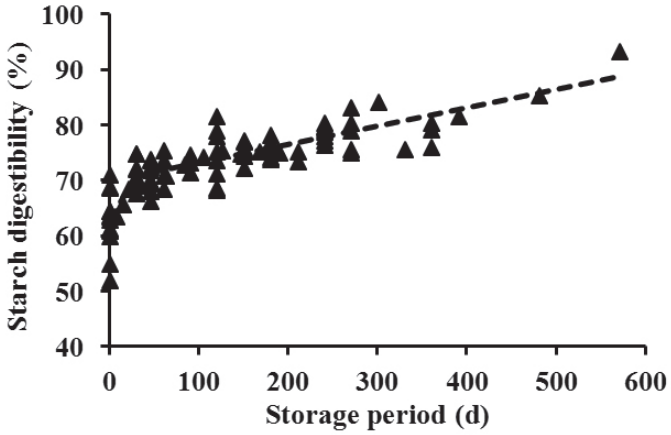

Figure 1 Starch digestibility (D_{starch}) of corn silages across the storage period (t), including a random effect of experiment. If $t < 30$, $D_{starch} = 62.7 + 0.28 \times t$; if $t \geq 30$, then $D_{starch} = 71.0 + 0.033 \times (t - 30)$; $P < 0.01$; RMSE = 2.73.

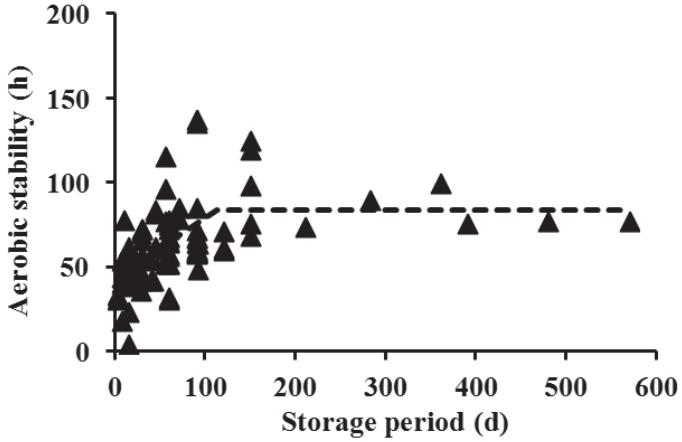

Figure 2 Aerobic stability (AS) of corn silages across the storage period (t), including a random effect of experiment. If $t < 113$, $AS = 83.5 - 0.36 \times (113 - t)$; if $t \geq 113$, then $AS = 83.5$; $P < 0.01$; RMSE = 10.8.

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Use of thermographic images for evaluation of superficial and subsurface temperature in the exposed area of corn silos

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Keywords temperature, thermography, silage, corn, aerobic spoilage

Introduction From total dry matter losses occurring in silage corn, more than 40% occur at the time of extraction and supply, the aerobic degradation in extraction and supply stage produces losses in quantity and quality. The temperature of the exposed area of the silo, compared with the ambient temperature can be indicator of the aerobic deterioration. Thermography is a passive measurement in real time, without contact with the object. The thermographic images show only the temperature distribution on the object surface, and do not show the temperature *inside* the object or through of it (López Jimeno, 2011). The thermography consists in capturing the infrared radiation of the electromagnetic spectrum, which allows you to convert the energy radiated in temperature information. The thermographic camera management requires study and training in the interpretation of images, the image alone is not sufficient to diagnose, but requires of fieldwork, direct visualization of the area and measurements with complementary elements (thermometers). Thermography technique was evaluated with the objective to identify possible aerobic deterioration areas in bunkers of corn silage and to determine whether thermographic images of the front surface exposed to air correlate with the subsurface of the silage exposed (5 and 20 cm deep). *"Silage heating may be evident at the feed out face but more likely it is more evident at a distance behind the feed out face where active aerobic deterioration is occurring. Thus temperature measurements at the feed out face surface may be deceiving. Oxygen has been measured up to 90 cm behind feed out faces in well-packed*" (Holmes et al. 2012).

Materials and methods In the province of Córdoba and Santa Fe (Argentina), 20 silos were sampled over their exposed fronts, the silo front was considered from 0 cm to 20 cm deep. The silages had more than 100 days since were made, and these were open in use stage. The temperature was taken by thermography camera (TESTO 875i) at 0 cm deep, and through digital contact thermometers at 0 cm, 5 cm and 20 cm deep (Kung, 2010) in 6 points of each silo; ambient temperature was also recorded. The thermographic images were analyzed by Testo IRSoft 3.2 software, these images were decoded and expressed in spreadsheets. The information was evaluated using Pearson correlation with Infostat 2011 (Di Rienzo, 2011) between the temperatures of the different depths of the front, and the ambient temperature; and the correlation between temperatures at 0 cm and the temperature at 20 cm deep. Moreover, the difference between the ambient temperature and the temperature of the different depths (0, 5 and 20 cm) was calculated to determine the aerobic stability status that was considered as it had lost its aerobic stability when the silage showed 2°C over ambient temperature (Muck, 2002).

Results and discussion The ambient temperature was positively correlated with the temperature at 20 cm ($r = 0.50$, $p = 0.0003$), with the temperature at 5 cm ($r = 0.90$, $p < 0.0001$), and the temperature at 0 cm ($r = 0.95$, $p < 0.0001$). It shows that the temperature at 0 and 5 cm is determined by the ambient, while the temperature at 20 cm is influenced by the ambient, but it is also influenced by other factors different to ambient temperature. The temperature at 0 cm was correlated significantly and positively with the temperature at 20 cm ($r = 0.55$, $p < 0.0001$). This indicates that the temperature at 0 cm, which was taken with thermographic camera, did not identify the thermal variance in the silo subsurface area. Therefore, the thermographic images (0 cm) would not provide accurate information to identify aerobic spoilage sites. Finally, 80% of the silos that were sampled were over 2° C above ambient temperature in the 20 cm deep, this could indicate a possible aerobic degradation.

Table 1 Ambient temperature and temperature in different depth of the silage front (ºC)

Position	Mean	±SD
0 cm	23.45	9.32
5 cm	23.17	7.34
20 cm	31.29	8.07
Ambient	24.75	8.15

Conclusions The temperature at 0 cm had low correlation with the temperature at 20 cm depth, therefore the thermographic images would not represent what happens inside the silo. The ambient temperature had high correlation with temperature at 0 and 5 cm depth, therefore, to determine subsurface temperature by thermographic images; it should explore the front at least 20 cm deep before taking the picture. This means remove 20 cm of the entire silage front. Thermography is a complementary tool to other methods to give a "visual" representation, and can help identify areas of the silage with aerobic degradation. The use of this technology requires training and knowledge.

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On-farm corn silage investigation: multi-analysis on silage practices, silage quality and its effect on density

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Keyword corn silage, silage quality, silage practices, density

Introduction Poor silage quality has a major impact on production costs, especially when it represents more than 50% of the daily intake for ruminants (Paragon et al., 2004). Silage practices, from harvest to bunker management, greatly influences the forage quality and thus the profitability of the farms. A silage investigation kit [a toolbox and its accompanying software, Corn Silage Investigation (CSI)] was designed to assess silage quality on one hand and silage practices on the other hand. This information enables us to define areas for improvement (Andrieu et al., 2012). The objective of this article is to combine the results of different surveys made using the CSI made in Europe into one multi analysis.

Materials and methods During the springs and summers of 2012 and 2013, corn silage on 149 dairy farms located in France, Italy and Greece were investigated using the standardized "CSI" method (Andrieu et al., 2012). One part of the survey collected information on the harvest practices, whilst the other part investigated the silage quality, based on measurement of the silo density (using a drilling core sampler of 747 cm^3, QUAS SRL, Italia) at 6 points in the silo face and silage particle size by using the Penn State Forage Particle Separator.

Results and discussions Results from this study show that on average the density of silos are lower than the optimal target of 240 kg DM/m^3.Only 36% of all the silos were correctly packed - (average of 6 sampling points ≥ 240 kg DM/m^3) (Figure 1). This confirms the previous observation made by Holmes (2006).

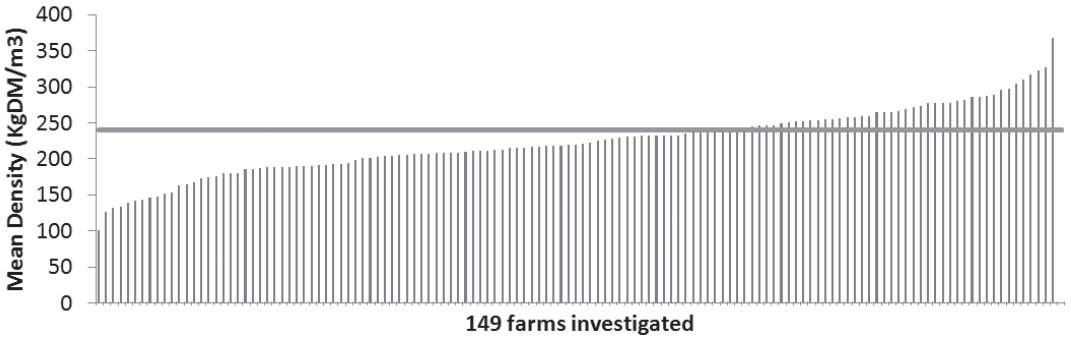

Figure 1 Silage density (kg DM/m3) measured on different farms.

The compaction method of the silo, horizontal vs. progressive wedge layers, has a significant impact on mean density (232 ±46 kg DM/m^3 vs 212±53 kg DM/m^3, P<0. 05). This is in opposition to common beliefs, although it should be noted that results of the effect of this parameter are not consistent between researches (Muck and Holmes, 2000). The type of silo also has an influence on the mean silage density. Both its design and its height are correlated to the average density. Data from this study (Figure 2) confirms

findings from d'Amours and Savoie (2005) namely that an increased silo height results in a higher density (P<0.05). This can be explained due to the self-compaction of the mass. Bunker-type silos have significantly higher densities compared with drive over piles (237.7 kg DM/m3 vs 184.6 kg DM/m3, P <0.05).

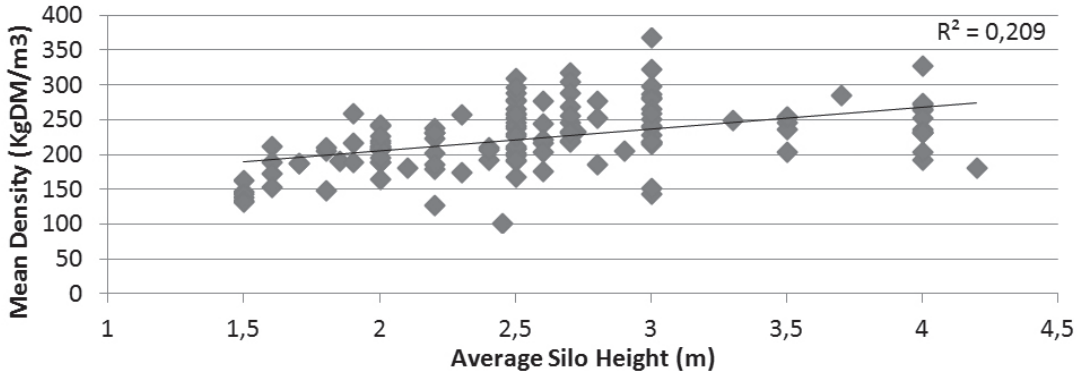

Figure 2 Silo density correlated to the silo height.

The DM density of the bunkers increased with the DM content of the corn silage (P<0.05, r^2=0.245), as also found by Muck et al. (2000). Silage particle size also influences the compaction potential of the silage at ensiling. The percentage of big particles (>19 mm) seems especially important. Among the 149 farms investigated, a significant negative correlation was observed (P<0.05; r^2=-0.131) between the presence of large particles and density. The feeding out method/equipment can also influence the silo face densities. In the present study, a correlation was found between front face densities and the type of cutter used. Use of a rotary cutter compared to a loader resulted in higher average silos densities (235.8 ±40 kg DM/m^3 vs 209.7 ±41 kg DM/m^3 respectively, P<0.05). Similar results were recorded by D'Amours and Savoie (2005). This implies that the upward movement associated with a loader tends to decrease the density on the front of the bunker. This may potentially degrade forage value and quality.

Conclusions A multi-analysis of surveys performed in 3 European countries on a total of 149 corn silos, revealed that silo densities are quite low with only 36% of the bunkers being packed correctly (>240 kg DM/m^3). High densities are obtained when good silage practices (compaction method, silage particle size) are combined with the correct silo parameters (silo design, silo height). The use of proper defacing equipment is also of importance in order to maintain good front face density.

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Effects of different harvest and storage methods on the quality of forage mixtures

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Keywords silage, haylage, Caramba, Berseem clover, vetch, triticale, forage quality

Introduction In forage harvesting, different techniques are used such as drying hay, direct cutting silage and haylage. Many researches have been done to investigate the quality of the forage. But, the numbers of the studies which examine the harvest-storage techniques and forage mixtures collectively are limited. This research was conducted to determine the effects of different harvesting and storage methods on the quality of legume and grass mixtures.

Material and methods Two forages mixtures namely, vetch-triticale (*Vicia sativa* L. -*Triticasecale wittmack*) and caramba-berseem clover (*Lolium multiflorum* cv Caramba - *Trifolium alexandrinum* L.) were used as material in the experiment. Mixture rates were 70% and 30% for vetch-triticale and 50% and 50% for caramba-berseem clover, respectively. Plants were harvested at the end of the flowering stage. The harvesting and storage systems investigated in the research were given in Table 1. All bales were wrapped with 0.025 mm plastic film in white color as four layers. The tests were conducted in three replications.

Table 1 Harvesting and storage systems investigated

System code	Machines used in harvesting	Storage technique
S1	Mower + round baler	Dry hay
S2	Disc mower + round baler	Dry hay
S3	Mower + round baler + wrapping machine	Haylage
S4	Disc mower + round baler + wrapping machine	Haylage
S5	Silage machine	Silage (Traditional)

The quality of the material was evaluated in terms of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and relative feed value (RFV). The dry matter (DM) content of plants was determined by drying to constant weight at 105 °C according to the ASAE standards. Nitrogen (N) content was measured using the Kjeldahl method. The pH values of plants were obtained as reported by Chen et al. (1997). The CP was calculated as N×6.25 (AOAC, 1990). The NDF and ADF were determined as suggested Van Soest et al. (1991) by using ANKOM fiber analyzer. The RFV was calculated by using Equation 1 given below (Mayouf and Arbouche, 2014).

$$\%RFV=(88.9-(0.779\times\%ADF))\times((120/\%NDF)/1.29) \quad (1)$$

The results were evaluated as fed by using standards assigned by Hay Market Task Force of American Forage and Grassland Council (Mayouf and Arbouche, 2014). The systems were compared with JMP Statical (Version 7) software.

Results and discussion The test results were presented in Table 2. Since, the CP content and RFV are used commonly for evaluating forage quality, the results are preferably discussed from this point of view. Dry matter content influenced the forage quality in

Haylage. This situation can be observed in S3 and S4 systems. If the mixtures were analyzed together, when the dry matter content decreased, the CP content and RFV also decreased. It was found that all systems investigated in this work have significant effect on quality factors for vetch-triticale mixture. Higher CP values obtained for S3 and S5 systems and there were no significant differences in between them. The RFV was the highest for S3 system and evaluated as the first quality according to the standards. For caramba-berseem clover mixture, the systems have no significant effects on the CP. Moreover, S3 and S5 systems have the highest RFV which was evaluated in the same group statically. These values were obtained as second quality according to the standards. Comparing the forage mixtures, there is no significant effect in terms of pH, ADF, NDF and RFV statically. However, caramba-berseem clover mixture has higher CP values than vetch-triticale mixture (Table 3).

Table 2 Effect of harvesting and storage systems on the quality of forage mixtures

	Vetch-Triticale mixture						Caramba-Berseem Clover mixture					
	DM** (%)	pH** (%)	CP** (%)	ADF** (%)	NDF** (%)	RFV**	DM** (%)	pH** (%)	CPns (%)	ADF* (%)	NDF** (%)	RFV**
S1	89.0^{a}	5.77^{a}	6.70^{c}	41.54^{a}	56.42^{a}	93.19^{c}	88.6^{a}	6.02^{a}	13.55	39.86^{a}	54.80^{a}	100.16^{c}
S2	89.3^{a}	5.74^{a}	7.91^{bc}	38.26^{b}	48.11^{b}	114.26^{b}	88.6^{a}	5.73^{ab}	13.01	37.38^{ab}	53.84^{ab}	103.59^{bc}
S3	54.6^{b}	5.85^{a}	10.18^{a}	30.03^{c}	46.45^{b}	131.17^{a}	42.6^{b}	6.10^{a}	13.78	33.80^{c}	50.26^{b}	115.79^{a}
S4	57.6^{b}	5.57^{a}	9.27^{ab}	31.33^{c}	48.70^{b}	123.21^{a}	39.3^{c}	5.25^{b}	12.49	34.86^{bc}	53.19^{ab}	107.94^{b}
S5	29.6^{c}	4.26^{b}	10.21^{a}	41.76^{a}	57.89^{a}	90.95^{c}	28.3^{d}	4.28^{c}	13.77	39.86^{a}	45.33^{c}	118.66^{a}

**Significant at 0.01 probability level; * Significant at 0.05 probability level; ns Non-significant
Values with different letters are significantly different according to the multiple range tests

Table 3 Comparing of mixtures in terms of forage quality

	pHns (%)	CP** (%)	ADFns (%)	NDFns (%)	RFVns
Caramba-Berseem Clover mixture	5.48	13.32a	36.85	51.48	109.23
Vetch-Triticale mixture	5.44	8.86b	36.59	51.51	110.55

**Significant at 0.01 probability level; * Significant at 0.05 probability level; ns Non-significant
Values with different letters are significantly different according to the multiple range tests

Conclusions The results showed that both forage mixtures provide satisfactory forage quality. Considering CP values, it can be said that the caramba-berseem clover mixture can be alternative forage instead of vetch-triticale mixture in Turkey conditions. From the point of storage technique, Haylage comes to the fore. However, during tests, it was observed that some plants which has woody structure such as triticale is less suitable for wrapping. Because of decreasing time needed, reducing the loss of leaves, simplifying the transportation, haylage can be considered more commercial than the other techniques.

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Plastic container a practical silo for small silage producer in Malaysia

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Keywords silo type, aerobic stability, spoilage

Introduction Silage making has been practiced in Malaysia since the 60's, but at small scale and using manual methods. Since the 80's silage production has become a popular option among farmers to provide conserved forage for ruminants. Various types of silos have been used for silage production depending on the availability of facilities and the animal numbers. The use of plastic container is preferable compared to the other types of silos such as bunker, stack, wrapped bale and bag. They are convenient to fill, pack and seal, are easy to handle, and can conveniently be used when feeding out. Unlike plastic bags or plastic film wrappings, the container cannot be gnawed through by rats, which would cause spoilage (Chin, 2002). The plastic container of either 100 or 128 L capacity, are the recycled containers from the chemical industry. They have to be washed and cleaned before use for silage fermentation. The invention of the OTOSIL®, a silage production machine comprising of a chopper, a compactor and an inoculants injector has enhanced silage production using plastic container in Malaysia (Hussin, 2015). An experiment was conducted to compare the quality and aerobic stability of silage produced using plastic container with other types of silo.

Materials and methods Ninety-day-old corn of Super Silage variety was harvested using forage harvester and packed into four types of silos: i. Plastic container, ii. Mini bunker, iii. Well Silo, and iv. Silosil. The plastic container is a cylindrical polyethylene container with capacity of 120 L with a lid that can seal the container with the aid of a rubber ring. The chopped corn was packed into the plastic container and closed with the lid and finally tightened with a metal clip. The concrete mini bunker consists of a concrete structure 304 cm x 60 cm dimension. It has two open end and two side 90 cm concrete wall. The chopped corn was placed and packed on a layer of polyethylene plastic film in the concrete bunker and finally covered with the same material. The well silo consists of an underground cylindrical structure constructed using stacks of concrete culverts and equipped with a basal metal plate at the bottom. About 45 cm of the structure is above the ground level and the rest is underground. The bottom plate was connected by a 3 m chain to a mechanized hoist. The mechanized hoist was attached to an H beam bar and supported by two H beam poles. After the silo is filled with the chopped corn, a sheet of canvas was used to cover and seal the culvert. The edge of canvas is submerged into the water around the culvert to prevent the air from entering the silo. Silosil consists of two PVC water tanks of unequal size. The smaller tank is placed into the bigger tank. Water was filled into the outer tank to about 20 cm level. The chopped corn was packed into the smaller water tank until full. A piece of canvas used to seal the smaller water tank and tightened in a similar procedure as for the well silo. Each silo type was repeated in four replications. At 30 days after fermentation, the silos were opened and exposed to the air. Estimation of spoilage rate (percentage) was carried out based on the colour changes of silage. The silage that has turned dark in colour is considered to be spoiled. The temperature of the silage was measured using a temperature probe connected to a data logger. The probe was placed into the silage mass at 30 cm from the surface of silage for each silo. The temperature

was recorded daily until 30 days exposure. Aerobic stability (days) was determined based on the number of days it took for the silage to reach temperatures exceeding 2°C above ambient temperature. The longer it took to reach this temperature the higher is the aerobic stability.

Results and discussion Plastic container showed the highest (27 days) aerobic stability, followed by well silo (10 days), silosil (7 days) and mini bunker silo (0 days) (Figure 1). Mini bunker silo had the shortest aerobic stability because the spoilage process started from the day one of fermentation period. This was also reflected by the highest rate of spoilage in the mini bunker silo. The plastic container showed the longest aerobic stability and no spoilage was observed. The plastic container has a relatively small surface area of silage exposed to the air compared to the other types of silo. Surface area plays a vital role in spoilage, because it determined the rate of oxygen entering the silage matrix causing spoilage of the silage. Losses and quality changes are typically considered to be more extreme in bunker silos than in tower silos because of greater surface area for oxygen penetration, less perfect sealing, and greater dependence on management practices during filling and feedout (Bolsen et al., 1993). High temperature and relative humidity in tropical weather promotes the growth of aerobic, acid-tolerant micro-organisms such as yeasts and moulds. These microbes oxidized the fermentation products present in the silage (Danner et al., 2003).

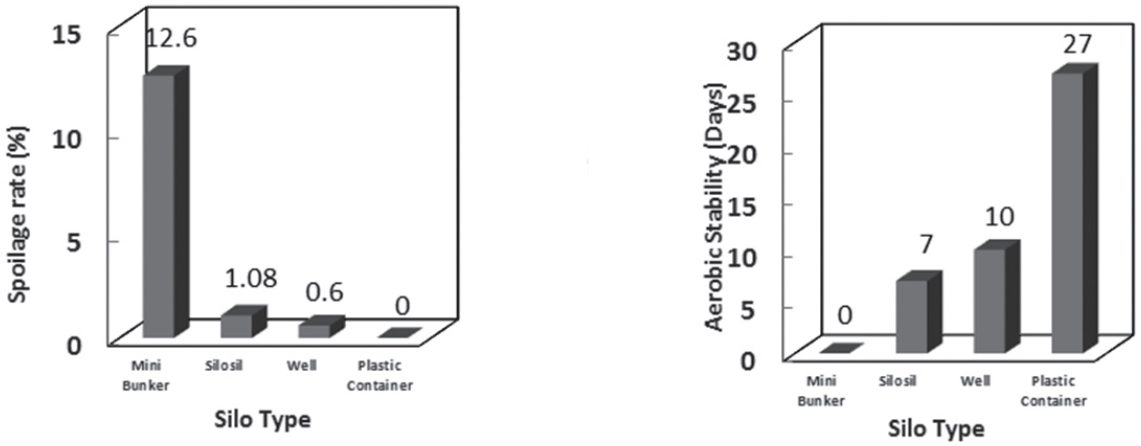

Figure 1 Aerobic stability and spoilage rate of corn silage in different type of silo.

Conclusions Based on the aerobic stability and spoilage rate observed in this experiment, plastic container was the suitable silo for silage production in the Malaysia, especially for small scale farmers.

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Effect of different bulk densities on temperature profiles and microbial respiration activities during reheating of corn silage

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Keywords silage quality, aerobic-induced silage reheating, bulk density, energy losses

Introduction Silage has reached great importance as a feeding material for livestock. Since the process of silage making is fully understood, the conditions needed for obtaining high silage quality are defined (Woolford, 1984). High compaction of plant material and airtight coverage are two of the major conditions for preventing and reducing aerobic deterioration accompanied by energy losses (Maack et al., 2007). The aerobic deterioration of silage is a worldwide problem for feed quality and profitability of farms (Tabacco et al., 2011). Oxygen entering into silage is utilized by microorganisms' respiration in conjunction with dry matter losses and heating of the material (Rotz, 2003). The objective of this study was to investigate the effect of the influencing factor 'bulk density' on the temperature profiles and microorganisms´ respiration activities during reheating of corn silage under controlled conditions.

Materials and methods Eight buckets made out of polyethylene with a volume of 60-L have been filled with 40 kg (low-density; n = 4) or 50 kg (high-density; n=4) of corn silage ensiled for 210 days. After filling, the buckets have been resealed airtight for three days. Three temperature sensors have been inserted at defined positions (150 mm, 250 mm, 350 mm distance from opening) into each bucket. These sensors were connected to a data logger for the next seven days. Thermographic images have been taken. To take gas samples out of the buckets cannulas were inserted. Gas samples were taken by evacuated injection vials at two defined sampling points and analysed for CO_2 contents in an external laboratory. For starting the experiment the covers of the buckets have been opened. The air could diffuse into the unsealed buckets unhindered and lead to deterioration by silage reheating. Figure 1 shows an illustration of the experimental setup.

Results and discussion There was a reheating effect observed in each of the eight buckets. The obtained temperature increases have been higher in the low-density buckets than in the high-density buckets. The maximum temperature rise is observed in a low-density bucket (from 19.23 °C up to 44.01 °C) and the minimum temperature rise in a high-density bucket (from 21.36 °C up to 32.23 °C). There is no difference in the starting time of reheating between the variations but in all buckets it took some time after opening before reheating started. In this time the microorganisms switch from anaerobic to aerobic metabolism. They are not able to use the oxygen that diffuses inside right from the beginning, because the microorganisms´ change in metabolism is independent of silage density. In all buckets the temperature rises measured by the sensors next to the opening of the buckets were faster and rose higher, than the temperature rises measured by the sensors with greater distance to the opening. The greater porosity of the low-density buckets facilitates the diffusion of air compared to the high-density buckets. As a result the air diffuses into the low-density buckets easier. This leads to a higher temperature rise caused by the consumption of high amounts of oxygen. Figure 1 (B) shows a thermographic picture of one high-density bucket and one low-density bucket taken at the last day of the experimental period. The results of the CO2 concentration measurements are graphically represented in Figure 2 A for the low-density-treatment buckets and in Figure 2 B for the high-density-treatment buckets. These results show, that the CO_2, which is inside the

buckets before opening flows out after opening caused by a concentration gradient which gets balanced by diffusion. Afterwards CO_2 concentrations increase until they find a balance at a level lower than the initial value. The CO_2 measured in the buckets is produced by microorganisms´ respiration. The measured increase of CO_2 concentrations starts a few hours before the temperature increase starts. This course confirms the findings regarding temperature progression initiated by respiration.

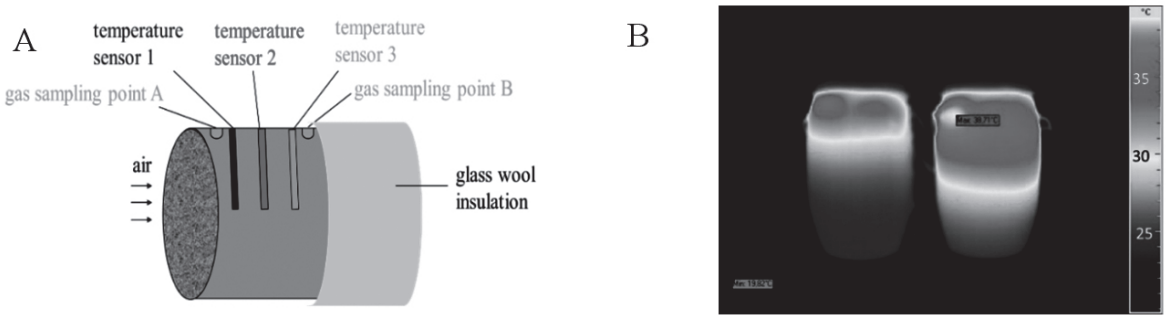

Figure 1 Schematic depiction of the experimental setup (A) and thermographic picture of one high-density bucket (left) and one low-density bucket (right) at day seven of the experiment (B).

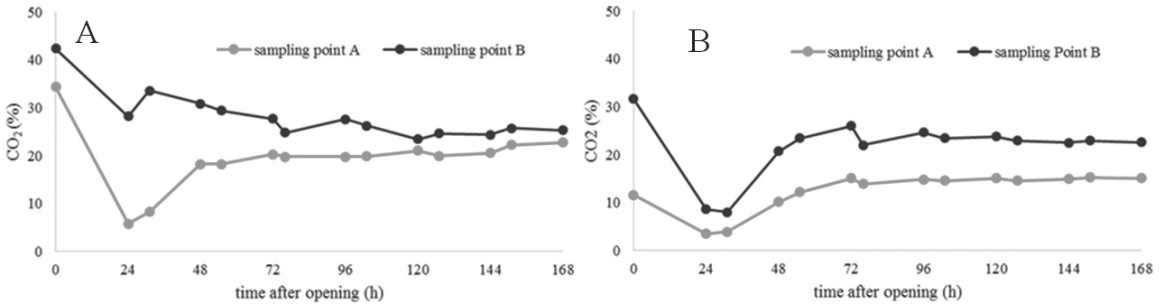

Figure 2 CO2 concentrations inside the low-density- (A) and high-density-treatment buckets (B)

Conclusions Recapitulatory the findings confirm, that dense compaction of plant material is an important influencing factor, which stabilizes silage by restraining the growth of microbial populations and their metabolism. This preserves energy in the aerobic feed out period.

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Testing feed improvement through ensiling for smallholders to bridge the dry season in Laos

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Keywords *Stylosanthes guianensis, Manihot esculenta* leaves, *Aeschynomene histrix*, pigs, beef cattle, dry season

Introduction Laos is a largely agricultural country with very traditional agricultural practices. Livestock is mostly kept as living asset and targeted feeding practices are mostly absent. Especially during the prolonged dry season from November to May most livestock is just released to take care of itself, though monogastrics are generally supplemented with some broken rice, maize or cassava. During this time of the year, livestock generally loses 10-40% of weight mainly due to poor quality of feed, body mass which is then recovered during the rainy season. Additional to being uneconomic and exposing the weakened animals to higher disease risk, the increased variation of rainfall patterns put an additional strain on livestock in years of increased drought. As silage making without mechanization is very labor intensive and could not be realized during the late rainy or early dry season due to the main crop harvest, it was tested whether ensiling still available material during the mid-dry season could improve its feed quality enough to be seen as suitable feedstuff for the mid and late dry season. Such improvement in animal nutrition would reduce risks and allow for additional livestock based economic activities.

Materials and methods Experiment A: Silage made from available sources during the mid-dry season (January-February) was offered to pigs in a controlled environment to assess the potential benefit of this approach. Rice bran, maize and soybean grain meal were purchased at a local market, while cassava leaves (*Manihot esculenta*), *Stylosanthes guianensis* (CIAT 184), and *Aeschynomene histrix* were harvested from existing stands at the Livestock Research Center in Vientiane, Lao PDR. Chopped *S. guianensis*, *A. histrix* and cassava leaf (50:25:25) with 4% molasses on fresh matter (FM) base were compacted into buckets, holding around 35 kg each. The top of the buckets was wrapped with plastic and sealed airtight before storing at ambient temperature for 30 days of fermentation. Four local growing pigs, 6-8 months of age with average live weights of 26.7±1.5 kg were confined in separate pens, vaccinated and de-wormed. The animals were arranged in a 4×4 single Latin square design with 4 replicates and 4 treatments: negative control (NC) low protein diet, positive control (PC) with soybean as protein source, replacing 50% soybean with silage, replacing 100% soybean with silage. Each experimental period consisted of 9 days for adaptation and 5 days of data collection during which the feed offer was restricted to 4% of body weight BW (DM basis). Feed intake as well as average daily gain was determined by weighing animals at day 9 and 14 of each period. Experiment B: Similar to exp. A, silage was offered to cattle. Rice straw was used as basic feed and supplemented with a 70% *Pennisetum purpureum* + 30% cassava leaves silage, with added lactic acid

bacteria at 10^5 cfu/g fresh matter (FM) and 4% (FM) sugar, incubated for 30 days in a concrete pit. Three native cattle weighing 115-140 kg, at the age of 2-2.5 years were offered one of three diets per treatment at a rate of 4% of body weight once a day. Animals had an adaptation period of 14 days followed by 14 days of experimental period in a Latin square design with the following treatments: Rice straw only (control), Rice straw 80% + silage 20%, Rice straw 60% + silage 40%.

Results and discussion In both experiments, ensiling did not improve the feed quality of available material sufficiently to justify this approach. For pigs, ensiling the already mature material did not result in a suitable feed source, leading to substantial weight loss even compared to the nominally lower quality diet. The higher fiber content in the silage (Table 1) could not be compensated by higher nitrogen contents and the overall nutrient availability in silage diets was apparently lower than in better digestible traditional diets with low protein content. For cattle, refusal of silage was high but animals given a 40% silage supplementation did show improved weight gains compared to the other treatments. Average daily gains were nevertheless small with 215 g on average. Treatments with only rice straw (as is farmer's practice) and 20% silage supplementation resulted in weight losses.

Table 1 Feed quality of used components and diets in Experiment A (% of DM)

	DM	CP	NDF	ADF
Ingredients				
Rice bran	90.5	5.9	67.3	66.6
Maize	88.5	6.9	33.2	16.9
Soybean	88.4	45.2	14.5	14.2
Cassava leaf	29.8	-	34.9	44.3
S. guianensis	40.2	13.6	62.7	54.3
A. histrix	36.6	16.2	50.1	58.1
Silage	48.0	12.8	62.3	56.6
Diets				
negative control	89.2	6.8	47.7	36.7
positive control	89.4	11.9	49.0	40.2
50% silage replacement	89.3	11.0	48.1	43.1
100% silage replacement	88.8	10.3	52.5	47.4

DM: dry matter (% of FM), CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber.

Conclusions Overall, ensiling of mature plant material as a means of feed improvement cannot be recommended as a labor efficient way to improve income security and reduce economic risks for smallholder farmers.

Effects of sealing time post-filling and sealing material on fermentation, nutritional quality, and organic matter loss of whole-plant maize ensiled in a drive-over pile

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Keywords sealing, oxygen barrier film, drive-over pile, surface spoilage

Introduction Standard white-on-black polyethylene (std. plastic) has been the most common material used to seal bunker silos and drive-over piles, but organic matter (OM) loss in the original top 0.91 meters can exceed 25% (Bolsen et al., 1993). The use of an oxygen barrier (OB) film (www.silostop.com) as an alternative to std. plastic was first reported at the XII International Silage Conference (Degano, 1999). Wilkinson and Rimini (2002) reported no visible surface mold or spoilage for OB film-sealed small-scale silos compared to single and double std. plastic-sealed silos. Borreani et al. (2007) confirmed that OB film was a promising tool minimize visible spoilage and DM losses, especially in critical farm conditions. Bolsen et al. (1993) showed OM losses in the original top 30 cm of lucerne and maize silages increased by 70 to 90 percent when sealing with std. plastic was delayed by 7 days. The trial presented here compared sealing time and sealing material using **a** drive-over pile of maize silage.

Materials and methods On August 21 and 22, 2013 about 600 tonnes of whole-plant maize was chopped at Maddox Dairy, Riverdale, CA (www.maddoxdairy.com). The maize was in the two-thirds milk line stage, contained 32% DM, and was inoculated at the forage harvester. The forage was ensiled at the California Polytechnic State University dairy farm in a drive-over pile, which was 18 m wide x 60 m long x 1.8 m apex height and an east to west orientation. About one-half of the forage was delivered the first day, packed with a payloader, and not sealed. On the second day, the remainder of the maize was chopped, inoculated, transported to the farm, and packed with a payloader. One-half of each day's forage surface was sealed with a sheet of std. plastic, and the other half sealed with a sheet of OB film. The silage was undisturbed for 90 days, and the sealing materials were removed from the south half of the pile. Samples were collected at 0 to 15, 15 to 30, and 30 to 45 cm depths from the surface at three north-south locations, which were equal distance from the east and west boundary of each of the four sealing treatments.

Results and discussion The results are presented for the mean of the three sampling depths. The effects of sealing time and sealing material on fermentation, nutritional quality, and OM loss are shown in Table 1. The immediate sealed silages had a lower ($P<0.05$) pH value than the delay sealed silages. The silage that was delay sealed with std. plastic had higher ($P<0.05$) ash and NDF contents and lower ($P<0.05$) NDF digestibility than the silage that was delay sealed with OB film and the silages that were sealed immediately with std. plastic or OB film.

Conclusions The OB film was more effective than std. plastic in preventing the entry of oxygen into the surface of the maize silage during the 90-day storage period. Delay sealing increased OM loss in the original top 45 cm of maize silage by 27.2% compared to immediate sealing (15.64 vs. 12.30%). However, delay sealing with OB film decreased OM loss in the original top 45 cm of maize silage by 20.6% compared to immediate sealing with std. plastic (12.33 vs. 15.54%).

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Table 1 Effects of sealing time (delay and immediate) and sealing material (std. plastic and OB film) on fermentation, nutritional quality, and estimated OM loss at 0 to 45 cm from the surface

	Delay seal		Immediate seal		SEM
Item	Std. plastic	OB film	Std. plastic	OB film	
Dry matter, %	30.27	29.51	30.13	30.11	
pH	4.07[a]	4.05[a]	3.99[b]	3.92[b]	0.022
Estimated OM loss, %[1]	18.94	12.33	15.54	9.06	
	----------		% of the DM	----------	
Neutral detergent fiber	47.4[a]	46.3[b]	46.0[b]	44.2[c]	0.51
NDF digestibility	61.7[a]	64.3[c]	62.9[b]	63.6[c]	0.38
Starch	27.8	28.1	27.9	29.6	
Ash	5.01[a]	4.65[b]	4.77[b]	4.49[b]	0.094
Lactic acid	2.86	3.20	2.62	3.33	

[1]Ash content of the pre-ensiled whole-plant maize was 4.10 percent, and estimated OM loss was calculated using equations by Bolsen et al. (1993).

[a,b,c,d] Means differ ($P<0.05$).

Improved crop compaction in bag silos

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Keywords silage bagger, bulk density, brake system, distance sensor, film extension

Introduction The system of silage bags can be used for dry cereals to prevent the crops of environmental influence without any building, as well as for conservation of silage crops by lactic fermentation at anaerobic conditions. The hermetically sealed bag provides anaerobic conditions during the closed storage and so different field crops and coproducts of food industry can be stored (Rössel and Wagner, 2011). The crop compaction using a press rotor in order to ensilage gras and maize has been proved as an effective tool. The bag can be filled with high performance and sufficient crop density. The press process has to be controlled manually by adapting the brake pressure. Density tests made by Muck and Holmes, at different positions at the silo face of bags show differences in crop density according to the positon. In the upper part of the bag the density was about 30% lower than near the ground or in the centre (Maack, 2011). The measurement on well pressed silages showed a better crop compaction and a more consistent film extension. To avoid the risk of ripping the bag film after pressing, the maximal extension of the film should not exceed 10% This has to be manually measured by the machine operator during the press process. To optimize the crop compaction and to exculpate the machine operator one aim of the project was to develop an automatic brake pressure adjustment according to the current conditions during the press process. The effect on crop density across the silo face has been observed by detailed density measurements at the open silo during feed out.

Materials and methods To carry out the tests a self-propelled bagger BUDISSA BAG RM 8000 (2.7m press tunnel diameter) has been equipped with a hydraulic brake driven by the machine system. To control the brake pressure, distance sensors observing the bag surface are connected with a laptop (Figure 1). According to the distance from the bag to the three sensors the film extension of the bag is calculated. The control unit gives a signal to the hydraulic performance valve. The brake effort is adapted by this closed control loop. The maximal film extension has to be adjusted in the software by the machine operator. If necessary the brake pressure can be manually adjusted in the software.

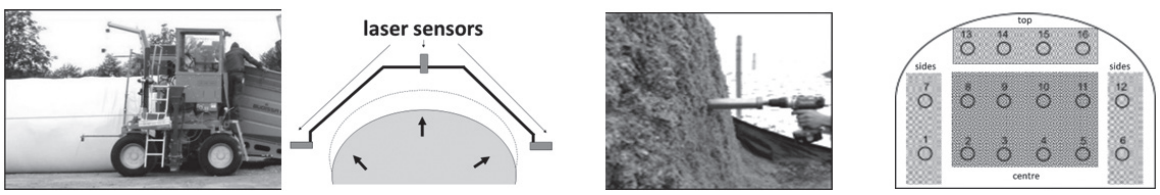

Figure 1 Distance laser sensors around the bag and sample points at the silo face.

The parameters brake pressure, film extension and the covered distance of the bagger during pressing are logged over time. All pressure adjustments and the following effects

are mapped and can be analyzed after the tests. In the tests the desired value of film extension was given between 7.5 and 9.5%. In order to analyze the effect of the different press intensities on the crop compaction in the bag a detailed density controlling was made during feed out of the silage. Samples of defined volume were taken at 16 different positons at the silo face.

Results and discussion The effect of a maximal compaction achieved by a film extension of 9.5% is higher in the centre than at the sides and the top of the bag (Figure 2). The bag volume of the high pressure variant is around 5% more per meter. In addition both effects (5% more volume and 8% higher density) lead to 14% more crop mass per meter silo bag. In comparison especially the density at the top and at the upper sides are on are evidently lower level. This can not be completely balanced by an automatic controlled brake pressure.

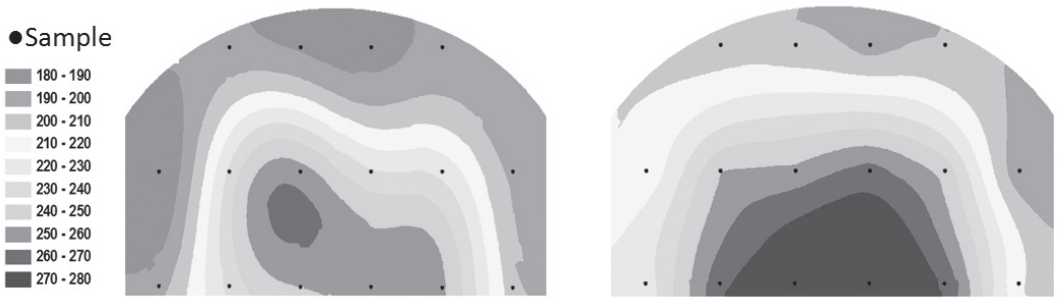

Figure 2 Crop density [kg DM m^{-3}] of maize across the silo face LD 7% film extension (left) and HD 9.5% film extension (right).

Conclusions Over all the automatic controlling of brake effort in case of silage bagging gives the opportunity to compact the silage with a constant maximal film extension. This leads to a higher crop density in the bag among the current conditions. The handling of the machine can be made easier for the operator, because he does not need to observe the film extension manually all the time. In order to a more improved compaction effect at the sides and the upper part of the bags further investigations and improvements of the pressing tools in the bagger seem to be a suitable way.

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The effect of harvester type (self propelled, trailed or forage wagon) and storage method (bunker or drive over pile) on the forage density of grass silage across Finland

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Keywords density, self propelled harvester, loader wagon, trailed forager, silo, pile, bale

Introduction Density and pore spaces of ensiled forages have direct correlation to dry matter (DM) loss through fermentation and storage (Ruppel, 1992), by affecting the degree to which air can penetrate into and behind the face of the bunker or the drive over pile (Losand, 2003), therefore extending or shortening the period in which the epiphytic organisms are active. The aim of this trial was...

Materials and methods Density of ensiled grass (primarily Timothy, mean DM 28.4%, SD 8.3%) at feed out was determined using the Dairy One Master Forage Probe in conjunction with a Stihl BT40 and defined method of Dairy One Laboratory, USA. Over 300 commercial farms across the entirety of Finland were assessed. Height of walled silo or pile, DM content and foraging system were recorded, with density cores being drilled at a central height of the storage system (piles and bunker varied between 2 and 3m high (8 silos were at 1.5m), with the core being therefore taken between 1 and 1.5m from the top of the storage system. Density was reported on a DM basis. DM content was determined via NIR report or standard microwave method. Data was subject to One-Way Analysis of Variance using Minitab v16. Significance was declared at $P<0.05$.

Results and discussion A total of 387 cores were taken from walled silos and drive over piles as described in Table 1.

Table 1 Description of storage system, method of harvesting and density analysis

Storage System	n	Harvesting System	n	Density Kg/m^3 DM	Density Kg/m^3 DM			
					Mean	SD	Min	Max
Walled Silo	323	Self Propelled	139	183.91[a]	193.7[a]	37.7	82.8	289.1
		Trailed Forager	74		184.3[a]	42.6	90.5	279.9
		Loader Wagon	110		168.9[b]	31.8	95.0	250.0
Drive Over Pile	64	Self Propelled	29	161.52[b]	162.8[b]	34.8	103.0	231.3
		Trailed Forager	8		162.9[b]	23.2	127.7	196.6
		Loader Wagon	27		148.5[b]	37.1	79.4	211.3

[a,b] Means with a different suffix are statistically different at 95%.

When comparing the total data of walled silos against drive over piles, the density of the grass silage in walled silos was statistically significantly greater than that in the drive over piles ($P<0.05$). Mean density values were not high, but where acceptable based on the DM. Comparison of the forager method of harvesting for the walled silos showed that the self

propelled forager and trailed forager silos had significantly higher density than the silos produced with loader wagon harvested forage (P<0.05), with the self propelled forager having both the highest mean density and maximum density. The loader wagon cut grass producing the lowest average density in the walled silos and also the lowest maximum density.

Table 2 Comparative density of walled silos and drive over piles made with the same foraging equipment

	Density of Silage Kg/m^3 DM produced by:		
	Self Propelled	Trailed Forager	Loader Wagon
n	n= 168	n= 82	n= 137
Drive Over Pile	162.8^b (SD 23.2)	162.9 (SD 34.8)	148.5^b (SD 37.1)
Bunker	193.7^a (SD 37.7)	184.3 (SD 42.6)	168.9^a (SD 31.8)

^{a,b} Means with a different suffix are statistically significantly different at 95%.

Density achieved in bunkers was consistently higher than the density achieved in drive over piles, with this difference being statistically significant with the self propelled forage harvester and loader wagons (P<0.05). Air penetrates deeper into the silage with reduced packing density (Losand, 2003) leading to higher DM losses (Ruppel 1992). Within Finland, the data set shows that walled silos avoid DM losses through storage than drive over piles owing to higher density, and that grass harvested with self propelled forage harvesters and trailed forage harvesters will likely avoid DM losses than grass harvested with a loader wagon (due to higher packing density). The large sample size partly nullifies the variance in chop length and exact sample position.

Conclusions Walled silos are more efficient at protecting DM through storage than drive over piles due to the statistically higher density achieved (P<0.05). This will further protect DM at feed out due to air being able to penetrate less deeply into the silage and support spoilage organism growth. Grass silage produced with a loader wagon is expected to be subject to higher DM losses through storage and feed out than grass silage produced with a self propelled or trailed forager.

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Comparing the effects of the number of wrap layers applied to bales on silage preservation

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Keywords bale, stretch film, dry matter, mould coverage

Introduction The aim of this study was to compare the effects of the number of wrap layers (2, 4, 6, and 8 layers) on silage quality, dry matter losses, mould coverage and nutritive value of silage.

Materials and methods The trial was conducted under farm conditions in the Experimental Farm of IMUZ in Falenty, Poland. Silages were made from a first cut of permanent meadow sward mown in May 2007. The herbage was wilted for 24 hours, raked and baled. The dry matter (DM) content in meadow sward at ensilage was 461 g/kg. Forty experimental big bales (about 400 kg/bale) were produced. The bales were wrapped after transport to their place of storage using 2, 4, 6 or 8 layers of a 25 micron thickness plastic film produced via blown film coextrusion process. The film composition is based on DOWLEX™ 2045S, an octene-based linear low density polyethylene specifically developed for blown silage film fabrication. Bales were wrapped with 500 mm wide stretch film. After 3 weeks storage period each bale was tested to evaluate the effectiveness of film seal by creating a vacuum within the bale and measuring the time taken for the pressure to drop. After 190 days of storage, bales were opened. Immediately after unwrapping each bale was assessed for mould coverage. Representative samples were taken for chemical analyses from each bale. Silage DM loss was estimated by difference between fresh forage DM weight (at ensiling) and silage DM weight.

Results and discussion Applying different number of wrap layers affected the film seal. In bales wrapped with two layers the film seal was less effective, while increasing the number of layers significantly improved the film seal: the time taken for air to re-enter the four and six layers bales was five to seven times longer compared to the two layers bales. The effectiveness of film seal had an effect on visible moulds coverage. Bales wrapped with two layers of film recorded 45% of mould coverage on the bale surface. Increasing the number of wrap layers reduced the risk of air penetrating the bale and significantly decreased moulding. As a result, the mean mould cover was only 9.5% and 1.5% of surface on bales wrapped respectively with six and eight layers. An additional result of interest is the significant reduction of DM losses as the numbers of layers increased from two to eight. Applying four layers of films resulted in three times lower DM losses then when wrapped with only two layers and ten times lower DM losses when wrapped with six layers. Bales wrapped with eight layers, DM losses were very small and in many bales were nil. Generally the number of layers had no significant influence on the silage chemical composition. Butyric acid, crude protein and water soluble carbohydrates concentrations increased significantly as the number of layers increased from two to eight. No significant differences were observed for lactic acid concentration in bales with higher number of

wrap layers. Ammonia, lactic acid and acetic acid concentrations decreased with the increase in the number of layers. A trend indicating an increase in net energy of lactation (Mcal/kg) in silage was noticed when the number of layers was increased.

Conclusions The study proves that wrapping silage bales in four to six layers of film provides a more robust oxygen barrier resulting in reduced loss of dry matter in silage from yeasts and moulds, as well as significant improvements in the quality and nutritive value and a more stable and consistent silage at feed out. The data confirms that applying two layers of silage wrap film does not guarantee an effective film seal on bales, whereas six layers of a plastic film based on a linear low density polyethylene resin has shown to be effective.

Comparison of two ensiling systems: trench silo × bale

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Keywords corn silage, density, fermentation, losses, temperature

Introduction The main factor responsible for the high milk productivity is the animal nutrition, where corn silage is the major feedstuff (Janssen, 2009). In recent years the techniques of cultivation of corn for silage evolved considerably (Carvalho, 2013). In the summer season 2013/2014, the producers of the ABC Group (Cooperatives Arapoti, Batavo and Castrolanda) cultivated an area of 14,305 ha of corn for silage (Fundação ABC, 2014). This silage can feed around 70,000 cows for one year. However, the chain of corn silage production not only understands the field phase. It is necessary that the mass produced be harvested, ensiled, stored and used properly, ensuring that the plant nutrients achieve to the animals. There are several ways for the storage of silage depending on the characteristics of each farm. Each type of silo has its advantages and disadvantages in relation to compaction, storage, sealing and removal of silage. The forage conservation goal is have minimal losses of dry matter and energy (Van Soest, 1994). Therefore, it is essential to study the different ensiling systems in order to minimize the quantitative and qualitative losses.

Materials and methods The study was conducted from February to May 2014, on the Santo Antonio do Iapó farm, in the city of Castro, Paraná, Brazil. The corn hybrid used was Pioneer 32R22H, harvested at 32% dry matter, with Krone Big X 700 machine. The particle size of silage was adjusted with Penn State particle separator. At ensiling, a commercial inoculant based on *Lactobacillus plantarum* MA 18/5U and *Propionibacterium acidipropionici* MA 26/4U was applied at 7.5×10^4 cfu/g of fresh forage. At the same time, two ensiling systems were proceed: trench silo and bale silo. The experimental design was completely randomized. Each experimental unit consisted of nylon bags with 1.6 kg of chopped forage. At moment of ensiling, four bags were placed in each position in the trench silo (top, middle and bottom), totaling 12 bags. In bale silos, there were four bales with three bags in each bale, also totaling 12 experimental bags. The silos remained closed for 91 days. The density of the original mass of the trench silo was measured through a drill in 12 set points, according to the methodology proposed by D'Amours and Savoie (2005). The density of the bales was determined by its weight and volume (1.19 m^3). A sample was taken from each experimental bag and shipped to the laboratory for analysis of pH (Silva and Queiroz, 2006), dry matter (AOAC, 1998) and *in vitro* digestibility of dry matter (IVDDM) (Tilley and Terry, 1963). The loss of original mass was calculated by the difference between the initial weight (ensiling) and final weight of bags at opening the silos. The silage temperature was evaluated with laser thermometer at the opening the silos and every 12 hours, up to 120 hours after opening. Aerobic stability of silage was defined as the time to raise 2°C above ambient temperature (Borreani and Tabacco, 2010). Statistical analysis was performed with SAS 9.3 program. When there was significance by F test, means were compared by Tukey test at 5% probability.

Results and discussion The bale system had greater fresh mass and dry mass density in relation to trench silo (Table 1). In trench silo, evaluations were carried out near the ramp at the beginning of the silo, which may have prejudiced the evaluation of density of this type of silo. The best compaction and sealing of the bale may have favored the fermentation, that resulted in smaller pH (3.73 vs. 3.80), low temperature (17.5 *ºC* vs. 27.4) and higher aerobic stability than the average of the trench silo (120 hours vs. 81 hours, respectively). In the bottom position of the trench silo, where the compression was better (192 kg DM m^{-3}), the results were close to the bale (pH 3.73 vs. 3.74, respectively). There was no difference in IVDDM of silage between two types of silos and positions in the silo. There was an increase of DM content of the silage in the top of trench silo, which resulted in negative dry matter loss, in other words, gain of dry matter. We don't know because occurred this. Interesting fact and that must be further studied.

Conclusions Ensiling in bales resulted in silages with higher density, lower pH, lower temperature and higher aerobic stability compared with that stored in trench silos.

Table 1 Variables analyzed according to the type of silo and position

Item	Bale silo	Trench silo				CV (%)	SEM
		Top	Middle	Bottom	Average		
Density (kg FM m^{-3})	706a	422b	439b	629a	497	7.8	48.0
Density (kg DM m^{-3})	225a	143c	141c	192b	158	7.3	14.3
FM losses (%)	4.5a	1.3a	3.6a	5.2a	3.4	2.8	2.7
DM losses (%)	7.6a	-3.6b	5.4a	10.8a	4.2	4.6	4.3
DM (%)	31.9b	34.3a	32.2ab	30.5b	32.3	3.6	1.2
pH	3.73b	3.88a	3.87a	3.74b	3.8	1.0	0.0
IVDDM (%)	71.2a	71.0a	70.6a	71.1a	70.9	1.7	1.2
Mean temperature (ºC)	17.5b	26.3a	28.0a	28.0a	27.4	11.3	2.5
Temp.12h after silo opening (ºC)	21.3b	23.3a	21.8b	21.4b	22.1	3.1	0.7
Maximum temperature (ºC)	25.7c	31.5a	27.8bc	28.8b	29.3	4.6	1.3
Aerobic stability (h)	120a	27b	102a	114a	81	19.0	19.1

[a-c] Means followed by the same letter on the line do not differ by Tukey test at 5% probability.
FM: fresh matter; DM: dry matter; IVDDM: *in vitro* digestibility of dry matter.
Temp. 12h: temperature of the silage, 12 hours after opening the silo.

Applied technologies for corn silage production in the Campos Gerais region - Brazil

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Keywords ensiling, conserved forages, dairy farms, nutrition, productive chain

Introduction Brazil is the fourth largest producer of milk in the world (FAO, 2011) and the first in beef exports (MAPA, 2014). Despite this large volume of production of dairy and beef products, there are few surveys about production practices, especially in relation to the production and use of conserved forages (Bernardes, 2012). Among the cities in Brazil, Arapoti, Castro and Carambeí (Campos Gerais region) have production of milk and productivity per cow much higher than the national average (IBGE, 2012). In addition to the high-tech employment in milk production, with big investments in genetics and management (Carvalho, 2013), 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 largest quantity (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 productive chain.

Materials and methods Through years 2009-2014 were visited 290 farms, in 18 cities of Campos Gerais region (Center East of Paraná State and south of São Paulo State). Silages were evaluated at 613 silos that were used in animal feeding. At visited silos, in-site evaluations were: type of silo (trench or surface), color of plastic film used for sealing (white and black, black, white), protection of the plastic film (without, soil, others), method of silage removal (manual with fork, by tractor with scoop, mechanic cutter, silage block cutter) and presence of effluent (without, little or much). A survey was ministered to producers regarding the presence or not of hybrid Bt technology for insect control, harvest of silage (own or custom service), type of forage harvester (self-propelled or by tractor), if there was fungicide application in the fields and if inoculant was used during ensiling process. Data were compiled per year and frequencies calculated for each of the variables. Regression analysis was performed using SAS 9.3 software with PROC REG procedure at 5% probability.

Results and discussion In the first year of survey, 100% of the farmers used conventional hybrids for silage. Over the years there was a linear increase in the use of Bt technology ($r^2 = 0.9163$; $P = 0.0027$). Between 2009 and 2014, there were no changes in the use of custom services for harvesting (70% of farms), mainly with self-propelled machines (64%). Approximately 45% of farmers applied fungicide at the maize plants and only 19% used inoculants at ensiling. Most (80%) of the silos were trench type (revested of concrete or no), which provides better compacted silage compared to silages stored in surface silos. In 2009, none silo was covered with black-on-white plastic sheets. Over the years there was an increase in its use ($r^2 = 0.8702$; $P = 0.0207$) and in 2014, more than half of the silos (56%) were sealed using this kind of material. In all the years, soil was the principal material for covering the plastic film (86%). Utilization of soil cover is considered a

positive aspect, because it promotes greater adhesion between the plastic sheet and the mass of silage and protects against sun radiation, resulting in lower oxygen influx. However, soil may be an impediment for handling (Bernardes, 2012). Most (56%) farmers used forks to remove the silage and 20% use tractor with scoop. There was an increase in the use of silage removal with mechanic cutter between 2011 and 2014 (r^2 = 0.9068; P = 0.0477).

Conclusions Most of farmers of studied region used custom harvesting services, self-propelled machines, trench silo, soil to coverage the plastic film, silage removal with forks and don't have effluent in silo and don't utilize silage inoculats. It has been increased the use of Bt technology hybrids, silo cover with black-on-white polyethylene films and mechanic cutter for silage removal.

Table 1 Technologies applied by farmers at ensiling process over the years in Campos Gerais region - Brazil (%)

Variable	Technology	Years							Year Regression	
		2009	2010	2011	2012	2013	2014	$\bar{x}$	Pr > F	r^2
Hybrid	Conventional	100	98	58	19	6	5	48	0.0027	0.9163
	Bt	0	2	42	81	94	95	52	0.0027	0.9163
Harvest	Own	26	34	36	28	22	33	30	0.8361	0.0120
	Custom-service	74	66	64	73	78	67	70	0.8361	0.0120
Machine	Self-propelled	70	55	54	68	74	62	64	0.7240	0.0347
	By tractor	30	45	46	32	26	38	36	0.7240	0.0347
Fungicide	No	57	62	53	55	52	50	55	0.0667	0.6099
	Yes	43	38	47	45	48	50	45	0.0667	0.6099
Inoculant	No	77	84	81	74	84	83	81	0.6203	0.0670
	Yes	23	16	19	26	16	17	19	0.6203	0.0670
Silo Type	Trench	-	77	75	85	78	84	80	0.3063	0.3353
	Surface	-	23	25	15	22	16	20	0.3063	0.3353
Plastic Film Color	Black-on-white	-	0	39	52	66	69	45	0.0207	0.8702
	Black	-	61	50	40	26	29	41	0.0108	0.9149
	White	-	39	10	8	8	2	14	0.0849	0.6821
Plastic Film Coverage	Without	-	8	7	3	7	13	8	0.4346	0.2124
	Soil	-	87	83	92	88	81	86	0.7587	0.0364
	Others	-	6	9	5	4	5	6	0.4674	0.1868
Removal	Fork	-	-	63	63	46	54	56	0.2998	0.4903
	Tractor scoop	-	-	21	18	25	17	20	0.7531	0.0610
	Mechanic cutter	-	-	14	18	28	28	22	0.0477	0.9068
	Block	-	-	2	1	1	1	1	0.1931	0.6511
Effluent	Without	-	71	75	69	61	83	72	0.7468	0.0401
	Little	-	25	16	30	37	17	25	0.9027	0.0059
	Much	-	4	9	1	3	0	3	0.1954	0.4790
n		53	89	107	120	114	130			

Level of technology used on silage production on dairy farms in southern Brazil

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Keywords clustering, principal components, silage production, silage quality

Introduction The silage is the main feed for cattle, mainly for dairy cow. Corn silage is specially used as energy and fiber source for these animals. Obtaining good silage quality depends on several factors, among which, the choice and management of the crop, associated with the production technology, are of great importance. In this sense, the objective was to take stock of the practices of production and use adopted for corn silage in dairy farms of southern Brazil.

Material and methods Visits were carried out in 40 farms milk producers in the states of Paran*á*, Santa Catarina and Rio Grande do Sul, with questionnaires and data collection. These dairy farms were selected in order to cover the different profiles of the farms and the production systems adopted. Starting at a matrix of 40 farms and 31 indicators obtained, the 18 indicators with a coefficient of variation of more than 45% were selected. After, these indicators were submitted to new selection criteria, through correlations analysis, excluding indicators with highest correlation than 75%, resulting in 14 indicators. These were submitted to factorial analysis of reducing data, using the method of principal components, verifying the explanation of 84, 12% of the variance through four indicators (location/weather, hybrid corn company, crop management and use of inoculant) and two principal components, which were submitted to cluster analysis, obtaining groups with common characteristics. The groups were submitted to variance analysis and the differences between the averages analyzed by Tukey test (P<0.05) through statistics program SPSS (Statistical Package for the Social Sciences - 2008).

Results and discussion As result of cluster analysis four distinct groups were obtained, hierarchized according to its similarity characteristics, and arranged on the two compounds axes (Figure 1). It was observed that the major number of properties was characterized by group 1, representing 40% of total. The group 2 was responsible for 20% of the farms, group 3 represented 30% and group 4 with the lowest amount corresponding to 10%. All the farms arranged in group 1 were localized in Paraná state; whereas 100% of the farms of group 4 were localized in Rio Grande do Sul and Santa Catarina state. The farms of group 2 were located in Paraná and Santa Catarina state. The groups 1 and 2 were the ones that more invested in silage/silage emptying processes (use of propelled machine, daily maintenance of the silage machine, use of inoculants, better protection of the silo and use of silo emptying machine). Among the visited farms, different criteria were adopted to choose the hybrid corn. In 27.5% productivity was the priority, 22.5% nutritive value and only 12.5% considered both characteristics. Silages were produced using 17 different

corn hybrids. Among the declared hybrids, 30% were sold by Pioneer, with major inset in groups 1 and 2, representing 31.3% and 75.0% respectively. In all studied properties some kind of technology is applied in crop growing management, which includes the direct seeding that is used in 80% of the cases. Among them 33% applied organic fertilizer and 60% used chemical fertilizer associated. Furthermore, 100% of the farms included in groups 3 and 4 employed the use of chemical fertilizer. Inoculants were applied in 65% of the silages studied, being the enzyme-bacterial inoculant Lalsil®Corn (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*) present in 30% of these. In groups 2 and 4 the percentage reached even 100%. According to Bernardes and Do Rêgo (2014) data, only 27.7% of Brazilians producers use additive in silage, being that, among the southern properties, only 18% of the additives are bacterial inoculant.

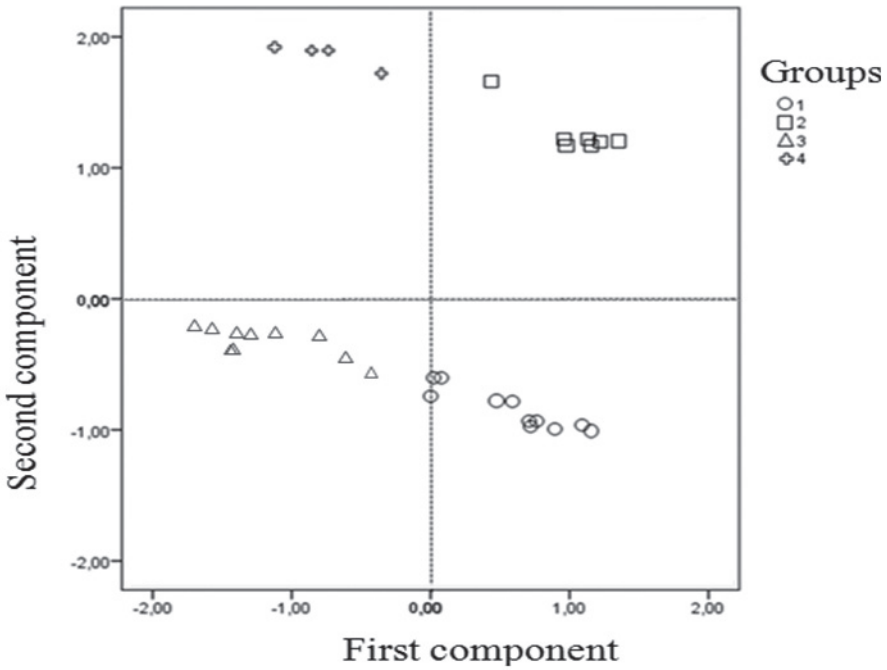

Figure 1 Spatial distribution of farms according to the two principal components: First component (explained variance: 59.6%) = higher values indicate better farm location (favorable climatic conditions), use of hybrid corn indicated to silage and adoption of better production management techniques. Second component (explained variance: 24.5%) = high values indicate the use of more effective inoculants in the silage quality.

Conclusions The dairy farms in southern Brazil, regardless of their size area, invest in various stages of silage production, being characterized as high technologies properties. The technologies used in the production of silage are more associated with management of corn culture, especially on farms in the state of Paraná.

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The influence of air ingress and additives on fermentation pattern, volatile organic compounds (VOC) and aerobic stability of maize silage

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Keywords air ingress, silage additive, maize silage, volatile organic compound

Introduction Volatile organic compounds (VOC), e.g. alcohols, organic acids and esters thereof, are frequently found in silages (Weiss and Auerbach 2013) and may detrimentally affect feed intake by dairy cattle. As the knowledge of the formation of VOC in silages and measures to reduce it is still very limited, it was the aim of this study to test the effects of air ingress and silage additives on fermentation pattern, production of VOC and aerobic stability of maize silage.

Materials and methods Forage maize (330 g/kg DM) was harvested and chopped in September 2014. The maize had 30.5% of starch in DM, log 6.4 cfu/g FM yeasts and log 5.5 cfu/g FM moulds. Fresh treated forage were packed into 1.5 L glass jars, all in triplicates, and stored for 49 days at 25 °C. The silos were further divided into two treatments during fermentation, no air ingress (NAI) or air ingress (AI) in compliance with the official guidelines of the DLG (German Agricultural Society) for silage additive testing of aim-of-action 2 – improving aerobic stability for AI. The following treatments were tested: Control (CON); homofermentative lactic acid bacteria (LABho), applied at 2.5×10^5 cfu/g FM; heterofermentative lactic acid bacteria (LABhe), applied at 1×10^5 cfu/g FM; homo- and heterofermentative LAB (LABho+he), applied at 2.5×10^5 cfu/g FM; LABho+PS, containing homofermentative LAB 3×10^5 cfu/g FM and potassium sorbate applied at 4 L/t FM; BPS containing sodium benzoate, calcium propionate and potassium sorbate, applied at 2.5 kg/t FM. The fermentation characteristics (pH, acids, alcohols, esters) were analysed in accordance with Weiss and Auerbach (2013). Losses of DM during fermentation were calculated as described by Weissbach (2005). Aerobic stability was measured by employing the temperature method (Honig, 1990). Data were analyzed with PROC MIXED/CORR of SAS 9.4.

Results and discussion Air ingress and silage additives affected fermentation parameters and aerobic stability (AS) and interactions were observed between the two factors (Table 1) except for ethyl lactate concentration. Air infiltration during fermentation process reduced lactic acid and AS. Losses of DM were higher ($P<.0001$) with air ingress (5.5% *vs* 4.6%) when compared with strict anaerobic storage (data not shown). Although the fermentation length was only 49 days, the additives LABho+PS, BPS and LABhe improved AS regardless of air treatment, and reduced visible moulding of silages at the end of the AS test (data not shown). The concentrations of acetic acid, ethanol and ethyl acetate were greater with LABhe especially with air ingress. This additive showed also the highest values of n-propanol, which may have been formed by *Lactobacillus diolivorans* (Kroonemann et al., 2002). Propyl acetate was not found in the silages. There was a positive correlation between the concentrations of ethanol and total esters of $r^2=0.628$ ($P<0.001$, $N=36$). The most pronounced reduction in ethanol and ethyl lactate levels was caused by the chemical mix BPS with and without air ingress.

Conclusions It can be concluded that silage additives containing sodium benzoate, calcium propionate and potassium sorbate were superior to all the other treatments regarding suppression of ethanol and ester formation as well as improvement of AS. These findings are in line with other results of Weiss and Auerbach (2012, 2013).

Table 1 Effects of aeration (no air ingress, NAI vs. air ingress, AI) and additives on fermentation and aerobic stability of maize silages

AE	Additive[1]	LA[2]	AA[3]	Eth[4]	Prop[5]	EA[6]	EL[7]	AS[8]
NAI	CON	54.8^{ce}	14.1^{b}	9.0^{c}	533^{a}	132^{bd}	120^{ce}	5.^{17b}
	LABho	54.8^{ce}	13.6^{ab}	9.3^{c}	904^{a}	105^{bc}	115^{be}	6.17^{bc}
	LABhe	36.3^{b}	22.7^{c}	12.1^{de}	9065^{c}	201^{cde}	74^{ab}	10.00^{de}
	LABho+he	54.7^{ce}	14.0^{b}	10.0^{cd}	1368^{a}	166^{d}	121^{ce}	7.92^{cd}
	LABho+PS	55.6^{de}	14.4^{b}	5.7^{abc}	542^{a}	73^{acd}	61^{a}	10.00^{de}
	BPS	58.1^{e}	13.1^{ab}	8.0^{ac}	435^{a}	117^{bd}	87^{abc}	10.00^{de}
AI	CON	35.0^{b}	11.7^{a}	9.6^{c}	128^{a}	27^{a}	134de	2.08^{a}
	LABho	38.0^{b}	12.4^{ab}	11.0^{bd}	212^{a}	81^{ab}	148^{e}	2.13^{a}
	LABhe	26.4^{a}	25.1^{d}	12.8^{e}	4091^{b}	280^{e}	125^{ce}	10.00^{e}
	LABho+he	52.4^{cd}	13.7^{ab}	10.2^{cd}	313^{a}	74^{ab}	140^{e}	2.42^{a}
	LABho+PS	49.8^{c}	13.8^{ab}	5.5^{acde}	70^{a}	105^{acde}	92^{abcd}	10.00^{e}
	BPS	55.3^{de}	14.1^{b}	7.1^{a}	190^{a}	79^{ab}	94^{abcd}	10.00^{e}
	SEM	0.6…1.2	0.4	0.2…0.4	4…128	7…39	1…22	0.2…0.5
Effects								
	AE	*<.0001*	*.4614*	*.0880*	*.0001*	*.0627*	*.0017*	*<.0001*
	SA	*<.0001*	*<.0001*	*<.0001*	*.0003*	*.0008*	*.0178*	*<.0001*
	AE x SA	*<.0001*	*<.0002*	*.0234*	*.0027*	*.0076*	*.3134*	*<.0001*

[1]CON, untreated; LABho, homofermentative lactic acid bacteria; LABhe, heterofermentative lactic acid bacteria; LABho+he, homo- and heterofermentative lactic acid; LABho+PS, homofermentative lactic acid bacteria and potassium sorbate; BPS, sodium benzoate, calcium propionate and potassium sorbate; [2]Lactic acid, g/kg DM; [3]Acetic acid, g/kg DM; [4]Ethanol, g/kg DM; [5]n-Propanol, mg/kg DM; [6]Ethyl acetate, mg/kg DM; [7]Ethyl lactate, mg/kg DM; [8]days; means in columns bearing unlike superscripts differ ($P \leq 0.05$; Tukey´s test); NS = not significant. 2-way ANOVA with variance heterogeneity.

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Lactic acid bacteria and *Bacillus subtilis* as inoculants for corn silage produced in tropical climate: chemical composition and fermentation

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Keywords *Lactobacillus* spp., Molds, Nutritive value, Yeasts

Introduction *Lactobacillus plantarum* is often used as silage inoculant due to rapid production of lactic acid and the consequent decrease in pH preserving the forage from spoilage microorganisms (Filya, 2003). Conversely, *L. buchneri* can increase the aerobic stability due to high production of acetic acid (Kleinschmit and Kung, 2006), whereas *Bacillus subtilis* can produce antifungal compounds and inhibit the growth of yeasts (Basso et al., 2012). Thus, the association between these bacteria should preserve more nutrients during fermentation, as well as result in adequate fermentative process. So, our aim was to evaluate the effect of homofermentative lactic acid bacteria (LAB) combined with heterofermentative LAB or *B. subtilis* on characteristics of corn silages produced in tropical climate.

Materials and methods A corn hybrid (Impacto Víptera, Syngenta, Sao Paulo, SP, Brazil) was harvested with the grains in the two thirds of milk line of the mature stage, using a Premium Flex forage harvester (Menta Mit, Cajuru, Sao Paulo, Brazil) and chopped without wilting to achieve a length of 10 mm. The corn plants were treated with water (2 L/t; control) or with either 1×10^5 cfu/g of fresh forage of *Lactobacillus plantarum* MA18/5U and 1×10^5 cfu/g of fresh forage of *L. buchneri* CNCM I-4323 (Lallemand Animal Nutrition, Milwaukee, WI, USA; LBLP), and treated with either 1×10^5 cfu/g of fresh forage of *L. plantarum* MA18/5U and 1×10^5 cfu/g of fresh forage of *Bacillus subtilis* AT553098 (Fatec Animal Nutrition, Sao Paulo, SP, Brazil; BSLP). The inoculant was dissolved in water (2 L/t) and then sprayed on piles of fresh forage under constant mixing. Forty tons of forage from each treatment was conserved in stack silos, which remained closed for 88 d. Silage samples were oven dried (55°C for 72 h) and ground in a knife mill to pass through a 1 mm screen, and analyzed for dry matter - DM (105°C for 12 h), neutral and acid detergent fiber (NDF and ADF), which were determined using the method of Van Soest et al. (1991) in an ANKOM 2000 Fiber Analyzer without sodium sulfite. Heat-stable α-amylase was used in the NDF assay. Lignin concentration was measured after hydrolysis of the cellulose in ADF residues in a 72% H_2SO_4 (Van Soest and Robertson, 1985). Total carbohydrate (CHO) and non-fibrous carbohydrates (NFC) concentrations were estimated according to Sniffen et al. (1992) and Detmann and Valadares Filho (2010), respectively. A water extract was made from fresh silage samples (Kung et al., 1984) and an electrode (MA522 model, Marconi Laboratory Equipments, Piracicaba, SP, Brazil) was used to measure the pH. Volatile fatty acids (VFA) were measured in a gas chromatograph (GC2014, Shimadzu Corporation, Kyoto, Japan) using a HP-INNO wax capillary column (30 m × 0.32 mm; Agilent Technologies, Colorado, USA) at an initial temperature of 80ºC and a final temperature of 240ºC. The experiment was developed in a completely randomized design with 12 replicates. Data of corn silages were analyzed by ANOVA using MIXED procedure of SAS (v. 9.2 SAS Institute Inc., Cary, NC) using the PDIFF ($P < 0.05$).

Results and discussion The inoculated silages exhibited greater DM content, which possibly is associated with DM recovery (not showed in this study). As known, LAB needs carbohydrates as energy and carbon sources and these microorganisms can metabolize NFC into organic acids (Rooke and Hatfield, 2003). This could explain the reduction on NFC for LBLP silage compared to control silage, as well as the higher NDF contents (Table 1).

Table 1 Chemical composition and fermentative profile of corn silages inoculated with lactic acid bacteria and *Bacillus subtilis* after silos opening

Item	Forage	Control	LBLP	BSLP	SEM[1]	*P*-value
Chemical composition, g/kg DM						
DM	354.4	345.6^{c}	362.1^{b}	392.0^{a}	3.43	<0.0001
CHO	-	821.2^{b}	818.2^{b}	836.1^{a}	3.52	0.0028
NDF	303.2	372.4^{b}	396.9^{a}	382.9^{ab}	5.98	0.0274
ADF	149.4	203.4^{ab}	223.9^{a}	193.8^{b}	5.61	0.0036
NFC	-	492.3^{a}	468.0^{b}	484.9^{ab}	6.31	0.0316
Lignin	48.2	49.9	62.7	29.9	9.86	0.1120
Fermentative profile, g/kg DM						
pH	-	3.97^{b}	4.10^{a}	4.17^{a}	0.04	0.0032
Acetic	NA	20.8	21.9	19.4	1.52	0.5089
Propionic	NA	0.19	0.17	0.15	0.16	0.9871
Butyric	NA	0.29	0.35	0.56	0.10	0.2337
Microorganisms profile, log_{10} cfu/g						
LAB	-	6.9	7.4	7.5	0.19	0.1196
Yeasts	-	6.6	7.2	6.6	0.31	0.3798
Molds	-	5.1^{b}	5.4^{b}	6.3^{a}	0.29	0.0199

[1]Standard errors of least squares means and *P*-values represent statistical comparison among silages by PDIFF test and do not include comparison with the unensiled crop.
NA = not applicable.

Even though the inoculants increased the pH of silages compared to control silage, all values are within the optimum range expected for corn silage, which is 3.7 to 4.2 (Kung and Shaver, 2001). One of the main goals to use inoculants is associated to their capacity to reduce the yeasts and molds populations, especially the *L. buchneri* (Kleinschmit and Kung, 2006) and *B. subtilis* (Basso et al., 2012) in the case of this study. However, the inoculated silages exhibited similar populations of LAB and yeasts, whereas there was greater molds population for BSLP silage. Such as the LAB population was similar, this reflected in no difference for acetic and propionic acid concentration, which have antifungal effect and reduce the population of spoilage microorganisms (Moon, 1983).

Conclusion The use of silage inoculants composed by associations between *L. plantarum* and *L. buchneri* or *B. subtilis* applied on corn silage no improves the chemical composition and fermentation profile of corn silages produced in tropical climate.

Lactic acid bacteria and *Bacillus subtilis* as inoculants for corn silage produced in tropical climate: fermentative process

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Keywords fermentative losses, *Lactobacillus* spp., organic acids

Introduction *Lactobacillus plantarum* and *Pediococcus acidilactici* are often used as silage inoculant due to rapid production of lactic acid and decrease of pH preserving the forage against spoilage microorganisms (Filya, 2003; Fitzsimons et al., 1992). Conversely, *L. buchneri* can increase the aerobic stability due to high production of acetic acid (Kleinschmit and Kung, 2006), whereas *Bacillus subtilis* produces antifungal compounds and inhibit the yeasts growth (Basso et al., 2012). Thus, the association between these bacteria should result in better fermentation, as well as higher aerobic stability of silages. So, our aim was to evaluate the effect of *L. plantarum* applied in combination with *L. buchneri* or *B. subtilis*, as well as *P. acidilactici* applied alone on the fermentative process of corn silages produced in tropical climate.

Materials and methods Corn hybrid (cv. 2B688Hx) was harvested at one-third milk-line of the mature stage. The corn plants were treated with water (2 L/t; control), 1×10^5 cfu/g fresh forage of *Lactobacillus plantarum* MA18/5U and 1×10^5 cfu/g fresh forage of *L. buchneri* CNCM I-4323 (LBLP); 1×10^5 cfu/g fresh forage of *L. plantarum* MA18/5U and 1×10^5 cfu/g fresh forage of *B. subtilis* AT553098 (BSLP); or 1×10^5 cfu/g fresh forage of *Pediococcus acidilactici* (PA) (Fatec Health and Animal Nutrition, Arujá, São Paulo, Brazil; and Lallemand Animal Nutrition, Milwaukee, WI, USA). The inoculants were dissolved in water (2 L/t) and then sprayed on piles of fresh forage under constant mixing. An amount of 3.6 kg of chopped forage from each treatment was packed into mini-silos (5 L plastic bucket silos) in quadruplicate, and sealed with a lid and adhesive tape, and remained closed for 2, 7, 14, 21 and 45 d. Mini-silos were weighed after filling and at the end of the ensiling period to determine gas and effluent losses (each mini-silo had 0.25 kg of sand at the bottom, and a thin plastic screen was used to prevent contact between the sand and forage). Water extract was made from fresh silage samples (Kung et al., 1984) and an electrode was used to measure the pH. Volatile fatty acids (VFA) were measured in a gas chromatograph. Water-soluble carbohydrates (WSC) also were determined (Nelson, 1944). The experiment was organized in a completely randomized design with four replicates, and data were analyzed using the MIXED procedure of SAS (version 9.4), considering the inoculants and fermentation days as fixed effects and the residual error as a random effect. Differences between means were determined using the DIFF procedure. Contrasts were constructed, and the single degree-of-freedom orthogonal comparisons included the linear, quadratic and cubic effects of fermentation days. Differences were declared significant at $P \leq 0.05$.

Results and discussion The association between *B. subtilis* and *L. plantarum* increased the gas losses for all times evaluated, which probably is related to higher production of CO_2 by *B. subtilis* due to metabolic pathways less desirable. Conversely, effluent losses were not changed by treatments. Inoculated silages exhibited lesser WSC content compared to control silage after 14, 21 and 45 d of ensiling. The fermentative process is based on LAB converting WSC into organic acids under anaerobic conditions (McDonald et al., 1991), and could be expected higher utilization of WSC by LAB to produce organic acids,

which occurred in later days during fermentation in this study (Table 1). However, only the production of propionic acid increased when inoculants were used, which occurred by combination between *L. plantarum* and *L. buchneri* after 14 and 21-d of fermentation. The presence of propionic acid in the LBLP silages could be explained by anaerobic conversion of lactic acid into acetic acid and 1,2 propanediol by *L. buchneri* (Oude Elferink et al., 2001), and whether *L. diolivorans* is present in the silage, this microorganisms is capable of converting 1,2 propanediol to propionic acid (Krooneman et al., 2002). Overall, due to lower production of acids, the BSLP silage exhibited greater pH values than control silage in all ensiling times evaluated.

Table 1 Fermentation (g/kg of DM) of inoculated corn silages produced in tropical climate

Time, d	Silage	Gas	Effluent	WSC	pH	Acetate	Propionate	Butyrate
2	Control	7.3^{b}	0.73^{a}	8.94^{a}	3.86^{c}	5.87^{a}	0.13^{a}	0.27^{a}
	LBLP	6.2^{b}	0.70^{a}	11.25^{a}	3.83^{c}	8.52^{a}	0.08^{a}	0.00^{a}
	BSLP	73.8^{a}	1.21^{a}	11.22^{a}	4.21^{a}	15.10^{a}	0.00^{a}	0.00^{a}
	Pediococcus	8.4^{b}	2.07^{a}	8.37^{a}	4.02^{b}	15.14^{a}	0.00^{a}	0.00^{a}
7	Control	9.2^{c}	2.30^{a}	13.61^{a}	3.93^{c}	5.22^{a}	0.00^{a}	0.00^{a}
	LBLP	19.5^{b}	1.58^{a}	13.27^{a}	3.50^{d}	3.57^{a}	0.00^{a}	0.00^{a}
	BSLP	79.9^{a}	1.97^{a}	11.73^{a}	4.22^{a}	6.22^{a}	0.00^{a}	0.00^{a}
	Pediococcus	8.5^{c}	3.95^{a}	8.88^{a}	4.01^{b}	13.32^{a}	0.00^{a}	0.00^{a}
14	Control	8.3^{c}	1.41^{a}	26.48^{a}	3.85^{c}	12.27^{a}	0.00^{b}	0.07^{a}
	LBLP	9.0^{bc}	1.44^{a}	17.44^{b}	3.61^{d}	11.07^{a}	0.27^{a}	0.00^{a}
	BSLP	79.0^{a}	2.44^{a}	11.22^{b}	4.13^{a}	20.83^{a}	0.00^{b}	0.00^{a}
	Pediococcus	13.1^{b}	3.14^{a}	5.10^{c}	3.95^{b}	20.28^{a}	0.00^{b}	0.00^{a}
21	Control	13.1^{b}	4.29^{a}	30.13^{a}	3.82^{c}	11.23^{a}	0.20^{b}	0.00^{a}
	LBLP	10.5^{b}	3.06^{a}	20.78^{b}	3.90^{b}	16.22^{a}	0.40^{a}	0.00^{a}
	BSLP	73.9^{a}	3.43^{a}	13.42^{c}	4.15^{a}	23.58^{a}	0.00^{c}	0.00^{a}
	Pediococcus	12.9^{b}	1.23^{a}	6.54^{d}	3.94^{b}	23.38^{a}	0.00^{c}	0.00^{a}
45	Control	13.9^{bc}	4.13^{a}	39.59^{a}	3.78^{c}	8.47^{a}	0.58^{a}	0.47^{a}
	LBLP	12.6^{c}	6.27^{a}	23.41^{b}	4.20^{a}	18.32^{a}	0.45^{a}	0.37^{a}
	BSLP	70.2^{a}	4.19^{a}	12.06^{c}	4.15^{a}	15.95^{a}	0.20^{b}	0.42^{a}
	Pediococcus	32.2^{b}	5.34^{a}	9.89^{c}	3.96^{b}	13.95^{a}	0.00^{c}	0.55^{a}
SEM		2.71	0.88	2.70	0.03	2.30	0.07	0.08
Silage (S)		<0.0001	0.7056	<0.0001	<0.0001	<0.0001	<0.0001	0.2954
Day (D)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S x D		0.0018	0.1298	0.0002	<0.0001	0.1332	0.0032	0.7261
Contrast[1,2]		C*	L**	L**	C**	C*	L**	Q**

[a-d]Means in the same column with different superscripts differed ($P < 0.05$).
[1]* $P < 0.05$; ** $P < 0.01$. [2]L = linear effect; Q = quadratic effect; C = cubic effect.

Conclusion No markedly changes were observed due to use of silage inoculants, and the association between *L. plantarum* and *B. subtilis* had a negative effect on fermentative process.

A meta-analysis of the effects of *Lactobacillus buchneri* associated or not with *L. plantarum* on the fermentation and aerobic stability of whole-crop corn silages in tropical weather

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Keywords digestibility, lactic acid, short-chain fatty acids, temperature, yeasts

Introduction *Lactobacillus plantarum* is often used to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH (Filya, 2003). But these silages can have low aerobic stability once lactic acid is not an antifungal compound (Moon, 1983), which is exacerbated in tropical weather (Ashbell et al., 2002). Thus, *L. buchneri* has been used as an additive to improve the aerobic stability of corn silages due to production of acetic acid (Oude Elferink et al., 2001; Queiroz et al., 2012). Consequently, could be expected lower fermentative losses and higher aerobic stability in corn silages inoculated with the combination of *L. plantarum* and *L. buchneri*, ensuring better nutritive value for this feed. So, our aim was to evaluate the effect of *L. buchneri* associated or not with *L. plantarum* on the fermentation and aerobic stability of whole-crop corn silages produced in tropical weather.

Materials and methods It was performed a meta-analysis considering trials which had examined the fermentation and aerobic stability of whole-plant corn silage inoculated with *L. buchneri* alone or combined with *L. plantarum*. The strains of *L. buchneri* (NCIMB 40788 and CNCM I-4323) were included in the same data set because the data in this review indicated similar responses on the studied variable. The treatments were classified into the following categories: 1) corn silage with no inoculant applied (untreated), 2) corn silage treated with 1×10^5 cfu/g of fresh forage of *L. buchneri* (LB), and 3) corn silage treated with LB treatment plus 1×10^5 cfu/g of fresh forage of *L. plantarum* MA18/5U (LBLP; Lallemand Animal Nutrition, Milwaukee, WI, USA). Data were taken from 8 trials conducted at São Paulo State University (Jaboticabal, SP, Brazil) including published (Basso et al., 2012, 2014; Salvo et al., 2013) and unpublished data. In these trials, the silos remained closed between 21 and 229 d. Overall for all trials, fermentative profile was measured in the water extract obtained from fresh silage samples (Kung et al., 1984), whereas short-chain fatty acids (SCFA) were measured by gas chromatograph; lactic acid was determined by a colorimetric method (Barker and Summerson, 1941), ammonia N by distillation (AOAC, 1996, method no. 941.04), and water-soluble carbohydrates (WSC) by a colorimetric method (Nelson, 1944). The coefficients of *in vitro* organic matter (IVOMD) digestibility were calculated from *in vitro* gas production (Menke and Steingass, 1988). Aerobic stability was defined in all trials as the length of time required for the temperature of the silages to increase 2°C above the baseline after exposure to air. The meta-analysis was performed using the MIXED procedure of SAS (version 9.4), and the statistical model with treatment being considered as a fixed effect and study as a random effect. Differences between means were determined using the DIFF option of LSMEANS statement. Differences were declared significant at $P \leq 0.05$.

Results and discussion The use of silage inoculants reduced the neutral detergent fiber (NDF), as well as increased the coefficients of IVOMD, which may be associated to ability of some *L. buchneri* strains to produce fibrolytic enzymes (Addah et al., 2011).

The LB silage exhibited lower yeasts counts due to higher production of acetic acid, which is an antifungal compound (Moon, 1983). As expected, the LBLP silage had higher lactic acid concentration, but this silage exhibited higher pH compared to other silages. A probably explanation for this result is associated to capacity of own LAB uses lactic acid as a substrate to growth whether WSC is limiting, which also is observed for yeasts (Pahlow et al., 2003). The LB silage exhibited higher concentration of butyric acid, which may be associated to lower initial reduction of pH leading to production of butyric acid by enterobacteria and clostridia (Pahlow et al., 2003). The LB silage no showed production of propionic acid. The *L. buchneri* is able to convert lactic acid into acetic acid and 1,2 propanediol (Oude Elferink et al., 2001), but the conversion of 1,2 propanediol into propionic acid is dependent of *L. diolivorans* (Krooneman et al., 2002), and if this bacterium is not present in the silage, could be expected no production of propionic acid. No differences for ammonia N and fermentative losses (gas and effluent) were observed in the corn silages. Conversely, the inoculated silages had lower heating rate, as well as higher aerobic stability, which is due to higher production of acetic acid.

Table 1 Chemical composition, fermentative profile and aerobic stability of whole-crop corn silage inoculated with *L. buchneri* (LB) alone or combined with *L. plantarum* (LBLP)

Item	Untreated	LB	LBLP	SEM	*P*-value
DM, g/kg as fed	309^{b}	318^{a}	308^{b}	12.4	0.0328
NDF, g/kg of DM	406^{a}	384^{b}	389^{b}	27.3	0.0014
ADIN[1], g/kg of DM	96	86	103	16.3	0.0992
NFC[2], g/kg of DM	428^{b}	452^{a}	428^{b}	42.9	0.0004
IVOMD, g/kg of OM	649^{c}	682^{b}	706^{a}	19.1	<0.0001
LAB, cfu/g of fresh silage	7.22	7.15	7.43	0.14	0.3259
Yeasts, cfu/g of fresh silage	4.29^{a}	3.09^{b}	4.92^{a}	0.89	0.0005
Molds, cfu/g of fresh silage	4.36	4.04	4.55	0.32	0.3111
WSC, g/kg of DM	24.56^{a}	26.67^{a}	12.04^{b}	104.8	0.0011
Lactic acid, g/kg of DM	65.2^{b}	62.4^{b}	89.9^{a}	6.9	0.0008
Acetic acid, g/kg of DM	25.0^{b}	34.1^{a}	30.8^{ab}	7.5	0.0011
Lactic: acetic ratio	3.9^{a}	2.8^{b}	3.8^{a}	1.1	0.0002
Propionic acid, g/kg of DM	2.1^{a}	0.0^{b}	2.0^{a}	1.5	0.0351
Butyric acid, g/kg of DM	1.2^{b}	2.4^{a}	1.2^{b}	0.7	0.0019
pH	3.90^{b}	3.93^{b}	4.02^{a}	0.08	0.0005
Ammonia N, g/kg of total N	40.1	40.3	46.3	5.0	0.2837
Gas, g/kg of DM	18.3	20.1	20.3	7.7	0.5127
Effluent, kg/t	7.1	8.6	6.5	2.5	0.3038
Heating rate, °C/h	0.21^{a}	-	0.09^{b}	0.01	<0.0001
Aerobic stability, h	34.3^{b}	60.9^{a}	72.9^{a}	9.6	<0.0001

$^{a-c}$Means in the same row with different superscripts differed ($P < 0.05$).
[1]ADIN = nitrogen insoluble in acid detergent. [2]NFC = non-fiber carbohydrates.

Conclusion The association between *L. buchneri* and *L. plantarum* seems to be more appropriated to improve the fermentation and aerobic stability of whole-crop corn silages produced in tropical weather.

Effect of additives on fermentation and chemical composition of maize silage

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Keywords maize silage, additives, fermentation, nutrients

Introduction Silage additives are represented mainly by chemical additives, enzyme additives and microbial additives (Zhang et al., 2014). Additives are used to improve silage quality (Xu et al., 2009). However, additives have different effects (Li et al., 2014). Some additives could restrain secondary aerobic oxidation of silage and some could increase the content of organic acid in maize silage. The objective was to compare the fermentation profile and chemical composition of maize silage treated with chemical, enzyme and *Lactobacillus buchneri* additives.

Materials and methods Maize was seeded at a planting density of 63,000 plants per hectare. Whole plant maize was harvested at dough stage. The four treatment were: control, disodium stannous citrate (0.5 g/kg of maize silage), α- amylase (0.5 g/kg of maize silage) and *Lactobacillus buchneri* (5×10^5 cfu/g of fresh forage), respectively. After application, additives were homogeneously mixed with the forage, packed in vacuum bags and stored for 90 days. Each treatment had 3 replicates. At opening CP was measured with Kjeldahl method, NDF and ADF measured with Van Soest method and lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA) were determined by High Performance Liquid Chromatography (HPLC).

Results and discussion The pH and the contents of $N\text{-}NH_3$, LA, AA, PA and BA of maize silages are shown in Table 1. Adding *Lactobacillus buchneri* increased the silage pH. The content of AA at *Lactobacillus buchneri* treatment was significantly ($P<0.01$) higher than in control, citrate and amylase treatments. The *Lactobacillus buchneri* also decreased the content of $N\text{-}NH_3$ ($P<0.01$). The content of PA at *Lactobacillus buchneri* group was higher than that of citrate and amylase additives, but no difference was observed with the control group. This experiment did not detect the BA at all treatment groups. Results showed that the chemical composition of maize silage was similar across the treatments (Table 2).

Conclusions The *Lactobacillus buchneri* increased the content of acetic acid and decrease the content of $N\text{-}NH_3$ in maize silage.

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Acknowledgements The authors wish to thank all the staves of the Institute of Grassland Science, China Agricultural University, China, for their help. This research was supported by the Special Fund for Agro-scientific Research in the Public Interest (Contract no. 201303061) and the National Key Technologies R&D Program (Contract no. 2011BAD17B02-02).

Table 1 Effects of additives on the fermentation profile of maize silages

Additive	pH	N-NH_3 (%)	LA (%)	AA (%)	PA (%)	BA (%)
Control	3.48±0.09[a]	1.77±0.11[a]	4.57±0.08	0.25±0.02[b]	0.23±0.14[a]	n.d.
Citrate	3.51±0.04[a]	1.57±0.23[a]	4.59±0.06	0.21±0.07[b]	0.13±0.03[b]	n.d.
Amylase	3.58±0.06[a]	1.87±0.15[a]	4.46±0.77	0.28±0.03[b]	0.15±0.05[b]	n.d.
L. buchneri	3.81±0.21[b]	0.82±0.25[b]	4.30±0.50	0.43±0.06[a]	0.21±0.03[a]	n.d.

Different letters in the same column means significant difference at $P<0.05$. N-NH_3= Ammonia nitrogen, LA= Lactic acid, AA= Acetic acid, PA=Propionic acid, BA=Butyric acid, n.d.=Not detected.

Table 2 Effects of additives on the chemical composition of maize silages

Additive	Dry matter recovery (%)	DM (%)	CP (%)	NDF (%)	ADF (%)	Ash (%)
Control	96.75±1.49	31.89±2.35	8.19±0.75	54.72±2.03	26.36±3.46	4.11±0.65
Citrate	97.14±1.31	31.17±1.72	8.42±0.61	55.07±2.37	25.20±1.31	4.31±0.09
Amylase	97.11±1.54	31.40±1.99	8.15±1.03	54.73±2.14	26.49±3.39	4.12±0.06
L. buchneri	97.43±1.68	30.44±1.49	8.12±0.68	54.59±3.04	25.54±2.65	3.79±0.04

Different letters in the same column means significant difference at $P<0.05$. DM= Dry matter, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber.

Microbial inoculant and ensiling time effects on fermentation profile, nitrogen fractions, and ruminal in vitro starch digestibility in corn shredlage and late-maturity corn silage

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Keywords length of storage, inoculant, starch digestibility, corn shredlage

Introduction Starch digestibility in whole-plant corn silage increases with longer ensiling times. However, data evaluating ensiling time effects on starch digestibility of well-processed corn shredlage or late-maturity corn silage are unavailable in the literature. In addition, microbial inoculants may either accelerate fermentation or increase acid production and thus increase proteolysis or solubilization of prolamins. Therefore, the objective of this study was to evaluate the impact of microbial inoculants and ensiling time on fermentation profile, nitrogen fractions, and starch digestibility of corn shredlage or late-maturity corn silage.

Materials and methods Two experiments were conducted simultaneously to evaluate either well-processed corn shredlage (Experiment 1; 76.0% of starch passing through a 4.75 mm screen, 39.3% DM, and 32.3% NDF) or late-maturity corn silage (Experiment 2; 48.0% DM and 25% NDF). For Experiment 1, unfermented corn shredlage was obtained from the University of Wisconsin – Madison Agricultural Research Station (Arlington, WI) during September, 2012. Samples were homogenized, allocated into 24 samples of 600 g each, and randomly assigned to 6 treatments so each treatment had 4 replications. Six treatments were a combination of corn shredlage non-inoculated (**CON**) or inoculated with the recommended inoculation rate (**1X**; 4.5 x 10^4 CFU/g of corn shredlage) or twice the recommended dose (**2X**; 9 x 10^4 CFU/g of corn shredlage) of a microbial inoculant and ensiled for either 30 or 120 d. The microbial inoculant contained *Lactobacillus plantarum, Lactobacillus casei, Streptococcus faecium* and *Pediococcus* (Silo Charger "D", NU-AG Bosko, Inc., Oskaloosa, IA, USA). All 24 samples, including the non-inoculated corn shredlage samples, received the same amount of double distilled water to ensure protocol similarity among all samples. After inoculation samples were ensiled in vacuum-sealed bags and stored in the dark at room temperature (approximately 20°C) until reaching the targeted ensiling time. Experiment 2 used the same experimental methodology except for evaluating treatments within later-maturity corn silage rather than corn shredlage. All samples were analyzed in duplicate for DM (% as fed), CP (% DM), ammonia-N (% CP), starch (% DM), ruminal in vitro starch digestibility at 7 h (% of starch), pH and fermentation profile. Data for both experiments were analyzed as a completely randomized design in a 3 × 2 factorial arrangement of treatments using Proc Mixed of SAS with inoculation, ensiling time and their interaction as fixed effects.

Results and discussion In Experiment 1, DM and starch concentrations were unaffected ($P > 0.10$) by treatments and averaged 39.6% and 39.0%, respectively. Although not affected ($P = 0.41$) by inoculation, content of CP increased from 30 to 120 d of ensiling ($P = 0.01$; 7.2% vs. 7.8%, respectively). Measurements of pH were reduced from 3.96 at 30d to 3.88 after 120 d of ensiling. This was related to increased ($P < 0.05$) acetate and total acid concentrations. However, concentrations of lactate and ethanol did not differ ($P > 0.10$). Ammonia-N concentration ($P = 0.001$; 3.25% vs. 4.54%) and starch digestibility ($P = 0.01$; 69.3% vs. 72.0%) increased from 30 to 120 d of ensiling. Fermentation profile, including ammonia-N, and starch digestibility of corn shredlage were unaffected ($P > 0.10$) by inoculation. In Experiment 2, ensiling time did not affect ($P > 0.10$) concentrations of DM, CP and starch (47.7%, 7.5%, and 43.0%, respectively). However, DM and starch contents were ($P < 0.05$) 2.5%- and 3.4%-units greater for 2X than other treatments, but CP content did not differ ($P = 0.34$). Concentrations of lactate and total acids were ($P = 0.01$) greater for CON and 1X compared with 2X. In addition, propionate and ethanol concentrations tended ($P < 0.10$) to be greater for CON than the other treatments. Despite the lower ($P = 0.01$) ammonia-N concentration for 2X compared with other treatments, starch digestibility was unaffected ($P = 0.69$) by microbial inoculation. Ensiling time affected fermentation profile similarly to Experiment 1 with greater ($P < 0.01$) lactate, acetate and total acid concentrations after 120 d of ensiling. Furthermore, reductions in pH and ethanol concentration were also observed ($P < 0.05$) for 120 d compared with 30 d. Late-maturity corn silage fermented for 120 d had greater ammonia-N ($P = 0.001$; 5.39% vs. 3.99%) and starch digestibility ($P = 0.04$; 66.7% vs. 61.7%) compared with 30 d.

Conclusions Organic acid production and corresponding pH decline with extended ensiling time occurred in both corn shredlage and late-maturity corn silage. Ammonia-N concentration and starch digestibility were greater after 120 d of fermentation in both experiments, suggesting that extended ensiling time is advantageous in both scenarios. Inoculation with lactate-producing bacteria, however, was not beneficial in either experiment. In addition, microbial inoculation did not increase starch digestibility.

Effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn silage in farm scale silos

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Keywords acetic acid, heterofermentative bacterium, silage inoculant

Introduction *Lactobacillus buchneri* is a heterolatic bacterium able to produce significant amounts of acetic acid, which has antifungal activity and improves aerobic stability of silages. Thus, the aim of this work was to evaluate the effects of *L. buchneri* on fermentation and aerobic stability of corn silage in farm scale silos.

Materials and methods The experiment was carried out at the Department of Animal Science, College of Agriculture "Luiz de Queiroz", Piracicaba, Brazil. The corn was harvested at 35% of dry matter (DM) and packed into eight bags with capacity of 40 t (4 bags per treatment). At ensiling, two treatments were imposed: Control (C) and *L. buchneri* applied at 1×10^5 ufc/g fresh matter (LB). The microbial inoculant containing *L. buchneri* (CNCM I-4323- Lallemand Animal Nutrition strain) was diluted in distilled water (4 L/t as fed) and sprayed onto the chopped forage. The control was sprayed with the same volume of distilled water. After 247 days of storage silos were opened and silages were evaluated. Silage temperature was measured in five points of the silo working face using a bulb thermometer. Silage samples were used to prepare aqueous extract and evaluate the pH, soluble carbohydrates and fermentation end-products. Silage samples were also collected for enumeration of yeasts, molds and lactic acid bacteria (LAB). The aerobic stability was tested for 10 days and was defined as the time (hours) until silage temperature reached 2°C above ambient temperature.

Results and discussion The LB silage showed higher counts of LAB compared with the control silage, whereas no effects were observed on the fermentation profile (Table 1). Because the counts of yeasts and molds and the concentrations of acetic acid were similar across the treatments, only a weak tendency ($P = 0.12$) was detected for higher aerobic stability in inoculated silages. Kleinschmit and Kung (2006) demonstrated that effects of *L. buchneri* are dose-dependent and that doses $\leq 1 \times 10^5$ cfu/g were more inconsistent than doses $>1 \times 10^5$ cfu/g to improve silage quality.

Conclusions Inoculation with *L. buchneri* at a dose of 1×10^5 cfu/g fresh matter has no major influence on the quality and aerobic stability of corn silage storage in farm scale bags.

Reference

Kleinschmit, D.H. and L. Kung, 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. J. Dairy Sci. 89: 4005-4013.

Table 1 Fermentation profile and microbial counts in corn silages inoculated with *L. buchneri*

Item	Treatments[1]		SEM	*P*-value
	C	LB		
Dry matter, % as fed	33.6	33.9	0.56	0.51
Soluble carbohydrates, % DM	3.29	2.63	0.23	0.13
pH	3.85	3.80	0.04	0.46
Lactic acid, % DM	3.27	3.33	0.52	0.91
Acetic acid, % DM	1.30	1.38	0.40	0.54
N-NH_3, % N	8.44	7.54	0.32	0.14
LAB[2], log cfu/g FM	5.89	6.33	0.06	0.02
Yeast, log cfu/g FM	3.38	3.39	0.04	0.83
Molds, log cfu/g FM	2.74	2.35	0.39	0.55
Panel temperature, ºC	29.3	28.9	0.17	0.21
Density, kg FM/m^3	422	391	14.4	0.23
Aerobic stability, h	15.8	24.5	3.35	0.12

[1]C: control, LB: *L. buchneri* applied at 1×10^5 cfu/g fresh matter.
[2]LAB: lactic acid bacteria.

Effects of selected lactic acid bacteria isolated from epiphytic microbiota of a range environments on the fermentation profile and aerobic stability of corn silage

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Keywords corn silage, microbial inoculants, LAB isolation

Introduction Lactic acid bacteria (LAB), which ferments water soluble carbohydrates into mainly lactic acid, are the most important microorganisms in order to achieve a desirable fermentation of silage. Inoculation of corn silage with homolactic acid bacteria has some advantages such as decreasing pH level, increasing lactic acid (LA) production and dry matter digestibility (Muck and Kung 1997), although some disadvantages such as promoting dry matter (DM) losses, poor aerobic stability and increasing ammonia production (Muck 2004; Lindsey and Kung, 2010). Every LAB strains have their own characteristics in terms of fermentability and antimicrobial compound production for protecting silage against yeast and mold (Moon, 1983). These microorganisms are effective not only for initial silage fermentation but also for aerobic stability when silage is exposed to air. The objective of this study was to determine the effects of new LAB strains isolated from various range environments on ensiled corn fermentation and aerobic stability.

Materials and methods A second crop season corn (Truva cv., Limagrain) was harvested and chopped at low DM content (about 210 g/kg) stage. Previously isolated and selected 10 high lactic acid producing bacteria strains were used as microbial inoculants with a target of 5×10^5cfu/g fresh crop. Two strains (1 *L.buchneri* and 1 *L. brevis*) were heterolactic while the others (1 *L. gasseri*, 4 *L. plantarum*, 1 *L.bifermentans*, 1 *P.pentosaceus* and 1 *L. citerum*) were homolactic. Silage was opened at 0, 6, 12, 24, 48 and 60 days after ensiling (T0, T1, T2, T3, T4 and T60, respectively). Silages were examined at T0, T1, T2, T3 and T4 to determine the effect of time intervals on microbial composition. Lactic acid, acetic acid (AA), propionic acid (PA), ethanol and ammonia-N were determined to explain fermentation profile of resultant silages (T60) in addition to cell wall components and aerobic stability.

Results and discussion The LAB counts in control silages at all opening times were significantly lower than that of inoculated silages. Yeast and mold counts were higher in control silages at T0, T1 and T2 opening times but they were not at countable rates at T3, T4 and T60 opening times (data not shown). The extent and rate of decreases in pH of inoculated silages were significantly higher than that of control silage. The DM recovery of all inoculated silages was higher than that of control (Table 1). Generally, inoculation significantly increased LA production compared to control silage. However, AA production were higher in two inoculants (LS6521 *L. bifermentans* and LS81 *P. pentosaceus*, both which were homolactic) than control (Table 2). These two inoculants also enhanced aerobic stability of silages significantly possibly due to increase in AA production. It is known that

L. bifermentans has the capability of converting the previously produced LA into AA to some extent. This conversion of LA into AA might have resulted in improvement in DM recovery and aerobic stability.

Table 1 Fermentation products of resultant silages (%) (T60)

Isolate	Isolate species	Ammonia	LA	A A	PA	Ethanol	ADF	NDF	CP
	Control	1.95 c	3.95 def	1.08 c	0.03 de	0.00 e	36.49 b	62.87	11.28 c
LS5522	*L.brevis*	1.71 d	4.08 de	0.13 fg	0.10 d	1.08 c	32.81 c	61.32	11.33 c
LS5121	*L.gasseri*	1.45 e	4.61 d	0.47 d	0.44 b	0.00 e	35.53 b	62.94	9.56 f
LS7123	*L.plantarum*	1.68 d	3.39 f	0.10 fg	0.00 e	0.00 e	35.51 b	64.11	10.57 e
LS241	*L.plantarum*	1.34 e	4.09 de	0.00 g	0.00 e	1.70 b	36.95 b	60.54	10.66 e
L7061	*L.citerum*	1.42 e	3.76 ef	0.18	0.31 c	0.00 e	35.77 b	64.02	9.81 f
LS81	*P.pentosaceus*	1.38 e	11.52 b	2.68 a	1.72 a	0.59 d	36.72 b	64.58	11.69 b
LS6521	*L.bifermentans*	1.68 d	17.81 a	2.10 b	0.00 e	2.61 a	35.89 b	64.3	10.94 d
LS33	*L.plantarum*	1.36 e	4.60 d	0.55 d	0.06 de	0.00 e	36.37 b	61.95	10.92 d
LS722	*L.plantarum*	5.71 a	4.06 def	0.00 g	1.72 a	0.00 e	40.59 a	63.47	12.10 a
LS3114	*L. buchneri*	2.17 b	5.28 c	0.28 e	0.33 c	0.00 e	35.68 b	62.45	10.75 de

ADF: Acid Detergent Fiber, NDF Neutral Detergent Fiber, CP:Crude Protein

Table 2 Silage pH changes and DM recovery

	pH						DM (%)		
Inoculants	T0	T1	T2	T3	T4	T60	T0	T60	DM Rec.
Control	5.73	5.81	5.78 b	5.44 a	4.43 a	3.84 a	21.88 a	21.08	96.34
L.brevis	5.86	5.86	5.82 b	4.12 g	3.97 b	3.77 c	21.00 b	20.58	98.00
L.gasseri	5.79	5.87	5.88 ab	4.23 f	3.84 cd	3.64 e	20.91 b	20.56	98.33
L.plantarum	5.82	5.89	5.85 ab	4.99 b	3.91 bcd	3.72 d	20.97 b	20.55	98.00
L.plantarum	5.82	5.92	5.79 b	4.12 g	3.94 bc	3.81 b	21.03 b	20.54	97.67
L.citerum	5.83	5.81	5.83 ab	4.72 c	3.88 bcd	3.61 f	21.02 b	20.6	98.00
P.pentosaceus	5.76	5.87	5.90 ab	4.21 fg	3.82 d	3.67 e	20.93 b	20.38	97.37
L.bifermentans	5.86	5.8	6.00 a	4.47 d	3.86 bcd	3.62 f	20.99 b	20.5	97.67
L.plantarum	5.87	5.83	5.83 b	4.45 d	3.88 bcd	3.63 f	20.90 b	20.41	97.66
L.plantarum	5.79	5.85	5.89 ab	4.35 e	3.87 bcd	3.66 e	20.92 b	20.57	98.33
L. buchneri	5.87	5.79	5.85 ab	4.44 d	3.87 bcd	3.58 f	20.95 b	20.53	98.00

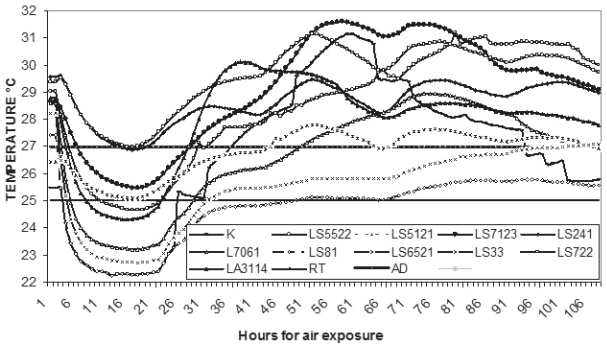

Figure 1 Aerobic stability of silages.

Conclusions Homolactic strains LS6521 *L. bifermentans* and LS81 *P.pentosaceus* seems to be promising to improve silage quality in terms of fermentation profiles, DM recovery and aerobic stability of silage. However, these two strains should be compared with commercially available inoculants before large implication.

Acknowledgement This study is a part of project 110O694 supported by TUBITAK.

Does the silage absorb air during its fermentation? A lab trial on maize silages added with natamycin

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Keywords gases, losses, maize, oxygen

Introduction The detrimental effects of air in silage are long time reported (Woolford, 1990). However the capability of silages to absorb air is still unknown. In this trial we aimed to simulate infiltration of atmospheric air in maize silages through low quality polyethylene sheets, when compared to hermetic sealing. The effect of natamycin, a bacteriocin that inhibits the growth of yeasts, was also assessed.

Materials and methods Maize plants (286 ± 5.3 g kg^{-1} DM) were harvested and chopped with a pull-type harvester. Thirty PVC lab silos (15 cm diameter × 50 cm height) were allocated with five replicates in a factorial arrangement with three treatments (C: no additive; N4: 4 g t^{-1} natamycin and N8: 8 g t^{-1} natamycin) and two conditions (A: daily injection of 40 mL of air; B: no air injection). All treatments were applied with non-chlorinated water (2 L t^{-1} forage). After filled, plastic polymer glue was applied around the cap of the silos to become them hermetically sealed. Pilot trials (pressurized air into the silo submerged in water) were performed to assure the hermetic sealing of the silos. A plastic 2-cm height platform was placed in the bottom of each silo to assess effluent losses (EL). Gas production (Gprod) was daily measured during all of the 66-d storage period through a graduate chamber immerged in water, and coupled to a 3-way valve. Air injections into the silos were made through this valve. Silos were weighed before and after fermentation to assess gravimetric DM losses (DML) and gas losses (GL) according to Jobim et al. (2007). Samples of forage and silage were taken after homogenization for DM determinations in duplicates (65 °C for 72 h plus 105 °C for 12 h). Silage pH was determined in water (25 g silage and 225 mL distilled water, homogenized by 1 minute). For aerobic stability assessment, 3 kg of silage were placed in 20-L buckets, kept in a controlled temperature room (24.1 ± 1.9°C). Data-loggers recorded silage temperature every five minutes for 168 hours. Aerobic stability (AS) was regarded as the time for silage to reach 2°C above room temperature. Statistical analyses were performed using SAS 9.3, for a completely randomized factorial design (3 treatments × 2 conditions). Normality was assessed through Shapiro-Wilk test, and variance homogeneity was evaluated by Box-Cox methodology. Data were submitted to analysis of variance and means were compared by Tukey test ($P<0.05$).

Results and discussion The only interaction between treatment and condition was observed for silage pH. Silage density averaged 638 ± 3.7 kg m^{-3} and did not differ between treatments or condition ($P>0.05$). The DM content of silages decreased after the ensiling (273, 263 and 265 g kg^{-1} for C, N4 and N8, respectively). Natamycin did not influence AS or GProd. Effluent losses were low, and were affected only by treatment, being higher for N4 when compared to C and N8. All silos presented negative values for gravimetric DML and GL. Although it is a usual finding in silage trials, negative values

for DM losses are commonly considered as experimental errors and rarely discussed. Air injection did not affect those variables. Neither the treatments nor the condition affected Gprod. Gas production happened mainly in the first 5 days after sealing, and decreased until 21 days. During the storage period we have injected 2.6 L of air into each B silos, and this volume of gas was not recovered. Mean Gprod was 4.77, 4.68 and 4.63 L t^{-1} DM for C, N4 and N8, respectively. Our hypothesis that forced air injection could lead to greater gas production, mainly as CO_2, was not confirmed. The mechanism of air absorption in silages is unknown and needs to be better studied. Our previous studies have showed that CO_2 is the main gas produced during silage fermentation at very high concentrations, about 25.000 ppmv (Schmidt et al., 2013). The anaerobic fixation of CO_2 by acetogenic bacteria is well reported in literature (Fuchs, 1986). However the presence of these bacteria in silages was not reported yet. Moreover, considering that air is composed by about 78% of nitrogen, mechanisms of N fixation can be happening into the silo. Air injection reduced the average AS from 70 to 66 h, indicating that some aerobic microorganisms might have proliferated during ensilage due to the presence of oxygen. The greater initial population of yeasts, as well as the slightly lower content of lactic acid could decrease AS of the silages that were air injected.

Conclusions Forced air injection into lab silos of maize silages do not increase DM losses nor gas production. The volume of air applied into the silo was not recovered and seems to be incorporated into the silage mass. This mechanism is still unknown.

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Table 1 Means of variables according treatments1 and forced air injection2 in maize silages

	Air injection			No air injection				
	C	N4	N8	C	N4	N8	Sign[3]	SEM
Dry matter losses, %	-5.97	-1.53	-6.07	-5.68	-1.76	-5.28	T	0.43
Gas losses, %DM	-6.23	-2.08	-6.35	-5.94	-2.40	-5.55	T	0.40
Gas production, L t^{-1} DM	4.66	4.70	4.31	4.88	4.67	4.94	-	0.10
Effluent, kg t^{-1}	2.40	5.39	2.59	2.41	6.25	2.59	T	0.44
pH	3.67	3.66	3.63	3.66	3.66	3.67	I	0.00
Acetic acid, g kg^{-1} DM	17.6	17.6	16.7	30.0	15.6	17.9	-	1.0
Lactic acid, g kg^{-1} DM	68.8	77.0	69.2	66.0	83.8	76.2	T/C	1.4
Aerobic stability, h	65.8	66.9	64.2	68.2	70.5	73.4	C	1.1

[1]C = no additives; N4 = natamycin, 4 g t^{-1}; N8 = natamycin, 8 g t^{-1}.
[2] Forced air injection (40 mL per silo per day) during a 66-d storage period.
[3] Significant difference ($P<0.05$) for treatment (T), condition (C), interaction (I), or no significant difference (-).

Developing a novel dual purpose silage inoculant

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Keywords maize, spoilage strains, silage inoculant, aerobe stability, dual purpose

Introduction 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 aerobe spoilage strains at feed-out. One would therefore expect that combining homo and heterofermentative strains would result in an additive effect. However, since lactic acid bacteria have different growth rates, and some LAB have even the ability to produce bacteriocins, such an additive effect is not guaranteed. Knowing the phenotypic characteristics of single strains and testing strain combinations seems crucial in order to ensure an additive effect. The current study therefore aimed at testing various dual purpose combinations both in a sterile *in vitro* medium and a mini-silo set in order to test the additive effect of homo and heterofermentative LAB.

Materials and methods Different test inoculants were formulated and blended in different ratios (table 1). Strains were tested in sterile medium containing various carbohydrates to simulate forage. The medium was inoculated with 150.000 CFU/mL in sterile 10 mL test tubes and samples were analyzed for pH and organic compounds at different time intervals up to 48 hours. For the mini-silo trial, maize was harvested and inoculated with different dual purpose or homofermentative silage inoculants (inoculant, B, C, D, H and I, see Table 1), and a control group (tap water) was included. The maize was inoculated with 150.000 CFU/g, vacuum packed and stored under anaerobe conditions at 25 °C for three months. After opening the bags, the silage was exposed to aerobe challenge in insulated containers with a hole (diameter 1 cm) in the top and bottom. Temperature was measured continuously.

Table 1 Inoculants used for *in vitro* medium and mini-silo trial

Inoculant	*In vitro* batch	Mini silo	*L. buchneri*	*L. plantarum*	*E. faecium*	*L. lactis* A	*L. lactis* B
A	X		100%				
B	X	X	70%	10%			20%
C	X	X	50%	20%	30%		
D	X	X	50%				50%
E	X		50%	20%			30%
F	X		33%	33%			33%
G	X		25%	25%		25%	25%
H	X	X		40%	30%	30%	
I	X	X					100%

Results and discussion The *in vitro* experiment showed that inoculants containing *L. plantarum* resulted in a pH below 3.8 after 24 h, while Inoculants D and I had a pH of 4.0 after 24 h while Inoculant A, containing only *L. buchneri*, had a pH of 4.7. The less

L. plantarum was included in the inoculant, the more acetic acid was accumulated in the medium.

Table 2 Mini-silos: Organic acid composition, pH, microbial count of vacuum packed maize. pH weight loss, temperature and microbial count after 7 days of aerobe challenge

	Control	B	C	D	H	I	P-value
After anaerobe fermentation							
pH	3.69^{c}	3.83^{b}	3.79^{b}	4.15^{a}	3.77^{b}	3.78^{b}	<0.001
Lactic acid, ppm	8305	7237	7859	6076	8116	8702	NS
Acetic acid, ppm	8651^{b}	9901^{ab}	8570^{b}	11932^{a}	5829^{b}	7561^{b}	<0.01
Lactic acid bacteria, CFU/g	7.6^{b}	8.9^{b}	8.8^{b}	9.3^{a}	8.6^{b}	8.6^{b}	<0.001
Yeast, CFU/g	0.9^{b}	0.0^{b*}	1.3^{b}	0.6^{b}	4.2^{a}	3.1^{a}	<0.001
Aerobe Spores, CFU/g	2.24^{a}	2.22^{a}	0.48^{b}	1.57^{ab}	0.96^{b}	2.48^{a}	<0.05
After exposure to air							
pH	6.74^{a}	5.67^{ab}	5.11^{bc}	4.05^{cd}	6.84^{a}	6.59^{a}	<0.001
Weight loss incl. water	2.25^{a}	2.15^{ab}	1.75^{b}	0.86^{c}	2.16^{ab}	2.61^{a}	<0.001
Stability, h	75^{b}	83^{b}	141^{a}	162^{a}	80^{b}	80^{b}	<0.001
Max temperature, °C	33.0^{a}	29.8^{ab}	25.2^{cd}	22.7^{d}	27.9^{bc}	29.0^{bc}	<0.01
Lactic acid bacteria, CFU/g	9.2^{a}	8.8^{b}	8.8^{b}	8.6^{b}	9.4^{a}	9.4^{a}	<0.05
Yeast, CFU/g	3.4^{b}	4.5^{ab}	5.8^{ab}	1.15^{b}	6.2^{ab}	8.4^{a}	<0.05
Aerobe Spores, CFU/g	2.8	2.7	1.4	1.7	2.4	3.1	NS

**All four samples below detection level of log =1*

The results on acetic acid in the mini-silo correlated with the results in the *in vitro* experiment. Inoculant D produced significantly (P<0.01) higher acetic acid concentration (ppm fresh silage) during anaerobe fermentation compared to inoculant C, which was inoculated with the same concentration of *L. buchneri*. There was no significant difference between Inoculant D compared to Inoculant B, which contained 70% *L. buchneri*. The pH of Inoculant D was significantly higher (P<0.001) compared to the other treatments after anaerobe fermentation. However, this treatment was the only one which kept all replicates (n=4) stable during 7 days of aerobe challenge. Furthermore, inoculant D had a significantly (P<0.001) lower weight loss compared to any of the other treatments.

Conclusions The results showed that strains in a silage inoculant product can affect each other, and that combinations and formulations of strains have to be investigated thoroughly. The *in vitro* experiment and mini silo trial showed that the combination of *L. lactis* and *L. buchneri* (Inoculant D) had a fast pH reduction, but without decreasing pH to such an extent that the growth of *L. buchneri* was inhibited.

Chemical additives reduce yeast count and enhance aerobic stability in high dry-matter corn silage

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Keywords chemical additive, corn silage, fermentation pattern, yeast, aerobic stability

Introduction Although heterofermentative lactic acid bacteria (LAB) are widely used to improve the bunk-life of corn silages (Kleinschmit and Kung, 2006), their effect is limited under suboptimal conditions, e.g. fermentation length shorter than six weeks and high dry matter (DM) concentrations at ensiling. Also, they may not be effective in warm climates as their activity depends on environmental conditions and forage characteristics (Muck, 2013). Therefore, this study aims at investigating the effects of novel liquid chemical additives on the fermentation and aerobic stability of high DM corn silage.

Materials and methods Corn [40% DM, 7% CP and 7% water-soluble carbohydrates of DM; log 6.7 cfu LAB and log 6.9 cfu yeast per g fresh matter (FM)] was chopped by a Claas Jaguar precision chopper to 20 mm particle size. From a larger amount of forage, six equal portions received the following treatments in three replicates: untreated control, 10 mL of water/kg FM, Xtrasil excel HD containing potassium sorbate, sodium benzoate and ammonium propionate, applied at 1 mL/kg (HD1) and 2 mL/kg FM (HD2), Xtrasil excel at 3 ml/kg FM (EXC3), composed of sodium benzoate, sodium propionate and ammonium propionate and Xtrasil stabilizer at 3 mL/kg FM (STAB3), containing propionic acid, ammonium propionate, sodium benzoate and potassium sorbate. All additives were provided by KONSIL Scandinavia, Sweden, and diluted prior to use to give 10 mL of liquid per kg FM. The treated forage was packed into 1.7-L glass jars, using three jars per treatment. The jars were equipped with a valve to enable release of fermentation gases and stored at 20°C for 58 days. Fermentation products and yeast counts were determined by routine analytical procedures. Aerobic stability was measured for 14 days by placing thermo loggers in the centre of the insulated silage samples, which recorded the temperature. Silages were considered unstable if the sample temperature was >2 °C higher than that of ambient (20°C). A completely randomized design was used and data were statistically evaluated by the SAS procedures GLM and REG. The yeast count was log-transformed using a value of log 0.7 cfu/g for all observations below the detection limit of 10 cfu/g. Significance was declared at $P < 0.05$ and multiple comparisons among LSMEANS were performed by employing the Tukey´s test.

Results and discussion All corn silages were well fermented (Table 1). Treatment had an influence on pH and lactate content but the differences were of no practical relevance. The concentration of acetate was unaffected by treatment. Typically for high DM corn silage, concentrations of ammonia-N and propionic acid were low, and the observed increases in comparison to untreated silage can be attributed to the added amounts contained in the products. In support of data by Auerbach and Nadeau (2013), chemical additives inhibited undesired fungal activity during fermentation, which was reflected by lower ethanol and

higher residual sugar concentrations compared to the untreated silage. Moreover, all additives reduced yeast counts and the yeast count was below the limit of detection for HD2, EXC3 and STAB3. As a result, the additives markedly enhanced the aerobic stability of the silages (Auerbach and Nadeau, 2013). A strong negative linear regression existed between the yeast count and aerobic stability (Figure 1).

Table 1 Effects of chemical additives on fermentation characteristics, yeast counts and aerobic stability of corn silage ensiled for 58 days (n=3)

Parameter	CON	HD1	HD2	EXC3	STAB3	SEM	*P*-value
pH	3.57^{b}	3.57^{b}	3.67^{a}	3.60^{b}	3.61^{b}	0.021	<0.05
NH_3-N (% total N)	6.9^{b}	6.4^{b}	6.4^{b}	6.5^{b}	8.0^{a}	0.22	<0.01
Lactic acid (% of DM)	4.95^{a}	4.74^{ab}	4.26^{b}	4.09^{b}	4.65^{ab}	0.147	<0.05
Acetic acid (% of DM)	0.96	0.95	0.95	0.93	0.88	0.047	0.78
Propionic acid (% of DM)	0^{d}	0.03^{c}	0.02^{c}	0.05^{b}	0.28^{a}	0.001	<0.001
Ethanol (% of DM)	0.36^{a}	0.25^{b}	0.29^{b}	0.29^{b}	0.26^{b}	0.014	<0.01
WSC (% of DM)	0.93^{b}	1.76^{a}	1.95^{a}	1.71^{a}	1.63^{a}	0.069	<0.001
Yeasts (log cfu/g)	3.1^{a}	1.6^{b}	$<1.0^{b}$	$<1.0^{b}$	$<1.0^{b}$	0.19	<0.001
Aerobic stability (days)	4.9^{c}	9.4^{b}	14.0^{a}	13.9^{a}	14.0^{a}	0.47	<0.001

CON = control, HD1 = Xtrasil excel HD at 1 L/t, HD2 = Xtrasil excel HD at 2 L/t, EXC3 = Xtrasil excel at 3 L/t and STAB3 = Xtrasil stabilizer at 3 L/t. WSC = water-soluble carbohydrates; means in rows bearing unlike superscripts differ (Tukey´s test).

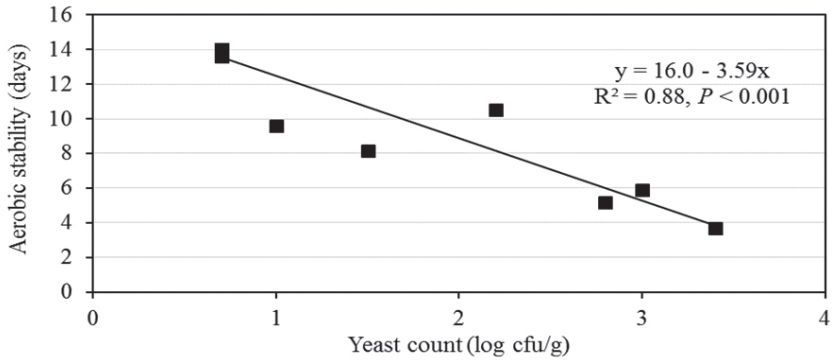

Figure 1 Relationship between yeast count and aerobic stability in corn silage.

Conclusions Regardless of composition and application rate, the tested chemical additives were highly effective in reducing yeast count, thereby enhancing the aerobic stability of corn silages. Their use to reduce DM losses during feed-out under challenging conditions, therefore, can be highly recommended.

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The effect of treating whole crop wheat with a bacterial inoculant on aerobic stability on farm scale silo

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Keywords whole crop wheat, heterofermentative, inoculant, aerobic stability

Introduction Whole-crop cereals have become increasingly popular in many winter diets in the UK. The rapid expansion in the use of wholecrop is due to the nutritional, agronomic and environmental benefits that this crop provides. Whole-crop is a good alternative forage in marginal maize growing areas and a useful complementary forage for grass and maize systems (Keady, 2005). However, due to its higher starch and sugar content, whole-crop silage is vulnerable to aerobic spoilage; therefore additives that protect the silage from heating can be very valuable. The objective of this trial was to examine the effects of treating whole-crop wheat with a bacterial inoculant at ensiling on aerobic stability (AS) on a farm silo scale.

Materials and methods A crop of spring sown wheat was harvested in a total of 12 loads. Loads 1-6 were harvested without being treated with an additive, whereas loads 7-12 were treated with a bacterial inoculant (*Biotal Wholecrop Gold* at 2.5 g per tonne fresh herbage) at the time of harvest. This application rate was designed to deliver *Pediococcus pentosaceus* at 1×10^5 cfu/g, *Lactobacillus buchneri* at 3×10^5 cfu/g, ß-glucanase (9804 IU/g) and xylanase (51269 IU/g). The untreated crop (Control) and additive treated crop (Additive) were ensiled in trench silos (nominal capacity of 90 t). The walls of the silos were lined with plastic sheet prior to filling. The crop was ensiled for 230 days before being opened. At opening, a sample of each silage was analysed for dry matter, nitrogen, ammonia-N, pH, volatile fatty acid, lactic acid and ethanol concentrations using wet chemistry. The AS of each of the two silages was measured. For this, 30 kg of silage was taken from each silo face, and subsamples (n = 6) placed in six polystyrene boxes, in a temperature controlled room. Temperature probes, each linked to automated loggers (ThermaData logger – model TDF), were placed into the centre of each pile. A lid was placed loosely on each box to prevent the silage from drying, but to allow air to enter the box. In addition, ambient temperature was recorded. Parameters describing the AS of the silages were analysed using ANOVA, with each of the six boxes per treatment used as replicates. Repeated measures ANOVA was undertaken using the mean temperature data for each two-hour period during the 14-day aerobic exposure period, with treatment, time and treatment × time interactions examined. All data were analysed using Genstat, Version 6.

Results and discussion From the results presented in Table 1, it appears that the addition of the inoculant had an effect on silage fermentation characteristics. The control silage had a numerically higher lactic acid concentration than the additive treated silage, whereas the additive treated silage had higher acetic acid and propionic acid concentrations than the control silage. This was reflected in the numerical difference in pH between the two silages.

Table 1 Fermentation characteristics of the control and additive treated silages

	Control	s.d.	Additive	s.d.
Volatile corrected dry matter (g/kg)	361	15.2	353	9.2
Crude protein (g/kg DM)	81	4.3	84	3.8
Ammonia-N (g/kg total N)	144	24.0	158	14.6
pH	4.13	0.14	4.40	0.05
Lactic acid (g/kg DM)	22.4	13.98	3.5	3.59
Acetic acid (g/kg DM)	25.2	6.74	38.0	1.74
Propionic acid (g/kg DM)	3.3	2.26	9.5	1.18
Butyric acid (g/kg DM)	0.9	0.70	0.4	0.23
Ethanol (g/kg DM)	5.1	0.60	6.9	0.49
Propanol (g/kg DM)	3.9	1.37	6.1	0.97

s.d., Standard deviation

Table 2 gives an overview of the results of the AS test. Commencing 24 hours after the start of incubation, the time taken for the temperature of the silages to increase by 3°C was 74 and 152 hours for the control and additive treatments resp. ($P<0.001$), whereas the maximum temperature during the aerobic exposure period was 31.3 and 22.0°C for the control and additive treatment silages resp. ($P<0.001$). Time taken for the maximum temperature to be reached was 89 and 169 hours, resp. ($P<0.001$), whereas the mean temperature during the incubation period was 20.3 and 18.6°C for the control and additive treated silages, resp. ($P=0.004$). This analysis clearly demonstrates that treating the crop with the additive at the time of harvest had a very dramatic effect on reducing the susceptibility of the ensiled crop to aerobic deterioration.

Table 2 Effect of treatment (additive vs. control) on aerobic stability of the silages

	Control	Additive	SEM	P-Value
Hours until 3ºC temperature rise‡	74	152	3.3	<0.001
Maximum temperature during incubation period (ºC)	31.3	22.0	1.06	<0.001
Hours until maximum temperature‡	89	169	3.73	<0.001
Average temperature during 9-day incubation period	20.3	18.6	0.33	0.004

The lower AS was evident both within the laboratory measurements and through observations at the silo face. The lower AS of the control silage resulted in a larger quantity of control silage being disposed of due to deterioration during the 56 day feed out period. As a result, the total number of 'cow feeding days' (a reflection of the quantity of edible silage removed from the pit) was 7.8% higher with the additive treatment.

Conclusions The additive treatment had a positive effect on the fermentation characteristics of the silages (significant higher acetic and propionic acid content), with the fermentation of the control silage being dominated by lactic acid. The additive treatment also had a significant effect on the AS of the silages, with the additive treated silage being much more stable than the control silage.

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Aerobic stability of whole plant corn silage inoculated with *Lactobacillus buchneri* in three maturity stages

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Keywords aerobic deterioration, inoculant, pH, acetic acid

Introduction Although there are several factors that determine the nutritive value of corn silage, the maturity stage at harvesting is the major (Johnson et al., 2003). The main goal in silage is to maintain the original quality of the preserved crop as much as possible (Wilkinson and Davies, 2012). The correct preservation of silage depends on absence of oxygen and the acidification of the material (McDonald et al., 1991). Once opened for delivery is exposed to oxygen deterioration due to aerobic microbial activity, which could influence negatively silage quality and profitability of production systems (Tabacco et al., 2011). Inoculants containing homofermentative bacteria have been developed with the purpose of producing lactic acid and reducing the pH quickly (Wilkinson and Davies, 2012). However, homofermentative bacteria have also been responsible for reducing aerobic stability in corn silage due to low production of volatile fatty acids which inhibit fungal activity (Muck and Kung, 1997). For these purposes, bacterial inoculants containing heterofermentative bacteria have been developed, such as *Lactobacillus buchneri*, which improve the aerobic stability of silage (Tabacco et al., 2011; Wilkinson and Davies, 2012). Recently, inoculants containing homofermentative and heterofermentative bacteria have been developed to overcome the limitations of inoculants containing only one type of bacteria. The aim of this trial was to evaluate the effect of inoculating *Lactobacillus buchneri* in whole plant corn silage at different maturity stages on aerobic stability.

Materials and methods Maize crop was harvested at three maturity stages: 25, 35 and 45% DM. The crop was harvested with a machine Cibus F Wintersteiger. Chopped material was ensiled in 20 L buckets (six silos for stage of maturity), sealed and opened after 80 d. Before the preparation of micro-silos, three were inoculated with *Lactobacillus buchneri* that also contained *Enterococcus faecium* and *Lactobacillus plantarum* (1.5×10^5 cfu/g of fresh forage) and the other three remained as Control (the same amount of water was applied to the treatment inoculated as placebo). After opening samples were taken and analyzed for pH, N-NH_4, water-soluble carbohydrates and volatile fatty acids (lactic, propionic and acetic acid). To determine aerobic stability, 3 kg of silage were placed in plastic buckets and maintained in an enclosed space at controlled temperature (average 20°C). The temperature of the silage was measured every 6 hours with a datalogger placed in the center of the mass during aerobic exposure (Basso et al., 2012). Aerobic stability is defined as the number of hours that silages maintains temperature before increasing more than 2°C above ambient temperature (Taylor and Kung Jr., 2002). Data were analyzed with the GLM procedure of the SAS system (SAS Institute, Inc., 2008). Means were compared by Tukey test ($P < 0.05$).

Results and discussion The interactions between maturity stage and inoculants treatments were not significant for all variables analyzed (Table 1). For the evaluated variables, there

was no difference among inoculated treatments, possibly due to the high content of acetic acid observed. But there was a difference between the maturity stages, where 25% DM silages had higher WSC content and acetic acid, which allowed a greater aerobic stability than with 35 and 45% DM. Dry matter losses were similar between inoculated treatments and the 45% DM treatment had the highest value compared to 25 and 35% DM.

Table 1 Chemical analyses and aerobic stability of the inoculant treatments at three maturity stages of corn silage

	Inoculant Treatments		Maturity Stages			Inoc	Maturity	Inoc ×
Item	Control	Inoculant	25%	35%	45%	Treatments	Stages	MS
pH	3.84	3.86	3.85^{b}	3.68^{c}	4.03^{a}	ns	*	ns
WSC, %	0.63	0.69	0.99^{a}	0.54^{b}	0.46^{b}	ns	*	ns
Ammonia N, %	0.05	0.06	0.06	0.06	0.05	ns	ns	ns
Acetic ac., %	1.79	1.83	2.63^{a}	1.66^{b}	1.15^{c}	ns	*	ns
Propionic ac., %	0.33	0.30	0.37^{a}	0.37^{a}	0.20^{b}	ns	*	ns
Lactic ac., %	8.42	7.86	10.0^{a}	7.80^{ab}	6.59^{b}	ns	*	ns
Total ac., %	10.5	9.98	13.0^{a}	9.82^{b}	7.94^{b}	ns	*	ns
AS, h	79	70	116^{a}	59^{b}	49^{b}	ns	*	ns
Hs to max temp, h	89	75	126^{a}	67^{b}	53^{b}	ns	*	ns
Max. temp, ºC	30.4	29.8	28.6^{b}	33.7^{a}	27.9^{b}	ns	*	ns
DM losses, %	14.5	14.3	13.6^{b}	13.2^{b}	16.5^{a}	ns	*	ns

$^{a-c}$ Means in line with different superscript letters differ ($P < 0.05$). NS = not significant; DM = Dry matter; WSC = Water-soluble carbohydrates; AS = Aerobic stability; MS = Maturity stages.

Conclusions There was no effect of *Lactobacillus buchneri* but there was an effect of harvesting time on the aerobic stability of silage. As the harvesting is delayed the aerobic stability of silage worsens.

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Fermentative profile and bacterial diversity in corn silages inoculated with epiphytic tropical lactic acid bacteria

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Keywords acetic acid, HPLC, *Lactobacillus buchneri*, PCR-DGGE

Introduction Corn (*Zea mays L.*) is the forage plant that is most often used for the production of silage. Due to the high concentration of substrates that are potentially oxidizable by opportunistic microorganisms in corn silages, DM losses might occur during the entire fermentation process. Investigators have recommended the use of microbial inoculants in corn silages as a way to increase the efficiency of the fermentation and reduce the losses originating from the process. The use of culture independent molecular techniques, like the PCR-DGGE followed by DNA sequencing, has allowed great advances in the knowledge of the microbiota present in silages, since it allows for the detection of microorganisms that are difficult to cultivate in the laboratory, as well as those that are not cultivable. In countries with a tropical climate, these studies are still rare. This work aimed to evaluate the effects of the inoculation of different tropical lactic acid bacteria (LAB) strains on the fermentative profile and bacterial diversity of corn silages.

Materials and methods Nine lactic acid bacteria strains isolated from sugarcane silage and screened for corn silage were used. A total of three strains of *Lactobacillus buchneri* (UFLA SLM11, UFLA SLM103, and UFLA SLM108), five strains of *L. plantarum* (UFLA SLM08, UFLA SLM41, UFLA SLM45, UFLA SLM46, and UFLA SLM105), and one strain of *Leuconostoc mesenteroides* (UFLA SLM06) were studied in experimental PVC tubes. Samples were collected from fresh forage prior to treatment and treated (0 day) and from the silages after 10, 30, 60, and 90 days of fermentation. Part of each sample was used to make a water extract to evaluate the microbial population, and detect the fermentation end products by HPLC. Yeasts and molds were enumerated by Dichloran Rose Bengal Chloramphenicol Medium (DRBC). The plates were incubated at 28ºC for 72 h. For LAB enumeration, pour plating onto DeMan-Rogosa-Sharpe agar (MRS) was used. The plates were incubated at 30ºC for 72 h. The silages inoculated with the strains which produced the highest concentrations of acetic acid and 1,2 propanediol were evaluated for bacterial diversity by PCR-DGGE during all fermentation periods. The DGGE bands were excised from the acrylamide gels. The DNA fragments were purified, and re-amplified with the primer 968rGC and 1401f. The PCR products were sequenced and the obtained sequences were compared with those available in the GenBank database.

Results and discussion The inoculation of LAB strains did not exert a significant influence ($P > 0.05$) over the lactic, propionic, and butyric acids, and ethanol concentrations of the silages ($P > 0.05$). However, significant differences were found ($P < 0.05$) in the concentration of these metabolites over the five fermentation times. The concentration of lactic acid increased from 8.9 to 51.6 g kg^{-1} DM up to 30 days of fermentation. Furthermore, the ethanol concentration increased during the first 10 days of fermentation reaching 30.0 g kg^{-1}. From these periods, a tendency towards the stabilization of these

metabolites was observed until the end of the fermentative period. During the first 10 days of fermentation the concentration of propionic acid decreased, although it showed an increase in relation to the fresh forage of 4.0 to 4.7 g kg^{-1} DM. The concentrations of butyric acid were reduced from 1.4 to 0.6 g kg^{-1} DM after 90 days of fermentation. For the concentration of acetic acid, a significant interaction was observed ($P < 0.05$) between the treatments and fermentation time. At the end of 90 days of fermentation, the silages inoculated with *L. buchneri* UFLA SLM11, UFLA SLM103, and UFLA SLM108 strains had the highest concentrations of this acid, producing 86.7% more acid than the homofermentative strains. For 1,2-propanediol concentration, a significant interaction ($P< 0.05$) was observed between the silage treatments and fermentation time. The silages inoculated with *L. buchneri* UFLA SLM11, UFLA SLM103, and UFLA SLM108 showed concentrations of 1,2-propanediol four-times higher than the others. Significant differences were not observed ($P> 0.05$) between the silage treatments for yeast and mold counts. However, the fermentation time did have a significant effect on the populations of these microorganisms ($P< 0.05$) (Figure 1a).The addition of inoculants and the fermentation periods influenced ($P< 0.05$) the LAB population of silages, without any interaction between the factors ($P> 0.05$). Analyzing the average of all treatments, it was observed that silages inoculated with *L. buchneri* UFLA SLM11 and UFLA SLM103 strains showed the highest LAB populations, with 8.77 and 8.64 log CFU g^{-1}, respectively (Figure 1b). The other silages showed an average LAB population of 8.11 log CFU g^{-1} (Figure 1b). In the evaluation of bacterial diversity using the DGGE technique in the silages inoculated with *L. buchneri* UFLA SLM11, UFLA SLM103, and UFLA SLM108 strains, it was clear that the inoculation did not affect the epiphytic microbiota and few differences were observed among them during the fermentation periods. Bacteria belonging to the *Enterobacteriaceae* family (*Klebsiella pneumoniae* subsp. *pneumoniae*, *Klebsiella oxytoca, Kluyvera ascorbata* and *Serratia marcescens*), and the *Clostridium* genus were detected in silages by DGGE analysis. In addition to the inoculated LAB, *Weissella confusa*, a heterofermentative LAB was also found in all of evaluated silages.

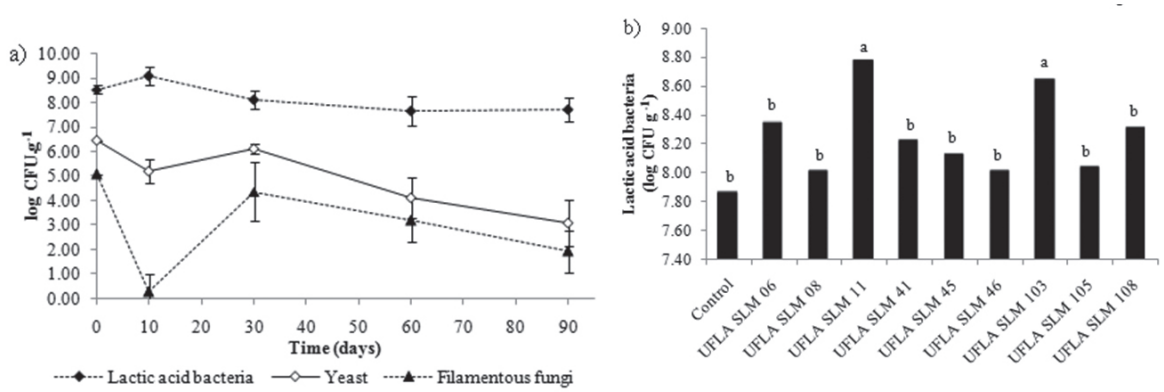

Figure 1 Microbial populations in different silages according to fermentation time (a). Counts of lactic acid bacteria (average of all treatments) in corn silages (b).

Conclusions A higher diversity of undesirable bacteria was detected by DGGE throughout the fermentative process in silages inoculated with UFLA SLM103 and UFLA SLM108 strains. Due to the fermentative and microbial character, UFLA SLM11 strain is considered to be promising for use as an inoculant in corn silages.

The effects of *Lactobacillus buchneri* and length of storage on the nutritive value of corn silage

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Keywords aerobic stability, microbial additive, yeasts

Introduction Previous studies have shown that yeast counts are negatively related to aerobic stability in silages and that inoculation with *Lactobacillus buchneri* inhibits yeast growth mainly because of the conversion of lactic acid to acetic acid and 1,2-propanediol. Recently, studies have also shown that some microbial processes occur during prolonged storage and this may affected the quality of silages. For instance, Kleinschmit and Kung (2006) reported a decline in number of yeasts and an improvement in aerobic stability of corn silage stored over a year. They also reported that *L. buchneri* remained fairly active for prolonged periods of time. Thus, the objective of this trial was to evaluate the effects of increasing doses of *L. buchneri* and length of storage on the microbial population, fermentation profile and aerobic stability of corn silages.

Materials and methods Whole plant corn was harvested at the experimental farm of the University of São Paulo in Piracicaba-Brazil when the dry matter content was approximately 33%. The experiment was carried out in a completely randomized design, with five dosages, four storage periods and four replications, totalizing 80 laboratory-scale silos (20 L). The following dosages were applied prior to ensiling: 1) control (no additive); 2) *L. buchneri* at 1×10^5 cfu/g; 3) *L. buchneri* at 3×10^5 cfu/g; 4) *L. buchneri* at 5×10^5 cfu/g; and 5) *L. buchneri* at 1×10^6 cfu/g of fresh forage. Silos were prepared with approximately 14 kg of forage and kept in a closed barn at ambient temperature for 15, 60, 90 and 150 days. After silo opening, representative samples were collected for analysis. Measurements included pH, fermentation end products, microbial counts and aerobic stability. The Mixed procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC) was used for statistical analysis.

Results and discussion The population of LAB and yeasts, pH, acetic acid concentration and aerobic stability were affected by both dosage and storage period (Table 1). The population of LAB markedly decreased from 15 to 150 days of storage in control silages (approximately 3 log reduction), whereas in treated silages the average reduction was 1.1 log cfu/g. Yeast numbers were not affected by levels of microbial additive when silos were opened at 15 days of storage. The lower concentration of acetic acid and the higher yeast population detected at this time point, may explain the lack of response of the microbial additive. Conversely, at 90 and 150 days of storage the population of yeasts were reduced, especially for silages treated with *L. buchneri* at rates $\geq 5 \times 10^5$ cfu/g of fresh forage. At 150 days, inoculation with the highest dose of *L. buchneri* slightly increased silage pH and increased the concentration of acetic acid. Doses of the inoculant $\geq 5 \times 10^5$ cfu/g fresh forage were also effective in improving the aerobic stability of silages stored for more than 90 days.

Table 1 The influence of *Lactobacillus buchneri* and length of storage on the nutritive value of corn silage

Storage period (d)	Dosage[1]					SEM
	Control	LB 1×10^5	LB 3×10^5	LB 5×10^5	LB 1×10^6	
	Lactic acid bacteria (log_{10} CFU/g of fresh forage)					
15	8.28^{Ac}	8.63^{Ab}	8.74^{Aab}	8.92^{Aab}	8.99^{Aa}	0.03
60	7.25^{A}	7.84^{A}	8.53^{AB}	8.73^{AB}	8.44^{AB}	0.14
90	5.18^{Bb}	7.82^{ABa}	8.33^{ABa}	8.92^{ABa}	8.51^{ABa}	0.12
150	5.35^{Bb}	7.54^{Ba}	7.83^{Ba}	7.94^{Ba}	7.88^{Ba}	0.04
	Yeasts (log_{10} CFU/g of fresh forage)					
15	5.63^{Aa}	5.48^{Aa}	5.57^{Aa}	5.64^{Aa}	5.52^{Aa}	0.03
60	4.96^{Ba}	4.14^{Bb}	< 3.0	< 3.0	< 3.0	0.40
90	4.83^{ABa}	4.23^{Ba}	2.78^{Bb}	1.86^{Bb}	1.98^{Bb}	0.11
150	4.43^{Ba}	3.84^{Ba}	2.77^{Bb}	2.27^{Bb}	2.33^{Bb}	0.08
	pH					
15	3.73^{Ba}	3.72^{Ba}	3.68^{Cb}	3.72^{Ba}	3.73^{Ba}	0.01
60	3.70^{B}	3.71^{B}	3.76^{B}	3.70^{B}	3.70^{B}	0.02
90	3.98^{Ab}	4.02^{Aab}	4.01^{Aab}	4.03^{Aab}	4.05^{Aa}	0.02
150	3.80^{Ab}	3.83^{Bb}	3.86^{Bab}	3.93^{Aab}	3.98^{Aa}	0.01
	Acetic acid (% of DM)					
15	0.69^{b}	0.91^{Bab}	1.15^{Ba}	1.32^{Ca}	1.29^{Ca}	0.04
60	0.72^{d}	1.33^{ABc}	1.62^{Ab}	2.27^{Aa}	1.88^{ABb}	0.03
90	0.98^{a}	1.05^{Ba}	1.29^{ABa}	1.44^{BCa}	1.59^{BCa}	0.05
150	1.18^{b}	1.82^{Aab}	1.86^{Aab}	2.10^{ABab}	2.43^{Aa}	0.08
	Aerobic stability (hours)					
15	38.1^{a}	20.6^{Bb}	21.3^{Cb}	32.2^{Cab}	39.3^{Ca}	1.30
60	44.8^{c}	52.2^{Abc}	61.9^{Bab}	68.0^{Ba}	64.5^{Bab}	1.29
90	41.5^{b}	51.1^{ABb}	83.0^{ABb}	173.2^{Aa}	169.7^{Aa}	6.48
150	45.9^{c}	55.0^{Ac}	96.2^{Ab}	160.7^{Aa}	153.6^{Aa}	2.03

[1] LB 1 × 10^5 = *L. buchneri* applied at 1 × 10^5 cfu/g of fresh forage; LB 3 × 10^5 = *L. buchneri* applied at 3 × 10^5 cfu/g of fresh forage; LB 5 × 10^5 = *L. buchneri* applied at 5 × 10^5 cfu/g of fresh forage; LB 1 × 10^6 = *L. buchneri* applied at 1 × 10^6 cfu/g of fresh forage.

^{a,b,c,d} Means within a row without a common superscript differ ($P < 0.05$).

^{A,B,C} Means within a column without a common superscript differ ($P < 0.05$).

Conclusion Longer storage period of corn silages resulted in a microbial profile favourable to the fermentation and aerobic stability of silages. Higher doses of *L. buchneri* may partly compensate the shorter storage periods, however further studies should be conducted to confirm this hypothesis.

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The effect of application of two chemical additives on the surface of ensiled maize

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Keywords sodium nitrite, sodium benzoate, potassium sorbate, poly-beta-hydroxy butyric acid

Introduction When silage is exposed to air on opening the silo, or after its removal from the silo, fermentation acids and other substrates are oxidized by aerobic bacteria, yeasts and moulds. The use of additives to increase aerobic stability is advisable (Wilkinson and Davies, 2012). Many of biological or chemical additives were tested when they were applied during the harvesting/chopping operation or during filling of the silo (Kleinschmit et al. 2005). The objective of our study was to assess the impact of the application on the top layer of maize silage of two chemical preservatives, commercially identified as Albit and Safesil, on the nutritive value and indicators of fermentation.

Material and methods The experiment took place in the experimental farm of The Institute of Animal Science Prague. The Ronaldinio (KWS OSIVA, s.r.o., Velké Meziříčí, CZ) hybrid maize chaff was stored into one bunker silos (1500 m^3).The top of silo was split into three parts, each 10 m^2. The control part was untreated with preservative (C), next two ones was treated with chemical preservatives applied by tractor sprayer on the surface of ensiled mass. The first additive Albit (JET COMPANY, s.r.o., Prague, CZ), based on poly-beta-hydroxy butyric acid, was applied in dose of 0.1 litre (in 1 litre of water) per m^2 (A). The second additive Safesil (Ab Hanson & Möhring, Salinity Agro, Sweden), based on sodium nitrite E251, sodium benzoate E211 and potassium sorbate E202, was applied in dose of 1.5 litre per m^2 (S). After the application of preservatives, the ensiling mass was covered with transparent varnish film and black and white canvas Dualen, which was loaded by gravel bags and tires. After 7 month of the fermentation the samples were collected for analysis from the top of silage (to the depth 5 cm) and under the top (depth 15 and 30 cm, respectively). The samples were analysed accordance with the methods of AOAC (1990). The 6 replications of samples were taken for each option. The statistical procedures were performed using Statistica 9.1 (StatSoft, Tulsa, OK, USA), by method of one-way analyse variance ANOVA, Tukey HSD test ($\alpha = 0.05$).

Results and discussion The effect on the nutritive and fermentative characteristic caused by treating the silage by the chemical additive is shown in the Table 1. The hypothesis, that the application of additives to the surface of the silage did not affect the fermentation at 30 cm depth under the surface, was confirmed, in all cases, the differences between variants in the depth of 30 cm were insignificant ($P>0.05$). In C silage was significant ($P<0.05$) difference in the DM, protein, ADF, NDF and AA at the depth between 30 and 5 cm. The application of S and A had positive effect ($P<0.05$) on DM and ash, corresponding to a greater reduction of losses of DM and organic matter. The application of both preservatives

(S more than A) positively affected the result of fermentation in terms of lactic acid to acetic acid ratio (C 1.82, A 2.94, S 2.47, respectively). The main advantage and innovative element of A product might be that it has antioxidant properties and activates enzymes, of S product that the interaction benzoate and sorbate with sodium nitrite (Knicky and Spörndly, 2009). Loucka et al. (2013) published the experiment with chemical additive S applied by two ways of application, by sprayer in a bunker silo or by sprayer in a cutter. There were obtained similar positive results.

Table 1 Chemical composition and fermentative profile

Variant	Depth (cm)	DM (g/kg)	Starch	Protein	ADF	NDF	Ash	Fat	LA	AA	pH
					(g/kg DM)				(%)		
Control	5	315.4 ^{a}	302.6	81.0^{abc}	260.6 ^{b}	503.3 ^{b}	46.9 ^{d}	26.1^{ab}	1.14	0.63^{ab}	4.11
	15	328.6^{ab}	302.0	83.6 ^{cd}	247.2^{ab}	476.0^{ab}	45.2^{cd}	27.0^{ab}	1.98	0.87^{bc}	4.07
	30	346.6 ^{b}	314.4	84.0 ^{d}	236.9 ^{a}	461.4 ^{a}	44.9^{cd}	27.6^{ab}	2.08	1.29 ^{c}	4.05
Albit	5	341.6 ^{b}	309.6	78.4 ^{a}	242.9^{ab}	479.1^{ab}	43.6^{bc}	25.7^{ab}	1.22	0.41 ^{a}	4.09
	15	333.9 ^{b}	307.3	81.2 ^{bc}	244.6^{ab}	476.1^{ab}	43.8^{bc}	26.4^{ab}	1.38	0.43^{ab}	4.03
	30	335.5 ^{b}	297.7	82.3 ^{cd}	248.3^{ab}	476.1^{ab}	43.5^{bc}	26.2^{ab}	1.78	0.64^{ab}	4.02
Safesil	5	342.4 ^{b}	315.8	78.8 ^{ab}	237.9 ^{a}	471.0^{ab}	41.0 ^{a}	25.4 ^{b}	1.11	0.45^{ab}	4.19
	15	344.3 ^{b}	309.5	79.6 ^{ab}	247.4^{ab}	479.7^{ab}	41.9^{ab}	25.9^{ab}	0.95	0.32 ^{a}	4.18
	30	336.0 ^{b}	292.6	83.2 ^{cd}	246.2^{ab}	482.3^{ab}	44.7^{cd}	26.3^{ab}	1.58	0.50^{ab}	4.03
SEM		1.595	2.932	0.249	1.729	2.624	0.213	0.159	0.10	0.06	0.02

DM, dry matter; ADF, acid detergent fibre; NDF, neutral detergent fibre; LA, lactic acid; AA, acetic acid; SEM, standard error of mean; Different letter superscripts within a column indicate statistical differences for Tukey HSD, $\alpha = 0.05$.

Conclusions The application of both tested chemical additives on the surface of ensiled maize had positive effect ($P<0.05$) on the reduction of losses of dry matter and organic matter, and increased the ratio lactic acid to acetic acid.

Acknowledgement Supported by project MZe QJ1510391 and MZERO0714.

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Wilkinson J. M. and D. R. Davies. 2012. The aerobic stability of silage: key findings and recent developments. Grass Forage Sci. 68:1–19.The objective of our study was to assess the impact of chemical preservatives (Safesil) surface layers on the quality of corn silage and its aerobic stability.

Effect of sodium benzoate, potassium sorbate, and sodium nitrite on the aerobic stability of corn silage with air stress

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Keywords aerobic stability, corn silage, potassium sorbate, sodium benzoate, sodium nitrate

Introduction Exposure of silage to air allows for the growth of yeasts that ultimately results in spoilage and reduced nutritive value. Although the time when silages are most exposed to air is usually considered to occur during removal from the silo for feeding and in the feed bunk, silages may be exposed to air at any time during storage. For example, leaks in doors of up right silos, and damage to the integrity of plastic from bag silos or silo covers, allows air to penetrate into the silo mass during storage. Various antifungal compounds have been added at the time of ensiling (Knicky and Sporndly, 2011; Kung et al., 2000) to impede the growth of yeasts. Overly mature corn silage (> 40% DM) is particularly prone to aerobic spoilage because of poor packing densities and restricted fermentations. The objective of this study was to evaluate a chemical additive applied to mature, whole-plant corn to improve aerobic stability and to establish its effectiveness after an air stress challenge.

Materials and methods Whole plant corn was harvested at about 45% DM with a pull-type chopper with kernel processor and chopped to a theoretical length of 19 mm. Five individually prepared replicate silos were prepared for each of the following additive treatment and air stress combinations: 1) untreated (**CON**), no air stress, 2) CON, air stress, 3) Safesil (**SFE,** potassium sorbate, sodium benzoate, and sodium nitrite) 3 L/t of fresh forage (Salinity/Agro, Halmstad, Sweden), no air stress, and 4) SFE, air stress. Forages were packed in 7.5 L buckets silos with a packing density of about 224 kg of DM/m^3 and sealed with plastic lids with O-ring seals. Silos used for air stress contained a total of three 1.60 cm holes plugged with rubber stoppers sealed with silicon glue; two located on the bottom of the bucket at 180° from each other and one on the lid of the bucket (see figure). The lid of the bucket also contained a 0.64 cm hose splicer attached to a 60 cm plastic hose that was placed in a container of water to allow for gas release but inhibit air from entering the silo. The air stress challenge was conducted by removing stoppers in the silos for 2 h/d once per wk during wk 8 to 14 of storage. Silos were opened after 14 wk of storage (22 ± 2°C) and analyzed for DM, pH, lactic acid bacteria, yeasts and molds, fermentation end products, and aerobic stability (hours before a 2°C rise in baseline temperature after exposure to air). The data were analyzed as a 2 (no additive or SFE) ´ 2 (no stress or air stress) factorial arrangement of treatments using the Fit Model procedure of JMP (SAS Institute Inc., Cary, NC) and differences are reported as significant when $P \leq$

0.05 using Tukey's test.

Results and discussion The composition of corn silages is shown in Table 1. Air stress tended to increase silage pH and increased the concentration of acetic acid, but decreased the concentration of ethanol. Addition of SFE increased silage pH, it had no effects on the concentrations of lactic or acetic acids, but it decreased the concentration of ethanol in silage. Unexplainably the population of yeasts was not affected by addition of SFE in unstressed silage compared to unstressed silage with no additive, but it was lower when SFE was added to silages stressed with air. Air stress numerically decreased the aerobic stability of silages without SFE (61 vs. 33 h) but this difference was not statistically significant. Addition of SFE improved the aerobic stability of silages without air stress (61 to >387 h) or with air stress (33 to >384 h).

Conclusions The addition of Safesil, containing potassium sorbate, sodium benzoate, and sodium nitrite, markedly improved the aerobic stability of mature corn silage even when it was challenged to air during storage. Whereas silage additives should not be used in lieu of good silage management, this study shows that Safesil may have the potential to overcome some challenges to air during storage.

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Table 1 The DM (%) content, pH, fermentation end products (% DM basis), yeasts (log cfu/g fresh weight basis), and aerobic stability (h) corn silage after 14 wk of ensiling

	CON[1]		SFE[1]		SEM	*P*-values		
Item	No Air	Air Stress	No Air	Air Stress		TRT[2]	AS[3]	TRT × AS
pH	3.56^{b}	3.58^{b}	3.60^{ab}	3.67^{a}	0.02	<0.01	0.06	0.17
Lactic acid	3.18	2.79	2.80	3.08	0.15	0.76	0.70	0.04
Acetic acid	0.78^{ab}	0.67^{bc}	0.62^{c}	0.87^{a}	0.03	0.62	0.04	<0.01
Ethanol	1.31^{a}	1.07^{ab}	0.96^{b}	0.80^{b}	0.08	<0.01	0.02	0.57
Yeasts	4.05^{ab}	5.45^{a}	4.35^{ab}	3.02^{b}	0.47	0.04	0.94	0.01
AerSt[4]	61^{b}	33^{b}	>387^{a*}	>384^{a*}	10	<0.01	0.14	0.22

$^{a-c}$Means in rows with unlike superscripts differ ($P < 0.05$).

[1]CON = untreated, SFE = treated with Safesil.

[2]Effect of treatment with SFE.

[3]Effect of air stress.

[4]Aerobic stability, h before a 2°C increase in baseline temperature after exposure to air at 22°C.

*Stability was > than the values noted because silages had not spoiled when recording was stopped.

The effects of packing density and air stress on corn silage inoculated with *Lactobacillus buchneri* 40788

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Key words aerobic stability, *Lactobacillus buchneri*, packing density, silage

Introduction Air is a detriment to silage fermentation because it allows for continued metabolism of undesirable aerobic microbes during the early stages of ensiling and it stimulates the growth of lactate assimilating yeasts responsible for initiating aerobic spoilage. Packing forages to a higher density can help remove air from the silo, resulting in better fermentations and less aerobic instability. *Lactobacillus buchneri* (**LB**) has also been added to forages at ensiling resulting in an improvement in aerobic stability of a variety of silages (Kleinschimt and Kung, 2006). However, silages can still be exposed to air during storage because of compromises to silo integrity. The objective of this study was to determine the effects of packing density, air stress, and treatment with LB on the fermentation and aerobic stability of corn silage.

Materials and methods Whole plant corn was harvested with a pull-behind chopper with kernel processor at about 35% DM and chopped to a theoretical length of 19 mm. Five replicated piles of forage were prepared for the following 8 treatments: Control, untreated (**CTRL**) with either low (176 kg DM/ m^3) or high (240 kg of DM/m^3) packing and no air stress; CTRL with either low or high packing density and air stress; Biotal Buchneri 500 (**LB**; *Lactobacillus buchneri* 40788, 400,000 cfu/g fresh forage and *Pediococcus pentosaceus,* 100,000 cfu/g; Lallemand Animal Nutrition, Milwaukee, WI) with low or high packing density and no air stress; or LB with low or high packing density and air stress. Treatments with air stress had silages exposed to air for 24 h at d 28, 42, and 84 of ensiling. Forage from each pile was packed into 7.5-L laboratory silos and sealed with plastic lids with O-ring seals. Silos subjected to air stress had 3 holes of 1.60 cm diameter that were plugged with rubber stoppers and sealed with silicon glue. Two holes were located 5 cm above the bottom of the silo at 180° from each other. The third hole was on the lid of the silo. Silos were opened after 91 d of storage at 22 ± 2°C. Silages were analyzed for standard end products of fermentation, microbial populations, and aerobic stability. Data were analyzed as a 2 × 2 × 2 factorial was used with the main effects of inoculant, density, and air stress, their interactions, and residual error included (Fit Model procedure of JMP, SAS Institute Inc., Cary, NC). Means were separated using Tukey's test ($P \leq 0.05$).

Results and discussion Analysis of corn silage is shown in Table 1. The concentration of lactic acid was not affected by the main effects of inoculation or air stress, but was higher in silage packed tightly (5.55%) vs. loosely (4.40%). The concentration of acetic acid was greater in silages treated with LB (2.39%) compared to untreated silages (1.42%) and in silages with air stress (2.13%) compared to no air stress (1.68%). Numbers of yeasts were lower in silages treated with LB (2.46 log cfu/g) compared to untreated silages (3.97 log cfu/g) but higher in silages exposed to air stress (4.77 log cfu/g) vs. no air stress (<2 log

cfu/g). There was a significant interaction between inoculation × air stress as the effect of inoculation with LB was greater when silage was not exposed to air stress. There was also a significant interaction between inoculation × density because treatment with LB was more effective when silage was packed densely rather than loosely. Although numerically better, treatment with LB did not statistically improve the aerobic stability of silage that was packed loosely and subjected to air stress.

Conclusions These findings reinforce the fact that good silo management and the use of LB 40788 can result in high quality silages but inoculation alone should not be considered an alternative to proper silo management.

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Table 1 The DM (%), lactic (LA) and acetic acids (AC) (% DM), lactic acid bacteria (LAB) and yeasts (cfu/g wet basis) and aerobic stability (h) of corn silage after 91 d of ensiling

Treatments[1]	DM	LA	AC	LAB	Yeasts	AS[2]
No Air Stress						
Low Density						
CTRL	35.86^{a}	4.87^{ab}	1.21^{c}	7.09^{b}	<2.00^{d}	135^{b}
LB	35.71^{a}	4.37^{ab}	1.83^{abc}	8.58^{a}	<2.00^{d}	252^{a}
High Density						
CTRL	35.45^{a}	5.93^{a}	1.51^{bc}	6.87^{bc}	2.70^{cd}	96^{bcd}
LB	35.91^{a}	5.79^{a}	2.17^{abc}	8.56^{a}	<2.00^{d}	274^{a}
Air Stress						
Low Density						
CTRL	34.93^{a}	4.67^{ab}	1.41^{c}	7.13^{b}	5.82^{a}	27^{e}
LB	36.09^{a}	3.68^{b}	2.74^{ab}	8.77^{a}	4.17^{abc}	71^{cde}
High Density						
CTRL	35.79^{a}	5.05^{ab}	1.56^{bc}	6.36^{c}	5.43^{ab}	30de
LB	36.19^{a}	5.46^{ab}	2.82^{a}	8.76^{a}	3.68^{bcd}	124^{bc}
SEM	0.57	0.46	0.30	0.18	0.81	16
Main effects			*P*-values			
Air stress	0.96	0.08	0.02	0.86	<0.01	<0.01
Density	0.61	<0.01	0.26	0.03	0.94	0.36
Inoculant	0.20	0.30	<0.01	<0.01	<0.01	<0.01

$^{a-e}$Means in columns with unlike superscripts differ ($P < 0.05$). [1]See descriptions in materials and methods. [2]Hours before a 2°C rise in temperature above baseline after exposure to air at 22°C.

Evaluation of microbial inoculants for improving the aerobic stability of corn silage

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Key words aerobic stability, corn silage, *Lactobacillus buchneri*, silage

Introduction Yeasts that assimilate lactate are common initiators of aerobic spoilage of corn silage in the US. Various strains and application rates of *Lactobacillus buchneri* have been used to improve the aerobic stability of silages because they can produce moderate amounts of acetic acid, which reduces the numbers of yeasts in silages (Muck, 2004). *Lactobacillus buchneri* has also been combined with various homolactic acid bacteria and used as silage inoculants (Reich and Kung, 2010). The objective of this study was to evaluate the effectiveness of combination of *L. plantarum* MTD1 and *L. buchneri* NCIMB 30139 to improve the aerobic stability of corn silage under North American conditions.

Materials and methods Whole plant corn from (2 different fields and with different hybrids) was harvested with a pull-type chopper with kernel processor and chopped to a theoretical length of 19 mm and used in 2 experiments (expt). In expt 1, five individual replicated piles were prepared for each of the following treatments: 1) untreated (**CTRL**), 2) *L. plantarum* MTD1 (**LP**, final application rate of 100,000 cfu/g of fresh forage), 3) Combo 1 – (**CMB1**, LP + *L. buchneri* NCIMB 30139, 200,000 cfu/g), 4) Combo 2 – (**CMB2**, LP + *L. buchneri* NCIMB 30139, 400,000 cfu/g. In expt 2 treatments were CTRL, LP and CMB1, as previously described. Forages were packed in 7.5-L lab silos and allowed to ensile for 30 and 92 (expt 1) or for 90 d (experiment 2). Inoculants were from Volac Intl., Ltd., UK. Samples were analyzed for standard chemical and microbiological components. Silages were also analyzed for aerobic stability (h before a 2°C increase in ambient temperature after exposure to air at 22°C). Data for expt 1 was analyzed as a 2 ´ 4 factorial arrangement of treatments whereas data for expt 2 was analyzed as completely randomized design using JMP version 11 (SAS Institute, Cary, NC). Main effects for expt 1 included the effect of time of ensiling, the effect of additive, and their interactions. Means were deemed significant using Tukey's test when $P < 0.05$.

Results and discussion Data from both experiments are shown in Table 1. In expt 1, after 30 d of ensiling there were no differences in numbers of yeasts between untreated and treated silages. However, aerobic stability was greater in CMB1 and CMB2 silages compared to other treatments. After 92 d of ensiling treatment with CMB1 and CMB2 resulted in silages with lower yeasts and nearly a three-fold improvement in aerobic stability compared to CTRL and LP. In expt 2, yeasts were not detected at the lowest dilution tested (2 log cfu/g) in silage treated with CMB1 after ensiling. Moreover, treatment with CMB1 markedly the improved aerobic stability (>250 h) when compared to CTRL (44 h) or LP (46 h). In fact, silage treated with CMB1 was still stable at 250 h when measurements ceased.

Conclusions The results of this study show that the combination of *L. plantarum* MTD1 and *L. buchneri* NCIMB 30139 improves the aerobic stability corn silage compared to

untreated silage or silage treated with only *L. plantarum* MTD1. In experiment 1 the improvement was greater at 92 vs. 30 d of ensiling agreeing with previous research showing that the effect of *L. buchneri* improves with time in the silo. The low and high rate of application of *L. buchneri* NCIMB 30139 resulted in similar improvements in aerobic stability in experiment 1. In experiment 2, the low rate of application was able to markedly improve stability.

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Table 1 The DM content, pH, yeasts and LAB (log cfu/g fresh weight basis), and aerobic stability (h) of corn silage after ensiling

Day and Treatment[1]	DM, %	pH	Yeasts	Aerobic stability[2]
30 d		Experiment 1		
CTRL	31.26^{ab}	3.68	3.68^{ab}	42^{c}
LP	31.11^{ab}	3.76	3.28^{abc}	45^{c}
CMB1	31.16^{ab}	3.76	3.18^{abc}	63^{b}
CMB2	31.28^{ab}	3.77	3.15^{abc}	62^{b}
92 d				
CTRL	30.38^{bc}	3.76	4.26^{a}	39^{c}
LP	30.14^{c}	3.76	4.04^{a}	45^{c}
CMB1	29.82^{c}	3.74	2.15^{bc}	106^{a}
CMB2	30.01^{c}	3.77	2.20^{c}	118^{a}
SEM	0.19	0.02	0.41	4
			P-values	
Additive	0.39	0.11	<0.01	<0.01
Day	<0.01	0.28	0.45	<0.01
Additive × Day	0.57	0.09	<0.01	<0.01
		Experiment 2		
90 d				
CTRL	36.22^{ab}	3.54^{c}	5.06^{a}	44^{b}
LP	36.60^{a}	3.62^{b}	4.93^{a}	48^{b}
CMB1	35.50^{b}	3.74^{a}	< 2.00^{b}	>260^{a}
SEM	0.28	0.02	0.07	1

[a-c] Means in columns within an experiment with unlike superscripts differ ($P < 0.05$).

[1] See materials and methods for additive description.

[2] Hours before a 2°C increase above ambient temperature after exposure to air at 22°C.

Emissions of ethanol and acetic acid in corn silages inoculated with *Lactobacillus buchneri*

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Keywords ambient temperature, energy losses, reactive organic gas, volatile organic compounds

Introduction Corn silage is one of the most important sources of forage fed to dairy cows worldwide. However, corn silage and its total mixed rations have been recognized as the main source of volatile organic compounds (VOC) in dairy farms (Mitloehner et al., 2009). In the presence of sun light, VOC react with nitrogen oxides to form tropospheric ozone, an air pollutant. Furthermore, the emission of VOC represents nutrient losses (Daniel and Nussio, 2011). In several studies, ethanol and acetic acid were reported as the major VOC in corn silages (Mitloehner et al., 2009; Weiß et al., 2009). Thus, the aim of this study was to evaluate the emissions of acetic acid and ethanol in corn silages inoculated with increased doses of *L. buchneri* in tropical conditions.

Materials and methods Corn crop was grown at the "Luiz de Queiroz" Campus, Piracicaba, and mechanically harvested at 35% DM. Chopped forage was treated and packed in 16 bag silos (1.5 m i.d.) (4 bags of 10 t FM per treatment). Forages received no additive (control) or were inoculated with *L. buchneri* CNCM I-4323 at theoretical application rates of 1×10^5 cfu/g fresh forage (LB1), 5×10^5 cfu/g fresh forage (LB5), and 1×10^6 cfu/g fresh forage (LB10). After 150 d of storage, four silos (one per treatment) were opened every three weeks for sampling. Collections were performed on the silo working face (undisturbed silage) at 0, 4 and 8 h after silage feedout. Sampling times corresponded to 7:00h, 11:00h and 15:00h. The VOC emissions were measured using surface isolation flux chambers (Odoflux, Odotech) coupled to a photoacoustic field gas-monitor (INNOVA 1412, LumaSense). Sweep air flow (ultra zero compressed air) was set at 10 mL/min and recorders were performed continuously during 15 min at steady-state (30 min after the flow start up) (Krauter and Blake, 2009). Flux rates were calculated in mg/m^2 per minute. Silage samples were also collected and analyzed for ethanol and acetic acid concentrations by gas chromatograph-mass spectrometry. Data were analyzed using the Mixed procedure of SAS, including random effect of week and fixed effects of treatment, hour and treatment×hour. Treatment means were compared by linear and quadratic contrasts.

Results and discussion There was no treatment effect on ethanol emission ($P = 0.20$). Contrary to expectation, ethanol emissions were higher ($P < 0.01$) at 4 h (74 mg/m^2.min) and 8 h (68 mg/m^2.min) after feedout compared with the emission measured immediately after silage unload (44 mg/m^2.min). Previous studies have showed that VOC emission decay after silage feedout (Montes et al., 2010). However, in the current study the temperature was not controlled and might have been higher at 11:00h and 15:00h than at 7:00h. As observed for ethanol, the acetic acid emission was higher at 4 h and 8 h after silage feedout (Figure 1). Therefore, it seems that VOC emissions are more influenced by the ambient temperature than the time after silage feedout. However, acetic acid emission was linearly increased ($P = 0.05$) with the dose of *L. buchneri*, due to the higher ($P < 0.01$)

acetic acid concentration in treated silages (C = 3.3%, LB1 = 4.6%, LB5 = 4.4% and LB10 = 5.1% DM). In this way, emissions of acetic acid (r = 0.57) and ethanol (r = 0.66) were positively correlated with their concentrations in silages.

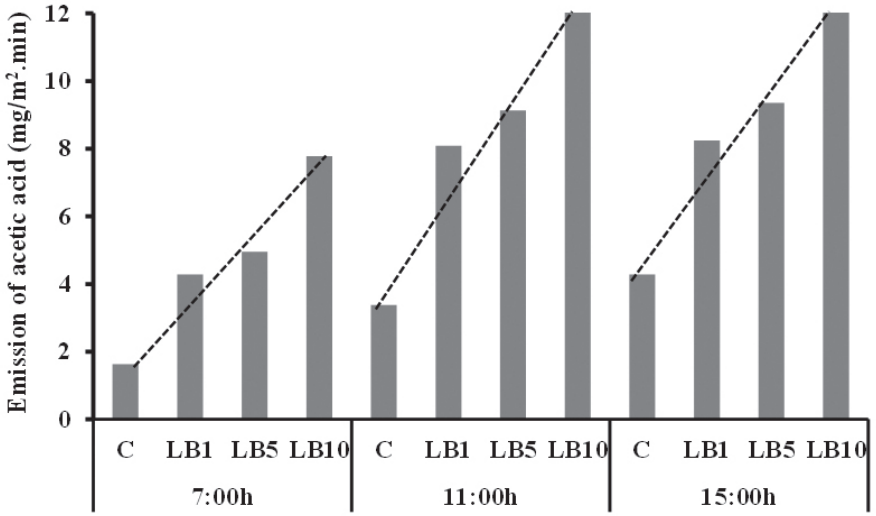

Figure 1 Influence of inoculation with *L. buchneri* on the emission of acetic acid in corn silages. C: control, LB1: *L. buchneri* applied at 1×10^5 cfu/g, LB5: *L. buchneri* applied at 5×10^5 cfu/g, LB10: *L. buchneri* applied at 1×10^6 cfu/g. $P<0.01$ for treatment effect; $P<0.01$ for hour effect; $P=0.63$ for treatment × hour effect. Dotted lines indicate the linear trend for treatment effect.

Conclusion Increasing the dose of *L. buchneri* inoculation led to a linear increase in acetic acid concentration as well as acetic acid emission. Emissions of ethanol and acetic acid from the silo working face (undisturbed silage) were more influenced by the ambient temperature than the time after silage feedout, and were positively correlated with their concentrations in silage.

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Effects of homo- and heterolactic bacteria on the dynamics of gas production during the fermentation of corn silage

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Keywords acetic acid, carbon dioxide, lactic acid, volatile organic compounds

Introduction In silages, gases are formed as by-products of microbial metabolism, especially in heterofermentative pathways. On the other hand, inoculating silages with homolactic bacteria is claimed to improve the fermentation efficiency, through a faster pH drop at the onset of fermentation, which ultimately would decrease gas production and nutrient losses. Therefore, the objective was to evaluate the effects of homo- and heterolactic inoculants on the dynamics of gas formation during the fermentation of corn silages.

Materials and methods Whole-corn plants (38%DM) were mechanically harvested (theoretical length of cut 12 mm) and treated as follows (as fed basis): **Control** (without additive), **Hetero** (*Lactobacillus buchneri*, at 4×10^5 cfu/g), **Homo** (*L. plantarum, Pediococcus acidilactici, L. salivarius* and *Enterococcus faecium,* at 4×10^5 cfu/g, total count), **ComboHigh** (Hetero at 4×10^5 cfu/g plus Homo at 4×10^5 cfu/g) or **ComboLow** (Hetero at 2×10^5 cfu/g plus Homo at 2×10^5 cfu/g). All inoculants were diluted in distilled water (5 mL/kg) and sprayed onto the forage. The same amount of water was applied to the control. Afterwards, treated forages were packed (0.40 porosity) into 1.96 L gas-tight silos (4 replicates/treatment). Silos were stored inside the lab at 25±1°C. The internal pressure of the silos was measured by using a pressure transducer connected to a data displayer. Readings were taken every 12 h until d-3, every 24 h from d-4 until d-21, every 3 d from d-22 until d-30, and every week until d-152 (end of storage). Pressure was converted to volume and presented as accumulated gas production per kg DM (see Daniel and Nussio, 2015). Gas and DM losses were also measured by the gravimetric method (weight difference). The DM was determined in an oven and corrected for volatile compounds (DM_{corr}) (Weissbach, 2009). Fermentation end-products were analyzed by gas chromatograph-mass spectroscopy, except lactic acid determined by colorimetry. Silage samples were exposed to air for 10 d to determine the aerobic stability (2°C rise in temperature above the ambient). In addition to the 1.96 L silos, vacuum-sealed bags (500 g) were stored during 1, 2, 3, 5, 7 and 16 d for measuring the pH drop at the onset of fermentation (4 bags/treat per time). Data were analyzed with the Mixed procedure of SAS.

Results and discussion The cumulative gas production is presented in Figure 1, whereas the fermentation profile and aerobic stability are shown in Table 1. The homolactic inoculant had no effects on silage pH drop (not showed) neither altered the final fermentation profile and aerobic stability of corn silages. On the other hand, the higher dose of *L. buchneri* (alone or in combo) increased gas production after 3 weeks of storage and the DM losses at the end of storage, although resulted in corn silages with higher aerobic stability. The lower dose of the heterolactic bacterium (2×10^5 cfu/g) combined with the homolactic inoculant was not enough to improve the aerobic stability.

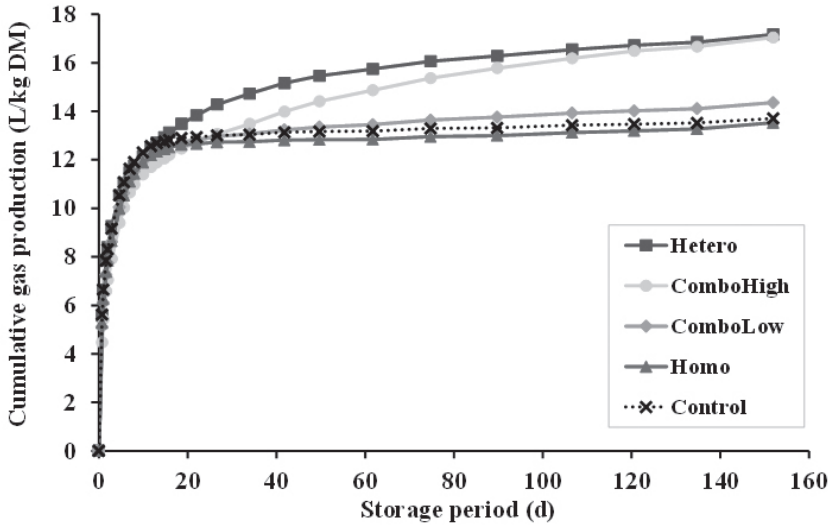

Figure 1 Influence of homo- and heterolactic inoculants on the dynamics of gas production in corn silages (P<0.01 for interaction treatment × storage period). See the text for legend.

Table 1 Fermentation profile and aerobic stability of corn silages inoculated with homo- and heterolactic bacteria

Item	Control	Homo	Hetero	ComboHigh	ComboLow	SEM	*P*-value
DM_{corr}, g/kg as fed	373	375	370	374	372	1.2	0.08
pH	3.75^{b}	3.75^{b}	3.79^{a}	3.80^{a}	3.77^{b}	0.01	<0.01
Lactic acid, g/kg DM_{corr}	42.9^{a}	34.8^{b}	32.9^{b}	31.1^{b}	32.6^{b}	1.6	<0.01
Acetic acid, g/kg DM_{corr}	11.4^{c}	13.6^{c}	21.4^{b}	27.8^{a}	18.7^{b}	0.8	<0.01
Ethanol, g/kg DM_{corr}	8.8^{a}	9.2^{a}	6.5^{b}	7.3^{b}	7.1^{b}	0.3	<0.01
1,2-Propanediol, g/kg DM_{corr}	0.1^{d}	0.2^{d}	9.8^{b}	12.3^{a}	4.1^{c}	0.3	<0.01
Propionic acid, mg/kg DM_{corr}	228	384	222	420	274	76.9	0.21
Ethyl lactate, mg/kg DM_{corr}	179^{ab}	199^{a}	125^{c}	155^{b}	171^{ab}	6.6	<0.01
Butyric acid, mg/kg DM_{corr}	135^{ab}	193^{a}	45^{b}	49^{b}	124^{ab}	32.9	0.03
Ethyl acetate, mg/kg DM_{corr}	58^{ab}	51^{b}	79^{a}	75^{a}	45^{b}	5.3	<0.01
1-Propanol, mg/kg DM_{corr}	12^{b}	39^{b}	141^{ab}	294^{a}	103^{ab}	58	0.03
Gas losses[1], g/kg DM	27.5^{c}	27.6^{c}	32.2^{b}	33.4^{a}	28.2^{c}	0.3	<0.01
Gas production[2], g/kg DM	26.8^{b}	26.4^{b}	33.5^{a}	33.3^{a}	28.0^{b}	0.4	<0.01
DM_{corr} losses, g/kg DM	33.7^{ab}	30.0^{b}	43.9^{a}	34.1^{ab}	36.5^{ab}	3.0	0.05
DM_{oven} losses, g/kg DM	58.5	49.1	72.9	61.9	67.2	7.6	0.28
Aerobic stability, h	120^{b}	125^{b}	183^{a}	171^{a}	142^{b}	6.7	<0.01

$^{a-d}$Means within a row with different superscripts differ (Tukey-Krammer, α = 0.05). [1]Determined by the gravimetric method. [2]Determined by the volumetric method (Daniel and Nussio, 2015).

Conclusions *L. buchneri* at 4×10^{5} cfu/g, alone or in combination with a homolactic inoculant, improved the aerobic stability upon silage exposure to air, whereas increased gas production and DM losses during the fermentation. The homolactic inoculant did not enhance the fermentation process in corn silages.

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Effects of different additives on the silage quality of sorghum-sudangrass hybrid

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Keywords sorghum-sudangrass, lactic acid bacteria, cellulase silage

Introduction Sorghum-sudangrass hybrids (*Sorghum bicolor* L.×*Sorghum sudanense* L.) has many good characters, such as broad leaves, strong tillering and generativity, good palatability, high digestibility and high forage value (Han *et al*. 2013). At present the main use of Sorghum-sudangrass hybrid in China is directly feeding, its short usage period causes a large amount of waste though the high yield, Ensiling is a preferable method of preserving fresh forage crops by fermentation for future use as a ruminant animal feed. Successful silage production requires sufficient lactic acid bacteria and water soluble carbohydrates to rapidly decrease the pH. Therefore, the objective of this study was to evaluate the effect of cellulose, lactic acid bacteria inoculants and their combination on silage quality of Sorghum-sudangrass hybrid.

Materials and methods Test materials were harvested at the early-heading stage of the first crop of Sorghum-sudangrass hybrid (Jicao No.2). Chopping and drying the mowed grass to 60% of the moisture content, mixing the chopped sample manually and then canning silage. The polyethylene can packed approximately 360 g per 0.5 L. The forage crops was assigned to one of the following treatments: (i) CK: untreated; (ii)CF: *cellulose enzyme*(a solid enzyme of multi-component compound biological catalyst, the standard unit of enzyme activity (CMC enzyme) is 1060 $U.g^{-1}$)at 0.0015%g per gram of fresh crop; (iii)LC: *Lactobacillus casei* at 1×10^6CFU per gram of fresh crop; (iv)LP: *Lactobacacillus plantarum* at 1×10^6CFU per gram of fresh crop; (v) LC+CF: a mixture of *Lactobacillus casei* (1×10^6CFU per gram of fresh crop)and *cellulose enzyme*(0.0015%g per gram of fresh crop); (vi)LP+CF: a mixture of *Lactobacacillus plantarum* (1×10^6CFU per gram of fresh crop)and *cellulose enzyme* (0.0015%g per gram of fresh crop) (Zhang *et al.* 2014). Each treatment had three replicates. Stored at room temperature (20±5.0°C) for 30 d. After this, the silage cans were opened to determine the value of pH, organic acids, ammonia nitrogen and crude protein, *et al* (Ji *et al*. 2012).

Results and discussion The silage quality of Sorghum-sudangrass hybrid is shown in table 1. Additive treatment influenced the pH value of the ensiled forage ($P < 0.05$). The silages treated with additives had lower pH values than the control silage ($P < 0.05$), and the lactic acid concentration was higher in the LP, LC+CF and LP+CF compared with the control group ($P < 0.05$). High NH3-N/TN ratios in CK suggested the degree of protein decomposition is high. Additive treatment can reduce ammonia concentration, and the ammonia nitrogen content in the LP, LC+ CF and LP+ CF was significantly lower than in CK ($P < 0.05$). Treated with CF lonely does not reduce the content of digestible fiber significantly, but it dose when mixed it with lactic acid bacteria. The crude protein content have no significant differences in the treatment groups, which is inconsistent with previous research and need further study. Cellulase reduces the fiber content by hydrolyzing plant

cell walls, which improves digestible organic matter on silage. The influence of cellulose for the silage quality of Sorghum-sudangrass hybrid is not significant, which may be associated with the adding amount of cellulase.

Table 1 Effects of additives on silage quality of Sorghum-sudangrass hybrid

	CK	CF	LC	LP	LC+CF	LP+CF	SEM
pH	4.26^{a}	4.15^{ab}	4.04^{bc}	3.95^{c}	3.98^{c}	3.88^{c}	0.04
Lactic acid (g kg^{-1} DM)	36.1^{b}	42.1^{ab}	34.8^{b}	58.4^{a}	54.9^{a}	54.5^{a}	0.30
Acetic acid (g kg^{-1} DM)	13.2^{ab}	16.3^{a}	5.0^{b}	12.9^{ab}	2.7^{b}	7.7^{ab}	0.17
Butyric acid (g kg^{-1} DM)	1.7^{ab}	2.6^{ab}	0.5^{b}	6.0^{a}	0.2^{b}	3.4^{ab}	0.07
NH_3-N (g kg^{-1}TN)	83.2^{a}	81.6^{ab}	62.4^{abc}	44.8^{c}	60.2^{bc}	41.5^{c}	0.50
Neutral detergent fiber (g kg^{-1} DM)	618.0^{a}	572.3^{bc}	593.0^{ab}	587.0^{ab}	549.0^{c}	547.5^{c}	0.77
Acid detergent fiber (g kg^{-1} DM)	368.4	343.0	345.1	341.8	337.6	318.8	0.67
Acid detergent lignin（g kg^{-1}DM)	45.9^{a}	42.0^{ab}	37.7^{ab}	35.0^{b}	38.3^{ab}	34.6^{b}	0.23
Crude protein（g kg^{-1} DM）	86.4	82.6	82.9	81.7	75.3	87.4	0.15

DM, dry matter; CK, untreated; CF: *cellulose enzyme*; LC: *Lactobacillus casei*; LP: *Lactobacacillus plantarum*; Means within the same row with different superscripts differ ($P < 0.05$).

Conclusion Inoculation with lactic acid bacteria showed positive effects on fermentation quality of Sorghum-sudangrass hybrid silage. The addition of Cellulase decreased the NDF content and enhanced the fermentation quality of Sorghum-sudangrass hybrid silage.

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Trichoderma spp. as silage inoculant: Effects on aerobic stability and fiber digestibility

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Keywords aerobic stability, antimicrobial activity, *Brachiaria*, cellulase, NDF digestibility, *Sorghum bicolor*, *Trichoderma spp*.

Introduction Ensiled fodder crops high in sugar such as sweet sorghum (*Sorghum bicolor*) and high dry matter (DM) grasses are particular prone to aerobic deterioration in tropical climates in the feed-out phase or when the silo is damaged. A company for biopesticides in Colombia promoted the use of its product consisting of different *Trichoderma* species for enhancing aerobic stability of e.g. sugarcane silages, and recommended its application together with lactic acid bacteria (LAB). *Trichoderma spp.* are fungi known for their antimicrobial behavior and also for their cellulolytic activity. Thus, the objective of this study was to evaluate whether the aerobic fungi could express these properties under ensiling conditions where aerobic phases are usually short and thus improve both the aerobic stability of silage and the fiber digestibility for animal benefit.

Materials and methods Two varieties of sweet sorghum, V2 and V9 (ICRISAT 614 and 615) and the *Brachiaria* hybrid Mulato II (*Brachiaria ruziziensis x B. decumbens x B. brizantha*) were harvested in 2012 in Palmira, Colombia, pre-wilted and chopped before undergoing 6 ensiling treatments in quadruplicate. *Trichoderma* was tested against an untreated control, an obligatory and a facultative heterolactic acid bacteria and combinations as follows: 1) Control, 2) Trichoplant® (mixture of *Trichoderma harzianum, T. lignorum, T. viridae and, T. koningii*), 3) *L. brevis* (DSM 13201), 4) *L. plantarum* (CIAT S66.7), 5) S66.7+Trichoplant and 6) S66.7+*L. brevis*. Additionally, the highly pre-wilted Mulato II underwent the treatment both without and with addition of 20 g sucrose (SU)/kg fresh matter (FM). The material was ensiled as Rostock Model Silages in vacuum sealer bags. From each forage material, 5 independent samples were taken before ensiling for chemical analysis. During the 49 d of storage air was allowed to enter for 24 h after 4 and 6 weeks through two little wholes (Ø ~5mm). After storage, DM and pH were determined and subsamples were monitored in the aerobic stability test according to Honig (1990) during 7 days. Temperature rise, pH increase, DM loss and fungi score from 0 (free of visible mold and yeasts) to 4 (totally contaminated) (Pahlow, unpublished) were used as indicators for aerobic changes. Neutral detergent fiber (NDF), NDF digestibility (NDFD) and acid detergent fiber (ADF) were determined before and after ensiling. Univariate analysis was performed using IBM SPSS Statistics, Version 19 (Table 1) and GLM procedure by SAS (Table 2).

Results and discussion The NDF digestibility was in no case increased by ensiling which might be explained by a slight rise of the ADF concentration (Table 1) (Pearson´s correlation between NDFD and ADF in Mulato II was -0.602, $P<0.05$). Behavior of the

sorghum silages and Mulato II haylage upon exposure to air was considered separately. Whereas the treatments *L. brevis* and S66.7+Trichoplant gave the best results with regard to temperature and pH increase and fungal growth in Mulato II (Table 2), there were no significant differences in these parameters in sorghum silages. Apart from little yeast growth, in sorghum the clearest sign for aerobic changes was the temperature increase, which was nominally highest in Trichoplant, which also ranked first in the haylage, where mold growth was predominant in Trichoplant and the control.

Table 1 Fiber (% of DM) and fiber digestibility (%) before and after ensiling

	Sorghum V2		Sorghum V9		MulatoII		MulatoII+SU			
	NDF	NDFD	NDF	NDFD	NDF	NDFD	ADF	NDF	NDFD	ADF
Before ensiling	59.4b	58.9a	54.8b	52.9	56.9	55.5a	27.7b	56.9	55.5a	27.7b
Control	62.1a	57.2ab	58.9a	53.5	56.3	51.0ab	29.3ab	53.5	49.3b	28.2ab
L. brevis	61.1ab	57.0ab	58.7a	52.4	57.1	48.4b	29.8a	52.8	52.3ab	28.4ab
S66.7	62.0a	54.3b	59.6a	54.1	56.3	48.3b	29.7a	54.9	49.7b	29.0ab
Trichoplant	60.5ab	56.0ab	59.2a	51.5	56.3	49.6b	29.4a	54.6	48.8b	29.4a
S66.7+*L.brevis*	61.3ab	56.9ab	59.4a	53.6	56.4	51.0ab	28.9ab	54.5	50.6ab	28.7ab
S66.7+Trichoplant	61.8ab	56.8ab	58.5a	53.8	57.1	51.9ab	29.6a	54.9	49.1b	28.5ab

Values with different letters within the same column differ by α=0.05 (Tukey-HSD).

Table 2 Silage DM and pH at opening and parameters of deterioration after 7 d aerobic stability test (AST)

		DM	pH (49d)	pH after AST	acc. Temp. °C	Fungi score
Sorghum (V2, V9)	Control	20.9 (4.4)	4.0 (0.2)	4.6 (0.6)	3.2 (3.0)	0.5 (1.0)
	L. brevis	20.8 (4.3)	3.8 (0.0)	4.0 (0.1)	3.2 (2.8)	0.3 (0.5)
	S66.7	20.5 (3.5)	3.8 (0.0)	4.1 (0.6)	9.0 (15.1)	0.3 (0.4)
	Trichoplant	20.4 (4.5)	3.9 (0.1)	4.0 (0.2)	13.4 (10.1)	0.1 (0.2)
	S66.7+*L.brevis*	20.7 (4.9)	3.8 (0.0)	4.0 (0.4)	3.3 (3.8)	0.3 (0.7)
	S66.7+Trichoplant	21.5 (4.5)	3.8 (0.0)	4.3 (0.7)	2.4 (1.5)	0.3 (0.3)
Mulato II (without, +SU)	Control	58.1 (1.9)	6.0 (0.1)a	8.0 (0.3)a	2.8 (1.0)ab	2.3 (1.2)a
	L. brevis	56.7 (0.7)	4.5 (0.1)d	4.6 (0.1)b	0.2 (0.3)c	0.9 (0.6)b
	S66.7	58.2 (0.4)	4.6 (0.1)c	4.7 (0.3)b	1.1 (2.1)bc	0.5 (0.0)b
	Trichoplant	58.2 (0.8)	5.6 (0.1)b	7.7 (1.1)a	3.5 (2.0)a	2.2 (1.3)a
	S66.7+*L.brevis*	59.9 (0.8)	4.6 (0.2)c	4.9 (0.6)b	0.7 (1.8)bc	1.1 (1.2)ab
	S66.7+Trichoplant	61.6 (1.0)	4.7 (0.1)c	4.7 (0.2)b	0.2 (0.6)c	0.7 (0.3)b
P (MulatoII)	INOCULUM		<0.001	<0.001	<0.001	<0.001

Values with different letters within the same column and plant species differ by α=0.05 (Ryan-Einot-Gabriel-Welsch-Test). Acc. Temp. accumulated temperature above ambient for 7 d.

Conclusions *Trichoderma spp.* did not improve fiber digestibility. In high DM silages, a combination of homofermentative LAB with *Trichoderma spp.* can have a similar positive effect on aerobic stability as the use of a heterofermentative LAB.

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Fermentation quality and aerobic stability of sorghum-sudangrass hybrid silage treated with Artemisia extract

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Keywords Artemisia, sorghum-sudangrass hybrid silage, fermentation quality, aerobic stability

Introduction The objective of this study was to investigate the effects of adding Artemisia extract on the fermentation quality and nutritional value of Sorghum-Sudangrass hybrid silage.

Materials and methods Whole Sorghum-sudangrass hybrid with dry matter(DM) content of 15.82% was harvested at the maturity of jointing stage, chopped to 1-2 cm, and ensiled with Artemisia extract at 0, 1.25, 2.5, 5 and 10 mL/kg of fresh matter (represented by CK, 1, 2, 3 and 4 respectively), in small silage bags. After 50 d of ensiling, the silages were sampled for analysis of pH, DM, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), water soluble carbohydrate (WSC), lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) contents and ammonia nitrogen(AN) and subjected to an aerobic stability evaluation for 10 days, in which the temperature of the silage were measured to determine the aerobic deterioration. Data analysis was performed by one-way analysis of variance using SPSS22.0 and differences among treatments were tested using the Duncan's Multiple Range Test.

Results and discussion After 50 days of ensilage, all silages were well-preserved, the Artemisia extract significantly improved silage quality, with reduced NDF contents and increased LA and AA contents ($P<0.05$). However, the pH, PA and ADF did not differ between the silages. The concentration of PA was the highest in the treatments with the highest doses of Artemisia extract ($P<0.05$). The concentrations of LA and AA were highest in 1.25 mL/kg treatment. No BA was detected among treated silages, showed that adding Artemisia extract strongly inhibited the growth of clostridia. The DM content was lower in 1.25 mL/kg, 2.5 mL/kg and 5 mL/kg treatments when compared with the control, because of intense microbial fermentation. The concentration of CP was lower in 1.25 mL/kg treated silage ($P<0.05$) presumably due to a high proteolysis in this silage evinced by the increase of ammonia-N (Table 1). Silages treated with Artemisia extract showed a greater aerobic stability ($P<0.05$) (Figure1). Therefore, Artemisia extract is effective against yeasts and improves the aerobic stability of sorghum-sudangrass hybrids silage.

Conclusion Sorghum-sudangrass hybrid ensiled with Artemisia extract improved silage fermentation quality, nutrition value and aerobic stability. Overall, the adding rate of 10 mL/kg Artemisia extract as fresh weight showed the best effect.

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Table 1 Fermentation quality and chemical composition of sorghum-sudangrass hybrid silage treated with Artemisia extract

Treatment	pH	WSC(%)	LA(%)	AA(%)	PA(%)	BA(%)	AN/TN(%)	DM(%)	CP(%)	NDF(%)	ADF(%)
CK	5.29a	0.58b	4.33a	2.33a	7.75a	1.16b	16.93abc	15.27bc	15.88a	51.58b	31.16a
1	5.14a	0.45ab	7.28b	3.75c	10.11a	0.00a	18.91c	14.44a	15.75a	51.04ab	31.06a
2	5.22a	0.38a	6.47ab	3.50bc	10.31a	0.00a	18.29bc	15.07b	16.09ab	50.61ab	30.83a
3	5.33a	0.49ab	4.36a	3.15abc	9.92a	0.00a	16.40ab	15.13b	16.40b	51.21ab	30.83a
4	5.18a	0.42ab	5.05ab	2.76ab	10.40a	0.00a	15.92a	15.73c	16.57b	50.25a	30.57a

DM: dry matter, FW: fresh weight, WSC: water soluble carbohydrate, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid, AN/TN: ammonia nitrogen to total nitrogen. Different letters in the same column indicate significant differences (P<0.05).

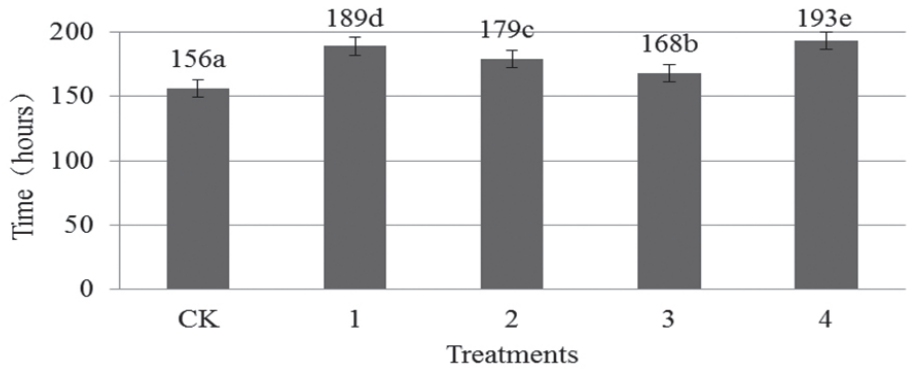

Figure 1 Aerobic stability of sorghum-sudangrass hybrid silage treated with Artemisia extract. Different letters on the bars indicate significant differences (P<0.05).

Fermentation characteristics and microbiological variables of whole crop barley ensiled in big bales with or without an inoculant

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Keywords bacteria, bale, spring barley, fermentation, inoculant

Introduction Bale silage is usually made of non-chopped or restricted cut herbages. Such kind of forage may be difficult to ensile due to delay in fermentation or material heterogeneity (Lättemäe, 2001). Microbial inoculation is a common practice to accelerate the fermentation resulting in good quality silage, that depends largely on the type and number of lactic acid bacteria (LAB) applied to crops (Hargreaves et al., 2009). Moreover, LAB inoculants can provide substantial benefit by reducing the risk of growth of spoilage organisms and produce anti-microbial and antifungal compounds (Muck, 2012). The objective of this study was to compare fermentation and microbiological quality of baled whole crop barley silage made with or without the LAB inoculant (SiloSolve MC).

Materials and methods A homogenous plot of whole crop spring barley at the doughy stage (401.1 g/kg DM) was divided into two blocks and was mown with a disk mower-conditioner harvester and set to place windrows (windrows covered approximately 40% of the ground area). The crop was bailed into 1.2 m wide and 1.2 m diameter cylindrical bales and wrapped with 8 layers of white coloured stretch film (width of 750 mm and thickness of 0.025 mm). The following additive treatments were applied to forage: T1: control and T2: SiloSolve MC (*Lactobacillus plantarum* DSM16568, *Enterococcus faecium* DSM 22502 and *Lactococcus lactis* NCIMB30117, Chr. Hansen A/S, Denmark). The additive was applied at the dose of 4 L/t fresh forage to achieve a target of at least 150 000 colony forming units (cfu) per 1 g herbage. Five big bales from each treatment were chosen at random, weighed when prepared and weighed again after 120 days of storage for measuring dry matter (DM) losses. On removal of the plastic film and after 14 days of exposure to air, samples were taken for microbiological analyses. Each bale was core sampled for chemical and microbial analyses. Data were statistically analyzed as a randomized complete block by using the GLM procedure of SAS.

Results and discussion Dry matter of whole crop barley at ensiling averaged 401.1 g/kg, and was relatively uniform across the field and no differences in DM content among treatments was observed. The CP, WSC, ADF, NDF, and starch content of initial forage were 162.7, 61.9, 283.4, 527.9 and 242.0 g/kg DM basis, respectively. Mean weights of bales were 700.4 and 707.6 kg for T1 and T2 treatments at ensiling, respectively. Inoculation of whole crop barley prior to ensiling had a significant effect on the ensilaging process and microbial composition of silage (Table 1). pH, butyric acid and alcohols concentrations were all lower ($P < 0.05$) while lactic acid concentration was higher ($P < 0.05$) in the silage prepared with additive SiloSolve MC which is in agreement with previous studies (Muck, 2012).

Table 1 Effect of SiloSolve MC treatment on the fermentation variables and microbial composition of big bale whole crop barley forage ensiled for 120 days

Treatment	T1	T2	$LSD_{0.05}$
DM (corrected for volatiles), g/kg	373	381*	4.46
WSC, g/kg DM	4.46	5.58	3.02
pH, 120 day of storage	4.36	4.20*	0.078
pH, 14 days exposure to air	5.18	4.34*	0.169
N-NH_3, g/kg total N	48.5	37.9*	7.87
Lactic acid, g/kg DM	22.3	35.9*	6.08
Acetic acid, g/kg DM	13.6	12.0	1.96
Butyric acid, g/kg DM	4.98	1.92*	1.55
Propionic acid, g/kg DM	0.412	0.366	0.195
Alcohols, g/kg DM	7.94	4.85*	1.21
DM loss, g/ DM	88.5	61.8*	12.3
Bale weight at day of ensiling (0 day of storage), kg	700	708	57.3
Bale weight 120 day of storage, kg	688	698	58.3
Bale weight 14 day exposure to air, kg	685	696	58.1
LAB**, 120 day of storage, log cfu/g silage	4.99	7.12*	0.530
Yeast, 120 day of storage, log cfu/g silage	1.42	1.12*	0.249
Moulds, 120 day of storage, log cfu/g silage	1.45	1.12*	0.240
Yeast, 14 day exposure to air, log cfu/g silage	4.23	1.85*	0.977
Mould, 14 day exposure to air, log cfu/g silage	4.95	1.84*	1.02

*statistically significant difference *vs* control (T1) $P < 0.05$
**LAB: Lactic acid bacteria

Inoculation did not affect acetic acid and propionic acid concentrations, but had a significant effect on protein degradation as measured by ammonia-N concentration with significantly lower value compared with control silage. The homofermentative additive SiloSolve MC, designed to improve fermentation, resulted in 30.1 % less ($P < 0.05$) DM loss. The treated T2 silage had a significantly decreased yeast and mould count, compared with the T1 silage.

Conclusion SiloSolve MC can enhance the hygienic value and decrease nutrient losses in baled whole crop barley silage.

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Effect of different additives on the silage quality of the highland barley straw

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Keywords highland barley straw, silage, *Lactobacillus delbrueckii*, formic acid

Introduction With the energy and environmental problems being increasingly serious, crop waste has attracted intensive interest as biomass feedstock for feed production because of its considerable availability, such as crop straw. Highland barley straw is a forage crop with high nutritive value and is often a major component of diets for high-producing dairy cows. However, proper ensiling of this forage crop can be difficult because its high contents of organic acids, salts, proteins, and minerals result in a high buffering capacity. Ensiling, which is an anaerobically fermented method, is used as a way of the storage of crop straw throughout the world. The main goal of ensiling crop straw is to prevent deterioration, conserve biomass and rapidly fermentation of water soluble carbohydrates into organic acids, preferably lactic acid. Therefore, in order to reduce the pH, some chemical additives (e.g., formic acid, acetic acid and sulfuric acid) and inoculants (e.g., *Lactobacillus delbrueckii, L. buchneri* and *L. rhamnosus*) have been used to control the fermentation pattern to avoid undesirable growth of spoilage microorganisms. Therefore, this experiment was conducted to investigate the effects of inoculating the highland barley straw silage with formic acid and *Lactobacillus delbrueckii* alone or as a combination of both on fermentation end-products of highland barley straw.

Materials and methods Whole-plant highland barley straw, cultivar Ganqin No.4, wilted to 60% DM, and chopped to a theoretical length of 0.90 cm by a New Holland FP 240 pull-type forage harvester (New Holland, PA). Within 30 min of harvesting, chopped highland barley straw was divided into four 500 g piles. Each pile was assigned to one of the following treatments: 1) highland barley straw, untreated (CK), 2) *L. delbrueckii* to achieve 5×10^5 cfu/g of fresh forage (LD), 3) 0.3% formic acid (FA), and 4) *L. delbrueckii* to achieve 5×10^5 cfu/g of fresh forage and 0.3% formic acid (LF), and then put into 500 mL triangle flask. Triplicate experimental silos for each treatment were allowed to ensile for 50 d at 28° in a constant temperature and humidity incubator (Guohua, Changzhou, China). After the ensiling period, silos were opened, their contents were thoroughly mixed, and samples were randomly obtained for the determination of fermentation end-products for chemical and microbiological analyses. The carboxylic acids (lactic, acetic, propionic, and butyric) were determined by HPLC in the silage extract. Another portion of the water extracts was filtered through a double layer of cheesecloth into 2 sets of sterile tubes for microbial analyses. One set was used for enumeration of lactic acid bacteria by pour plating 10-fold serial dilutions on MRS agar (Oxoid CM0361), and the second set was used for the population of yeasts and molds by pour plating on malt extract agar (Oxoid

CM0059) that had been acidified with 85% lactic acid (0.5% vol/vol) after autoclaving. A 1-mL aliquot was collected from the spray bottles before inoculation and also pour-plated on MRS agar to confirm the targeted application rate of the inoculants. Agar plates were incubated in a 32°C oven for 48 to 72 h and counted for numbers of viable colony-forming units. All microbial data was transformed to log10 and are presented on a wet weight basis. Chemical data are presented on a DM basis. All data were analyzed by ANOVA using the GLM procedures of SAS (SAS Institute, 1999). Tukey's test was used to test treatment means ($P < 0.05$) for different treatments.

Results and discussion The content of organic acids are shown in Table1. All organic acids content were higher compared with the CK, except the pentanoic acid. Microbial compositions of fresh chopped forage are shown in Figure 1. Counts of *L. buchneri* were greater in inoculated silages compared with untreated silages and the lower numbers of yeast and mould. So, the three treatment methods could keep the lower pH, which can avoid undesirable growth of spoilage microorganisms, especially the group LF.

Table 1 Content of organic acid in highland barley straw silage

Treatment	Lactic acid (%DM)	Formic acid (%DM)	Acetic acid (%DM)	Propionic acid (%DM)	Butyric acid (%DM)	Pentanoic acid (%DM)
CK	34.35a	1.12a	11.65a	4.65a	1.45a	0.32a
LF	45.65b	1.32a	19.32b	9.21b	2.65b	0.31a
LD	42.32c	1.65b	16.98c	6.36c	2.22c	0.42a
FA	44.32b	1.23a	17.29c	7.65d	2.32c	0.23a
SEM	2.65	0.12	1.67	0.93	0.25	0.037
P-value	0.347	0.314	0.701	0.051	0.404	0.828

Different letters (a, b, c) in the same column shows statistical difference ($P < 0.05$).

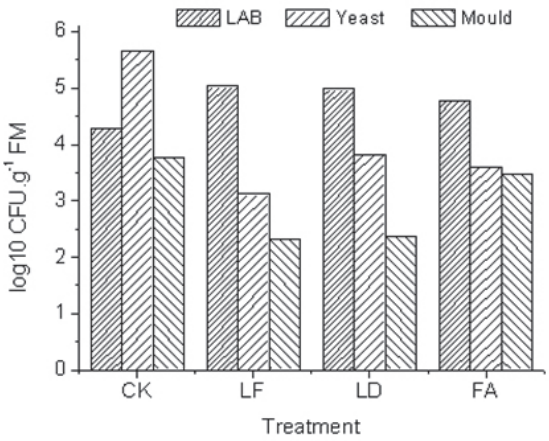

Figure 1 Microbe number of each treatment. FM: fresh matter, CK: highland barley straw, untreated, LD: *L. delbrueckii* to achieve 5×10^5 cfu/g of fresh forage, FA: 0.3% formic acid, and LF: *L. delbrueckii* to achieve 5×10^5 cfu/g of fresh forage and 0.3% formic acid.

Conclusion The different bacterial inoculants or chemical additives on the fermentation can avoid undesirable growth of spoilage microorganisms and keep the silage good quality.

Effects of different additives on the quality of baled oat silage with high moisture content

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Keywords oat, silage, additive, quality

Introduction Oat (*Avena sativa L.*) is an important feed in high altitude regions of China, especially Qinghai-Tibet Plateau. It is usually used to make hay. But frequent rain during autumn makes it difficult to get high quality hay in these areas. Oat is more suitable to make silage. Ordinary silage is made with 65%-70% moisture content, silage made with higher moisture content (>70%) materials were also reported. This kind of silage can be made just after cutting and does not need wilting, thus reduce the field loss and weather influence. However, too high water content is harmful for silage making, and additives are often used for this kind of silage. The objective of this trial was to study the effects of different additives on the quality of baled oat silage with higher moisture content.

Materials and methods The experiment was conducted in Xiahe county, which is located in Gannan Tibetan Autonomous Prefecture (E102°83′, N35°23′, 2517m altitude). The maximum temperature is 29.7°C, the minimum is -24.1 °C; annual rainfall is 489mm. Oat was harvested at filling stage, containing 78-83% moisture. The treatments were: corn flour (T1), urea (T2), Synlac Dry (T3), Sila-Max 200 (T4), and no additives (CK). The amount of additives were based on fresh weight of raw material, namely 4%, 0.4%, 0.0002% and 0.00025%, respectively; then wrapped with 4 layers plastic film. Samples were taken 40d after baling. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), NH_3-N and soluble carbohydrate (WSC) were tested. HPLC (Agilent 1260 and G1321B ultraviolet fluorescence detector) was used to determine lactic acid (LA), acetic acid (AA), acrylic acid (PA) and butyric acid (BA) contents.

Results and discussion The nutritive quality of oat silage was significantly affected by additives (Figure 1). Comparing with the control, CP contents of oat silage with T1 and T3 treatments had slight decrease, while T1 and T4 increased CP by 22.78% and 11.65%. The change of NDF was slight. The ADF had similar trend with NDF, T2 had the highest ADF (7.19% higher than the control), and T4 was the least (7.59% lower than the control). The T3, T1 and T4 increased WSC of oat silage by 24.90%, 18.41% and 16.69%, respectively; while T2 decreased WSC by 9.14%. Additives had significant effects on fermentation quality of oat silage. Table 1 showed that under T2 treatment, pH of oat silage declined slowly to 4.5 after 40 d; meanwhile, T3 and T4 had pH value as low as 4.1. The T1 had no significant influence on pH. The T2 helped to reduce LA by 10.73%; other 3 treatments increased LA, especially T4 (increased 56.39% compared with the control). All the additives significantly improved AA while reduced PA content. BA was not detected. The T2 had the highest NH_3-N content (3.8 fold of the control), T1 had no apparent effect, T3 and T4 decreased NH_3-N content by 17.25% and 33.26%. Our results showed that adding urea reduced WSC and increased NDF and ADF. Urea can decompose to NH_3 which

dissolve in water to form ammonium hydroxide, weaken the connection between cellulose and xylogen. But this reaction may need a longer period than 40d during later autumn to winter in alpine area. It is generally believed that remaining oxygen in silage makes proteinase retain vitality, decompose protein into ammonia and amine, causing protein loss. In addition, under aerobic condition, the aerobic bacteria such as clostridia decompose amino acid, resulting in increasing protein loss. The T3 and T4 treatments significantly increased the lactic acid bacteria quantity at the beginning of the fermentation when oxygen is still remaining; as lactic acid bacteria multiplied, the growth of other aerobic microbial were significantly inhibited, pH reduced and the content of NH_3-N decreased significantly. The T4 had a substantially higher LA , CP and volatile fatty acids (VFA), and lower NDF and ADF. In this study, BA was not detected in all treatments including the control, indicating successful silage.

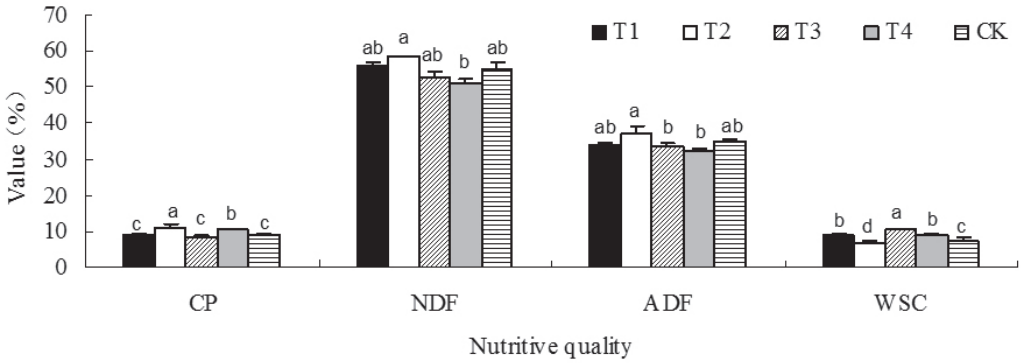

Figure 1 The effects of additives on nutritive quality of oat silage.

Table 1 The effects of additives on fermentation quality of oat silage

Item	PH	LA（%DM）	AA（%DM）	PA（%DM）	BA（%DM）	NH_3-N（% TN）
T1	4.3	7.98	2.32	0.03	0.00	4.94
T2	4.5	6.57	2.94	0.01	0.00	18.68
T3	4.1	8.49	3.09	0.01	0.00	4.03
T4	4.1	11.51	4.33	0.01	0.00	3.25
CK	4.2	7.36	2.07	0.04	0.00	4.87

Conclusion In Xiahe County of Gannan Tibet Autonomous Prefecture, oat can be harvested at grain filling stage with 78%-83% moisture and directly ensiled. However, additives can accelerate the fermentation process, reduce losses and improve silage quality. Compared with corn and urea, bio-additives gave much better performance, of which, Sila-Max 200 was better than Synlac Dry.

Lactobacillus parafarraginis ZH1 producing anti-fungal compounds to improve the aerobic stability of silage

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Keywords anti-fungal compound, *Lactobacillus parafarraginis* ZH1, silage, aerobic stability

Introduction When a silo is opened, anaerobic conditions are no longer maintained, and aerobic microbes have the opportunity to grow. These aerobic microbes produce heat through the consumption of nutrients from silage, thereby causing spoilage. Once aerobic deterioration of silage starts, large loss of dry matter in silage is inevitable. In addition, a vast number of toxic substances might appear with aerobic deterioration of silage, which affect animal health and decrease their productivity (McDonald et al., 1991). Therefore, improving the aerobic stability of silage can confer a substantial advantage to producers. *L. buchneri* was found to improve aerobic stability of ensiled forage by increasing acetic acid during fermentation and has been widely used in silage preparation. However, some silages still deteriorate when acetic acid was directly added at ensiling (Zhang et al., 2006). This might indicate that the other substitutes besides acetic acid, produced by some lactic acid bacteria (LAB), also play a main role in improving the aerobic stability. Recently, we isolated a strain of *Lactobacillus parafarraginis* ZH1 and found it could improve the aerobic stability of silage (Liu et al., 2014). The purpose of the current work was to investigate the anti-fungal compounds produced from *L. parafarraginis* ZH1 in silage.

Materials and methods Light wilted oat (*Avena sativa* L.) was used as silage material. The material was inoculated with ZH1, *L. plantarum* or *L.buchneri* at 1×10^6 cfu/g FM, and the control was sprayed with the same amount of distilled water alone. After treating and thorough mixing, 300 g of material was filled into a plastic film bag in triplicates, degassed and sealed using a vacuum sealer. Thereafter, they were kept in the incubators at 15° for ensiling 45 days. After silo opened, the fermentation quality and aerobic stability was evaluated. Aerobic bacteria were counted on nutrient agar (Nissui-Seiyaku Ltd, Tokyo, Japan), while yeasts and molds were counted on potato dextrose agar (Nissui-seiyaku Ltd.,) acidified with sterilized tartaric acid solution to pH of 3.5. Anti-fungal compounds were extracted according to the method of Ström *et al*. (2002) and measured using GC-MS.

Results and discussion Propionic acid, butyric acid and molds were not detected in all the silages. ZH1 inoculation did not reduce pH value over the control ($P>0.05$), but significantly reduced the numbers of aerobic bacteria and yeasts and lactic acid ($P<0.05$), increased contents of acetic acid, benzoic acid, tetradecanoic acid, palmitic acid and octadecanoic acid, compared to the other treatments (Table 1). ZH1 inoculation significant improved the aerobic stability of silage compared to the other treatments ($P<0.05$). Benzoic acid is a common weak acid and sodium benzoate is used as preservatives in beverage and silage production, which inhibits yeasts through acidifying the cytosol, increasing ATP depletion and causing oxidative stress (discrete intracellular membrane trafficking

pathways in yeast, including macro autophagy). Tetradecanoic acid, palmitic acid and octadecanoic acid are the saturated fatty acid, which alone or together could inhibit yeasts and molds. In this study, benzoic acid and palmitic acid were far more than tetradecanoic acid and octadecanoic acid, which might play an important role in inhibiting yeasts. This was confirmed by our pure culture experiment, where both benzoic acid and palmitic acid inhibited the growth of yeasts at the concentration of 0.5 to 1.0 g/kg. The similar results were also reported by other workers. *L. plantarum* and *L. buchneri* inoculation reduced pH and increased lactic acid content. Although they also increased acetic acid content to the some extent compared to the control, but the aerobic stability of their silages was still poor.

Table 1 The effects of LAB inoculation on chemical and microbial characteristics of oat silage

Items	pH	Lactic acid	Acetic acid	Benzoic acid	Palmitic acid	Octadecanoic acid	Aerobic bacteria	Yeast	Aerobic stability
		g/kg DM					log cfu/g FM		h
Control	4.19 a	50.11 c	0.00 d	0.07 b	0.14 b	0.03 b	4.78 a	3.99 a	32.9 c
ZH1	4.15 a	29.75 d	22.92 a	0.97 a	1.89 a	0.46 a	2.82 b	<2.00 c	144 a
LP	3.61 c	94.93 a	10.54 b	0.09 b	0.17 b	0.04 b	4.66 a	4.06 a	29.8 c
LB	3.97 b	59.09 b	9.13 c	0.07 b	0.14 b	0.03 b	4.68 a	3.20 b	38.6 b

Values within the same column with different lowercase differ significantly from each other at $P<0.05$; LP, *Lactobacillus plantarum*; LB, *L. buchneri*; DM, dry matter; FM, fresh matter.

Conclusions The silage inoculated with *L. parafarraginis* ZH1 produced more benzoic acid, tetradecanoic acid, palmitic acid and octadecanoic acid, as well as acetic acid. All these compounds benefited to the aerobic stability of silage, especially benzoic acid and palmitic acid might play an important role.

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High ensiling density and lactic acid bacteria inoculant improved fermentation quality of *Leymus chinensis* silage

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Keywords lactic acid bacteria, ensiling density, *Leymus chinensis*, silage quality

Introduction Chinese leymus (*Leymus chinensis (Trin.) Tzvel*.), the dominant steppe communities, is an important source of animal feed in China. Due to its high yield, palatability, and ability to meet livestock nutritional requirements, this species is cultivated as one of the principal forage grasses in northern China, the Mongolia plateau, and western and eastern Siberia. Conserving this forage as silage is a better approach than traditional hay-making due to lower cost and less nutrition loss. However, it is difficult to obtain high quality *L. chinensis* silage without additives. On the other hand, the hollow and hard stems impede compressing of the mass and the creation of anaerobic conditions for successful ensiling. So this study was conducted to evaluate the effects of ensiling density and lactic acid bacteria (LAB) inoculant on fermentation quality of *L. chinensis* silage.

Materials and methods *Lactobacillus plantarum* (LP1) was isolated from *L. chinensis* silage according to the procedure described by Cai et al. (1998). The strain was selected and identified by 16S rDNA according Zhang *et al*. (2014).*L. chinensis* was harvested on 8 July 2014from Hebei (China). LAB inoculation (LP1 and control) and ensiling density levels (500, 600 kg m^{-3}) were designed. The material was ensiled without a LAB inoculant (CK), with the LP1 inoculant (LP1) at the two ensiling density levels respectively. LP1 was inoculated at 1×10^5 cfu g^{-1} fresh material (FM). The control was sprayed with the same volume of distilled water. After inoculation and a thorough mixing, the material was packed into 5.0 L plastic jars. Chemical and microorganism compositions were analyzed after 60 days of ensiling. Twenty grams of each silage sample was homogenized in a blender with 180 mL of distilled water for 1 min and then filtered through four layers of cheesecloth. The filtrate was used to measure pH, NH_3-N and organic acids content.

Results and discussion The effects of LAB strains on the fermentation characteristics of *L. chinensis* silage are shown in Table 1. The LP1 increased lactic acid content and the lactic: acetic ratio, decreased butyric acid and NH_3-N content. Coliform bacteria count was less than 2.00 log cfu g^{-1}FM when LP1 was added. These may be because the reduction of pH by the isolates addition reduced the growth and proteolytic activity of microorganisms like clostridia (Arriola et al. 2011). The increase in ensiling density was an effective method to improve the fermentation quality. Compared the untreated silages in two ensiling density levels in this study, pH, lactic acid content were higher and butyric acid content, NH_3-N content, coliform bacteria were lower at 600 kg m^{-3}ensiling density level. No significant difference of the fermentation quality was observed between the two ensiling density levels when the isolated strains were added. It indicates high quality *L. chinensis* silage could also be obtained at 500 kg m^{-3} ensiling density with the addition of LAB inoculants.

Conclusions The fermentation quality of *L. chinensis* silage was better at 600 kg m^{-3} ensiling density. *Lactobacillus plantarum* LP1 improved the fermentation quality of *L. chinensis* silage at the two ensiling density levels. High quality *L. chinensis* silage could also be obtained at 500 kg m^{-3} ensiling density with the addition of LP1.

Table1 Effects of LAB inoculant and ensiling density on the fermentation quality of *Leymus chinensis* silage

	500 kg m^{-3}		600 kg m^{-3}			Significance		
Item	CK	LP1	CK	LP1	SEM	Density	LAB	Interaction
pH	5.47^{a}	4.05^{c}	4.74^{b}	3.94^{c}	0.22	*	**	NS
Lactic acid (g kg^{-1} DM)	11.07^{b}	43.30^{a}	18.54^{b}	35.88^{a}	4.92	NS	**	NS
Acetic acid(g kg^{-1} DM)	7.09^{b}	13.97^{a}	6.03^{b}	7.10^{b}	1.29	*	*	NS
Butyric acid (g kg^{-1} DM)	3.17^{a}	ND	0.54^{b}	ND	0.78	*	-	-
Lactic: Acetic ratio	1.51^{b}	3.30^{ab}	2.93^{b}	5.30^{a}	0.51	NS	*	NS
NH_3-N(g kg^{-1} FM)	0.85^{a}	0.28^{b}	0.65^{a}	0.28^{b}	0.09	NS	**	NS
Lactic acid bacteria (log cfu g^{-1}FM)	8.00^{a}	6.11^{b}	7.88^{a}	5.83^{b}	0.31	NS	**	NS
Coliform bacteria (log cfu g^{-1}FM)	5.67^{a}	< 2.00	3.22^{b}	< 2.00	0.62	**	NS	NS
Yeast and molds (log cfu g^{-1}FM)	< 2.00	< 2.00	3.78	< 2.00	0.24	-	-	-

DM, dry matter; FM, fresh matter; LP1, *L. plantarum* LP1. Means within the same column with different superscripts differ significantly from each other (P< 0.05)

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Effect of *Lactobacillus plantarum* and Mongolian herbal extracts on the fermentation quality of native grass silages

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Keywords *Lactobacillus plantarum,* Mongolian herbal extract, native grass silage, fermentation

Introduction In order to protect the ecological environment of the pasture in Inner Mongolia region, grazing prohibition and Hugh ranch now has been common used ways. Making silages had become a more and more important way for seasonal adjustment of forages. Native grasses are difficult to ensile because of the lower concentration of water soluble carbohydrate and number lactic acid bacteria. *Lactobacillus plantarum* is usually expected to produce more lactic acid and decrease the pH and concentration of ammonia nitrogen. Addition of Mongolian herbal extracts as feed additives can benefit the immune ability, metabolism and production performance of animals, but there are little research about the effect of this extract on the fermentation quality of silages. The aim of this experiment was to evaluate the effect of *Lactobacillus plantarum* inoculant and Mongolian herbal extracts on fermentation quality of native grass silages.

Materials and methods The native grasses were harvested at the pasture in jarud banner, Tong Liao city, Inner Mongolia. The fresh grasses harvested were wilted to 40% moisture and ensiled. The treatments were as follows: (1) *Lactobacillus plantarum* (LP), added at 1×10^6 CFU/g; (2) Arora (TCR), an extract made from the fruit of *Terminalia chebula Retz.*, added at the rate of 1.33% of fresh matter; (3) LP+TCR, combination of (1) and (2); (4) Jirora (GJE), an extract made from the fruit of *Gardenia jasminoides Ellis*, added at the rate of 1.33% of fresh grasses; (5) LP+GJE, combination of (1) and (4); (6) Barora (MTS), an extract made from the fruit of *Melia toosendan Sieb. et Zucc.*, added at the rate of 1.33% of fresh grasses; (7) LP+MTS, combination of (1) and (6); (8) Dogolebsu (SFA), an extract made from the root of *Sophora flavescens Ait.*, added at the rate of 1.33% of fresh grasses; (9) LP+KZ, combination of (1) and (8); (10) control group, added with same volume (1 mL) distilled water as other treatments. The Mongolian herbal was cooked and then filtered to obtain the extract. All forages ensiled in bags for 45 days before the opening. The pH, lactic acid, volatile fatty acids and ammonia nitrogen were detected according to the method of Sun *et al.* (2012). The variance analysis and multiple comparisons were performed using the PROC GLM of SAS.

Results and discussion The LP-treated silages, as expected, had lower ($P < 0.05$) pH value and higher ($P < 0.05$) lactic acid content than the control. For the four Mongolian herbal extract additives compared to control, only MTS treatment decreased ($P < 0.05$) the pH, while treatments TCR, GJE and MTS increased ($P < 0.05$) the lactic acid content. On the other hand, the silages treated with TCR, GJE, SFA had lower ammonia nitrogen content than the control. The GJE treatment preserved the best in ammonia nitrogen compared to

other Mongolian herbal additives and similar to that of LP treatment. The compound of LP and the four chemical additives had the lowest ($P < 0.05$) pH and highest ($P < 0.05$) content of lactic acid and acetic acid in most cases. For the decrease of ammonia nitrogen content, LP+GJE preserved the best in the four compound additives but the effect just similar to that of LP and GJE treatment used alone (Table 1).

Conclusions Addition of LP decreased the pH value and ammonia-N content by increasing the lactic acid content. The effects of Mongolian herbal extract additives added alone were poorer than or closed to that of LP. Combination of LP and Mongolian herbal additives increased lactic acid production and decreased silage pH.

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Table 1 Effect of *Lactobacillus plantarum* and Mongolian herbal extract on the fermentation quality of native grass after 45 days of ensiling

	pH	LA[2] (g kg^{-1}DM)	AA (g kg^{-1}DM)	PA (g kg^{-1}DM)	BA (g kg^{-1}DM)	AN/TN (g kg^{-1}TN)
Control	4.49^{a}	5.0^{d}	1.0^{c}	0	0	19.2^{a}
LP[1]	4.32^{b}	11.8^{b}	2.5^{b}	0.2	0	7.30^{c}
TCR	4.44^{a}	12.2^{a}	3.0^{a}	0	0	13.3^{b}
LP +TCR	4.13^{c}	24.6^{a}	3.3^{a}	0	0	12.4^{b}
GJE	4.46^{a}	8.2^{c}	1.4^{c}	0	0.5	8.20^{c}
LP+GJE	4.17^{c}	27.9^{a}	2.3^{b}	0	0	7.90^{c}
MTS	4.31^{b}	11.1^{b}	2.4^{b}	0.2	0	15.1^{ab}
LP+MTS	4.14^{c}	29.0^{a}	3.7^{a}	0	0	9.60^{b}
SFA	4.45^{a}	4.5^{d}	1.0^{c}	0	0	10.3^{b}
LP+SFA	4.11^{c}	28.7^{a}	3.4^{a}	0	0	14.8^{ab}

[1]LP, *Lactobacillus plantarum*; TCR, *Terminalia chebula Retz.*; GJE, *Gardenia jasminoides Ellis*; MTS, *Melia toosendan Sieb. et Zucc*; SFA, *Sophora flavescens Ait*;

[2]DM, dry matter; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; AN, ammonia nitrogen; TN, total nitrogen.

$^{a-d}$ Means within a row with different superscripts differ ($P < 0.05$).

Effect of lactic acid bacteria on the tropical silage preparation in Thailand

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Keywords lactic acid bacteria, silage, tropic

Introduction Recently, silage is an increasingly important source of animal feed in tropic, in general, suitable plants for silage preparation may be including the permanent grass and the nonpermanent such forage crops. Guinea grass (*Pannicum maximum* Jacq.) and sorghum (*Sorghum bicolor* (L.) Moench) are major forage crops and grasses that are widely used to make hay or silage for ruminant feed in the tropical area. Lactic acid bacteria (LAB) play an important role in silage fermentation and silage is now the most common preserved feed for cattle production in many countries. Therefore, the characteristics of LAB species including commercial inoculants and selected strains in the silage environment require further study. In the present study, selected strains of LAB were isolated from forage crops and their application for tropical silage preparation were studied.

Materials and methods Purple Guinea grass (Cultivar TD 58) and sorghum (Cultivar MOENCH) were obtained from an experimental farm located in faculty of agriculture, Khon Kaen University, Khon Kaen, Thailand. *Lactobacillus casei* TH 14, *L. plantarum* TH 21 and *L. plantarum* TH 64 were isolated from tropical silages prepared by using a small-scale system (Cai et al., 1998) in Thailand. These strains were selected by low pH growth range and high productivity of lactic acid than other isolates during silage fermentation. The silage treatments were designed as follows: untreated control, selected strains TH 12, TH 14 and TH 64, and two commercial inoculant strains CH (Chikuso-1, *L. plantarum*, Snow Brand Seed Co., Ltd, Sapporo, Japan) and SN (Snow Lact L, *L. rhamnosus*, Snow Brand Seed Co., Ltd). Three replicate samples of each treatment was analyzed for silage quality analysis.

Results and discussion Overall, fresh and wilted Guinea grass and sorghum were 10^3 to 10^5 LAB and yeasts, 10^6 to 10^7 coliform bacteria and aerobic bacteria, 10^3 to 10^4 molds in cfu/g FM. Sorghum silages at d 30 of fermentation were all well preserved with a low pH level (below 3.7) and high lactate (> 5.9% of DM). Guinea grass silage inoculated with LAB more effectively inhibited the growth of aerobic bacteria and coliform bacteria, increased lactic acid content, and decreased pH values, contents of butyric acid and ammonia nitrogen compared with the control silage. Strain TH 14 was more effectively to improve Guinea grass and sorghum silage quality than commercial inoculant and other strains (Table 1).

Conclusion Selected strains of LAB were isolated from forage crops and their application

for tropical silage preparation were studied in Thailand. The results confirmed that strain *L. casei* TH 14 is considered suitable as a potential inoculant for tropical silage preparation.

Table 1 Fermentation product of Guinea grass and sorghum silages at 30 d of ensiling

		DM	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ammonia-N
		g/kg		g/kg DM				
Guinea grass								
Fresh	Control	227.50^{ef}	6.58^{a}	26.81^{fg}	12.17^{d}	1.03^{c}	3.20^{a}	1.42^{b}
	TH 14	246.00^{e}	4.55^{def}	61.86^{cd}	11.78^{d}	0.40^{efg}	0.18^{de}	0.37^{de}
	TH 21	237.50^{ef}	4.62^{cde}	48.35^{de}	13.64^{d}	0.41^{efg}	0.71^{d}	0.50^{ef}
	TH 64	238.60^{ef}	4.68^{efg}	49.00^{de}	12.57^{cd}	0.48^{def}	0.19^{b}	0.25^{d}
	CH	233.70^{ef}	4.72^{cd}	36.94^{ef}	15.04^{c}	0.43^{defg}	0.30^{de}	0.49^{d}
	SN	225.80^{f}	4.84^{c}	32.16^{fg}	17.53^{b}	0.43^{defg}	0.76^{cd}	0.50^{d}
Wilted	Control	342.50^{c}	5.93^{b}	18.18^{g}	3.97^{g}	0.57^{de}	0.33^{de}	0.50^{d}
	TH 14	380.60^{ab}	4.40^{g}	69.64^{bc}	5.67^{fg}	0.25^{g}	0.00^{e}	0.15^{f}
	TH 21	338.30^{d}	4.48^{efg}	62.05^{cd}	9.14^{e}	0.28^{fg}	0.00^{e}	0.30^{ef}
	TH 64	322.30^{cd}	4.54^{g}	56.37^{cd}	9.11^{e}	0.28^{fg}	0.12^{de}	0.29^{ef}
	CH	366.20^{b}	4.49^{fg}	64.26^{c}	9.18^{e}	0.27^{g}	0.00^{e}	0.25^{ef}
	SN	393.10^{a}	4.42^{g}	64.56^{c}	7.80^{ef}	0.24^{g}	0.00^{e}	0.29^{ef}
Sorghum	Control	238.80^{ef}	3.70^{h}	59.51^{cd}	22.51^{a}	1.61^{a}	2.07^{b}	1.64^{a}
	TH 14	245.20^{ef}	3.38^{j}	108.30^{a}	15.34^{bc}	0.61^{d}	0.49^{de}	0.58^{d}
	TH 21	228.40^{ef}	3.58^{hi}	64.79^{c}	3.49^{g}	1.29^{b}	1.16^{b}	0.92^{c}
	TH 64	233.30^{ef}	3.64^{hi}	82.64^{b}	3.89^{g}	1.40^{b}	1.44^{de}	0.86^{c}
	CH	227.20^{ef}	3.55^{hi}	60.39^{cd}	4.43^{g}	1.27^{b}	1.43^{b}	0.87^{c}
	SN	235.00^{ef}	3.52^{ij}	65.48^{c}	5.47^{fg}	1.25^{b}	1.38^{b}	0.88^{c}
	SEM	0.067	0.063	4.242	0.895	0.065	0.094	0.060

$^{a\text{-}f}$ Means within columns with difference superscript letters differ ($P < 0.05$).

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The influence of additives on fermentation pattern, volatile organic compounds (VOC) and aerobic stability of grass silage

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Keywords silage additive, grass silage, volatile organic compound, ester, aerobic stability

Introduction Volatile organic compounds (VOC), e.g. alcohols, organic acids and esters thereof, are frequently found in silages (Weiss and Auerbach, 2013) and may detrimentally affect feed intake by dairy cattle. However, the knowledge of the formation of VOC in grass silages and the effects of additives thereon is still very limited. Therefore, this study aim at testing different types of commercial additives regarding their effects on fermentation pattern, production of VOC and aerobic stability of grass silage.

Materials and methods A fourth-cut natural grassland crop was mowed on a dairy farm in East Germany, wilted overnight to 26.8% DM (9.6% crude ash, 16.1% crude protein, 23.6% crude fibre, 17.8% water-soluble carbohydrates based on DM), chopped to a theoretical particle size of about 2 cm by a Claas Jaguar. After packing the material into 1.5-L glass jars the silages were stored at 25 °C for 72 days. Forages received the treatment with one of a total of 21 commercial additives which were obtained from the German marketplace and used according to the instructions of the manufacturers. These included: 6 homofermentative (Ho) and 1 heterofermentative (He) inoculants and 5 combinations of homo- and heterofermentative (HoHe) lactic acid bacteria (LAB). Additionally, 5 combinations of Ho with antimycotic substances (HoCh, benzoate or sorbate or mixtures thereof) and 4 chemical (Ch) mixtures (nitrite, hexamine, benzoate and/or sorbate or buffered formic and propionic acids) were used. All silage treatments were produced in triplicates, and untreated forage served as control (Con). The fermentation characteristics (pH, acids, alcohols, esters) were analysed with routine analytical procedures (Weiss und Auerbach, 2013). Aerobic stability (AS) was determined by measuring temperature for 15 days. Silages were considered unstable once the sample temperature had reached 2 °C above ambient (20 °C). Statistical analysis by was performed by PROC MIXED of SAS, comparing the means of untreated with each additive treatment. Significance was declared at $P<0.05$ (Dunnett test). The PROC CORR of SAS was employed to evaluate the relationship between ethanol and ethyl esters.

Results and discussion Grass silages were well fermented as reflected by low pH (Table 1) and no butyric acid was found (data not given). The production of lactic acid was stimulated by some additives of the types Ho, HoHe and HoCh whereas the pure He inoculant as well as two chemicals reduced it. The treatment with homofermentative LAB, either applied alone or in combination with antimycotic chemicals, always resulted in lower acetate levels. The lowest ethanol and ethyl ester contents were detected in silages that had received chemical additives. There was a strong positive linear correlation between these two parameters (r^2=0.72, $P<0.001$), which substantiates data by Weiss and Auerbach (2013). The production of 1-propanol was highest in silages treated with the heterofermentative inoculant. All additives containing LAB increased acetone formation, but the reason for this and the relevance of this compound for silage quality remains to be elucidated. The concentrations of methanol and 2-butanol were largely unaffected by treatment. The AS of grass silage was consistently enhanced by the application of

chemical additives regardless of their composition. One combination of homofermentative LAB and an antimycotic compound also showed an improvement. On the contrary, 5 of the 6 tested pure homofermentative inoculants reduced the AS due to the reduction in acetic acid.

Table 1 Effects of additives on fermentation pattern, volatile organic compounds and aerobic stability of grass silage stored for 72 days

Treatment	pH	Lactic acid[1]	Acetic acid[1]	Ethanol[1]	EE[2,3]	Propanol[3]	Acetone[3]	Methanol[3]	2-Butanol[3]	AS[4]
Con[5]	4.0	82.0	14.1	10.2	344	236	0	697	205	7.4
Ho[6]	3.9*	85.1	7.9*	8.7	301	0*	119*	789	224	3.3*
Ho[6]	3.9*	96.0*	11.0§	9.0	316	23*	131*	844§	222	7.0
Ho[6]	3.8*	87.2	7.9*	7.5#	258	0*	109*	796	212	2.3*
Ho[6]	3.8*	90.9§	8.5*	8.2§	284	0*	108*	845§	128§	1.8*
Ho[6]	3.8*	91.2§	7.6*	7.5*	309	0*	99*	686	152	2.7*
Ho[6]	3.8*	88.6	8.9*	7.7#	261	0*	89*	694	160	4.3§
He[7]	4.1*	61.1*	22.8*	13.4*	353	1080*	100*	817	195	8.8
HoHe[8]	3.9#	85.2	14.0	10.5	384	44*	126*	828	208	6.3
HoHe[8]	3.9*	79.6	11.3	8.7	342	100§	76*	816	197	7.2
HoHe[8]	3.9*	86.8	11.3	8.8	411	72#	111*	878#	130§	6.4
HoHe[8]	3.9*	96.2*	10.2§	7.8#	329	0*	108*	853§	214	5.4
HoHe[8]	3.9*	89.0	12.3	8.8	327	78#	99*	786	196	6.7
HoCh[9]	3.9*	93.2§	10.3§	9.8	300	0*	18	641	155	7.4
HoCh[9]	3.9*	81.7	10.2§	8.9	292	0*	24	660	187	8.1
HoCh[9]	3.9*	85.0	9.5#	8.6	272	0*	69*	692	179	6.8
HoCh[9]	3.9*	87.0	7.7*	7.5*	208	0*	51*	598	202	7.3
HoCh[9]	3.9*	84.2	8.6*	7.5*	265	0*	97*	729	241	10.9§
Ch[10]	4.0#	72.4§	16.5	2.3*	80*	499*	0	809	161	15.0*
Ch[11]	4.0	77.9	15.6	4.2*	143*	165*	0	611	153	15.0*
Ch[12]	4.0	61.0*	11.6	4.5*	105*	0*	0	583	164	14.1*
Ch[12]	3.9*	78.1	11.5	3.2*	61*	0*	0	630	209	15.0*

Means of each additive treatment in columns bearing unlike superscripts differ compared with untreated; *$P<0.001$, #$P<0.01$, §$P<0.05$; [1]g/kg DM; [2]ethyl lactate+ethyl acetate; [3]mg/kg DM; [4]aerobic stability, days; [5]untreated; [6]homofermentative LAB; [7]heterofermentative LAB; [8]combination of homo- and heterofermentative LAB; [9]combination of homofermentative LAB and antimycotic chemical(s); [10]nitrite, hexamine, sorbate; [11]nitrite, benzoate, sorbate; [12]buffered formic and propionic acid blends.

Conclusions Chemical additives were superior to all additive types regarding their effect on ethanol and ethyl ester formation in grass silages. Therefore, their use is strongly encouraged to prevent the formation of VOC in grass silages.

Acknowledgement The authors would like to thank ADDCON GmbH, Bonn, Germany, for partial funding of this study.

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Effect of biological additives on ultrastructure along with changes of fibre of *Leymus chinensis* silage

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Keywords *Leymus chinensis,* cell wall, biological additives, ultrastructure

Introduction *Leymus chinensis* is an important forage used for grazing or feeding in northern China. Due to its high fiber content and relatively low soluble carbohydrate content, it is difficult to be satisfied with conventional silage fermentation. Biological additives like lactic acid bacteria can be easy to produce more lactic acid for *Leymus chinensis* silage; as a result, to conduct a successful ensiling. However, it hasn't been found which part of the cell wall was degraded by the fibrolytic enzymes which originally added or produced by LAB, and so, LAB could use substrates from degradation of cell-wall provided by enzymes. So, this research was conducted to find the relationship between biological additives and the changes of cell wall after treatment.

Materials and methods Cellulase treatment is added cellulase which is a solid enzyme multi-component biocatalyst; its standard enzyme activity unit is (CMC enzyme) 1060 Ug^{-1}. LC (*Lactobacillus casei*) was isolated from *L. chinensis* silage according to the procedure described by Zhang et al. (2014). The strain was selected and identified by 16S rDNA according to Zhang et al. (2014). The LC (*Lactobacillus casei*) was inoculated at 1×10^5 CFU g^{-1} fresh material. *L. chinensis* was harvested at 8 July 2014 from Hebei (China). The control was sprayed with the same volume of distilled water. *Leymus chinensis* were chopped into particles of approximately 2 cm. After inoculation and a thorough mixing, 3000 g of material were packed into 5.0 L plastic jars. Chemical nutrient and ultrastructure of stem of the *Leymus chinensis* were analyzed after 60 days of ensiling. The procedures for preparation of samples for transmission electron microscope (TEM) were described by Liu *et al*. (2005).

Results and discussion Nutrient composition and changes parenchyma cell of treated and untreated *L. chinensis* silage are displayed below. The contents of NDF (Neutral Detergent Fiber) in the treatment LC and Cellulase were the lowest, followed by contents in the treatment LC, Cellulase and the untreated (P<0.05). Hemicellulose in the *L. chinensis* silage treated by LC and Cellulase, and Cellulase was effectively lower than those treated by LC and the untreated. However, WSC (Water Soluble Carbohydrate) content in the *L. chinensis* silage were highest when treated by LC and Cellulase, followed by LC, and Cellulase, and the untreated (P<0.05; Table 1). The reason why the nutrients in the *L. chinensis* silage change like above is described below. With TEM, the parenchyma cell can be observed. There is large amount of easy colored materials in the parenchyma cell of the *L. chinensis* in the untreated, while less in the parenchyma cell of the *L. chinensis* after treatments by Cellulase and approximately none after treatments by LC and Cellulase (Figure 1). It is easy to find the clearly seen cells wall in the parenchyma cell of the untreated, while less after treatments by Cellulase and nearly none after treatments by LC

and Cellulase. With the degradation of the cells wall after treatments by LC and Cellulase, and Cellulase, so the WSC contents increase and the NDF and hemicellulose contents decrease, because that fibre was degraded by fibrolytic enzymes and transformed to WSC; and the effect of LC and Cellulase is better followed by Cellulase and LC.

Conclusion Both LC and Cellulase can degrade NDF and ADF and increase WSC. The combination of LC and Cellulase is better than the single additive by degrading cell wall and utilizing cell contents.

Table 1 Composition of treated and untreated *Leymus chinensis* silage

	none	Cellulase	LC	LC and Cellulase
NDF	589.03±3.39^{a}	558.45±2.94^{c}	577.14±3.7^{b}	545.14±2.26^{d}
ADF	307.61±6.14^{a}	288.12±2.15^{bc}	296.35±2.12^{b}	285.32±1.43^{c}
Hemicellulose	281.42±2.76^{a}	270.33±0.85^{b}	280.79±1.93^{a}	259.82±1.11^{c}
ADL	47.77±2.56^{a}	53.22±0.52^{a}	48.59±1.29^{a}	49.67±2.65^{a}
WSC	14.47±0.87^{c}	21.83±1.59^{bc}	24.75±1.89^{b}	34.97±3.75^{a}

ADL, Acid Detergent Lignin. Means within the same row with different superscripts differ significantly from each other ($P < 0.05$).

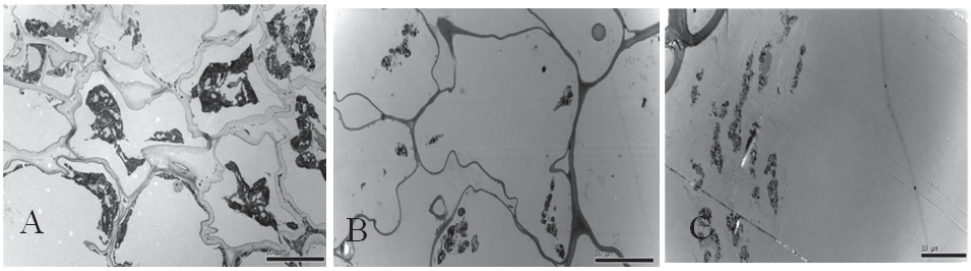

Figure 1 Changes transmission electron microscopy parenchyma cell after treatments by Cellulase (B), LC and Cellulase (C) and the untreated (A), respectively.

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The effect of isolated lactic acid bacteria on fermentation of Italian ryegrass silage at different dry matter content

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Keywords Italian ryegrass silage, fermentation, lactic acid bacteria, dry matter

Introduction Italian ryegrass (*Lolium multiflorum*) has been widely used for silage making because of its fast growth, high yield, palatability and digestibility. However, it is difficult to ensure good fermentation quality from fresh material directly due to the higher moisture, and wilting is not suitable in high humidity season. Therefore, LAB additive was developed which improved ryegrass silage fermentation quality significantly. Microbial silage inoculants containing lactic acid bacteria have long been used to improve silage fermentation. Epiphytic lactic acid bacteria (LAB) convert water-soluble carbohydrates into organic acids, mainly lactic acid. As a result, the pH decreases and the forage is preserved. The aim of this study was to examine the effect of isolated lactic acid bacteria on fermentation of Italian ryegrass silage at different dry matter content.

Materials and methods The Italian ryegrass was harvested at Chengdu, Sichuan province, China in 2013. After harvesting, the fresh forage was chopped and wilted to three DM contents, 25% (Low), 35% (Medium) and 45% (High). Four lactic acid bacteria (LAB, isolated from alfalfa silage) were identified and added as inoculants in this experiment. One is identified as *Lactobacillus casei* (LC), the other three were identified as *Lactobacillus plantarum* (LP1, LP2 and LP3). The addition level of the four LAB was all 10^6 CFU g^{-1} fresh forage. The control was sprayed with the same volume of distilled water. All silages were ensiled for 30 days. After silos opening, dry matter (DM), pH value and ammonia nitrogen contents were determined. Lactic acid (LA), volatile fatty acids (acetic acid, AA; propionic acid, PA; butyric acid, BA) were detected by high performance liquid chromatography. Treatment effects were evaluated using analysis of variance, the GLM procedure of SAS ver.9.1.

Results and discussion The effect of different isolated LAB and dry matter content treatments on fermentation of Italian ryegrass silage is shown in Table 1. The increase of DM content inhibited the activity of epiphytic LAB in raw material and decreased the concentration of lactic acid in silages significantly ($P<0.01$) and finally increased ($P<0.01$) the pH value. Application of LAB inoculants (LC, LP1 and LP3) increased ($P<0.05$) the content of lactic acid and decreased the pH value of silages of all DM contents. There were interactive effects ($P<0.01$) between DM content and LAB inoculants. In low DM content silages, LP2- treated silages had no significant difference to control silages in pH value and lactic acid content. In Medium DM content silages, LP2 produced more ($P<0.05$) lactic acid than the control but had no significant effect on pH value. But in high DM content silages, LP2 increased the lactic acid content and decreased the pH value significantly ($P<0.05$) compared to control and was similar (no significance) to the other three inoculants. High DM content silages had lower ($P<0.05$) ammonia nitrogen and butyric

acid contents than the low DM content silages. LC, LP1 and LP3 treated silages had lower ($P<0.05$) ammonia nitrogen content than control just in Medium DM content silages.

Table 1 Fermentation characteristics of Italian ryegrass silage inoculated with LAB at different dry matter content

DM content	LAB Treatment	pH	DM[1] (%)	AN (%TN)	LA (%DM)	AA	PA	BA
Low	Control	4.09^{cd}	25.52^{ef}	2.58^{abcde}	8.22^{d}	0.6^{d}	4.92	0.04
	LC	3.72^{fg}	27.12^{e}	3.28^{a}	17.9^{a}	1.23^{cd}	7.91	0
	LP1	3.82^{defg}	25.82^{ef}	2.22^{bcdef}	13.2^{b}	1.59^{abcd}	6.08	0
	LP2	3.98^{cdef}	24.53^{f}	3.04^{ab}	9.34^{cd}	1.13^{cd}	5.89	0
	LP3	3.61^{g}	26.26^{e}	2.32^{bcde}	17.85^{a}	1.62^{abcd}	6.96	0
Medium	Control	4.38^{b}	36.48^{c}	2.64^{abcd}	5.46^{e}	2.02^{abc}	4.17	0
	LC	3.77^{efg}	36.16^{c}	1.38^{fgh}	13.49^{b}	1.89^{abc}	7.40	0
	LP1	3.87^{cdefg}	36.36^{c}	0.72^{h}	11.52^{bc}	2.29^{abc}	5.70	0
	LP2	4.11^{bc}	33.04^{d}	2.95^{abc}	9.44^{cd}	1.39^{bcd}	5.07	0
	LP3	3.70^{fg}	35.40^{c}	1.73^{efg}	15.67^{a}	2.38^{abc}	8.17	0
High	Control	5.09^{a}	46.31^{ab}	0.99^{gh}	2.57^{f}	2.84^{a}	7.49	0
	LC	4.01^{cde}	47.59^{a}	0.91^{gh}	9.02^{d}	1.78^{abcd}	6.72	0
	LP1	4.15^{bc}	47.49^{a}	1.05^{gh}	8.75^{d}	2.63^{ab}	6.91	0
	LP2	4.15^{bc}	46.48^{ab}	2.13^{cdef}	8.21^{d}	2.22^{abc}	6.51	0
	LP3	3.89^{cdefg}	45.71^{b}	2.05^{def}	9.45^{cd}	1.53^{bcd}	5.66	0
Significance								
	M[2]	**	**	**	**	**	NS	-
	L	**	**	**	**	NS	NS	-
	M×L	**	NS	**	**	NS	NS	-

[1]DM, dry matter; AN (%TN), ammonia nitrogen (% total nitrogen); LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid;

[2]M, moisture Content effect; L, LAB Treatment effect; M×L, interaction effect of M and L.

Conclusions The increase of DM content had benefit for the Italian ryegrass silages due to the decrease of ammonia nitrogen and butyric acid content, although the content of lactic acid decreased and the pH value increased. Lactic acid bacteria inoculants LC, LP1 and LP3 had positive effect on pH value decrease and lactic acid production in all DM content silages. LP2 did not have any effect on low DM content silages.

Effects of *Emsilage* and *Silosolve* bacterial inoculants on the fermentation and aerobic stability of ensiled ryegrass

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Keywords ryegrass, ensiling, inoculants, fermentation, aerobic stability

Introduction Ryegrass is considered as a high quality forage that have high nutrient digestibility. This forage is rich in sugar and suitable for ensiling. Ensiling of forages pertains the use of silage additives to improve the preservation of forages. Bacterial inoculants are used to improve the nutritive value of silage, reduce some secondary fermentation during ensiling with subsequent improvement in animal performance (Kung, 1996). If sufficient lactic acid bacteria (LAB) are not present on the crop during ensiling, a slow rate of pH decrease will happen (Muck et al., 2007). The present study was conducted to evaluate the effects of bacterial inoculation on the fermentation and aerobic stability of ryegrass silage.

Materials and methods Italian ryegrass (Enhancer cultivar) (50kg) was harvested and finely chopped (2 cm length) and ensiled in 1.5 litre anaerobic jars for 90 days. The forage was treated with or without bacterial inoculants by mixing 0.5g of inoculant with 45 ml deionized water to treat 50 kg of chopped material. The chopped materials were thoroughly mixed and ensiled in 1.5 L anaerobic jars. After 90 days of ensiling, 3 jars per treatment were opened to determine the fermentation characteristics and nutritive values of silage. In addition, silage samples were subjected to an aerobic stability test, which lasted for 5 days following the procedure of Ashbell et al (1991).

Results and discussion Silage pH was reduced ($P<0.05$) with *Emsilage* compared to other treatments (Table 1). Inoculation increased ($P<0.05$) the content of lactic acid, and reduced ammonia-N compared to control. The content of acetic acid was increased ($P<0.05$) with *Silosolve* compared to other treatments. This increase in acetic acid led to improved ($P<0.05$) aerobic stability of silage, as indicated by reduced CO_2 production and increased number of hours in the *Silosolve* treatment, compared to the treated inoculant. *Silosolve* is typical of a heterofermentative inoculant, since high content of acetic acid was produced compared to other treatments (Weinberg et al., 1993).

Conclusions Inoculation of *Emsilage* to ryegrass improved the fermentation of silage by increasing the concentration of lactic acid. The aerobic stability of silage was improved with *Silosolve* while it was worsen with *Emsilage* inoculation. Work to evaluate effects of ryegrass silage treated with these inoculants on ruminant growth performance is needed.

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Table 1 Effects of treatments on the fermentation and aerobic stability of ensiled ryegrass (n = 3)

		Treatment			
Parameter	Control	Emsilage	Silosolve	SEM	P
DM, g/kg	282.2[a]	244.2[b]	239.7[c]	1.82	0.001
GE MJ/kg DM	16.4[b]	16.5[a]	15.6[c]	0.041	0.001
CP, g/kg DM	224.5[c]	247.4[b]	289.5[a]	1.52	0.010
WSC g/kg DM	32.6[b]	48.7[a]	47.0[a]	1.33	0.032
pH	4.3[a]	3.8[c]	4.5[a]	0.03	0.001
LA, g/kg DM	92.6[c]	145.9[a]	133.4[b]	2.38	0.001
AA, g/kg DM	28.5[b]	16.9[c]	56.7[a]	4.72	0.001
NH_3-N %TN	32.6[a]	13.9[c]	18.3[b]	1.42	0.001
Aerobic stability (5 days aerobic exposure)					
Hours	53[b]	42[c]	85[a]	3.62	<0.001
CO_2 g/kg DM	54.3[b]	69.6[a]	36.4[c]	2.42	<0.001

[a-c] Means with different letters in a row differ significantly (P<0.05)

DM, dry matter; GE, gross energy; CP, crude protein; WSC, water soluble-carbohydrates; LA, lactic acid; AA, acetic acid; NH-$_3$, Ammonia Nitrogen; CO_2, Carbon dioxide

Effects of the silage additive Sil All Fireguard ™ in grass silage

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Keywords additive, fermentation quality, aerobic stability, losses, silage

Introduction Besides poor fermentation, aerobic instability is the most common challenge in wilted grass silages. One possibility to avoid aerobic instability is the use of an additive. Therefore the efficacy of the silage additive SIL-ALL FIREGUARD™ was determined by: (1) analyzing the aerobic stability after opening of aerobically challenged and non-aerobically challenged grass silages treated with SIL-ALL FIREGUARD™ against untreated control silages and (2) analyzing fermentation characteristics and weight losses to make sure that the additive did not markedly deteriorate these parameters. The trial was designed to conform to and to obtain DLG approval 'Improving Aerobic Stability' (DLG, 2013). While the DLG guidelines were followed throughout and took precedence, the trial was also performed with the aim to meet the guidelines for EU registration of silage additives (technological additives; EC No. 1831/2003).

Materials and methods The grass mixture of *Lolium perenne* varieties was harvested on August 28, 2013 with an approximate dry matter (DM) content of 34% and water soluble carbohydrates (WSC) of 5.9 % of fresh matter (FM). The silage additive SIL-ALL FIREGUARD™ (Table 1) was applied in freeze-dried form. For application the powder was dissolved in water and sprayed onto the grass. The dosage was 37.5 g/250 kg FM, dissolved in 2 L of sterile water according to the manufacturer›s instructions. The chopped grass with a mean length of 40 mm was ensiled in 1.5 L capacity glass laboratory-scale silos. For each treatment 6 silos were filled, which allowed for 3 to be opened on each of days 49 and 90 (end of the trial). For determination of in-silo DM losses during fermentation the laboratory silos were weighed before and after the ensiling process. The silos were stored in a temperature controlled room at 25 ± 2°C. The determination of aerobic stability was conducted according Honig (1990). All laboratory procedures and analytical methods were conducted according DLG guidelines (DLG 2013). For statistical evaluation the data were examined by SAS evaluation including Kruskal-Wallis Test for significant differences ($P < 0.05$) between the control and the treatment group.

Table 1 Composition of the additive SIL-ALL FIREGUARD™ used in grass silage of 2013

Ingredients	
Lactobacillus plantarum CNCM I-3235	> 4.00 x 10^8 cfu*
Pediococcus pentosaceus NCIMB 12455	> 6.67 x 10^7 cfu*
Pediococcus acidilactici CNCM I-3237	> 2.00 x 10^8 cfu*
Alpha-amylase (EC 3.2.1.1) from *Bacillus amyloliquefaciens* SD 80	> 12 BAU**
Cellulase (EC 3.2.1.4) from *Trichoderma reesei* ATCC SD 6331	> 0.39 CMC***
Sodium Benzoate , Potassium Sorbate , Dextrose	up to 7.5 kg

* Colony forming unit ** Bacterial Amylase Unit *** Carboxymethycellulose Activity Units.

Results and discussion The ensilability of the grass mixture with a WSC-content of 5.9 % FM fulfils the EU requirements of >3 % FM in the category easy to ensile. At day 90 the fermentation quality was significantly improved by the parameters pH, butyric acid, NH_3-N, the proportion of lactic and acetic acid (LA/AA) and ethanol (Table 2). However DM losses were not reduced by the treatment. The nutritional characteristics were not significantly different between control and treated silages (results not presented). The aerobic stability of the treated silages at day 49 was enhanced by nearly 9 days and at day 90 by 1 day. In line with these results the pH-out was also significantly lower for treated silage. The positive effect on the aerobic stability is probably due to the content of sodium benzoate and potassium sorbate in the product.

Table 2 Fermentation characteristics of grass silage without and with SIL-ALL FIREGUARD™ treatment

Fermentation characteristics		Days of storage	Control		SIL-ALL FIREGUARD™		P-value
			$\bar{x}$	SD	$\bar{x}$	SD	
DM	(%)	90	36.07	1.43	34.57	0.47	0.127
pH		90	4.60	0.00	4.20	0.00	0.025
Lactic acid	(% FM)	90	2.47	0.86	3.51	0.49	0.127
Acetic acid	(% FM)	90	0.79	0.12	0.70	0.06	0.184
Butyric acid	(% FM)	90	0.32	0.13	0.03	0.01	0.046
Propionic acid	(% FM)	90	0.02	0.03	0.00	0.00	0.317
LA/AA		90	3.07	0.70	5.00	0.70	0.050
Ethanol	(% FM)	90	0.42	0.05	0.12	0.00	0.037
NH_3-N / total-N	(%)	90	12.00	1.00	8.00	0.00	0.037
Yeasts	(log CFU/g FM)	90	< 3	0.00	< 2	0.00	1.000
Moulds	(log CFU/g FM)	90	2.33	0.74	< 2	0.00	0.317
Aerobic Stability	(d)	49	2.43	0.93	11.83	0.29	0.046
Aerobic Stability	(d)	90	7.93	0.51	8.90	0.00	0.037
pH after AST**		90	5.37	0.46	4.50	0.00	0.034
Dry matter loss*	(% DM)	90	4.80	3.03	5.43	0.06	0.057

* Dry matter loss after 90 days of airtight storage ** AST= aerobic stability test
P-values ≤ 0.05 stand for significant differences. (FM: fresh matter. DM: dry matter)

Conclusions To ensure fermentation quality and if aerobic instability is expected, it is recommended to apply SIL-ALL FIREGUARD™ to wilted grass silage.

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Effect of silage additives on aerobic stability and fermentation quality of ryegrass silages at two harvesting times

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Keywords lactic acid bacteria, aerobic stability, silage management

Introduction During the past ten years it was observed, that the grass silages were increasingly reliable for aerobic instability and reheating in Germany. With this regard, Spiekers et al. (2009) indicated aerobic deterioration as a major source for dry matter (DM) losses. One reason might be the rising use of high sugar grass breeds based on perennial ryegrass varieties, which often resulted in residual contents of water soluble carbohydrates (WSCH) in the grass silages > 10 % of DM after several months of ensiling. On the other hand, strong technological progress for harvesting machines has generally increased the performance and thus reduced the filling time of the farm scaled bunker silos markedly. Unfortunately, compaction time of the harvested forage has not been synchronized to the harvest machinery performance resulting in lower densities especially in the silo upper layer leading to negative effects on aerobic stability (AS). Köhler and Spiekers (2012) analysed DM losses on farm scaled bunker silos of grass, maize and alfalfa silage and observed on average 9-12 % of DM losses for all investigated crops. DM losses can be reduced by improved silage making process and also by strategic use of silage additives (biological and/or chemical). Thereby, silage additive industry is looking for new possibilities in order to deal with low forage compaction densities in silos and grasses with high WSCH content. This study focusses on the potential of different biological and chemical silage additives to improve both AS and silage fermentation pattern of ryegrass silages of different harvesting times.

Materials and methods In 2014, two second cut grass varieties (mainly perennial ryegrass) were ensiled at the Chamber of Agriculture of Niedersachsen in triplicate laboratory silage minisilos. The two grass varieties differed in stage of vegetation during cutting (a) normal and b) over-mature, Table 1). Laboratory minisilos were analysed after 49 days of storage with air stress and low density (160 kg DM/m^3) and after 90 days of storage without air stress and high density (240 kg DM/m^3) at 20 °C. Silage additive variants were: I) untreated control, (II) chemical silage additive: 500 g Sodium benzoate + 285 g Potassium sorbate/ ton of fresh forage (FM)), (III) *Bonsilage Twin GS*: Four different homo- and heterofermentative lactic acid bacteria with the dosage of 2.5×10^5 cfu/ tons FM; IV) *Bonsilage Twin GF*: Three homo- and heterofermentative lactic acid bacteria with the dosage of 2.5×10^5 cfu/ tons FM). *Bonsilage Twin GS* is a new commercial product with mainly lactic fermentation pattern used in the under layer of the silo. *Bonsilage Twin GF* is a new commercial product with mainly acetic fermentation pattern, which is only used in the upper layer of the silo. Determination of plant nutrients, pH-value and fermentation pattern was carried out. Tests of AS were performed according to Honig (1990). They were operated by periodic temperature measurements for eight days of aerobic exposure. Both yeasts and moulds were counted according to VDLUFA method (III 28.1.2.) before and after the eight days of stability tests. Statistical analysis was performed using ANOVA with the fixed effects of treatment and harvesting time (SAS 9.4).

Results and discussion After 90 days of storage no differences were observed for

AS between control and silage additive variants. Significant differences ($p<0.05$) in fermentation pattern and AS occurred after 49 days of storage with air stress. For both ryegrass harvesting times by far the highest amount of acetic acid, 1,2-propandiol and sum of volatile fatty acids and alcohols were found for silage additive IV (mainly heterofermentative fermentation pattern) resulting to the longest AS and lowest count of yeasts and moulds before and after the AS test. Chemical treatment II also improved AS for both the young and over-mature ryegrass. The mainly homofermentative silage additive III even yielded the highest amounts of lactic acid and lowest amount of acetic acid, resulting to the lowest AS for the young grass and only a marginal better AS for the over mature grass.

Table 1 Forage quality parameters of the different harvesting times young and over mature

Harvesting time	DM %	Sugar (DM, %)	ADF_{org} (DM, %)	LAB (log cfu/g FM)	Yeasts (log cfu/g FM)	Moulds (log cfu/g FM)
Young	41.8	16.8	27.5	6.3	5.2	2
Over mature	42.7	8.1	35.5	8.8	6.3	3

LAB= Lactic acid bacteria

Table 2 Silage quality parameters for the different silage additive treatments I to IV and for the different harvesting times young (a) and over-mature (b) after 49 days of storage

Parameter	Treatment/Harvesting time							
	I		II		III		IV	
	a	b	a	b	a	b	a	b
DM_c (%)	41.6	44.0	41.8	46.7	41.7	46.4	43.2	44.7
LA (% DM)	4.5	3.7	5.2	3.3	6.0*	5.5*	5.4	4.1
AA (% DM)	1.7	0.9	2.0	1.0	0.4*	0.8*	5.7*	2.5*
PD (% DM)	0.02	0.02	0.07	0.02	0.05	0.11	4.3*	1.5*
SAA (% DM)	6.5	5.1	7.5	4.6	7.1	6.8	18.8*	8.6*
pH	3.88	4.24	3.88	4.24	3.89*	4.02*	3.84	4.17
AS (days)	8.0	1.2	8.0	2.6*	1.0*	2.1*	8.0	7.0*
YnA (log cfu/ g FM)	4.0	n.n.	3.6	n.n.	9.8	n.n.	2.0*	n.n.
MnA (log cfu/ g FM)	3.6	n.n.	3.4	n.n.	5.5	n.n.	3.0	n.n.

DM_c = DM corrected, LA=Lactic acid; AA= Acetic acid; PD= 1,2-Propandiol; SAA= Sum of volatile fatty acids and alcohols; AS= Aerobic stability; YnA= Yeasts after Aerobic Stability Test; MnA Moulds after Aerobic Stability Test; n.n.=not analysed; * symbolize significant differences to control treatment I ($p < 0.05$)

Conclusions The results of the present study indicated a clear effect of storage length, air stress during storage and density for AS. Furthermore differences between silage additives were observed. Ryegrass with high WSCH content is most suitable for a treatment with a mainly heterofermentative LAB mixture. In order to reduce DM losses due to reheating a heterofermentative LAB mixture is a better strategy to use as silage additive in farm scaled bunker silos compared to homofermentative LAB mixture especially in the upper layer of the silo.

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Combining the beneficial effects of homo- and heterolactic bacteria

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Keywords grass silage, silage additives, lactic acid bacteria, fermentation quality, aerobic stability, fermentation losses, proteolysis

Introduction Homolactic bacteria are well known to enhance fermentation quality of grass silage with a faster decline of pH-values, higher concentrations of lactic acid, reduced concentrations of acetic acid and reduced proteolysis resulting in lower concentrations of NH_3-N (Kung et al, 2003). Heterolactic bacteria increase the aerobic stability of silages but this beneficial effect often is accompanied by a more poor fermentation quality like increased NH_3-N concentrations and higher fermentation losses (Kung et al, 2001). The aim of this study was to determine the right ratio between two commercial bacterial inoculants to combine the beneficial effects of both: increased fermentation quality of the homolactic product (LAB-ho) without a decrease of aerobic stability due to the influence of the heterolactic product (LAB-he).

Materials and methods Ryegrass pasture (*Lolium perenne*, ssp. "Aberavon") was used for this trial. Fresh grass was chopped, wilted and immediately after applying the treatments ensiled in 1.5 L glass jars and stored for a 90 days period at 25°C according DLGregulations (DLG, 2013). The following fermentation parameters were determined: lactic-, acetic- butyric- and propionic acids, ethanol, 1,2-propanediol and ammonia-N. Additional glass jars were filled to measure the aerobic stability after 49 days of storage with twice aerobic stress (DLG, 2013). Aerobic stability was tested too after the 90 day storage period. In the first trial (1st cut material) ratios between LAB-he (*Lactobacillus buchneri*; DSM 13573) and LAB-ho (*Lactobacillus plantarum*; DSM 8866; DSM 8862) of a: 2/3 vs. 1/3 and b: 3/4 vs. 1/4 (Colony forming units (CFU): 66.667 vs. 100.000 and 75.000 vs. 75.000) were tested. Application rate was in total 1 gram of these mixtures per ton fresh material (FM) as both commercial products were applied at this dosage. In the second trial (3rd cut material) the ratio was changed to 1 g LAB-he and 0.1 g LAB-ho/t FM (CFU: 100.000 vs. 30.000) and 1 g LAB-he and 0.2 g LAB-ho/t FM (CFU: 100.000 vs. 60.000). In all trials each treatment was done in three repetitions. Mixtures have been tested in both trials versus untreated control and pure LAB-ho (1 g/t FM; 300.000 CFU /g FM).

Results and discussion Composition of the fresh materials is shown in Table 1. Table 2 shows the fermentation parameters of the silages after a storage period of 90 days and the test results for aerobic stability after 49 and 90 days of storage. Except the untreated control silages of trial 1 all silages have been free of butyric acid. Compared to untreated control silages all treatments showed higher concentrations of lactic acid, reduced acetic acid, lower concentrations of NH_3-N and in sum reduced fermentation losses. As shown in Table 2 silages treated with mixtures of LAB-he and LAB-ho showed comparable fermentation properties like pure LAB-ho treatments but, differing to them, a minor decrease of aerobic stabilities was determined.

Table 1 Composition of the fresh material

	Trial 1	Trial 2
Dry matter (g/kg FM)	341	413
Ash (g/kg DM)	96	81
Crude protein (g/kg DM)	140	119
Crude fiber (g/kg DM)	216	224
Water soluble carbohydrates (g/kg DM)	190	207
Buffering capacity (g lactic acid /100g DM)	8,7	4,6
Fermentation coefficient (FC)	52	77

Table 2 Organic acids, alcohols, ammonia-N concentrations, pH-values, aerobic stability and fermentation losses of the fermented material (n=3)

	Trial 1				Trial 2			
Treatment	Control	100 % LAB-ho	LAB-he / LAB-ho 2/3 / 1/3	LAB-he / LAB-h 3/4 / 1/4	Control	100 % LAB-ho	LAB-he / LAB-ho 1g+0.1g /t FM	LAB-he / LAB-ho 1g+0.2g /t FM
Lactic acid (g/kg DM)	43	85	81	85	61	76	75	71
Acetic acid (g/kg DM)	16	8	12	12	32	5	12	10
Propionic acid (g/kg DM)	0	0	0	0	0	0	0	0
Butyric acid (g/kg DM)	7	0	0	0	0	0	0	0
Ethanol (g/kg DM)	17	13	11	12	7	18	4	10
1,2-Propanediol (g/kg DM)	3	0	0	0	13	0	0	0
NH_3-N (% of total N)	7.5	4.9	5.1	5.2	6.6	4.5	5.1	4.9
pH 90 days	4.5	4.1	4.1	4.1	4.5	4.2	4.2	4.3
Aerobic stability (days), 49 days	5.8	1.2	1.8	2.1	18.4	1.9	8.6	3.2
Aerobic stability, (days) 90 days	16.5	5.1	8.2	8.7	18.5	5.2	17.0	10.8
DM losses during ferment.* (%)	7.6	5.2	5.3	5.5	6.5	5.4	4.4	5.0

*Weissbach (1998)

Conclusions Applied mixtures of hetero- and homolactic bacteria lead to comparable silage qualities like silages treated with pure LAB-ho. With increased numbers of LAB-ho in mixtures the fermentation effects become more alike the effects of pure LAB-ho treatment. Compared with 100% LAB-ho these mixtures produced nearly identical fermentation qualities with a distinctly minor decrease of aerobic stabilities than 100% LAB-ho.

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The effect of a multi strain and enzyme silage inoculant on fermentation characteristics and aerobic stability of grass silage

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Keywords *L. plantarum, P. pentosaceus, P. acidipropionici, P. acidilactici,* ryegrass, silage

Introduction 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 objective of the trial was to determine the effect of a commercial multi strain silage inoculant on the DM loss, fermentation characteristics and aerobic stability (AS) of wilted grass silage.

Materials and methods The trial consisted of 2 groups: Control (C) (no additive) and Treatment (T) (Sil-All 4x4 + WS, Danstar Ferment, Switzerland). The inoculant contains 4 bacteria and 4 enzymes (*Lactobacillus plantarum* CNCM I-3235 (500 000 CFU/ g fresh forage), *Pediococcus pentosaceus* NCIMB 12455 (200 000 CFU/ g fresh forage), *Propionibacterium acidipropionici* CNCM MA/26 4U (200 000 CFU/ g fresh forage), *Pediococcus acidilactici* CNCM I-3237 (100 000 CFU/ g fresh forage), α-amylase , β-glucanase, cellulase and glucanase (enzymes included at EU 1831/2003 efficacy application rate) . A third cut grass mixture (mainly ryegrass) was cut and wilted on field (48 h) (Table 1). The chopped herbage (4-6 cm) was sprayed with water or inoculant solution and packed in 12 L mini silos (4 silos/treatment). The silos were opened after 90 days and analysed for nutritional value (DM, Ash, CP, NDF, ADF, WSC, Soluble-N, *in vitro* DM digestibility), fermentation parameters (pH, N-NH_3, organic acids, ethanol) and microbial counts [lactic acid bacteria (LAB), yeasts, moulds] and DM losses were calculated. Metabolizable energy (ME) was also estimated (INRA, 2007). The AS was assessed by Honig method (1990) (7-day test at +22˚C). A rise of ≥ 3˚C above room temperature was taken as an indicator of instability. After the stability test pH, DM and microbial counts were measured. Data were subjected to one-way analysis of variance [GLM procedure in SPSS (v. 22.0)]. Significances were declared at $P<0.05$.

Table 1 Chemical composition of wilted grass

DM	Ash	CP	NDF	ADF	WSC	Soluble-N	dDM [1]	LAB	Yeast	Molds
g/kg	g/kg DM							log_{10} CFU/g FM		
624	88.6	145	570	330	89.4	6.6	813	6.22	6.55	5.18

[1] *In vitro* dry matter digestibility

Results and discussion The nutritional value of both silages is shown in Table 2. The inoculant had no effect on the chemical composition of grass silages. Fermentation profile (Table 3) shows significantly decreased pH for T compared to C ($P< 0.05$). Ethanol content was also significantly lower in T than in C ($P<0.05$), which is in line with the lower yeast count for T (3.31 vs. 3.64 log CFU/g silage, $P<0.05$). The inoculant decreased the lactic acid content, but the lactic acid:acetic acid ratio tended to be improved (116 vs 46; $P<0.1$).

None of silages contained butyric acid. The DM losses during ensiling were 9% lower for the T group compared to C. To simulate what these results could represent on farm scale, the obtained data were entered in the Milk 2000 spreadsheet developed by university of Wisconsin (Milk 2000 version 7.54). A 1.1% increase in yield (milk/acre) would be obtained according to this simulation. All silages were aerobically stable as no heating occurred during the 7 days. However, pH at the end of AS test was significantly lower for T compared to C (4.28 vs. 5.05, P<0.05).

Table 2 Nutritional value of control and treatment silage after 90 d of ensiling

Item	C	T	SEM
Dry matter, g/kg	582 [a]	587 [b]	1.3
Ash, g/kg DM	93.1	91.4	1.0
Crude protein, g/kg DM	137	136	1.3
Soluble-N, g/kg DM	8.8	8.2	0.4
NDF, g/kg DM	560	561	2.7
ADF, g/kg DM	345 [a]	332 [b]	3.2
WSC, g/kg DM	63.0 [a]	53.9 [b]	1.2
dDM, g/kg DM	795 [a]	785 [b]	4.6
ME, MJ/kg DM	2315	2360	

[a–b] Means within row with different superscripts differ ($P < 0.05$).

Table 3 Fermentation characteristics of silage after 90 d of ensiling

Item	C	T	SEM
pH	4.90 [a]	4.29 [b]	0.05
N-NH_3, g/kg N	31.8	34.6	1.7
Lactic acid, g/kg DM	27.0 [a]	19.7 [b]	0.4
Acetic acid, g/kg DM	0.9	0.2	0.2
Ethanol, g/kg DM	6.7 [a]	3.1 [b]	0.4
DM losses, g/kg DM	67.2	61.1	2.6

[a–b] Means within row with different superscripts differ ($P < 0.05$).

Conclusions The multi strain and enzyme silage additive improved the fermentation characteristics (P<0.05) and reduced DM losses (P<0.05) of wilted grass silage.

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Chemical composition of elephant grass silage with different levels of soybean molasses and enzyme-microbial inoculant

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Keywords dry matter, ensilage, forage conservation, tropical forage

Introduction Forage production seasonality is one of the serious obstacles in the Brazilian livestock, which causes fluctuations in the animal production. The cultivation of forage for cutting, grass stocking piles and silage are alternatives to reduce the food shortage problem in the scarcity period. Among the plant species, elephant grass *(Pennisetum purpureum* Shum.) stands out due to its high yield and good acceptance by the animals. In this context, the aim of this study was aimed to evaluate the chemical composition of silage from elephant grass with soy molasses and enzyme-microbial inoculant addition.

Materials and methods The experiment was conducted in UFMT/Sinop campus in partnership with Embrapa Agrosylvipastoral. Elephant grass (*Pennisetum purpureum*) cv. Roxo was ground and ensiled in 30 PVC silos, with diameter of 0.1 m and 0.35 m high, with a volume of 2,75 liters, provided with Bunsen valves. We used a completely randomized design, with three replicates per treatment, with or without addition of enzyme-microbial inoculant, associated with 5 soy molasses levels (0, 4, 8, 12 e 16% in the fresh matter). The enzyme-microbial inoculant Sil All C4 contained homofermentative bacteria (*Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactobacillus salivarius*) and heterofermentative bacteria (*Enterococcus faecium*), as well as enzymes (amylase, cellulase, xylanase and hemicellulase). Soy molasses (SM) contained 79.62% dry matter (DM); 86.87% organic matter (OM); 0.59% ether extract (EE); 10.69% crude protein (CP). Samples were predried in an oven, with forced air ventilation at 55 ºC and ground to a 1.0 mm diameter. The MS analyzes were determined by AOAC (1990) method. The crude protein (CP) was obtained by determining total nitrogen, according to the micro-Kjeldahl method. The ether extract (EE) was obtained by the ANKOM XT15 method (AOCS official procedure Am 5-04). The fiber analysis in neutral detergent fiber (NDF) and acid detergent fiber (ADF) were performed according to Van Soest and Robertson (1985), while the hemicellulose levels were calculated by the difference between the NDF and ADF. The effect of the addition or not of enzyme-microbial inoculant within each soy molasses' inclusion level was evaluated using Tukey's test. Evaluation of the effect of soy molasses' inclusion levels, with and without the addition of enzyme-microbial inoculant, was adjusted by the linear, quadratic and cubic regression models, considering 0.05 significance level for type I error.

Results and discussion There was no interaction between enzyme-microbial inoculant and soy molasses levels (Table 1). The inclusion or not of enzyme-microbial inoculant did not affect levels of DM, NDF, ADF, hemicellulose or EE. Inoculation increased the CP content,

as the inoculant provides fermentation improvements, with a quick drop in pH and lower proteolysis, preventing nitrogen losses. The DM content in creased linearly according to the addition of SM (P<0.05), quantitatively an increase of 0.45 % for each 1% of SM added.

Table 1 Effect of the addition or not of enzyme-microbial inoculant and of the inclusion levels of soy molasses over the chemical composition of silage from elephant grass

	Inoculant		*P*-value	Soy Molasses levels					*P*-value	SEM[2]	Int[3]
	Without	With		0%	4%	8%	12%	16%			
DM (%)	19.39a	19.66a	0.1495	15.98	17.71	19.13	21.55	23.26	<0.0001	0.50	0.1142
OM[1]	89.42a	88.96b	<0.0001	88.47	89.17	89.55	89.45	89.32	<0.0001	0.32	0.1324
CP[1]	9.74b	10.18a	0.0018	9.32	9.61	9.87	10.35	10.65	<0.0001	0.42	0.1927
EE[1]	4.20a	4.02a	0.6792	3.22	3.76	4.05	4.03	5.49	0.0206	1.74	0.9836
NDF[1]	49.70a	49.58a	0.8728	60.94	54.04	47.77	44.35	41.04	<0.0001	1.12	0.3714
ADF[1]	30.13a	29.59a	0.3800	37.58	32.99	28.64	27.42	22.65	<0.0001	0.97	0.5065
HEM[1]	18.98a	19.83a	0.3461	23.36	21.04	19.13	16.93	16.57	0.0005	1.11	0.8211

[1] % DM; [2] standard error mean; [3] Interaction between variables; Means in the same row, followed by lowercase letters did not statistically differ, according to Tukey's Test with 5% probability for Type I error I.. $\hat{Y}_{DM} = 15.84 + 0.4554*SML$ ($r^2 = 96.75$); $\hat{Y}_{OM} = 88.55 + 0.1938*SML - 0.0094*SML^2$ ($R^2 = 59.00$); $\hat{Y}_{CP} = 9.28 + 0.0813*SML$ ($r^2 = 63.87$); $\hat{Y}_{EE} = 3.15 + 0.1264*SML$ ($r^2 = 39.46$); $\hat{Y}_{NDF} = 59.53 - 1.1274*SML$ ($r^2 = 89.98$); $\hat{Y}_{ADF} = 36.94 - 0.8855*SML$ ($r^2 = 89.92$); $\hat{Y}_{HEM} = 23.02 - 0.4620*SML$ ($r^2 = 61.93$)

Addition of soy molasses had a quadratic effect on OM content, with a maximum content of 89.5% at 10.31% SM level. However, the decrease with the inoculation can be understood as an increase in bacterial population, and the consequent increase in consumption of soluble carbohydrates, which is part of the OM. The CP levels linearly raised (P<0.05) according to the increasing concentration of SM, which is explained by the CP content of the SM, changing positively the chemical composition of silage. The values of NDF, ADF and hemicellulose linearly decreased (P<0.05) according to the increased SM concentration, due to the low content of fibrous compounds. Gradual inclusion of SM linearly increased the EE content (P<0.05) of the silage, possibly because the EE content of the additive is higher than the EE grass content, which may interfere with the animal consumption at the highest level of SM inclusion.

Conclusion It is recommended the inclusion of 4% soy molasses (% natural material) of elephant grass silage for improved chemical composition of the silage, with increasing dry matter and crude protein.

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Fermentative profile of elephant grass silage with different levels of soy molasses and enzyme-microbial inoculant

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Keyworks dry matter, ensilage, forage conservation, pH

Introduction Forage production seasonality is one of the serious obstacles in the Brazilian livestock, which causes fluctuations in the animal production. Among the plant species, elephant grass *(Pennisetum purpureum* Shum.) stands out due to its high yield and good acceptance by the animals. However, the silage from unconventional grasses generally have low nutritional value, as they have low dry matter content, high buffering capacity and low soluble carbohydrates content, compromising the conservation process. In this context, the objective was to evaluate the fermentation profile of silage from elephant grass with soy molasses inclusion and enzyme-microbial inoculant.

Materials and methods The experiment was conducted in UFMT/Sinop campus in partnership with Embrapa Agrosylvipastoral. Elephant grass (*Pennisetum purpureum*) cv. Roxo was ground and ensiled in 30 PVC silos, with diameter of 0.1 m and 0.35 m high, with a volume of 2.75 liters, provided with Bunsen valves. We used a completely randomized design, with three replicates per treatment, with or without addition of enzyme-microbial inoculant, associated with five soy molasses levels (0, 4, 8, 12 e 16% in the fresh matter). The enzyme-microbial inoculant Sil All C4 contained homofermentative bacteria (*Lactobacillus plantarum*, *Pediococcus acidilactici* and *Lactobacillus salivarius*) and heterofermentative bacteria (*Enterococcus faecium*), as well as enzymes (amylase, cellulase, xylanase and hemicellulase). Soy molasses (SM) contained 79.62% dry matter (DM); 86.87% organic matter (OM); 0.59% ether extract (EE); 10.69% crude protein (CP). We evaluated titratable acidity (TAC) and the pH levels, according to methodologies from Silva & Queiroz (2002). The evaluation of ammoniacal nitrogen (NNH_3) was performed by the method proposed by Chaney and Marbach (1962), sampling silage diluted in water and trichloroacetic acid (10%). Soluble carbohydrates (SC) determinations were performed by the technique described by Johnson et al. (1966). The effect of the addition or not of enzyme-microbial inoculant within each soy molasses' inclusion level was evaluated using Tukey's test. Evaluation of the effect of soy molasses' inclusion levels, with and without the addition of enzyme-microbial inoculant, was adjusted by the linear, quadratic and cubic regression models, considering 0.05 significance level for type I error.

Results and discussion There was an interaction effect (Table 1) for pH, TAC, NNH_3 and SC. The DM content increased linearly according to the addition of SM ($P<0.05$), quantitatively an increase of 0.45 % for each 1% of SM added. The pH ($P> 0.05$), by the use or not of inoculant, and pH increased linearly ($P<0.05$) with the inclusion of SM, with a value of 3.85 with 16% of MLS. For all treatments, pH was within the optimal range for

silage preservation, which is between 3.8 and 4.2 (McDonald et al., 1991), and despite the reduced DM content of the material and low osmotic potential, the SC content ensured the rapid drop of pH, with substrate availability for the fermenting bacteria.

Table 1 Effect of the addition or not of enzyme-microbial inoculant and of the inclusion levels of soy molasses on silage from elephant grass

	Inoculant		*P*-value	Soy Molasses Levels					*P*-value	SEM[4]	Int[5]
	Without	With		0%	4%	8%	12%	16%			
DM (%)	19.39a	19.66a	0.1495	15.98	17.71	19.13	21.55	23.26	<0.0001	0.50	0.1142
pH	3.83	3.84	0.2997	3.80	3.79	3.87	3.87	3.85	<0.0001	0.11	0.0082
TAC[1]	19.63	18.61	0.0195	14.03	17.50	18.63	21.23	24.21	<0.0001	0.77	0.0278
NNH_3[2]	8.49	9.10	0.0634	7.16	8.77	9.04	10.13	8.88	0.0004	0.67	0.0005
SC[3]	14.75	11.72	0.0079	2.82	8.65	16.19	20.16	18.38	<0.0001	1.21	0.0215

Interaction effects between the levels of soy molasses and inoculant.

	Inoc[6]	Soy molasses levels					Model
		0%	4%	8%	12%	16%	
pH	Without	3.76a	3.81a	3.87a	3.85a	3.84a	$\hat{Y}_{pH} = 3.79 + 0.0050*SLM$ ($r^2 = 41.56$)
	With*	3.83a	3.77a	3.86a	3.89a	3.85a	$\hat{Y}_{pH} = 3.84$
TAC [1]	Without	14.36a	17.66a	19.20a	23.27a	23.67a	$\hat{Y}_{TAC} = 14.79 + 0.6050*SLM$ (r^2=88.78)
	With	13.70a	17.33a	18.06a	19.20b	24.76a	$\hat{Y}_{TAC} = 13.81 + 0.6377*SLM$ (r^2=2.07)
NNH_3[2]	Without	6.93a	7.40b	7.74b	10.92a	9.43a	$\hat{Y}_{N\text{-}NH3} = 6.78 + 0.2127*$ SLM (r^2 =4.60)
	With	7.39a	10.12a	10.33a	9.33a	8.32a	$\hat{Y}_{N\text{-}NH3}$=7.58+0.69*SML+0.0407*SML^2 (R^2=78.10)
SC[3]	Without	2.96a	9.16a	15.31a	25.57a	20.77a	$\hat{Y}_{CHOS} = 4.35 + 1.30*SML$ ($r^2 = 76.46$)
	With	2.67a	8.14a	17.06 a	14.75b	15.99a	$\hat{Y}_{CHOS} = 5.08 + 0.8374*SML$ (r^2=62.21)

[1] Expressed in mL of 0.1N NaOH until pH reached 7.0; [2] % total nitrogen; [3] % DM; [4] standard error mean; [5] interaction between variables; [6] inoculant. Averages in the same row followed by same lowercase letters do not differ according to Tukey's test at 5% probability for type I error. * No model adjusted to the fermentation period.

The TAC values increased linearly with the inclusion levels of SM, with or without inoculant. The concentration of NNH_3 in silage, without the use of inoculant, increased linearly, however, with inoculant, the SML had a quadratic effect of NNH_3 concentrations, achieving a maximum at the level of 8.48% SML and a value of 10.50% NNH_3, which is considered ideal for good quality silage. Regardless the use of inoculant, the SC levels increased linearly with the SM inclusion levels, mainly due to the chemical composition of SM, which is high in soluble carbohydrates.

Conclusion It is recommended the inclusion of 4% soy molasses (% natural material) of elephant grass silage for improved the fermentation of elephant grass silage, with potential use in animal feed.

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How fibrolytic enzyme additives applied during ensiling influence the fibre, preservation and digestibility of two grass species at two harvest dates in the primary growth

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Keywords silage, fibrolytic enzyme additives, fibre, digestibility, fermentation characteristics

Introduction Slow or restricted degradation of plant cell walls limits the productivity of grassland biomass used as a renewable energy source. Although fibre may have nutrition and health benefits for ruminant animals, in anaerobic digestion (AD) the cell wall complex is a source of energy that is not sufficiently exploited. During ensiling of grass biomass there is an opportunity to upgrade the silage feedstock by initiating the breakdown of cell wall constituents with fibrolytic enzymes. Thus, the objective of this experiment was to investigate the effect of an array of fibrolytic enzyme additives on the fibre, ensiling characteristics and digestibility of two contrasting grassland species at two harvest dates, in the primary growth.

Materials and methods Two grassland species (perennial ryegrass (PRG) and timothy (TIM)) were each grown in four field plots. These were harvested at two dates in the primary growth (Harvest 1 = 28th May and Harvest 2 = 18th June), precision chopped and sub-samples (nine units of 6 kg per plot) were randomly assigned to nine treatments prior to ensiling in laboratory silos for 120 days. Treatments included four enzyme additives at two dose levels as follows: (1) control (i.e. no enzyme additive) (2) phytase low (3) phytase high (4) cellulase-a low (5) cellulase-a high (6) cellulase-b low (7) cellulase-b high (8) xylanase low (9) xylanase high. On average, enzyme additives were applied at 0.5 ml kg^{-1} and 5 ml kg^{-1} of fresh herbage for low and high doses, respectively. Representative silage samples were taken for chemical analysis and data were analysed as a split-split-split plot design using the mixed procedure in SAS v. 9.3.

Results and discussion Results are presented in Table 1. Interactions that were not significant were omitted. With later harvest date, dry matter (DM) and neutral detergent fibre (NDF) contents increased while digestibility (DMD) decreased (PRG only). All silages underwent a successful preservation except for Harvest 1 of timothy. The high ammonia-N content of this silage indicates that a secondary fermentation took place. Secondary fermentations can have a negative effect on the total net energy output due to greater dry matter losses (McEniry et al., 2014; Flynn, 1981). In comparison to control silages, a significant reduction in the NDF content was observed with cellulase-b and xylanase additives. The higher enzyme dose had a greater effect than the lower dose for DM, NDF and lactic acid. Based on these results enzyme additives with cellulase-b and/or xylanase activity, applied at higher doses, were most effective at initiating cell wall degradation within the silo prior to anaerobic digestion or ingestion by ruminants.

Conclusions Additives such as cellulase-b and xylanase activity were observed to be the most promising treatments NDF without adversely affecting successful preservation of

grass silage.

Table 1 Effect of harvest, species, enzyme and dose on silage chemical composition

Harvest	Species	Enzyme	Dose	DM (g/kg)	DMD (g/kg DM)	NDF (g/kg DM)	pH	Lactic acid (g/kg DM)	Ammonia-N (g/kg N)
1	PRG	Control		160	743	554	4.10	77	68
1	PRG	Phytase	low	175	736	540	4.25	87	69
1	PRG	Cellulase a	low	171	733	542	3.82	74	79
1	PRG	Cellulase b	low	175	744	529	3.67	125	68
1	PRG	Xylanase	low	178	713	527	3.75	131	73
1	PRG	Phytase	high	174	722	521	4.14	125	70
1	PRG	Cellulase a	high	175	738	539	4.23	105	71
1	PRG	Cellulase b	high	181	722	500	3.76	159	62
1	PRG	Xylanase	high	193	721	474	3.57	156	65
1	TIM	Control		161	685	649	4.78	16	94
1	TIM	Phytase	low	167	683	649	4.62	11	88
1	TIM	Cellulase a	low	167	693	652	4.69	18	82
1	TIM	Cellulase b	low	165	704	623	4.36	27	69
1	TIM	Xylanase	low	177	691	602	4.20	31	78
1	TIM	Phytase	high	174	685	626	4.57	22	62
1	TIM	Cellulase a	high	165	693	638	4.68	19	77
1	TIM	Cellulase b	high	174	691	599	4.37	30	78
1	TIM	Xylanase	high	187	697	555	4.07	68	68
2	PRG	Control		187	646	622	3.62	149	56
2	PRG	Phytase	low	180	643	661	3.54	156	55
2	PRG	Cellulase a	low	190	659	602	3.56	143	46
2	PRG	Cellulase b	low	180	645	600	3.50	157	48
2	PRG	Xylanase	low	191	661	585	3.37	188	41
2	PRG	Phytase	high	190	661	629	3.41	163	50
2	PRG	Cellulase a	high	191	668	603	3.47	155	60
2	PRG	Cellulase b	high	190	646	573	3.33	194	40
2	PRG	Xylanase	high	208	620	555	3.28	177	42
2	TIM	Control		220	694	724	3.58	127	63
2	TIM	Phytase	low	222	643	764	3.56	134	57
2	TIM	Cellulase a	low	209	600	679	3.47	163	66
2	TIM	Cellulase b	low	222	626	728	3.53	125	54
2	TIM	Xylanase	low	229	605	631	3.41	150	51
2	TIM	Phytase	high	232	639	656	3.52	145	62
2	TIM	Cellulase a	high	216	658	687	3.58	122	54
2	TIM	Cellulase b	high	243	621	647	3.46	125	52
2	TIM	Xylanase	high	234	631	630	3.33	164	58
Standard error of the mean									
Harvest (H)				2.6	1.5	9.9	0.02	8.1	7.4
Species (S)				2.3	2.4	13.1	0.04	7.1	10.8
Enzyme (E)				2.6	4.6	12.9	0.04	7.4	8.2
Dose (D)				2.0	2.5	8.9	0.02	6.2	6.1
H x S				3.2	3.3	18.5	0.06	10.0	15.2
H x E				3.7	6.5	12.2	0.06	10.5	11.8
Level of significance									
Harvest (H)				**	***	**	***	**	*
Species (S)				**	**	**	*	***	NS
Enzyme (E)				***	*	***	***	***	***
Dose (D)				***	NS	**	NS	*	NS
H x S				***	**	NS	*	**	NS
H x E				NS	NS	NS	*	NS	*

Harvest, 1 = 28th May. Harvest 2 = 18th June. PRG, perennial ryegrass. TIM, timothy. DM, dry matter. DMD, dry matter digestibility. NDF, neutral detergent fibre. * = P<0.05, ** = P<0.01, *** = P<0.001, NS = not significant.

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Ryegrass and elephant grass ensiled with chemical and microbial additives

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Keywords aerobic stability, chemical composition, fermentation losses, gases, LAB

Introduction The fermentation of tropical and temperate grasses is not as intense as that observed in corn or sugarcane silages. In this study we aimed to evaluate two inoculants, being one associated to a chemical component, as additives for ryegrass (RG) and elephant grass (EG) silages.

Materials and methods Two trials were separately carried on: elephant grass (2014, feb-apr) and ryegrass (2014, sept-nov). Fresh forages were pre-wilted for 24 and 72 h, respectively. The EG was chopped by a pull-type harvester, and RG was ensiled without chopping. For each trial, three treatments with five replicates were applied: control – no additives (**C**), Sil-All 4x4 (**S**) or Sil-All Fireguard (**F**). Additive compositions were: S – *Lactobacillus plantarum*, *L. salivarius*, *Pediococcus acidilactici*, *Enterococcus faecium*, amylase, cellulase, hemicellulase and xylanase (2×10^5 CFU g^{-1}); F – *L. plantarum*, *P. acidilactici*, *P. pentosaceus*, sodium benzoate, amylase, xylanase and β-glucanase (1×10^5 CFU g^{-1}). All treatments were sprayed onto the forage diluted in non-chlorinated water (2 L t^{-1}). The lab silos were made by PVC pipes (15 cm diameter × 50 cm height). A 2-cm height plastic platform was placed in the bottom of each silo to assess effluent losses (EL). Gas production (GP) was measured daily during all of the storage period (65 and 62 d for EG and RG, respectively) through a graduate chamber immerged in water, connected to the silo by a hose and a 3-way valve. The valve was used to empty the chamber as needed. Silos were weighted before and after fermentation to assess gravimetric DM losses (DML) and gas losses (GL) according to Jobim et al. (2007). Samples of forages and silages were taken for determining pH (25 g silage and 225 mL distilled water, homogenized by 1 minute) and DM content (65 °C for 72 h plus 105 °C for 12 h). Lactic acid bacteria (LAB), yeasts and molds counts were done in silage water extract (25 g silage and 225 mL distilled water, blended for 1 minute, filtered in gauze and paper filter) sequentially diluted and plated in Petrifilm (AC and YM, 3M, USA). Ash content was determined at 600°C for 3 hours. Neutral and acid detergent fiber contents (NDF and ADF, respectively) were determined by sequential ANKOM methodology. Crude protein (CP) was determined by Dumas method (CP = total nitrogen x 6.25). For assessing aerobic stability (AS), silage from each silo was kept in open buckets in a controlled temperature room (EG: 3 kg for 120 h; RG: 2 kg for 140 h). Data-loggers were placed in the center of the mass, recording silage temperature every five minutes. Aerobic stability was regarded as the time for silage to reach 2°C above room temperature. Statistical analyses were performed separately for each trial, using SAS 9.3, as completely randomized designs with 3 treatment and 5 replicates. Normality was assessed through Shapiro-Wilk test. Variance homogeneity was evaluated and corrected through variable transformation, if necessary, by Box-Cox methodology. Data were submitted to analysis of variance and means were compared by Tukey test ($P<0.05$).

Results and discussion Forage DM and pH after wilting were 22.8 ± 0.43% and 6.2 ± 0.02 for EG and 28.9 ± 0.50 and 7.6 ± 0.16 for RG. Bulk density and results for silage variables are presented in Table 1. Regarding microbial counts, replicates with null count for all dilutions were assumed to contain half of the minimum detectable concentration. Aerobic stability of the replicates which did not reach 2°C above ambient temperature during aerobic exposure period was considered 120 and 140 h for EG and RG, respectively. Both additives improved AS and reduced yeasts population of EG silages, but did not affect RG silages so markedly. The GP was decreased by both additives in RG silages, but only by F in EG silages. Other variables were not affected in EG silages. Improvement on silage quality and fermentation was evident in RG silages: the S treatment decreased silage pH, which indicates favored homolactic fermentation. The reduction in LAB count in S treatment might be due to competition with epiphytic microbiota. The F treatment reduced the DML and GL when compared to C, probably due to inhibitory effect of sodium benzoate on yeasts. Both additives reduced NDF and ADF content in RG silages compared to control silages. When reducing losses, the additives probably conserved soluble carbohydrates, proportionally reducing fiber and protein contents. Overall, microbial additives can improve grass silage fermentation leading to better nutritive values of silages.

Conclusions Both additives were capable of reducing fermentative DM losses and gas production during fermentation and improve the aerobic stability of grass silages.

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Table 1 Elephant grass and ryegrass silages treated with Sil-All 4x4 (S) and Sil-All Fireguard (F)

	Elephant grass				Ryegrass			
Variables[1]	C	S	F	SEM[2]	C	S	F	SEM
Density, kg m^{-3}	524.0^{A}	527.5^{A}	531.2^{A}	10.2	445.6^{a}	468.5^{a}	466.38^{a}	12.62
pH	4.35^{A}	4.34^{A}	4.31^{A}	0.02	4.64^{a}	4.22^{b}	4.63^{a}	0.06
DM, %	22.46^{A}	22.68^{A}	22.60^{A}	0.11	27.12^{a}	28.56^{a}	28.75^{a}	0.31
DML, % DM	1.28^{A}	0.40^{A}	0.72^{A}	0.46	6.66^{a}	3.73^{ab}	0.25^{b}	1.12
GL, % DM	1.15^{A}	0.25^{A}	0.59^{A}	0.46	6.59^{a}	3.69^{ab}	0.23^{b}	1.11
EL, kg t^{-1}	1.34^{A}	1.55^{A}	1.35^{A}	0.12	0.77^{a}	0.49^{a}	0.22^{a}	0.16
Gp, L kg^{-1} DM	5.87^{A}	4.91^{AB}	4.14^{B}	0.26	9.14^{a}	3.85^{b}	4.72^{b}	0.79
LAB, log cfu g^{-1}	7.55^{A}	7.49^{A}	7.67^{A}	0.29	8.09^{a}	7.31^{b}	7.97^{a}	0.10
YEA, log cfu g^{-1}	3.35^{A}	1.76^{B}	1.82^{B}	0.32	3.97^{a}	3.00^{a}	3.42^{a}	0.29
MOL, log cfu g^{-1}	1.76^{A}	1.70^{A}	1.70^{A}	0.02	2.28^{a}	2.20^{a}	2.24^{a}	0.15
Ashes, % DM	11.08^{A}	11.15^{A}	11.14^{A}	0.05	10.68^{a}	10.00^{b}	10.34^{ab}	0.09
NDF, % DM	69.12^{A}	68.31^{A}	68.8^{A}	0.22	48.16^{a}	46.06^{b}	45.52^{b}	0.38
ADF, % DM	42.26^{A}	41.52^{A}	41.23^{A}	0.25	29.03^{a}	27.40^{b}	27.27^{b}	0.26
CP, % DM	7.61^{A}	7.65^{A}	7.20^{A}	0.13	19.80^{a}	19.54^{ab}	19.08^{b}	0.11
AS, hours	78.3^{B}	120.0^{A}	120.0^{A}	7.8	67.2^{a}	123.7^{a}	105.8^{a}	11.1

Means followed by different superscript within each forage, in the same row, differ by Tukey test ($P<0.05$).
[1]DM = dry matter; DML = DM losses; GL = gas losses; EL = effluent losses; Gp = gas production; LAB = lactic acid bacteria; YEA = yeasts; MOL = molds; NDF and ADF = neutral and acid detergent fiber, respectively; CP = crude protein; AS = aerobic stability.
[2]Standard error of the mean.

Effects of biological additives on silage quality of erect milkvetch

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Keywords erect milkvetch, silage, lactic acid bacteria inoculant, cellulase

Introduction Erect milkvetch (*Astragalus adsurgens Pall.*) is a perennial legume. Due to its characteristics of high quality, high yields, extensive-adapted, well-tolerant and so on, it is grown in many parts of China (Yu et al., 2008). Ensiling is an important option of erect milkvetch, which not only can avoid the weather damage, but also can reduce nutritional losses. However, it is difficult to obtain high-quality erect milkvetch silages without any additive, because this legume contains a low amount of water soluble carbohydrates (WSC) and has a high buffering capacity. Biological additives, such as bacterial inoculants and enzymes, have been developed to improve the ensiling properties of forage by increasing the number of lactic acid bacteria and fermentation substrates on early fermentation.The objective of this study was to assess the effect of inoculant, enzymes and their mixture during ensiling on the fermentation quality of silage from erect milkvetch forage.

Materials and methods The erect milkvetch was harvested at bud stage from Inner Mongolia (China). There were 4 treatments: (i) LP (*Lactobacillus plantarum* isolated from *L. chinensis* silage inoculated at 1×10^6 CFU g^{-1} fresh material; (ii) E (cellulose degradation enzymes, and cellulase added at 0.005% of fresh material); (iii) LP + E (a mixture of *Lactobacillus plantarum* inoculated at 1×10^6 CFU g^{-1} fresh material and lignocellulose degradation enzymes, and cellulase added at 0.005% of fresh material); and (iv) Control (sprayed with the same volume of distilled water) and 3 replicates of each treatment. Chemical compositions were analyzed after 40 days of ensiling. Twenty grams of each silage sample was homogenized in a blender with 180 mL of distilled water for 1 min and then filtered through four layers of cheesecloth. The filtrate was used to measure pH value, ammonia nitrogen and organic acids contents.

Results and discussion The effects of biological additives on erect milkvetch silages are shown in the Table 1. The untreated erect milkvetch silage had a high pH value and ammonia nitrogen content and low lactic acid content. These may be due to the lower natural number of lactic acid bacteria on erect milkvetch forages, higher buffer capacity and lower WSC content (Cai et al., 1994). All treatments decreased the pH value. The ammonia nitrogen contents ($P < 0.05$) were lower in LP and LP+E treated silages than control. Only LP decreased the content of crude protein ($P < 0.05$) compared with the control. The E and LP+E treatments increased lactic acid content significantly ($P < 0.05$) and numerically decreased the butyric acid content. This indicated that the additives can improve the erect milkvetch silage quality (Yu et al., 2011).

Conclusion It is difficult to produce high quality erect milkvetch silages without additives.

Biological additives can improve erect milkvetch silage fermentation quality. Lactic acid bacteria combined with cellulase could be an efficient strategy to improve the fermentation quality of erect milkvetch.

Table 1 The influence of biological additives on the quality of erect milkvetch silage

	Control	LP	E	LP + E	SEM
pH	5.11^{a}	4.84^{b}	4.69^{bc}	4.52^{c}	0.05
Lactic acid (g kg^{-1} DM)	27.0^{b}	26.0^{b}	42.1^{a}	46.1^{a}	0.25
Acetic acid (g kg^{-1} DM)	26.2	31.9	38.7	35.6	0.19
Butyric acid (g kg^{-1} DM)	4.7	3.7	2.9	3.1	0.27
Ammonia nitrogen (g kg^{-1}TN)	237.4^{a}	87.8^{b}	211.4^{a}	104.2^{b}	1.70
Neutral detergent fiber (g kg^{-1} DM)	287.0	305.3	310.6	275.5	0.65
Acid detergent fiber (g kg^{-1} DM)	210.0	226.8	232.5	207.8	0.51
Acid detergent lignin (g kg^{-1} DM)	46.4	44.4	49.3	39.7	0.13
Crude protein (g kg^{-1} DM）	163.3^{b}	183.3^{a}	176.7^{ab}	180.0^{ab}	0.01
Water soluble carbohydrates (g kg^{-1} DM)	9.8	9.3	10.4	11.3	0.18

LP, *Lactobacillus plantarum*; E, cellulose degradation enzymes; LP+ E, mixture of LP and E; DM, dry matter; TN, total nitrogen;

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The influence of Lalsil Dry and Sil-All inoculation on the fermentation and aerobic stability of ensiled whole crop soybean

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Keywords forage, grains, inoculant, proteins, ruminants, silage

Introduction Although soybean (*Glycine max* (*L.*) *Merr.*) forages are grown primarily for grain or oil production worldwide, adverse weather conditions may cause reduced seed yields or quality, forcing soybean producers to seek alternative uses for the crops. In order to have enough feeds to sustain ruminant production during winter or drought months, forage soybeans should be ensiled. The ensiling of forages includes the use of bacterial inoculants to improve the fermentation dynamics of ensiled forages by accelerating the decline in pH and improving dry matter (DM) and nutrient retention (Muck, 2010). With its low content of water soluble carbohydrate (WSC) and high buffering capacity, whole crop soybean (WCS) is difficult to ensile without additives. The present study aimed to evaluate the effect of Lalsil Dry (LD) and Sil-All inoculation on the fermentation and aerobic stability of ensiled WCS forage.

Materials and methods The WCS (cultivar Link LF 6466, 268 g DM/kg, 7.04 pH, 72 g WSC/kg DM, 170 crude protein (CP)/kg DM, 453 g NDF/kg DM and 313 g ADF/kg DM) was harvested at the R6 stage and chopped to a theoretical cut length of 25 mm. The chopped WCS was treated with: no inoculant (control), LD (*Lactobacillus plantarum*, *Enterococcus faecium*, *Lactobacillus salivarius*, *Pediococcus acidilactici*, *Trichoderma longibrachiatum*, *Beta glucan*, calcium silicate, *Aspergillus niger* and *Bacillus subtilis)* and Sil-All (*Pediococcus acidilactici* (*CNCM MA 18/5M*), *Lactobacillus buchneri* (*NCIMB 40788*) and ensiled in 1.5 L anaerobic jars (3 jars/treatment) and kept at room temperature of 24-28^{0}C for 90 days. Both inoculants were applied to achieve a theoretical application rate of 3.5 x 10^5 colony forming units (CFU)/g of fresh forage. After 90 days of ensiling, jars 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 5 days following the procedure of Ashbell et al. (1991). Carbon dioxide and number of hours were determined after 5 days exposure.

Results and discussion Sil-All inoculation improved ($P<0.05$) DM and CP contents, reduced fibre content and increased lactic acid compared to other treatments, consistent to Sucu and Filya (2006). In addition, Sil-All inoculation had higher ($P<0.05$) residual WSC, which led to poor aerobic stability of silage (Weinberg et al., 1993) as indicated by higher CO_2 production and lesser hours of aerobic stability compared to other treatments. Inoculation with LD increased ($P<0.05$) the content of acetic acid and improved silage aerobic stability compared to other treatments.

Conclusion The fermentation of WCS was improved with Sil-All while the silage aerobic stability was improved with LD inoculation.

Table 1 Chemical composition of two bacterial inoculants on the fermentation and aerobic stability of ensiled whole crop soybean (n=3)

	Treatments				
Parameter	Control	Lalsil	Sil-All	SEM	*P*-value
Chemical composition					
DM, g/kg	303.7^{b}	228.3^{c}	313.7^{a}	2.08	0.001
CP, g/kg DM	154.6^{b}	142.1^{c}	172.5^{a}	1.85	0.001
GE, MJ/kg DM	18.42	18.12	18.50	0.160	0.296
EE, g/kg DM	63.30^{b}	67.73^{a}	62.47^{b}	0.987	0.019
aNDF, g/kg DM	474.0^{a}	450.6^{b}	398.5^{c}	1.085	0.001
ADF, g/kg DM	393.4^{a}	393.2^{a}	314.7^{b}	1.037	0.001
ADL, g/kg DM	75.07^{a}	74.57^{a}	62.03^{b}	0.471	0.001
Fermentation characteristics					
WSC, g/kg DM	2.66^{b}	6.47^{a}	7.28^{a}	0.462	0.001
pH	4.40^{b}	5.06^{a}	4.2^{b}	0.108	0.03
LA, g/kg DM	54.8^{b}	38.2^{c}	69.7^{a}	1.329	0.001
AA, g/kg DM	41.0^{b}	91.2^{a}	24.6^{c}	1.571	0.001
PA, g/kg DM	6.9^{b}	37.1^{a}	4.3^{c}	0.504	0.001
BA, g/kg DM	31.83^{a}	15.77^{b}	0.00^{c}	0.870	0.001
NH_3-N, g/kg DM	12.77^{a}	10.26^{b}	6.74^{c}	0.434	0.001
Aerobic stability					
CO_2, g/kg DM	23.63^{b}	6.98^{c}	27.9^{a}	0.724	0.001
Aerobic stability, h	55.63^{b}	75.57^{a}	33.23^{c}	1.478	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; CO_2, Carbon dioxide.

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Potential effects of lactic acid bacteria with antimicrobial activity on spoilage microorganisms in alfalfa silage

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Keywords antimicrobial capacity, lactic acid bacteria, 16S rDNA

Introduction Alfalfa is a plant of global interest and is considered to be one of the most important crops due to the large cultivated area, its high nutritive value, high CP content, and high DM digestibility. However, alfalfa, such as other legumes, has some limitations to the ensiling process such as low DM and water soluble carbohydrates, and a high buffering capacity. In addition, there is a low epiphytic population of lactic acid bacteria (LAB). Studies evaluating the diversity of LAB and its succession in tropical silages are scarce for both silages of grass and legumes, thereby indicating a need to isolate as well as genetically and phenotypically characterize the epiphytic populations. The use of LAB isolated from the same plant can result in more significant effects on fermentation characteristics and therefore improve silage quality. The objective of this study was to isolate, identify, and evaluate the antimicrobial capacity of LAB in alfalfa silages in different fermentation periods.

Materials and methods The alfalfa was harvested at the beginning of the flowering stage and was wilted for 6h. After, it was chopped into a 1.5-cm particle size and packed in bags, with three replications. The bags were opened after 1, 3, 7, 14, 28, and 56 days after ensiling. Samples of fresh alfalfa (day 0) and its silages from the different fermentation periods were homogenized in 225 mL of sterile Ringer's solution. The water extract was submitted to serial dilutions and these solutions were inoculated by the *Pour Plate* plating technique while using plates containing MRS-agar with added bromocresol purple, which were then incubated for 48 hours at 37ºC. After the tests to identify possible-LAB (Gram differential coloration test and a test to determine the capacity to produce acid), 102 isolates were selected. The DNA from the isolates was extracted by using a commercial kit (Wizard® Genomic DNA Purification kit, Promega, Madison, USA) and the coding region of the 16S rDNA gene was amplified by PCR using the primers p027F and 1492R. The antimicrobial activity from each isolate on the indicator bacteria *Listeria monocytogenes* ATCC 7644, *Escherichia coli* K12, *Pseudomonas aeruginosa*, *Bacillus cereus* ATCC 4904, and *Staphylococcus aureus* ATCC 25923 was determined according to the differed activity method (Taag et al., 1976).

Results and discussion Based on the analysis of 16S rRNA sequences for each isolate, we observed the succession of LAB during fermentation (Figure 1). *Weissela cibaria* prevailed at the beginning of the fermentation period, whereas *Pediococcus acidilactici* prevailed during all fermentation period. Bacteria from this genus are found in plants, although it is also present in humans and animals. The presence of *P. pentosaceus* and *P. acidilactici*, which are more common in silages, was also verified in the saliva of humans and feces of animals and humans (Holzapfel et al., 2006). Thus, part of this population is probably from other sources. From the 102 isolates that were obtained in this study, 78 presented

with sequences with an identity greater than 97% when using the program BLAST. All isolates presented antagonist activity against at least one indicator microorganism that was utilized in the antimicrobial test, and 50% of them showed activity against all indicator microorganisms that were utilized. Some studies have reported that the antimicrobial activity of LAB on gram-negative bacteria is not effective due to the presence of the lipopolysaccharide layer on its external membrane, which acts as an additional barrier against antimicrobial substances (Cleveland et al., 2001). However, in this study, the gram-negative bacteria *P. aeruginosa* was the bacteria that was most affected by antimicrobial activity of the most isolates.

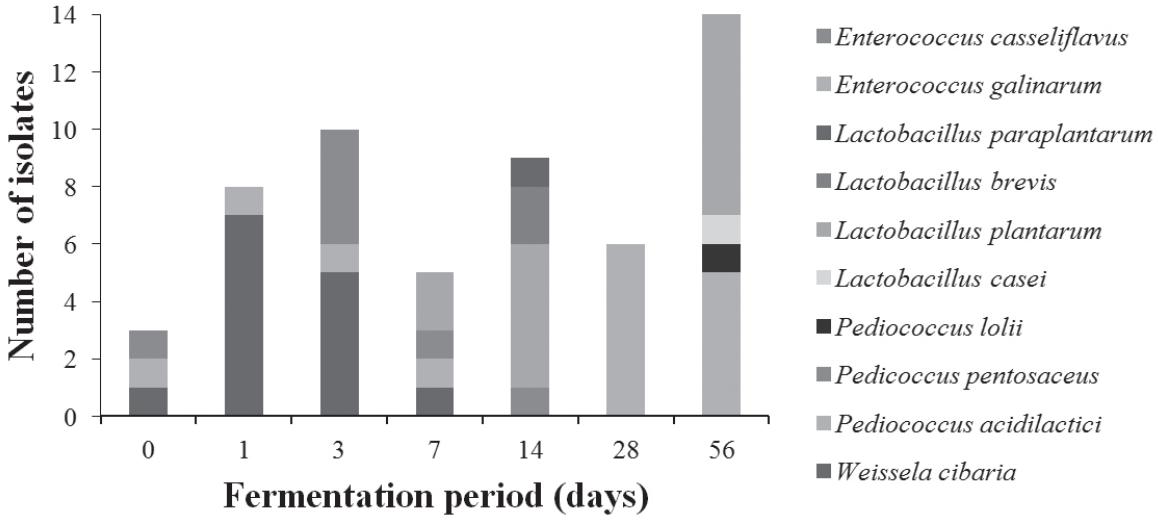

Figure 1 Succession of lactic acid bacteria during alfalfa fermentation.

Conclusions The microorganisms *Weissela cibaria*, *Pediococcus pentosaceus*, *P. acidilactici*, *P. lolli*, *Lactobacillus plantarum*, *L. casei* and *L. paraplantarum* were predominant among the LAB species in alfalfa silage in tropical conditions. *Pediococcus acidilacti* was predominant from the beginning to the end of the fermentation period. Among the 78 isolates, all presented antimicrobial activity against at least one indicator microorganism utilized in this study, thus arousing interest of using them as inoculants in future studies.

Acknowledgments This research was supported by the FAPEMIG, CNPq and CAPES foundations.

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The effect of a multi strain and enzyme silage inoculant on fermentation characteristics and aerobic stability of legume silage

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Keywords *L. plantarum, P. pentosaceus, P. acidipropionici, P. acidilactici,* lucerne, silage

Introduction 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 objective of the trial was to determine the effect of a commercial multi strain silage inoculant on the DM loss, fermentation characteristics and aerobic stability (AS) of wilted lucerne silage.

Materials and methods The trial consisted of 2 groups: Control (C) (no additive) and Treatment (T) (Sil-All 4x4+ WS, Danstar Ferment, Switzerland). The inoculant contains 4 bacteria and 4 enzymes (*Lactobacillus plantarum* CNCM I-3235 (500 000 CFU/ g fresh forage), *Pediococcus pentosaceus* NCIMB 12455 (200 000 CFU/ g fresh forage), *Propionibacterium acidipropionici* CNCM MA/26 4U (200 000 CFU/ g fresh forage), *Pediococcus acidilactici* CNCM I-3237 (100 000 CFU/ g fresh forage), α-amylase, β-glucanase, cellulase and glucanase (enzymes included at EU 1831/2003 efficacy application rate). Lucerne of a third cut was cut and wilted on field (24 h) (Table 1).The chopped herbage (3-4 cm) was sprayed with water or inoculant solution and packed in 12 L mini silos (4 silos/treatment). The silos were opened after 90 days and the silages were analysed for nutritional value (DM, Ash, CP, NDF, ADF, WSC, Soluble-N, *in vitro* DM digestibility), fermentation parameters (pH, N-NH_3, organic acids, ethanol) and microbial counts [lactic acid bacteria (LAB) yeasts, moulds] and DM losses were calculated. Metabolizable energy (ME) was also estimated (INRA, 2007). The AS was assessed by Honig method (1990) (7-day test at +22˚C). A rise of ≥3˚C above room temperature was taken as an indicator of instability. After the stability test pH, DM and microbial counts were measured. Data were subjected to one-way analysis of variance [GLM procedure in SPSS (v. 22.0)]. Significances were declared at $P<0.05$.

Table 1 Chemical composition of wilted lucerne

DM	Ash	CP	NDF	ADF	WSC	Soluble-N	dDM [1]	LAB	Yeast	Molds
g/kg	g/kg DM							log_{10} CFU/g FM		
354	109	207	402	364	63.2	12.8	829	5.34	7.11	0.00

[1] *In vitro* dry matter digestibility

Results and discussion The nutritional value of both silages is shown in Table 2. The T silage had significantly more WSC compared to C silage ($P<0.05$). Table 3 shows that T silages had a significantly lower pH compared to the C ($P< 0.05$). The contents of lactic acid and acetic acid were significantly higher in T silages compared to C ($P<0.05$). Ethanol content was 30% lower in T silage compared to C ($P<0.05$). None of the silages contained butyric acid. The DM losses during ensiling were significantly lower for the T

group compared to C (P<0.05). To simulate what these results could represent on farm scale, the obtained data were entered in the Milk 2000 spreadsheet developed by university of Wisconsin (Milk 2000 version 7.54). A 3.2% increase in yield (milk/acre) would be obtained according to this simulation. All silages were aerobically stable as no heating occurred during the 7 d test. After the AS-test, yeast counts were significantly lower in T silage (3.12 vs. 3.93 log CFU/g silage, P<0.05). After the AS test, the pH remained low for both silages (T: 4.27 vs. C: 4.38, P<0.05).

Table 2 Nutritional value of Control and Treatment silage after 90 d of ensiling

Item	C	T	SEM
Dry matter, g/kg	324 [a]	351 [b]	2.3
Ash, g/kg DM	110	107	1.1
Crude protein, g/kg DM	206	207	1.3
Soluble-N, g/kg DM	20.3 [a]	18.1 [b]	0.3
NDF, g/kg DM	424 [a]	410 [b]	1.8
ADF, g/kg DM	393 [a]	371 [b]	5.1
WSC, g/kg DM	7.6 [a]	11.2 [b]	0.4
dDM, g/ kg DM	787	786	4.6
ME, MJ/kg DM	2126	2158	

[a–b] Means within row with different superscripts differ ($P < 0.05$).

Table 3 Fermentation characteristics of silage after 90 d of ensiling

	C	T	SEM
pH	4.42 [a]	4.27 [b]	0.02
N-NH_3, g/kg N	62.7 [a]	51.2 [b]	1.9
Lactic acid, g/kg DM	37.1 [a]	55.7 [b]	1.7
Acetic acid, g/kg DM	15.8 [a]	21.7 [b]	0.8
Ethanol, g/kg DM	12.8 [a]	8.9 [b]	0.6
DM losses, g/kg DM	48.8 [a]	42.9 [b]	4.4

[a–b] Means within row with different superscripts differ ($P < 0.05$).

Conclusions The multi strain silage and enzyme additive improved fermentation characteristics (P<0.05) and reduced DM losses (P<0.05) during ensiling of lucerne.

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The effect of enzymes on the fermentation of Lucerne silage

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Keywords lucerne, amylase, cellulase, β-glucanase, xylanase, fermentation dynamics

Introduction Polysaccharide-hydrolysing enzymes can be used as silage additives. Several studies observed that the most beneficial effects are obtained when enzyme preparations are combined with bacterial inoculants (Nadeau et al., 2000; Sheperd et al., 1995; Tengerdy et al., 1991). The role of specific enzymes used as complementary additives is to produce quickly fermentable sugars from complex carbohydrates that will ultimately be available for silage microorganisms. This can be of particular interest during the early stages of ensiling when sugar availability is limiting (Muck et al., 1997).The objective of the present trial was to determine the effect of 4 enzyme preparations (amylase, cellulase, β-glucanase and xylanase) in conjunction with a homolactic bacterium (*Pediococcus pentosaceus)*, on the fermentation characteristics of a difficult to ensile forage.

Materials and methods Lucerne was harvested in budding-early blooming stage of maturity (24 % DM). The chopped raw material (2-3 cm) was pooled; thoroughly homogenised and separated into 6 equal piles. The trial consisted of 6 treatments: control, PC (*Pediococcus pentosaceus* at 3×10^5 CFU/ g fresh forage), PC + amylase (18 BAU/ kg FM), PC + cellulase (0.3 CMCU/kg FM), PC + *β*-glucanase (4 IU/kg FM) and PC + xylanase (7.5 IU/kg FM). Treatments were applied, homogeneously mixed. Fifteen micro-silos (1.7 L) per treatment were filled-up and packed. Micro-silos were stored at ambient temperature and opened at 1, 5, 10, 28 and 90 days. Three mini-silos of each treatment were opened at each opening time and analysed for dry matter (DM), water soluble carbohydrates (WSC), pH, lactic acid (LA) acetic acid (AA) and NH_3. Data were analysed as one-way ANOVA with SPSS v. 21.0. Significance was declared at $P<0.05$.

Results and discussion The drop in pH was affected by all the treatments during the first 28 days of ensiling (Table 1). The pH was significantly lower in the PC compared to the control at day 5 post ensiling ($P<0.05$), indicating a faster acidification. This was confirmed by a higher LA content in the PC vs. control (Table 1) whatever the time points, except on day 5. The beneficial effect of adding enzymes was measured at the start of the ensiling process (d 1 and d 5) with a numerical lower pH and a higher LA content for all enzyme treated silages compared to the PC. These results suggest a better availability of fermentable sugars due to the action of the enzymes. The faster acidification also affected the final quality of the silages (Table 2). All treated silages had a significant higher concentration of LA, compared to the control at 90 d ($P<0.05$). Furthermore, the addition of fibrolytic enzymes (cellulase, *β*-glucanase or xylanase) showed significant higher LA than the PC at d 90 ($P<0.05$). All 4 enzyme treated silages also had a significant lower NH_3-N than the control and PC at d 90, indicating a lower proteolysis. These results are in line with Tengerdy et al. (1991) and Sheperd et al. (1995).

Table 1 Dynamics of pH and LA of silage treated with different enzymes (on DM base ± s.d.)

	1 d		5 d		10 d		28 d	
	pH	LA(%)	pH	LA (%)	pH	LA (%)	pH	LA (%)
Control	5.18 ^{a}	2.16 ^{a}	4.88 ^{a}	4.57 ^{a}	4.90 ^{a}	7.27 ^{a}	4.86 ^{ab}	6.27 ^{a}
PC	5.17 ^{ac}	2.74 ^{bc}	4.78 ^{b}	5.52 ^{a}	4.86 ^{ab}	8.15 ^{b}	4.82 ^{ab}	11.48 ^{b}
PC + Amylase	5.11 ^{abc}	2.73 ^{bc}	4.75 ^{c}	7.47 ^{ab}	4.90 ^{ab}	6.14 ^{a}	4.85 ^{b}	11.58 ^{b}
PC + Cellulase	5.11 ^{abc}	2.48 ^{ab}	4.78 ^{b}	9.95 ^{ab}	4.86 ^{b}	8.26 ^{b}	4.83 ^{ab}	8.45 ^{c}
PC + β-glucanase	5.10 ^{b}	2.40 ^{ab}	4.73 ^{c}	11.72 ^{b}	4.84 ^{ab}	7.93 ^{b}	4.86 ^{ab}	9.69 ^{bc}
–PC + Xylanase	5.15 ^{c}	3.01 ^{c}	4.78 ^{b}	12.09 ^{ab}	4.82 ^{b}	8.95 ^{b}	4.81 ^{a}	11.26 ^{b}
SEM	0.01	0.24	0.01	1.35	0.01	0.60	0.01	1.07

PC: *Pediococcus pentosaceus* at 3×10^{5} CFU/ g fresh forage.

Table 2 Fermentation characteristics of silages after 90 d (DM base ± s.d.)

	DM	WSC (%)	pH	LA (%)	AA (%)	NH 3-N (%total N)
Control	20.7	0.69	4.69 ^{a}	5.63 ^{a}	4.66	19.02 ^{a}
PC	20.5	1.52	4.69 ^{a}	10.21 ^{b}	5.76	18.47 ^{a}
PC + Amylase	21.0	1.41	4.72 ^{b}	11.05 ^{b}	4.98	16.58 ^{bd}
PC + Cellulase	20.5	1.01	4.70 ^{a}	15.14 ^{c}	5.79	16.77 ^{b}
PC + β-glucanase	20.1	3.05	4.73 ^{b}	16.03 ^{c}	5.86	15.19 ^{c}
–PC + Xylanase	19.9	2.16	4.67 ^{a}	16.55 ^{c}	6.17	15.68 ^{cd}
SEM	0.4	0.70	0.01	1.27	0.59	0.48

PC: *Pediococcus pentosaceus* at 3×10^{5} CFU/ g fresh forage.

$^{a–d}$Means within column with different superscripts differ ($P < 0.05$).

Conclusions The addition of enzymes combined with a bacterial inoculant can significantly improve fermentation characteristics and significantly decrease proteolysis of lucerne silage.

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Metabolomic profiles of alfalfa silage inoculated with or without *Lactobacillus plantarum* and *L. buchneri*. II. Metabolites distribution patterns

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Keywords Silage metabonomics, *Lactobacillus plantarum*, *Lactobacillus buchneri*

Introduction Metabolites in silage, such as organic acids (lactic, acetic, propionic and butyric acids), ethanol and 1,2-propanediol, are detected conventionally to evaluate the fermentation quality of ensiled forage. During fermentation, however, lactic acid bacteria also can produce many other metabolites, *e.g.*, amino acid, fatty acid, oligosaccharide, vitamin, small peptide, flavoring agent and aromatic compound, etc. Little information is available regarding whole picture of metabolites in ensiled forage. Thus, the present study was performed to investigate the metabolomic profiles of alfalfa silage inoculated without or with *Lactobacillus plantarum* or *L. buchneri*.

Materials and methods See **"**Metabolomic profiles of alfalfa silage inoculated with or without *Lactobacillus plantarum* and *L. buchneri*. I. Fermentation quality and differentially expressed metabolites".

Results and discussion In 280 detected substances, a total of 102 kinds of metabolites were identified and their concentrations were determined. According to PCA and Heatmap analysis (Figures 1 and 2), an obvious separation between samples within treatments was detected. The sample of control, *L. plantarum* and *L. buchneri* were clearly separated by the PC1, which represented 47.4% of variation among samples. The PC2 distinguished the samples from the three treatments, explaining 22.6% of the variation.

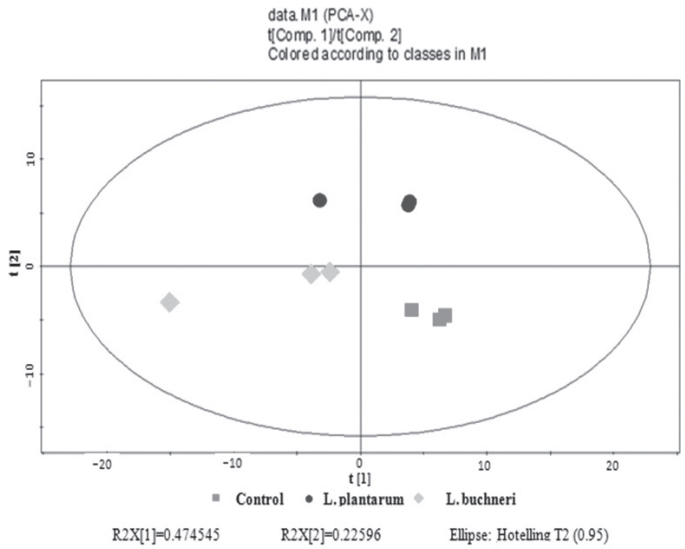

Figure 1 Principal component analysis (PCA) of metabolic profiles in alfalfa silage with or without inoculation of *L. plantarum* or *L. buchneri.*

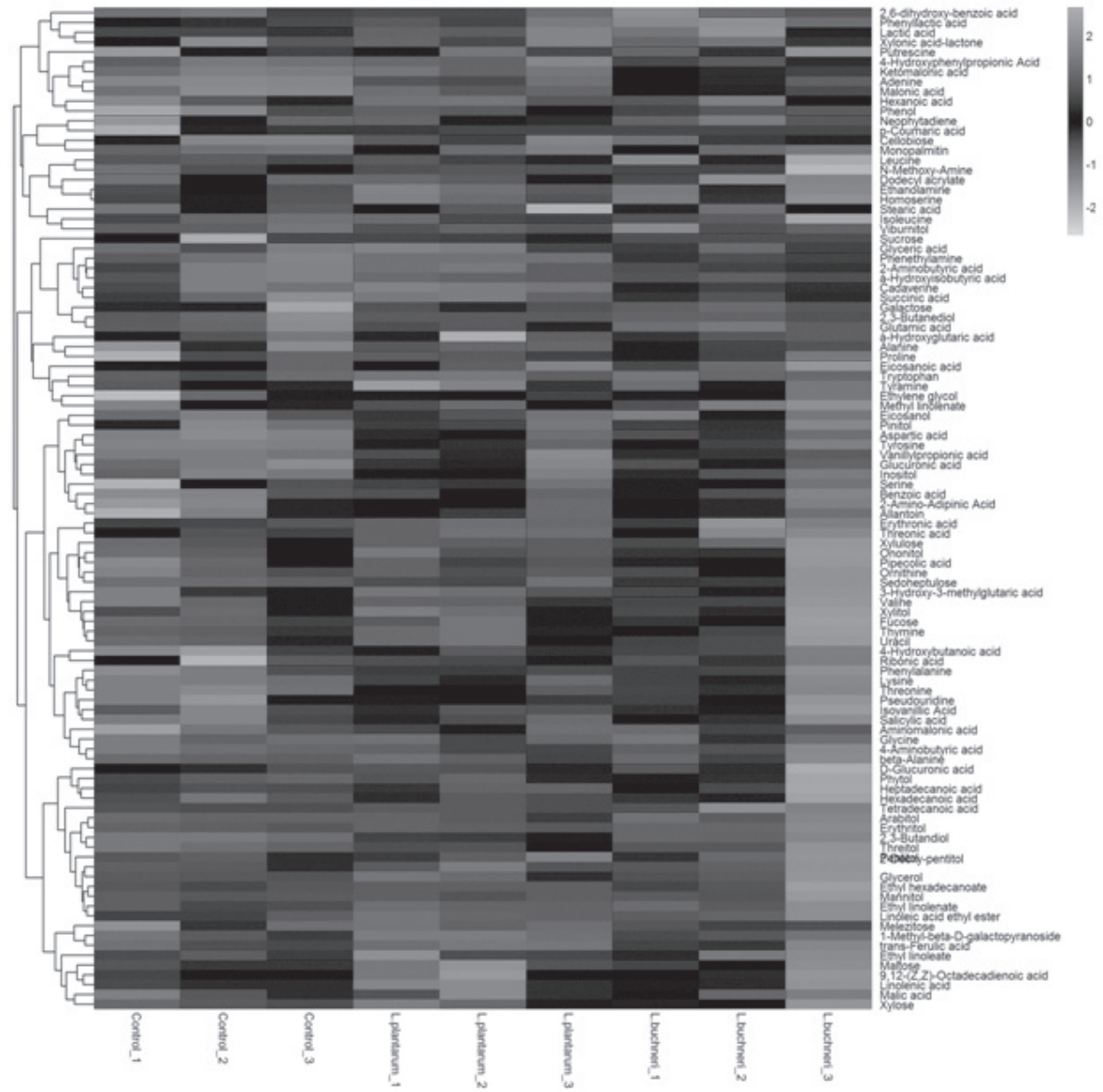

Figure 2 Heatmap analysis combined with hierarchical cluster analysis (HCA) of 102 metabolites.

Conclusion Metabolomics profiling analysis provides a deep insight in metabolites that we ever do not know in silage after fermentation. Inoculation of *L. plantarum* or *L. buchneri* changes metabolites composition pattern of ensiled forage, and improved concentrations of some amino acids, flavoring agent of butandiol, and prebiotics of 4-aminobutyric acid by fermentation metabolism of these inoculants in the present study.

Evaluation of wild strains of lactic acid bacteria for enhancing the aerobic stability of sugarcane silage

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Keywords yeast, *Lactobacillus hilgardii, Lactobacillus brevis, Lactobacillus plantarum*

Introduction Homofermentative lactic acid bacteria (LAB) have been selected to increase lactic acid concentration in the silo, but aerobic stability may be impaired because lactic acid can be easily oxidized by yeasts when the silage is exposed to air (Pahlow et al., 2003). There is a correlation between the yeast population in silage and the loss of aerobic stability; therefore it is necessary to identify the predominant yeast species present during the exposure of silage to air. This study aimed to identify the major yeast species associated with the aerobic deterioration process of sugarcane silages and to select bacterial strains, which have the potential to improve the aerobic stability after silo opening.

Materials and methods Seven strains of facultative heterofermentative *Lactobacillus plantarum* (CCMA 0172, CCMA 0176, CCMA 0178, CCMA 0179, CCMA 0181, CCMA 0180 and CCMA 0182), five strains of obligatory heterofermentative *L. brevis* (CCMA 0177, CCMA 0171, CCMA 0173, CCMA 0174 and CCMA 0175) and two strains of obligatory heterofermentative *L. hilgardii* (CCMA 0183 and CCMA 0170) were used as inoculants, and a control treatment without inoculants was evaluated. After 126 days of anaerobic fermentation the silos were opened, and the temperature was evaluated every 30 min, during 216 h. Silage samples were collected at 0, 96 and 216 h. The yeasts were enumerated by surface inoculation in Dichloran Rose Bengal Chloramphenicol Medium agar. The yeast colonies (231 isolates) were microscopically (cell size, cell shape, type of reproduction) and physiologically characterized by fermentation of carbohydrates (sucrose, fructose and glucose) and the assimilative capacity of the lactic acid as described by Kurtzman et al. (2011).

Results and discussion The highest maximum temperatures were observed in the treatment inoculated with *L. brevis* strains (Table 1). The maximum temperatures were lower and similar to each other in all of the other evaluated silages. Silages treated with *L. plantarum* CCMA 0176, *L. plantarum* CCMA 0178, *L. hilgardii* CCMA 0183 and *L. hilgardii* CCMA 0170 took more time to achieve the maximum temperature (Table 1). Ten species were identified, including *Candida diversa, C. ethanolica, Hanseniaspora opuntiae, Issatchenkia orientalis, Pichia fermentans, P. kudriavzevii, P. manshurica, Schizosaccharomyces pombe, Debaryomyces etchellsii* and *Zygosaccharomyces bailii.* The homology of the sequences reported in the GenBank was within 98–100%. The species *C. diversa* and *C. ethanolica* isolated from sugarcane silage were able to utilize lactate even when it was present in high concentrations. These species were found in larger population in the silages inoculated with CCMA 0181, CCMA 0177, CCMA 0174 and CCMA 0175 strains. It seems that the higher population of *Candida* species led to lower aerobic

stability, whereas presence of *S. pombe* and *Z. bailii* were related to higher aerobic stability as observed in silages treated with CCMA 0183 and CCMA 0170 strains.

Conclusions The growth and metabolism of LAB strains modified the diversity of yeast species in the sugarcane silages during aerobic exposure. The diverse behaviour of LAB strains of the same species during ensiling reinforces the need of using specific strains for each forage. Among the evaluated LAB strains, the obligatory heterofermentative *L. hilgardii* CCMA 0183 and CCMA 0170 strains provided the silages with lower temperatures and additional unheated time and are suitable for use in sugarcane silage.

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Table 1 Dynamics of temperature during aerobic exposure of sugarcane silages without inoculants and inoculated with strains of lactic acid bacteria (LAB) and identification of LAB strains evaluated

Treatment	Maximum temperature (ºC)	Time to reach maximum temperature (h)	Aerobic stability (h)	Molecular identification[2]
Control	47.0 [A1]	22.0 [B]	14.2	
CCMA 0172	45.2 [A]	26.0 [B]	17.0	*L. plantarum* 98% (FJ669130.1)[3]
CCMA 0176	43.5 [B]	42.2 [A]	20.2	*L. plantarum* 99% (HM218291.1)
CCMA 0178	43.0 [B]	48.7 [A]	21.0	*L. plantarum* 98% (HM218291.1)
CCMA 0179	41.2 [B]	35.7 [B]	22.0	*L. plantarum* 98% (HM218291.1)
CCMA 0181	43.8 [B]	30.3 [B]	19.8	*L. plantarum* 99% (HM218291.1)
CCMA 0180	44.2 [B]	28.5 [B]	17.5	*L. plantarum* 99% (HM218291.1)
CCMA 0182	43.2 [B]	27.5 [B]	17.8	*L. plantarum* 99% (HM218291.1)
CCMA 0177	46.0 [A]	27.2 [B]	17.8	*L. brevis* 98% (FJ227316.1)
CCMA 0171	46.3 [A]	28.8 [B]	18.2	*L. brevis* 97% (FJ532364.1)
CCMA 0173	45.0 [A]	33.3 [B]	18.2	*L. brevis* 99% (FJ227316.1)
CCMA 0174	43.8 [B]	27.5 [B]	16.0	*L. brevis* 98% (FJ227316.1)
CCMA 0175	47.2 [A]	28.0 [B]	18.3	*L. brevis* 98% (FJ227316.1)
CCMA 0183	41.8 [B]	58.0 [A]	21.5	*L. hilgardii* 98% (HM217953.1)
CCMA 0170	43.8 [B]	54.0 [A]	30.3	*L. hilgardii* 98% (HM217953.1)
P	0.03	0.02	0.05	
SEM†	1.22	6.70	2.03	

[1] The mean values with different capital letters are significant at $P < 0.05$ according to the Scott–Knott test. [2] Sequencing of 16S rDNA (Ávila *et al*., 2013). [3] The number in parentheses refers to the access code in Gen-Bank. † Standard error of the means.

Glycerin as an additive for sugarcane silages

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Keywords glycerol, microbial inoculum, aerobic stability

Introduction Growth of yeast in sugarcane silages induces a large loss of dry matter (**DM**) and energy (Kung and Stanley, 1982), increasing the content of neutral detergent fiber (**NDF**) wich has low digestibility (Corrêa et al., 2003). Although the use of starter cultures and chemical additives have been tested, but the results from using these additives have been inconsistent. Brazil is among the largest producer of biodiesel in the world. Each 100 kg of biodiesel generates approximately 10 kg of crude glycerin (Santibanéz et al., 2011). However, crude glycerin contains variable amounts of methanol, which may result in animal disturbances (Nie et al., 2007). Microbial fermentation during the storage may be able to detoxifying methanol crude glycerin (Hartner and Gliender, 2006). Inoculation of sugarcane with lactic acid bacteria with obligatory heterofermentative strains generally results in high concentration of acetic acid that reduces yeast population. The objective of this work was to evaluated the chemical and microbiological characteristics of sugarcane silages containing glycerin in different dosages in the response to inoculation with wild and commercial strains.

Materials and methods The effects of adding glycerin and microbial inoculants in sugarcane silages were evaluated in a completely randomized design with factorial arrangement (4×2) on the trial 1. The treatments were: without microbial inoculum (**WMI**), or with *Lactobacillus hilgardii* CCMA 0170 (**LH**), or with *L. plantarum* + *Enterococus faecium* + *L. buchneri* (**LP+EF+LB,** Biomax LB, Chr Hansen, Milwaukee, USA), or with *L. plantarum* + *Propionibacterium acidipropionici* (**LP+PA,** Kera-Sil cana, LNF Latino Americana, Bento Gonçalves, Brazil) combined with 10% purified glycerin or without glycerin. Five silos were constructed for each one of eight possible treatments. Silos remained closed for 72 days. Data were analyzed with PROC GLM of SAS by model containing effect of microbial inoculum, glycerin and their interactions. Treatments were compared by pre-planned contrasts WMI *vs.* LH; WMI *vs.* LP+EF+LB and WMI *vs.* LP+PA. The trial 2, the effect of different inclusions of purified glycerin (0, 3, 6 and 9% of fresh matter) with 1.1% addition methanol during sugarcane ensilage with two opening times (45 and 90 days), was evaluated in a completely randomized design with factorial arrangement (2×4). Six mini silos were constructed for each one of eight treatments combination; three silos were opened after 45 days and the last three opened with 90 days. Data were analyzed with PROC GLM of SAS by model containing effect of glycerin dosage, days of ensiling and their interactions. Means were tested for linear, quadratic and cubic trends by pre-planned polynomial contrasts.

Results and discussion Trial 1. The addition of glycerin reduced the NDF concentration in 22.3% (659 *vs*. 512 g/ kg of DM, $P<0.01$) and kept the DM content higher (259 *vs*. 210 g/kg of DM, $P<0.01$) compared to silages without glycerin addition. Glycerin has high DM concentration than sugarcane resulting in silages with higher DM concentration when this additive was added. The NDF concentration was lower for glycerin treatments, probably due to the dilution effect. The LH treatment reduced DM losses with or without glycerin compared with WMI (15.7 *vs*. 28.6%, $P<0.01$). However, glycerin by itself did not reduce dry matter losses. The positive results obtained from the use of *L. hilgardii* may be due to the capacity to produces acetic acid (Ávila et al., 2014). The end products of silage fermentation, lactic acid (53 *vs.* 81 g/kg of DM), acetic acid (29.5 *vs*. 26.2 g/kg of DM) and ethanol (41.5 *vs*. 83.2 g/kg of DM) were affected by glycerin addition ($P<0.01$). When glycerin was combined with microbial inoculum the response of LP+PA treatment was more intense. Lactic acid bacteria with obligatory heterofermentative metabolism can produce acetic acid in addition to lactic acid. The higher acetic acid concentrations is related with better preservation of sugarcane silages and result in lower DM losses and lower ethanol production (Carvalho et al., 2014). *Lactobacillus* strains, when cultured in a medium containing glycerol as the sole carbon source are able to produce lactic and acetic acids (Garai-Ibabe et al., 2008). There was no statistically effect of glycerin on silage pH. On the Trial 2, concentrations of DM and water soluble carbohydrates (**WSC**) increased linearly ($P<0.01$) with the increase in the glycerin dosage. The treatment with 9% glycerin showed a 13.5% higher DM concentration compared to the treatment with 0 % glycerin. The NDF values ranged from 716 to 596 g/kg of DM in treatments 0% and 9 % of glycerin respectively ($P<0.01$). The effect of glycerin addition increased DM losses with linear effect ($P<0.04$), with a 9.8% variation between treatment with 9% and 0 % glycerin. Forages ensiled for 45 and 90 days showed in average DM losses of 26.8 and 29.4%, respectively ($P=0.02$) indicating increase losses by time effect. Methanol content increased along time ($P=0.01$) which may have been result of higher DM losses at 90 days causing a concentration effect of this compound. There was a linear reduction on lactic acid concentration ($P=0.04$) and increase in glycerol and succinic acid concentrations ($P<0.01$) with the increase in glycerin dosage. Succinic acid is a dicarboxylic acid produced as an intermediate of the tricarboxylic cycle and is one of the products of fermentation (Song and Lee, 2006). Succinate has been shown to increase propionate production and to act as a glycogenic precursor for protein synthesis in the rumen (Zeiukus et al., 1999). The count of lactic acid bacteria was not affected by the increase in the glycerin (7.5 log cfu/g silage, $P=0.45$). The lowest number of yeast (4.96 log cfu/g silage) was observed in the control treatment and there was no statistically difference between 45 and 90 days.

Conclusions The inclusion of glycerin with methanol in sugarcane silages was effective in reduce NDF concentration and associated with *L. hilgardii* or *L. plantarum* + *Propionibacterium acidipropionici* could be a plausible strategy for reducing dry matter losses.

Fermentative profile and dry matter losses of sugarcane silage with different levels of calcium oxide and crude glycerin

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Keywords ensilage, fermentation, forage conservation

Introduction Forage conservation is an essential management tool to provide feed and meet animal requirements in periods of forage shortage. The use of sugarcane silage is a viable option for shortages of forage in pastures. However, the use of additives is required due to the high level of soluble carbohydrates, as well as the large number of yeasts that promote alcoholic fermentation with high CO2 production, increasing losses during the fermentation process. This experiment aimed to evaluate the effects of calcium oxide and crude glycerin on fermentative profile and losses in sugarcane silage.

Materials and methods The experiment was conducted in Federal University of Mato Grosso in partnership with Embrapa Agrosylvipastoral. The sugarcane (*Saccharum officinarum* L.) cultivar RB 72-454, was chopped and ensiled in 36 PVC silos with a volume of 2.75 liters, provided with Bunsen valves. We used a factorial design (3×4) in a completely randomized desing, with three replicates per treatment, consisting of: three levels of calcium oxide (0, 0.5 and 1%) and four crude glycerin levels (0, 4, 8 and 12%) in fresh matter (FM). The composition of crude glycerin was 82% glycerol, 0.52% (w/w) methanol and 70.34 g/kg of mineral. The titratable acidity (TAC) and pH were determined according to the technique described by Silva and Queiroz (2002). The evaluation of ammonia nitrogen (NNH3) was performed by the method proposed by Chaney and Marbach (1962). The dry matter losses (DML) were quantified by the method Jobim et al. (2007) as the difference in the weight of the set before and after ensiling. The effect of adding calcium oxide within each level of glycerin inclusion was assessed using Tukey's test, and for the evaluation of the effect of glycerin inclusion we made regression models, linear, quadratic and cubic, both considering a significance of 5% for type I error.

Results and discussion There was significant difference (P>0.05) between DM values, by increasing their levels following the addition of calcium oxide, and with linear effect for the addiction of crude glycerin. The pH values increased with the addition of calcium oxide (CaO) with significant interaction, due to the alkaline nature of these compounds. To the level of 0.5% of calcium oxide, there was no model that would fit the fermentation period, and to the level of 1% of calcium oxide, there was a linear decrease in pH values with the addition of crude glycerin. The pH was within the optimal range for silage, between 3.8 and 4.2 (McDonald et al., 1991). For titratable acidity there was a linear reduction to the interaction effect between treatments at levels of 0% and 0.5% calcium oxide with added glycerin. For the 1% level was obtained a quadratic response, according to the regression model, with the maximum point of 3.72, with the addition of crude glycerin levels. The NNH_3 values linearly increased with the addition of calcium oxide and crude glycerin, with an average of 6.38% and a maximum value of 10.64%, in the treatment with inclusion of

0.5% calcium oxide and 12% glycerin, what is considered acceptable for a fermentative process, without excessive losses by proteolysis (Van Soest, 1994).

Table 1 The DM, pH, TAC, NNH3 and DML of sugarcane silages

	CaO (% FM)			*P*-	Glycerin (% FM)				*P*-	SEM[5]	Int[6]
	0	0.5	1.0	value	0	4	8	12	value		
DM[1]	24.14c	28.10b	30.97a	<0.0001	23.61	27.21	29.20	30.93	<0.0001	0.58	0.1167
pH	3.60	3.94	4.19	<0.0001	3.92	3.87	3.91	3.95	0.1146	0.20	0.0017
TAC[2]	12.61	16.58	17.90	<0.0001	16.11	16.87	15.63	14.18	<0.0001	0.44	<0.0001
NNH_3[3]	5.19	6.79	7.15	<0.0001	4.97	5.66	6.66	8.22	<0.0001	0.56	<0.0001
DM_L[4]	21.95	13.60	9.14	<0.0001	21.05	12.95	13.22	12.37	<0.0001	0.83	<0.0001
Interaction effect between the levels of addition of calcium oxide and glycerin											
pH	0	3.50 c	3.47 c	3.68 c	3.72 c	$\hat{Y} = 3.48 + 0.02*G$ ($r^2 = 66.70$)					
	0.5	3.98 b	3.94 b	3.93 b	3.94 b	$\hat{Y} = 3.95$					
	1.0	4.29 a	4.18 a	4.13 a	4.19 a	$\hat{Y} = 4.25 - 0.0091*G$ ($r^2 = 24.36$)					
TAC[2]	0	14.07b	13.53b	11.27c	11.57b	$\hat{Y} = 14.07 - 0.24*G$ ($r^2 = 78.58$)					
	0.5	17.07a	18.53a	16.47b	14.25b	$\hat{Y} = 18.09 - 0.2384*G$ ($r^2 = 49.45$)					
	1.0	17.20a	18.53a	19.17a	16.74a	$\hat{Y} = 17.53 + 0.4370*G - 0.0588*G^2$ ($R^2 = 90.55$)					
NNH_3[3]	0	4.63 a	4.81 a	5.44 b	5.91 c	$\hat{Y} = 4.53 + 0.11*G$ ($r^2 = 91.56$)					
	0.5	5.05 a	5.30 a	6.16 b	10.64a	$\hat{Y} = 4.24 + 0.4018*G$ ($r^2 = 70.65$)					
	1.0	5.22 a	6.88 a	8.40 a	8.10 b	$\hat{Y} = 5.58 + 0.2727*G$ ($r^2 = 64.10$)					
DM_L[4]	0	26.83a	19.23a	22.01a	19.73a	$\hat{Y} = 24.73 - 0.4400*G$ ($r^2 = 44.00$)					
	0.5	24.8 a	9.93b	10.43b	9.19 b	$\hat{Y} = 20.81 - 1.2470*G$ ($r^2 = 62.19$)					
	1.0	11.45b	9.70b	7.23b	8.17 b	$\hat{Y} = 11.06 - 0.3367*G$ ($r^2 = 64.12$)					

[1] %; [2] Expressed in mL of 0.1N NaOH until pH reached 7.0; [3] % total nitrogen; [4] % DM; [5] error of the means; [6] Interaction of variables. $\hat{Y}_{MS} = 24.36 + 0.5583*G$ ($r^2 = 41.13$); Averages in the same row followed by same lowercase letters do not differ according to Tukey's test at 5% probability for type I error.

Observed DML values linearly decreased with the addition of glycerin and within each level of calcium oxide, whereas from 4% added glycerin levels, DML did not differ between the 0.5 and 1.0% added calcium oxide. The DM losses in sugar cane silage are very variable, reaching 31% which is well above the values found with addition of 0.5% calcium oxide and 4% crude glycerin (Freitas et al. 2004).

Conclusion It is recommended the inclusion of 0.5% calcium oxide and 4% crude glycerin in sugarcane silage, because they promote improvements in fermentation profile and reduction of dry matter losses, with potential use in animal feed.

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Chemical composition of sugarcane silage with different levels of calcium oxide and crude glycerin

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Keywords ensilage, forage conservation, glycerol

Introduction The use of sugarcane silage is a feasible option for shortage of forage in pastures, increasing the losses during the fermentation process. However, the use of additives is required due to the high level of soluble carbohydrates, as well as the large number of yeasts that promote alcoholic fermentation with high CO_2 production, increasing losses during the fermentation process. This experiment aimed to evaluate the effects of calcium oxide and crude glycerin on the chemical composition of sugarcane silage.

Materials and methods The experiment was conducted in Federal University of Mato Grosso in partnership with Embrapa Agrosylvipastoral. The sugarcane (*Saccharum officinarum* L.) cultivar RB 72-454, was chopped and ensiled in 36 PVC silos with a volume of 2.75 liters, provided with Bunsen valves. We used a factorial design (3×4) in a completely randomized design, with three replicates per treatment, consisting of: three levels of calcium oxide (0, 0.5 and 1%) and four crude glycerin levels (0, 4, 8 and 12%) in fresh matter (FM). The composition of crude glycerin was 82% glycerol, 0.52% (w/w) methanol and 70.34 g/kg of mineral. Samples were pre-dried in an oven with forced ventilation of air at 55ºC and ground to a diameter of 1 mm. DM analysis was determined by AOAC method (1990). The crude protein (CP) was obtained by determining total nitrogen, according to the micro-Kjeldahl method. The fiber analysis in neutral detergent (NDF) and in acid detergent (ADF) were performed according to Van Soest and Robertson (1985), while hemicellulose content (HEM) was calculated by the difference between the NDF and ADF. Total carbohydrates were calculated according to Sniffen et al. (1992). The effect of adding calcium oxide within each level of glycerin inclusion was assessed using Tukey's test, and for the evaluation of the effect of glycerin inclusion we made regression models, linear, quadratic and cubic, both considering a significance of 5% for type I error.

Results and discussion The values of dry matter (DM), organic matter (OM), crude protein (CP), total carbohydrates (TC), neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicellulose (HEM) are presented in Table 1. There was a significant difference ($P<0.05$) between DM values, with increased levels following the addiction of calcium oxide (CaO), and linear effect for crude glycerin addition, since calcium oxide is an absorbent additive (Santos et al. 2008), promoting increased DM in the ensiled material, and glycerin, though fluid, has a high level of DM. For OM, there was interaction between treatments, observing a linear reduction in the levels of 0 and 0.5% of calcium oxide inclusion, and for each 1% added glycerin, there is a reduction of 0.96% of OM. This reduction can be explained by the high mineral content of the crude glycerin, which provided increased mineral content of treatments. For the inclusion of 1% calcium oxide we did not find any model that would fit to the level of glycerin ($\hat{Y}_{MO} = 93.91$).

Table 1 Average and of interaction of chemical variables of sugarcane silage with inclusion of calcium oxide and crude glycerin

	CaO (%FM)			*P*-value	Crude Glycerin (% FM)				*P*-value	SEM[3]	Int.[4]
	0	0.5	1.0		0	4	8	12			
DM[1]	24.14c	28.10b	30.97a	<0.0001	23.61	27.21	29.20	30.93	<0.0001	0.58	0.1167
OM[2]	95.91	94.98	93.91	<0.0001	95.15	95.25	94.66	94.69	<0.0001	0.27	<0.0001
CP[2]	2.19a	1.96b	1.89b	<0.0001	2.27	2.13	1.92	1.73	<0.0001	0.22	0.5704
TC[2]	92.23a	92.23a	91.86a	0.7193	92.46	92.16	91.64	92.17	0.6177	0.83	0.1838
NDF[2]	53.48a	43.34b	36.40c	<0.0001	49.77	46.12	40.44	41.31	<0.0001	1.40	0.3589
ADF[2]	30.14a	26.74b	20.72c	<0.0001	29.45	26.96	23.14	23.92	<0.0001	1.03	0.1003
HEM[2]	23.34a	18.41b	16.78b	<0.0001	20.32	20.03	17.30	20.38	0.0654	1.22	0.2887

Interaction effect between the levels of addition of calcium oxide and glycerin.

	CaO	Glycerin (% FM)				Model
	(%FM)	0	4	8	12	
OM[2]	0	96.69a	96.38a	95.49a	95.09a	$\hat{Y}_{MO} = 96.76 - 0.1424*G$ ($r^2 = 93.64$)
	0.5	95.15b	95.27b	94.67b	94.87a	$\hat{Y}_{MO} = 95.21 - 0.0335*G$ ($r^2 = 41.91$)
	1.0	93.60c	94.11c	93.82c	94.11b	$*\hat{Y}_{MO} = 93.91$

[1] %; [2] % of DM; [3] error of the means; [4] interaction of variables. $\hat{Y}DM = 24.36 + 0.5583*G$ ($r^2 = 41.13$); YHEM = 19.50; $\hat{Y}TC = 92.11$; $\hat{Y}DNF = 49.09 - 0.7844*G$ ($r^2 = 79.01$); $\hat{Y}CP = 2.28 - 0.0416*G$ ($r^2 = 56.25$); $\hat{Y}ADF = 28.98 - 0.5133*G$ ($r^2 = 19.41$); Averages in the same row followed by same lowercase letters do not differ according to Tukey's test at 5% probability for type I error. *No model adjusted to the fermentation period.

The CP values showed a decrease with the addition of calcium oxide, possibly due to proportionate dilution of the cell wall components. The values also reduced with the inclusion of glycerin levels, due to the low concentration of nitrogen compounds in glycerin. The TC showed no significant difference (P> 0.05) for addition of calcium oxide and glycerin. Values of NDF, ADF and HEM linearly decreased with the addition of calcium oxide (P<0.05). These results, according to Klopfenstein (1978), can be explained by the partial solution of the fibrous fraction, with the addition of alkaline agents, as well as the lack of fibrous compounds in crude glycerin. However, the addition of crude protein showed no effect in hemicellulose contents, presenting an average value of 19.50%.

Conclusion It is recommended the addition of 0.5% calcium oxide and 4% crude glycerin in sugarcane silage, because it improved the chemical composition, with potential use in animal feed.

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The effects of *Lactobacillus kefiri* and *L. brevis* on the fermentation and aerobic stability of sugarcane silage

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Keywords aerobic stability, *L. kefiri*, *L. brevis*, *Saccharum officinarum* L., sugarcane silage

Introduction The objective of this study was to evaluate the effects of the two heterolactic bacteria *L. kefiri* DSM 19455 and *L. brevis* DSM 23231 on the fermentation and aerobic stability of sugarcane silage.

Materials and methods The experiment was carried out at the University of São Paulo, College of Agriculture "Luiz de Queiroz". Sugarcane variety IAC 93-3046 was manually harvested from five locations in one plot, after 14 months of regrowth (fourth cut), in 2013-2014. Sugarcane tillers from each location were chopped to a theoretical cut length of 8 mm. After chopping, each forage portion (derived from one location) was separated in four piles and treated as follows: no additives (Control), *L. brevis* DSM 23231 at 2×10^5 cfu/g, *L. kefiri* DSM 19455 at 2×10^5 cfu/g, *L. brevis* DSM 23231 at 1×10^5 cfu/g plus *L. kefiri* DSM 19455 at 1×10^5 cfu/g. Plastic buckets (20 L) were used as experimental silos.

Results and discussion Sugarcane is notorious for its high soluble carbohydrates (SC) content and large yeast population (Alli et al., 1982; Ávila et al., 2010). Therefore, when sugarcane is ensiled, SC are converted into fermentation end-products, especially ethanol (Kung Jr. and Stanley, 1982; Pedroso et al., 2005; Daniel et al., 2013b). Consequently, large amounts of DM and net energy are lost (Daniel and Nussio, 2011; Daniel et al., 2013a). In the current study, we hypothesized that heterolactic inoculants containing *L. kefiri* alone or in combination with *L. brevis* would increase the production of acetic acid during the fermentation phase and inhibit yeast population, resulting in better conserved silages. As expected and shown in Table 1, ensiling sugarcane with heterolactic inoculants (*L. kefiri* and *L. brevis*) decreased nutrient losses during the anaerobic storage and reduced ethanol levels significant. The inoculation with solely *L. kefiri* reduced yeast counts while the two treatments with *L. brevis* increased yeast counts (Table 1). The aerobic stability of *L. kefiri* silage was with 52.7 hours the highest compared to the other treatments, but it did not significantly differ from control with 42.2 hours. Based on results from Kleinschmit and Kung (2006) possibly a higher dose of *L. kefiri* could improve the aerobic stability to a greater extend.

Conclusions Both *L. kefiri* and *L. brevis* applied at 2×10^5 cfu/g were able to mitigate the formation of volatile organic compounds and decreased the losses of nutrients during the anaerobic storage of sugarcane silages. The *L. kefiri* also tended to decrease heat

accumulation during air exposure.

Table 1 Fermentative profile of sugarcane silages inoculated with heterolactic bacteria

Item	Unit	Control	*L. brevis*	*L. kefiri*	*L. brevis* + *L. kefiri*	*P*-value
DM losses in oven	g/kg DM_{oven}	250[a]	172[b]	183[b]	174[b]	<0.01
DM losses corr. for volatiles	g/kg DM_{corr}	137[a]	102[b]	101[b]	101[b]	<0.01
Gas losses	g/kg DM_{corr}	131[a]	85[c]	95[b]	91g[c]	<0.01
Yeast	log cfu/g	3.41[a]	4.75[b]	2.72[c]	4.97[b]	<0.01
Mold	log cfu/g	1.74	1.62	1.48	1.48	0.90
pH		3.84	3.85	3.86	3.83	0.32
Ethanol	g/kg DM_{corr}	134[a]	66[b]	73[b]	67[b]	<0.01
Lactic acid	g/kg DM_{corr}	34[a]	30[b]	26[c]	29[bc]	<0.01
Acetic acid	g/kg DM_{corr}	20[a]	22[a]	26[b]	23[a]	<0.01

Means within a row with different superscripts differ ($P<0.05$).

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Effect of sodium nitrite on clostridium control during the fermentation of sugarcane silages treated with lime

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Keywords butyric acid bacteria, chemical additive, spore forming bacteria

Introduction Naturally fermented sugarcane results in large losses of DM and net energy. Among the additives used to inhibit epiphytic yeast populations and ethanol formation, lime has been reported with positive effects on DM recovery. However, the alkaline origin of lime increases silage pH, enabling the development of undesirable microorganisms, such as clostridia. Sodium nitrite is well-known for its capacity to suppress spore forming bacteria (Woolford, 1975). Therefore, the aim of this study was to combine lime with different sodium nitrite doses which might prevent clostridium growth in sugarcane silages.

Materials and methods Fresh chopped sugarcane added with 15 g of lime per kg fresh matter (FM) was treated with increased dosages of sodium nitrite, as follows: 0, 0.5, 1.0, and 1.5 g/kg FM. Sodium nitrite was diluted in distilled water and sprayed onto the forage (5 mL/kg FM). After the treatment, approximately 300 g of forage was packed into nylon-polyethylene bags, vacuumed and heat sealed (5 bags per treatment). All bags were stored for 90 d (at 25°C). At opening, samples were collected for *Clostridium* enumeration and analysis of DM, pH, ammonia-N, organic acids, alcohols, esters, and acetone by standard methods. Statistical analysis was performed using the MIXED procedure of SAS as a completely randomized design. Degrees of freedom for treatment were partitioned into two single degree of freedom orthogonal contrasts: linear effect and quadratic effect of sodium nitrite dose. Contrasts were declared significant at $P \leq 0.05$.

Results and discussion Contents of DM, ammonia-N and acetic acid increased linearly with sodium nitrite levels. Lactic acid concentration was unchanged, whereas silage pH, propionic acid, 1,2-propanediol, and acetone follow a quadratic trend with higher values for intermediate doses. Although sodium nitrite quadratically decreased ethanol, all treatments had low concentrations. Sodium nitrite did not alter *Clostridium* counts, whereas the concentrations of butyric acid, valeric acid, and 2,3-butanediol were significantly reduced with sodium nitrite, indicating an inhibition of *Clostridium* metabolism. However, the butyric acid concentration (5.9 g/kg DM) achieved with the highest dose of sodium nitrite (1.5 g/kg FM) was just above the target value for well-fermented silages (Haigh and Parker 1985). At ensiling, the concentration of nitrite (NO_2) supplied by the highest dose of sodium nitrite was 2.9 g/kg DM (i.e., 1.5 g $NaNO_2$ per kg FM = 4.4 g $NaNO_2$ per kg DM = 2.9 g NO_2 per kg DM). Even if NO_2 was not analyzed in silages, it is likely that the residual concentrations would be lower than the toxic levels capable to cause poisoning in cattle. In high pH silages most NO_2 is converted to nitrogenous gases by silage microorganisms within a few days after ensilage (Spoelstra, 1985). In this way, ammonia-N concentration

increased with nitrite supplementation, indicating that most of the added NO_2 should be converted to ammonia and volatile forms of N, such as nitric oxide.

Conclusions Sodium nitrite decreased *Clostridium* activity in sugarcane silages treated with lime. However, none of evaluated doses were able to provide completely butyric acid free silages.

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Table 1 Influence of sodium nitrite on fermentation of lime-treated sugarcane silages (mg/kg DM, unless otherwise stated)

	Sodium nitrite dose (g/kg FM)					*P*-value†	
Item	0	0.5	1.0	1.5	SEM	L	Q
Clostridia, log cfu/g FM	5.3	5.9	5.1	5.3	0.17	0.30	0.27
DM, g/kg FM	303	308	320	339	2.8	<0.01	0.04
pH	4.76	5.09	4.93	4.71	0.05	0.16	<0.01
Ammonia-N, g/kg N	4.6	4.4	23.5	42.6	6.43	<0.01	0.26
Lactic acid, g/kg DM	51.2	47.1	48.3	50.7	2.9	0.99	0.28
Butyric acid, g/kg DM	42.0	52.9	33.4	5.9	4.1	<0.01	<0.01
2,3-Butanediol, g/kg DM	32.9	18.6	10.9	7.2	1.9	<0.01	0.02
Acetic acid, g/kg DM	25.9	25.3	41.2	42.2	2.8	<0.01	0.79
Ethanol, g/kg DM	7.2	3.4	1.8	4.4	0.98	0.04	<0.01
Lactic:acetic ratio	2.0	1.9	1.2	1.2	0.12	<0.01	0.47
1,2-Propanediol	178	330	446	321	47.3	0.02	0.01
Valeric acid	142	92	61	51	30.1	0.04	0.51
Propionic acid	134	266	233	185	287	0.36	<0.01
i-Valeric acid	80	151	24	49	66.4	0.47	0.73
Ethyl lactate	19	32	29	51	11.8	0.10	0.73
1-Propanol	11	60	7	106	34.7	0.16	0.48
i-Propanol	11	15	15	18	3.3	0.23	0.94
Acetone	8	16	16	12	28.3	0.29	0.06
Ethyl acetate	4	14	4	18	75	0.35	0.81
2-Butanol	2	6	2	8	2.7	0.29	0.79
Propyl acetate	2	16	8	14	5.3	0.26	0.52

†L: linear effect of sodium nitrite dose, Q: quadratic effect of sodium nitrite dose.

The effects of chemical and microbial additives on the emissions of ethanol and acetic acid in sugarcane silages

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Keywords energy losses, reactive organic gas, volatile organic compounds

Introduction Sugarcane silage is an attractive forage source in tropical areas. However, ensiling sugarcane results in the conversion of most of the water-soluble carbohydrates into fermentation end-products, which are rich in volatile organic compounds (VOC) (Daniel et al., 2013). In the presence of sun light, VOC react with nitrogen oxides to form tropospheric ozone, an air pollutant (Krauter and Blake, 2009). Furthermore, the emission of VOC represents nutrient losses (Daniel and Nussio, 2011). As in corn silages (Montes et al., 2010), ethanol and acetic acid are the major VOC in sugarcane silages (Daniel et al., 2013). Thus, the aim of this study was to evaluate the effects of chemical and microbial additives on the emissions of ethanol and acetic acid in sugarcane silages.

Materials and methods Sugarcane crop (IAC 93-3046) was grown at the "Luiz de Queiroz" Campus, Piracicaba, and mechanically harvested after 10 months of regrowth (3rd cut). Chopped forage was treated and packed in 8 bag silos (1.5 m i.d.) [2 bags of 10 t fresh matter (FM) per treatment]. Treatments were: no additive (control), urea applied at 10 g/kg FM, *Lactobacillus buchneri* CNCM I-4323 applied at 5×10^5 cfu/g FM, and sodium benzoate applied at 2 g/kg FM. After 90 d of storage, the silos were opened and the collections were performed on the silo working face (undisturbed silage) at 0 and 4 h after silage feedout. The VOC emissions were measured using surface isolation flux chambers (Odoflux, Odotech) coupled to a photoacoustic field gas-monitor (INNOVA 1412, LumaSense). Sweep air flow (ultra zero compressed air) was set at 10 mL/min and sample collections were performed during 15 min at steady-state (30 min after the flow start up) (Krauter and Blake, 2009). Flux rates were calculated in mg/m^2 per minute. Silage samples were also collected and analyzed for ethanol and acetic acid concentrations by gas chromatograph-mass spectrometry. Data were analyzed using the Mixed procedure of SAS, including random effect hour and fixed effect of treatment. Means were compared using the Tukey-Kramer test ($\alpha = 0.05$ and 0.10).

Results and discussion As expected, the control silage (without additive) had higher concentrations of ethanol and acetic acid (Daniel and Nussio, 2011). On the other hand, sodium benzoate applied at 2 g/kg fresh matter significantly decreased the concentrations of ethanol and acetic acid (Table 1). Urea and *L. buchneri* had high concentrations of acetic acid like the control, whereas the inoculation with *L. buchneri* at 5×10^5 cfu/g FM led to intermediary levels of ethanol. Due to the emission rates were positively correlated with the VOC concentrations (Figure 1), the silages preserved without additive or treated with urea or *L. buchneri* had higher emission rates than the silage treated with sodium benzoate. Additionally, sodium benzoate was the most effective additive to mitigate ethanol emission, whereas *L. buchneri* results in intermediary values.

Table 1 Influence of additives on the concentrations and emissions of ethanol and acetic acid in sugarcane silages

Compound	Control	Urea	*L. buchneri*	Benzoate	SEM	*P*-value
	Concentration (g/kg DM)					
Ethanol	69.7^{a}	59.9^{a}	25.4^{b}	13.6^{c}	4.1	0.04
Acetic acid	60.9^{x}	57.7^{x}	69.2^{x}	38.7^{y}	7.2	0.09
	Emission (mg/m^2.min)					
Ethanol	334^{a}	290^{ab}	152^{bc}	63^{c}	33	<0.01
Acetic acid	11.4^{ab}	19.8^{a}	14.3^{ab}	6.5^{b}	2.5	0.02

Means with different letters differ, α = 0.05 for a, b, and c; α = 0.10 for x and y.
SEM: standard error of the mean.

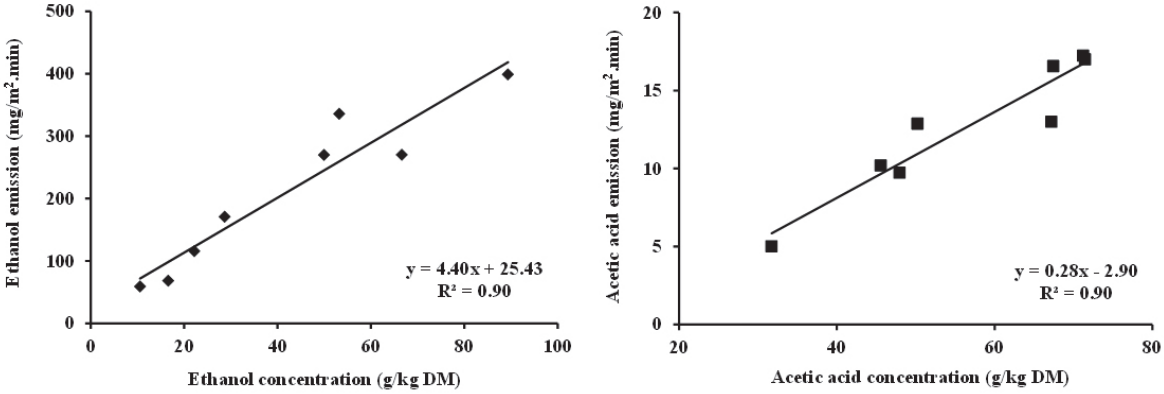

Figure 1 Relationship between emissions and concentrations of ethanol and acetic acid in sugarcane silages.

Conclusion 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. Sodium benzoate applied at 2 g/kg fresh matter was very effective to prevent the formation and the emission of ethanol and acetic acid in sugarcane silages.

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Effect of chemical and microbial additives on the dynamics of gas production during the fermentation of sugarcane silage

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Keywords ethanol, gas loss, sodium benzoate, volatile organic compounds

Introduction During the silage fermentation, most water-soluble carbohydrates are mainly converted to organic acids, alcohols, esters, ketones, aldehydes, water and gases. Therefore, gases are the main sink of dry matter (DM) loss. In sugarcane silages preserved without additives, the prevalence of alcoholic fermentation leads to high DM losses (up to 30%). Thus, the objective was to evaluate the effects of silage additives on the dynamics of gas formation during sugarcane fermentation.

Materials and methods Sugarcane variety IAC 93-3046 (22.3°brix, 36%DM) was manually harvested, mechanically chopped (8 mm) and treated as follows (as fed basis): control (without additive), *Lactobacillus buchneri* CNCM I-4323 (5×10^5 cfu/g), urea (10 g/kg), lime (10 g/kg), or sodium benzoate (2 g/kg). All additives were diluted in distilled water (5 mL/kg) and sprayed onto the forage. The same amount of water was applied to the control. Afterwards, treated forages were packed (0.40 porosity) into 1.96 L gas-tight silos (4 replicates/treatment). Silos were stored inside the lab at 25±1°C. The internal pressure of the silos was measured by using a pressure transducer connected to a data displayer. Readings were taken every 12 h until d-3, every 24 h from d-4 until d-21, every 3 d from d-22 until d-30, and every week until d-124 (end of storage). Pressure was converted to volume and presented as accumulated gas production per kg DM (see Daniel and Nussio, 2015). Gas and DM losses were also measured by the gravimetric method (weight difference). Silage fermentation end-products were analyzed by gas chromatograph-mass spectroscopy, except lactic acid which was determined by colorimetry. Clostridium counts were determined by pour-plating standard procedures. The DM was determined in an oven and corrected for volatile compounds (DM_{corr}) (Weissbach, 2009). Soluble carbohydrates were determined by the phenol-sulfuric method. Silage samples were exposed to air for 10 d to determine the aerobic stability (2°C rise in temperature above the ambient). Data were analyzed with the Mixed procedure of SAS.

Results and discussion The cumulative gas production is presented in Figure 1, whereas the fermentation profile and aerobic stability are shown in Table 1. As expected, sugarcane ensiled without additives (control) had highest gas production and highest DM losses. Urea had a discrete effect on the fermentation, although sodium benzoate and lime were very effective to control the alcoholic fermentation, prevent the formation of volatile organic compounds and mitigate nutrient losses. *L. buchneri* led to intermediate responses. All additives improved the aerobic stability, however better improvements were obtained with lime and sodium benzoate. Although lime seems a more attractive option due the lower cost, the alkaline origin of lime increases silage pH, enabling the development of undesirable microorganisms, such as clostridia, which was followed by increased butyric acid concentration.

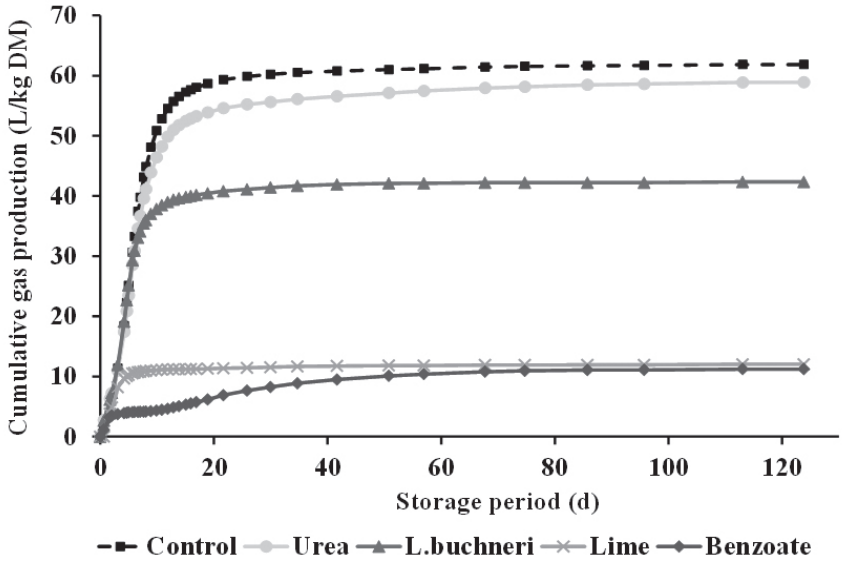

Figure 1 Influence of chemical and microbial additives on the dynamics of gas production in sugarcane silages ($P<0.01$ for interaction between treatment and storage period).

Table 1 Fermentation profile and aerobic stability of sugarcane ensiled with chemical and microbial additives

	Treatment						
Item	Control	Urea	*L. buchneri*	Lime	Benzoate	SEM	*P*-value
DM_{corr}, g/kg as fed	339^{c}	345^{bc}	341^{c}	358^{a}	353^{ab}	2.1	<0.01
Soluble carbohydrates, g/kg DM_{corr}	117^{d}	107^{d}	182^{c}	214^{b}	250^{a}	3.2	<0.01
pH	3.72^{c}	3.89^{b}	3.64^{d}	4.23^{a}	3.67^{cd}	0.01	<0.01
Ethanol, g/kg DM_{corr}	111^{a}	92^{b}	57^{c}	8.3^{d}	3.1^{d}	3.2	<0.01
Lactic acid, g/kg DM_{corr}	38^{b}	31^{b}	38^{b}	74^{a}	42^{b}	4.7	<0.01
Acetic acid, g/kg DM_{corr}	14^{c}	20^{b}	30^{a}	34^{a}	26^{b}	1.6	<0.01
1,2-Propanediol, g/kg DM_{corr}	3.4^{c}	7.6^{b}	11.7^{a}	0.6^{d}	1.4^{d}	0.8	<0.01
Ethyl lactate, mg/kg DM_{corr}	1001^{a}	983^{a}	709^{b}	89^{c}	47^{c}	29.3	<0.01
Ethyl acetate, mg/kg DM_{corr}	581^{a}	657^{a}	600^{a}	98^{b}	30^{b}	56.8	<0.01
Butyric acid, mg/kg DM_{corr}	11^{b}	7.3^{b}	4.3^{b}	345^{a}	9.7^{b}	72	0.04
Clostridia, log cfu/g as fed	2.6^{bc}	3.0^{b}	2.7^{bc}	5.4^{a}	1.9^{c}	0.20	<0.01
Gas losses[1], g/kg DM	112^{a}	110^{b}	76^{c}	26^{d}	13^{d}	5.2	<0.01
Gas production[2], g/kg DM	120^{a}	115^{b}	82^{c}	23^{d}	21^{d}	5.4	<0.01
DM_{corr} losses, g/kg DM	102^{a}	86^{b}	83^{c}	19^{d}	28^{d}	5.0	<0.01
DM_{oven} losses, g/kg DM	210^{a}	184^{b}	148^{c}	22^{d}	12^{d}	5.1	<0.01
Aerobic stability, h	153^{c}	192^{b}	201^{b}	225^{a}	>240^{a}	6.4	<0.01

$^{a-d}$Means within a row with different superscripts differ (Tukey-Krammer, $\alpha = 0.05$). [1]Determined by the gravimetric method. [2]Determined by the volumetric method (Daniel and Nussio, 2015).

Conclusion Sodium benzoate at 2 g/kg (as fed) is a feasible additive to preserve sugarcane as silage.

Reference

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The effects of adding molasses to sugar beet pulp on the silage quality

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Keywords sugar beet pulp, molasses, silage quality, aerobic stability

Introduction Sugar beet pulp and molasses are by-products from sugar production. In the sugar factory, molasses are added to the sugar beet pulp, which can be used ensiled or dried as an animal feed. In this experiment, two different amounts of molasses were added to sugar beet pulp and the effect on the silage quality, as well as on the aerobic stability, was investigated.

Materials and methods In 2013, round bales only with sugar beet pulp (T-0), sugar beet pulp with 7% molasses (T-7) and sugar beet pulp with 14% molasses (T-14) were produced. The bales had a volume of 1.4 m^3 and a weight of 1088, 1163 and 1196 kg for the three treatments T-0, T-7 and T-14. After a storage period of 120 days samples were taken. Dry matter (DM) and nutrient contents were analysed in the fresh and ensiled material. Furthermore, pH, silage acids, aerobic stability, as well as the microbiological quality (aerobic bacteria, molds and yeasts), were investigated in three bales per treatment. Data were analysed using analysis of variance and Bonferroni-Test (Systat 13).

Results and discussion The addition of molasses significantly increased the DM, ash and water-soluble carbohydrates content (WSC) in the fresh pulp. On the other hand the ADF and NDF contents decreased with increasing proportion of molasses (Table 1). The WSC was degraded during the fermentation process . The more WSC was in the fresh material, the more WSC was degraded and lactic acid was produced (Table 2). But the pH-values were not influenced by the addition of molasses. All silages showed a very good silage quality and reached all the maximum DLG points of 100. With increasing proportion of molasses the silages had a better aerobic stability, but the values were statistically not different. All silages showed a good microbiological quality (Figure 1). The two silages with molasses showed less molds.

Table 1 DM and nutrient contents of the fresh material

		T-0 0% molasses	T-7 7% molasses	T-14 14% molasses	SE	P-value
DM	%	32.4[a]	34.8[b]	37.4[c]	0.17	< 0.001
Ash	g/kg DM	83[a]	93[b]	96[b]	1.0	0.006
Protein	g/kg DM	81[a]	89[b]	95[c]	0.9	0.004
ADF	g/kg DM	239[a]	204[b]	180[c]	2.1	< 0.001
NDF	g/kg DM	484[a]	382[b]	329[c]	5.1	< 0.001
WSC	g/kg DM	18[a]	107[b]	171[c]	7.7	0.002

DM: dry matter; ADF: acid detergent fiber; NDF: neutral detergent fiber; WSC: water soluble carbohydrates; SE: standard error

Table 2 DM, nutrient contents and silage parameters of the different silages

		T-0 0% molasses	T-7 7% molasses	T-14 14% molasses	SE	P-value
DM	%	32.7[a]	35.3[b]	37.9[c]	0.12	< 0.001
Ash	g/kg DM	78[a]	91[ab]	98[b]	4.1	0.041
Protein	g/kg DM	82[a]	91[b]	99[c]	1.0	< 0.001
ADF	g/kg DM	247[a]	222[b]	197[c]	3.7	< 0.001
NDF	g/kg DM	482[a]	412[b]	370[b]	10.1	< 0.001
WSC	g/kg DM	13[a]	28[b]	49[c]	3.1	< 0.001
pH		4.3	4.4	4.4	0.02	0.217
Lactic acid	g/kg DM	22[a]	37[b]	51[c]	0.4	< 0.001
Acetic acid	g/kg DM	5[a]	14[b]	18[c]	0.2	< 0.001
Butyric acid	g/kg DM	0.1[a]	0.4[b]	0.8[c]	0.01	< 0.001
Ethanol	g/kg DM	8[a]	21[b]	26[c]	0.9	< 0.001
NH_3-N/N tot	%	1.6	1.6	1.8	0.33	0.965
DLG Points		100	100	100		
Aerobic stability	hours	66	78	110	16.1	0.218

DM: dry matter; ADF: acid detergent fiber; NDF: neutral detergent fiber; WSC: water soluble carbohydrates; SE: standard error of the means.

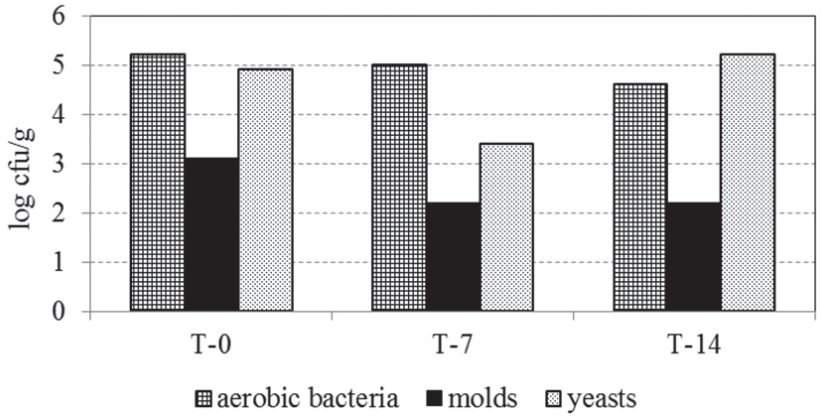

Figure 1 Microbiological quality of the different sugar beet pulp silages with 0, 7 and 14% molasses (cfu: colony format units).

Conclusions The addition of molasses to sugar beet pulp increased the content of DM and water-soluble carbohydrates. However, the latter were rapidly fermented to lactic acid. All silages showed a good silage quality, and the aerobic stability was positively influenced by the addition of molasses.

Effect of additives on the quality of corn straw and sugar beet mixed silage

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Keyword additives, corn straw, sugar beet, fermentation quality, mixed silage

Introduction Sugar beet (*Beta vulgaris* L.; SB) is a main economic crop in the northeast region of China. Ensiling sugar beet mixed with other crops is a strategy to supply feedstuffs for livestock during the spring and winter seasons. Ensiling could avoid mildew and rot of fresh forages and would decrease environmental pollution. The aim of this study was to evaluate the effect of additives on the quality of corn straw and sugar beet silage.

Material and methods Corn (*Zea may* L.) straw (CS), SB, and three silage additives [Lalsil Fresh (LF; *Lactobacillus buchneri*), cellulose (CE) and beet pulp (BP)] were used in this study. Corn straw was chopped into 1 to 2 cm, and SB was shredded with a rubbing filament machine, and then both materials were mixed in the ratio of 3:1. The mixed material was ensiled in laboratory silos (25 cm high with an internal diameter of 7.6 cm, at the density from 700 g L^{-1}), with no additives (CK), LF [5 g t^{-1} fresh forage (FM); 5×10^5 CFU/g], CE (2.5 g t^{-1} FM), BP (5 g t^{-1} FM), LF+CE (5 g t^{-1} + 2.5 g t^{-1} FM), and CE+BP (2.5 g t^{-1} + 5 g t^{-1} FM), respectively. Each treatment had three replicates. The fermentation quality and nutrient compositions of the silages were determined after 50 days of storage. Data were processed and analyzed by one-way ANOVA with SAS.

Results and discussion The nutrient compositions of the mixed silages are presented in Table 1. The crude protein (CP) content of the mixed silages added with CE and LF+CE was higher than other treatments ($P<0.05$). The mixed silages with no additives (CK) had higher crude fiber (CF) than additives treatments ($P<0.05$) except the BP. The neutral detergent fiber (NDF) concentrations of CK and BP were higher than that of LF and CE +BP ($P<0.05$) and no difference with that of CE and LF+CE. There was no difference in the acid detergent fiber (ADF) and ether extract (EE) concentrations between all treatments. The organic acid concentrations are presented in Table 2. The treatments of LF and LF+CE had higher lactic acid (LA) concentration than other treatments ($P<0.05$), and CK, BP and CE +BP higher than CE ($P<0.05$).The acetic acid (AA) of CE was the highest in all treatments ($P<0.05$), and CK, BP and CE +BP higher than LF and LF+CE ($P<0.05$). The mixed silages with BP had highest propionic acid (PA) ($P<0.05$) in all treatments, LF+CE had lowest ($P<0.05$), there was no difference between other treatments ($P>0.05$). Butyric acid (BA) was not detected in all treatments.

Conclusion Adding LF, BP and LF+CE to the corn straw and sugar beet mixed silage can improve the fermentation. The effect of LF+CE on quality is better than other additives.

Table 1 The nutrient compositions (g kg^{-1} DM) of the mixed silage

Treatments	CP	CF	NDF	ADF	EE
CK	96.7b	261.1a	632.0a	320.3	6.4
LF	97.2b	234.2b	614.0b	324.0	5.0
CE	102.3a	247.8b	625.2ab	340.6	4.6
BP	95.2b	253.7ab	635.3a	345.1	5.7
LF+CE	100.5a	239.8b	629.7ab	340.5	5.9
CE +BP	96.1b	247.1b	619.6b	349.4	5.5
SEM	2.67	2.98	4.67	4.92	0.78

Different letters in each column shows significant differences ($P < 0.05$).

Table 2 The organic acid (g kg^{-1} DM) of the mixed silage

Treatments	LA	AA	PA	BA
CK	78.3b	16.4b	5.2b	n.d.
LF	90.2a	3.7c	6.0b	n.d.
CE	70.2c	23.8a	5.9b	n.d.
BP	75.6b	15.7b	8.6a	n.d.
LF+CE	92.4a	5.6c	1.9c	n.d.
CE +BP	77.7b	16.6b	5.5b	n.d.
SEM	1.75	0.59	0.46	-

Different letters in each column shows significant differences ($P < 0.05$). n.d.: not detected.

Effects of microbial additives on fermentation and aerobic stability of alfalfa, perennial ryegrass and red clover/perennial ryegrass silage

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Keywords lactic acid bacteria, grass, alfalfa, fermentation, aerobic stability

Introduction New lactic acid bacteria (LAB) strains and mixtures are emerging and are believed to improve silage quality by reducing pH and shifting the fermentation end products profile. However, there is still little information available on how significant the new species are to silage preservation. An in vitro experiment was performed to investigate whether the addition of new LAB cultures can affect the fermentation end product profile and aerobic stability of different forages.

Materials and methods Alfalfa at early-flower stage, perennial ryegrass at early-boot stage and red clover/perennial ryegrass at early bloom stage of red clover were harvested and wilted. Forages were chopped to about 2.5 cm and ensiled in 3 liter silos to a target density of 0.2 kg DM/L. The additives applied to the different forages were (C) no additive (sterile water); (T1) Feedtech™ Silage F22 (*L. lactis, P. acidilactici, E. faecium, L. plantarum, xylanase, sodium benzoate,* targeting 5.0×10^5 g/kg forage); (T2) Feedtech™ Silage M25AS (*L. plantarum, P. pentosaceus, L. buchneri,* targeting 2.0×10^5 g/kg forage); (T3) Feedtech™ Silage F600 (*L. buchneri, L. plantarum,* targeting 2.3×10^5 g/kg forage), with 5 replicates per inoculant/forage combination. Silages nutrient content, fermentation parameters and aerobic stability were analysed after 90 d of storage at 20°C. Aerobic stability was determined by monitoring the temperature increase in silages stored in insulated PVC-tubes until silages were 3°C above the ambient temperature. Fermentation and aerobic stability (incubation time where the silo temperature exceeded the ambient temperature with at least 3°C) data were statistically analyzed by using respectively PROC MIXED and PROC LIFETEST of SAS.

Results and discussion Mean forages DM, WSC and buffering capacity for alfalfa, perennial ryegrass and red clover/perennial ryegrass were 398, 350 and 327 g/kg; 48.9, 78.0 and 79.2 g/kg DM and 375, 260 and 265 mEq/kg DM, respectively. Inoculation affected fermentation profile of all ensiled forages (Table 1). Inoculant treatments produced larger ($P < 0.05$) reductions in pH compared with control treatment, and the largest pH reduction was obtained with T1 treatment for all three types of silages. In all three forages, treatment T1 resulted in higher ($P < 0.05$) lactic acid concentrations when compared to the C and treatments T2 and T3. Acetic acid concentration increased ($P < 0.05$) in perennial ryegrass and red clover/perennial ryegrass for treatment T3 compared with control (C) and treatments T1 and T2. Except for red clover/perennial ryegrass T2 and T3 silages, inoculants reduced ($P < 0.05$) ammonia-N formation. Alcohols were significantly lower in all experimental treatments compared with the control ($P < 0.05$). There was a significant reduction ($P < 0.05$) in DM losses during the whole storage time in all inoculated silages, and treatment T2 gave the lowest DM loss for the all three forages. Treatment with T3 treatment improved aerobic stability with all silages, while treatment T1 improved aerobic

stability of alfalfa and perennial ryegrass silages. Treatment with T2 treatment did not affect silages aerobic stability. This is in agreement with previous findings in which sodium benzoate improved aerobic stability of silage when combined with inoculants (Saarisalo et al., 2006) and silages with higher acetic acid content were more resistant for the aerobic deterioration than those with lower acetic concentration (Hu et al., 2009).

Table 1 Chemical composition and aerobic stability of silages

Silage	Item	C	T1	T2	T3
A	DM*	38.5 ^{a}	39.6 ^{b}	39.1 ^{ab}	39.5 ^{b}
	pH	5.03 ^{a}	4.60 ^{b}	4.71 ^{c}	4.68 ^{c}
	Lactic acid, % DM	3.40 ^{a}	5.99 ^{b}	4.60 ^{c}	4.94 ^{bc}
	Acetic acid, % DM	1.96 ^{a}	2.04 ^{a}	2.59 ^{bc}	2.70 ^{bc}
	N-NH3 fraction, % total N	8.34 ^{a}	5.40 ^{b}	6.55 ^{cd}	6.52 ^{cd}
	Alcohols, % DM	0.57 ^{a}	0.21 ^{b}	0.29 ^{c}	0.25 ^{bc}
	DM loss, %	6.80 ^{a}	3.22 ^{b}	4.40 ^{c}	3.68 ^{cb}
	Aerobic stability, hours	127 ^{a}	240 ^{b}	160 ^{ad}	240 ^{bc}
P	DM*	33.2 ^{a}	34.4 ^{b}	34.1 ^{b}	34.0 ^{b}
	pH	4.54 ^{a}	4.02 ^{b}	4.13 ^{cd}	4.22 ^{cd}
	Lactic acid, % DM	3.48 ^{a}	7.55 ^{b}	5.59 ^{cd}	4.74 ^{cd}
	Acetic acid, % DM	1.97 ^{a}	2.28 ^{a}	2.10 ^{a}	3.75 ^{b}
	N-NH3 fraction, % total N	7.48 ^{a}	5.00 ^{bc}	5.70 ^{bc}	6.16 ^{abc}
	Alcohols, % DM	0.94 ^{a}	0.46 ^{b}	0.52 ^{b}	0.71 ^{c}
	DM loss, %	6.83 ^{a}	2.43 ^{b}	3.45 ^{bc}	4.04 ^{c}
	Aerobic stability, hours	108 ^{a}	216 ^{bc}	175 ^{ab}	232 ^{c}
RP	DM*	31.3 ^{a}	32.1 ^{a}	31.8 ^{a}	31.8 ^{a}
	pH	4.59 ^{a}	4.21 ^{b}	4.30 ^{c}	4.44 ^{d}
	Lactic acid, % DM	6.18 ^{a}	9.01 ^{b}	6.05 ^{a}	6.41 ^{a}
	Acetic acid, % DM	2.26 ^{a}	1.95 ^{b}	2.47 ^{a}	3.10 ^{c}
	N-NH3 fraction, % total N	4.83 ^{a}	3.54 ^{b}	4.02 ^{ab}	4.27 ^{ab}
	Alcohols, % DM	0.66 ^{a}	0.30 ^{b}	0.31 ^{b}	0.33 ^{b}
	DM loss, %	5.88 ^{a}	3.25 ^{b}	4.28 ^{bc}	4.59 ^{c}
	Aerobic stability, h	97.2 ^{a}	154 ^{ab}	155 ^{ab}	179 ^{b}

* Dry matter, fermentation parameters are corrected for volatiles. $^{a-d}$ Means within a row with unlike superscripts differ ($P < 0.05$). A=Alfalfa; P=Perennial ryegrass; RP=Red clover/perennial ryegrass, C= Negative control, T1= Feedtech™ Silage F22, T2= Feedtech™ Silage M25AS, T3= Feedtech™ Silage F600

Conclusions In this study, the addition of combined LAB strains inoculants improved fermentation characteristics of alfalfa, perennial ryegrass and red clover/perennial ryegrass silage. In particular, *L. buchneri* in combination with *L. plantarum* consistently improved silages aerobic stability.

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The effect of silage additive on volatile organic compounds of white lupin-wheat silage

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Keywords silage additive, ethanol, ethyl ester, volatile organic compound

Introduction Only a few studies of silage as source of volatile organic compounds (VOC) and their impact on animal health and feed intake are available. The produced amount of VOC in silages might depend on fermentation type and ensiling material (Hafner et al., 2013). The aim of this experiment was to study the effect of different silage additives on VOC production, especially ethyl acetate and ethyl lactate esters.

Materials and methods White lupin (*Lupinus albus*, var. Ludic) and spring wheat (*Triticum aestivum, var.* Amaretto) were harvested in 2012 at the University of Helsinki at two growth stages: 96 days (G1) and 110 days (G2) after sowing. At G1 wheat was at the beginning of the dough stage and at G2 at the end of the dough stage. Two mixtures of lupin and wheat were composed for ensiling: 1/3 lupin + 2/3 wheat and 2/3 lupin + 1/3 wheat in fresh weight. The mixtures were treated with three different silage additives: 1) formic acid (FA) 4 L (100%)/t; 2) mixture of sodium nitrite (0.75 kg/t) and hexamethylentetramine (hexamine) (0.5 kg/t) (NaHe); 3) *Lactobacillus plantarum* 1×10^6cfu/g fresh forage (LAB). The control (CON) was without any treatment. Silos were opened after 100 days of storage. The VOC-analyses were made at the University of Berlin. Normally distributed variables were tested with ANOVA (SAS 9.3). Non-normal distributed data were tested with Kruskall-Wallis analysis (SPSS, version 21).

Results and discussion The performance of the additives in limiting VOC varied between the different growth stages and mix ratio of white lupin and wheat (Table 1). Additives reduced the concentration of ethanol and total esters compared with the control ($P<0.001$) in all mixtures. Chemical additives FA and NaHe decreased the formation of ethanol and total esters significantly compared with LAB. Increased proportion of lupin increased concentration of VOC. There were no significant differences between the chemical additives concerning the production of VOC. In this experiment FA did not activate yeasts to produce ethanol. Highest ethanol values were found in the control silages.

Conclusions Application of silage additives did decrease the concentration of VOC. In this study chemical additives were superior to biological additives due to their influence on fermentation patterns.

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Table 1 The effect of additive on silage volatile organic compounds (mg/kg DM, unless otherwise stated)

	Silage additive[1]					Statistical significance[1]		
	CON	FA	NaHe	LAB	SEM	CON vs. additives	LAB vs. FA and NaHe	FA vs. NaHe
MIX 1								
Dry matter, g/kg	290	311	313	309	6.3	0.02	0.68	0.80
pH	4.53	4.28	5.01	3.75	0.028	<0.001	<0.001	<0.001
VFA total, g/kg DM	42.8	11.1	10.5	4.8	1.42	<0.001	0.01	0.77
Ethanol, g/kg DM	25.2	3.8	2.3	6.4	0.55	<0.001	<0.01	0.10
Other alcohols, g/kg DM	3.2[a]	1.9[ab]	1.8[ab]	1.6[b]	0.26	non-normally distributed		
Acetone	93	193	147	112	27.5	0.10	0.12	0.29
Ethyl acetate	92[a]	0[b]	0[b]	0[b]	9.6	non-normally distributed		
Ethyl lactate	78	24	31	171	7.8	0.79	<0.001	0.55
Ethyl esters total	170	24	31	171	12.2	<0.001	<0.001	0.70
MIX 2								
Dry matter, g/kg	226	237	240	245	4.1	0.02	0.24	0.56
pH	4.60	4.06	4.67	3.83	0.094	0.01	<0.01	<0.001
VFA total, g/kg DM	55.1	19.2	12.5	7.27	4.69	<0.001	0.17	0.35
Ethanol, g/kg DM	28.3	5.9	5.4	12.9	0.001	<0.001	<0.01	0.82
Other alcohols, g/kg DM	8.3	3.8	3.8	3.4	0.31	<0.001	0.35	0.99
Acetone	122	173	234	210	63.0	non-normally distributed		
Ethyl acetate	273	0	0	220	16.4	<0.001	<0.001	1.00
Ethyl lactate	41	0	27	219	18.1	0.09	<0.001	0.32
Ethyl esters total	315	0	27	439	16.7	<0.001	<0.001	0.29
MIX 3								
Dry matter, g/kg	315	311	317	327	5.3	0.58	0.09	0.43
pH	4.05	4.69	4.20	4.08	0.047	<0.01	<0.001	<0.001
VFA total, g/kg DM	10.8	21.9	9.4	10.0	1.71	0.180.028	0.03	<0.001
Ethanol, g/kg DM	5.8	4.0	1.6	4.4	0.01	<0.01	<0.01	<0.01
Other alcohols, g/kg DM	1.6	1.5	1.8	1.3	0.12	0.41	0.03	0.08
Aceton	156	142	158	161	14.4	0.87	0.56	0.46
Ethyl acetate	101	0	0	0	28.1	non-normally distributed		
Ethyl lactate	107	0	0	69	6.3	<0.001	<0.001	1.00
Ethyl esters total	207[a]	0[b]	0[b]	69[a]	31.0	non-normally distributed		
MIX 4								
Dry matter, g/kg	218	226	229	245	9.5	0.20	0.16	0.86
pH	3.93	4.20	3.96	4.00	0.146	0.50	0.69	0.28
VFA total, g/kg DM	15.5	23.5	12.5	20.3	6.85	0.69	0.79	0.29
Ethanol, g/kg DM	11.3	3.4	3.6	7.6	0.87	<0.001	<0.01	0.90
Other alcohols, g/kg DM	3.7	2.6	3.2	2.8	0.39	0.11	0.78	0.35
Acetone	176	272	197	194	32.7	0.27	0.34	0.15
Ethyl acetate	190	0	0	83	42.7	non-normally distributed		
Ethyl lactate	180[a]	0[b]	0[b]	95[a]	12.0	non-normally distributed		
Ethyl esters total	371[a]	0[b]	0[b]	178[a]	41.1	non-normally distributed		

[1]CON=no additive, FA=formic acid, NaHe=hexamethylentetramine and sodium nitrite mixure, LAB=*Lactobacillus plantarum*
MIX 1:growth stage 1, white lupin / spring wheat ratio 1:2 of fresh weight; MIX 2:growth stage 1, white lupin/ spring wheat ratio 2:1 of fresh weight; MIX3: growth stage 2, white lupin/ spring wheat ratio 1:2 of fresh weight; MIX4: growth stage 2, white lupin/ spring wheat ratio 2:1 of fresh weight.
Other alcohols: methanol, propanol, butanol, 2-butanol.
Means in the same row with different superscript letters differ significantly ($p<0.05$).

In vitro gas production kinetics of *Tithonia diversifolia* and *Pennisetum purpureum* silage mixtures enriched or not with lactic acid bacteria strains

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Keywords gas production, inoculum, *Pennisetum purpureum*, ruminant fermentation *Tithonia diversifolia.*

Introduction Nutritional requirements should be met by local resources. There is a special interest in research for unconventional species which can be supplied as hay or silage throughout the year, especially in times of scarcity. Tropical legumes and non-leguminous shrubs are an option to increase crude protein concentration in the diet (Botero et al., 2014). However, it is necessary to know the extent of inclusion and the possible blending with grasses to obtain the maximum benefit for the animal (La O et al., 2009). We evaluated the association of *Tithonia diversifolia* (TD) and *Pennisetum purpureum* (PP) for silage making and the dynamics of gas production of these forages to select the best fermentation parameters of the materials favoring the availability of nutrients for rumen microorganisms.

Materials and methods *Tithonia* and *Pennisetum* were harvested, wilted and chopped and underwent the following three treatments per forage: control, lactic acid bacteria (LAB) strain T735 and Silall 4x4 ™. The treatments were ensiled in vacuum sealer bags as Rostock Model Silages in triplicates. They were stored at about 25°C for 90 d. Silages were lyophilized and ground to 1 mm for the gas test. The TD and PP were arranged in four different proportions [T1: 100/0; T2: 67/33; T3: 33/67; and T4: 0/100, fresh matter (FM) basis], resulting in 12 treatments in total. The *in vitro* gas production was measured at 3, 6, 12, 24, 33, 48, 60, 72, 96, 120 and 144 h in triplicate. A Gompertz equation was used to model gas accumulation from different mixtures used in the silages, where the parameters a, b and c, were estimated by analyzing nonlinear regression, using the software Infostat. We used a factorial design experiment, where the first factor was the inclusion level of the PP in the mixtures and the second factor was the inoculant used: $Y_{ij} = \mu + PP_i + I_j + I \times PP_{ij} + \varepsilon$; where Y = is the target variable, μ is the overall mean = I = inoculant (control; T735; SilAll4x4 ™), PP = proportion of grass in the silage (0/100, 33/67, 67/33, and 100/0) and ε = random experimental error. Analysis of variance was performed and statistical differences were detected by Duncan mean comparisons ($P<0.05$).

Results and discussion The results showed lower gas production accumulated at 144 h in T1 (167.62 mL) with statistical differences ($P<0.0001$) compared to the other treatments. An increased gas production was found in T4 (0/100 TD/PP) at 144 h (204.12 mL). There was no effect of the inoculum in the production of gas ($P=0.28$). The results of the interaction mixture (TD/PP) × inoculum indicate that silages prepared with higher

inclusion of TD independent of LAB inoculation produced fewer gas, ranging between 166.2 and 169.7 mL (Table 1), meaning that larger amount of inclusion of PP in ensiling process increase gas production. In the present study, a higher silage digestibility can be expected with higher proportion of PP, at the expense of TD.

Table 1 Cumulative gas production per g of organic matter

	Fermentation (hours)							
Tretments	3	12	24	48	60	96	120	144
T1+ without inoculum	3.1abc	55.6cd	96.9abcd	136.6ab	148.6a	160.6a	164.6a	166.2a
T1+ T-735	3.5abc	64.8d	101.7bcd	138.1ab	149.2a	160.4a	164.8a	166.8a
T1+SilAll ^{4X4}	5.0bc	60.8cd	101.2bcd	140.0ab	151.2ab	163.6a	167.9a	169.7a
T2+ without inoculum	2.0a	52.2bc	91.1abc	135.1a	149.1a	162.4a	167.9a	170.4a
T2+ T735	5.1bc	65.2d	107.6d	152.0ab	165.3ab	179.1ab	184.1abc	187.1abc
T2+SilAll ^{4X4}	5.6c	64.3d	106.7d	144.5ab	156.8ab	169.8ab	174.6ab	177.7ab
T3+ no inoculo	3.1abc	55.7cd	102.6cd	155.6b	171.6b	189.6b	195.4bc	199.6c
T3+ T735	4.0abc	56.2cd	101.9bcd	155.5b	171.9b	189.3b	195.7bc	200.2c
T3+SilAll ^{4X4}	2.7ab	51.3bc	97.5abcd	151.4ab	167.3ab	185.2b	191.2bc	195.2bc
T4+ no inoculo	1.7a	37.8a	84.0a	142.2ab	161.6ab	186.7b	196.2bc	202.7c
T4+ T735	2.2a	42.8ab	90.5abc	146.4ab	165.6ab	189.2b	198.2c	204.8c
T4+SilAll ^{4X4}	2.1a	40.8ab	88.0ab	145.0ab	163.7ab	188.8b	198.1c	204.7c

T1: 100/0; T2: 67/33; T3: 33/67; and T4: 0/100 *Tithonia diversifolia* / *Pennisetum purpureum*; Different letters in the same column mean significant differences between treatments (P<0.05).

The highest rate of gas production were: T4+Silall; T4+T735 and T4 without inoculum with 3.32, 3.31 and 3.39 mL/h, respectively. Importantly, these treatments are those with the largest proportion of PP. Lower values (2.95, 2.99, 3.04 mL/h) were reported for the treatments with higher proportion of TD (T2+SilAll; T2+T735 and T1+T735, respectively).

Conclusions The treatments with a high proportion of *Tithonia diversifolia* presented the lowest values of gas production, while treatments with higher grass inclusion produced more gas. The silage inoculum did not influence the *in vitro* gas production.

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Effect of ethanol or *Lactobacillus plantarum* on total mixed ration silage fermentation characteristics and aerobic stability in Tibet

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Keywords ethanol, *Lactobacillus plantarum*, fermentation quality, aerobic stability

Introduction In Tibet, it is common practice to make and relocate TMR silages before feeding due to the uneven distribution of forages in temporal and spatial. TMR prepared as silage had a series of advantages to reduce the loss of nutrients, preserve for a longer time, and improve odors and flavors of unpalatable by-products. In this study, the effects of ethanol and *L. plantarum* on fermentation quality and aerobic stability of TMR silages in Tibet were tested.

Materials and methods The TMR were prepared using whole-crop corn, hulless-barley straw and concentrate at a rate of 40:20:40, on a fresh weight (FW) basis. TMR were treated without additive (control), or with ethanol (E, 2.5% FW), *L. plantarum* (L, 6 log cfu g^{-1} FW) and ethanol combination with *L. plantarum* (EL). The fermentation quality and aerobic stability of TMR silage were determined, experimental procedures and analytical methods were conducted according to Chen *et al.* (2014). The TMR silage fermentation quality and aerobic stability data were subjected to two-way analysis of variance with treatments and storage periods as main factors (SAS, 1990).

Results and discussion After 45 days of ensiling (Table 1), the pH value decreased ($P<0.05$) and lactic acid concentrations increased ($P<0.05$) in L and EL compared with control and E. Differences in acetic acid content of TMR silage with various treatments were not significant ($P>0.05$). Little concentrations of propionic acid and butyric acid were detected in all TMR silages. The pH value increased and concentrations of lactic acid, water-soluble carbohydrates and NH_3-N in TMR silages decreased after exposure to air (Table 3). At end of aerobic period, pH value of control and L was 5.61 and 5.71, which increased by 36.8% and 47.2% compared with initial days of aerobic period, following lactic acid concentrations decreased by 61.5% and 69.5%, respectively. The pH value and lactic acid concentrations were 4.68 and 36.3 g kg^{-1} DM in E respectively, which were different ($P<0.05$) from control. The pH value of EL-treated silage remained stable (about 4.20), and lactic acid concentration was 40.8 g kg^{-1} DM by d 9. After 9 days of aerobic exposure, water-soluble carbohydrates concentrations in EL was higher ($P<0.05$) than that of control and E, while NH_3-N concentrations in control were lower ($P<0.05$) than that of other silages. Previous research has confirmed that *L. plantarum* could promote homolactic fermentation with more lactic acid concentrations and much lower pH value (Contreras-Govea, 2013). Zhang et al. (2011) found that ethanol could be used as a silage additive to inhibit the utilization of WSC by undesirable bacteria, reducing silage losses during ensiling and resulting in more fermentable substrate for lactic acid bacteria. This result showed the combinational beneficial effects of *L. plantarum* and ethanol was found in EL silages, indicated by intermediate fermentation quality and higher aerobic stability.

Conclusions The combination of *L. plantarum* with ethanol not only improved the fermentation quality, but also had benefit effect on the aerobic stability of TMR silages.

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Table 1 Change in chemical composition of TMR silages after exposure to air

Item	treatments	Days of aerobic exposure				Analysis of variance[1]			SEM[2]
		0	3	6	9	T	D	T*D	
pH	control	4.10cA	4.82bA	5.39abA	5.61aA	**	**	**	0.034
	E	4.18bA	4.56aAB	4.47abB	4.68aB				
	L	3.88cB	4.43bcAB	4.74bAB	5.71aA				
	EL	3.86aB	4.25aB	4.36aB	4.20aB				
lactic acid (g kg^{-1} DM)	control	43.9aB	32.6aC	24.2bC	16.9bC	**	**	**	0.869
	E	46.3aB	40.4abBC	39.6bcAB	36.3cB				
	L	66.5aA	45.2bAB	33.2cBC	20.3dC				
	EL	62.3aA	52.8bA	47.7bA	50.8bA				
Water-soluble carbohydrates (g kg^{-1} DM)	control	42.6aB	39.8abA	30.6bcB	25.3cC	**	**	*	0.493
	E	43.1aB	51.5aA	41.5bA	38.4bAB				
	L	50.5aAB	44.1abA	36.9bcAB	31.1cB				
	EL	56.2aA	49.3aA	42.4aA	43.7aA				
NH_3-N (g kg^{-1} DM)	control	45.6aA	41.2aAB	17.3bBC	13.3bC	**	**	*	0.757
	E	44.3bA	52.6aA	25.7cA	28.0cA				
	L	38.0aA	33.3aB	13.2cC	22.3bAB				
	EL	38.1aA	43.7aAB	22.3bAB	20.3bB				

Values in the same row (a~d) or in the same column (A~D) with different following letters are significantly different. *, $P<0.05$; **, $P<0.01$; N, No significant.

Improving protein quality of roughages in ruminant nutrition by using silage additives on the basis of condensed tannins

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Keywords mimosa, proteolysis, quebracho, silage, tannin

Introduction Condensed tannins can bind to proteins and reduce nitrogen availability to rumen microorganisms (McSweeney, 2001). This ability of tannins to bind to proteins can be utilized to reduce the proteolysis during ensilage. Colombini et al. (2009) and Tabacco et al. (2006) made investigations on the use of hydrolysable chestnut tannins during ensilage of alfalfa. Colombini et al. (2009) found that silage with tannins had a smaller A fraction (non-protein nitrogen (NPN)) (563 g/kg crude protein (CP)) than the control (624 g/kg CP). At the same time the true protein fractions (B1, B2 and B3) increased in the silage with tannin addition (30; 289 and 46 g/kg CP) compared to the control (8; 248 and 15 g/kg CP). The results from Tabacco et al. (2006) showed a decrease of NPN and ammonia-N in the silage with 4 % tannin addition. In sacco treatments with cows fed grass silage with tannins (30 g/kg DM) resulted in 230 g rumen undegradable protein (RUP)/ kg crude protein (CP) instead of 180 g RUP/kg CP in the control. The protein solubility is reduced during the first 8 h and is afterwards comparable to the control (Roscher and Steinhöfel, unpublished). The objective of our study was to detect the most effective tannin extract and the optimal amount of tannin to conserve a high proportion of true protein. By using two different dry matter levels the best zone of action of tannins should be evaluated. A standard digestion trial was realized to detect the influence of the tannin on digestion of protein, energy and the organic matter in ruminants (adult wethers).

Materials and methods Alfalfa was cut in June 2013 in Saxony, Germany, and wilted to 360 and 420 g DM/kg. Four levels of Quebracho (QUE) and Mimosa (MIM) extract were added to the plant material: 0 (control), 5, 15 and 30 g/kg dry matter (DM) in triplicate. The tannin content from QUE was 677 g/kg DM with 133g condensed tannins/kg DM and from MIM 570 g/kg DM with 245 g condensed tannins/kg DM. The samples before and after ensiling were analyzed for their chemical composition (DM, crude fiber, CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), protein fractions) using methods according to near infrared spectroscopy (NIR) and VDLUFA book of methods. The RUP was estimated according to Kirchhof et al. (2010). Cornell net carbohydrate and protein system (CNCPS) was adapted according to Licitra et al. (1996), modified by Shannak et al. (2000). The fermentation quality of the silages was evaluated by sensory evaluation (smell, colour, structure) and by measuring pH in relation to DM and NH_3-N. A standard digestion trial with 2 × 4 adult wethers fed alfalfa silage with 30 g/kg DM Mimosa and control ration was realized in autumn 2013. The guidelines for the treatment are based on GfE (1991). Data were analyzed with SAS 9.3 using proc mixed. Differences were declared significant at $P < 0.05$.

Results and discussion All silages had a good fermentation quality regardless of the treatment. No butyric acid was detected in the silages. The CP content was similar in all treatments (CP at 360 g DM/ kg fresh matter (FM): 156 ± 6.2 g/kg DM and CP at 420 g DM/ kg fresh matter: 159 ± 7.3 g/kg DM). The protein fractions differed between treatments. The fraction A was smaller in the treatments with the higher tannin concentration in contrast to the fraction B2 (true soluble protein). These results confirm those from Colombini et al. (2009). The effect of tannin addition was more pronounced in the silage lower in DM. The content of the RUP was lower in the control (360 g DM / kg FM) with only 160 g/kg CP in contrast to the other control (420 g DM/ kg FM) with 205 g/kg CP. The use of 30 g MIM /kg DM or QUE in the samples with 360 g DM/kg FM was comparable to the samples with 420 g DM/kg FM. The protein digestibility was significantly reduced in the group containing tannin extract (digestibility 64 vs. 72 % in the control; P= 0.003). The digestibility of the organic matter was low in both groups (Tannin group 62 %; control 63%), but similar. The energy content was not affected by tannin addition either.

Conclusions Tannins reduced proteolysis in silages with lower dry matter contents. The utilized tannin products had different tannin contents (see above). With rising tannin content proteolysis was increasingly inhibited. More evaluations are necessary to give practical recommendations.

Acknowledgements The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support program.

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Efficacy of different additives in ensiling whole crops of faba bean - wheat and pea - wheat mixtures

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Keywords additive, aerobic stability, *Fabaceae*, formic acid, lactic acid bacteria, legume, silage

Introduction Organic forage production is dependent on the ability of legumes to fix nitrogen. Mixtures of non-leguminous cereals and legume grains are cultivated to gain benefits of intercropping. Suitable mixtures may result in large yields even in short growing season when harvested as whole crop silage. Increasing the proportion of legume in the mixture will challenge the ensiling process due to higher buffering capacity and lower dry matter (DM) content of the legumes compared to cereals (Pursiainen et al., 2008), which may lead into poor fermentation quality of the silage (e.g. Borreani et al., 2009). Silage additives can be used to aid the ensiling process. However, the legislation restricts the choice of silage additives in organic production (EU 889/2008). An ensiling experiment with four additive treatments was conducted to clarify the challenges related to ensiling legume-cereal mixtures.

Materials and methods Two legume-cereal mixtures, faba bean (*Vicia faba*, cv. Fuego) + wheat (cv. Anniina) (FBW) and pea (*Pisum sativum*, cv. Florida) + wheat (*Triticum aestivum*, cv. Anniina) (PW), were harvested in Siikajoki, Finland (64°N, 25°E) with direct cut precision chopper (Claas Jaguar 870) when wheat was at early dough stage. The yield of FBW was 6783 and PW 6839 kg DM/ha. The proportion of legumes in the mixtures was exceptionally high (0.84 for FBW and 0.89 for PW) and DM content was low (173 g/kg for FBW and 181 g/kg PW). The material was sprayed with additives as follows: Control without additive; LAB1 2.5×10^5 CFU/g containing *Lactobacillus plantarum, L. paracasei, L. buchneri* and *Lactococcus lactis*; LAB2 1.0×10^6 CFU/g containing *Lactobacillus plantarum, Pediococcus acidilactici, P. pentosaceu, Propionibacterium acidipropionici* and enzymes; and ACID 5 L/t containing formic acid 590, propionic acid 200, ammonium formiate 40 and benzoic/sorbic acid 25 g/kg. For each treatment, three 12 liter silos were filled and compacted during filling. Silos were closed and a 10 kg weight was added on top of each silo. Effluent was not removed from the silos during ensiling. Silos were opened after 105 days and analyzed for fermentation quality and aerobic stability.

Results and discussion The ensiled forage had high (> 10^6 CFU/g) initial number of lactic acid bacteria, and the only significant effect of LAB treatments was the reduced acetic acid and the total volatile fatty acids content of PW silage treated with LAB1 (Table 1). Generally Control and LAB silages had high amount of lactic acid while the amount of water soluble carbohydrates was low. However the amount of butyric acid was low (< 0.8 g/kg DM) in all silages although the high amount of ammonium nitrogen of PW silages indicates poor quality. ACID treatment was able to restrict fermentation especially in FBW silages. Typically higher dose of ACID is recommended for materials difficult to ensile (6

L/t instead of 5 L/t, which was used in this experiment). Further, the high ethanol content of PW ACID silages suggests strong role of yeasts during fermentation although they were not alive at the time of silo opening as demonstrated by both the good aerobic stability and low yeast numbers (maximum 7500 CFU/g) of those silages.

Table 1 Fermentation quality and aerobic stability of the whole crop silages from mixed crops

	Pea – wheat (PW)				Faba bean – wheat (FBW)				SEM
	Control	ACID	LAB1	LAB2	Control	ACID	LAB1	LAB2	
Dry matter (DM), g/kg	181	188	189	185	170	171	174	172	2.81
pH	4.06^{B}	4.20^{A}	4.05^{B}	4.07^{B}	4.01^{B}	4.24^{A}	4.05^{B}	4.02^{B}	0.012
Acetic acid, g/kg DM	27.1^{A}	22.0^{B}	22.5^{B}	25.8^{A}	27.4^{A}	8.0^{C}	26.1^{A}	27.5^{A}	0.57
VFA, g/kg DM	29.7^{A}	28.8^{A}	23.9^{A}	27.2^{A}	28.8^{A}	15.8^{A}	27.7^{A}	29.0^{A}	0.60
Lactic acid, g/kg DM	140^{A}	81.4^{C}	135^{AB}	133^{AB}	130^{AB}	17.0^{D}	124^{B}	130^{AB}	3.3
WSC, g/kg DM	10.3^{B}	21.2^{B}	16.9^{B}	9.87^{B}	12.0^{B}	146^{A}	15.2^{B}	14.1^{B}	3.61
Ethanol, g/kg DM	34.7^{B}	68.5^{A}	31.7^{B}	32.6^{B}	21.7^{C}	10.1^{D}	20.6^{C}	17.9^{C}	1.29
Amm. N, g/kg N[1]	92.4^{AB}	84.3^{B}	84.7^{AB}	94.3^{A}	67.7^{C}	50.7^{D}	69.2^{C}	69.8^{C}	1.99
Aerobic stability, h	56.8^{C}	$>235.0^{A}$	56.8^{C}	65.4^{C}	56.2^{C}	59.4^{C}	88.3^{BC}	101.2^{B}	7.31

Treatments: Control without additive; ACID formic acid based additive, LAB1 lactic acid bacteria strains, LAB2 lactic acid bacteria strains and enzymes. SEM = Standard error of the means. VFA = sum of volatile fatty acids C2 – C6. WSC = Water soluble carbohydrates

[1]Amm. = Ammonium. Ammonium derived from ACID treatment (8.6 and 10.5 g/kg N respectively for PW and FBW) subtracted from the analyzed ammonium content.

Differences within the same row without same superscript differ significantly from each other ($p < 0.05$, Tukey test). The factors (type of forage, additive treatment) and their interaction had statistically significant ($p < 0.05$) effect on most of the listed variables in Table 1. Only dry matter content was not affected by the additive treatment and the forage type had no significant effect on pH.

Conclusions Organic production is dependent on the nitrogen fixing ability of legumes. The potential of those crops is challenged by the difficulty of ensiling. Organic acids have potential to improve the quality of silage despite the challenges of legumes as demonstrated by the results of this experiment.

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Testing of a novel dual purpose silage strain combination on various different crops

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Keywords aerobic stability, fermentation, lactic acid bacteria, silage additive, oxygen scavenging

Introduction Homo- and heterofermentative strains are used for fast pH reduction at the beginning of the ensiling process and secure long aerobic stability, respectively. Combining those two groups of lactic acid bacteria is not always delivering the expected result. Recently, a novel *Lactococcus lactis* O224 DSM11037 strain has been introduced, which is superior in oxygen scavenging and also relatively fast in reducing pH (Hindrichsen *et al.*, 2012). Combining this *L. lactis* O224 DSM11037 strain with *Lactobacillus buchneri* DSM 22501 have shown better results on aerobic stability than previously seen with combinations of *L. buchneri* and high lactate producing strains, such as *L. plantarum*. Our objective with the current study was to evaluate the efficacy of the new *L. lactis* O224 DSM11037/*L. buchneri* DSM 22501 combination on various forages by measuring both fermentation characteristics and aerobic stability. The additive was compared to an untreated reference in laboratory-scale experiment.

Materials and methods Silages were prepared from grass (32.82 % DM; 2.03 % water soluble carbohydrate (WSC) of fresh matter (FM), alfalfa (34.11 % DM; 1.45 % WSC FM), alfalfa/grass (35 % DM; 2.34 % WSC FM) and whole crop maize 38.55 % DM; 2.53 % WSC FM) after treatment with the following LAB combination, SiloSolve FC (TA) *L. buchneri* DSM22501 and *L. lactis* O224 DSM11037, Chr. Hansen A/S, Denmark. The application rate was 4 ml inoculant suspension/kg forage targeting 150,000 cfu/g forage. The untreated control (UT) received 4 ml water/kg forage. Herbages were ensiled in 3 l laboratory silos (each treatment and crop was replicated 5 times) and analyzed after 90 d of storage at 20°C. Aerobic stability (AS) was determined by monitoring the temperature increase in silages stored in insulated PVC-tubes at 20 ºC ambient temperature and defined as a temperature increase of 3°C above the ambient temperature. Data were statistically analyzed as a randomized complete block by using the GLM procedure of SAS.

Results and discussion The silage fermentation quality parameters measured on very different silages with the silage inoculant TA resulted in better preserved silage with a significantly lower pH, concentration of ammonia-N, butyric acid, ethanol and dry matter loss compared with untreated silages UT (see Table 1). The main indicators of clostridia infection are high levels of butyrate and ammonia-N. TA significantly reduced butyric acid and ammonia-N in all various silages compared to untreated silage. Silage additives applied to herbage during silage making can reduce respiration and/or proteolysis by plant enzymes, manipulate fermentation, as well as inhibit the activity of clostridia and aerobic microorganisms such as yeast and mold (Kung *et al.*, 2003). On an average, the use of additive TA reduced fermentation losses by 3.3 % units ($P < 0.05$) compared to the

untreated control silages. At the same time more ($P < 0.05$) lactic acid and more ($P < 0.05$) acetic acid (except additive TA for Grass) was produced in the inoculated silages compared to untreated silages. The higher amount of acetic acid in the TA inoculated silages was expected because the heterofermentative lactic acid bacteria strain *L. buchneri* can result in high levels of acetic acid (Oude Elferink *et al.*, 2001). Acetic acid is a fungicidal agent and can inhibit growth of yeasts and molds, leading to increased aerobic stability of silages. Silage inoculated with additive TA had lower temperatures ($P < 0.05$), when exposed to air, and aerobic stability was increased from average of 5 days for untreated silages to an average of 9.5 days for additive TA treated silages ($P < 0.05$).

Table 1 Silages parameters of trials with various crops, ensiled with or without silage additive

Item	Grass		Alfalfa		Alfalfa/grass		Maize	
	UT	TA	UT	TA	UT	TA	UT	TA
90 d anaerobe fermentation								
DM, %	30.5	31.4*	32.1	33.2*	32.3	33.4*	36.7	37.6*
DM loss, %	7.90	4.94*	6.99	3.32*	9.28	5.60*	6.74	3.90*
pH, 3 d**	4.75	4.40*	5.50	5.08*	5.24	4.82*	4.36	4.17*
pH, 90 d	4.38	4.17*	5.06	4.77*	4.68	4.41*	4.04	3.92*
pH, 10 day aerobic challenge	7.93	5.35*	6.97	4.89*	7.66	4.89*	8.29	4.39*
NH_3-N, % total N	5.38	3.79*	6.35	4.73*	7.03	5.03*	5.18	3.92*
Lactic acid, % DM	4.55	6.28*	4.68	5.66*	6.71	7.60*	2.78	3.47*
Acetic acid, % DM	2.42	2.38	2.51	2.96*	2.15	3.27*	1.11	2.68*
Butyric acid, % DM	0.24	0.01*	0.29	0.05*	0.45	0.02*	0.03	0.01*
Ethanol, % DM	0.94	0.71*	0.97	0.50*	1.11	0.68*	1.00	0.50*
10 d aerobe challenge								
pH, 10 d aerobe challenge	7.93	5.35*	6.97	4.89*	7.66	4.89*	8.29	4.39*
AS, (days)	3.80	7.95*	6.5	10*	7.2	10*	2.75	10*

UT = untreated, TA = *L. buchneri* DSM22501 and *L. lactis* O224 DSM11037, AS = Aerobic stability. *Significantly different from untreated control (P<0.05). **pH maize after 2 days

Conclusions In this study, the novel combination of a hetero- and homofermentative strain *L. buchner*i DSM22501 and *L. lactis* O224 DSM11037 (TA) was efficient in improving fermentation, reducing protein breakdown, and nutrient losses of various crops. Inoculating with additive TA, resulted in higher levels of acetic acid and thereby significantly enhanced aerobic stability in all treated crops compared to the untreated crops.

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Effects of added lactic acid bacteria and enzymes on fermentation of liquid feeds for pigs

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Keywords controlled fermentation, undesired degradation, liquid feed, amino acids

Introduction The fermentation of liquid feeds is a new feed preservation method with a special relevance in pig production. In practice, several lactic acid bacteria strains (LAB) are partially used as starter cultures for fermentation of liquid feeds (Heinze and Rau, 2011). Furthermore it is known from the fermentation process, that selected LAB are able to avoid the degradation of lysine to cadaverine due to undesired growth of E-coli-bacteria (Niven et. al., 2006). The present study aimed to investigate the ability of LAB and enzymes to optimize the fermentation process of liquid feed. The effects on the reduction of pH and short chain fatty acids yield were considered. In addition, it was investigated whether the fermentation could result in an increase of amino acid chemical reduction.

Materials and methods A liquid feed for fattening pigs composed by dry substrate (barley, wheat and soybean meal) and water adjusted to 25% dry matter was inoculated with the following treatments: 1) control without additive., 2) LAB mixture: Three strains of homofermentative lactic bacteria LAB (L.paracasei, P.pentosaceus, L.rhamnosus-*Schaumalac Feed Protect*®), 3) enzyme mixture: 50 % Amylase, 50 % pectinase and 4) LAB mixture plus enzyme mixture. The fermentation took place in three replicate minisilos during 24 hours at different temperatures (20°C, 30°C, 37°C). The pH-value and the short chain fatty acids yield via HPLC were recorded. Amino acid analysis was performed before and after fermentation process according to VDLUFA method EG 152/2009, i.e. ion exchange chromatography with postcolumn derivatization with ninhydrin. Statistical analysis (SAS software 9.4.) was performed using a multiple ANOVA with fixed effects of treatment and time.

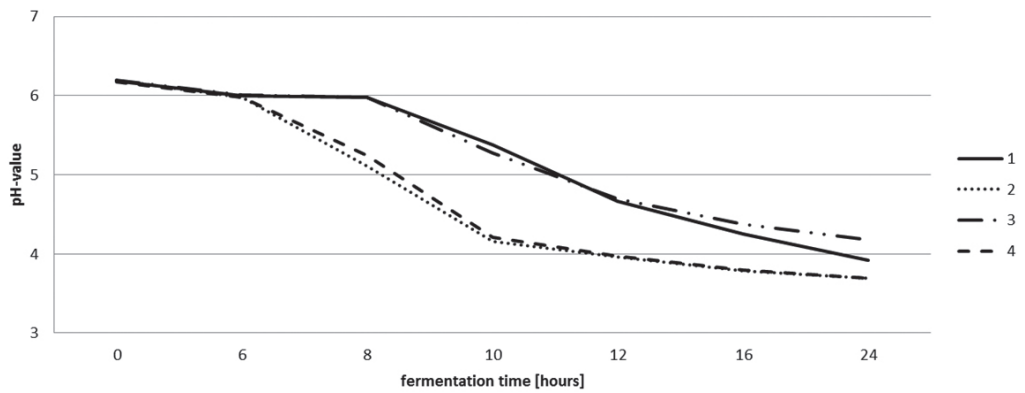

Figure 1 Effect of starter culture on pH- value during fermentation process of 24 hours at constant temperature of 37°C depending on the test design; 1: control; 2: LAB mixture; 3: enzyme mixture; 4: LAB mixture & enzyme mixture.

Results and discussion The results show that the fermentation temperature has influenced the fermentation rate. A fermentation temperature of 37 °C led to faster dropping of the pH-value of the liquid feed below 4 (after 12 h) in contrast to the temperature of 30 °C (after 16 h) and 20 ° (not until 24 h). Furthermore, the addition of LAB mixture yielded to a faster pH reduction (Figure 1) and higher amount of short chain fatty acids (not shown). This was also observed by Heinze and Rau (2013). The enzyme mixture did not affect the fermentation rate based on pH values (Figure 1). Nevertheless, the results of amino acid analysis showed that there was no reduction of the individual amino acids during the fermentation process, as demonstrated for lysine (Figure 2). Conversely, a slightly numerous increase of lysine content was observed for the addition of LAB mixture, but this might be due to margin analysis. The results are partial according to Niven et al. (2006), who also observed no degradation of Lysin with addition of suitable LAB, while they found strong degradation of Lysin during fermentation without addition of LAB.

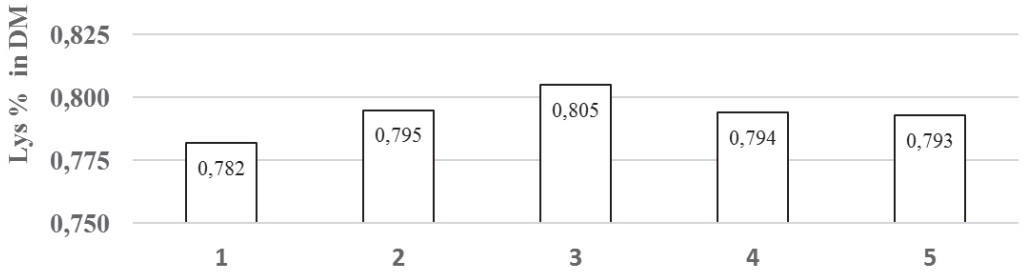

Figure 2 The concentration of lysine in dry matter before and after 24h fermentation depending on the test design; 1: not fermented liquid feed before fermentation; 2: control; 3: LAB mixture; 4: enzyme mixture; 5: LAB mixture & enzyme mixture.

Conclusions The results of the present study show that the fastest reduction of the pH-value was observed with inoculation of LAB at a fermentation temperature of 37°C. Furthermore, no negative effect of the fermentation process on the content of native amino acids was indicated. Thus, selected LAB are useful as starter cultures.

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Nutrient composition and digestibility of whole-plant corn silage and high-moisture corn straw silage in Tianjin

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Keywords whole-plant corn silage, high-moisture corn straw silage, nutrient composition

Introduction Whole-plant corn (WPC) silage and high-moisture corn straw (HMCS) silage are the main source of livestock fodder for cattle in Tianjin, and fully understanding the fermentation quality and the nutrition quality of those silages can play an important role on the development of animal husbandry. The objective of this research was to study the nutrient composition and digestibility of WPC and HMCS silages.

Materials and methods One corn (*Zea mays*. L) variety (Jingke) was sown on Tianjing Dairy Farm. The WPC and HMCS were harvested at 2/3 milk line and bloom stage, respectively, immediately chopped into 1 to 2 cm, and then packed into laboratory silos (25 cm high with an internal diameter of 7.6 cm; 700 g L^{-1} density), with 3 replicates per treatment. Silages were placed in a dark room at 18° to 21° for 60 days. The dry matter (DM), crude protein (CP), ether extract (EE), water soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, calcium (Ca), phosphorus (P), *in vitro* DM digestibility (*IV*DMD), *in vitro* NDF digestibility (*IV*NDFD) and *in vitro* ADF digestibility (*IV*ADFD) of silages were determined. The data were analyzed by one-factor ANOVA by using the GLM procedure of SAS.

Results and discussion Because that WPC was harvested at later stage than HMCS, the concentrations of DM, EE, WSC, NDF and ADF in WPC silage were higher than those in HMCS silage ($P<0.05$). Ash concentration was lower in WPC silage than in HMCS silage ($P<0.05$). There was no difference in CP, Ca and P between WPC and HMCS silages ($P>0.05$). Higher fiber concentrations may lead to reduction in *IVD*MD of forages (Contreras-Govea, *et al.*, 2009). Furthermore, forage DM and fiber digestibility have strong negative correlation with lignin concentration (Hatfield, 1993). So, the HMCS silage had higher *IV*DMD, *IV*NDFD and *IV*ADFD than WPC silage, and the nutrient value of HMCS silage might be better than WPC silage.

Conclusions High-moisture corn straw silage has lower NDF and ADF concentrations and better nutritive value than whole-plant corn silage.

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Table 1 Nutrient composition (g kg^{-1} DM) and *in vitro* digestibility (g kg^{-1}) of WPC and HMCS silages in Tianjin

Item	WPC silage	HMCS silage
DM	347.3±24.4a	196.7±24.1b
CP	92.2±6.3a	103.0±7.7a
EE	22.8±1.3a	14.7±3.7b
WSC	47.7±7.1a	30.7±11.7b
NDF	547.4±40.4a	512.3±65.3b
ADF	428.4±18.0a	325.1±31.2b
Ash	61.3±8.2b	85.6±11.8a
Ca	5.8±0.3a	5.9±0.4a
P	1.6±0.3a	1.3±0.4a
*IV*DMD	622.8±56.4b	725.7±64.2a
*IV*NDFD	314.6±23.2b	530.57±32.4a
*IV*ADFD	243.4±21.5b	497.53±19.4a

Means in the same row whit different letters differ significantly ($P<0.05$).

Digestibility and degradability of starch and cell walls in maize silage

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Keywords maize silage, ruminants, digestibility, degradability, starch, cell walls

Introduction Maize silage, commonly used in the diet of high yielding ruminants, provides two energetic fractions (starch and cell-walls or NDF). With the stage of maturity at harvest, type of hybrid and climatic conditions, the proportion of the two energetic fractions in the whole plant varies greatly and so, the nature of energy provided by maize silage to the animal differs. New hybrid genetics and late harvest practices (>350g/kg of DM) lead to the use of high-starch content silages. A better characterization of the digestibility of energetic fractions in maize silage is required to improve its use in ruminant diets. The aim of this study was to investigate the impact of maturity stage and hybrid on chemical composition, *in vivo* digestibility and *in sacco* degradability of maize silage.

Materials and methods Thirty-two maize silages were obtained from 4 hybrids (F_1: flint grain/high NDF digestibility, F_2: flint grain/low NDF digestibility, FD: flint-dent grain/ high NDF digestibility, D: dent grain/ low NDF digestibility) that were cultivated for 2 consecutive years in the same location (France) and harvested at 4 stages of maturity from milk-dough stage to vitreous stage. *In vivo* digestibility of OM (OMD), NDF (NDFD) and Starch (StarchD) were measured by total faeces collection over 6 days on eight castrated sheep in individual metabolic crates according to repeated Latin squares designs. *In sacco* starch and cell wall degradability in the rumen was measured in cows for the 32 maize silages with an adapted methodology for high starch content forages (Peyrat et al., 2014). Effective degradability of dry matter (ED_{DM}), NDF (ED_{NDF}) and starch (ED_{Starch}) were calculated with the step by step method, assuming a particle outflow rate of $0.04h^{-1}$ for DM, $0.06\ h^{-1}$ for starch and $0.02\ h^{-1}$ for NDF. The effects of hybrid, maturity stage, year of harvest and their interaction on *in vivo* digestibility and on *in sacco* degradability were analysed using the MIXED procedure of SAS 9.3.

Results and discussion From first to the last maturity stage studied, respectively 283 and 407g DM/kg, starch significantly increased by 138 g/kgDM and NDF significantly decreased by 69 g/kg DM (Table 1). The OMDonly slightly changed: it increased ($P<0.01$) from maturity stage 1 to 3 and decreased ($P>0.01$) at last maturity stage. The relative stability of OMD with stage of maturity was explained by the compensation between the increase in starch content with high digestibility and the decrease in NDF digestibility. While NDFD decreased significantly from the first to the last maturity stage, StarchD, averaging 99.0%, slightly increased with advancing maturity. Hybrid FD with the highest starch content had the highest OMD and ED_{DM} while hybrid D had the highest StarchD and ED_{Starch}. In 2012, climatic conditions (late massive rains, temperature and light deficit) lead to maize with lower starch content and OMD than in 2011. Significant interactions

were observedbetween maturity stage, hybrid and climatic conditions, on all measured digestibility and degradability (except for NDFD, for which only interaction between maturity and climatic conditions was observed). The ED_{DM}, ED_{NDF}, ED_{starch}, decreased ($P<0.01$) from the first to the last maturity stage.While in the whole tract OM and starch digestibility slightly changed, DM and starch degradability in the rumen significantly decreased with advancing maturity. The lower ruminal starch degradability of maize harvested at late stage could be explained by a low core grain vitreousness of mature grain limiting the action of hydrolytic enzymes (Philippeau and Michalet-Doreau, 1997). Concerning hybrids, differences on NDF digestibility and degradability could be related to the structure of cell-wall components while differences on starch degradability could be related to the type of grain. (Philippeau and Michalet-Doreau, 1997).

Table 1 Impact of maturity stage, hybrid and climatic conditions (represented by year of harvest) on chemical composition, *in vivo* digestibility and *in sacco* degradability of maize silage

	Maturity stage (M)				Hybrid (H)				Year (Y)			P-value		
	1	2	3	4	F_1	F_2	FD	D	2011	2012	SEM	M	H	Y
Components contents (g/kg)														
DM	283^{a}	328^{b}	337^{b}	407^{c}	339^{a}	330^{ab}	362^{c}	324^{b}	333^{a}	345^{b}	13.4	<0.01	<0.01	<0.01
NDF	405^{c}	366^{b}	357^{b}	336^{a}	375^{b}	393^{c}	331^{a}	364^{b}	335^{a}	397^{b}	19.9	<0.01	<0.01	<0.01
Starch	276^{a}	320^{b}	355^{c}	414^{d}	334^{b}	307^{a}	382^{c}	341^{b}	376^{b}	305^{a}	24.9	<0.01	<0.01	<0.01
***In vivo* digestibility (%)**														
OMD	72.5^{a}	73.6^{b}	73.4^{b}	72.2^{a}	72.3^{a}	71.9^{a}	74.7^{b}	72.8^{a}	74.4^{b}	71.5^{a}	2.33	<0.01	<0.01	<0.01
NDFD	55.1^{c}	51.5^{b}	50.0^{b}	43.4^{a}	50.4	51.1	49.7	48.7	51.4^{b}	48.6^{a}	5.37	<0.01	0.51	<0.01
StarchD	98.9^{b}	98.7^{a}	98.8^{b}	99.4^{c}	98.4^{a}	99.0^{c}	98.9^{b}	99.6^{d}	98.8^{a}	99.2^{b}	0.14	<0.01	<0.01	<0.01
***In sacco* effective degradability (%)**														
ED_{DM}	64.6^{c}	63.8^{bc}	62.6^{b}	58.3^{a}	57.4^{a}	60.1^{b}	67.0^{d}	64.6^{c}	62.2	62.4	1.32	<0.01	<0.01	0.48
ED_{NDF}	57.0^{b}	57.3^{b}	56.1^{b}	52.0^{a}	52.0^{a}	56.2^{c}	59.8^{d}	54.4^{b}	54.8^{a}	56.4^{b}	1.75	<0.01	<0.01	<0.01
ED_{Starch}	75.6^{c}	65.2^{b}	65.9^{b}	60.5^{a}	59.6^{a}	61.4^{b}	70.4^{c}	75.8^{d}	66.3^{a}	67.3^{b}	0.90	<0.01	<0.01	<0.01

For each effect (M, H orY), a, b, c and d represents significant differences at $P<0.01$.

Conclusion Regardless of climatic condition, this study showed that maturity stage has a small but significant impact on the total tract digestibility of OM with a maximum reached around 30-35% of DM. However, with advancing maturity, the ratio of NDF/starch potentially degradable in the rumen significantly decreased from 1.1 to 0.7 between early to late maturity stage as a consequence of the increase in starch content and the decrease in NDF digestibility. According the stage of maturity and type of hybrid and their impact on digestibility and rumen degradability, maize silages could be classified in relation to the rumen degradable NDF/starch ratio for a better use in ruminant diets. These new references on *in vivo* digestibility and degradability of maize silage will allow better evaluation of the nutritive value of maize silage in the future feed evaluation systems.

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Characterization of the starch fraction of corn silage stored under dairy farm conditions

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Keywords corn silage, digestibility, starch, structural characteristics

Introduction Starch digestibility of corn silage is affected by length of fermentation (LOF) and need to be considered in order to properly balance rations. It has been indicated that non-starch components, such as the protein matrix, which binds starch granules together, may limit attack by microbes and enzymes, thus decreasing starch digestion. Hoffman *et al*. (2011) concluded that the degree of starch encapsulation by zein at ensiling combined with fermentation intensity and length of the fermentation, appear to regulate the disassociation of starch granule clusters in high moisture corn. The objective of this study was to assess the effect of LOF on starch content, starch digestibility and its structural changes in inoculated corn silage stored on-farm.

Materials and methods Whole-corn plants (P0636AMX, Pioneer, Johnston, IA) were chopped using a self-propelled harvester (Claas Jaguar 980) fitted with an after-market cross grooved crop-processing rolls (Shredlage, LLC, Tea, SD). Fresh forage was inoculated at the chopper using a homo-fermentative microbial inoculant (Biomax 5, CHR. Hansen, Milwaukee, WI). Forage was ensiled using an Ag Bagger MB90-1012 HyPac (Warrenton, OR) and stored in an Ag Bag (Klerks Hyplast Inc. , Chester, S.C.) measuring 3.66 m in diameter x 152.44 m long with an estimated storage capacity of 1000 metric tons of fresh forage. Duplicate forage samples were taken at ensiling (day 0), then at 240 d and 385 d post-ensiling. Samples were analyzed at a properly accredited commercial laboratory (Rock River Laboratory, Inc., Watertown, WI). Analysis included dry matter (DM), starch content and 7 h *In Situ* ruminal starch digestibility. Corn kernel samples were micrographed using a scanning electron microscope (Jeol model JSM 5410LV, Jeol USA, Inc. Peabody, MA). Statistical analysis was performed as a completely randomized design with repeated measures over time. Tukey-test was used for mean separation. Nutritional composition and fiber digestibility of the silage were reported in a companion two-page abstract.

Results and discussion Silage DM content decreased ($P<0.05$) by 9.7 percentage units after 385 d of ensiling (Table 1). Although not statistically significant ($P>0.05$), the starch content of the silage decreased 8.5 percentage units after being ensiled for 385 d. Starch digestibility increased ($P<0.05$) almost 24 percentage units due to ensiling. Calculating the amount of starch and digestible starch provided by 10 kg DM of corn silage shows that the increase in starch digestibility results in a numerical increase ($P>0.05$) of digestible starch being supplied despite the decrease in starch content of silage. The 2,000x micrograph at ensiling (d 0) shows smooth surface, spherical starch granules surrounded by protein bodies (Figure 1). After 240d of ensiling, the starch granules have lost the smooth surface

and sphericity. The protein bodies were broken down into small pieces. This dissociation of the starch granule with the protein bodies was similar to that reported by Hoffman et al. (2011) for high moisture corn ensiled for 240d. After 385d of ensiling, the surface of the starch granule is no longer smooth, it appears pitted due to the hydrolytic action of amylase and protein bodies are hardly visible. The 10,000x micrograph taken after 385 d of ensiling clearly shows the roughness of the surface of the starch granule and the pitting presumably due to the hydrolytic action of enzymes.

Conclusions Structural changes in the starch-protein matrix and in the starch granule during ensiling result in increased starch digestibility**.**

Reference

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Table 1 DM, starch, digestibility and supply of corn silage ensiled for 0, 240 or 385 d

	Length of ensiling, d		
Item	0	240	385
DM[1], %	36.7[a,b]	39.9[a]	27.05[b]
Starch, %	42.87[a]	37.38[a]	34.39[a]
7 h *in Situ* ruminal starch digestibility, % starch	65.43[b]	90.72[a]	87.97[a]
Starch supplied by 10 kg DM of corn silage	4.29[a]	3.74[a]	3.44[a]
Digestible starch supplied by 10 kg DM of corn silage	2.80[a]	3.39[a]	3.03[a]

Fresh basis; all others reported on a DM basis unless otherwise specified.
[a, b] Means with unlike superscripts in the same row differ P<0.05 .

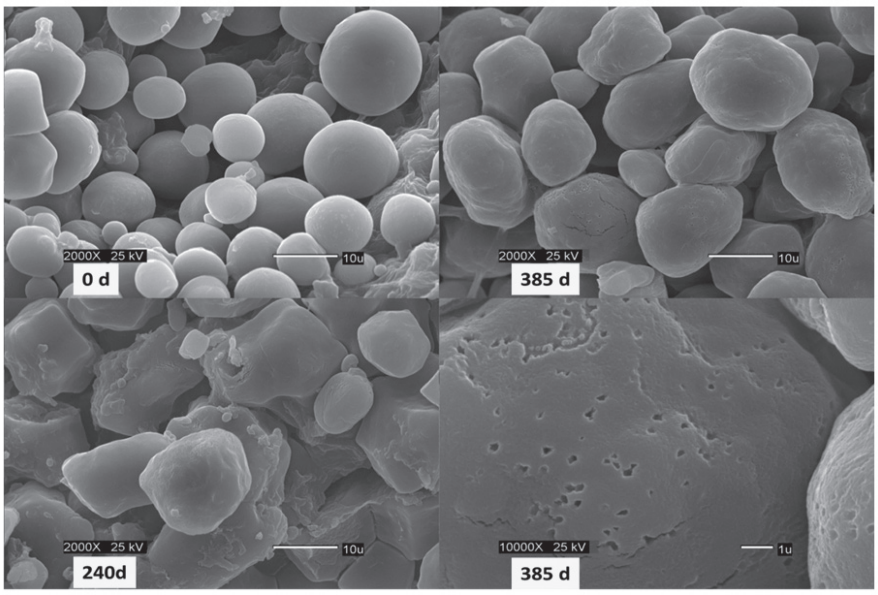

Figure 1 Scanning electron micrographs of inoculated corn silage ensiled for 0, 240 or 385d.

Nutritional characterization and fiber digestibility of corn silage stored on-farm

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Keywords corn silage, fiber digestibility, length of ensiling, nutrient content

Introduction The nutritional value and fiber digestibility of corn silage may be affected by ensiling time and it needs to be accounted for when balancing rations. Cows fed "green" corn silage (<4 mo ensiling) usually do not perform well and cows seem to milk best after silage is fully fermented. The objective of this study was to assess the effect of length of ensiling (LOE) on nutrient content and ash-free neutral detergent fiber (aNDF) digestibility of inoculated corn silage stored in a large volume silage bag at the farm.

Materials and methods Whole-corn plants (P0636AMX, Pioneer, Johnston IA) were chopped using a self-propelled harvester (Claas Jaguar 980) fitted with an after-market cross grooved crop-processing rolls (Shredlage, LLC, Tea, SD). The chopper was set for a theoretical length of cut (TLC) of 26-33 mm, which is longer than normal TLC. Chop length was determined using a Penn State Particle Size Separator. Fresh forage was inoculated at the harvester using a homo-fermentative microbial inoculant (Biomax 5, CHR. Hansen, Milwaukee, WI). Forage was ensiled using an Ag Bagger MB90-1012 HyPac (Warrenton, OR) and stored in an Ag Bag (Klerks Hyplast Inc. , Chester, S.C.) measuring 3.66 m in diameter x 152.44 m long with an estimated storage capacity of 1000 metric tons of fresh forage. Duplicate forage samples were taken at ensiling (day 0), then at 240 d and 385 d post-ensiling. Samples were analyzed at a properly accredited commercial laboratory by wet chemistry (Rock River Laboratory, Inc., Watertown, WI) for nutritional characteristics, fermentation end products and 30 h *In vitro* ruminal aNDF digestibility. Statistical analysis was performed as a completely randomized design with repeated measures over time. Tukey-test was used for mean separation. A companion two-page abstract focusses on the starch fraction.

Results and discussion The main effect of using the shredlage processor on the chopper was to increase the particle size retained on the top screen above current recommendation (Table 1). This increase in particle size should be beneficial for the ruminal health of dairy cows**.** The dry matter (DM) content of the silage decreased ($P<0.05$) after 385 d of ensiling (Table 2). The content of aNDF increased ($P<0.05$) after 240 d post-ensiling, presumably due to the consumption of sugars ($P<0.05$) and possibly other nutrients during the ensiling process. As expected, ensiling resulted in a significantly ($P<0.05$) lower pH, higher lactic acid, propionic acid, and total volatile fatty acids (VFA). Lactic acid decreased ($P<0.05$) from 240 to 385 d post-ensiling. The content of acetic acid increased non-significantly ($P>0.05$) between fresh and silage ensiled for 240 d, but significantly increased ($P<0.05$) after being ensiled for 385 d. Butyric acid was not detected until 385 d post-ensiling. The changes observed in lactic, acetic and butyric acids are probably due to the silage management during feed-out. The digestibility of aNDF did not differ ($P>0.05$) at the different LOE, despite digestibility numerically decreasing by 5.7 percentage units after 240 d of ensiling. This observation, although not statistically significant, is biologically and

economically important. A major obstacle to conducting on-farm research is the very low level of replication possible. Interpretation of on-farm results must be conducted with care, as results without statistical significance may have major implications on animal health and performance and on the economic performance of the enterprise.

Conclusion Ensiling for 240 or 385 d resulted in significant changes in fermentation characteristics, which appear to be the result of sugar consumption. The differences in fermentation profile between 240 and 385 d of ensiling are probably due to silage feed-out management and not to ensiling per se. After 385 of ensiling, the content of DM decreased, aNDF increased and its digestibility decreased numerically. These changes indicate the need to closely monitor the nutritional and fermentation characteristics of silage in order to properly balance rations and feed cows.

Table 1 Corn silage particle size using a shredlage processor compared to recommended size, % of as fed retained

Penn State Particle Separator Pore size, mm	Recommended	Shredlage Processor
19	3-8	45.9
7.9	45-65	30.4
4	20-30	22.1
Bottom pan	<10	1.6

Table 2 Chemical and fiber digestibility characteristics of corn silage ensiled for 0, 240 or 385 d

	Length of ensiling, d		
Item	0	240	385
DM[1]	36.7[a,b]	39.9[a]	27.05[b]
CP, %	7.19[a]	7.13[a]	7.41[a]
ADF, %	25.04[a]	22.88[a]	23.67[a]
aNDF, %	36.5[a]	39.55[a,b]	42.82[b]
ADF bound protein, %	0.52[b]	0.68[a]	0.75[a]
Lignin, %	1.38[a]	2.48[a]	2.59[a]
Ether extract, %	1.95[a]	1.78[a]	1.95[a]
Sugar, %	8.7[a]	1.79[b]	1.67[b]
NFC, %	51.52[a]	49.49[a]	45.57[a]
Ash, %	3.74[a]	3.30[a]	3.71[a]
pH, %	5.75[a]	4.23[b]	4.41[b]
Lactic acid, %	0.12[c]	2.47[a]	1.45[b]
Acetic Acid, %	0.08[b]	0.43[b]	1.49[a]
Propionic Acid, %	0[b]	0.32[a]	0.24[a]
Butyric acid, %	0[b]	0[b]	0.15[a]
Total VFA, %	0.19[b]	3.21[a]	3.32[a]
Ammonia-N, %	0.01[a]	0.07[a]	0.08[a]
In vitro 30 h ruminal aNDF digestibility, % aNDF	53.74[a]	48.03[a]	48.34[a]

[1]Fresh basis; all others reported on a DM basis unless otherwise specified.
[a, b] Means with unlike superscripts in the same row differ $P<0.05$.

Evolution of corn silage quality in the Campos Gerais region - Brazil

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Keywords analysis, nutrition, particle size, silo

Introduction Due to the seasonality in the production of forages, silage is an important source of conserved feed for ruminants. The use of silage in diets for dairy cows has increased in recent years in Brazil (Senger et al., 2005), as well as the milk production and the number of cows, 5% and 3% per year, respectively (IBGE/ Search National Livestock, 2014). Thus, we can expect that demand for silage continue increasing. 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). The particle size affects the amount of effective fiber that is associated with the chewing activity, rumen pH and milk fat content (Mertens, 1997). So, measuring the chemical and physical quality of corn silage is fundamental for the correct balance of diet and increased of animal productivity.

Materials and methods Through the years 2009-2014 were visited 290 farms, in 18 cities of Campos Gerais region (Centre East of Paraná State) and south of São Paulo State - Brazil. Silages were evaluated of 613 silos that were used in animal feed. The distribution and average particle size (APS) were determined using the Penn State Particle Separator, which consist of three sieves with diameters of 19.0, 8.0 and 1.18 mm, plus a bottom box (Lammers et al., 1996). The chemical analysis of the silage were dry matter (DM) by AOAC (1998), crude protein (CP) by Dumas combustion method (Nelson and Sommers, 1980), insoluble acid detergent fiber (ADF) and insoluble neutral detergent fiber (NDF) by Van Soest (1991), *in vitro* dry matter digestibility (IVDMD) and *in vitro* NDF digestibility (IVNDFD) by Tilley and Terry (1963), starch by Pereira and Rossi (1995) and pH. The total digestible nutrients content (TDN) were estimated by the equation TDN = 87.84 – (0.7 x ADF) by Undersander et al. (1993) and relative feed value (RFV) by the equation RFV = (DMI x DMD)/1.29, where DMI = 120/NDF e DMD = 88.9 – (0.779 x ADF) by Rohweder et al. (1978). A descriptive statistical analysis and regression analysis between the years at 5% probability was conducted. The software used was SAS 9.3.

Results and discussion The chemical and physical 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 = 0.7691; P = 0.0218), and the average was close the technical indication which is 30 to 35% DM (Nussio et. al, 2001). The ADF content reduced (r^2 = 0.8244; P = 0.0123) and TDN increased (r^2 = 0.8243; P = 0.0123) with the time, which means that the silages are improving their quality of fiber and energy. The ADF average was 25% and TDN 70%, similar to those found in US silages, 26% and 71, respectively (Dairy One, 2015). Between years, there was a reduction in the amount of particles retained on the sieve 3 (r^2 = 0.7174; P = 0.0333), which is related to kernel processing and this can result in lower utilization of starch by animals. The average particle size (APS) increased with the time (r^2 = 0.7158; P

= 0.0337). According to the report of farmers, they have sought to work with larger particle size of silage due to use of mechanic cutter, which reduces the particle size at loading and mixing the diet ingredients. On the other hand, long particles impair silage packing and increase particle sorting at the feedbunk.

Conclusions Corn silages from the studied region had good chemical composition, but it is necessary to improve the particles size in most of the silages.

Table 1 Chemical and Physical Characteristics of silage over the years (%)

	Year									Standard	Year Reg	
Item	2009	2010	2011	2012	2013	2014	Average	Min.	Max.	deviation	Pr > F	r^2
DM (%)	29	31	31	31	32	33	31	21	49	4,.1	0.0218	0.7691
CP (%)	7	7	8	7	7	7	7	5	10	0.9	0.5633	0.0901
ADF (%)	26	26	25	25	24	25	25	17	36	3.3	0.0123	0.8244
NDF (%)	45	44	46	46	45	46	45	34	62	5.0	0.2376	0.3249
TDN (%)	70	70	70	71	71	71	70	62	76	2.3	0.0123	0.8243
Starch (%)	33	34	33	33	33	31	33	15	47	4.7	0.2087	0.3591
pH	3.9	3.9	3.9	3.8	3.8	3.8	3.8	3.5	4.5	0.1	0.1649	0.4186
S1 (%)	5	6	8	7	6	9	7	1	48	5.1	0.1730	0.4069
S2 (%)	55	60	66	69	70	65	64	28	84	10.0	0.0621	0.6226
S3 (%)	38	32	25	23	23	24	28	9	67	9.5	0.0333	0.7174
S4 (%)	2.2	1.8	1.0	0.8	0.9	1.2	1.3	0.0	5.6	1.0	0.0969	0.5384
APS (mm)	8	8	10	10	10	10	9	5	16	1.4	0.0337	0.7158
RFV	143	146	142	143	145	143	144	92	204	19.7	0.6856	0.0453
IVDMD (%)	-	-	72	68	68	70	69	55	79	3.5	0.6418	0.1283
IVNDFD (%)	-	-	-	55	52	55	54	43	67	4.2	0.9305	0.0119
n	53	89	107	120	114	130						

DM: dry matter; CP: crude protein; ADF: insoluble acid detergent fiber; NDF: insoluble neutral detergent fiber; TDN: total digestible nutrients; S1: particles retained at 19.0 mm sieve; S2: particles retained at 8.0 mm sieve; S3: particles retained at 1.18 mm sieve; S4: bottom box; APS: average particle size; RFV: relative feed value; IVDMD: *in vitro* dry matter digestibility; IVNDFD: *in vitro* NDF digestibility; n: number of samples.

Dry matter losses evaluation in winter cereals silage

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Keywords barley, effluent, gas losses, triticale silage

Introduction The winter cereals has its importance for the production of silage as they represent an alternative to the use of land, improving livestock production while reducing production costs. Among these cereals, some are great relevance in south Brazil, for example, the oats, wheat, barley and triticale. Considering the characteristics of temperate grasses, it is possible may occur secondary events of silage fermentation process. Such events may cause losses of dry matter that directly affect the final nutritional quality of silage. Among these losses, it possible cite losses by effluent and gases, which occur due to the low dry matter content of the material at the time of ensiling and operational failure on silo loading. Thus, the objective of this study was to evaluate the dry matter losses from the silage winter cereals.

Materials and methods The experiment was conducted in Núcleo de Produção Animal (NUPRAN) of the Center for Agricultural and Environmental Sciences. University of the Midwest (UNICENTRO) in Guarapuava - PR. The treatments consisted of five winter cereals: wheat (*Triticuma estivum* cv. BRS Crow Blue), barley (*Hordeum vulgare* cv. BRS Brau), oat (*Avena sativa* cv. URS Guara), oat (*Avena strigosa* cv. Embrapa 139) and triticale (*X Triticosecale* cv. IPR 11). The harvest for silage was done when the plants were milky grains. After cutting with an average particle size of 10 mm, the materials were stored in PVC silos. After 60 days of storage, the silos were opened and the silages were evaluated for dry matter losses. Total losses due to silage production were evaluated using the methodology and equations described by Jobim et al. (2007). Gas losses were evaluated using the equation predicted by Schmidt (2006). The experimental design was randomized blocks composed of five cereal species and five replications. The statistical analysis was conducted using ANOVA procedure and the means were compared by Tukey test at 5% significance level.

Results and discussion The results of dry matter losses by effluents, gases and the dry matter recovery index of winter cereals silages are shown in Table 1.

Table 1 Losses of effluent, gas and dry matter recovery (DMR) of winter cereals silage

Treatment	Dry matter losses		DMR
	Effluent ($kg.ton^{-1}$)	Gases (%)	(%)
Triticum aestivum	26.298a	9.758a	79.40b
X Triticosecale	22.334a	8.216b	75.60b
Hordeum vulgare	11.224b	4.722c	92.40a
Avena sativa	12.162b	4.760c	88.60ab
Avena strigosa	10.370b	5.256c	83.80ab
EMS	17.887	0.547	0.0052

Means followed by different letters on the line were statistically different ($P<0.05$) by Tukey test; EMS = Error mean square.

Among the cereals evaluated in this study, it was observed that the greatest losses by effluents and by gases were in wheat silages (26.298 $kg.ton^{-1}$ and 9.758%, respectively), while barley showed the lowest losses and higher dry matter recovery rate (11.224 $kg.ton^{-1}$, 4.722% and 92.41%, respectively). Similar results for dry matter recovery of silage from barley and oat silage, was observed by others authors. However, for wheat and triticale silage recovery rates were higher than in this study. This fact showing that the ensiled in milk stage shows a marked loss of CO_2 due to a greater amount of epiphytic yeasts. The activity of yeasts contributes to the increase of gas losses and consequently to the reduction in dry matter recovery rate. This may be the explanation for the findings in this work. For the triticale, other authors evaluated that in the milk stage, there are low levels of acetic. Indeed, this leads to a proliferation of yeasts, and this could be the reason of the results of this present study.

Conclusions The triticale and wheat silages had higher losses by effluent and gases, with consequent lower dry matter recovery.

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Mineral composition and quality of grass silages contaminated with various types of soil with different iron level

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Keywords contamination, grass silage, iron, minerals, soil

Introduction Iron supply exceeding cows' requirements is common when feeding forages. Especially grass silages, contaminated with soil are considered as its main source in diets. Intensive conditions of use of grasslands by means of harvesting machines, heavy organic fertilization in farming with high stocking rates as well as wet weather result in higher amounts of dirt and soil, which get on the surface of plants. Iron as an antagonist of copper decreases its availability in the animal and can lead to metabolic disorders. In addition, acidic conditions during ensiling increase the availability of minerals from contaminating soil. Moreover, soil deteriorates effects of forage conservation and enhances butyric fermentation as carrier of clostridial spores. Thus, the aim of this study was the evaluation of the mineral composition and quality of grass silages from material at two levels of dry matter content and contaminated with four diverse types of soil from locations which were particularly scarce, moderate and particularly abundant in iron.

Materials and methods Temperate grass dominated pasture was cut on 1st July, 2014 and used in fresh (248 ±22 g kg^{-1} DM) and prewilted form (444 ±66 g kg^{-1} DM) to prepare silages contaminated with 10 % addition (on DM basis) of 4 types of soil with different iron level: low, 4100 mg, intermediate, 6700 and 8400 mg, and high, 11300 mg kg^{-1} DM, respectively from locations in Naundorf, Großolbersdorf, Köllitsch and Memmendorf. Treatments without soil addition (W/O) were also prepared at both levels of DM. The chemical composition of silages prepared in triplicate was determined according to standard methods (AOAC, 2007). Silages underwent an aerobic stability test as well as sensory and ensiling quality evaluation in accordance with DLG (1990, 2004, 2006). Data obtained in the study were subjected to a two-way analysis of variance by means of STATISTICA 10 (StatSoft) and the significance of differences was estimated by the Tukey test at $\alpha = 0,05$.

Results and discussion Content of crude ash exceeded considerably 150 g/kg DM in all silages treated with soil, what proves ash level as a good indicator for soil contamination of forage. Mineral composition of studied silages differed significantly within studied treatments, however content of sodium, chloride, sulphur and potassium was not affected resulting in similar dietary cation-anion difference (DCAD) (results not shown). Addition of soil caused increase ($P<0.01$) in content of magnesium, iron, manganese, silicon and aluminium and in zinc, copper in initial materials ($P<0.05$) (data not shown). Their content was lower in silages than in corresponding initial materials, excluding W/O treatments. However, the availability of minerals rises considerably under ensiling process and may

cause symptoms of Cu-deficiency in cows. It is caused by antagonistic action of iron, which is assumed at 450 mg/kg DM. It was surpassed manifold in the study when soil was added. Content of magnesium and all of the studied trace elements in silages was higher ($P<0.01$) in 25 % DM variants, especially with Memmendorf soil (Table 1). Fermentation parameters were not affected by the treatments; although results of ensiling quality were worse ($P<0.01$) in lower dry matter silages with addition of soil, still they were marked as very good. Nonetheless heavy soil contaminated silages may be hazardous in feeding of dairy ruminants since they may be a carrier of *Clostridium* and *Listeria* bacteria and occurrence of listeriosis and botulism as well as deterioration of quality of cheese produced from milk. While presence of soil in grass is not completely unavoidable it may be reduced through appropriate harvesting, storage and application of fertilizers followed by a waiting period.

Conclusions Content of crude ash was high in all silages, especially after soil treatment. It was a source of minerals such as magnesium, iron and other trace elements in silages. Their highest level presented silages with Memmendorf soil. Although soil had little impact on grass silage quality, they should be always analysed for content of Cu-antagonists like Fe.

Table 1 Chemical composition, parameters of fermentation and evaluation of silage quality (g kg^{-1} DM, unless otherwise stated)

		Dry matter		Soil type					
Item	*Ref.*	25%	45%	W/O	Naun.	Groß.	Köll.	Mem.	SEM
Nutrient									
Dry matter, g kg^{-1}	*248.3*	266.5^{A}	480.9^{B}	358.7	376.3	477.6	481.4	400.8	23.0
Crude ash	*97.7*	159.8	159.8	114.3^{B}	171.7^{A}	176^{A}	181.6^{A}	172.5^{A}	5.6
Acid insoluble ash		74.3	77.6	33.9^{B}	90.3^{A}	92^{A}	99.1^{Ab}	84.6^{Ab}	5.2
Minerals									
Ca	*6.5*	6.9^{a}	6.4^{b}	6.8	6.6	6.4	6.5	6.6	0.1
P	*3.5*	3.6^{a}	3.3^{b}	3.5	3.4	3.3	3.2	3.4	0.0
Mg	*1.7*	1.9^{A}	1.8^{B}	1.7^{C}	1.8^{B}	1.9^{B}	1.8^{B}	2.0^{A}	0.0
Fe mg kg^{-1} DM	*139*	2099^{A}	1933^{B}	235^{C}	1813^{Bb}	2450^{Ba}	2173^{Ba}	3381^{A}	247
Mn mg kg^{-1} DM	*29*	84^{A}	78^{B}	34^{C}	92^{Ba}	79^{Bb}	91^{Ba}	106^{A}	6
Zn mg kg^{-1} DM	*35*	45^{A}	42^{B}	40^{B}	41^{B}	45	40^{B}	49^{A}	1
Cu mg kg^{-1} DM	*7*	9^{A}	9^{B}	8^{B}	8^{Bb}	9^{Ba}	9^{Ba}	11^{A}	0
Si	*23.5*	34.6^{A}	32^{B}	25.5^{C}	37.2^{Aa}	30.9^{Bc}	33.9^{Ab}	36^{A}	1.0
Al mg kg^{-1} DM	*136*	2802^{A}	2541^{B}	60^{C}	2764^{B}	3005^{B}	3219^{B}	4322^{A}	337

Fermentation quality of tropical forage legume silages

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Keywords *Vigna unguiculata, Canavalia brasiliensis, Ipomoea batatas, silage quality*

Introduction Legumes are considered difficult to ensile, due to three main factors: they are highly buffering, and low in water-soluble carbohydrates and dry matter (DM). For this reason, it is necessary to add a source of soluble carbohydrates, so that the lactic acid bacteria (LAB) accelerate the fermentation process, increase the production of organic acids improving the quality of silage. The objective of the study was to evaluate the quality of silages from tropical forages (*Vigna unguiculata* CIAT4555, *Canavalia brasiliensis* CIAT17009) and the sweet potato tuber (*Ipomoea batatas*, variety Tainun) and their mixtures at different cutting interval.

Materials and methods The legumes *Vigna unguiculata* and *Canavalia brasiliensis* were evaluated at four different cutting intervals: *Vigna* was cut at 6, 8, 10 and 12 weeks of growth; *Canavalia* was cut at 8, 12, 16 and 20 weeks of growth. The forage and *Ipomoea batatas* tubers were dried in the sun to approximately 350 g DM/kg and then chopped. They were ensiled in PVC tubes (1.8 L volume) in three replicates. Six treatments were applied: *Vigna* or *Canavalia* or *Ipomoea* alone; *Vigna* + *Canavalia* in the proportion 1:1 on fresh matter (FM) base; and *Vigna* or *Canavalia* mixed with sweet potatoes in the proportion 1:1 on FM base. Silos were stored for about 3 months at ~25°C. A completely randomized design with a factorial arrangement and 3 sources of variation was used and analyzed by the GLM procedure (SAS). Organoleptic properties (color, smell and texture) were evaluated when uncovering the micro silos (up to 4 points is considered rotten, 5-9 bad, 10-13 average, 14-17 good, 18-20 excellent quality) according to DLG (2006). The dry matter content and pH were determined. Organic acids (acetic, propionic and butyric) were determined by HPLC, according to the methodology proposed by Siegfried, et al., (1984) NH_3-N of total N was determined by the micro-diffusion technique described by Voigt et al. (1967).

Results and discussion The fermentation quality was better in mixtures of *Ipomoea* (Table 1). NH_3-N of total N as indicator of proteolysis was relatively high, exceeding 80 g/kg total N as a threshold value.

Conclusions The inclusion of *Ipomoea* improved the silage quality of the legumes (*Vigna, Canavalia*) mainly by the contribution of soluble carbohydrates thus favoring an increase in bacterial population lowering the pH and resulting in good organoleptic characteristics.

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Table 1 Chemical composition of the silages of *Vigna unguiculata*, *Canavalia brasiliensis* and sweet potato and their mixtures

Cutting intervals	*Vigna*	*Canavalia*	*Ipomoea*	*Vigna-Ipomoea*	*Canavalia-Ipomoea*	*Vigna-Canavalia*	Means
pH							
6 weeks	5.49	--	--	4.38	--	--	4.93^{a}
8 weeks	6.04	5.30	3.63	4.17	4.34	5.34	4.8^{b}
10 weeks	5.19	--	3.98	4.37	--	--	4.51^{de}
12 weeks	4.61	5.05	3.84	4.59	4.77	4.6	4.58^{cd}
16 weeks	--	5.25	4.21	--	4.45	--	4.64^{c}
20 weeks	--	5.15	3.81	--	4.3	--	4.42^{e}
Means	5.33^{a}	5.19^{b}	3.89^{f}	4.37^{e}	4.46^{d}	4.97^{cd}	
NH3-N g/kg total N							
6 weeks	14.94	--	--	21.03	--	--	17.98^{b}
8 weeks	27.62	15.58	47.79	36.83	14.38	16.6	26.47^{a}
10 weeks	38.47	--	8.73	8.82	--	--	8.74^{d}
12 weeks	5.49	10.79	4.14	7.74	8.67	7.83	15.73^{bc}
16 weeks	--	15.95	16.57	--	14.67	--	7.44^{d}
20 weeks	--	12.89	14.16	--	11.4	--	12.82^{c}
Means	14.2^{b}	13.8^{b}	18.3^{a}	18.6^{a}	12.28^{b}	12.21^{b}	
DLG quality points (color, smell, and texture)							
6 weeks	19	--	--	9.7	--	--	14.3^{a}
8 weeks	11	12.6	13	13	13	17	13.3^{a}
10 weeks	1.8	--	19	13	--	--	15. 7^{a}
12 weeks	13	9.3	9	19	13.6	11.3	12.5^{a}
16 weeks	--	18	--	--	17	--	14.9^{a}
20 weeks	--	14.6	9.7	--	13.3	--	12.6^{a}
Means	14.6^{a}	13.7^{a}	12.1^{a}	13.7^{a}	14.2^{a}	14.2^{a}	
Acetic acid g/kg DM							
6 weeks	0.6	--	--	0.4	--	--	0.5^{c}
8 weeks	1.4	0.7	0.5	0.6	0.6	1.16	0.8^{a}
10 weeks	0.2	--	0.6	0.6	--	--	0.7^{ab}
12 weeks	0.5	1.2	0	0.3	0.5	0.6	0.5^{c}
16 weeks	--	0.9	0.4	--	0.5	--	0.6^{bc}
20 weeks	--	1.3	0.3	--	0.7	--	0.8^{a}
Means	0,9^{a}	1,02^{a}	0,4^{cdef}	0,51^{bc}	0,6^{b}	0,87^{a}	

Data with different letters indicate significant differences according to Duncan test ($P < 0.05$)

Investigations on the ensilability of whole-crop soybean and the feeding value of its silage

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Keywords Glycine max, silage additives, nitrogen fractionation

Introduction Cultivation of soybean plants becomes more and more popular in Southern Germany and Austria. Due to climates of these regions, weather conditions can limit ripening of soybeans to a threshable stage. Thus, production and feeding of whole-crop soybean silages in a late growth stage becomes an interesting research field, especially because information about this is currently rare (Mustafa and Seguin, 2003). Therefore, silage trails were conducted in the years 2013 and 2014 to get information about (1) the ensilability of whole-crop soybean at late maturity stages, (2) the benefit of using silage additives during the silage making process and (3) the feeding value of produced silages.

Materials and methods Non-GMO soybeans (variety Merlin) were cultivated at the Grub Research Station (Bavarian State Research Center for Agriculture), 20 km east of Munich. In 2013, soybeans were harvested directly at three different reproductive stages R5-R6 (August 24th), R6-R7 (September 11th) and R8 (October 13th), and ensiled into 1.75 L jars, according to guidelines of German Agricultural Society (DLG) for silage additive-testing. Two additives were applied to the split base material, one based on lactic acid bacteria [*L. plantarum* (NCIMB 30083+30084), *P. acidilactici* (NCIMB 30086+30085), *E. faecium* (NCIMB 11181), Siloferm, Agravis] and the other on sodium nitrate plus hexamine (Kofasil liquid, Addcon). Silages were analyzed for fermentation parameters and additionally, pooled samples were taken for nitrogen fractions according to Licitra et al. (1996, modified method). In 2014, soybeans were harvested at one reproductive stage (R5-R6, August 21st) and ensiled in 1.75 L jars and 300 L barrels. The silage trial included the same silage additives used in 2013. Silages from barrels, which were stored at 10-15 °C for 42 days, were used for a digestibility trial with wethers.

Results and discussion Fermentability of base materials in 2013 differed widely, reaching fermentation coefficients (Weißbach et al. 1974) of 38-62. Consequently, silages showed great differences regarding fermentation parameters (Table 1). Fermentation quality improved as a consequence of late maturity stages and increasing dry matter (DM) contents of the plants. While the addition of lactic acid bacteria resulted in rather a slight improvement of the fermentation quality, the addition of the chemical additive largely prevented butyric acid fermentation at all reproductive stages. However, regarding results of nitrogen fractionation, no clear beneficial effect of the application of silage additives could be observed (Table 2). The experimental silages in 2014 showed great similarities to the results for reproductive stage R5-R6 in 2013 (data not shown). However, silages produced in barrels simultaneously did not exhibit any sign of failed fermentation. Therefore, important mean parameters for feeding values (2014) were 26 % of DM, 36 % of neutral detergent fiber (NDF_{om}), 22 % of crude protein (CP), 7 % of crude lipids (CL), digestibility of organic matter (OM) of 73 % and 11.4 MJ/kg DM of metabolizable energy

(ME), without significant beneficial effects of additive application.

Table 1 Fermentation parameters, DM-losses and aerobic stabilities of whole-crop soybean silages at three reproductive stages, without and with addition of silage additives (2013)

Harvest/ treatment	pH	Lactic acid	Acetic acid	Butyric acid	Ethanol	NH_3-N/Nt	DM-losses	Aerobic stability
		g/kg DM				%	%	days
1/control	5.5[a]	8[a]	9[b]	56[d]	22[f]	10.3[e]	10.3[e]	7.0
1/LAB_{homo}	5.5[a]	30[c]	6[a]	34[c]	13[d]	6.8[d]	7.8[d]	7.0
1/chemical	4.6[e]	74[f]	17[d]	2[a]	8[b]	6.4[d]	3.5[c]	6.2
2/control	5.6[a]	6[a]	8[b]	37[c]	15[e]	5.4[c]	7.7[d]	7.0
2/LAB_{homo}	4.8[d]	41[d]	12[c]	8[b]	5[a]	4.0[b]	4.1[c]	7.0
2/chemical	4.6[e]	50[e]	14[c]	0[a]	5[a]	4.1[b]	3.0[b]	7.0
3/control	5.3[b]	19[b]	5[a]	5[ab]	10[c]	4.1[b]	3.7[c]	7.0
3/LAB_{homo}	4.7[e]	38[d]	10[b]	1[a]	4[a]	3.0[a]	2.1[a]	7.0
3/chemical	4.9[c]	31[c]	9[b]	0[a]	6[a]	4.9[c]	2.3[a]	6.2

NH3-N/Nt=portion of ammonia-N of total N; aerobic stability=opened 49 days after ensiling, days until silage temperature is >3°C above room temperature, max. 7.0 days, means of n=3; LAB_{homo}=homofermentative lactic acid bacteria; means of n=3, $P<0.05$, statistical model included harvest treatment harvest×treatment effects.

Table 2 Shift of nitrogen fractionation from base material to whole-crop soybean silages depending on reproductive stage and use of silage additives (2013)

Harvest/ treatment	DM g/kg	Nitrogen fractions (% CP)					UDP5
		A	B1	B2	B3	C	
1/control	277	23	0	-13	-9	-1	-14
1/LAB_{homo}	291	26	-3	-13	-9	-2	-14
1/chemical	306	29	-2	-17	-9	-2	-16
2/control	381	13	2	-10	-3	-2	-11
2/LAB_{homo}	426	19	6	-18	-4	-2	-17
2/chemical	408	20	4	-17	-4	-2	-16
3/control	581	8	3	-1	-5	-5	-22
3/LAB_{homo}	613	2	7	2	-3	-7	-24
3/chemical	583	13	-1	-5	0	-7	-24

Fraction A=Nonprotein N, B=soluble protein, C=acid detergent insoluble protein, UDP=undegradable protein at 5 % h^{-1} passage rate, LAB_{homo}=homofermentative lactic acid bacteria, pooled samples of n=3.

Conclusions Whole-crop soybean silage is a feedstuff of potential high feeding value but ensilability of the whole-crop appears limited. Therefore, chemical silage additives which improve the fermentation process should be used. A shift within nitrogen fractions from B to A due to ensiling was independent of fermentation success.

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In situ rumen degradation kinetics, protein and carbohydrates fractionations of tropical legume (*Stylosanthes* cv. Campo Grande) and corn silages

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Keywords *Stylosanthes* silage, neutral detergent fiber, dry matter degradation.

Introduction In the tropics, the dry season undertakes the production of ruminant in the pastures, because of the decrease in the forage production. Making silage is an alternative to solve this problem. Recently, it has grown in Brazil interest in legume silages. The *Stylosanthes* cv. Campo Grande (*Stylosanthes capitata* plus *Stylosanthes macrocephala*) is adapted to tropical conditions, exhibit a nutritional value comparable to other high efficiency feeds being used in the tropics and has good characteristics of fermentation and animal performance (Souza et al., 2014). The objective of this study was to evaluate the ruminal degradation kinetics and fractions of carbohydrates and protein of *Stylosanthes* (**SS**) and corn silages (**CS**).

Materials and methods The silages samples were subjected to preliminary drying for 72 hours in the oven at 55° C and ground to 2 mm particle size for degradability and 1 mm for fractionations. For *in situ* incubations, approximately 5 g of sample were weighed into nylon bags in duplicate for each silage in each animal that represented a repetition. Two rumen-cannulated steers were used, with an average body weight of 400 kg, fed SS and concentrate (13% CP). The incubation times were 0, 2, 4, 8, 16, 24, 48, 72, 96, 120 and 144 hours. We used the asymptotic model of first order proposed by Orskov and McDonald (1979) to estimate of the degradation of the DM and CP. The exponential decay model, adjusted for the lag time, proposed by Mertens and Loften (1980) was used to estimate NDF degradability. The Marquardt regression procedure was used to fit the models by using the SAS software. The protein and carbohydrates fractionation were determined according to the methods described by Licitra et al. (1996) and Sniffen et al. (1992), respectively. Data were submitted to analysis of variance and test F.

Results and discussion The SS had higher non-degradable fraction of NDF and lower DM and CP degradation than the CS (Table 1). Tropical legumes generally have higher lignification of the cell wall and lower digestibility. Despite the higher content of total nitrogen (**TN**), SS has the highest fractions of non-protein nitrogen, unavailable nitrogen (% TN) and lower total carbohydrates than the CS (Table 2).

Conclusions The *Stylosanthes* cv. Campo Grande silage has higher nitrogen content than corn silage, but less potentially degradable fraction of DM, CP and NDF compared to the corn silage.

Acknowledgements The authors are thankful to CNPq and FAPEMIG for providing financial support.

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Table 1 In situ degradation kinetic parameters of Stylosanthes cv. Campo Grande (SS) and corn silages (CS)

Neutral Detergent Fiber Degradation				
Itens[1]	SS	CS	SEM	P-value
B	40.20	70.83	8.87	0.0036
U	54.80	26.87	8.10	0.0046
L	2.91	1.07	0.55	0.0272
kd	0.03	0.01	0.01	0.0208
Crude Protein Degradation				
a	10.00	4.38	1.67	0.0310
b	22.31	58.46	10.44	0.0008
kd	0.07	0.04	0.01	0.0110
Dry Matter Degradation				
a	11.90	16.79	1.41	0.0019
b	33.64	56.57	6.64	0.0024
kd	0.04	0.01	0.01	0.0013

[1]B = potentially degradable fraction of the fiber (%); kd = rumen degradation dynamics of fraction (h^{-1}); L = lag time (h); U = undegradable fraction of the neutral detergent fiber (%); a = soluble nitrogen (%); b = potentially degradable fractions (%).

Table 2 Protein and carbohydrates fractionations (%) of Stylosanthes cv. Campo Grande (SS) and corn silages (CS)

Proteins				
Fractions[1]	SS	CS	SEM	P-value
A	38.14	21.85	4.72	0.0050
B1	25.60	25.83	0.36	0.8165
B2	17.88	37.08	5.55	0.0018
B3	14.29	13.68	0.18	0.0208
C	10.09	3.76	1.83	0.0002
TN	1.91	1.28	0.26	0.0079
Carbohydrates				
A+B1	23.17	36.04	3.76	0.0123
B	30.95	39.97	2.61	0.0010
C	45.88	23.99	6.36	0.0056
TC	75.15	86.26	3.21	0.0040

[1]A = non-protein nitrogen (% TN); B1, B2 and B3 = protein nitrogen (% TN); C = unavailable fractions (% TN or % TC); TN = total nitrogen; A+B1 = non-fiber carbohydrates (% TC); B = available fraction of the fiber (% TC); TC = total carbohydrates.

Effects of different ratios of mixed alfalfa and corn silages on *in vitro* fermentation

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Keywords alfalfa, corn, mixed silage, *in vitro*

Introduction It is well known that alfalfa is a forage crop with high nutritive value and is often a fermentation major component of diets for high-producing dairy cows. Alfalfa is an excellent perennial leguminous grass, which is widely planted in China. However, this forage crop is difficult to ensile due to its low fermentable carbohydrate and high buffering capacity. So it is considered necessary to add something high in sugars into alfalfa silage to create the nutrient equilibrium conditions, including mixing alfalfa with sugary grass silage. Corn is characterized by high water soluble carbohydrate content. Alfalfa can produce 4 crops a year, and the optimum mowing period of alfalfa is in accord with spring-sown corn and summer-sown corn. The information on the ability of *in vitro* fermentation of mixed silages is lacking. The objective of this study was to assess the effects of different ratios of mixed alfalfa and corn silages on *in vitro* fermentation.

Materials and methods The experiment was carried out at the Agricultural Research Station of Zhuozhou of Hebei Province in China. Alfalfa and corn respectively in the early bloom and late milk stage were chopped into particles of approximately 2 cm. The ratios of alfalfa and corn at ensilage were 10:0, 8:2, 6:4, 4:6, 0:10 on the natural basis. The silage was ensiled in triplicate for each treatment in 5 L laboratory PVC silos (packing density were 750 kg/m^3). The silos were capped tightly, until opened for sampling following 60 d preservation at room temperature (20-25 ºC). Silage samples were oven-dried at 65ºC for 48 h, and ground through a 1-mm screen. Basal medium were prepared by the method of Menke and Steingass(1988). Rumen fluids were obtained from three rumen-cannulated lactating Holstein dairy cows at 2 h prior to the morning feeding. All bottles were individually connected with medical plastic infusion pipes to the gas inlets of an automated gas production recording system as used by Zhang and Yang (2011). Data of cumulative gas production, obtained from the automated gas production recording system, were fitted to the exponential equation as described by Ørskov and McDonald (1979). Treatment effects were evaluated using one-way analysis of variance, the GLM procedure of SAS (v 9.1, SAS Institute, Cary, NC, 2002). Duncan's multiple comparison test was used to evaluate differences in means from those treatments determined to be significant ($P<0.05$).

Results and discussion The content of the *in vitro* dry matter disappearance (IVDMD) in alfalfa silage alone were higher than for the substrate high in corn silage alone ($P <0.05$), and the IVDMD in silage was decreased by the addiction of corn ($P<0.05$, Table 1). The contents of the cumulative gas production (ml/g DM) after 48 h fermentation (GP_{48h}), ideal maximum gas production were increased linearly with the proportions of corn. Compare to the alfalfa silage alone, the use of corn in silage reduced IVDMD by 26.1 to 53.6% and increased GP_{48h} by 89.2 to 161.1%. Considering that acid detergent fiber

is the component that most relates to digestibility, this performance can be explained by the increase in corn. The GP_{48h} is differed between alfalfa and corn, and other degradation characteristics differed between grain varieties and were also influence by feed processing. Ideal maximum gas production likely explanation for this difference was the degradation of protein, which is known to yield less gas per unit of substrate than carbohydrates. No significant difference occurred in gas production speed and Halftime due to whatever use of corn ($P < 0.05$). Neither linear effect of the corn addition occurred on lag.

Table 1 *In vitro* rumen digestibility, gas production kinetics and fermentation characteristics of alfalfa-corn hybrid silages incubated with rumen fluid for 48 h

	Alfalfa: corn ratio						
Items	10:0	8:2	6:4	4:6	0:10	S.E.M	P value
IVDMD (%)	70.32^{a}	67.07^{b}	63.94^{c}	57.71^{d}	45.78^{e}	1.10	<0.0001
GP_{48h} (ml/g)	52.70^{c}	99.71^{b}	111.58^{ab}	112.65^{ab}	137.58^{a}	0.98	<0.0001
Kinetic gas production							
ideal maximum gas production (ml)	58.72^{c}	107.41^{b}	111.59^{b}	112.65^{b}	137.62^{a}	0.68	0.0001
gas production speed (h^{-1})	0.24	0.22	0.22	0.24	0.20	0.03	0.4471
lag time of gas production (h)	0.01^{ab}	0.03^{ab}	0.12^{a}	0.09^{ab}	-0.02^{b}	0.15	0.0909
Half time (h)	2.16	2.23	2.35	2.22	2.27	0.56	0.4976
AGPR[1] (ml/h)	19.54^{b}	34.19^{a}	33.74^{a}	38.05^{a}	40.13^{a}	0.48	0.0001

[1]AGPR is the gas production speed when gas production is 1/2 of the maximum.

Conclusion Addition of corn to alfalfa silage affected in vitro fermentation characteristics in terms of reduced IVDMD and increased GP_{48h}, but did not reduced AGPR.

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Mineral composition and quality of grass silages with inclusion of common dandelion (*Taraxacum officinale*)

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Keywords dandelion, fertilization, grass silage, minerals, potassium

Introduction A slight overbalance of anions in transition cows' diets may reduce the occurrence of hypocalcemia preventing metabolic alkalosis. The practical measure is the dietary cation-anion difference (DCAD, (Na+K)-(Cl+S)), which should show negative values and is primarily dependent on the potassium content in the whole diet. However, grassland feeds usually provide excess of potassium and its level is especially high in common dandelion. Agronomic measures such as mowing frequency and fertilization may affect its mineral composition and proportion in grasslands. Its bioactive substances and chemical composition other than those from grasses may change the ensiling process. Dandelion's importance in modifying the mineral content of the entire sward and its ensiling properties are not yet well-known. Thus the aim of this study was to investigate the mineral composition and the quality of grass silages containing or not dandelion obtained from meadows under low- and high-input management.

Materials and methods Forages were harvested from two plots, which were treated intensively (INT) with mowing and organic-fertilization or extensively (EXT) without fertilization. They were prewilted to prepare silages in triplicate in their original botanical mixture (orig.) and from sorted forages: without dandelion (W/O dand.) and from dandelion only (dand.). The chemical composition of the material before and after ensiling was determined according to standard methods (AOAC, 2007). Silages underwent an aerobic stability test and sensory and ensiling quality evaluation according to Honig (1990) and DLG (2004, 2006). Data obtained in the study were subjected to a two-way analysis of variance by means of STATISTICA 10 (StatSoft) and the significance of differences was estimated by the Duncan's test at $\alpha = 0.05$.

Results and discussion Only orig. crop was evaluated on EXT plot, because of dandelion's absence. It shows a possibility of its control in crops by intensity of grassland management. Mean share of dand. on INT plot was 6.4% in DM. Lower ($P< 0.01$) DM content presented dand. silage, what indicates need of additional wilting time of forage with high inclusion of herb to obtain optimal DM content for ensiling (Table 1). INT silages were richer ($P<0.01$) in crude protein caused by higher supply of nitrogen by fertilizer and crude ash ($P<0.05$) from dirt contamination caused by agricultural machines during fertilization preceding harvest, but lower ($P<0.01$) in crude fibre. Dandelion had the best ($P<0.01$) nutritive value among all silages in terms of higher protein content and lower content of fibre and its fractions. Content of chloride and sulphur was higher in silages from INT plot resulting in lower DCAD in comparison with EXT. DCAD of silages differed also among forage composition. Dand. showed the ability to accumulate high concentrations of potassium, resulting in higher values of DCAD in comparison to orig. and W/O dand. swards. However, its inclusion in orig. silages did not affect their DCAD values. Content of potassium amounted to approximately 30 g kg^{-1} in all studied grass silages what can limit their application in dry cows' diets close to parturition. Dandelion was generally richer in

all macrominerals. It contained also higher amounts of copper what confirms its ability to accumulate metals as a good indicator of environmental pollution caused by agricultural and industrial activity. Moreover, herbs may be a source of minerals in naturally occurring forms, which show higher availability for animals than from traditional additives.

Conclusions Content of potassium and DCAD values were high in all studied silages, especially in EXT variants and in dand.. However, its inclusion did not affect DCAD of orig. silages. Dandelion was the richest in all macrominerals and copper and did not affect quality of silages.

Table 1 Chemical composition, fermentation parameters and evaluation of silage quality [g kg^{-1} DM, unless otherwise stated (mean values)]

	Fertilization		Material			
Item	INT	EXT	orig.	W/O dand.	dand.	SEM
Nutrient						
Dry matter, g kg^{-1}	337,2^{B}	523,9^{A}	436,3^{A}	406,5^{A}	256,4^{B}	30,4
Crude ash	126,9^{A}	93^{B}	104,9^{B}	106,6^{B}	157,2^{A}	7,4
Crude protein	124,0^{a}	122,3^{b}	115,7^{B}	108,9^{B}	154,0^{A}	5,7
Crude fibre	268,3^{B}	280,3^{A}	297,4^{Ab}	324,5^{Aa}	166,0^{B}	19,2
ADFom	302,3	302,3	316,8^{Ab}	335,5^{Aa}	240,1^{B}	11,6
aNDFom	451,5	539,6	528,3^{A}	524,7^{A}	312,7^{B}	29,6
Minerals						
DCAD, mEq kg^{-1} DM	377,7^{b}	468,9^{a}	393,3	334,7^{b}	480,7^{a}	28,8
Na	0,5^{a}	0,1^{b}	0,3	0,8	0,3	0,1
K	32,5	27,2	27,7^{B}	26,1^{B}	43^{A}	2,1
S	2,1	2,0	1,9B^{a}	1,7B^{b}	2,8^{A}	0,1
Cl	12,3^{a}	3,8^{b}	7,4	9,4	16,5	1,6
Ca	12,9^{a}	4,4^{b}	6,4^{B}	8,1^{B}	22,4^{A}	2,1
P	3,8	3,1	3,2^{B}	3,1^{B}	5,1^{A}	0,3
Mg	2,5	1,3	1,4^{B}	1,5^{B}	4,5^{A}	0,4
Mn mg kg^{-1} DM	34,8^{B}	54,8^{A}	46,7^{A}	35,9^{Ba}	29,9^{Bb}	2,9
Zn mg kg^{-1} DM	26,6^{B}	47,5^{A}	36,3	25,1	29,8	2,9
Cu mg kg^{-1} DM	7,9	7,2	6,5^{Ba}	5,6^{Bb}	12,4^{A}	0,8
Ferm. parameters						
Storage losses, %	0,8	1,0	1,1a	1,1a	0,0b	0,2
Aerobic stability, h	121,6^{a}	98,0^{b}	117,7	118,3	84,0	7,6
NH_3-N of total N in %	7,6^{a}	6,4^{b}	7,5^{a}	8,5^{A}	5,8^{Bb}	0,4
pH	4,4^{B}	5,7^{A}	5,0	4,5	4,5	0,2
Butyric acid	2,0^{a}	0,7^{b}	1,9	1,8	1,2	0,4
Acetic acid	6,0	3,1	4,9	6,2	5,2	0,7
Ethanol	0,8	2,3	1,6	0,9	0,7	0,3
DLG sensory quality[1]	2,4	6,8	4,9	2,0	2,3	0,8
DLG ensiling quality[2]	93,6	91,7	92,8	96,7	90,0	1,6

[1]DLG (2004), 0 - maximum and 'very good' and <11 - minimum and 'very bad'. [2]DLG (2006), 100-point scale with 0 - minimum and 'very bad' and 100 - maximum and 'very good'. Treatments: INT, EXT intensive and extensive fertilization; orig., original crop; W/O dand., crop without dandelion; dand., dandelion; ADFom and aNDFom acid detergent fibre and neutral detergent fibre on basis of organic matter; Different letters within the same row and treatment mean: lower case – statistically significant difference at $P < 0,05$; upper case – statistically significant difference at $P < 0,01$.

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Chemical composition, fermentative losses and aerobic stability of grass-legume silages

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Keywords dry matter loss, *Gliricidia sepium*, nutritive value, spoilage microorganisms

Introduction Tropical-grass silages are widely used for ruminant nutrition, but fermentation process is usually inadequate due to low amount of water soluble carbohydrates (WSC), high buffering capacity, low dry matter (DM) and protein contents at cutting, beside low energy (Bernardes et al., 2007; Rodrigues et al., 2005). Thus, forage legumes can be used to improve the quality of grass silage by increasing the contents of protein and WSC (Tjandraatmadja et al., 1993). As example, Gliricidia (*Gliricidia sepium*) has been used during ensiling and after wilting to improve Pangola grass fermentation (Tjandraatmadja et al., 1993). However, the ensiling of Aruana grass associated to Gliricidia has not been studied. Therefore, our objective was to investigate the effect of the replacement of Aruana grass by Gliricidia on the chemical composition, fermentative losses and aerobic stability of silages.

Materials and methods Gliricidia and Aruana grass were harvested at 150 and 75 d of growth, respectively. For Gliricidia, we harvested only the leaves and stem with a diameter lower than 1 cm, whereas the *Panicum maximum* Jacq. cv. Aruana was harvested cutting the plants at 20 cm above the soil surface. Gliricidia was wilted under the sun for one day. The treatments evaluated were different Aruana grass: Gliricidia ratios: 100:0, 75:25, 50:50, 25:75 and 0:100 based on fresh matter. Mini-silos (polyvinyl chloride tubes with a 5-L capacity) were used in the process and remained closed for 40 d. Mini-silos were weighed after filling and at the end of ensiling period to determine effluent, gas losses and DM losses. After silos were opened, the assay of aerobic stability was carried in ambient temperature by placing 4 kg of silages into buckets and the temperature was measured every 6 h during 3-d. For microbiological analyses, nutrient agar was used to count the total microorganisms present in the silage, whereas Rogosa agar was used to count Lactobacilli, and BDA agar was used to count yeasts and molds (these last were measured together). Aerobic stability was defined as the length of time required for the temperature of the silages to increase to 2°C above the baseline after exposure to air. The heating rate also was evaluated (Ruppel et al., 1995). A water extract was made from the wet silage samples (Kung et al., 1984) to evaluate the pH. Silage samples were analyzed for DM (105°C for 12 h). After determination of total nitrogen - TN (AOAC, 1996; method 954.01), crude protein (CP) content was determined by TN × 6.25. Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) contents were determined sequentially (Van Soest et al., 1991). The *in vitro* dry matter digestibility (IVDMD) also was determined (Tilley and Terry, 1963) using rumen fluid from a ruminally-cannulated bull fed Aruana and Gliricidia silages for 15 d. The experiment was organized in a completely randomized design with four replicates. All data were analyzed with a mixed model using the MIXED procedure of SAS (v 9.4), considering the Gliricidia replacement as a fixed effect and the residual error as a random effect. Contrasts were constructed and single degree-of-freedom orthogonal comparisons included the linear, quadratic and cubic effects. The differences were declared significant at $P \leq 0.05$.

Results and discussion The CP and aNDF contents linearly increased and decreased due to the replacement of Aruana grass by Gliricidia, respectively (Table 1). Comparing Aruana grass and Gliricidia ensiled as a single crop, the CP content of Gliricidia silage was 43.30% higher than Aruana grass silage. Addition of Gliricidia reduced aNDF content of Aruana grass silages because carbohydrates of tropical forages have low solubility (cell-wall components), higher aNDF indigestible and low ruminal degradability rates compared to temperate forages or legumes (Van Soest, 1994). Similarly to aNDF, the ADF content decreased quadratically when the Aruana grass was replaced with Gliricidia. Consequently, the IVDMD linearly enhanced due to addition of Gliricidia. The lactic acid bacteria counts showed a quadratic response, whereas a cubic response was reported for yeasts and molds and total bacteria. The pH values quadratically reduced due to replacement of Aruana grass by Gliricidia and the lowest value was reported for Gliricidia silage as a single crop (4.86). No differences were reported for DM recovery, but all silages showed great losses during the fermentation process because the elevated effluent losses, which are consequence of low DM content at ensiling (approximately 270 g/kg as fed). The heating rate of silages during aerobic exposure was similar among the treatments. As expected, the temperature of silages increased during the aerobic phase, but this increase was not greater than 2ºC for each treatment. However, the Gliricidia ensiled as a single crop exhibited the highest aerobic stability.

Table 1 Chemical composition, microbial counts, fermentative losses and aerobic stability of grass-legume silages

Item[1]	Aruana grass replaced by Gliricidia, %					SEM[2]	*P*-value	Contrast[3]
	0	25	50	75	100			
Chemical composition, g/kg of DM								
DM	281.6	282.5	280.8	274.5	275.9	1.28	0.0013	C*
CP	89.5	100.7	110.6	120.0	128.3	3.84	<0.0001	L**
aNDF	671.4	609.4	537.4	465.4	394.6	11.46	<0.0001	L**
ADF	413.2	400.6	372.4	303.7	256.4	6.33	<0.0001	Q*
IVDMD	573.1	576.2	598.6	605.3	619.7	4.97	<0.0001	L**
Microbial counts (log_{10} cfu/g) and fermentative losses								
LAB	7.81	8.38	9.16	8.29	7.77	0.04	<0.0001	Q**
Yeasts and molds	7.50	8.38	6.66	7.08	8.29	0.03	<0.0001	C**
Total bacteria	7.51	8.09	8.45	7.02	8.41	0.03	<0.0001	C**
pH	5.76	5.35	5.00	4.90	4.86	0.10	0.0002	Q*
Effluent, kg/t	35.76	78.36	74.9	76.9	64.55	8.30	0.0127	Q*
Gas, g/kg	4.22	4.26	4.27	4.51	3.89	0.87	0.9913	ns
DM recovery, g/kg	855.6	811.9	822.5	810.0	845.3	34.77	0.8432	ns
Aerobic exposure phase								
Heating hate, ºC/h	0.19	0.22	0.15	0.05	0.05	0.06	0.2225	ns
Aerobic stability, h	42.0	42.0	22.5	42.0	72.0	2.97	<0.0001	C*

[1]DM = dry matter; CP = crude protein; aNDF = neutral detergent fiber; ADF = acid detergent fiber; IVDMD = *in vitro* dry matter digestibility; LAB = lactic acid bacteria. [2]Standard errors of least squares means and *P*-values represent the statistical comparison among the silages. [3]L = linear; Q = quadratic; C = cubic; ns = no significant. * $P < 0.05$; ** $P < 0.01$.

Conclusion We recommended the replacement of approximately 75% of Aruana grass by Gliricidia as an alternative to improve the fermentation process of tropical-grass silages.

Total mixed ration silage containing elephant grass enhances feeding management on small-scale dairy farms

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Keywords total mixed ration silage, elephant grass silage, smallholder

Introduction Elephant grass (*Pennisetum purpureum* Schum.) silage is an important forage source for small-scale dairy farmers in tropical areas. However, elephant grass silage requires attention because the high moisture content causes loss of nutrients due to effluent production and undesirable fermentation. Furthermore, smallholders lack information on nutrient requirements of animals and management practices for optimizing the use of feed ingredients. Thus, preserving elephant grass associated with other ingredients as a total mixed ration (TMR) silage can overcome the obstacles outlined above. The adoption of this technology may improve fermentation profile, reduce effluent production in elephant grass silage, and maximize animal nutrition on small dairy farms. The objective of this study was to evaluate the effect of ensiling elephant grass associated with two types of concentrates on the fermentation profile, microbial count, effluent losses, chemical composition and aerobic stability.

Materials and methods The treatments were as follow: exclusive elephant grass silage (EG), EG with ground corn and soybean meal (EGCS), and EG with ground corn and sunflower cake (EGCSF), a by-product from biodiesel industry. Ingredients were combined to meet the requirements of dairy cows with milk production of 15 kg per day (NRC, 2001). The EGCS silage consisted of 57% EG, 12% soybean meal and 31% ground corn, whereas EGCSF silage consisted of 48% EG, 25% sunflower cake and 27% ground corn (DM basis). A vitamin-mineral premix was included (0.2% DM) in both TMR. As experimental silos were used plastic buckets with 15 L capacity, containing sand on the bottom to quantify effluent production. After 94 d of storage, silages were evaluated for fermentation end products, microbial counts, chemical composition and aerobic stability. Aerobic stability was defined as the number of hours that the silage was stable before it reaches 2°C above ambient temperature. Data were analyzed as a completely randomized design in PROC GLM of SAS 9.2, and means were compared with Tukey test at 5% probability.

Results and discussion The characteristics of EG and their TMR are shown in Table 1. As expected, the EGCS and EGCSF treatments increased the DM concentrations and improved chemical composition (i.e., lower NDF and higher CP concentrations). The EG treatment showed higher pH than EGCS silage, whereas EGCSF had an intermediate value. Higher moisture present in EG silage creates condition for growing of undesirable microorganisms, such as clostridia and this fact may explain poorer fermentation in control silages in terms of pH and NH_3-N concentration compared to the TMR silages. The concentration of lactic acid was markedly greater in EGCS and EGCSF treatments. For

other organic acids, there was no difference among treatments, although the concentration of butyric acid was numerically higher in the EG treatment. The DM loss was lower in EGCS treatment (8.10%) compared with EGCSF and EG treatments (24.6 and 21.7% respectively). Greater effluent production was observed in EG silage compared to other treatments. This finding may be explained by the positive effect of concentrate ingredients in holding water throughout conservation period. Aerobic stability was long in all treatments since silages were stable for more than 240 h. High concentrations of total volatile fatty acids inhibit yeasts, increasing aerobic stability of silages at feeding.

Table 1 Characteristics of elephant grass TMR silage after 94 d of storage

Item	EG	EGCS	EGCSF	SEM	*P*-value
DM, % as fed	13.5^{c}	25.1^{b}	29.1^{a}	0.47	<0.01
NDF, % DM	73.4^{a}	35.3^{c}	46.8^{b}	0.85	<0.01
CP, % DM	5.54^{c}	13.3^{a}	10.4^{b}	0.34	<0.01
EE, % DM	1.64^{c}	3.92^{b}	6.99^{a}	0.16	0.01
pH	5.01^{a}	4.43^{b}	4.56^{ab}	0.10	<0.01
N-NH_3 (% of total N)	5.41^{a}	1.83^{b}	2.26^{b}	3.05	<0.01
Lactic acid, % DM	0.37^{c}	13.9^{a}	7.15^{b}	1.19	0.25
Acetic acid, % DM	3.43	0.92	1.17	0.54	0.10
Propionic acid, % DM	0.23	1.94	1.79	0.40	0.19
Butyric acid, % DM	2.90	1.30	1.64	0.63	0.07
BAL, log cfu g^{-1}	4.82	4.31	5.38	0.95	0.35
Yeast, log cfu g^{-1}	<2	<2	<2		
Molds, log cfu g^{-1}	<2	<2	<2		
DM losses, %	24.6^{a}	8.09^{b}	21.7^{a}	2.42	<0.01
Effluent, g kg^{-1}	126^{a}	101^{b}	93^{b}	7.20	<0.01
Aerobic stability, h	>240	>240	>240	–[1]	–

[1]Statistical analysis was not performed because the silages did not exceeded 2 °C above the ambient temperature; EG: Elephant grass silage; EGCS: TMR containing EG, ground corn and soybean meal; EGCSF: TMR containing EG, ground corn, and sunflower cake.

Conclusion Overall, both TMR fermented well enough and reduced effluent production. As sunflower seed cake from biofuel industry have low cost, it may substitute partially or totally soybean meal in terms of silage fermentation and losses. More studies need to be performed to assess the effect of TMR silages on animal nutrition.

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Nutritional composition and *in vitro* digestibility of sorghum silage intercropped with legumes

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Keywords bean, bmr sorghum, mixed silage, quality silage, soybean

Introduction The production of sorghum silage has great importance, given the restrictions on the use of water for agriculture, due to its better adaptation to hydric deficit conditions. However, despite reasonable energy content, sorghum silage has protein levels lower than those required for good productive performance of dairy cows. Thus, sorghum intercropped with a legume can be an alternative to increase the crude protein content of silage and its nutritional value, provided that, observed the ratio of legumes in the forage to be ensiled, due to its low quality for silage production, which can to compromise the final quality. In this sense, the objective was to evaluate the composition and digestibility of mixed silages produced from sorghum plus legumes.

Materials and methods It was evaluated two silages produced from associated crops, being the dwarf grain sorghum (*Sorghum bicolor* (L.) cv. Surgho) with soybean (*Glycine max* (L.) var. Mitzuko NT), and giant grain sorghum (*Sorghum bicolor* (L.) cv. Sweet virginia BMR) with common beans (*Phaseolus coccineus* (L.) var. Neckargold). The silages were made in bunker silos with capacity of 216 $m^{3\ each,}$ so as to obtain the same characteristics of particle size, density and sealing. After 60 days, the silos were opened, followed by daily collects at different points of the silos, during all feed-out period. The samples were grouped weekly for forming a composite sample/week, to give twelve samples for each treatment (silo), which are pre-dried and triturated to 1 mm. The samples were analyzed for the dry matter (DM), ash, crude protein (CP), water-soluble carbohydrate (WSC), neutral detergent insoluble nitrogen (NDIN), ratio between acid detergent insoluble nitrogen/total nitrogen (ADIN/Total-N), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin and in vitro dry matter digestibility (IVDMD). Data were subjected to analysis of variance and means compared by F test ($P<0.05$) in a completely randomized design using the statistical program SAS (Statistical Analysis System - 2009).

Results and discussion The dwarf sorghum+soybean silage showed higher DM content ($P<0.05$) than the giant sorghum+bean silage (Table 1). However, in both treatments, the DM was lower than 300 $g.kg^{-1}$ of natural matter, value usually adopted as the minimum to avoid losses by effluent and undesirable fermentation. Likewise, the dwarf sorghum+soybean silage had higher concentrations of ash ($P<0.05$) compared to the giant sorghum+bean silage, with values of 77.17 and 74.57 g kg^{-1} of DM, respectively. In this trial, the addition of soybean in the production of silage significantly increased ($P<0.05$) the CP concentrations, in relation to addition of the beans. This increase in protein concentration was followed by the best protein profile of silages, observing higher

concentrations of NDIN and lower ratio ADIN/Total-N in dwarf sorghum+soybean silage. This implies in providing more protein available for animal metabolism. The WSC concentrations were also higher in silages with dwarf sorghum plus soybean, indicating the highest energy contribution of this silage. In contrast, it was observed lower NDF and ADF concentrations in dwarf sorghum+soybean silage. In general, the leguminous crops have a lower NDF and ADF than grasses, because of the lower concentration of celluloses and hemicelluloses in their cell walls, but the cell wall is little digestible due to the higher quantity of lignin. As a result, the dwarf sorghum+soybean silage had greater IVDMD, reflecting better use of nutrients of these silages and better nutritional value.

Table 1 Composition and in vitro dry matter digestibility of mixed silages of dwarf sorghum with soybean and giant sorghum with common beans

Item ($g.kg^{-1}$ of DM)	Silage		SEM
	dwarf sorghum+soybean	giant sorghum+bean	
Dry matter	281.1[a]	279.8[b]	0.15
Ash	77.17[a]	74.57[b]	0.40
CP	80.42[a]	60.50[b]	2.26
WSC	99.35[a]	48,95[b]	5.05
NDIN	1.87[a]	1.43[b]	0.05
ADIN/Total-N	0.049[b]	0.067[a]	0.002
NDF	594.07[b]	623.57[a]	5.35
ADF	366.15[b]	384.66[a]	3.43
Lignin	60.36	60.18	0.72
IVDMD	538.84[a]	517.11[b]	5.11

Means in the same row, followed by unequal letters, differ by F test ($P<0.05$).

Conclusions This trial show that silage of dwarf grain sorghum intercropped with soybean has better nutritional composition and higher digestibility than the giant grain sorghum+bean silages. It also allows a higher milk production (Da Silva et al., 2015). In order to definitely recommend this type of mixture for feeding ruminants further investigations have to be done, especially to improve the rate of legumes in the forage and to better control weeds during the plants growth.

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Leachate and pH changes in response to post ensiling treatment of macroalgae

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Introduction This study examines the preservation of macroalgae (seaweed) in order to provide a feedstock for liquid fuel production. The preservation of macroalgae is key to providing stable biomass between harvest periods. Though many species of terrestrial plant are ensiled for feeding animals and bio-digesters, the use of whole macroalgae as a biomass for large-scale silage production is a relatively unknown process. Bacterial and biochemical changes occurring in ensiled terrestrial plants are well understood. Macroalgae, however, are marine species and have, marine epiphytic flora and biofilms associated with them in conjunction with different structural biopolymers and no waxy cuticle. Owing to these differences, and wide species diversity, it is anticipated macroalgae will react differently to the ensiling process, showing different critical preservation parameters, such as pH changes and leachate production and thus loss of potentially valuable molecules and reaction mass.

Materials and methods The macroalgae *Laminaria digitata* (LD), *Porphyra umbilicalis* (PU) and *Ulva lactuca* (UL) were collected from Northumberland, UK in March 2014 and were subjected to differing chilling treatments before testing for the volume of leachate produced (Vol), the pH of the leachate and the mass loss (MassLost) due to ensiling. After rinsing in seawater and removal of epiphytes the three species were chopped by hand into roughly 4 cm^2 sections. Three 50 g samples from each species were taken as baseline samples and frozen. The remainder of the unfrozen material was ensiled (no additive treatments) in 100 g (*Laminaria digitata*) or 50 g (*Porphyra umbilicalis* and *Ulva lactuca*) portions within a vacuum pack. After 8 days the baseline samples were defrosted and any leachate was removed and the leachate pH tested (Fresh) and a 5 g subsample of the solids remaining was agitated, by stomacher, in 50 mL distilled water for 3 minutes and the pH of liquid tested (Solids). At 14 days post ensiling randomly selected subsets were subjected to treatments, Fridge (samples stored in the fridge at 4°C for 4 days), Freeze (Samples frozen at -18°C for 4 days) and NoStore (leachate removed on day of selection) before measuring the volume of leachate produced and the pH of the leachate. Subsequent statistical analysis was by ANOVA using species × treatment, with a post hoc Tukey test to examine the data for groupings.

Results and discussion The results show significant differences ($P<0.001$) between species and treatments for both the pH, volume of leachate produced and mass lost. Overall mean of the pH of the Solids tested and the liquid leached from Fresh was not significantly different. Additionally, overall, no significant differences were seen in pH on day 14 post ensiling in the three treatments with all being lower than the pH Solids and Fresh. For each species individually, this is repeated for *Laminaria digitata* but not for *Porphyra umbilicalis* and *Ulva lactuca* where the pH Solids and Fresh are different and the pH in *Ulva lactuca* increased from the baseline after ensiling (Table 1). Overall, leachate lost was lowest for *Ulva lactuca* (1.6 mL) and within its treatments this was driven by a higher

volume of leachate lost in Fresh (Table 1). For *Ulva lactuca* the overall MassLost at 46.2% was the least between the species. There was no treatment effect in *Laminaria digitata* but for *Porphyra umbilicalis* and *Ulva lactuca* more mass was lost in the freezing and defrosting of Fresh than the other treatments (Table 1).

Table 1 Overall, mean pH, volume of leachate per g lyophilised mass (Vol), mass lost from the original weight ensiled (MassLost)

	Species	pH	Vol	MassLost	
	LD	5.4[b]	3.0[a]	62.4[a]	
	PU	6.3[a]	3.2[a]	61.9[a]	
	UL	5.7[ab]	1.6[b]	46.2[b]	
				Species[1]	
	Treatments[2]	Overall	LD	PU	UL
pH	Solids	6.7[a]	6.4[a]	7.6[b]	6.0[a]
	Fresh	6.5[a]	5.9[ab]	8.2[a]	5.2[b]
	Fridge	5.3[b]	5.1[bc]	5.3[c]	5.7[ab]
	Freeze	5.3[b]	4.9[bc]	5.2[c]	5.6[ab]
	NoStore	5.2[b]	4.6[c]	5.1[c]	5.8[ab]
Vol	Treatments	Overall	LD	PU	UL
	Fresh	3.1[a]	3.3[ab]	2.4[a]	3.8[a]
	Fridge	2.4[a]	2.9[ab]	3.1[a]	1.4[b]
	Freeze	3.0[a]	3.9[a]	3.8[a]	1.3[b]
	NoStore	2.1[a]	2.1[b]	3.4[a]	0.7[b]
MassLost	Treatments	Overall	LD	PU	UL
	Fresh	69.2[a]	62.2[a]	72.8[a]	74.2[a]
	Fridge	52.3[a]	62.4[a]	52.7[b]	41.7[b]
	Freeze	53.9[a]	61.6[a]	61.0[ab]	39.0[b]
	NoStore	54.5[a]	63.2[a]	61.2[ab]	39.2[b]

[1]macroalgae species *Laminaria digitata* (LD), *Porphyra umbilicalis* (PU) and *Ulva lactuca* (UL); [2]Treatments consisted of a baseline where leachate was removed and the leachate pH tested (Fresh) and the solid' pH tested (Solids). After 14 days subsets were treated as Fridge (samples stored in the fridge), Freeze (Samples frozen at -18°C) and NoStore (leachate removed on day 14); numbers sharing a grouping letter within columns and analysis groups are not significantly different

Conclusions The pH reached after 14 days ensiled in *Laminaria digitata* and *Ulva lactuca* was lower than *Porphyra umbilicalis.* In *Ulva lactuca* the *pH* rose and suggests an alkali breakdown product. The volume of leachate produced and the mass lost to leachate was greater in *Laminaria digitata* and *Porphyra umbilicalis*. Overall, leachate produced from the baseline samples after it was frozen, before testing for ensiling, has not affected the baseline pH. Cold storage of macroalgae silage samples before testing does not affect the pH but does affect the leachate volume and this was lower in *Ulva lactuca*. In conclusion, although the response of each species needs to be examined separately, to facilitate sample analysis in macroalgae silage testing, samples can be examined fresh, or after cold storage.

Acknowledgements This work is funded through the UK based Engineering & Physical Sciences Research Council 'Macrobiocrude' consortium.

Silage dry matter influences rumen degradability

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Keywords grass silage, rumen degradability, wilting grass silage.

Introduction Wilting of grass before ensiling is often used as a method of improving forage quality (Marsh 1979). However, the reported effects of wilting on the animal performance are often rather contradicting (Gordon, 2000; Patterson, 1996; Wright, 2000). The mean dry matter (DM) content of grass silage in different countries over Europe shows that the common practice also uses different target values. The goal of this study was to understand why the optimal DM of silages seems to vary in practice. There is a correlation (-0.79) between lignin (ADL) and DM in silages; meaning that in countries with high ADL contents, best practice seems to be ensiling grass with low DM. As an explanation, we hypothesise that the silage DM influences the digestibility of silages.

Materials and methods A database with commercial analyses from Dutch, German and Danish dairy farmers has been used to investigate the effect of DM on the rumen-degradability of grass silage. All silages were ensiled and analysed in the year 2014. Analyses were performed by near infrared spectrometry (NIRS) and the standard laboratory techniques of BLGG (www.blgg.com) (ISO-12099). The fermentative parameters are also analysed by NIRS. To derive a clear effect of DM, a selection was made with silages of comparable chemical composition crude protein (CP), neutral detergent fiber (NDF) and ADL. The total database of 2014 had an average of 480 g kg DM^{-1} of NDF and 172 g kg DM^{-1} of CP. To create a representative sample, silages were selected within the range of 475-500 g kg DM^{-1} of NDF, 170-175 g kg DM^{-1} of CP and 20 g kg DM^{-1} of ADL. These silages were grouped in three levels of DM: low (200-300 g kg^{-1}), medium (350-450 g kg^{-1}) and high (500-600 g kg^{-1}). In total 498 silages were included, and statistics (Anova) were performed with IBM SPSS statistics version 20.

Results and discussion All differences in the Table 1 and 2 are significant ($P<0.05$). The lower DM resulted in a higher concentration of acids and a lower pH, indicating a more intensive fermentation, and higher degradation rate of CP (kd CP). The amount of washable CP (W-CP) was also high for low DM silages. The results of Edmunds (2014) and Repetto (2005) also showed that high fermented silages have more and faster degradable protein fractions. A low DM also led to an increased degradation rate of NDF. However, the remaining NDF is less digestible, according to the higher indigestible fraction (U-NDF). It is suggested to be an effect of the low pH in wet silages, leading to hydrolysis of cell-wall polysaccharides (Bakken, 2011).

Table 1 Mean chemical composition of silages (g kg DM^{-1})

Item	Low	Medium	High
DM, g kg^{-1}	278	405	543
pH	4.1	4.7	5.3
Lactic Acid	84	45	17
Acetic Acid	19	16	8
NH_3, %	11	10	7
CP*	173	173	172
Sugar	29	71	106
NDF	488	488	490
ADL	20	20	20
n	23	309	166

*Crude protein including NH_3.

Table 2 Digestibility and rumen degradation of silages (g kg DM^{-1})

Item	Low	Medium	High
D-OM, %	78	78	77
D-NDF, %	74	74	73
kd CP, % h^{-1}	10.3	9.4	8.9
WCP	93	83	69
UCP	3	3	3
kd NDF, % h^{-1}	5.1	4.7	4.4
UNDF	94	86	82
n	23	309	166

Conclusions Silages with low DM content have a more intensive fermentation process after ensiling. Rumen degradability of CP and NDF is higher in grass silages with a low DM content. In practice this means that it is possible to influence rumen protein degradation by steering the dry matter content of grass silage. The optimal DM content depends on the initial grass quality. Protein rich grass that already is highly fermentable, could be a risk to rumen acidosis and nitrogen losses. Therefore these grasses should preferably be wilted to a higher DM content before ensiling. Increasing the rumen degradation of low digestible grasses could lead to higher total digestibility. Therefore low digestible grasses should preferably be ensiled with a low DM content.

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Tropical forage legume silages and their *in-vitro* digestibility for pigs

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Keywords *Vigna unguiculata, Canavalia brasiliensis, Ipomoea batatas, in-vitro* digestibility, pigs

Introduction The use of silage can be of great benefit for the small producer to feed his pregnant sows and growing and finishing pigs (Artiles et al., 2012). The aim of this work was to assess the nutritional value of silages of the tropical forages *Vigna unguiculata* and *Canavalia brasiliensis* and the sweet potato tuber *(Ipomoea batatas)* and mixtures thereof at different cutting ages.

Materials and methods *Vigna unguiculata*, and *Canavalia brasiliensis* and *Ipomoea batatas* and their mixtures were ensiled at four different growth stages in PVC tubes (1.8 L volume) in three replicates (see more details in our company paper, Guerrero and Martens, 2015). The silages were evaluated after three months of storage for their *in-vitro* digestibility of dry matter (IVDMD) for pigs. The protocol for *in-vitro* digestion in pigs with pepsin/pancreatin as the main active agents was applied (Leterme, et al., 2008). Silage was hydrolyzed in pepsin solution at pH 2.0 for 2 hours and in a second step with pancreatin at pH 6.8 for 4 hours.

Results and discussion Ipomoea had the highest *in-vitro* digestibility, probably explained by the higher content of carbohydrates and less lignin compared to the forages. Thus, also mixtures with Ipomoea were better digestible. The neutral detergent fiber (NDF) was increased with the maturity of the forage (Table 2), leading to a decreased content of organic acids, which generated a decrease in digestibility (IVDMD) (Table 1).

Table 1 The IVDMD at different states of maturity and plant species (*Vigna unguiculata, Canavalia brasiliensis* and sweet potato) and their mixtures

Cutting intervals	*Vigna*	*Canavalia*	*Ipomoea*	*Vigna-Ipomoea*	*Canavalia-Ipomoea*	*Vigna-Canavalia*	Mean per interval
6 weeks	51.8	--	--	65.6	--	--	58.7^{a}
8 weeks	42.5	42.6	72.4	60.2	51.9	40.6	51.7^{b}
10 weeks	88.4	--	67.2	49.6	--	--	51^{b}
12 weeks	38.9	27.8	75.5	68.2	54.9	37.2	50.4^{b}
16 weeks	--	34.0	70	--	47	--	50^{b}
20 weeks	--	27.6	85.5	--	54.2	--	55.8^{a}
Mean per species	42.4^{d}	33.0^{f}	73.9^{a}	60.9^{b}	52^{c}	38.9^{e}	

Data with different letters indicate significant differences according to Duncan test ($P < 0.05$)

Table 2 The NDF content at different states of maturity and plant species (*Vigna unguiculata, Canavalia brasiliensis* and sweet potato) and their mixtures

Cutting intervals	*Vigna*	*Canavalia*	*Ipomoea*	*Vigna-Ipomoea*	*Canavalia-Ipomoea*	*Vigna-Canavalia*	Mean per interval
6 weeks	38.1	--	--	--	37	--	37.6^{e}
8 weeks	37.4	48.2	27.9	44.7	48	45	41.9^{d}
10 weeks	13.2	--	45	52.8	--	--	51.8^{b}
12 weeks	51.8	58.5	51.2	54.5	54	53.4	53.9^{a}
16 weeks	--	54.6	49	--	56	--	53.2^{ab}
20 weeks	--	62.7	27.4	--	48.2	--	46.1^{c}
Mean per species	46.2^{d}	55.9^{a}	40.1^{e}	47.3^{e}	51.6^{b}	49.2^{c}	

Data with different letters indicate significant differences according to Duncan test ($P < 0.05$)

Conclusions The inclusion of Ipomoea improved the digestibility of silages with legumes (*Vigna, Canavalia*). The IVDMD decreased with increasing maturity of the forages. For this reason, the optimum cutting time for pigs is between 6 and 8 weeks although yield is still low at this point of time (Martens et al., 2008).

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Impact of low temperature on lactic acid bacteria diversity in corn silage

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Keywords lactic acid bacteria, low temperature, corn, PCR-DGGE

Introduction High temperatures negatively affect lactic acid bacteria survival and activities during silage fermentation phase, as observed in several studies (Kim, 2006). In several countries, i.e. northern US, Canada, northern Europe, northern China, low temperature at whole-corn ensiling might be an issue for the fermentation as temperatures below 10°C are often experienced. In order to understand the effect of temperature on lactic acid bacteria population, we studied their profile under five temperatures, ranging from 25 to 5°C.

Materials and methods A total of 145 mini-silos were made from freshly harvested corn and incubated at 5, 10, 15, 20 or 25°C during 60 days. The population of lactic acid bacteria was monitored using a culture-independent approach from the mini-silos (Johnson, 2005). Destructive opening of silos were performed after 0, 1, 2, 3, 7, 28 and 60 days of incubation (4 repetitions). The DNA extraction was performed using the PowerFood DNA extraction kit (MoBio). The PCR-DGGE DNA fingerprint using primers L1GC/HAD2, specific to the lactobacillus-group (*Lactobacillus*, *Weissella*, *Pediococcus*, and *Leuconostoc*), were generated from amplification of 185bp fragment of the 16S rRNA genes.

Results and discussion When silos were opened after 60 days of incubation, final pH varied between 3.85 and 4.32. All silos were under pH threshold classically used to characterize anaerobic stability. A total of 14 operating taxonomic units (OTU) were detected. The principal OTU were related to *Lactobacillus plantarum*, *Lactobacillus buchneri, Lactobacillus brevis*, *Lactobacillus sakei*, *Lactobacillus coryniformis*, *Lactobacillus curvatus*, *Leuconostoc citreum,* and *Pediococcus pentosaceus.* Temperature played an important role in modulating the bacterial diversity profiles (Figure 1). The OTU related to *L. plantarum*, *L. brevis*, and *P. pentosaceus* appeared to be indicator species of silos incubated at 15°C, 20°C and 25°C. The OTU related to *L. sakei* and *L. curvatus* were indicator species for lower temperatures (5°C, 10°C and 15°C). The OTU related to *L. citreum* and *L. coryniformis* were mainly observed at mid temperature range, 10°C, 15°C and 20°C. Finally, for temperatures above 15°C, the OTU related to the heterofermentative *L. buchneri*, a species usually used as an inoculant to increase the aerobic stability of silages, was the most represented during anaerobic stability phase at 25°C. For temperature below 15°C, OTU related to *L. sakei* was dominant at later stage of ensiling.

Conclusion Low temperatures contribute to create different evolutions of patterns of lactic acid bacteria population responsible for whole-plant corn silage fermentation. Fingerprint techniques such as DGGE, are considered an interesting approaches to investigate the microbial population dynamics during the ensiling process.

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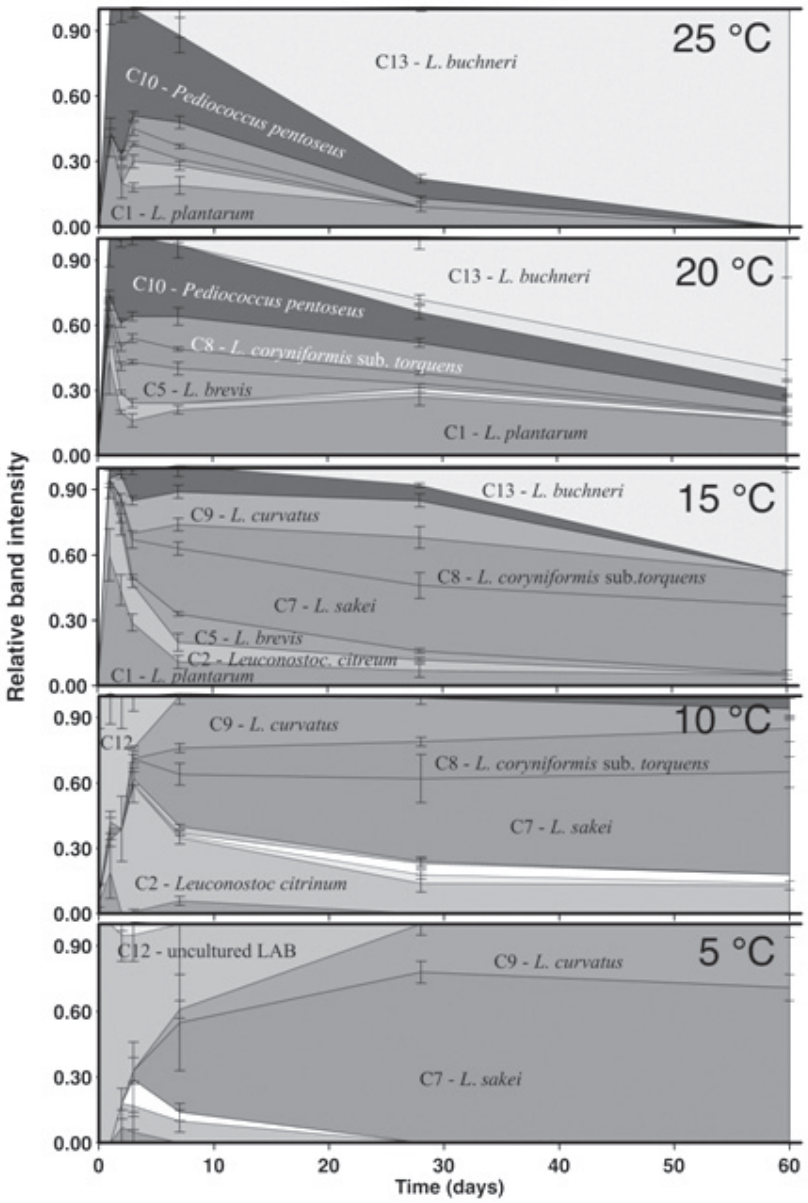

Figure 1 Population profiles by PCR-DGGE of lactic acid bacteria population at time of ensiling and after 1, 2, 3, 7, 28 and 60 days of incubation at different temperatures.

Change in microbial population in corn silage during anaerobic fermentation and aerobic challenge

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Keywords corn silage, metagenomics, DNA extraction

Introduction Silage has in the past been studied intensely focusing on chemical analyses, dry matter loss and aerobic stability, while whole microbial population has only been analyzed in a minor extent. Today we have an insight into the main fermentation process, which have supplied tools for significant improvements, but to fully understand the process we need to obtain a more complete picture of the microbiological flora during the ensiling process. New powerful methods within the field of DNA, called Next Generation Sequencing (NGS), have evolved the past two decades. These new technologies give the possibility to collect DNA sequence information of the total DNA present in a sample, the so called metagenomics approach. The FoodGenomics is a collaboration between Chr. Hansen and two of the leading academic groups within NGS work (Center for GeoGenetics, University of Copenhagen, and Center for Biological Sequence Analysis, Technical University of Denmark) and aims at increasing the quality of fermented food and feed. It utilizes the metagenomics approach to link microbial flora composition to measures of quality with emphasis on the role of the addition of specific microbes. Here we show an initial metagenomic analysis of a laboratory scale corn silage trial with whole corn from a farm in Denmark.

Materials and methods Whole corn was chopped and four 1-kg vacuum-packed bags were packed in the laboratory and stored for 91 days at 25 °C. A sub-sample of each bag was exposed to aerobic challenge for 7 days at room temperature (20-21°C). Pellet formation followed the protocol given by Eikmeyer et al. (2013), but with an initial homogenization for 2 min in a stomacher (Smasher, AES Laboratoir, FranceCells) were disrupted by bead beating 100 mg pellet together with 0.4 g of 0.5 mm Zirconia beads (BioSPec, Bartlesville, OK, USA) at maximum speed for 2 min using a TissueLyserII (Qiagen, Carlsbad,CA, USA). DNA was extracted with the QiAmp DNA Stool mini kit (Qiagen) according to manufacturer instructions. A blunt-end Library was built according to Meyer and Kircher (2010) using NEB next 6040 kit (New England BioScience) and amplified using Q7 Polymerase (New England BioScience). The libraries where run on HiSeq 2500; 100 paired-end reads at Center for GeoGenetics (University of Copenhagen) giving around 200 million reads in total. The metagenomic reads were mapped against a sequence database comprising 16S ribosomal RNA sequences of type strains of Bacteria and Archaea (http://www.arb-silva.de/projects/living-tree/) in a single end mode. Only reads with a match/read length fraction equal or higher than 0.8 was accepted for further analysis.

Results and discussion Library preparation succeeded in the non-fermented corn, in two samples after 91 days of anaerobic fermentation and in three samples from the aerobic challenge.
The non-fermented corn sample showed the most diverse bacterial flora with Cyanobacteria and Proteobacteria well represented, followed by members of the Bacteroidetes/Chlorobi group and minor amounts of the gram positive phyla Actinobacteria and Firmicutes. After 91 days of anaerobic fermentation, the bacterial and archaeal flora was dominated by *Lactobacillaceae*, which increased to around 45% in both samples, while Archaea accounted for 15% of the reads, which to our knowledge has not been reported previously for corn silage. One percent of the reads map to the *Acetobacteraceae*. The silage had an aerobic stability of 75 hours on average. The DNA samples after 7 days of aerobic challenge were dominated by the family *Acetobacteraceae*, especially in sample A and C. Also sample D had a high prevalence of *Acetobacteraceae,* but it was not as pronounced as the first two. In this sample, 15% *Lactobacillaceae* were found and to a smaller extent *Bacillaceae, Flavobacteriaceae*, Gammaproteobacteria and Archaea. *Acetobacter pasteurianus* (*Acetobacteraceae*) has previously been detected in corn silage inoculated with *L. buchneri*, *L. plantarum*, and *E. faecium* using DGGE method.

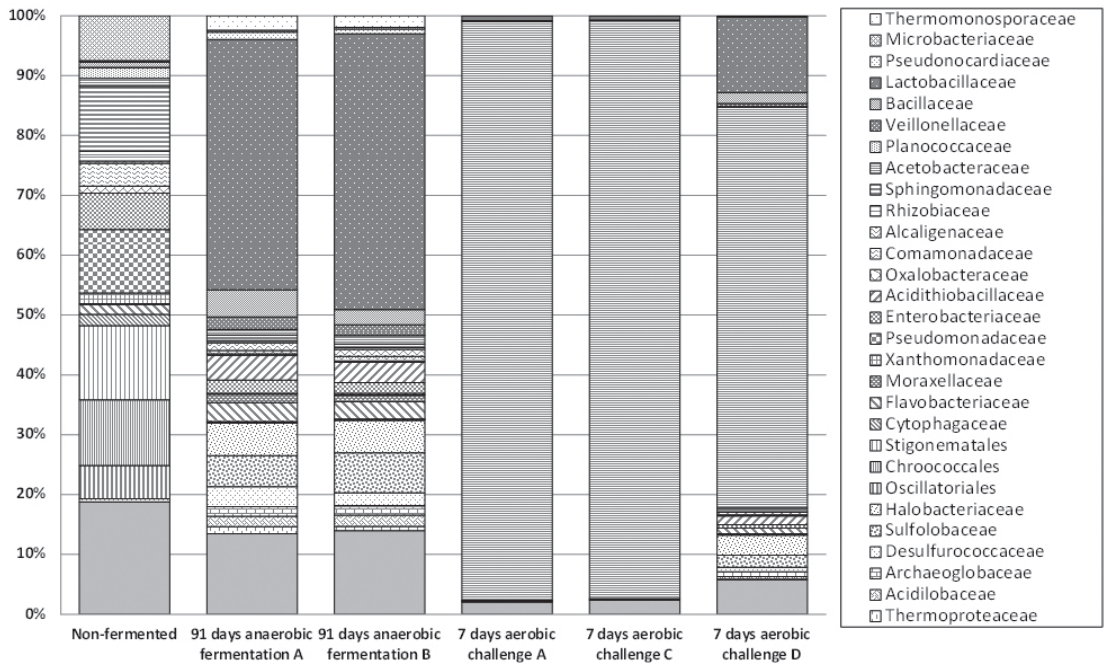

Figure 1 Distribution of read mapping to 16S ribosomal RNA sequence database grouped by family.

Conclusions Our results show a prevalence of *Lactobacillaceae* at the end of the anaerobic fermentation process. The following aerobic challenge led to a dominance of *Acetobacteraceae*. The method for extraction of DNA needs to be optimized in order to achieve a more consistent yield and purity from a large variety of silage samples.

The competition between lactic acid bacteria and spoiling *Bacillus* species in grass silage is strongly determined by the degree of compaction

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Keywords grass silage, bacterial community, 16S rRNA gene, silage compaction, aerobic stability

Introduction The optimal silage quality and aerobic stability depends not only on the harvesting and ensiling technology, but also on the development of a preservative microbial population. It is well known that insufficient compacted silage often leads to a reduced aerobic stability. As a consequence, a more rapid penetration of aerobic spoilage organism can occur on the silage. The intensity of spoilage is highly dependent on the number and activity of the undesirable microorganisms in the silage. Numerous studies examine the effects, mainly the influence of bacterial starter cultures, on the aerobic stability of silage to improve the quality. However, the microbial community responsible for spoilage processes remains largely unknown. Therefore, the aim of the study was to examine the microbial community dynamics of the silage fermentation in insufficient compacted grass silage to receive more insights into the spoilage process.

Materials and methods For the microbial community characterization, a cultivation independent molecular approach was applied involving terminal restriction fragment length polymorphism (TRFLP) analysis and cloning and sequencing of the 16S rRNA gene. Standard chemical analyses were carried out to examine the forage crop and silage variants. Perennial ryegrass was ensiled in laboratory glass jars in two compaction variants (optimal and insufficient). Insufficient compaction samples were air infiltrated (during ensilage for 24 hours after 28 and 42 days ensilage time). Triplicate replications of each ensiling treatment were sampled prior to ensiling (day zero) and after three, 10 and 49 days of ensilage. To determine the aerobic stability, after 49 days of storage silage samples were transferred to air permeable cups at constant room temperature. The temperature of the silage was measured during the aerobic exposure for two, four and seven days, and samples were taken for molecular and chemical analyses. The aerobic stability was considered as the time as long the temperature remains stable before increase by more than 3ºC above the ambient temperature.

Results and discussion TRFLP analysis revealed that the ensiling of ryegrass is a very dynamic microbial process even at optimal ensilage conditions and even without the addition of distinct starter cultures. The bacterial community in the grass before ensiling, was dominated by aerobic or aerotolerant microorganisms (Figure 1). Already after three days of ensiling, this initial community was completely replaced and now dominated by *Weissella cibaria* (*Leuconostocaceae*) (TRF 608, TRF 610) and *Lactobacillus sakei* (*Lactobacillaceae*) (TRF 605). Nonetheless, after 10 days of ensiling, a further intensive

community shift was detected toward a pure *Lactobacillus*-community also stable at aerobic conditions. Surprisingly, the *Lactobacillus*-community seems to proliferate also under these stress conditions as indicated by the increase in the lactic acid content (Figure 1). In contrast to optimal ensiling, in silage with insufficient compaction and air stress, a higher microbial diversity was found. Independent of compaction and air infiltration, the Bray-Curtis similarity of 78 % indicates that the bacterial communities were developed similarly after 49 days of ensiling. In contrast, only traces of the anaerobic community in the insufficient compaction silage were determined seven days after opening (Bray-Curtis index = 2%). Increasing temperature of the silage material was detected after two and a half days of aerobic exposure. After four days of aerobic stress, opportunistic *Bacillus* species completely suppress the lactic acid bacteria. The fast spoilage process was also characterized by decreasing lactic acid concentrations and increasing pH values.

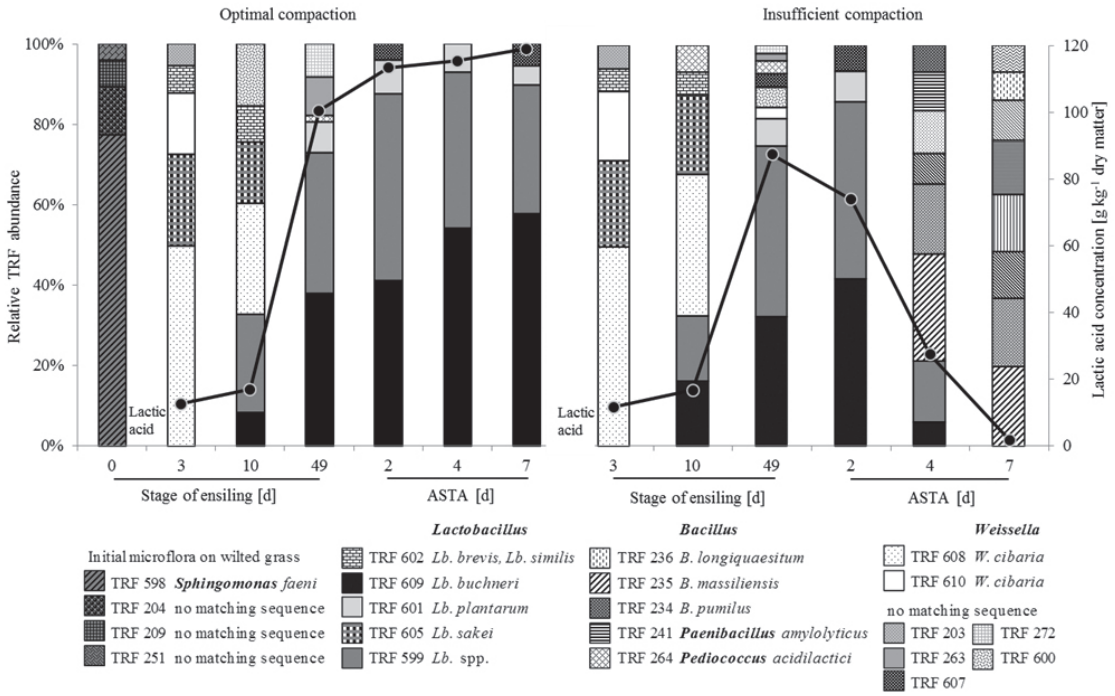

Figure 1 Bacterial community profiles of grass silage during ensiling and subsequent aerobic stability test (ASTA) determined by TRFLP analysis. Detected TRFs were phylogenetically assigned based on 16S rRNA gene sequence libraries.

Conclusions This study reveals that the bacterial community of the grass before ensiling differs completely from the community in the silage and in the aerobic spoilage process. This may indicate that the microbial community of the starting material has a minor role than previously thought. Further work is necessary to elucidate the origin of the aerobic spoilage microorganism and to clarify the relevance of the microbial population of the forage crop.

Isolation and identification of lactic acid bacteria in elephant grass silage

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Keywords PCR, tropical grass, *Lactobacillus plantarum*

Introduction Among tropical grasses, elephant grass is the most utilized for silage production due to its high forage yield and adequate water-soluble carbohydrate content, which favors a good fermentation. Inoculants based on lactic acid bacteria (LAB) are commonly used to increase the production of lactic acid, resulting in a fast decrease in pH of ensiled material. The use of specific LAB strains isolated from the same forage it is used to treat is a strategy that has been utilized by some researchers aiming to obtain better quality silage. Due to the specificity between the forage species and the epiphytic microflora, studies are necessary to isolate and to identify the main microbial groups in the main forages that are utilized for ensilage in tropical conditions. Therefore, the objective of the present study was to isolate and identify LAB through partial sequencing of the 16S rDNA gene in elephant grass silage with the ultimate goal of identifying species of LAB that could be developed into an inoculant for this type of silage.

Materials and methods Elephant grass (*Pennisetum purpureum*, Schum var. Cameroon) forage was harvested at approximately 70 d of growth (23.53% average DM) and then chopped and ensiled in eighteen 25.4 cm × 35.56 cm bags. Five hundred grams of chopped forage were put in each bag 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, and 56 d of fermentation. Samples of fresh forage and silages (25 g) were mixed with 225 mL of Ringer Solution and 10-fold serial dilutions were prepared. The dilutions (100 µL) were plated on MRS agar and the plates were incubated at 37ºC for 48 h. After incubation, random colonies were selected and plated on MRS agar containing 5 g/L of $CaCO_3$ and 0.04 g/L of bromocresol purple to evaluate their acid production. The isolates that were characterized as acid positive and catalase negative were selected and further identified by 16S rRNA gene sequencing. The isolates were evaluated for growth capacity by using MRS broth at different temperatures (15ºC and 45ºC), as well as different concentrations of NaCl (4.0% and 6.5%), pH (3.5, 4.0, 4.5, and 8.0), and CO_2 production. After 24 h of incubation at 37ºC, the absorbance at 630 nm was measured by using a plate spectrophotometer (Thermo Scientific®). Extraction of the DNA was performed by using the Wizard® Genomic DNA Purification kit (Promega), with modifications. The quantification of DNA was determined in the spectrophotometer NanoVue™ (GE Healthcare) and it was maintained at –20°C. The PCR was performed by using thermocycler [3]Prime (Techne) with DNA (80 ng) as the template, the set of primers 1492F/P027R, dNTP mix, $MgCl_2$, TaqDNA Polymerase, and buffer 10X (Promega). The product of the PCR, one fragment approximately of 1500 pb, was sent to Macrogen, Korea, for purification and sequencing. The sequences were compared to the database from GenBank using the BLAST algorithm (National Center for Biotechnology Information, Maryland, USA).

Results and discussion Seven strains of LAB were isolated from the samples of fresh forage and 162 strains were isolated from the silages. A large number of isolates had stronger growth when incubated at the highest temperature (45°C) than at 15°C. Most of the isolated strains (76%) were characterized as homofermentative. The growth of strains at the highest temperature and the low pH, in addition to being important for the culture predominance during silage fermentation, is also important when it comes to increasing the viability of these cultures when submitted to the lyophilization process since the inoculants are commercialized as "lyophilized". The 16S rDNA gene sequencing resulted in the identification of 156 isolates with similarity that was equal to or greater than 97% of the sequences that are available on GenBank. The main LAB present during the fermentation period were *Weissela cibaria, W. paramesenteroides*, *L. plantarum and L. pentosus* (Figure 1). The genus *Weissela* predominated in the first days of fermentation, while *Lactobacillus* predominated after the seventh day. The genus *Weissella* belongs to the group of LAB, however, few studies have reported the presence of this genus in silages. The other strains of LAB that were identified in this study were microorganisms commonly found in many forage species. For example, *L. plantarum* has been identified as the predominant species in majority of forages and is one of the main LAB that is often used as an inoculant for silages.

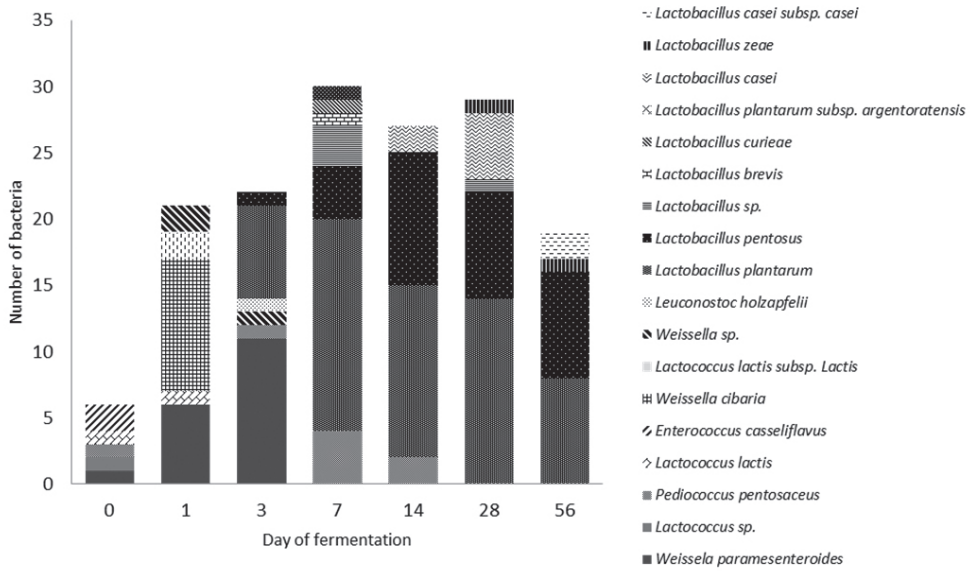

Figure 1 Lactic acid bacteria sucession during elephant grass fermentation.

Conclusion *Lactobacillus plantarum* and *L. pentosus* were the predominant species of LAB in elephant grass silages that were produced in tropical conditions. Future studies plan to evaluate these predominant species as inoculants to improve the fermentation process of elephant grass silages.

A**cknowledgments** The authors thank Brazilian agencies Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq), Fundação de Amparo a Pesquisa de Minas Gerais (FAPEMIG), and Coordenação de Aperfeicoamento de Pessoal de Nivel Superior (CAPES).

Effects of wilting, molasses addition, and lactic acid bacteria inoculation on fermentation and bacterial community of elephant grass silage produced in Vietnam

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Keywords fermentation, microbiota, silage additive, tropical grass

Introduction To feed the current and increasing stock of ruminants in the tropics, the utilization of forage crops needs to be optimized. Proper preservation is critical to enable stable feed supply for both small- and large-holder farmers. Acetic acid fermentation often occurs in tropical grass silage, and alcohols content may exceed acids content when sugar-rich crop is used. In addition, high ambient temperature can enhance aerobic deterioration after silo opening. Although attempts have been made to control tropical grass ensiling, sufficient data are not yet available on the bacterial community influenced by various treatments and additives. The present study was conducted to examine the effects of wilting, molasses addition, and lactic acid bacteria (LAB) inoculation on fermentation and bacterial community of elephant grass silage produced in Vietnam. Both homo-fermentative and hetero-fermentative LAB species were tested.

Materials and methods Direct-cut and wilted elephant grass (*Pennisetum purpureum* Schumach.) was stored in a laboratory-scale silo with and without molasses (50 g/kg) and LAB inoculants. The grasses were manually chopped using knives into 10–20-mm cuts before ensiling. Homo-fermentative LAB contained *Lactobacillus paracasei* and *Lactococcus lactis*, and hetero-fermentative LAB had *Lactobacillus buchneri*. Silages were stored in Hue, Vietnam, for 4 month at room temperature, and then transferred to Okayama, Japan. Fermentation products were determined by HPLC, and microbial analyses were carried out by plate-culture and denaturing gradient gel electrophoresis (Tu et al. 2013). Searches in the GenBank database with the BLAST program were performed to determine the closest relatives of partial 16S rRNA gene sequences.

Table 1 Fermentation products and microbial counts of direct-cut elephant grass silage prepared with and without molasses addition and lactic acid bacteria inoculation

	− Molasses				+ Molasses			
	Cont	LP+LC	LB	SE	Cont	LP+LC	LB	SE
Dry matter (g/kg)	156^{b}	189^{a}	159^{b}	6.03	164	170	165	2.11
pH	4.20^{a}	3.40^{b}	4.28^{a}	0.10	4.12^{x}	3.43^{y}	4.27^{x}	0.08
Lactic acid (g/kgDM)	15.8^{b}	96.8^{a}	2.21^{b}	5.60	23.4^{y}	108^{x}	6.48^{y}	5.83
Acetic acid (g/kgDM)	26.3^{b}	2.45^{c}	48.9^{a}	3.52	30.6^{y}	2.61^{z}	49.1^{x}	2.92
Ethanol (g/kgDM)	17.7^{a}	10.1^{c}	13.0^{b}	0.53	20.0^{x}	10.0^{y}	12.4^{y}	0.97
1,2-PD (g/kgDM)	3.17^{a}	0.58^{b}	2.97^{a}	0.47	3.44^{x}	0.95^{y}	3.19^{x}	0.25
LAB (log cfu/g)	7.61^{a}	5.52^{b}	6.96^{ab}	0.38	7.72^{x}	5.27^{y}	7.57^{x}	0.32
Yeasts (log cfu/g)	7.72^{a}	5.59^{b}	6.85^{ab}	0.43	7.57^{x}	7.78^{x}	5.50^{y}	0.08

Means of triplicate silages. Values in the same row with different superscript letters (a−c, x−z) are significantly different ($P<0.05$). LP, *Lactobacillus paracasei*, LC, *Lactococcus lactis*; LB, *Lactobacillus buchneri*. 1,2-PD, 1,2-propanediol; LAB, lactic acid bacteria.

Results and discussion Regardless of wilting, untreated silage had acetic acid as the predominant acid, and molasses addition increased ethanol fermentation. Homo-fermentative LAB inoculation greatly enhanced lactic acid production and suppressed acetic acid and ethanol production. Hetero-fermentative LAB inoculant inhibited aerobic deterioration, while decreasing ethanol content and increasing acetic acid content. The DGGE profiles indicated that indigenous bacteria appeared to be eliminated by homo-fermentative LAB inoculant, whereas no apparent changes were found with hetero-fermentative LAB. Alcoholic fermentation encouraged by molasses addition was suppressed in LAB-inoculated silage.

Table 2 Fermentation products and microbial counts of wilted elephant grass silage prepared with and without molasses addition and lactic acid bacteria inoculation

	− Molasses				+ Molasses			
	Cont	LP+LC	LB	SE	Cont	LP+LC	LB	SE
Dry matter (g/kg)	248^{B}	261^{A}	245^{B}	4.69	239^{Y}	262^{X}	248^{Y}	3.20
pH	4.42^{A}	3.43^{B}	4.24^{A}	0.05	4.61^{X}	3.47^{Z}	4.35^{Y}	0.05
Lactic acid (g/kgDM)	13.3^{B}	105^{A}	13.1^{B}	8.24	9.89^{Y}	152^{X}	6.81^{Y}	18.1
Acetic acid (g/kgDM)	19.6^{B}	3.79^{C}	48.8^{A}	2.24	22.7^{XY}	5.58^{Y}	42.1^{X}	5.00
Ethanol (g/kgDM)	22.0^{A}	11.6^{B}	17.9^{AB}	1.80	35.3^{X}	15.9^{Y}	14.4^{Y}	4.80
1,2-PD (g/kgDM)	12.0^{A}	2.03^{B}	11.7^{A}	1.69	19.0	3.09	14.0	3.77
LAB (log cfu/g)	7.75	6.86	7.57	0.44	8.11^{X}	4.26^{Y}	7.77^{X}	0.19
Yeasts (log cfu/g)	8.13^{A}	5.69^{B}	7.76^{A}	0.40	8.03^{X}	4.89^{Y}	7.77^{X}	0.16

Means of triplicate silages. Values in the same row with different superscript letters (A−C, X−Z) are significantly different ($P<0.05$). LP, *Lactobacillus paracasei*, LC, *Lactococcus lactis*; LB, *Lactobacillus buchneri*. 1,2-PD, 1,2-propanediol; LAB, lactic acid bacteria.

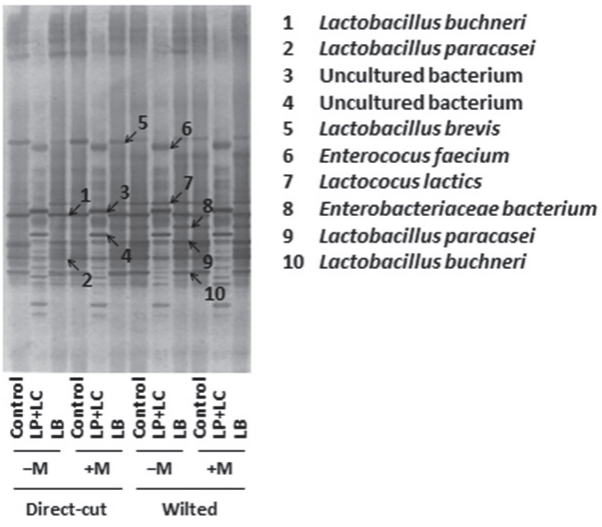

Figure 1 Bacterial communities of direct-cut and wilted elephant grass silages prepared with and without molasses addition and lactic acid bacteria inoculant. LP, Lactobacillus paracasei, LC, Lactococcus lactis; LB, Lactobacillus buchneri, M, molasses addition.

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Bacterial communities in tropical forage legume silages

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Keywords Single strand conformation polymorphism (SSCP), lactic acid bacteria, *Vigna unguiculata, Canavalia brasiliensis, Ipomoea batatas*

Introduction The success of ensiling from a biochemical point of view depends mainly on a sufficient amount of efficient lactic acid bacteria (LAB) and adequate carbohydrate availability. In our study the molecular technique PCR-SSCP was used to characterize bacterial communities in tropical forage silages *(Vigna unguiculata, Canavalia brasiliensis*) and sweet potato tuber (*Ipomoea batatas*) to assess the potential of the inherent microflora.

Materials and methods *Vigna, Canavalia* and *Ipomoea* alone or in mixtures were ensiled in micro-silos at different cutting intervals in triplicate for three months (see more details in our company paper, Guerrero and Martens, 2015). Silage samples were lyophilized starting at -40°C for 48 hours. For DNA extraction, the protocol according to Sanabria et al. (2009) was followed. For PCR amplification of the 16S rDNA region, the universal primers Com1 and Com 2-Ph were used, following amplification conditions suggested by Schwieger and Tebbe (1998). The amplified region was visualized by electrophoresis in agarose gels. To estimate the size of amplified products, a molecular weight marker of 100 bp was employed. The PCR products were purified using the Kit Wizard® *PCR* Preps DNA Purification System following the recommendation of the manufacturer. Digestion was performed using Lambda Exonuclease with a final concentration of 1× and proteins were extracted using phenol chloroform (Orita et al., 1989). Before the electrophoretic analysis, 8µl of loading buffer were added and the mixture was denatured at 94 ° C for 2 min, then immediately cooled on ice. The samples were loaded on MDE® gels with a concentration of 0.7× and electrophoresis (1× TBE) was performed at 9Ma for 16 h at 20°C. For visualization of the DNA fragments, the gels were silver stained (Bassam et al., 1991). As community marker *Rhizobuim trifolii*, *Flavobacterium johnsoniae*, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Agrobacterium tumefaciens* were used. The results were evaluated using GelCompar II (Applied Maths NV).

Results and discussion The cetyltrimethylammonium bromide (CTAB) procedure resulted in a fair amount of high molecular weight DNA with high purity free of inhibitors or contaminants. Amplified products with universal primers amplifying the 16S rDNA region had a molecular weight between 300 to 400 bp. In the SSCP analysis, a total of 17 different single DNA bands were identified. Mixtures of legumes with *I. batatas* (1:1) showed a higher number of bands, representing a wide variety of bacteria. The harvest age also affected the number of bands. The most common bacteria in the treatments were (Figure 1): **g** position - unknown bacteria, was found in 15 of 24 treatments, proving to be the dominant species independent of silage or cutting interval, suggesting that bacteria belonging to this band can suit different conditions. Other signs such as **f** position - *Lactobacillus plantarum*, was found in 13/24 treatments and **d** position - *L. brevis*, was found in 9/24 treatments, these bacteria are present in most but not all profiles, independent

of cutting interval and mixture, and other signs as at position **j** *Agrobacterium tumefaciens*, was found in 7/24 treatments. In position **c** *Flavobacterium johnsoniae* occurred in 6/24 treatments. These profiles showed 54.1% similarity among each other and reveal 3 groups that are influenced by the plant species.

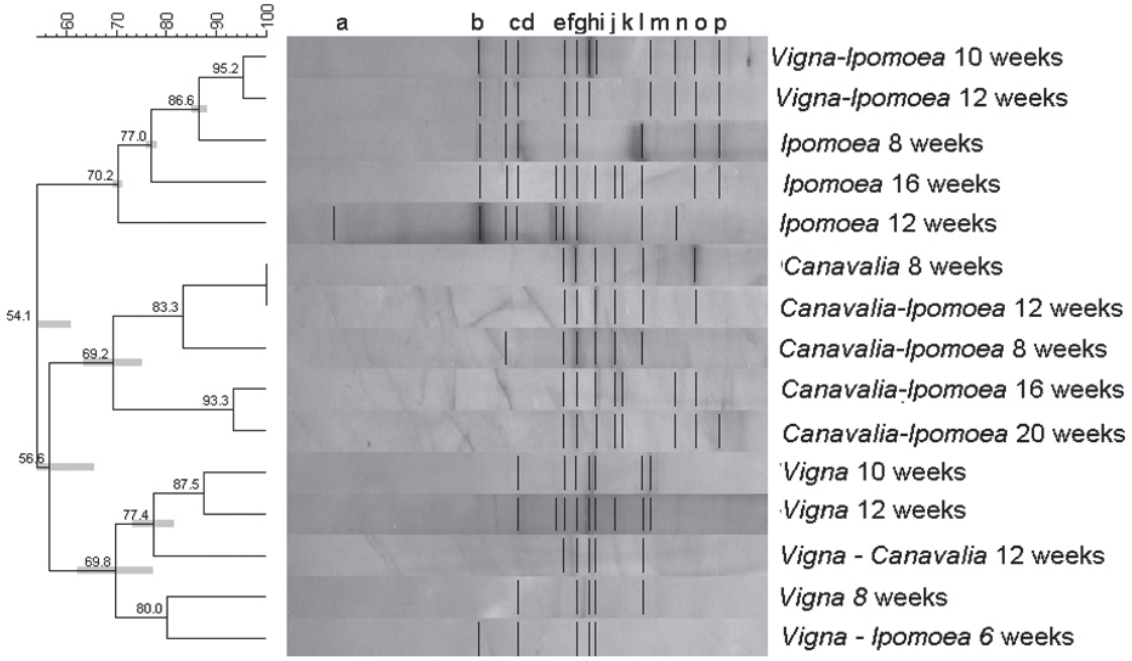

Figure 1 Similarity Analysis of SSCP profiles produced by PCR amplification of 16S rDNA silage samples of the tropical forages *Vigna unguiculata*, *Canavalia brasiliensis* and *Ipomoea batatas* tubers and mixtures thereof, at different cutting intervals. Community marker positions; a. *Rhizobuim trifolii,* c. *Flavobacterium johnsoniae,* d. *Lactobacillus brevis,* f. *Lactobacillus plantarum*, j. *Agrobacterium tumefaciens*.

Conclusions This study demonstrated the applicability and feasibility of PCR-SSCP to assess the diversity of bacteria found in tropical forage silages. It could also be used to study microbial factors affecting silage quality.

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Does wilting affect the succession of lactic acid bacteria in alfalfa silage?

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Keywords PCR, microbial population, molecular identification, *Lactobacillus plantarum*

Introduction The amount, diversity and activity of epiphytic lactic acid bacteria (**LAB**) direct the fermentation and the proportions of organic acids that will be produced, thereby influencing silage quality. The colonization of plants by LAB is a complex process that depends on several factors including plant species, stage of growth, weather, geographic localization, and type of fertilizers that are used (McGarvey et al., 2013). The reduction in moisture content through wilting alters the substrate concentration, which together with the increase in osmotic potential, provides varied effects in the microbiota (Woolford, 1984), depending on weather, conditions at the moment of the process and time of drying. However, studies are not conclusive, as some present an increase while others present a decrease in LAB populations in alfalfa silages that were submitted to wilting. A thorough understanding of the behavior of alfalfa grown in tropical conditions as well as the evaluation of changes in the epiphytic microbial populations can help to obtain strategies to improve the fermentation process and decrease nutrient losses. Thus, the hypothesis of this study was that the wilting process does not alter microbial diversity in alfalfa silage produced in tropical conditions.

Materials and methods Lactic acid bacteria were isolated from samples of fresh alfalfa plants without wilting, fresh forage (day 0) wilted for 6h and its both non-wilted and wilted silages in different fermentation periods (1, 3, 7, 14, 28, and 56 days). The DNA isolated from plants and silages was extracted by using a commercial kit (Wizard® Genomic DNA Purification kit, Promega). 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 BLASTn (*Basic Local Alignment Search Tool*) (http://www.ncbi.nlm.nih.gov/BLAST) for nucleotides. The 16S rRNA sequences that showed similarity equal to or greater than 97% were considered to be from the same Operational Taxonomic Unit.

Results and discussion The succession of LAB isolated from plants and silages of different fermentation periods, non-wilted and wilted, are shown in Figures 1 and 2, respectively. The presence of *Lactobacillus plantarum* was observed in all fermentation periods, except in the wilted forage before ensiling. The wilted forage showed high diversity of species before ensiling.
Although variations occurred in the appearance of species during the fermentation process, the microbial fermentation of the forage before ensiling and the respective unwilted and wilted silages showed small variations. According to Hartmann and Windmer (2006), significant changes in bacterial populations did not occur to increase diversity, but changes can occur in the appearance of some taxonomic groups which are offset by changes in other groups.

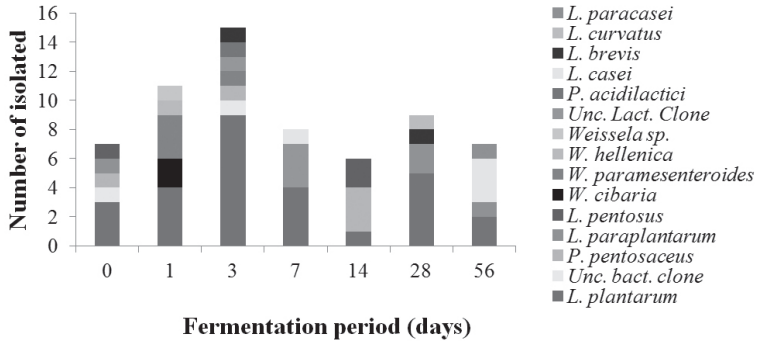

Figure 1 Succession of lactic acid bacteria in unwilted forage and alfalfa silage in different fermentation periods.

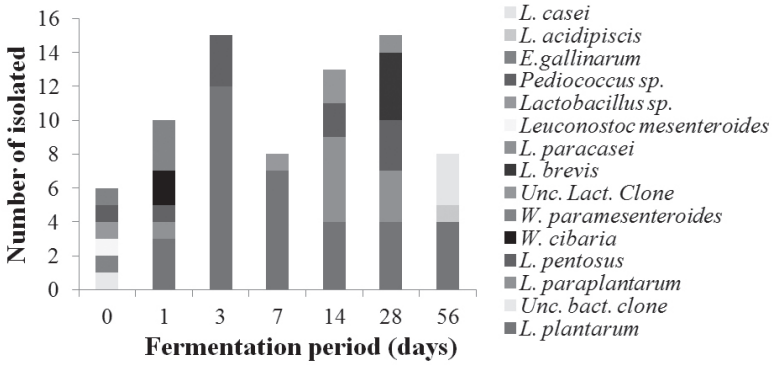

Figure 2 Succession of lactic acid bacteria in wilted forage and alfalfa silage in different fermentation periods.

Conclusions There is a high diversity of LAB species in both unwilted and wilted alfalfa (fresh forage and silage). *Lactobacillus plantarum* predominates among LAB species, although its behavior over the fermentation period is different between unwilted and wilted alfalfa silages.

Acknowledgments: This research was supported by the FAPEMIG and CNPq foundations.

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Microbial characterization of *Arachis pintoi* silage at different fermentation periods

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Keywords bacterial DNA, lactic acid bacteria, ion torrent

Introduction The forage peanut is a tropical legume that has been considered a good alternative to silage production because of its characteristics of persistence and high nutritive value. However, it presents high buffering capacity, low content of water soluble carbohydrates and DM, and a low epiphytic population of lactic acid bacteria (LAB), thereby requiring the use of additives in order to be ensiled and to improve the silage fermentation. Due to the lack of information about the epiphytic population of forage peanut and its ensiling process, this study was carried out to evaluate the microbial population through the new generation sequencing technique by using the Ion Torrent platform.

Materials and methods The forage peanut was harvested at the beginning of flowering period by using a backpack mower and it was chopped into 1.5-cm pieces and ensiled in triplicate in 25.40 × 35.56 cm bags (Doug Care Equipment, Springville, CA). The anaerobic condition of the bags was established by using a vacuum sealer. The samples of silage that were collected at different fermentation periods (1, 3, 7, 14, 28, and 56 days) were utilized to extract bacterial DNA. The bacterial DNA was extracted by using a commercial kit for DNA extraction (Wizard - Promega), quantified in a spectrophotometer Nanodrop (Thermo Scientific 2000), and stored at – 20 °C. The Polymerization Chain Reaction (PCR) was performed by using a pair of specific primers for the eubacteria dominance to amplify the 16S rRNA gene. The PCR products were adjusted for a final concentration of 10 – 15 pM and were adhered in ion spherical particles (ISP) by using the Xpress kit for fragments of 200 pb (Life Technologies, USA) while following the instructions of the manufacturer. The sequences were processed using the program MOTHUR v.23.0.

Results and discussion A total of 721,837 sequences of samples from plants and silages at different fermentation periods were generated by Ion Torrent platform. The total number of sequences and those of better quality were recovered from each sample. The silage at 3 days of fermentation presented the highest number of high quality sequences (56,148) in comparison to the other silages from different fermentation periods and fresh plants, while the silage at 56 days of fermentation presented lower numbers of sequences (26,606) in comparison to other silages and fresh plants. In this study, as the fermentation time increased, a smaller amount of DNA was obtained. The forage peanut fermentation was dominated by the genus *Weissela,* followed by a small participation from the genus *Pediococcus* in the final stages of fermentation (Figure 1A). The genus *Lactobacillus* appeared only in silages with 56 days of fermentation, presenting with a low number of

sequences. The genus *Weissela* is part of the composition of the epiphytic population of alfalfa, although there is no information in the literature that proves it dominates throughout storage time. In relation to the analysis of family, the *Enterococcaceae* family was low in number in the plants and disappeared during fermentation (Figure 1 B). This was expected due to the unfavorable conditions (i.e. decreasing pH) during fermentation, which inhibits the growth of bacteria from this family.

Conclusion The genus *Weissella* prevailed during all fermentation periods in forage peanut silage.

Acknowledgments This research was supported by the FAPEMIG and CNPq.

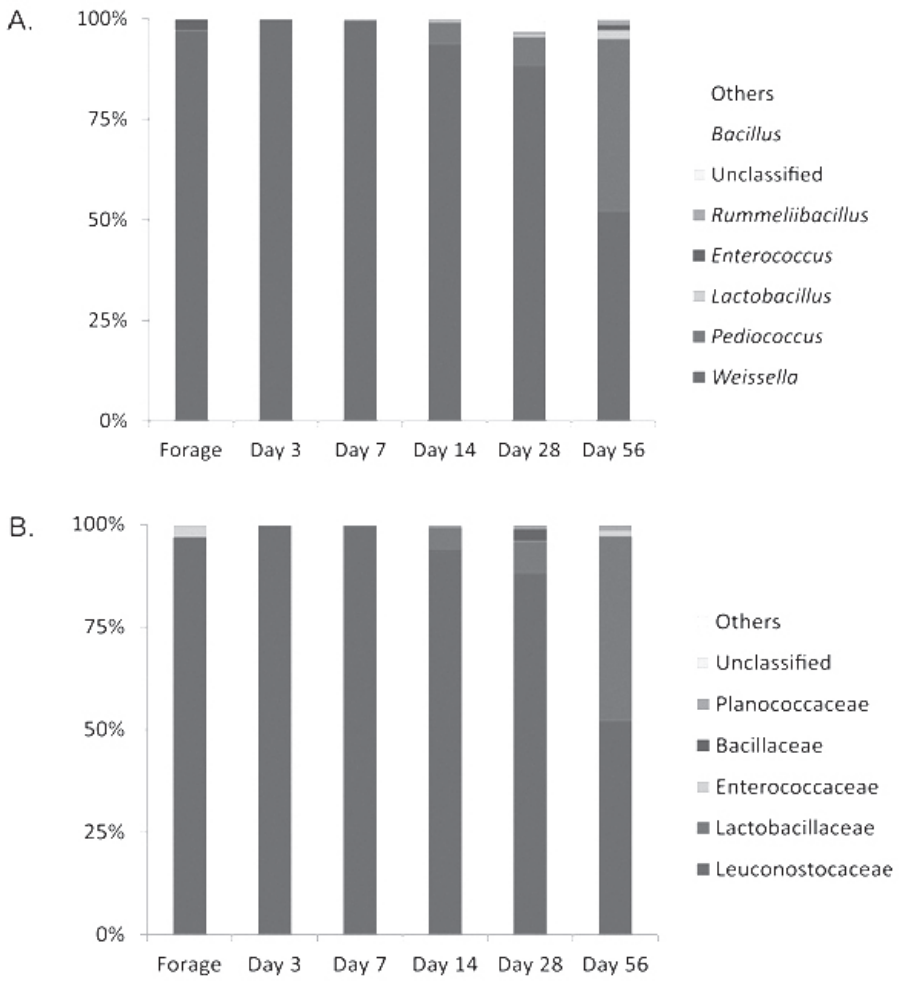

Figure 1 Phylogenetic taxonomic composition of the forage peanut silage microbiome during fermentation.

Isolation of *Lactobacillus fructivorans* from aerobically stable alfalfa silage

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Keywords fermentation, microbiota, silage additive, tropical grass

Introduction It is generally thought that differences exist between crop species in terms of susceptibility or resistance to aerobic deterioration of silage. Corn silage is known to spoil easily in the presence of air, and significant heating will often be seen 1−2 days after silo opening. Grass silage occasionally shows a resistance to aerobic deterioration, but such stability is not promising and hard to predict. In contrast, alfalfa silage can remain unheated for more than a week, and does not spoil despite lactic acid predomination over the fermentation, i.e. the stability is not a result of high concentrations of antifungal acetic, propionic, and butyric acids. Bacterial factors involved in the aerobic stability are thus worth examining. Using denaturing gradient gel electrophoresis (DGGE) analyses, we found out *Lactobacillus fructivorans* as the predominant lactic acid bacterium (LAB) in aerobically stable alfalfa silage. We then successfully isolated *L. fructivorans* and performed inoculation experiments using wilted grass and whole crop corn silages.

Materials and methods Wilted alfalfa (DM 491 g/kg) was ensiled in a plastic pouch with and without addition of molasses at 20 g/kg on a wet basis. The silo was opened at 5, 10, and 60 days after ensiling, and concentration of fermentation products and composition of the bacterial community were determined. The PCR was used to amplify a variable region of the bacterial 16S rRNA gene, and the PCR products were separated according to their sequences by DGGE. Select bands were excised from DGGE gels, and the DNA was cloned and sequenced to identify bacterial species. Liver infusion sake (LIS) medium, which is composed of Japanese rice wine and animal liver homogenate, was used instead of MRS medium to isolate *L. fructivorans*.

Results and discussion Aerobic deterioration took place in control silage stored for 5 and 10 days; heating due to spoilage was seen on day 5 after silo opening. No heating was found in molasses-added silage regardless of ensiling period, whereas the additive was intended to lower aerobic stability. Non-LAB species such as *Pantoea* sp., *Oceanobacillus* sp., and *Methylobacterium* sp. were seen as major bacteria in untreated silage (Figure 1). A number of bands indicative of *Pantoea* sp. became faint when ensiling was prolonged for 60 days. *L. fructivorans* was detected common for aerobically stable silages, and the bands appeared distinctive in molasses-added alfalfa silage. Although this finding suggested association of *L. fructivorans* with high aerobic stability, plate-culture using MRS medium failed to isolate the LAB species. Fortunately, this difficult-to-culture LAB grew well in LIS medium; about 60% of isolates were identified as *L. fructivorans*. This isolate was inoculated to wilted Italian ryegrass and whole crop corn silage at levels of 10^4, 10^5, and 10^6 cfu/g. Spoilage inhibition was seen for wilted grass silage regardless of the inoculation level, whereas the activity was small and restricted at the highest inoculation level for corn silage. These results indicated that, although *L. fructivorans* may be involved, the activity of spoilage inhibition can be insufficient and unclarified substance produced during the fermentation should also contribute to high stability of alfalfa silage.

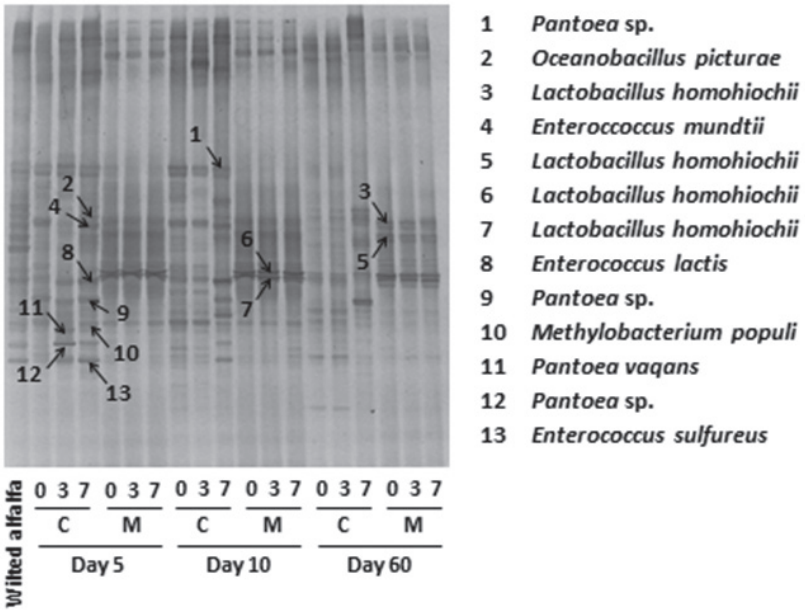

Figure 1 Bacterial community at silo opening and after 3-day and 7-day aerobic spoilage test of wilted alfalfa silage stored for 5, 10, and 60 days without (C) and with molasses (M).

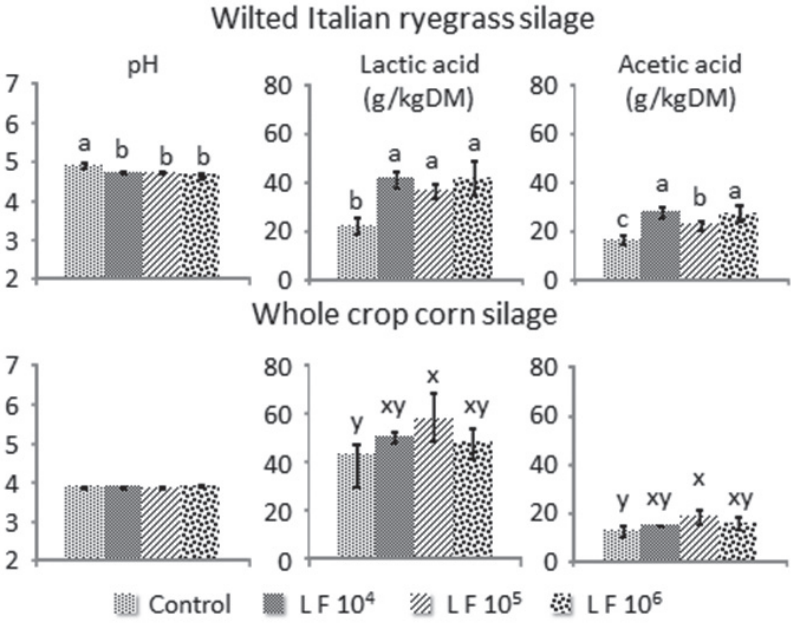

Figure 2 Fermentation products of wilted Italian ryegrass and whole crop corn silage inoculated with and without Lactobacillus fructivorans (LF) at 104, 105, and 106 cfu/g.

Fermentation characteristics and lactic acid bacteria succession of total mixed ration silages formulated with peach pomace

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Keywords aerobic stability, lactic acid bacteria, peach pomace, total mixed ration Silage

Introduction Peach juice is an internationally popular beverage due to its high nutritional value and health benefits. Owing to its high moisture content and water-soluble carbohydrates content, it is easy for microorganisms to propagate, which lead to great waste and potential environmental problems. Peach pomace could be a feed source for ruminants. This study was to evaluate the feasibility of total mixed ration (TMR) silages formulated with peach pomace.

Materials and methods The TMR were prepared using peach pomace, alfalfa (or *Leymus chinensis*) hay, corn meal, soybean meal, cotton meal, limestone, vitamin-mineral supplement, and salt in a ratio of 6.0:34.0:44.4:7.0:5.0:2.5:1.0:0.1% (DM basis) and sealed in plastic bags (800×1,500 mm) in triplicate. Silos were opened after 1, 3, 7, 14, 28 and 56 d of ensiling at about 20°C. Silages ensiled for 28 and 56 d were used for aerobic stability test, following procedures of Wang et al. (2012). Silage pH and organic acids were determined as described by Xu et al. (2007). Microbial counts were determined by plate count method (Xu et al., 2008). The LAB colonies were purified by repeated streaking. Genomic DNA was extracted and 16S rDNA gene was amplified for sequence analyses by Sunny Biotechnology Co., Ltd. Data were subjected to one-way analysis of variance using general linear model procedure of the SAS software package (SAS, 2004). Tukey's test was used to differentiate means and significance was set at $P<0.05$.

Results and discussion Regardless of the type of roughage, TMR silages were well fermented with low pH and high lactic acid contents. In this study, yeasts were not effectively suppressed even when ensiling was prolonged to 56 d, all above 10^4 cfu/g fresh matter. There was no significant difference in yeast counts between TMR silages with different hay. However, aerobic stability was extended. Therefore, yeast populations at silo opening had less effect on aerobic stability. In view of the difference in organic acid contents, the enhanced aerobic stability appears to be related to undissociated acids. In this study, *Lactobacillus buchneri* was isolated from TMR and multiplied rapidly after 28 d of ensiling. Although *L. buchneri* was not predominant in 28-d TMR silages, it grew rapidly during aerobic deterioration and became dominant. In 56-d TMR silages, *L. buchneri* comprised the majority of LAB and could be detected throughout the aerobic exposure. In addition, it was observed that *Pediococcus acidilactici* was also predominant during ensiling and aerobic exposure in TMR silages. In recent years, the pediocin produced by *P. acidilactici* has received much attention, and *Pediococcus* species are often used to preserve foods and feeds and can be utilized as probiotics (Digaitiene et al., 2012). In this study, *P. acidilactici* and *L. buchneri* were the predominant LAB in the later stage of ensiling and during aerobic deterioration of TMR silages. *P. acidilactici* might produce bacteriocin during ensiling that inhibits certain LAB and other harmful microorganisms,

and this bacteriocin can be effective during aerobic deterioration. Most previous studies have focused on the inhibition of yeast and harmful bacteria by organic acids and ignored the antagonism between microorganisms caused by bacteriocins. The roles of *P. acidilactici* during ensiling and aerobic exposure are worth examining in the future study.

Table 1 Fermentation quality and microbial dynamics during ensiling of ATMR[1] and LTMR[2] silages

Item[1]		Days of ensiling					
		1	3	7	14	28	56
Fermentation quality							
pH	ATMR	5.25^{a}	4.61^{b}	4.63^{b}	4.39^{c}	4.29^{c}	4.29^{c}
	LTMR	4.91^{a}	4.81^{a}	4.52^{b}	4.56^{b}	4.36^{bc}	4.24^{c}
Lactic acid, % DM	ATMR	1.43^{e}	3.77^{d}	5.24^{c}	6.53^{b}	6.54^{b}	7.11^{a}
	LTMR	2.40^{e}	2.64^{d}	5.48^{c}	5.94^{b}	6.24^{a}	6.21^{a}
Acetic acid, % DM	ATMR	0.60^{d}	0.83^{c}	1.07^{b}	1.16^{b}	1.18^{b}	1.38^{a}
	LTMR	0.84^{b}	0.88^{b}	1.09^{a}	0.96^{ab}	0.96^{ab}	0.90^{b}
Microbial component							
Lactic acid bacteria, log cfu/g	ATMR	8.64^{ab}	9.05^{a}	8.89^{ab}	8.55^{b}	7.25^{c}	6.74^{d}
	LTMR	6.94^{c}	8.80^{a}	8.45^{a}	8.67^{a}	7.94^{b}	6.63^{c}
Yeasts, log cfu/g	ATMR	5.72^{b}	6.73^{a}	6.86^{a}	5.73^{b}	4.56^{c}	4.21^{c}
	LTMR	5.88^{a}	6.75^{a}	5.98^{a}	5.83^{a}	4.86^{c}	4.27^{d}

$^{a\text{-}e}$Means within a row with different superscripts differ ($P < 0.05$).

[1]ATMR = total mixed ration with alfalfa hay; [2]LTMR = total mixed ration with *Leymus chinensis* hay.

Conclusions Peach pomace can be effectively utilized as a component of TMR silage. Yeast populations at silo opening had a marginal effect on aerobic stability. Although marked changes were seen in LAB community, *L. buchneri* and *P. acidilactici* may play a major role in aerobic stability, and further study is necessary to evaluate the effect of *P. acidilactici*.

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Bacterial community of grass, legume, and whole crop corn silage assessed by denaturing gradient gel electrophoresis and next generation sequencing

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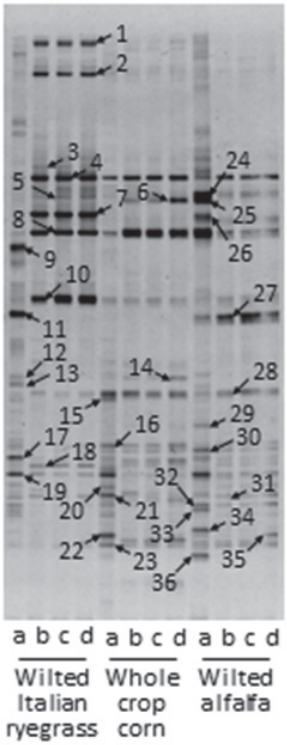
Keywords denaturing gradient gel electrophoresis, microbiota, next generation sequencing, silage

Introduction Bacteria play an important role in silage fermentation through their metabolism and competitive exclusion. Great efforts have been devoted to understand the complex community in pre-ensiled crop and silage, and integration of plate-culture and DNA-based microbial analysis has greatly progressed in the past decade. We have employed denaturing gradient gel electrophoresis (DGGE) in this regard, and identified a number of non-conventional bacteria involved in the ensiling process. It has been pointed out that assessment of microbial composition is affected by protocols and primers used for PCR amplification, resulting in possible underestimation of key component of bacteria. Recently the means of next generation sequencing (NGS) have become pivotal in facilitating the discovery of microbiota and their competitive and cooperative interaction. To evaluate its power in examining silage microbiota, grass, legume, and whole crop corn silages were subjected to DGGE and NGS analyses.

Materials and methods Pre-ensiled crop and 60-day silage of wilted Italian ryegrass, wilted alfalfa, and whole crop corn were used. Bacterial DNA was extracted and purified using a commercial kit with lysozyme pretreatment. For DGGE, we amplified a variable (V3) region of 16S rRNA gene that create approximately 200 bp, with the GC-clamp attached to the forward primer. For NGS analysis, we used the primer pair for V3 and V4 regions that create an amplicon of approximately 460 bp. We completed DGGE analysis, whereas NGS by Illumina MiSeq System is now underway.

Results and discussion We selected untreated silage to compare results between DGGE and NGS, because various lactic acid bacteria (LAB) and non-LAB species could be identified by our standard DGGE procedure. A total of 36 bands were successfully sequenced including 10 bands for uncultured bacteria (Figure 1). Only 3 bacterial species were common for 3 silage types; bands indicative of *Weissella paramesenteroides* (bands 4 and 8), *Pediococcus pentosaceus* (band 10), and uncultured bacterium (band 28) were seen in all silages. The identified LAB species belonging to the genus of *Weissella*, *Enterococcus*, *Leuconostoc*, and *Pediococcus* can be found frequently in naturally fermented silage. Besides, several unusual non-LAB species, such as *Staphycoccus saprophyticus* and *Terribacillus saccharophilus*, were also detected in this DGGE analysis. Each of 3 silage replicates showed similar bacterial community; hence, based on low resolving power of DGGE, analysis of one silage sample can be accepted to demonstrate the bacterial community. We are now trying NGS for the same samples to understand how NGS can improve and revise our knowledge and microbial analysis procedure.



1	Uncultured bacterium	19	Uncultured bacterium
2	*Weissella paramesenteroides*	20	Uncultured bacterium
3	*Weissella cibaria*	21	*Agrobacterium tumefaciens*
4	*Weissella paramesenteroides*	22	*Klebsiella variicola*
5	*Staphycoccus saprophyticus*	23	*Streptomyces* sp.
6	*Enterobacter* sp.	24	*Staphycoccus saprophyticus*
7	*Leuconostoc mesenteroides*	25	*Enterococcus* sp.
8	*Weissella paramesenteroides*	26	*Enterococcus gallinarum*
9	*Leuconostoc mesenteroides*	27	*Enterococcus mundtii*
10	*Pediococcus pentosaceus*	28	Uncultured bacterium
11	*Pseudomonas trivialis*	29	*Enterococcus* sp.
12	Uncultured bacterium	30	*Terribacillus saccharophilus*
13	Uncultured bacterium	31	*Paracoccus* sp.
14	*Methylobacterium* sp.	32	*Agrobacterium* sp.
15	*Pseudomonas putida*	33	Uncultured bacterium
16	Uncultured bacterium	34	*Sanguibacter* sp.
17	Uncultured bacterium	35	*Acetobacter* sp.
18	*Pediococcus pentosaceus*	36	*Klebsiella* sp.

Figure 1 Bacterial community determined by denaturing gradient gel electrophoresis of pre-ensiled crops and silages prepared from wilted Italian ryegrass, whole crop corn, and wilted alfalfa. a, pre-ensiled crop; b−d, triplicate silages.

Fermentation products and bacterial community of whole crop rice silage stored in a bunker silo with different chop length

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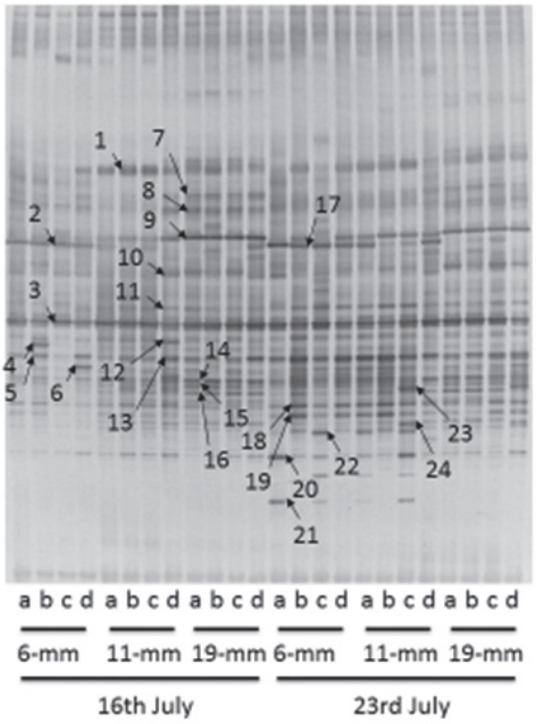
Keywords bacterial community, bunker silo, fermentation, whole crop rice

Introduction In Japan, whole crop rice has been proposed as a feed substitute for imported grass and legume hay. Round bale ensiling is practiced using various types of harvester and roll baler. Whole crop rice is usually insufficient in sugars and lactic acid bacteria, and the hollow stem of rice plant may increase remaining air in a silo. Silage making in a bunker silo, therefore, has been avoided for fear of fungal growth during fermentation and after exposure to air. Lately a new cultivar with high sugar content (cv. Tachisuzuka) and a new harvester that enables fine chopping (6 mm at shortest) have been developed. This improvement encourages ensiling whole crop rice in a bunker silo. In this study, we examined fermentation products and bacterial community of bunker-made whole crop rice silage prepared with 6-, 11-, and 19-mm chop length in combination of *Lactobacillus buchneri* inoculation.

Materials and methods Whole crop rice silage was produced at Livestock Technology Research Center, Hiroshima Prefectural Technology Research Institute. The crop was harvested at yellow ripe stage, while chopping into 6-, 11-, and 19-mm lengths by newly developed harvester. A commercial inoculant consisting of *L. buchneri* was applied at 10^5 cfu/g level, and then stored in a bunker silo for 9 months. We took samples 2 times from one bunker silo, and each time samples were collected from 4 different positions; outer-upper, outer-lower, inner-upper, and inner-lower. Fermentation products and bacterial community were determined using HPLC and denaturing gradient gel electrophoresis, respectively.

Results and discussion All silos were well preserved at acceptable pH ranging from 3.9 to 4.3. Higher level of lactic acid was found in 6- and 11-mm than in 19-mm silo, whereas the contents of acetic acid and 1,2-propanediol were greater in 19-mm than the other 2 silos. In addition, differences between upper and lower layers were seen in the contents of ethanol and 1,2-propanediol. Distinctive band for *L. buchneri* was detected in all samples, indicating *L. buchneri* could survive well in bunker-made whole crop rice silage. Although bands indicative of *Lactobacillus plantarum* and *Lactococcus lactis* were found regardless of chop length, they appeared faint compared to those of *L. buchneri. Enterobacter ludwigii* and *Pediococcus pentosaceus* were detected only in 19-mm silo, whereas *Lactobacillus acetotolerans* was detected exclusively in 6- and 11-mm silos. *Weissella paramesenteroides* was found solely in 11- and 19-mm silos.



1 *Lactobacillus plantarum*
2 *Lactobacillus acetotolerans*
3 *Lactobacillus buchneri*
4 *Pediococcus ethanolidurans*
5 *Enterobacter ludwigii*
6 Uncultured bacterium
7 *Enterobacter ludwigii*
8 *Enterobacter ludwigii*
9 *Weissella paramesenteroides*
10 *Pediococcus pentosaceus*
11 *Lactococcus lactis*
12 *Enterobacter* sp.
13 *Enterobacter* sp.
14 Uncultured bacterium
15 *Lactobacillus plantarum*
16 Uncultured bacterium
17 *Lactobacillus acetotolerans*
18 *Klebsiella varricola*
19 *Klebsiella pneumoniae*
20 Uncultured bacterium
21 Uncultured bacterium
22 Uncultured bacterium
23 *Enterobacter cloacae*
24 *Lactobacillus paracasei*

Figure 1 Bacterial community of whole crop rice silage stored in a bunker silo with 6-, 11-, and 19-mm chop length in combination of *Lactobacillus buchneri* inoculation. a, outer-upper; b, outer-lower; c, inner-upper; d, inner-lower.

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Lactic acid bacteria in silage: adhesion ability to plant surface and effects on adhesion of pathogenic bacteria

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Introduction There is evidences that some lactic acid bacteria can inhibit silage deterioration by blocking adherence of the pathogens to the plant surface cells. However, this effect of probiotics depends on both the specific probiotic strain and the pathogen. One of the common virulence strategies of pathogenic strains is adhesion to the host cells which provides a new target for treatment strategies. There are many approaches which inhibit bacterial attachment to the host cells. This study was to investigate the adhesive properties of several strains of lactic acid bacteria and the inhibition effects of these strains on the adhesion of *Escherichia coli* in silage.

Materials and methods Six isolates were obtained from spontaneous whole-crop corn silage by plating, paper diffusion and denaturing gradient gel electrophoresis. All these strains were used to determine the antibacterial activity of several strains of lactic acid bacteria selected from silage. The adhesion of different strains was evaluated by fluorescence labeling method. Pre-incubation, post-incubation and co-incubation tests were used to determine the inhibition to the six strains of pathogenic bacteria above.

Results and discussion The results are showed as follows: (1) Phylogenetic analysis of the six strains based on 16S rDNA gene sequence data indicated the presence of*Enterobacter cloacae*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Klebsiella terrigena* , which were all common pathogens or opportunistic pathogens in silage. (2) Lactic acid bacteria selected from silage had strong antibacterial activity. Antibacterial activities of seven different cell-free culture supernatants were no difference, the antibacterial substances were stable after heating and partly sensitive to protease K. (3) Except for *Lactococcus lactis*, the adhesion rate of the other lactic acid bacteria strains was higher than that of the two pathogenic bacteria strains, and the adhesion rate was *Lactobacillus* > *Enterococcus* > pathogenic bacteria > *Lactococcus* > (4) Most strains of lactic acid bacteria could inhibit the adhesion of *Enterobacter cloacae* and *Klebsiella pneumoniae* to plant surface cells through post-incubation test.

Conclusions Lactic acid bacteria strains isolated from silage possess high adhesion rate, especially *Lactobacillus*. Post-incubation is the main way which these strains inhibit adhesion of pathogenic bacteria.

Acknowledgements The work was supported by National Natural Science Foundation of China (Grant No. 31402136) and the Foundation for Sci & Tech Research Project of Heilongjiang Province of China (Grant No. WB13B111-2).

Table 1 Effects of lactic acid bacteria strains on adhesion ability (%) of *Enterobacter cloacae* to plant cells

Strains	Co-incubation test	Pre-incubation test	Post-incubation test
Enterobacter cloacae	39.2 ± 1.13	39.8 ± 2.03	40.8 ± 2.03
Lactobacillus rhamnosus	51.3 ± 0.09**	63.1 ± 2.02**	22.3 ± 1.08**
Lactobacillus plantarum	52.2 ± 1.57*	64.2 ± 1.14**	20.8 ± 1.01**
Enterococcus faecalis	46.5 ± 1.75	55.9 ± 3.06**	32.3 ± 1.18
Enterococcus faecium	40.8 ± 0.52*	48.5 ± 2.08**	25.2 ± 1.00**
Lactococcus lactis	35.7 ± 2.33	43.9 ± 3.02	20.6 ± 0.32**

Compared with *Enterobacter cloacae*, values with ** mean significant difference ($P < 0.01$), and with * mean significant difference ($P < 0.05$), while without markers mean no significant difference ($P > 0.05$).

Molecular tracking of lactic acid bacteria in piglets fed a fermented diet

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Keywords sow milk, lactic acid bacteria, RT-qPCR, fermented diet, piglet

Introduction Feeding fermented diets to suckling and weaning piglets is an alternative strategy to the use of so called growth promoters, i.e. the preventive use of antibiotics e.g. tetracyclines and sulfonamides. We isolated and identified a lactic acid bacteria (LAB) strain from sow milk, thus coming from the immediate environment of the piglet, and with potential to be used as inoculant for ensiling (Martens and Heinritz, 2012). The objective of the study was to track the strain molecularly to observe whether its traceability can explain possible health promoting effects on the gut of piglets.

Materials and methods The three experimental diets based on maize and lactose as main source of carbohydrates and fish meal + processed soy as main source of amino acids differed only in the anti- or probiotic additive. They were fed to three groups of 6 piglets each from day 9 to day 36 of life: a positive control with chlortetracycline hydrochloride (CON+), a negative control without growth promoter (CON-) fed as meal and a liquid diet fermented for 24 h inoculated with LABCIAT L605 (FER). Samples of feed and feces were obtained at around 15 (pre-weaning), 22 (weaning) and 26 (post-weaning) days of life of the six animals of each group. Potential probiotic traits of L605 were assessed by the mannose adhesion test (Pretzer et al., 2005). DNA of the inoculant was extracted using Wizard® Genomic DNA Purification Kit (Promega), and the conserved region 16S was amplified by different primers for the genus *Lactobacillus*. Highest similarity with documented gene sequences at NCBI (http://www.ncbi.nlm.nih.gov/) was given for *Lactobacillus plantarum*. Total DNA of the feces samples was extracted using QIAamp DNA Stool Mini Kit (QIAGEN) and amplified based on a method described by Han et al. (2011). The inoculant should be detected both on species and on strain level. Specific bands of the 16S region of the inoculant were cloned using the vector pGEM-T easy®. The primer Lpla-3 was used to quantify *L. plantarum* via RT-qPCR as suggested by Tamminen et al. (2012).Two primers out of 9with a high strain specifity verified by randomly amplified polymorphic DNA (RAPDs) served to develop primers for detecting strain L605.

Results and discussion Whereas there was a clear enrichment of *L. plantarum* in the fermented diet, no differences could be detected in the feces nor were there differences between ages (Table 1). Although strain L605 could successfully be distinguished from other *L. plantarum* strains by developing strain specific primers, it was detected in feces samples from all treatments (Figure 1). This can be probably explained by the origin of L605. Present in sow milk, this strain is probably ubiquitous for piglets. However, in our study it was not quantified for the different treatments.When appraising the occurrence of

diarrhea in the piglets, two out of six observations were positive in CON+, one in CON- and zero in FER. Despite the small sample size there might have been an effect of feeding the fermented diet even though no differences in the target species were detected in the feces which would confirm the probiotic trait expressed in the mannose-adhesion test.

Table 1 Quantity of *L. plantarum* (log_{10} ng/µl) in the diets and feces at the three sampling dates

	Day 15	Day 22	Day 26
Diet			
CON+	9.53 ± 0.56	9.15 ± 0.56	10.26 ± 0.56
CON-	9.34 ± 0.29	8.75 ± 0.29	9.12 ± 0.29
FER	13.43 ± 0.19	13.81 ± 0.19	13.71 ± 0.19
Feces			
CON+	10.33 ± 0.93	10.26 ± 0.87	9.95 ± 0.87
CON-	9.32 ± 0.17	9.88 ± 0.99	9.35 ± 0.81
FER	9.62 ± 0.75	10.24 ± 0.37	9.18 ± 1.0

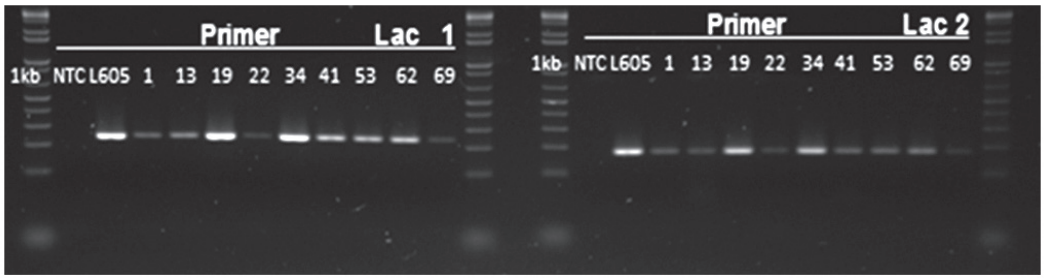

Figure 1 Detection of strain L605 (*L. plantarum*) in feces samples from the three different treatments at three different ages (NTC: control (water), L605: pure strain, No. 1, 22 and53: CON+, No. 13, 34 and62: CON-, No. 19, 41 and69: FER).

Conclusions The chosen methods allowed detecting the inoculated strain both in the diet and the feces. Due to similar numbers of the species in the different treatments it was not possible to relate animal health to the occurrence of *L. plantarum* in the piglets.

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Effect of additives on fungi and aflatoxin content of corn silage in north China

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Keywords Additive, corn silage, aflatoxin, fungi, fermentation quality, aerobic stability

Introduction Aflatoxins usually can be detected in the corn silages, which can induce negative effects on the health of animals, especially dairy cattle. The fungi and aflatoxins may accumulate on the raw materials, negatively impact the ensiling process and increase rapidly after opening the silo and contacting with the oxygen. The application of silage additives may have the ability to prevent the growth of fungi and limit the production of the aflatoxins. Thus, the objective of this experiment was to evaluate the effect of three kinds of silages additives on the fungi and concentration of aflatoxins and aerobic stability of corn silages and their relationship with the fermentation products.

Materials and methods Corn plants were grown at the density of 75000 plants/ ha in Zhuozhou station of China agriculture university in 2014 and harvested at DM concentration of 35%. Treatments were as follows: (1) *Lactobacillus plantarum* (LP), added at 1×10^6 CFU g^{-1}; (2) *lactobacillus buchneri* (LB), added at 1×10^6 CFU g^{-1}; (3) sodium propionate (SP) , added at a rate of 6.0 g kg^{-1}; and (4) control group, added with same volume distilled water. All forages were ensiled for 80 days before opened. The fermentation quality (pH value, organic acid and ammonia nitrogen), fungi count, aflatoxin concentration (HPLC method) and aerobic stability were evaluated.

Results and discussion The fermentation quality and aerobic stability of the silages are described in Table 1. The pH value ($P<0.05$), lactic acid ($P<0.05$), contents and rate of lactic acid to acetic acid ($P<0.01$) were significantly influenced by the silages additives, but no additives in this experiment had positive effect on these parameter when compared to control. LP-treated silages had the relatively higher pH value and lower aerobic stability than control. LB increased acetic acid contents ($P<0.05$), while sodium propionate increased the propionic acid ($P<0.05$) compared to control. Aerobic stability was not improved by any additives but was lower in LP-treated silages. The Dynamic changes of aflatoxin B1 (Figure 1) shown that AFB1 appeared in the corn silages and increased during aerobic exposure, addition of sodium propionate had lower AFB1 content than other treatments, even after14 days of exposure. The inefficiency of two inoculants was possibly due to the slow growth at low room temperature (20°) and intolerance to rapid decrease of pH value during ensiling.

Conclusions All silages were preserved well in this experiment. Addition of additive did not cause positive effect on the decline of pH value and ammonia nitrogen content and increase of lactic acid. Sodium propionate had the best effect on the reduction of aflatoxin B1.

Table 1 Fermentation quality and aerobic stability of the additive treated corn silages after 80d of ensiling

	Control	LP	LB	SP	SEM	*P*-value
pH	3.93^{b}	3.99^{a}	3.89^{b}	3.91^{b}	0.01	0.011
Lactic acid (%DM)	3.56^{ab}	2.90^{b}	4.20^{a}	3.18^{b}	0.18	0.038
Acetic acid (%DM)	0.91^{b}	0.57^{c}	1.28^{a}	0.85^{b}	0.09	0.003
Propionic acid (%DM)	0.04^{b}	0.00^{c}	0.03^{b}	0.09^{a}	0.01	0.013
Butyric acid (%DM)	0.00	0.00	0.00	0.00	0.00	
Citric acid (%DM)	0.13	0.12	0.23	0.17	0.02	0.386
Lactic acid/acetic acid	3.88^{b}	5.31^{a}	3.29^{b}	3.79^{b}	0.26	0.009
Ammonia nitrogen (%TN)	4.43	3.55	5.01	4.01	0.33	0.503
Aerobic stability (h)	105^{ab}	63^{c}	115^{a}	87^{b}	7.81	0.013
Mold count (log_{10} cfu/g)	3.15	2.50	2.03	2.26	0.19	0.176
Yeast count (log_{10} cfu/g)	4.97	5.12	4.64	4.77	0.18	0.841

DM, dry matter; TN, total nitrogen; LP, *Lactobacillus plantarum;* LB, *lactobacillus buchneri*; SP, sodium propionate; $^{a-c}$, Means within a row with different superscripts differ ($P < 0.05$).

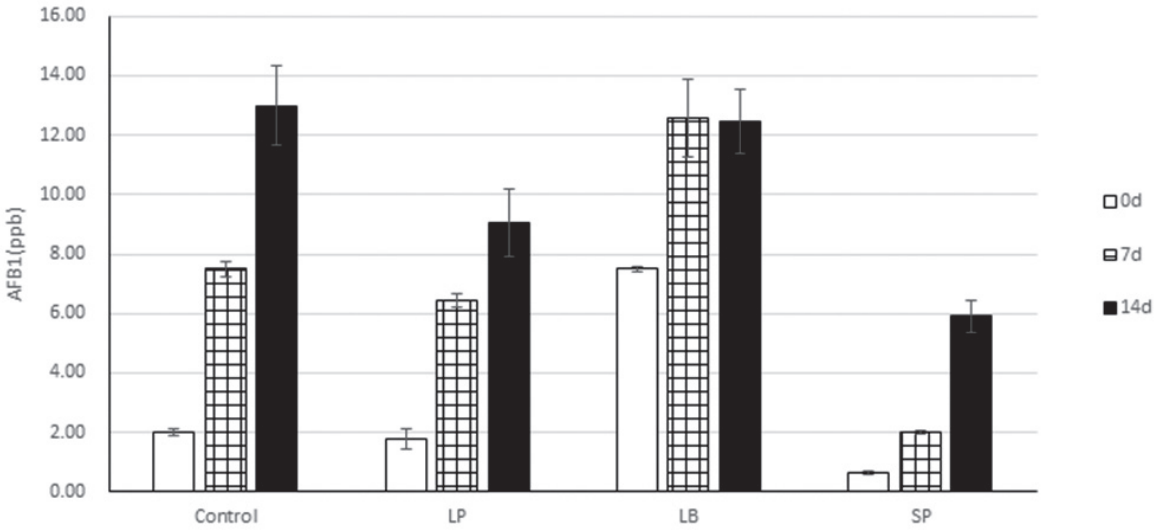

Figure 1 Dynamic change of AFB1 concentration in corn silages treated with additives when it is exposed to the air. LP, *Lactobacillus plantarum;* LB, *lactobacillus buchneri*; SP, sodium propionate; AFB1, Aflatoxin B1.

Mycotoxins and organic acids in corn silage on dairy farms in southern Brazil

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Keywords aflatoxins, silage fermentation, zearalenona, silage quality

Introduction The use of inappropriate practices during the process of production of the crop, ensilage technology and silo emptying are potential aggravating factors for the poor silage quality. The lack of technology favors the development of molds with their respective mycotoxins, among which, aflatoxins stand out, because they have carcinogenic, mutagenic and teratogenic properties. Also zearalenone deserves mention due its estrogenic effect in domestic animals, leading lower milk production, repetition of estrus, low conception rate and abortion. In this context, the objective was to evaluate the presence of mycotoxins and organic acids in silage on dairy farms in southern Brazil.

Materials and methods Samples of the silages were collected on 40 dairy farms in southern Brazil (Paraná, Santa Catarina and Rio Grande do Sul). The silages were analyzed for pH, temperature, organic acids (lactic, acetic, propionic and butyric), alcohol and mycotoxin levels (aflatoxin and zearalenone). The data were subjected to factorial analysis of data reduction by using the principal components (PC) method. The PC were submitted to cluster analysis, obtaining groups with common characteristics. The groups was submitted to variance analysis and the differences between the averages analyzed by Tukey test ($P<0.05$) for the chemical characteristics, using the PROCGLM of the statistical program SAS (2009).

Results and discussion There were no differences ($P>0.05$) for pH, obtaining as properties overall average, pH values equal to 3.98 (Table 1). This value can be indicative of suitable fermentation process, considering that the optimum pH range is between 3.6 and 4.5. The concentration of dry matter silage (DM) did not differ among silages of different groups, with average values of 330 $g.kg^{-1}$.There were no differences ($P>0.05$) between groups of dairy farms in amounts of alcohol, acetic acid, butyric acid and lactic acid, being observed mean values of 8.86, 9.22, 0.29 and 26.05, respectively. The values found for acetic acid, except in the farms of group 3, were higher than the recommended maximum level, that is <20 $g.kg^{-1}$ of DM (Cruz et al., 2001), which can be explained by the high use of inoculants with *Propionibacterium acidipropionici* (65% of the silage). The butyric acid concentrations were within the standard generally observed in corn silage (<1$g.kg^{-1}$ of DM). The butyric acid concentration reflects the extent of clostridial activity and is related to higher final pH values. Therefore, concentrations below 3$g.kg^{-1}$ of DM indicate lower losses in energy and silage dry matter. The lactic acid concentrations were lower than the values usually found in corn silage (60 to 80 $g.kg^{-1}$ of MS). For propionic acid, there were differences between farms in groups 3 and 4, with values of 2.57 and 7.95, respectively.

However, all groups had levels considered adequate (0 to 10 g.kg^{-1} of DM). As for concentrations of mycotoxins, differences were observed (P<0.05) for aflatoxins, with higher values in the silages of the groups 1 and 2. The levels of aflatoxin and zearalenone of the silage were within the maximum limits established by ANVISA (National Health Surveillance Agency - Brazil), which are 50 and 400 ppb, respectively.

Table1 Chemical characteristics of silage on different groups in dairy farms in southern Brazil (mean and standard deviation)

Item	Groups*				Overall average	P>f
	1	2	3	4		
pH	4.02 (±0.20)	3.76 (±0.11)	4.08 (±0.49)	3.95 (±0.25)	3.98 (±0.32)	0.17
Dry matter, g.kg^{-1}	326 (±60.7)	328 (±28.2)	344 (±54.8)	304 (±69.4)	330 (±53.9)	0.63
Alcohol, g.kg^{-1} of DM	10.1 (±5.70)	8.96 (±5.74)	8.24 (±4.18)	5.97 (±3.82)	8.86 (±5.08)	0.53
Acetic acid, g.kg^{-1} of DM	25.2 (±8.97)	22.0 (±8.76)	15.8 (±5.57)	28.6 (±14.6)	22.1 (±9.55)	0.03
Propionic acid,g.kg^{-1} of DM	3.20^{ab} (±1.98)	3.83^{ab} (±3.38)	2.57^{b} (±1.95)	7.95^{a} (±7.26)	3.67 (±3.40)	0.04
Butyric acid, g.kg^{-1} of DM	0.55 (±1.48)	0.20 (±0.21)	0.10 (±0.11)	0.09 (±0.04)	0.29 (±0.92)	0.62
Lactic acid, g.kg^{-1} of DM	24.6 (±6.94)	22.0 (±5.03)	29.7 (±9.97)	29.1 (±8.92)	26.1 (±8.13)	0.16
Aflatoxin, ppb	12.5^{a} (±1.08)	11.8^{a} (±2.30)	8.46^{b} (±2.30)	7.70^{b} (±0.94)	10.6 (±2.64)	<0.01
Zearalenone, ppb	80.6 (±108)	43.5 (±44.7)	58.5 (±135)	18.9 (±24.1)	60.4 (±102)	0.69

*1 - good farm location (favorable climatic conditions), use of corn hybrid indicated to silage and adoption of better production management techniques, but, with the use of less effective inoculants in the silage quality or absence of inoculant; 2 - good farm location (favorable climatic conditions), use of corn hybrid indicated to silage and adoption of better production management techniques, associated the use of more effective inoculants in the silage quality; 3 - bad farm location (unfavorable climatic conditions), use of corn hybrid no indicated to silage and adoption of bad production management techniques, associated the use of less effective inoculants in the silage quality or absence of inoculant; 4 - bad farm location (unfavorable climatic conditions), use of corn hybrid no indicated to silage and adoption of bad production management techniques, but, with the use of more effective inoculants in the silage quality.

Conclusions The corn silage produced on dairy farms in southern Brazil, present good fermentation characteristics and the levels of mycotoxin observed are safe for feeding to livestock.

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Can silage bacteria bind aflatoxin?

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Keywords aflatoxin B_1, binding, lactic acid bacteria, silage

Introduction Previous studies showed that lactic acid bacteria used in the human food and dairy industries bound mutagens and carcinogens in aqueous solution effectively. To our knowledge, no studies have examined if the lactic acid bacteria used as silage inoculants can also sequester aflatoxin. The aim was to evaluate the aflatoxin B_1 (**AFB_1**)-binding capacity of silage inoculant bacteria.

Materials and methods Experiment 1 examined the effects of bacteria species or strain and inoculation rate on the AFB_1-binding capacity of silage bacteria. The AFB_1-binding capacity of 10 commonly inoculated silage bacteria (Table 1) from Lallemand Animal Nutrition were examined at populations of 10^6 or 10^9 colony forming units (**cfu**)/mL. The bacteria were grown and enumerated on MRS broth and centrifuged. The respective pellets were suspended in 1.5 mL of AFB_1 solution (5 µg/mL of AFB_1 in phosphate-buffered saline, PBS) in quadruplicate for 24 h at 20°C. Bacteria and AFB_1 controls were also incubated. The AFB_1 in the supernatant was quantified by HPLC after centrifugation. Data were analyzed as a completely randomized design using the Glimmix procedure of SAS (SAS Inst., Cary, NC, USA). Experiment 2 examined the AFB_1-binding capacity of viable and nonviable forms of 3 of the most promising bacteria from Experiment 1 at ruminal, abomasal and duodenal pH values. Pellets of *Lactobacillus plantarum* R2014 (Lp), *L. buchneri* R1102 (Lb), and *Pediococcus acidilactici* EQ01 (Pa) prepared from populations of 10^9 cfu/mL as in Experiment 1 were incubated in PBS (viable cells) or 2 M HCl (nonviable cells) and centrifuged. The pellets were suspended for 24 h at 20°C in quadruplicate in AFB_1 solutions adjusted to pH 2.5, 6 and 8 using HCl, PBS and NaOH. The AFB_1 in the supernatant was quantified by HPLC. The treatments were arranged as a 3 x 2 x 3 factorial and the data were analyzed with the Glimmix procedure of SAS.

Results and discussion In Experiment 1, binding of AFB_1 was strain- and population-dependent (Table 1). At the 10^6 cfu/mL population, only one bacterium bound the toxin (4%) but at 10^9 cfu/ml, AFB_1 binding ranged from 18.2 to 33.0 %. The bacteria with the greatest AFB_1-binding capacity were Lp, Lb, Pa, *L. plantarum* EQ12, and *P. acidilactici* R2142. In Experiment 2, killing Lb and Lp with the acid increased AFB_1 binding but killing Pa did not (Table 2). Binding of AFB_1 was greatest at pH 2.5 and least at pH 8 across bacteria. The greatest proportions of AFB_1 were bound when nonviable cells of Lb and Lp or viable cells of Pa were acidified at pH 2.5 (60.5, 66.5 and 56.9%, respectively).

Conclusion Certain silage bacteria can bind AFB_1 but the extent varies with the bacterial population, strain, and viability and the prevailing pH.

Acknowledgement We are grateful to Lallemand Animal Nutrition for funding this study.

Table 1 Percentage of aflatoxin B_1 (AFB_1) bound by two populations of the ten bacteria examined in Experiment 1

Bacteria	Bacteria population, cfu/ml	
	10^6	10^9
	……..% AFB_1 bound……	
Lactobacillus plantarum R2014	0.83[ab]	33.0[a]
Lactobacillus plantarum EQ12	1.08[ab]	28.4[abc]
Lactobacillus plantarum PT5B	4.27[a]	19.3[cd]
Lactobacillus buchneri R1102	0.04[ab]	30.3[ab]
Pediococcus acidilactici R2142	0.00[b]	23.9[abcd]
Pediococcus acidilactici EQ01	0.66[ab]	25.4[abcd]
Pediococcus pentosaceus EQ44	0.23[ab]	21.6[bcd]
Pediococcus pentosaceus IA38	0.96[ab]	18.2[d]
Propionibacterium jensenii SE253	0.00[b]	19.7[cd]
Propionibacterium acidipropionici EQ42	1.82[ab]	18.9[d]
SEM	0.88	3.78
P value	0.04	<0.001

[a, b, c, d] Means within a column with different superscripts differ, $P < 0.05$.

Table 2 Effects of bacteria species, viability and prevailing pH on the percentage of aflatoxin B_1 (AFB_1) bound by three silage bacteria

	Bacterial species[1], B						
	Lp		Lb		Pa		SEM[3]
pH	Viable	Dead[2]	Viable	Dead	Viable	Dead	
	……AFB_1 bound, %.....						
2.5	56.2[b]	60.5[a]	51.5[b]	66.5[a]	56.9[a]	2.91[bc]	0.57
6.0	10.1[e]	15.8[d]	1.47[d]	34.1[c]	6.76[b]	4.40[bc]	0.57
8.0	8.05[e]	21.6[c]	0.32[d]	29.2[c]	2.05[c]	0.00[c]	0.58
SEM	0.80	0.80	0.80	0.85	0.80	0.80	
Contrast[4]	L, Q	L, Q	L, Q	L, Q*	L, Q	Q*	

[a, b, c, d]Means within a bacterium with different superscripts differ, $P < 0.05$.
[1]Lp = *Lactobacillus plantarum* R2014, Lb = *L. buchneri* R1102, and Pa = *Pe. acidilactici* EQ01. Each bacterium was applied at 10^9 cfu/mL; [2] Killed with 2 M HCL; [3] *P*-values for bacterial species, viability, pH and all interactions of these terms were 0.001; [4]Linear (L), quadratic (Q) effects ($P < 0.05$); Q*, Quadratic trend ($P < 0.10$).

Mycotoxin survey in American corn silage and Brazilian corn samples

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Keywords mycotoxins, LC-MS/MS, corn, corn silage

Introduction As part of its yearly Biomin mycotoxin survey a total of 34 Brazilian corn samples and 25 American corn silage samples were screened for mycotoxin contamination in 2014. The following study gives an overview of the mycotoxin occurrence in these samples.

Materials and methods The metabolites in these samples were analyzed with ELISA, HPLC and a multi-mycotoxin approach based on a combination of Liquid Chromatography and tandem Mass Spectrometry (LC-MS/MS) (Malachová et al., 2014). Besides the detection of the most common mycotoxins like aflatoxins (AFLA), deoxynivalenol (DON), zearalenone (ZEN), ochratoxins (OTA) or fumonisins (FUM) the LC-MS/MS method also has the advantage of finding masked mycotoxins and fungal metabolites. These metabolites commonly escape routine analysis; however can negatively affect performance and health of livestock animals.

Results and discussion A total of twenty-five (25) corn silage samples were screened from America. Out of these samples 68 % contained one mycotoxin and 28 % were co-contaminated with more than one mycotoxin. The detected mycotoxin levels in the corn silage samples are shown in Table 1.

Table 1 Mycotoxin contamination in the corn silage samples from America

	% of positives	average of positives (ppb)	maximum (ppb)
DON	55	558	1,800
ZEN	52	95	284
FUM	48	438	999
OTA	5	5	5
AFLA	0	-	-

Thirty-four (34) corn samples from Brazil were analyzed with the multi-mycotoxin approach in 2014. All tested corn samples were contaminated with FUM (average 6,141 ppb and maximum 52,437 ppb). Due to the high prevalence and high average concentration Fumonisins represent the highest risk in the Brazilian corn samples. Total type B trichothecenes, which include DON and its masked form DON-3-O-glucoside (D3G), occurred in 85 % of the samples (average of positives 1,403 ppb and maximum 17,712

ppb) and ZEN was present in 67 % of the samples with an average of positives of 348 ppb and maximum of 5,261 ppb. *Fusarium* and *Penicillium* metabolites were found in all corn samples from Brazil. Enniatins and beauvericins occurred in 97 % and *Aspergillus* toxins were present in 85 % of the samples. On average, 29 metabolites were detected in the samples.

Conclusions These results emphasize the importance of screening feed for the co-occurrence of fungal metabolites. The data also draw attention to the necessity of analyzing mycotoxin occurrence in silage not only in times of high aflatoxin contamination, and might also raise the awareness of dairy farmers against the risk of multiple mycotoxins.

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Investigations on the influence of the preservation method on the content of pyrrolizidine alkaloids in hay and silage from *Senecio aquaticus* infested grasslands

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Keywords marsh ragwort, PA, feedstuff, feed safety

Introduction *Senecio aquaticus* (*Jacobaea aquatica*, marsh ragwort) is a weed widely spread in grasslands of Southern Germany, Austria and Switzerland. Since it is a producer of hepatotoxic 1,2-unsaturated pyrrolizidine alkaloids (PA), its presence in forages concerns both animal and human health Thus, possible effects of different methods of forage preservation on the PA content of the feeds is a point of major interest, but investigations addressed to answer this question are scarce (Candrian et al., 1984, Becerra-Jimenez et al., 2013). To get more information about the stability of PAs during different preservation processes, comparative investigations on experimentally produced hay and silage from *S. aquaticus* infested grassland were conducted.

Materials and methods Natural marshy grassland with a dominance of *S. aquaticus* of 30 % was cut on June 3rd (first cut) with a sickle bar mower at 5 cm cutting height. The forage biomass of two spots (each 15 m²) was chopped using a laboratory scale chopper and subdivided into four equal portions which were either immediately frozen (-20 °C), dried (35 or 105 °C for 6 h) or ensiled into 1.75 l jars. The mini silos were stored at 25 °C in dark conditions for 60 days. Once the forage fermentation has been completed, the silage was taken out from the jars and frozen at -20 °C, subsequent homogenized using a meat chopper and refrozen immediately. Base material was processed equally. A portion of the homogenized silage was dried at 105 °C for 6 h. Hay (35 and 105 °C) was homogenized with a cutting mill (1 mm) and stored at 4 °C in the dark. For PA analysis, 2 g of each sample were weighed into a 50 ml centrifugation tube and extracted for 20 minutes with 2 % formic acid. An aliquot of the supernatant was filtered (0.45 µm) and injected into a HPLC-MS/MS system (PerkinElmer Series 200, AB Sciex API 3200). PAs were separated by a Synergi-RP-HPLC column. A total of 28 PA and PA N-oxides (PANO) were detected with electrospray ionization (ESI+), using external matrix matched standards for quantification.

Results and discussion Measured contents of 1,2-unsaturated PA (sum of single PA and PANO) in the analyzed forage biomass were high (62 and 95 mg/kg DM at spots 1and 2 respectively). Such levels in forages represent a health risk for dairy cattle and horses (Stegelmeier, 2004). Since ingested PA can be transferred into milk, there could be also a health risk (e.g. carcinogenic effects) for humans who consume large quantities of contaminated milk (EFSA, 2011). Fermentation quality of the experimental silos was good (pH 4.0-4.5, butyric acid < 3g/kg DM) indicating usual microbiological and biochemical processes. The proportion of PAs and their N-oxides vary depending on the forage preservation method (Figure 1). Thereby it showed differences regarding metabolism of

PA to an undetectable conformation. Drying at 35 °C did not lead to a statistical difference of the sum of PA and PANO compared to frozen base material, the proportion of PANO decreased. This decrease was even more pronounced at 105 °C drying temperature. In addition, the sum of PA and PANO decreased to 35-48 % of the initial amount. Ensiling process led to the most significant reduction of the sum of PA and PANO (77-89 %) and to an almost complete disappearance of PANO. Drying of silages at 105 °C induced an extra reduction of measurable PA and PANO contents. The recovery of initial PA and PANO contents after ensiling and drying amounted to 3-8 %.

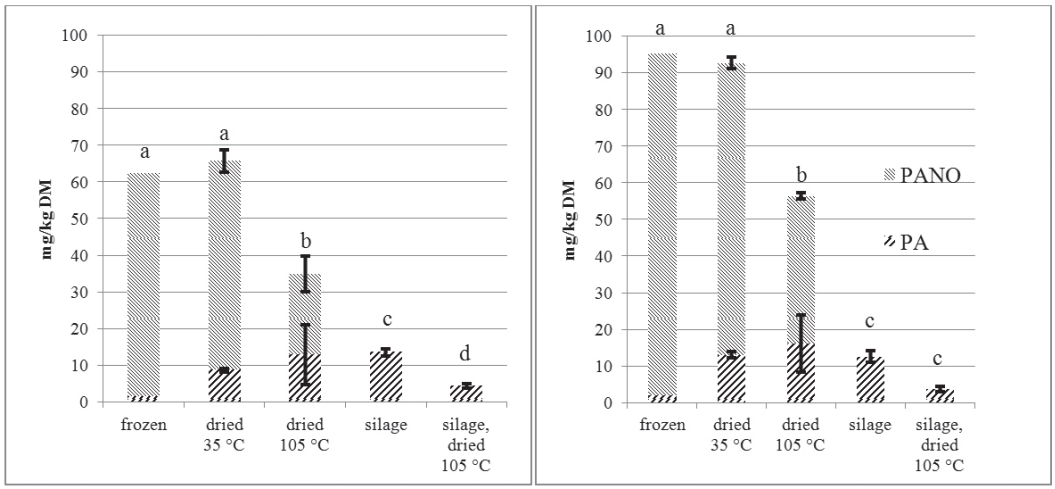

Figure 1 Sums of contents of 1,2-unsaturated pyrrolizidine alkaloids and N-oxides in frozen base materials (sample 1 left, sample 2 right) compared to their dried or ensiled products. Different letters indicate significant differences in PA+PANO (Student-Newman-Keuls method, $P<0.05$).

Conclusions The presence of the weed *Senecio aquaticus* in forage mixtures lead to PA toxic values for animals fed with it. Tested forage preservation methods, except drying at 35 °C contributed with a reduction of measurable 1,2-unsaturated PA and their N-oxides. However is unknown if the reduction was associated with detoxification of the feedstuffs. A temperature dependent reduction of the measurable PA and PANO contents in hay was observed. A deoxidization of N-oxides and the most pronounced reduction of the initial contents could be shown for the silage process but none of the tested methods led to a complete elimination of PAs from feeds.

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Effects of different chemical additives on the fermentation and aerobic stability of high-moisture corn ensiled in bags

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Keywords: chemical additives, high-moisture corn, fermentation pattern, yeasts, aerobic stability, bags

Introduction High-moisture corn (HMC) represents a monetarily and nutritionally valuable feed for a range of farm animals. Due to the low fermentation losses and the production of ready-to-use feed already at the time of harvest, storage of HMC in plastic bags has attracted significant interest world-wide. However, due to low stability upon exposure to air, it may rapidly undergo fungal deterioration after bag opening. Heterofermentative lactic acid bacteria inoculants may successfully be used to enhance aerobic stability (Kung et al., 2007) but the major constraint to its use are the required minimal storage length of about 8 weeks and the unpredictable effects of environmental conditions (e.g. climate) and crop characteristics on their activity. Therefore, antimycotic chemical additives have been widely used. The aim of this study was to test the effect of chemical additives on the fermentation and aerobic stability of HMC stored in bags.

Materials and methods The corn crop was grown on a dairy farm in the German State of Saxony in 2014. At harvest on October 10, the corn had 35.1% moisture and 76.4% of starch (DM basis). The crop was infested with epiphytic moulds at log 4.1 cfu/g and yeasts at log 6.7 cfu/g. After processing by a roller mill (CP2, RoMill, Brno, Czech Republic) to a particle size of <1 mm, the ground corn was directly packed into a polyethylene plastic bag (diameter: 1.95 m, thickness: 195 µm). The application of liquid additives was performed during milling by an applicator mounted on the machine. One section of about 8 m length for each of the following treatments was produced: untreated (CON), Xtrasil excel HD, containing potassium sorbate, sodium benzoate, ammonium propionate, applied at 1 L/t (HD1) or 2 L/t (HD2) and Xtrasil stabilizer, composed of propionic acid, ammonium propionate, sodium benzoate, potassium sorbate, applied at 1.5 L/t (STAB1.5) and 3 L/t (STAB3), respectively. All additives were provided by KONSIL Scandinavia, Sweden. The bag was stored outside for 62 days until December 15, 2014. On the day of opening, a total of six samples per treatment were taken by a hollow drill (33 cm length, 13 cm diameter) – 3 from the top and 3 from the side at 40 cm above the ground. Fermentation products and yeast count were determined by routine analytical procedures. Aerobic stability was measured by using thermo loggers to record the temperature in the insulated samples, which were kept in a barn at 21.6±1.3 °C for 288 hours. Silages were considered unstable if the temperature in the sample exceeded that of ambient by 3 °C. The data were statistically evaluated by PROC GLM of SAS. Significance was declared at $P<0.05$, and pair-wise comparisons among means were performed by employing the Tukey´s test.

Results and discussion In general, the HMC produced in bags was of very good quality (Table 1). Although differences were observed across treatments regarding pH, lactate and acetate they were of no practical relevance. The increased ammonia-N and propionate levels in treated silages can be attributed to the addition of these substances with the additives. Ethanol production was significantly restricted by all additives when compared

with the control. There was a dose-dependent decrease in yeast count, which resulted in enhanced aerobic stability. Although the aerobic stability of treatment STAB1.5 was only by 36 hours higher than that of untreated HMC, the extent of heat generation by microbial activity during the course of air exposure (aerobic deterioration) was far less pronounced (Figure 1).

Table 1 Effects of chemical additives on fermentation characteristics, yeast count and aerobic stability of high-moisture corn ensiled in bags for 62 days (% of DM unless otherwise stated)

Parameter	CON	HD1	HD2	STAB1.5	STAB3	SEM	*P*
pH	3.75^{b}	3.78^{a}	3.80^{a}	3.78^{ab}	3.80^{a}	0.007	***
NH_3-N (% N)	3.4^{c}	3.6^{bc}	3.8^{b}	4.3^{a}	4.4^{a}	0.05	***
Lactic acid	1.98^{a}	1.70^{b}	1.90^{ab}	2.09^{a}	2.01^{ab}	0.065	**
Acetic acid	0.18^{b}	0.19^{b}	0.21^{a}	0.21^{a}	0.21^{a}	0.005	**
Propionic acid	0^{c}	0.01^{c}	0.02^{c}	0.11^{b}	0.19^{a}	0.011	***
Ethanol	0.83^{a}	0.46^{bc}	0.41^{bc}	0.50^{b}	0.38^{c}	0.023	***
Yeasts (log cfu/g)	4.52^{a}	3.46^{bc}	2.76^{c}	4.07^{ab}	3.25^{bc}	0.231	***
ASTA[1] (hours)	85^{c}	186^{b}	288^{a}	118^{bc}	288^{a}	14.8	***

CON=untreated, HD1=Xtrasil excel HD (1 L/t), HD2=Xtrasil excel HD (2 L/t, STAB1.5= Xtrasil stabilizer (1.5 L/t), STAB3=Xtrasil stabilizer (3 L/t), [1]Aerobic stability, $*P<0.05$, $**P<0.01$, $***P<0.001$, means in rows bearing unlike superscripts differ (Tukey´s test).

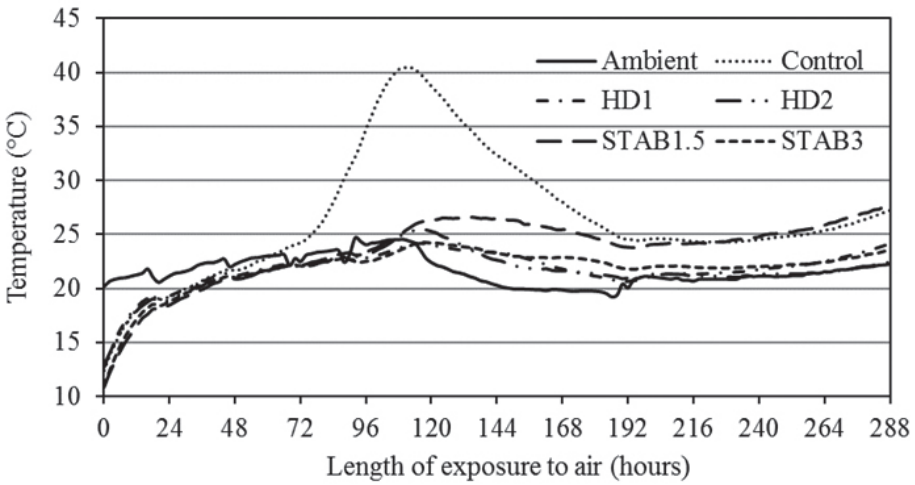

Figure 1 Course of temperature development in high-moisture corn during aerobic storage.

Conclusions The chemical additives inhibited fungal activity, thereby enhancing aerobic stability. Thus, they can be highly recommended for the treatment of HMC stored in bags. As the magnitude of the effect was dose-dependent, the use of the higher application rate is strongly advised to ensure consistently positive results, also under challenging conditions, e. g. short fermentation length and high temperature at feed-out.

Acknowledgement The authors are deeply indebted to Elisabet Nadeau, Swedish University of Agricultural Sciences, Skara, Sweden for running statistics.

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The effect of different treatments on fermentation of high moisture corn silage

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Keywords high moisture corn, silage additive, fermentation process

Introduction High moisture corn (*Zea mays* L.) is an important source of energy in feeding rations for ruminants due to the high content of starch. Corn grain silage is prone to aerobic spoilage with negative effects on its feeding value. To ensure proper fermentation growers may want to consider the use of an effective bacterial inoculant or chemical additive (Kung et al., 2006; Revello-Chion et al., 2012; Galik et al., 2008; Coudure et al., 2012). The aim of this study was to determine the effect of different treatments of high moisture corn grain on silage fermentation.

Materials and methods The crimped corn grain at content of dry matter on the level 657 g/kg (Table 1) was homogenized and then ensiled in 1.7-litre laboratory silos.

Table 1 Content of nutrients in ensilaged crimped high moisture corn (g/kg DM)

Item	Value	Item	Value
Dry matter, g/kg FM	657	Ash	13
Crude protein	95	Starch	682
Crude fibre	32	Total sugars	30
Fat,	36	Reducing sugars	12

At ensiling we used one control variant (C) without treatment of corn and experimental variants treated with preparations/ T1 - the homofermentative biological inoculant (*Lactobacillus plantarum* DSM 3676 and 3677, Propionic bacterium DSM 9576 and 9577);4. 10^5 CFU / g FM (application rate 2 l/t); 10^5 CFU / g FM (application rate 2 l/t) ; T3-the chemical additive (22.9% sodium benzoate, 8.3% sodium propionate); 1 application rate 4 IL/t of feed. The silos were opened after 150 days of storage. Chemical analysis was done in samples of crimped corn grain and its silages; results were statistically processed and evaluated with the Student t-test

Results and discussion The pH values were lower in all the treated experimental variants than in the control variant, which was confirmed by the statistical differences. The content of the lactic acid was higher in the treated variants than in the control variant without any treatment. The content of the acetic acid was the lowest in the variant treated with the chemical additives and the highest in the variant treated with the combination of the two lactic acid bacteria. Differences in the content of the propionic acid were small. The highest content was in the variant T1 and the lowest one in the T2. The statistically important difference was identified between variants T1 and T3. Significantly lower content of the butyric acid was found in all the treated variants than in the control variant. The lowest content of butyric acid was in the variant treated with the chemical additives. The highest content of all acids was found in the variant T1 treated with the combination

of the two lactic acid bacteria. On the other hand the lowest content of all acids was in the variant T3 treated with the chemical additives. The alcohol content in the control variant was higher than in the variants treated with the preparations. The pH changes on the third day after opening were the highest in the control variant and then in the variant T1 treated with the homofermentative lactic acid bacteria. Decrease in pH was the same in variants treated with the heterofermentative bacteria and with the chemical additives and it was lower in comparison to the other variants. Loss of dry matter was not high during the fermentation. The highest loss was found in the variant treated with the combination of the two lactic acid bacteria, the lowest one in the variant T2. Differences among the variants were not significant.

Table 2 Parameters of fermentation process in silage from crimped high moisture corn (n = 5)

Item	Treatment[1]							
	Control		T1		T2		T3	
	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
pH	3.88[a]	0.02	3.84[ab]	0.02	3.78[b]	0.02	3,81[b]	0.01
pH, 3rd day of air exposure	3.99[a]	0.04	3.93[a]	0.02	3.86[b]	0.01	3,89[c]	0.01
Acids, g/kg DM								
- lactic	24.17	0.33	25.13	1.72	24.60	1.50	24.20	0.66
- acetic	4.33[a]	0.65	3.26[b]	0.23	4.43[a]	0.42	2.83[c]	0.18
- propionic	0.84[a]	0.17	0.47[b]	0.02	0.94[a]	0.10	0.72[a]	0.06
- butyric + i.b	0.62[a]	0.06	0.20[b]	0.02	0.41[c]	0.08	0.10[b]	0.05
Total VFA, g/kg DM	6.20[a]	0.92	4.21[b]	0.30	6.17[a]	0.54	3.85[b]	0.16
Ethanol, g/kg DM	1.44[a]	0.07	1.21[b]	0.09	1.16[b]	0.09	1.07[b]	0.16
Dry mater, g/kg FM	651.84[a]	0.85	651.14[a]	0.33	654.48[b]	1.32	652.92[ab]	1.49
DM losses, %	0.75	0.13	0.40	0.20	0.79	0.08	0.70	0.29

a–c Means within a row with different superscripts differ (P< 0.01) ; [1] T1- *Lactobacillus plantarum* DSM 3676 and 3677, *Propionic bacterium* DSM 9576 and 9577; T2 – *Lactobacillus buchneri* DSM 13573; T3 – 22.9 % sodium benzoate, 8.3 % sodium propionate

Conclusions The treatment of the high moisture corn grain positively affected the fermentation process. We found lower content of butyric acid in treated feeds and slower rise of pH after anaerobic exposure compared to the non-treated feed.

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Ensiling crimped high moisture barley with organic acids or lactic acid bacteria strains

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Keywords aerobic stability, barley, feed, mould, quality, spoilage

Introduction Storage of high moisture grains is popular in areas of humid weather conditions during harvesting window. Benefits of the storage method include possibility to start the harvest earlier, less weather dependent harvest season and omission of drying costs. The method has become more common in conjunction of increasing herd size and uptake of total mixed ration (TMR) feeding. In this experiment, the effects of a formic acid based additive (FO) or lactic acid bacteria strains (LAB) on the quality of high moisture barley were tested.

Materials and methods The experiment was conducted at Luke (former MTT Agrifood Research Finland), Jokioinen, Finland. Spring barley (dough - yellow stage of ripening) was crimped using a farm scale crimper (Murska, Aimo Kortteen Konepaja, Ylivieska, Finland). The additive treatments (FO at 3 or 4 L/ton, LAB, and control without additive (CO)] were dosed on equal batches of crimped barley. Three replicate silos (4.5 kg fresh matter (FM) per silo) were filled per each treatment. The silos were compacted, sealed and weighted. Silos were opened after a 107-day storage period and sampled for analyses.

Results and discussion The crimped barley just before ensiling had a DM concentration of 605 g/kg, and concentrations of ash, crude protein, starch, and crude fiber were 32, 118, 567, and 50 g/kg DM, respectively. Prior to ensiling, the total counts of aerobic bacteria, yeasts, and molds were 7.78, 5.92, and 5.10 $\log_{10}$ cfu/g, respectively. Weight changes during ensiling were small as no effluent losses were detected. Without correction for the volatile components, the DM losses were smallest for FO treatments (15 g/kg), intermediate for CO (23 g/kg) and highest for LAB treatment (30 g/kg, Tukey's test $P<0.05$). At the time of silo opening, the only silos without any molds on the surface layer were those ensiled with FO at 4 L/t. However in the samples taken within the silos the counts of yeasts and molds were below detection limit (3 $\log_{10}$ cfu/g FM) in all the silos. The LAB treated silos had higher counts (> 8.5 $\log_{10}$ cfu/g FM) of aerobic bacteria than CO (6.99 – 7.05 $\log_{10}$ cfu/g FM) or FO treatments (<5.75 $\log_{10}$ cfu/g FM). The FO treatment restricted the fermentation compared to other treatments, and LAB treatment increased the production of acetic acid and reduced the concentration of ethanol (Table 1). Aerobic stability was enhanced by both additives compared to CO. The DM concentration of the ensiled barley was well within the general recommendations (550-650 g/kg, Palva et al. 2005) for this type of grain ensiling and the crimping procedure was trouble-free. The role of fermentation was clear, as the sum of fermentation products exceeded 35 g/kg DM in all treatments. The DM concentration is a crucial factor for the growth of microbes and should be carefully considered when selecting additive for the ensiling procedure. It has been demonstrated that when DM concentration in crimped barley is above 720 g/kg, the amount of fermentation products in the final product is minimal (Seppälä et al. 2012). In this experiment FO treatments restricted fermentation resulting in a lower concentrations of fermentation products, smaller losses during fermentation and higher water soluble carbohydrate content in the ensiled barley compared to LAB and CO treatments. The

role of fermentation of grain component on the intake and production potential of TMR should be evaluated. The enhanced aerobic stability of LAB treatment is most probably due to higher acetic acid content of the LAB treatment compared to other treatments. Heterofermentative type of fermentation is known to increase losses during fermentation, as also demonstrated in this experiment.

Table 1 Fermentation quality and aerobic stability of the crimped barley

Treatment	DM	pH	WSC	AA	VFA	LA	ETH	AMM	AER
Control	611	3.87	42	4.3	4.4	40	8.6	28	141 – 204
LAB	612	3.97	22	15.7	15.7	35	6.2	32	>300
FO 3 L/t	610	4.02	72	4.5	5.0	27	3.0	40	>300
FO 4 L/t	610	4.06	80	5.1	5.8	23	3.1	45	>255
SEM	0.5	0.003	0.4	0.20	0.20	0.3	0.11	0.01	

LAB treatment with *Pediococcus pentosaceus* and *Lactobacillus buchneri*, 612 000 cfu/g, Biocrimp, Biotal, UK; FO product containing formic acid 425, ammonium formate 303, propionic acid 100, benzoic acid 22 and water 150 g/kg, Kemira Oyj., Finland.
SEM standard error of the mean; DM dry matter g/kg; WSC water soluble carbohydrates, AA acetic acid; VFA sum of volatile fatty acids; LA lactic acid; ETH ethanol; AMM ammonium N g/kg total N; AER aerobic stability, hours.
The differences between treatments were statistically significant ($P<0.05$, Tukey's test) in all the other cases except the differences between FO 3 L/t and FO 4 L/t in the variables ETH and VFA.
Units g/kg DM unless otherwise stated.

Conclusion Formic acid based additive and the additive including *L. buchneri* were able to improve aerobic stability of crimped barley when DM was around 600 g/kg, but only the formic acid based additive was able to prevent the growth of molds on the silo surface. Formic acid restricted fermentation, spared soluble sugars and reduced losses during fermentation.

Acknowledgements We thank Kemira Oyj. (Silage additive business currently run by Eastman Chemical Company) for cooperation and funding in arranging this experiment.

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Effect of vitreousness, length of storage and inoculation on pH, NH_3-N, crude protein and dry matter of rehydrated corn silage

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Keywords pH, silage, corn, vitreousness, dry matter, protein

Introduction The mature corn grain (*Zea mays*) harvested with low moisture is the main component of energy of concentrates used for high production dairy cows. Given the importance of corn starch in dairy cow nutrition, its digestibility and storage has a strong impact on production systems. However, in Brazil the predominant cultivation of corn varieties with higher percentages of vitreous endosperm (Correa et al., 2002) results in lower use of corn by ruminants (Bal et al., 2000; Johnson et al., 2002). Thus, to increase the digestibility of corn starch, it is necessary to lower the barriers imposed by the protein matrix of corn grain. The grain silage when stored for long periods can degrade proteins that cover the starch granules (Hoffman et al., 2011). In this context, rehydration and ensiling of corn harvested with low moisture and mature allow more flexibility for ensiling and can improve the digestibility of corn starch. Therefore, it is important to evaluate the pH, CP, NH_3-N % of CP, and dry matter (DM) content to follow the quality of the silage stored for long periods.

Materials and methods Our aim was investigate the influence of vitreousness content, microbial inoculation and length of storage on pH and DM content. Three maize cultivars with different vitreousness levels (58, 71 and 80% of vitreous endosperm), split in two groups (inoculated or not inoculated) were harvested udderlly ripe, ground and rehydrated for different ensiling periods (0, 15, 30, 60, 120, 240 days). The harvested grains were dried at 40ºC until reaching 87% of DM content to be ground in a hammer mill fitted with a 2 mm sieve. Then, corn was rehydrated and one of the groups was subjected to microbiological inoculation whit 600,000 cfu/g (*Lactobacillus buchneri* NCNM I 4323 Lallemand®). The samples were rehydrated to achieve 650 g DM/kg and packed to reach 800 kg/m^3 density. The silage was stored in plastic bags of 120 × 100 mm wrapped in plastic sheet specific for silage. The arrangement of treatments was a 3×2×6 factorial, with 3 types of corn, 2 types of silage inoculation and 6 periods of storage. The design was completely randomized and the results were analyzed as repeated measures using PROC MIXED by the computer program Statistical Analysis System® (SAS, 2001), after verification of normal errors and homogeneity of variances.

Results and discussion The pH values (Figure 1B) suggest an effect of vitreousness ($P < 0.01$), period ($P < 0.01$) and inoculant ($P < 0.01$) and there was an interaction between vitreousness and inoculant ($P < 0.01$), vitreousness and period ($P < 0.01$), inoculant and period ($P < 0.01$) and vitreousness, inoculant and period ($P < 0.01$). According to Hoffman et al., 2011, lower pH values were also observed for silage inoculated high-moisture corn, compared to the same silage did not inoculated. According to the author this it happened because of the highest concentration of lactic acid and acetic acid in the silage inoculated with *Lactobacillus buchneri* versus uninoculated silage. The DM content (Figure 1D) also suggest that there was effect of vitreousness ($P < 0.01$), period ($P < 0.05$) and inoculant

($P = 0.04$) and there was an interaction between vitreousness and inoculant ($P < 0.05$), vitreousness and period ($P = 0.03$) and vitreousness, inoculant and period ($P < 0.01$). The results for concentrations of NH3-N % of CP (Figure 1A) and CP (Figure 1C) suggest vitreousness effect ($P < 0.01$), inoculant ($P < 0.01$), ensiling time ($P < 0.01$) interaction between vitreousness and inoculant ($P < 0.01$) vitreousness and time ($P < 0.01$) inoculant and time ($P < 0.01$) and vitreousness, inoculant and time ($P < 0.01$). Groups using inoculant ($P < 0.01$) showed minor concentration of NH3-N during the fermentation periods. The increase in concentration of NH3-N in uninoculated silage can be an explanation for the lower values of crude protein in the groups 58 and 71, as these silages exposed to the environment can be volatilized nitrogen which was in the form of NH3-N.

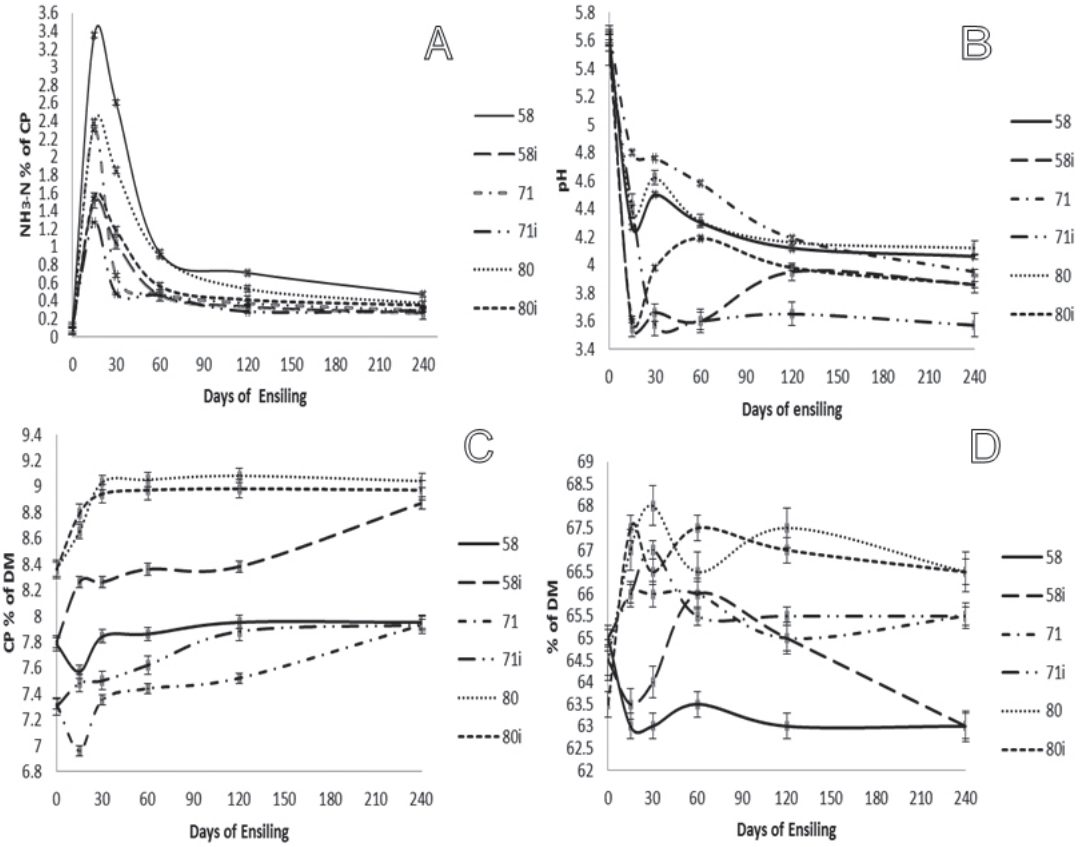

Figure 1 NH_3-N % of crude protein of silage (A) pH of silage (B), crude protein (CP) of silage of rehydrated corn grain (C) and DM of silage (D) in relation to the time of ensiling. Numbers followed by the letter "i" represents the use of microbial inoculant in its confection.

Conclusion The use of inoculants for rehydrated grain silage promoted lower pH values and lower concentration of NH_3-N in the initial fermentation period for all corn vitreousness. The use of inoculant promoted more crude protein values for corn groups with lower percentage of vitreous endosperm, without however no difference scheduled for the group with the highest percentage of vitreous endosperm. The values of dry matter were affected more markedly by vitreousness content.

Influence of storage time and use of inoculant *L. buchneri* on aerobic stability of high-moisture corn and rehydrated corn silages

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Keywords aerobic exposure, reconstitution, silage additive

Introduction The predominant type of corn in Brazil is flint, with high proportion of vitreous endosperm and lower starch digestibility (Correia et al., 2002). Storage of corn grain with high moisture content increases starch digestibility by promoting proteolysis of the protein matrix (Hoffman et al., 2011). New techniques such as rehydration of dry corn for later ensilage have been practiced, but few scientific studies are available. Another relevant aspect is that ensiled corn kernels are susceptible to aerobic deterioration. Thus, the objective of the present study is to evaluate the storage time and use of inoculants on aerobic stability of silages of high-moisture corn and rehydrated corn.

Materials and methods The trial was carried out at Agencia Paulista de Tecnologia dos Agronegócios (APTA), in Colina-SP, Brazil. High-moisture corn was harvested with 650 g/kg of dry matter (DM). Dry corn was ground and then rehydrated with water aiming to reach 650 g/kg of DM. High-moisture corn (HMC) and rehydrated corn (RC) were ensiled in plastic buckets (20 L) without (Control) or with (LB) *L. buchneri* (1×10^5 cfu/g as fed), with four replicates per treatment. Silos were opened at 15, 30, 60 and 90 d of storage. Yeast and mold counts were performed at the opening. Alterations during the aerobic exposure were evaluated for 12 d. Aerobic stability was defined as the number of hours the silage mass took to reach 2 ºC above the room temperature. Aerobic deterioration was defined as the sum of the daily temperature increases above the ambient temperature in the first 5 d of air exposure. Temperature peak (ºC) and hours to reach the temperature peak were also computed. The variables were analyzed as a completely randomized design with a 2×2 factorial arrangement with repeated measures using the MIXED procedure of SAS software.

Results and discussion There was an interaction between inoculant and storage time ($P < 0.01$) on the yeast count, which decreased from 30 d of storage in the silages with and without inoculant, wherein the greatest reduction was found in the inoculated silages (Figure 1). The yeast count in inoculated silages remained below the detection limit (< 2.00 log cfu/g of silage) after 60 d. For mold, an interaction was observed between silage and storage time ($P = 0.01$). The count of mold for the HMC increased from 30 d of storage, remaining higher than that in the RC silages, which remained below the detection limit (< 2.00 log cfu/g of silage). Aerobic stability, temperature peak, hours to reach the temperature peak, and aerobic deterioration showed to be significantly affected by an interaction ($P < 0.01$) among type of silage, inoculant, and storage time (Figure 1). The RC inoculated with LB silages had greater aerobic stability on 15, 60, and 90 d. Aerobic stability increased with storage time for all silages except HMC-Control. In this

way, aerobic deterioration reduced through storage time in all silages, but was highest in the HMC-Control silages on d 30, 60, and 90. The time to reach the temperature peak was shorter for the HMC-Control silages from 60 d of storage, wherein the highest peak at 60 d was achieved by the HMC-Control silages. The increased concentration of acetic acid, a powerful antifungal agent produced by *L. buchneri*, may be a plausible explanation for the enhanced aerobic stability of HMC-LB and RC-LB silages.

Conclusions Silages inoculated with *L. buchneri* showed greater aerobic stability. High-moisture corn silages are more prone to aerobic deterioration than rehydrated corn silages. Therefore, additives capable to improve the aerobic stability, such *L. buchneri*, are recommended for ensiling high-moisture corn.

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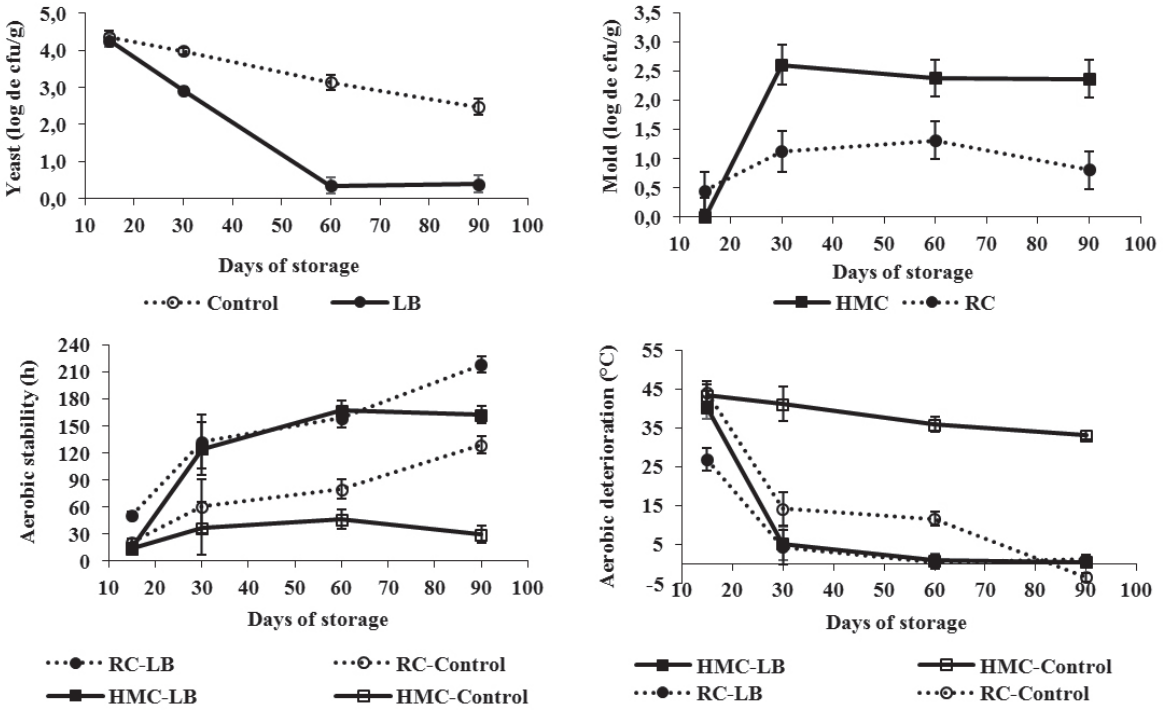

Figure 1 Counts of yeasts and molds, and aerobic stability of high-moisture corn (HMC) and rehydrated corn (RC) ensiled without (Control) and with (LB) *L. buchneri* (1×10^5 cfu/g as fed) at different storage times.

Ruminal degradability and aerobic stability of reconstituted corn grain silages treated with sodium benzoate

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Keywords aerobic deterioration, chemical additive, DM degradability, ruminal *in* situ

Introduction Ensiling high moisture grains typically increases the starch digestibility (Hoffman et al. 2011), whereas turns the grains more prone to aerobic deterioration (Taylor and Kung, 2002). Sodium benzoate is an antimicrobial compound (Woolford, 1975) capable to improve the aerobic stability of silages. In addition to its antifungal properties, sodium benzoate also restricts bacterial metabolism. Therefore, we hypothesized that treating reconstituted corn grains with sodium benzoate would increase the aerobic stability, but might decrease the proteolytic activity during silage fermentation and, in turn, reduce the ruminal degradability of starch.

Materials and methods Ground corn grains from a commercial source of flint hybrid with initial DM of 915 g/kg FM was rehydrated to reach 630 g DM/kg FM, using only water to control silages (RC) or water plus sodium benzoate 2g/kg of FM (RB). Silages were stored in plastic barrels with capacity of 200 L for 170 d. To determine aerobic stability, approximately 5 kg of silage was placed in plastic buckets, with three replications, and the mass temperature recorded with temperature data loggers every 15 min, during 10 d. Three non-lactating cows fitted with rumen cannula were used during the ruminal degradability assay. The total mixed ration used for feeding the cows contained (DM basis): 38% corn silage, 10% Coast-cross hay, 20.7% soybean meal, 11.5% citrus pulp, 2.5% premix mineral + vitamins, and 17.3% dry ground corn. Samples of RC, RB and dry ground corn (DG) (from the same grain source) were allocated in nylon bags and incubated in the rumen ventral sac for 0, 12, 24 and 48 h, in a reverse sequence and removed at once. Three incubation periods were carried-out with one-week intervals. Data were analyzed using the Mixed procedure of SAS. For the ruminal degradability the statistical model included the fixed effects of treatment, time and treatment × time and the random effects of period and cow. The interaction cow × treatment was used as the error term, whereas the covariance structure was the autoregressive(1). Means were compared by orthogonal contrasts: [DG vs (RC + RB)] and [RC vs. RB]. The aerobic stability was compared considering the fixed effect of treatment.

Results and discussion As expected, silage aerobic stability was greatly improved by sodium benzoate. Furthermore, grains treated with sodium benzoate showed lower overall heat accumulation, indicating lower deterioration upon air exposure (Table 1). The ruminal DM degradability was similar across silages (RC and RB), which were superior to DG, especially in short-incubation times (Figure 1). Ensiling rehydrated grains (with or without sodium benzoate) was effective to improve starch availability. Thus, both key issues of high moisture grain silages (digestibility and aerobic stability) can be ensured by using of sodium benzoate at 2g/kg of FM.

Table 1 Aerobic stability of rehydrated corn grain silages treated with sodium benzoate, after 170 d of storage

Item	RC	RB	SEM	*P*-value
Aerobic stability, h	122	236	10.7	<0.01
Accumulated temperature in 5 d, °C	3.73	0.97	2.78	0.26
Accumulated temperature in 10 d, °C	70.9	2.97	7.69	<0.01

RC: without additive (control); RB: treated with sodium benzoate.

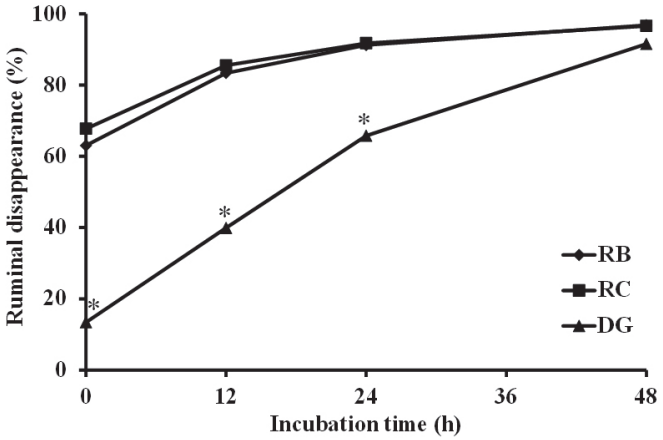

Figure 1 *In situ* ruminal DM degradability of dry ground corn (DG) and rehydrated corn grain silage without additives (RC) or treated with sodium benzoate (RB). *P*<0.01 for treatment × time interaction, SEM = 1.14, * *P*<0.01 for DG vs. (RC + RB).

Conclusion The rehydration of corn grains for silage significantly increased ruminal degradability of DM when compared with dry grains. Treating with sodium benzoate (0.2% w/w) led to improved aerobic stability without compromising the ruminal DM degradability.

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Effects of chemical and microbial additives on the fermentation and aerobic stability of rehydrated corn grain silages

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Keywords reconstituted corn grain silage, *Lactobacillus buchneri*, *Lactobacillus plantarum*, sodium benzoate, sodium nitrite

Introduction Ensiling dry corn grains after moisture reconstitution may result in poor fermented silage and more prone to spoilage. Mature grains have low content of soluble carbohydrates because the starch formation is completed and higher counts of yeasts and molds, among other undesirable microorganisms such as *Clostridium* spores (Carvalho, 2014). Thus, the use of additives is recommended to improve the fermentation and the aerobic stability of rehydrated corn grain silages, however still lacking more data on combination of additives and fine tuning dose-dependent trials. The aim of this study was to assess the impact of chemical and microbial additives on fermentation aspects and prevention of aerobic deterioration in rehydrated corn grain silages.

Materials and methods Corn grains from a commercial source of flint hybrid with initial DM of 985 g/kg FM was ground and rehydrated to reach 650 g DM/kg FM. The treatments were: control (without additive), *L. buchneri* at $5\times\mathbf{10}^5$ cfu/g FM (LB), *L. plantarum* at $5\times\mathbf{10}^5$ cfu/g FM (LP), LB + LP (Combo), LB + sodium nitrite at 1.5 g/kg FM (LBNit), and sodium benzoate at 2.0 g/kg FM (Benz). All additives were diluted in the distilled water used to rehydrate the grains. Initial counts of lactic acid bacteria (LAB), yeasts, and molds in dry grains were 6.5, 4.2 and 4.7 log cfu/g FM. Polyethylene buckets (6 L) were used as experimental silos (4 replications per treatment). The statistical analysis were performed by using the Mixed procedure of SAS and means compared with Tukey test ($\alpha = 0.05$).

Results and discussion Silages treated with *L. buchneri* showed higher LAB and pH values, leading to an effective control of yeast growth. Despite of minimum LAB population, sodium benzoate containing silages also presented a significant decrease on spoiling due to yeasts and molds, with reduced counts. Fermentative DM losses were negligible in all silages, despite the slightly higher DM losses in silages treated with *L. buchneri* (LB and combo), since the metabolism of this heterolactic bacteria result in release of CO_2 (Driehuis et al., 1999). Silages treated with *L. buchneri* (LB and combo) or sodium benzoate had higher aerobic stability. Inoculation of corn grains with *L buchneri* plus sodium nitrite resulted in lower yeast counts than the control silages upon air exposed, but still less effective than using *L. buchneri* exclusively. Rehydrated corn grain silages without additives or treated with homolactic bacteria were the less promising silages, because yeasts were not inhibited during the fermentation and after silos opening the silages showed a fast heating and increased aerobic losses.

Conclusion The use of *Lactobacillus buchneri* or sodium benzoate is effective to improve the aerobic stability of rehydrated corn grain silages.

Table 1 Fermentation traits and aerobic stability of rehydrated corn grain silages treated with chemical and microbial additives, after 90 d of storage

Item	Control	LB	LP	Combo	LBNit	Benz	SEM	*P*
Fermentation data								
pH	3.84^{c}	4.43^{a}	3.87^{c}	4.02^{b}	3.96^{b}	3.89^{c}	0.01	<0.01
DM, g/kg FM	673^{c}	663^{e}	683^{a}	668^{d}	679^{ab}	676^{bc}	0.10	<0.01
Fermentative Losses, %	0.60^{d}	1.22^{a}	0.67^{c}	0.96^{b}	0.18^{f}	0.27^{e}	0.01	<0.01
LAB, log cfu/g	4.5^{c}	8.1^{a}	5.2^{bc}	7.9^{a}	6.1^{b}	3.9^{c}	0.35	<0.01
Molds, log cfu/g	4.2	3.5	3.6	3.5	3.2	2.8	0.51	0.534
Yeasts, log cfu/g	4.4^{b}	<2.0^{c}	4.6^{a}	<2.0^{c}	<2.0^{c}	<2.0^{c}	0.04	<0.01
Aerobic stability data								
AS, h[2]	51^{d}	240^{a}	69^{c}	240^{a}	151^{b}	240^{a}	3.04	<0.01
Aerobic Losses,%	34.4^{a}	1.9^{c}	31.6^{a}	3.5^{c}	24.0^{b}	3.2^{c}	1.53	<0.01
Max. Temp.,°C[3]	46.3^{a}	26.0^{b}	43.5^{a}	26.1^{b}	45.2^{a}	26.2^{b}	0.66	<0.01
Time to Max. Temp., h	109^{b}	240^{a}	139^{b}	240^{a}	229^{a}	240^{a}	6.44	<0.01
Ac5d, °C[4]	37.6^{a}	0.0^{b}	22.9^{a}	0.0^{b}	0.3^{b}	0.0^{b}	3.21	<0.01
Ac10d, °C[5]	100.2^{a}	0.9^{d}	81.5^{b}	1.1^{d}	52.9^{c}	1.2^{d}	2.72	<0.01

[1]Additives applied onto fresh matter. Control: without additive, LB: *L. bucnheri* at 5×10[5] cfu/g, LP: *L. plantarum* at 5×10[5] cfu /g, Combo:LB + LP, LBNit: LB + 7 g of sodium nitrite/kg, Benz: 15 g of sodium benzoate/kg. [2]Time until silage temperature reached 2°C above ambient temperature (25°C). [3]Maximum temperature. Accumulated temperature in 5 days[4] and 10 days[5]. Different letters superscripts within a line indicate statistical differences (Tukey, α = 0.05).

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Shifts on bacterial population of high moisture corn silages and its correlation with fermentation end-products

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Keywords high moisture corn, bacterial diversity, 16S DNAr, sequencing, Illumina MiSeq™ System

Introduction Characterizing microbial communities present in silage may help on understanding of silage fermentation profile and aerobic stability, since establishment of distinct microbial communities in ensiled mass results in distinct fermentation patterns. Nowadays, the application of molecular techniques allows assessing the shifts caused on microbial communities by a culture independent approach. In this way, the objective of this study was to determine the composition of bacterial communities in high moisture corn silage as well as their fermentation end-products and their correlations.

Materials and methods Corn cultivars AG-1051 and IAC-8390 were harvested in three maturity stages: 1/2 milk line (ML), black line (BL) and dry grain, which was reconstituted to 32% of moisture (DR). Ground grains were ensiled in mini-bags and stored for 0, 7 and 120 days. Silage DNA was extracted using a laboratory protocol in which the samples were ground with liquid nitrogen and then weighed in a 2 mL Eppendorf tube, followed by addition of buffers and precipitation with phenol:chloroform:isoamyl alcohol solution (25:24:1). Later steps included serial centrifugations, addition of precipitation solutions and in the final step the DNA was resuspended in ultrapure water. The PCR products were sequenced by Illumina MiSeq™ system. The content of lactic acid was measured according to Pryce (1969) and ammonia nitrogen (NH3-N/N) according to Chaney and Marbach (1962). Furthermore the levels of alcohols, esters, acetone and volatile fatty acids were determined by gas chromatography-mass spectrometry. Data were analyzed with statistical software package of Canoco 4.5 (Biometris, Wageningen, Holand), Past 1.90 (Hammer et al., 2001) and QIIME (Quantitative Insights Into Microbial Ecology).

Results and discussion The ML and BL silages samples showed positive correlation with lactic acid content (Figure 2) which characterizes a desirable fermentation. However, after 120 days of storage, DR samples had more than 40% of sequences affiliated to the gender *Clostridium*, whereas *Lactobacillus* represented less than 7% (Figure 1). In this way, the content of butyric acid was the fermentation end-product that most contributed to differentiate DR silages from the others (Figure 2). Furthermore, butyric acid was negatively correlated to lactic acid content. Probably dried grains suffer more stress at field conditions, which in turn can interfere with the sanitary hygienic quality of silages obtained from those grains.

Conclusion Fermentation end-products found corroborate that clostridia dominated the fermentative process in DR silages. Kernel reconstitution did not ensure a desirable

fermentation. Treating DR with additives could be a possible strategy to prevent clostridium development in silages.

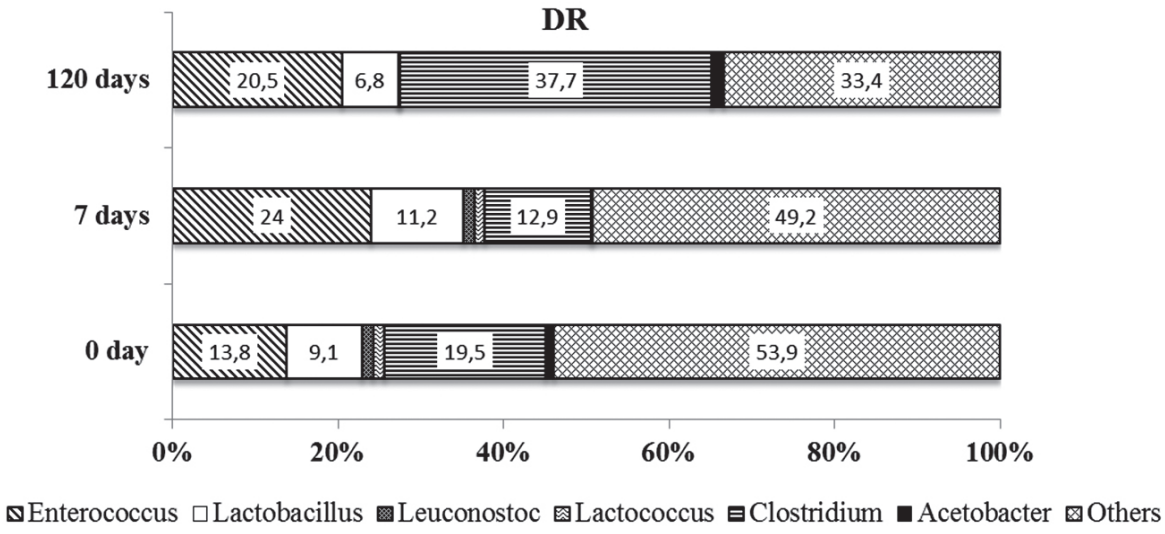

Figure 1 Diversity of main bacterial genera obtained from the OTUs matrix data in DR silage samples. Genera included in "Other" category, represent less than 1% of the total bacterial population in samples analyzed. Analysis was carried out with software QIIME.

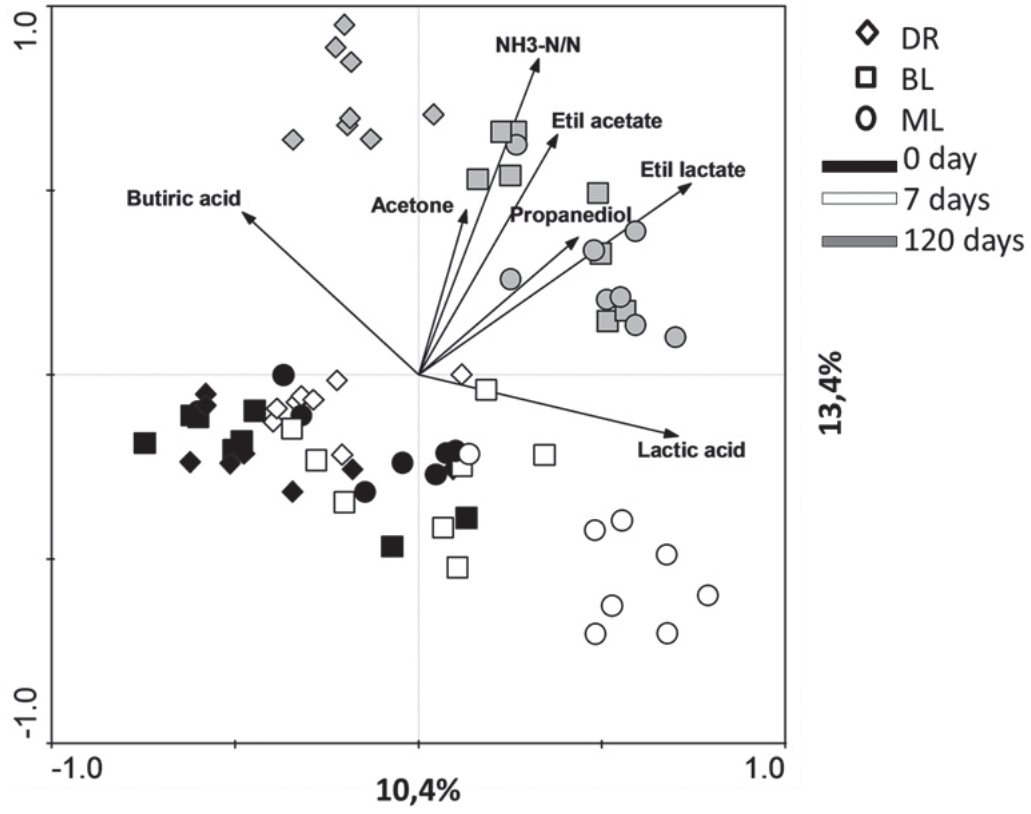

Figure 2 Redundancy analysis (RDA) using Monte Carlo test with 499 permutations. Symbols indicate maturity stage (DR, BL and ML) and colors shows storage period (0, 7 and 120 days).

Effects of exogenous protease addition and inoculation on fermentation profile, nitrogen fractions and ruminal in vitro starch digestibility in high-moisture corn

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Keywords protease, inoculant, starch digestibility, high-moisture corn

Introduction Fermented starch sources, such as high-moisture corn (**HMC**), have greater starch digestibility compared with unfermented sources. However, maximum starch digestibility may be only reached after 8 months of ensiling period (Ferraretto et al., 2014). Exogenous protease addition may be an alternative to increase proteolysis of prolamins and thus starch digestibility in HMC fermented for short periods. In addition, microbial inoculation may either accelerate fermentation or increase acid production and thus increase proteolysis or solubilization of prolamins. Thus, the experimental objective was to evaluate the impact of exogenous protease addition, microbial inoculation or both on fermentation profile, nitrogen fractions and starch digestibility of HMC.

Materials and methods On the day of harvest, 10 kg of unfermented HMC was obtained at the University of Wisconsin – Madison Agricultural Research Station (Arlington, WI) on October, 2013, homogenized and allocated into 24 samples of 300 g each. Samples were randomly assigned to eight treatments so each treatment had three replications. Eight treatments were a combination of HMC non-inoculated or inoculated with one of three microbial inoculants and with or without exogenous protease addition. The inoculant treatments followed the recommended application rate of microbial inoculant as follows: 1) 5.46 x 10^5 CFU of *Lactobacillus buchneri* 40788 and 1.36 x 10^5 CFU of *Pediococcus pentosaceus*/g of HMC (**LBPP**; Biotal Buchneri 500; Lallemand Animal Nutrition, Milwaukee, WI); 2) 5.46 x 10^5 CFU of *Lactobacillus buchneri* 40788/ g of HMC (**LB**; Biotal Buchneri 40788; Lallemand Animal Nutrition, Milwaukee, WI); and 3) 1.64 x 10^6 CFU of a mixture of *Pediococcus pentosaceus* and *Propionibacteria freudenreichii*/ g of HMC (**PP**; Biotal Plus II; Lallemand Animal Nutrition, Milwaukee, WI). The experimental exogenous protease (DSM Nutritional Products, Basel, Switzerland / Novozymes, Bagsvaerd, Denmark) was added at a rate of 1,825 mg of protease per kg of corn DM to protease treatments. All 24 samples, including the HMC without inoculation or protease addition, received the same amount of double distilled water to ensure protocol similarity among all samples. After inoculation, samples were ensiled in vacuum-sealed bags and stored in the dark at room temperature for 30 d prior to nutrient analysis. All samples were analyzed in duplicate for DM (% as fed), CP (% DM), borate-phosphate buffer soluble-CP (% CP), ammonia-N (% CP), starch (% DM), ruminal in vitro starch digestibility at 7 h (% of starch), pH and fermentation profile. Data were analyzed as a completely randomized designed in a 4 × 2 factorial arrangement of treatments using Proc Mixed of SAS with inoculation, protease addition and their interaction as fixed effects.

Results and discussion Concentrations of DM and CP were unaffected ($P > 0.10$) by protease addition and averaged 72.7% and 8.2%, respectively. Lactate and total acid

concentrations were increased ($P = 0.001$) and acetate concentration decreased ($P = 0.01$) with exogenous protease addition at ensiling. However, no effects on pH ($P = 0.12$; 4.27 on average) or ethanol concentration ($P = 0.29$; 0.27 on average) were observed. Starch digestibility was increased 7.5-% units by the addition of protease ($P = 0.02$; 44.4 vs. 51.9%) suggesting increased proteolysis. Increased concentrations of ammonia-N and soluble-CP with exogenous protease addition support this premise. Content of DM tended ($P = 0.06$) to be 1.6-% units higher for PP than other treatments, but CP concentration did not differ ($P = 0.37$). Measurements of pH were ($P = 0.001$) lowest for PP and LBPP, followed by LB, and highest for CON. Lactate concentration was greater ($P = 0.001$) and ethanol concentration lower ($P = 0.001$) for LBPP and PP than LB and CON. Acetate concentration was ($P = 0.001$) greatest for LB, followed by LBPP, CON and lowest for PP. Although both, ammonia-N and soluble CP, were increased ($P = 0.01$) by all three inoculants, starch digestibility was unaffected ($P = 0.64$; averaged 48.2%).

Conclusion Exogenous protease addition increased starch digestibility and may be an alternative when ensiling HMC for short periods. Although starch digestibility was not affected by inoculation, all three inoculants improved fermentation profile in HMC.

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Bacteriological profile of sorghum and corn grain rehydrated silages with the use of enzymes

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Keywords amylase, lactic acid bacteria, protease, rehydration

Introduction Rehydrated grain silages consists basically on hydration of mature grounded grain and ensure their storage by fermentation as silage. During the corn and sorghum grain ensiling, microbial enzymes hydrolyze the prolamine involving the starch granules and turn the rumen digestion easier. The effect of the fermentation on digestibility of starch is increased with fermentation time. The digestibility of starch can be increased by the use of enzymes in rehydrated grain during the fermentation. The microorganism's population influences the ensiling process, and is also important to maintain the silage quality and could alter the starch digestibility. This work aimed to evaluate the effects of the inclusion of amylase and protease or amyloglucosidase on the microbial population of sorghum and corn grain rehydrated silages.

Materials and methods The corn hybrid with flint endosperm (Dow 2B707) and the sorghum hybrid (Dow 1G220) were ground to achieve 3 mm diameter particles and rehydrated with water to obtain 35 % moisture content. The grains were ensiled in PVC tubes with 3 replicates. The treatments were: **Control** (without enzyme), amyloglucosidase (**AMG**, Kerazyme 4560), and amylase and protease (**AP**, Termamyl and Kerazyme 66495). After 30 and 180 days of fermentation the silos were opened and samples of approximately 3 kg were removed to evaluate the pH values and microbial population. Water extracts were used for enumeration of microorganisms. Sequential ten-fold dilutions were prepared to quantify the microbial groups. Yeasts and molds were enumerated by Dichloran Rose Bengal Chloramphenicol Medium (DRBC, Difco; Becton Dickinson, Sparks, MD, USA). The plates were incubated at 28ºC for 72 h. For the enumeration of lactic acid bacteria (LAB) and *Bacillus* spp, DeMan-Rogosa-Sharpe agar (M641I, HiMedia; Mumbai, India) plus nystatin (4 $mL.L^{-1)}$ and nutrient agar medium were used, respectively. The plates were incubated at 30ºC for 72 h. The colonies were counted on plates containing a minimum of 30 CFU and a maximum of 300 CFU. Data analysis was performed using the PROC GLM (SAS v. 9. 2), applying Tukey test for means comparison with 5% probability.

Results and discussion There was an interaction between type of grain and enzyme used. Corn silage showed higher LAB counts compared with sorghum control silages. The treated silages presented similar values (Table 1). The use of enzymes at ensiling of both rehydrated grains reduced LAB counts. After 30 days of fermentation the LAB count in corn silage was superior, and for both silages the LAB count decreased during fermentation. *Bacillus* spp, filamentous fungi and yeasts populations were below the detection limit (< 2 log cfu g^{-1}). For pH values there was a significant interaction between type of grain and enzymes used (Table 1). The sorghum silage pH was higher when compared to corn silage in the control treatment and with AMG enzyme. Using the AP

enzyme was not possible to verify differences between the pH values between the grains. The use of enzymes did not affect the pH values of corn silages; however, the use of AP enzyme reduced the pH of rehydrated sorghum silage. There was an interaction between type of grain and time of fermentation. At 30 days of storage, pH of sorghum silage was higher but there was a reduction in pH of both silages at 180 days. There was an interaction between length of storage and enzymes for LAB counts (Table 2). There was no difference in LAB counts at 30 days of fermentation with and without the use of enzymes, whereas after 180 days of storage, there was an increase in LAB counts in control silages.

Table 1 Count of LAB and pH in sorghum and corn grain rehydrated silages with or without enzymes, stored for 30 or 180 d

Grain	Enzyme					Time of fermentation (d)			
	Control[1]	AMG[2]	AP[3]	SE[4]	*P*-value	30	180	SE	*P*-value
	LAB (log cfu g^{-1})								
Corn	5.18 Aa	3.89 Ab	3.46 Ab	0.169	< 0.01	6.49 Aa	< 2.0 Ab	0.138	< 0.01
Sorghum	4.28 Ba	3.30 Ab	2.97 Ab	0.169		5.05 Ba	< 2.0 Ab	0.138	
	pH								
Corn	3.80 Ba	3.78 Ba	3.80 Aa	0.01	< 0.01	3.92 Ba	3.66 Ab	0.01	< 0.01
Sorghum	3.86 Aa	3.85 Aa	3.78 Ab	0.01		4.03 Aa	3.64 Ab	0.01	

[1]Without enzymes; [2]amyloglucosidase enzyme; [3]amylase and protease enzymes; [4] standard error of the mean; Values followed by same lowercase letter in line do not present significant difference by Tukey test (P > 0.05); Values followed by same uppercase letter in the column do not present significant difference by Tukey test (P > 0.05).

Table 2 Count of LAB and pH in sorghum and corn grain rehydrated silages with or without enzymes, stored for 30 or 180 d

Time of fermentation (days)	Enzyme				
	Control[1]	AMG[2]	AP[3]	SE	*P*-value
	LAB (ufc g^{-1})				
30	5.93 Aa	2.18 Aa	5.43 Aa	0.169	< 0.0001
180	3.54 Ba	< 2.0 Bb	< 2.0 Bb	0.169	
	pH				
30	3.99 Aa	3.99 Aa	3.94 Aa	0.01	0.1076
180	3.68 Ba	3.64 Ba	3.64 Ba	0.01	

[1]: Without enzymes; [2]: amyloglucosidase enzyme; [3]: amylase and protease enzymes; [4] Mean standard error; Values followed by same lowercase letter in line do not present significant difference by Tukey test (P > 0.05); Values followed by same uppercase letter in the column do not present significant difference by Tukey test (P > 0.05);

Conclusions Enzyme addition reduces the LAB counts with 180 days of fermentation in rehydrated corn and sorghum silages. The use of AP reduces pH values of sorghum silages.

Change in the starch granule structure by the ensiling process in rehydrated corn grains

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Keywords additives, electron microscopy, losses, natamycin, prolamin

Introduction Corn grain is the most important energetic feedstuff in ruminant diet. The difficulties related to grain storage in Brazil led the industry to choose *flint* varieties to avoid insect damage. This type of grain has a large proportion prolamin:starch and lower starch digestibility. The rehydrated corn grain silage can be an alternative to solve storage problems and increase starch degradability. This work aimed to evaluate the effect of ensiling process on the starch granule structure, fermentative parameters and *in vitro* degradability of rehydrated corn grains.

Materials and methods The trial was performed in the Centro de Pesquisas em Forragicultura (CPFOR), Pinhais, PR, Brazil, where the crop was grown. The corn hybrid (AG 8041) was harvested at 83% DM content. Corn kernels were milled (5 mm) and rehydrated (373 L/ton) to achieve 60% DM. Grains were ensiled in mini silos of PVC pipes (9.8 cm diameters and 50 cm height) equipped with bulsen valves. The treatments were: **Control** (no additives); **Buchneri** (*L. buchneri* 1×10^5 cfu/g FM); and **Natamycin** (8 g/t FM). Samples from each treatment were collected. Silos were filled in order to achieve bulk density of 1000 kg/m^3. After 45 days of storage, the silos were opened and the homogenized mass from each silo was sampled. Both pre- and post-fermentation samples were used for assessing DM content, pH, *in vitro* degradability (Table 1), and scanning electron microscopy of starch granules (Figure 1). The DM content was obtained in a forced-air oven at 60°C for 72 h. The pH was measured in water extracts (25 g of silage diluted in 225 mL of water). The *in vitro* degradability of DM was evaluated according to Theodorou et al. (1994) using the RF Gas Production System (Ankom Technology, Fairport, NY) and the data was recorded every 20 minutes during 24 h. The pressure inside of the bottle was measured in psi and convert to mL/g DM according to Mauricio et al. (1999) equation: Volume (mL) = -0.111813 + (3.60148×psi) + (-0.01247×(psi×psi)). The microscopy analysis was performed using a TESCAN VEGA3 LMU®. Data were statistically evaluated by ANOVA and treatments means were compared by Tukey test at 95% confidence level.

Results and discussion The DM content of silages was similar among all treatments (Table 1). The added water was sufficient to decrease DM at required level (60% DM) which allows the microbial growth. The mean pH value (3.93) indicates a satisfactory fermentation for both treatments, even in silage with no additives that was expected to present higher pH. These data suggests that epiphytic bacterial were able to drop pH and *L. buchneri* and natanycin didn't have any contribution. The additives increased the fermentative losses (Table 1), mainly in natamycin treatment. However, DM losses were based on oven drying, and volatiles losses could lead to misjudgment. The *in vitro*

degradability of silages was different across treatments ($P < 0.01$). Natamycin treatment showed the highest gas production (457 mL/g DM), followed by the control (387 mL/g DM), and Buchneri (325 mL/g DM). The pre-ensiled grain presented the lowest *in vitro* gas production (298 mL/g DM). The non-fermented grain spent 8 h to start gas production after incubation, whereas the ensiled grain initiated the produced gas production after 2 h of incubation. The ensiling process affected the structure of starch granules turning them more available for the rumen microorganisms, when compared to dry ground grain. The changes in physical interactions between the prolamin-protein matrix and the starch can be seen in Figure 1. Although no objective analyses can be performed from these microscopy findings, the difference from pre- and post-ensiled grains can be seen as the number of starch granules free from a matrix structure.

Conclusions Rehydrated corn grain silages change the structure of starch granules and improve the rumen degradability. Additives can contribute to this process, possibly by action of acids and bacterial enzymes on starch structure. More study should be done to understand this occurrence.

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Prolonged storage offset the negative effect of vitreousness on the degradability of high moisture corn silages

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Keywords high moisture corn, dent corn, flint corn, prolamin

Introduction Corn is one of the most used cereals in animal nutrition due to its nutritive value and high yield potential. Corn kernel endosperm consists of a translucent area that is referred to as vitreous or cornea and an opaque amorphous area characterized as soft or farinaceous. Especially in the vitreous portion, the protein matrix (rich in prolamin) is seen as a physico-chemical barrier for starch digestion. Therefore, interest in high moisture corn silage is rising among the types of processing. Ensiling high moisture grains increases ruminal starch degradability, not only for being harvested at a less maturity stage, but also because of the protein matrix is disrupted during the fermentation. Therefore, the aim of this work was compare the content of prolamin and the starch degradability of two corn hybrids, harvested in three maturity stages and stored as silage for different periods.

Materials and methods Kernels of two corn genotypes, IAC-8390 (flint) and AG-1051 (dent), were harvested in three maturity stages: 1/2 milk line (ML), black line (BL) and dry grain, which was reconstituted to 32% moisture (DR). Ground grains were ensiled in mini-bags (500 g) and stored for 0, 7, 21, 60 and 120 days, with four replications per treatment per storage period. The content of prolamin was measured according to Nellis et al. (2013). Ruminal *in situ* degradability of starch was determined in dried samples incubated for 12 hours in three non-lactating crossbred cows. The starch content was evaluated according to Hall (2009). Data were analyzed with the Mixed procedure of SAS.

Results and discussion As expected, the content of prolamin increased with the maturity advance in both hybrids, whereas IAC-8390 had a more vitrified endosperm than AG-1051 before ensiling. However, the concentration of prolamin decreased across the fermentation period. After 60 days of storage, the flint hybrid IAC-8390 had the same level of prolamin as compared to the dent hybrid AG-1051. Therefore, the ruminal degradability of starch (Figure 2) increased with the length of storage. When the grain is ensiled, regardless of maturity, the differences between vitreousness and degradability do not persist. Most starch degradation can be explained by the proteolysis of endosperm prolamin.

Conclusion When corn grains with vitreous or dent endosperm are ensiled, the prolamin present in the protein matrix are degraded, increasing the availability of starch granules and ruminal degradation of this nutrient.

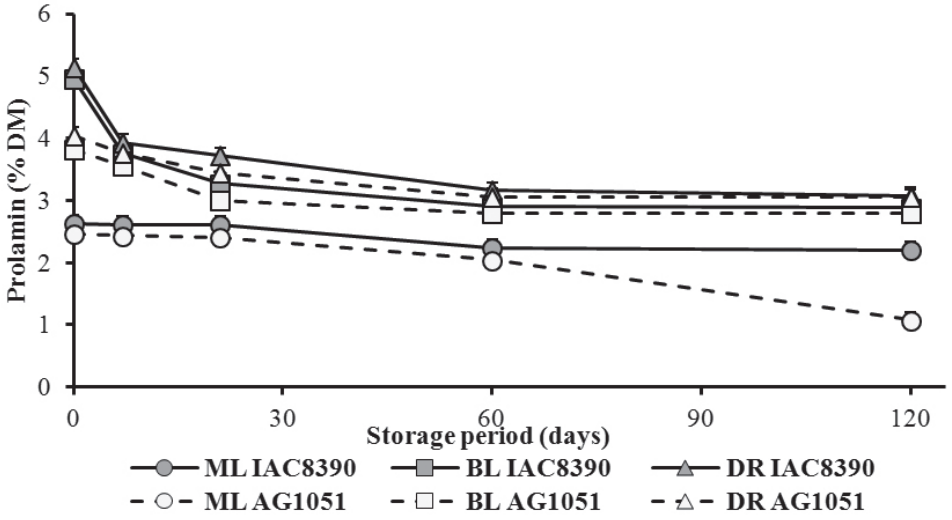

Figure 1 Concentration of prolamin in corn grain silage (AG1051 and IAC8390) according to maturity (ML, BL and DR) and storage period (0, 7, 21, 60 and 120 d). $P = 0.95$ hybrid × maturity. $P < 0.01$ hybrid × storage. $P < 0.01$ maturity × storage. $P < 0.01$ hybrid × maturity × storage.

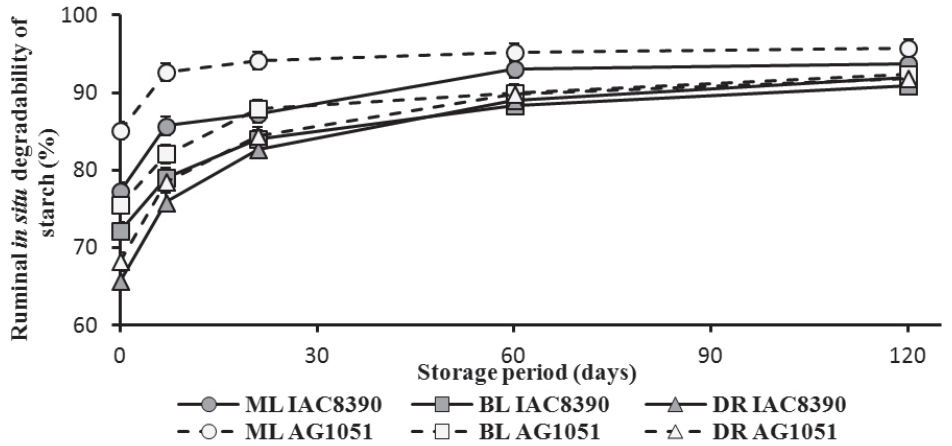

Figure 2 Ruminal *in situ* degradability of starch at 12 h in corn grain silage (IAC-8390 and AG-1051) according to maturity (ML, BL and DR) and storage period (0, 7, 21, 60 and 120 d). $P = 0.02$ hybrid × maturity. $P < 0.01$ hybrid × storage. $P < 0.01$ maturity × storage. $P = 0.11$ hybrid × maturity× storage.

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Contribution of proteolytic sources during fermentation of reconstituted corn grain silages

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Keywords corn kernel, prolamin, protein matrix, starch digestibility, zein

Introduction The protein matrix in corn kernel is a physicochemical impairment to starch digestion in ruminants (Owens et al., 1986). Ensiling high moisture corn kernels lead to degradation of hydrophobic zein proteins on the starch-protein matrix during the fermentation process (Hoffman et al., 2011). Several mechanisms are involved on breakdown of proteins in whole plant silages (Ohshima and McDonald, 1978; Heron et al.,1986; Hoffman et al., 2011), whereas in high moisture corn silages the activity of proteolytic sources are scarcely determined. Thus, the aim of this study was to determine the contribution of kernel enzymes, bacteria, fungi, and fermentation end-products on the mechanism of protein solubilization during fermentation of reconstituted corn grain silages.

Materials and methods Dried whole corn kernels was ground at 5 mm sieve, rehydrated to 350 g DM/kg FM, and treated as follows: no additives (**Ctrl**); gamma irradiation (32 kGy; **Irradiated**); Irradiated + fermentation end-products (lactic acid, acetic acid and ethanol at 10, 3 and 7 g/kg FM, respectively; **Irradiated+FEP**); and natamycin (10 g/kg FM; **Antifungal**). Ground kernels were ensiled in mini-bags (500 g FM per bag) with 5 replicates per treatment and stored for 90 d. Original ground corn kernels and silages were sampled to determine soluble protein concentration according to Krishnamoorthy et al. (1982). Protein solubilization (g/kg CP) was calculated for each treatment as: [soluble protein after fermentation (g/kg CP)] – [soluble protein before fermentation (g/kg CP)]. Then, the contributions of proteolytic sources (kernel enzymes, fermentation end-products, fungi and bacteria) to total proteolysis were calculated as:
Kernel enzymes (%) = 100 × [(protein solubilization in Irradiated silage) / (protein solubilization in Ctrl silage)];
Fermentation end-products (%) = 100 × [(protein solubilization in Irradiated+FEP silage) – (protein solubilization in Irradiated silage) / (protein solubilization in Ctrl silage)];
Fungi (%) = 100 × [(protein solubilization in Ctrl silage) – (protein solubilization in Antifungal silage) / (protein solubilization in Ctrl silage)];
Bacteria (%) = 100 – corn kernel enzymes – fermentation end-products – fungi.

Results and discussion The relationships between soluble protein concentrations in nonfermented grain (116 g/kg CP), ctrl (523 g/kg CP), irradiated (236 g/kg CP), irradiated+FEP (255 g/kg CP) and antifungal (501 g/kg CP) treatmens indicated that bacterial proteolytic activity is the major mechanism of protein degradation in reconstituted corn grain silages (Figure 1). Additionally, corn kernel enzymes contributed to 30% of

protein breakdown, whereas fungi and fermentation end-products revealed only minor contribution to protein solubilization (≈ 5%).

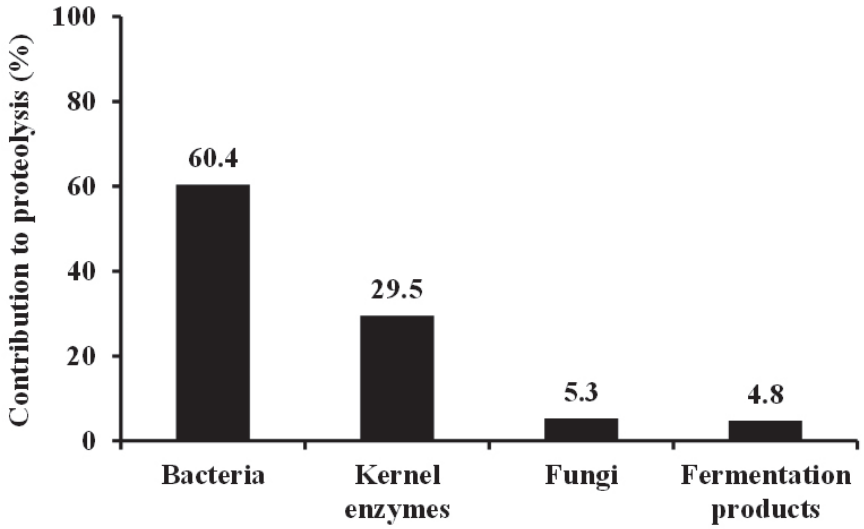

Figure 1 Contribution of corn kernel enzymes, microorganisms (bacteria and fungi) and fermentation end-products to proteolysis in reconstituted corn grain silages.

Conclusion Bacterial activity is the major proteolytic source in reconstituted corn grain silages.

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Effects of chemical and microbial additives on the conservation of reconstituted sorghum grain silage

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Keywords lactic acid bacteria, aerobic deterioration, fermentative loss, yeast

Introduction Reconstitution of grains by rehydration is feasible at farm level and may allow improvement in starch degradability, however, such silages still remains more prone to aerobic deterioration after silo opening, which may lead to high losses of dry matter and energy and impair animal performance. The aim of this study was to evaluate the influence of chemical and microbial additives on the conservation and aerobic stability of reconstituted sorghum grain silages.

Materials and methods Sorghum grains from a commercial source with initial DM of 987 g/kg FM were ground and reconstituted to reach 650 g DM/kg FM. The treatments were: (1) Control (without additives); (2) LB, *Lactobacillus buchneri* at 5×10^5 cfu/g FM (LalSil AS - Lallemand®); (3) LP, *L. plantarum* at 5×10^5 cfu/g FM (Sill All 4x4 WS - Altech®); (4) Combo, LB + LP; (5) Benz, sodium benzoate at 2 g/kg of FM; and (6) LBNit, LB plus sodium nitrite at 1.5 g/kg of FM. Additives were diluted in distilled water used to rehydrate the grain samples. Polyethylene buckets (6 L) were used as experimental silos, with four replicates per treatment. Initial counts of lactic acid bacteria (LAB), yeasts and molds in dry grains were 6.4, 4.2 and 4.7 log cfu/g FM. Statistical analysis was performed using the Mixed procedure of SAS and means compared with Tukey's test ($\alpha = 0.05$).

Results and discussion Sorghum grain silage treated with *L. buchneri* (LB, Combo and LBNit) showed higher pH values, as a result of the typical heterolactic pathway. Unexpectedly, the LP treatment decreased the LAB counts. The treatments LB, Combo, LBNit and Benz were effective to control the growth of molds and yeasts. Although fermentation losses were low in all silages, sodium benzoate was effective to reduce DM losses during fermentation. On the other hand, LB and Combo treatments increased the fermentative losses. Treatments containing *L. buchneri* (LB, Combo and LBNit) and sodium benzoate showed higher aerobic stability and lower aerobic DM losses, due to their antifungal attributes. Reconstituted sorghum grain silages without additive or treated with homolactic bacteria were highly susceptible to deterioration upon air exposure.

Conclusions Improved fermentation and aerobic stability might be achieved by either add *L. buchneri* or sodium benzoate in reconstituted sorghum grain silages. Microbial or chemical combinations with *L. buchneri* were more effective than control but not better than *L.buchneri* add exclusively.

Table 1 Fermentation and aerobic stability of reconstituted sorghum grain silages treated with chemical and microbial additives, after 90 d of storage

	Treatment[1]							
Item	Control	LB	LP	Combo	LBNit	Benz	SEM	*P*
Fermentation								
pH	3.96^{c}	4.34^{b}	3.96^{c}	4.40^{a}	3.98^{c}	4.01^{c}	0.01	<0.01
DM, g/kg FM	674^{ab}	664^{b}	674^{ab}	665^{b}	675^{ab}	679^{ab}	0.27	<0.01
LAB, log cfu/g	6.67^{ab}	6.98^{ab}	4.86^{b}	7.18^{ab}	7.92^{a}	6.64^{ab}	0.55	0.03
Molds, log cfu/g	4.23^{a}	3.87^{a}	4.3^{a}	4.08^{a}	4.32^{a}	4.48^{a}	0.47	0.96
Yeasts, log cfu/g	4.74^{a}	<2.0^{b}	4.6^{a}	<2.0^{b}	<2.0^{b}	<2.0^{b}	0.06	<0.01
Fermentative losses, %	0.73^{c}	1.40^{a}	0.62^{d}	1.34^{b}	0.52^{e}	0.20^{f}	0.01	<0.01
Aerobic stability								
AS[2], h	38.5^{b}	240^{a}	36.33^{c}	240^{a}	240^{a}	240^{a}	0.39	<0.01
Ac 5 d[3], ^{o}C	46.52^{a}	0.0^{b}	45.93^{a}	0.0^{b}	0.0^{b}	0.0^{b}	1.16	<0.01
Ac 10 d[4], ^{o}C	102.3^{a}	1.33^{b}	95.0^{a}	0.0^{b}	0.60^{b}	0.5^{b}	2.7	<0.01
Aerobic losses, %	28.46^{a}	2.35^{b}	27.43^{a}	2.34^{b}	1.92^{b}	2.19^{b}	3.11	<0.01

[1]Control: without additive, LB: *L. buchneri* at 5 × 10^{5} cfu/g, LP: *L. plantarum* at 5 × 10^{5} cfu/g, Combo: LB + LP, LBNit: LB + 1.5 g/kg of sodium nitrite, Benz: 2 g/kg of sodium benzoate. [2]Time elapsed before silage temperature increased by at least 2°C above the room temperature (25° C). [3]Accumulate temperature in 5 days. [4]Accumulate temperature in 10 days.

Co-fermentation of energy crop silages in batch fermentation test

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Keywords maize, sugar beet, dry matter losses, batch fermentation test, methane yield

Introduction Biogas production based on energy crops is very common in Germany. Often a mix of various energy crops is used as substrate. Within the last years sugar beets supplement the conventional substrates because of their excellent fermentation properties. Biogas plant operators reported higher gas yields after the first switch to sugar beet in the mixture. A joint project at Kiel University, which is sponsored by the German Federal Ministry of Education and Research from 2012 to 2015, investigates the impact of the application of digestates on the physical and chemical soil properties and on soil life. The digestates are produced on laboratory scale by mono- or co-fermentation of energy crops (maize, wheat, grass, sugar beet) in discontinuous fermentation tests. Because the required silages were prepared on laboratory scale level, the dry matter losses could be recorded as well as the losses of methane yield. The results of the fermentation tests allow to answer the question if there is an effect due to co-fermentation or not. The present research work focusses on results of fermentation of maize and sugar beet.

Materials and methods Sugar beets were prepared for ensiling (washed, defoliated and chopped <10 mm). Maize was harvested and chopped by field chopper (<10 mm). Afterwards maize was ensiled in preserving jars, sugar beets in plastic bags for 90 days. Losses were recorded by weighing, the silages were examined for pH, dry matter and organic dry matter, crude nutrients composition, fermentation acids and alcohols as well as ammonia. To determine the biogas and methane yield of fresh substrates and chosen silages under laboratory conditions a common fermentation batch test (min. four lab replicates; anaerobic fermentation for 28 days at 38 °C, max. of 48 batch-reactors parallel) was carried out in compliance with German Standard Procedure VDI 4630 (2006). The experimental procedure is described in detail in Ohl (2011). Silages were examined in mono-fermentation (100 %) or in co-fermentation with another energy crop silage (80 %-20 %, 50 %-50 %, 20 %-80 %). The silages were tested with respect to the specific methane yield of single substrates and mixtures, the estimation of losses of methane yield according to the scheme of Nussbaum et al. (2012) and the calculation of the theoretical methane production of the mixtures.

Results and discussion During ensiling of maize dry matter losses could be recorded (Table 1), which were on average 2.2% with a low deviation. These losses caused losses in methane yield up to 6.8%. Only one silage (no. 5) showed an increase of methane yield because of marginal dry matter loss and high specific methane production. Sugar beet silages had higher dry matter losses (mean 20.1%) with higher deviation, which corresponded to the different composition of fermentation acids, especially the presence of alcohols, e.g. ethanol (<1% DM at no. 2 and 3 up to 12.7 % DM). Due to the higher specific methane production of these silages, the losses in methane yield were at least halved. Maize silage (no. 1; Figure 1) reached a lower specific methane production

(334 L_N/kg_{oDM}) compared to sugar beet silage (no. 4; 381L_N/kg_{oDM}). Co-fermentation of both silages resulted in higher methane yields, depending on the portion of sugar beet in the mixture. The calculation of the theoretical methane production of the mixtures accorded with the measured data. An increasing effect by the co-fermentation could not be observed.

Table 1 Dry matter (DM) and methane (CH_4) losses caused by ensiling of maize and sugar beet

		losses [%]	
	No.	DM	CH_4
Maize	1	6.4	
	2	0.7	6.8
	3	3.5	
	4	1.2	3.6
	5	0.4	-4.7
	F1	0.8	2.5
	$\bar{x}$	2.2	2.0
	SD	2.2	4.2

		losses [%]	
	No.	DM	CH4
Sugar beet	1	23.9	11.2
	2	11.9	4.9
	3	12.0	
	4	26.1	9.7
	5	26.6	8.4
	$\bar{x}$	20.1	8.6
	SD	6.7	2.3

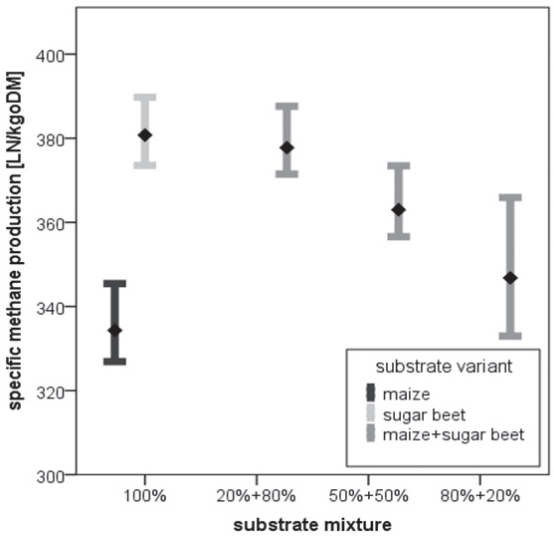

Figure 1 Specific methane production of maize and sugar beet silage in mono- and co-fermentation.

C**onclusions** Although no enhancing effect could be observed by the co-fermentation of maize with sugar beet, their use as co-substrate has advantages. Sugar beets have excellent fermentation properties, the gas formation starts rapidly and is completed in a few days whereas the gas formation of maize begins later and lasts longer. Due to the lower dry matter content of sugar beets the digestates are more fluid, so that the stirring is facilitated.

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Producing biogas from winter sugar beets in Germany

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Keywords winter beet, sugar beet, dry matter losses, biogas, batch fermentation test

Introduction The cultivation of winter beet offers a possibility to further increase the yield potential of sugar beet. Winter beet are sown in August and grown until the summer of the following year. This prolonged growing season can result in assets and drawbacks. During winter, the sugar beet plants shift from vegetative to reproductive growth and subsequently they produce bolters. Bolted beets are not suitable for sugar extraction and bolting resistant varieties of sugar beet are not available so far. Producing biogas from winter beet could be an appropriate option for utilizing the shoots and roots. Therefore a joint project, which is sponsored by the German Federal Ministry of Education and Research from 2009 to 2014, considered the whole value chain from breeding, cultivation systems and crop production to storage and use of winter beets for biogas production. The objectives of the presented study were to identify possibilities to make winter beets storable and to assess the suitability as biogas substrate compared to conventionally grown sugar beets.

Materials and methods Bolted winter beets (three genotypes), which were grown in plot trials in Kiel and Göttingen from 2009 to 2012, were harvested as whole plant, the roots were washed. Thereafter, the preparation for ensiling was carried out, four variants were formed: a) only roots without chopping (unground), b) only chopped roots (<10 mm, pulp), c) roots and shoots with coarse chopping (200 to 250 mm, unground) and d) chopped roots and shoots (<10 mm, pulp). Substrate samples were either frozen immediately (fresh samples) or ensiled under laboratory conditions (three replicates, preserving bags, storage temperature 20°C for 90 days) to identify their dry matter losses and silage quality. A subsample of each fresh substrate was sent to an external laboratory to determine the chemical composition by wet-chemical Weender-analysis. The silages were examined for pH, dry matter and organic dry matter, fermentation acids and ammonia. Conventionally grown sugar beets from another plot trial in Kiel served as reference material, because sugar beets are used as biogas substrate in Germany for some years. The procedure for preparation and ensiling was analogous to the winter beet variants a) and b). To determine the biogas and methane yield of fresh substrates and chosen silages under laboratory conditions a common fermentation batch test (four lab replicates; anaerobic fermentation for 28 days at 38°C, max. of 48 batch-reactors parallel) was carried out in compliance with German Standard Procedure VDI 4630 (2006). Silage variants a) and c) were chopped to pulp before testing. The experimental procedure is described in detail in Ohl (2011). The methane yield per hectare is calculated from the combination of the specific methane production with the dry matter yield of the plots. The dry matter loss which occurred during the ensiling is taken into account.

Results and discussion Fresh winter beets (root) had a similar dry matter content (DM 21 %) but they differed mainly in a higher fiber content (XF 13 %) from sugar beets (DM 22 %, XF 5 %). The whole plants of winter beet had lower dry matter content (17 %) and an even higher portion of fiber (25 %). Thus the part of Nitrogen free extractives (XX) is even lower in winter beets. During ensiling the pH declined under 4.0. Mainly fermentation products were lactic and acetic acid, indeed the content of ethanol varied

between undetectable and 15 % of DM (winter beets) or up to 26 %of DM at two sugar beet silages. During ensiling of all types of beets dry matter losses up to 31 % were observed, which were always particularly high if larger amounts of ethanol could be detected in the silage. It could be possible, that the losses have been overestimated because there was no way to determine the content of other alcohols such as methanol in this project. However, methanol is often detected in sugar beet silages (Weissbach and Strubelt, 2008). Ensiled winter beets (whole plant) achieved lower specific methane yields (330 L_N/kg_{oDM}) and lower methane yields per hectare (2931 Nm^3/ha) than conventionally grown sugar beets (373 L_N/kg_{oDM}; 5529 Nm^3/ha; figure 1). The composition of ensiled winter beet is less suited for biogas production and the dry matter yield is reduced, causing lower methane yields per hectare.

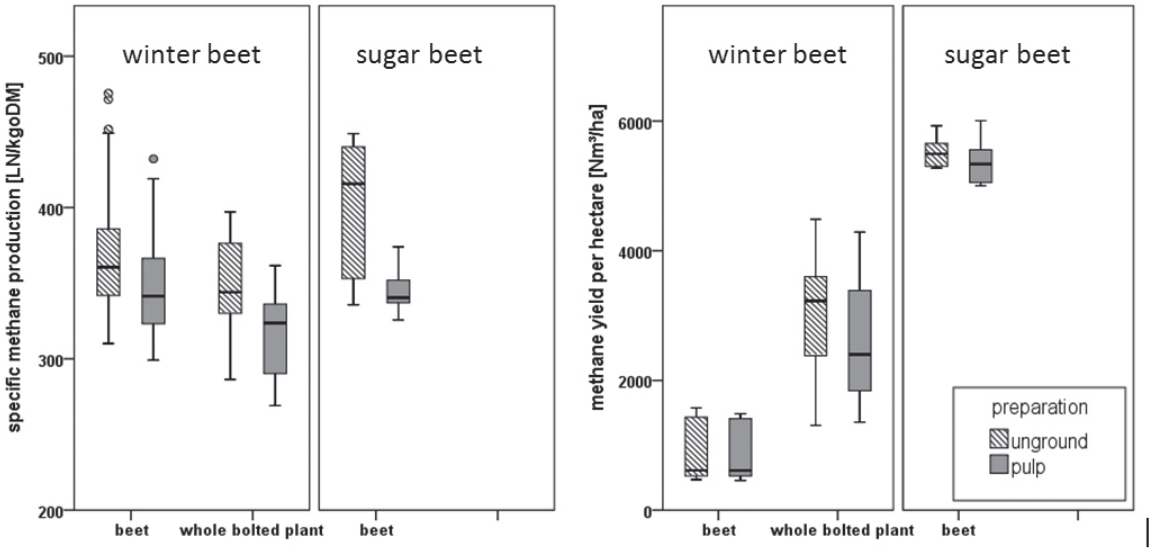

Figure 1 Specific methane production and methane yield per hectare of winter and sugar beets.

C**onclusions** The use of sugar beets as substrate was established in German biogas plants due to the excellent fermentation property. For year-round storage ensiling of pulp or whole roots of sugar and/or winter beets is possible. If the shoots of winter beets should also be ensiled, they need to be chopped before ensiling. Winter beets are generally suited for fermentation in biogas plants, however the methane production is lower compared to conventionally grown sugar beets. To increase the winter hardiness and the dry matter yield of winter beets are the most important steps to make them competitive to sugar beets and other biogas substrates such as maize silage. Additionally breeders make efforts to create non-bolting beet varieties.

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Grass silage for biogas – is the biogas and methane output enhanced after application of fibrolytic enzyme additives during ensiling?

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Keywords grass silage, fibrolytic enzyme additive, anaerobic digestion, methane

Introduction The main aims of additive application at ensiling are to maintain, but ideally enhance, the nutritive value of the biomass while restricting conservation losses. Fibrolytic enzyme additives partially digest the lignocellulosic cell wall of crops as measured by a reduction in neutral detergent fibre (NDF) concentration (Dehghani *et al.*, 2012). This reduction may in turn increase the availability of substrates for fermentation in either the silo or during subsequent anaerobic digestion (AD) (Vervaeren et al., 2010). Thus, fibrolytic enzyme additives may increase the energy captured from biomass when digested anaerobically within a specific timeframe. The objective of this experiment was to determine the effects of fibrolytic enzyme additives on the specific biogas and methane (CH_4) yields of silages produced from two grass species at two harvest dates in the primary growth.

Materials and methods At two harvest dates (Harvest 1 = 28 May and Harvest 2 = 18 June) in the primary growth, perennial ryegrass (PRG) and timothy (TIM) were each grown in quadruplicate plots. These were precision-chopped and subsamples (units of 6 kg/plot) were subjected to the following additive treatments prior to ensiling: (1) Control (i.e. no enzyme additive), (2) Phytase, (3) Cellulase-A, (4) Cellulase-B and (5) Xylanase. Additives were obtained from solid state fermentations, dissolved in 10 mL Tween20®/L solution and added at 5 mL/kg fresh herbage, in line with the producer's recommended rates. These were ensiled in laboratory pipe silos for 120 days after which a representative sample was taken and stored at -18°C. The biogas and CH_4 output of non-dried, milled samples were determined in an *in vitro* batch anaerobic digestion test according to VDI 4630 (2006) guidelines. Data were analysed with the MIXED procedure (SAS 9.3) as a split-spilt plot design with replicate blocks.

Results and discussion Results are presented in Table 1. Interactions that were not significant were omitted. In Harvest 1, the control TIM silage underwent a clostridial fermentation (data not presented). In comparison to the control PRG silage, that underwent a lactic acid dominant fermentation, there was an increase ($P<0.01$) in biogas and CH_4 yields for control TIM silage, in Harvest 1. This increase could be due to higher proportions of propionic and butyric acids formed during the clostridial fermentation. In Harvest 2, both species underwent a lactic acid dominant fermentation and led to increased ($P<0.01$) biogas and CH_4 yields for control PRG silage compared to control TIM silage. With advancing harvest date the biogas and CH_4 yields increased for control PRG silage ($P<0.05$) but not for control TIM silage. With both species, at both harvest dates, enzyme additives had contrasting effects in comparison to the controls. There was no significant benefit observed for Cellulase-A. The other three enzyme additives had a greater biogas

yield (P<0.012) and CH_4 yield (P<0.015) than Control treatment or Cellulase-A. The three successful enzyme additives were not significantly different from one another and all show the potential to enhance the biogas and CH_4 output from anaerobic digestion of grass silages per unit of volatile solids (VS) added.

Conclusions Harvest date and species had contrasting effects on silage fermentations, which had subsequent effects during AD. In comparison to their specific control treatments, biogas and CH_4 yields from grass silages were enhanced by some fibrolytic enzyme additive treatments such as Phytase, Cellulase-B and Xylanase.

Table 1 Effect of harvest, species and enzyme additive on biogas and methane yields in a 35 d *in vitro* batch anaerobic digestion test

Harvest	Species	Enzyme	Variable	
			Biogas yield (L biogas kg^{-1} VS)	Methane yield (L CH_4 kg^{-1} VS)
1	PRG	Control	493	252
1	PRG	Phytase	506	263
1	PRG	Cellulase-A	481	251
1	PRG	Cellulase-B	519	268
1	PRG	Xylanase	534	278
1	TIM	Control	550	274
1	TIM	Phytase	675	347
1	TIM	Cellulase-A	610	311
1	TIM	Cellulase-B	670	344
1	TIM	Xylanase	689	359
2	PRG	Control	591	298
2	PRG	Phytase	611	309
2	PRG	Cellulase-A	559	278
2	PRG	Cellulase-B	593	303
2	PRG	Xylanase	653	334
2	TIM	Control	496	244
2	TIM	Phytase	570	295
2	TIM	Cellulase-A	481	239
2	TIM	Cellulase-B	568	284
2	TIM	Xylanase	555	266
Standard error of the mean				
Harvest (H)			16.7	7.5
Species (S)			13.1	7.0
Enzyme (E)			16.0	8.1
H x S			18.7	10.0
Level of significance				
Harvest (H)			NS	NS
Species (S)			*	NS
Enzyme (E)			***	*
H x S			***	**

Harvest 1, 28 May. Harvest 2, 18 June. PRG, perennial ryegrass. TIM, timothy. * P<0.05 ** P<0.01 *** P<0.001 NS- not significant.

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A simple and reliable system for measuring gas production kinetics during silage fermentation in lab scale silos

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Keywords temperature, carbon dioxide, pressure, volume

Introduction During silage fermentation, gases are formed as by-products of microbial metabolism. Therefore, measuring gas production kinetics is a method to determine microbial activity and nutrient losses. Thus, the aim of this study was to develop a simple and reliable system for measuring the kinetics of gas production during silage fermentation in lab scale silos.

Materials and methods A semi-automated system was developed for measuring the kinetics of gas production during silage fermentation. Experimental silos (1.96 L) were built with acrylic tubes (10 cm i.d. × 25 cm height; 5 mm thick) and acrylic plates (20 mm thick) using glue for acrylic (S330, Plastecno, São Paulo, Brazil). The silo cap was adapted with a rubber septum 10-mm thick and fastened to the silo body using screws with wing nuts to facilitate silo load and unload. An O-ring was positioned under the cap to keep the silos air-tight (Figure 1A and 1B). Silo tightness was determined by producing a 5 psi pressure with an air compressor and recording the pressure after a week. At ensiling, mass porosity (Pitt, 1986) was equalized at 0.40 for whole-plant (corn, grass and sugarcane) and at 0.20 for grain (reconstituted corn) crops. Fresh crops were manually packed into the silos using a polyethylene stick. A portable temperature sensor (Novus, Porto Alegre, Brazil) was inserted in the mass to register the silage temperature every 12 h. Silos were stored inside the lab at 25±1°C. The internal pressure of the silos was measured by using a pressure transducer (DataPress, MPL, Piracicaba, Brazil) connected to a data displayer. Readings were taken every 12 h until d-3, every 24 h from d-4 until d-21, every 3 d from d-22 until d-30, and every week until the end of the storage. A hypodermic needle attached to a three-way Luer lock coupled to the transducer was used to puncture the cap septum and capture the internal pressure. After pressure recording, the internal pressure of the silos was equilibrated to the external pressure by open the Luer lock. A 60-mL syringe connected to the Luer lock was used to calibrate the system and convert pressure to volume (Figure 1C). Because all calibrations were made at 25°C (530 m above sea level), the actual pressure was corrected to 25°C, considering the temperature increasing inside the silo due to the heat generated by the fermentation [e.g. for whole-plant silages, Pressure at 25°C = Actual Pressure + (25 – Actual temperature, °C) × 0.138, where 0.138 is the increment of pressure caused by 1°C increase over 25°C in the present system]. Data were presented as accumulated gas production per kg DM. The total gas production was compared with the gas losses determined by the traditional gravimetric method (weight difference).

Results and discussion Silo tightness was confirmed after the silos holding the imposed pressure (5 psi) during a week. Separated calibrations were obtained for whole-plant [V_{wp} (mL) = 104.3 × Pressure (psi); R^2 = 0.997; n = 40] and grain silages [V_g (mL) = 59.6 × Pressure (psi); R^2 = 0.999; n = 40] due different porosities. The cumulative gas production

of whole-plant corn, sugarcane, elephant grass and reconstituted corn grain are presented in Figure 2A. As expected, sugarcane silage had the highest gas production, whereas corn silage, grass silage and reconstituted corn grain had comparable losses per kg DM. Afterwards, the total gas production was converted to mass of CO_2, considering the gas composition (99% CO_2; data not showed) and the mass of 1 L of CO_2 (1.96 g). The gas production measured with the novel system was accurate and precise (Figure 2B).

Conclusion The system is feasible for measuring the kinetics of gas production during silage fermentation. The strong correlation with gravimetric losses allows gas production to be tracked in real time and predicts the kinetics of DM losses properly.

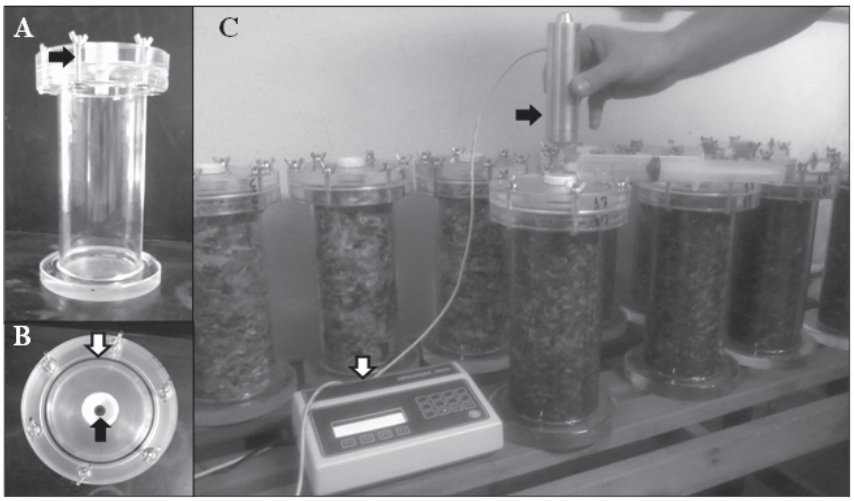

Figure 1 Semi-automated system for measuring the kinetics of gas production during silage fermentation. **A**: side view of the experimental silo, emphasizing the screws with wing nuts (black arrow). **B**: top view, emphasizing the rubber septum (black arrow) and the O-ring (white arrow). C: system in operation, emphasizing the data displayer (white arrow) and the pressure transducer (black arrow).

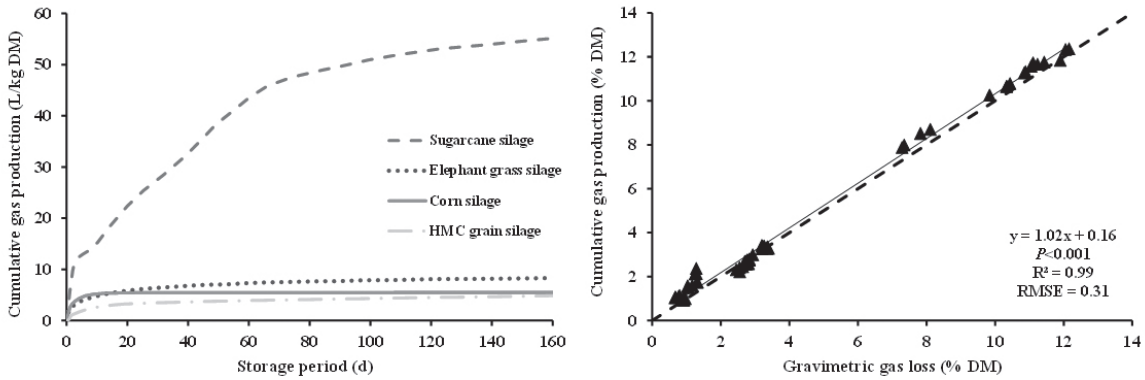

Figure 2 Kinetics of gas production during the fermentation of selected silages (A) and relationship between gas production (measured by the novel system) and gas loss (measured by the traditional gravimetric method) (B).

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Effect of different filters on the concentrations of neutral detergent fiber and acid detergent fiber in silages

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Keywords silage, neutral detergent fiber, acid detergent fiber

Introduction Silage is the main source of livestock fodder for cattle in Inner Mongolia. The nutritional value of livestock ration is closely related to the inclusion of fibrous feedstuffs. Determining accurately neutral detergent fiber (NDF) and acid detergent fiber (ADF) of silage plays important role in the development of animal husbandry in production practices. Using different filters for fiber determination may impact on NDF and ADF contents in silage. In this study, we compared three kinds of filters for NDF and ADF determination in different silages.

Material and methods Silage samples which included whole-plant corn silage, corn straw silage, high-moisture corn straw silage, *Beta vulgaris* silage, *Medicago sativa* silage, *Roegneria turzaninovii* silage, *Lespedeza hedysaroides* silage, and TMR silage were collected from Inner Mongolia. The concentrations of NDF and ADF were determined according to Van Soest's method and the filters included ANKOM bag, CAU bag that was made by Chinese Agricultural University (CAU), and FOSS glass crucible. The data were analyzed by one-factor ANOVA by using the GLM procedure of SAS.

Results and discussion The NDF concentration of different silages is presented in Table 1. The NDF concentration in all silages which was determined by FOSS glass crucible was the highest. FOSS glass crucible was higher than CAU bag ($P<0.05$), and higher than ANKOM bag ($P<0.05$), except in high-moisture corn straw silage ($P>0.05$) and *Roegneria turzaninovii* silage ($P>0.05$). There was no difference between ANKOM bag and CAU bag ($P>0.05$), except in *Medicago sativa* silage ($P<0.05$). The ADF concentration of different silages is presented in Table 2. The ADF concentration in all silages which was determined by FOSS glass crucible was higher than ANKOM bag ($P<0.05$), except in *Roegneria turzaninovii* silage ($P>0.05$) and *Lespedeza hedysaroides* silage ($P>0.05$), and higher than CAU bag ($P<0.05$) in whole-plant corn silage, corn straw silage, *Medicago sativa* silage and TMR silage. The CAU bag was higher than ANKOM bag ($P<0.05$) in corn straw silage and *Lespedeza hedysaroides* silage. There was no difference among three kinds of filters in *Roegneria turzaninovii* silage ($P>0.05$).

Conclusions In most silages, the NDF and ADF concentrations were higher by using FOSS glass crucible than ANKOM and CAU bags, whereas there was no difference between ANKOM bag and CAU bag.

Table 1 Effect of different filers on the NDF concentration of silages (g kg^{-1} DM)

Silages	ANKOM	CAU	FOSS
Whole-plant corn silage	565.8b	555.6b	593.9a
Corn straw silage	659.6b	656.6b	671.8a
High-moisture corn straw silage	592.2ab	587.9b	605.7a
Beta vulgaris silage	480.5b	482.8b	502.2a
Medicago sativa silage	357.0c	361.9b	374.8a
Roegneria turzaninovii silage	578.3ab	567.6b	593.6a
Lespedeza hedysaroides silage	406.0b	405.5b	433.9a
TMR silage	385.3b	375.4b	449.8a

Means in the same row whit different letters differ significantly ($P<0.05$)

Table 2 Effect of different filers on the ADF concentration of silages (g kg^{-1} DM)

Silages	ANKOM	CAU	FOSS
Whole-plant corn silage	318.5b	315.6b	328.9a
Corn straw silage	416.2c	431.7b	463.4a
High-moisture corn straw silage	382.6b	390.1ab	410.9a
Beta vulgaris silage	265.3b	279.9ab	291.1a
Medicago sativa silage	297.5b	296.9b	326.0a
Roegneria turzaninovii silage	379.5	364.8	378.7
Lespedeza hedysaroides silage	395.2b	457.6a	378.1b
TMR silage	187.2b	169.1b	226.1a

Means in the same row whit different letters differ significantly ($P<0.05$)

Higher proportion of corn silage to alfalfa hay in diets improves energy balance in early lactation Holstein dairy cows in hot climate condition

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Keywords Heat stress, energy balance, early lactation, body condition score, corn silage

Introduction Suppressed DMI is usually observed after calving in fresh lactating dairy cows. Moreover, hot climates results in heat stress which negatively affects the DMI. In the most dairy farms, corn silage and alfalfa hay are the main forages. The proportion of the two forages may affects DMI and consequently energy balance in dairy cattle especially in early lactation stage.

Materials and methods 120 cows with similar milk production (47.5 ± 0.5), days in milk (40 ± 15) and parity (2.48) were allocated to two treatments. In this study two proportion of CS to AH (LCS=50% alfalfa hay and 50% corn silage; HCS=25% alfalfa hay and 75% corn silage) were examined. In both treatments, the forage to concentrate ratio was 65 to 35. Diets were balanced with the same NE_L (1.7 Mcal/kg DM), CP (17± 0.2%), NDF (32 ± 0.2%) and NFC (42 ± 0.3%). Feed were offered as TMR in equal part (0500 and1700) as ad libitum. The experimental period was 40 days. The cattle were milked 4 times daily. Total tract digestion of nutrients and blood samples were analyzed on the last week of the experiment.

Results and discussion Mean Temperature Humidity Index (THI) was 80 during the experimental period and cattle were on heat stress (Figure 1). Digestibility of DM was higher (P<0.01) in cows received HCS diet compared with cattle on LCS. More NDF was digested in HCS diet (P<0.05) compared with cattle on LCS may be due to hydrolysis effect of low pH on degradability of NDF. Moreover, digestibility of CP was higher in HCS diet (P<0.01) compared with cattle on LCS may be due to greater proportion of NPN in corn silage when compared with alfalfa hay.

Table 1 Effects of low or high corn silage diets on nutrients digestionin total gastrointestinal tract of early lactation dairy cows in hot climate condition

	Treatments			
Item	LCS	HCS	SE	P-value
DM (%)	60.89	68.55	1.42	<0.01
CP (%)	59.10	65.57	1.86	0.02
NDF (%)	44.65	57.68	1.89	<0.01

LCS=Low corn silage diet; HCS=High corn silage diet; DM=Dry matter; CP=Crude protein; NDF=Neutral detergent fiber.

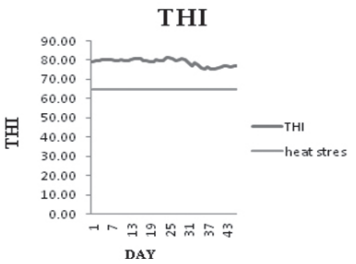

Figure 1 Climate condition during the experimental period.

Dry matter intake tended to be higher (P=0.06) in cows received 75:25 corn silage compared with cattle on 50:50 corn silage (Table 2) due to smaller particle size of corn silage compared with alfalfa hay which increases DM consumption in each meal. As a result, body condition score (BCS) of cows fed 75% corn silage were higher than cows fed 50% corn silage (P<0.01). In addition, cattle on HCS diet lost less BCS during the experimental period (P<0.05) because of greater DMI and lower negative energy balance during the early lactation.

Table 2 Effects of low or high corn silage diets on dry matter intake and body condition score of early lactation dairy cows in hot climate condition

Item	Treatments		SE	P-value
	LCS	HCS		
DMI (kg/d)	23.87	24.88	0.35	0.06
BCS	2.60	2.90	0.02	<0.01
BCS change	-0.69	-0.09	0.34	0.04

LCS=Low corn silage diet; HCS=High corn silage diet; DMI=Dry matter intake; BCS=Body condition score.

The results showed that (Table 3) there were not any significant differences between treatments in blood glucose or triglyceride concentrations. However, the concentration of blood urea nitrogen was lower in HCS diet compared with LCS (P<0.01).Lower BUN may increases reproductive performances in early lactation cows.

Table 3 Effects of low or high corn silage diets on blood parameters of early lactation dairy cows in hot climate condition

Item	Treatments		SE	P-value
	LCS	HCS		
Glucose (mg/dl)	51.42	50.85	1.79	0.82
TG (mg/dl)	9.54	8.70	0.63	0.37
BUN (mg/dl)	18.00	12.84	1.00	<0.01

LCS=Low corn silage diet; HCS=High corn silage diet; TG=Triglyceride; BUN=Blood urea nitrogen.

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Effects of high corn silage to alfalfa hay diets on productive performances of Holstein dairy cows in heat stress condition

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Keywords heat stress, milk yield, early lactation, corn silage, rumen fermentation

Introduction Suppressed DMI is usually observed after calving in fresh lactating dairy cows. Moreover, hot climates results in heat stress which negatively affects the DMI and consequensly milk yield. In the most dairy farms, corn silage and alfalfa hay are the main forages. The proportion of the two forages may affect DMI and consequently milk yield and composition in dairy cattle especially in early lactation stage.

Materials and methods A hundred and twenty (n = 120) cows with similar milk production (47.5 ± 0.5), days in milk (40 ± 15) and parity (2.48) were allocated to two treatments. In this study two proportion of CS to AH (LCS=50% alfalfa hay and 50% corn silage; HCS=25% alfalfa hay and 75% corn silage) were examined. Feed were offered as TMR in equal part (0500 and1700) as ad libitum. Chemical composition of the experimental diets is shown in Table 1. The experimental period was 40 days. The cattle were milked 4 times daily. DMI, rumen sample, milk yield and milk composition were analyzed on the last week of the experiment.

Table 1 Ingredients and chemical composition of experimental diets

	Treatments	
Item	LCS	HCS
Alfalfa hay	19.04	8.49
Corn silage	16.46	26.45
Beet sugar pulp	5.37	5.39
Barley	11.26	11.97
Corn grain	16.51	13.98
Wheat	3.48	3.49
Cottonsead	4.62	4.64
Wheat bran	0.00	1.59
Mollasses	2.41	1.55
Fat	1.59	1.59
Fish meal	1.47	1.47
Soybean meal	10.66	12.49
Soybean roasted	2.34	2.34
Basmitomacs (bypass soy protein)	1.07	1.07
Min-vit	3.41	3.41
Chemical composition		
NDF	31.14	32.58
Fat	4.91	4.91
NEl	1.68	1.72
CP	17.17	16.93
NFC	43.00	42.00

LCS=Low corn silage diet; HCS=High corn silage diet;

Results and discussion Mean temperature humidity index (THI) was 80 during the experimental period and cattle were on heat stress. Dry matter intake tended to be higher (P=0.06) in cows recived 75:25 corn silage compared with cattle on 50:50 corn silage (Table 2) due to smaller particle size of corn silage compared with alfalfa hay which increases DM consumption in each meal. As a result, milk yield was increasedin cows fed 75% corn silage in comparision with cows fed 50% corn silage (P<0.01). However, there were not any significant differences between treatments in milk fat or milk protein concentration.

Table 2 Effects of low or high corn silage diets on milk production and composition of dairy cows in hot climate condition

	Treatments			
Item	LCS	HCS	SE	P-value
DMI (kg/d)	23.87	24.88	0.35	0.06
Milk (kg/d)	44.23	47.22	0.68	<0.01
Milk fat (%)	3.73	3.56	0.16	0.46
Milk protein (%)	2.81	2.78	0.06	0.78

LCS=Low corn silage diet; HCS=High corn silage diet; DMI=Dry matter intake

The results showed that (Table 3) there were not any significant differences between treatments in rumen fermentation parameters. However, ratio of acetate to propionate tended to be lower (P=0.14) in HCS diet compared wwith LCS which consequently resulted in numerically lower milk fat percentage in HCS cattle.

Table 3 Effects of low or high corn silage diets on milk production and composition of dairy cows in hot climate condition

	Treatments			
Item	LCS	HCS	SE	P-value
pH	6.31	6.13	0.12	0.33
Total VFA	90.15	83.23	4.79	0.32
Acetate (mmol/L)	57.57	51.53	2.90	0.17
Propionate (mmol/L)	18.05	18.33	1.56	0.90
Butyrate (mmol/L)	10.46	9.65	0.43	0.21
Acetate/Propionate	3.18	2.87	0.18	0.14

LCS=Low corn silage diet; HCS=High corn silage diet;

Conclusions Increasing corn silage from 50% to 75% of forage portion of diet in early lactating cows in hot climate condition improved DMI and milk yield.

Effect of *Bt* maize and feed-out strategy on silage aerobic stability and performance of lactating dairy cows

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Keywords aerobic phase, animal production, manual unloading, mechanical unloading

Introduction The method chosen for unloading depend on the storage itself, type of silo, type of silage and scale of operation. Depending on silage characteristics (silage density, removal rate, fermentation and final products) the aerobic deterioration can take place or not. Corn silages from hybrids with *cry*1Ab gene can reduce aerobic deterioration due to higher defense of the plant against armyworm, whereas the insect damage of plants cause disruption of the protective cell wall and creates entry points for molds in the field. Mechanical unloading also can avoid extended silage aerobic spoilage for reducing the ingress of oxygen into the silage mass. Although the pitchfork may still be used, manual unloading has been replaced by mechanical systems in most farms. Nowadays, although some farmers use hybrids with *cry*1Ab gene (*Bt* maize) and have the skills to produce good silages, they keep making mistakes in management practices in silage feed-out. This study aimed to evaluate the milk yield and silages aerobic stability from different hybrids under manual or mechanical unloading.

Material and methods This research was carried out at the São Paulo State Agency Agribusiness Technology of Secretary of Agriculture and Food Supply. The hybrid DKB 390 from Dekalb and AG 8088 from Agroceres containing the *cry*1Ab gene from *Bacillus thuringiensis* (*Bt*) and its isogenic without the *cry*1Ab gene were used. Whole plants were harvested (35% DM) and packed into lab and farm scale silos to reach a bulk density of 600 kg m^{-3}. Two horizontal silos with side walls and capacity to store 120 t per hybrid, were used to make silages from DKB 390 (with or without *cry*1Ab gene). The removal rate was 0.20 m from both silo faces, measured by tape beside the silo. The management practices during feed-out by pitchfork or mechanical system (Totalmix®, Casale) were used for feeding two groups of the animals during 28 days each one. Twenty six Jersey cows in pairs according calving date, about 100 days after calving, were assigned into two groups: feeding with conventional DKB 390 silage (n=13) or feeding with transgenic DKB 390 silage (n=13) for 56 days to compare the milk yield, that was recorded twice daily in three consecutive days, every 14 days. A paired t-test was used to determine whether there were significant differences between the two treatments in factorial arrangement 2 × 2 (conventional or transgenic hybrid and silage unloading by pitchfork or mechanical system). To determine the silage aerobic stability, silages from hybrids DKB 390 and AG 8088 from lab silos, with or without the cry1Ab gene, were exposed to air after 186 days of fermentation and homogenized in a polystyrene box, in a climatic chamber kept at 25 ± 1ºC to determine the temperature, and samples were collected for pH measurement values. Thermometers were inserted 10 cm into the silage mass, the temperature were recorded at every 5 min and pH values twice daily lasting 5 days. The variables measured were: aerobic stability, defined as the time in hours to rise the temperature by 2ºC in relation to

environmental temperature (h T>2ºC), maximum temperature reached by the mass in ºC (T Max), hours to reach maximum temperature (h T Max) and heating rate measured as T Max / min T Max (HR). The data were analyzed according to a repeated measures model in a complete randomized design with five replications, in factorial arrangement 2 × 2.

Results and discussion There was significant effect of hybrid on silage aerobic stability (Table 1). The AG hybrids and transgenesis decreased the aerobic deterioration. No significant interactions were found between hybrids or feed-out system on daily milk yield. There was higher milk yield with mechanical unloading than manual unloading by pitchfork. There was higher milk yield of cows feeding silage from transgenic hybrid than cows feeding silage from conventional hybrid. These results showed that spoiled silage could have negative consequences with substantial energy loss and great economic impacts on feed quality and animal production. The total mix rations analysis, expressed on a 100% dry matter basis, to pitchfork or mechanical system feed-out, were: 11.43 vs 13.39 of crude protein; 1.34 vs 1.61 of ether extract; 30.82 vs 24.06 of acid detergent fiber (ADF); 54.92 vs 43.94 of neutral detergent fiber (NDF); 55.94 vs 60.09 of soluble carbohydrates content; and 60.29 vs 64.30 of total digestible nutrients. The NDF and ADF values increased during the aerobic exposure due soluble carbohydrates losses.

Conclusions Transgenic silages and mechanical feed-out resulted in decreased aerobic deterioration and improved milk yield.

Table 1 Milk yield and aerobic stability using different hybrids and feed-out systems

	Hybrid		GMO			Probability		
	DKB	AG	Control	*cry*1Ab	SEM	HYB	GMO	Inter
Aerobic stability (h)	20.1	26.0	18.9	27.2	3.31	<0.01	<0.01	0.16
T Max (ºC)	42.7	41.7	43.4	41.0	2.74	0.47	0.11	0.91
Time T Max (h)	27.0	33.4	28.2	32.2	4.80	0.02	0.12	0.09
Heating rate	1.59	1.29	1.54	1.34	0.19	<0.01	0.06	0.12
pH 32 h	3.90	3.72	3.90	3.73	0.26	0.13	0.16	0.23
pH 48 h	5.42	4.89	5.49	4.82	0.78	0.14	0.07	0.44
	Dekalb 390		Feed-out System			Probability		
	GMO	nGMO	Mechanical	Fork	SEM	GMO	Feed-out	Inter
Milk yield (kg/d)	16.8	14.6	16.3	15.0	2.18	<0.01	0.04	0.53

HYB: effect of the hybrids (DKB x AG); GMO: effect of transgenic versus conventional hybrid; Mechanical: effect of mechanical feed-out system; Inter: interaction between Hybrid and GMO or GMO and Feed-out system.

Effects of sealing strategies of corn silage and addition of sodium benzoate on the nutritive value of total mixed ration for dairy cows

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Keywords chemical additive, corn silage, aerobic deterioration, milk yield, milk quality

Introduction When exposed to air, silage is deteriorated by spoiling microorganisms. Therefore, sealing strategies are critical in order to preserve nutrients and hygienic quality of silages. Additionally, additives may reduce aerobic deterioration of silages and their total mixed rations (TMR). Thus, the objective of this study was to evaluate the performance of dairy cows fed diets based on corn silage from horizontal silos using two sealing strategies, with or without the addition of sodium benzoate onto the total mixed ration.

Materials and methods This trial was conducted at the Department of Animal Science, "Luiz de Queiroz" College of Agriculture, University of São Paulo. Corn crop was harvested at 35% of dry matter (DM) and packed in horizontal silos (40 t capacity). 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 a lactation trial started. Every day, the inedible silage was quantified and only the visually edible silage fed to dairy cows. Twenty late-lactation Holstein cows, housed in a Tie-stall barn, were allocated in five Latin squares (4 × 4) with periods of 21 days (14 d adaptation). Twice a day, four dietary treatments were prepared: 1) BG, 2) BZ, 3) BG plus sodium benzoate (1.5 g/kg TMR as fed), and 4) BZ plus sodium benzoate (1.5 g/kg TMR as fed). Dietary sodium benzoate was diluted in water (1:3) and sprayed onto the TMR during the mixture immediately before feeding (0800 and 1800h). The TMR were formulated to contain 53% corn silage, 8% whole cottonseed, 18% soybean meal, 9.5% citrus pulp, 9% dry corn meal, and 2.5 % vitamin and mineral premix. Dry matter intake (DMI), milk yield and composition were recorded from d-15 to d-21 in each experimental period. Data were analyzed using the MIXED procedure of SAS.

Results and discussion The BG silage was better preserved than BZ silage, since the visual top losses as a proportion of the silo working face was lower in BG (1.54% of inedible silage) than in BZ silage (6.73% of inedible silage) (data not shown). Bagasse provided a better adherence of plastic film to ensiled mass and protected the film from direct sunlight incidence, decreasing oxygen permeability. Even the antimicrobial effect of sodium benzoate applied only onto the mass surface was not enough to control silage deterioration. Contrary, Da Silva et al. (2014) reported a significant reduction in DM loss on 30-cm top layer treated with sodium benzoate (2 g/kg as fed) compared with the control. In that trial, the higher volume of applied solution (13 L/m^2) allows the benzoate to percolate and might be a plausible explanation for dissimilar results compared to the current study. Even after discard the inedible silage, BZ resulted in lower DMI compared with BG, whereas there were no interaction between silo sealing and benzoate addition

on TMR (Table 1). Milk yield and milk composition were similar across treatments. The lack of response in milk yield based on the increased DMI for BG, might be due the physiological state of cows, 252 days in milk at the beginning of the trial (late-lactation). The higher energy balance experienced by the cows fed BG diets (data not shown) may have be deposited as body reserves.

Conclusion Covering the plastic film with sugarcane bagasse decreased the aerobic deterioration of corn silages stored in bunker silos and improved the dry matter intake of total mixed rations containing those silages. Addition of sodium benzoate on total mixed rations had no effect on animal performance and milk composition.

Table 1 Influence of sealing strategy of horizontal silos and addition of sodium benzoate onto the TMR on the performance of dairy cows

	Bagasse over the film		Benzoate under the film			*P*-value[2]		
Item[3]	C[1]	Benz[1]	C	Benz	EPM	S	B	S × B
DMI, kg/d	21.8	21.31	20.5	20.52	0.45	<0.01	0.43	0.38
Milk, kg/d	25.70	25.99	25.83	26.13	25.9	0.76	0.50	0.98
FCM, kg/d	26.33	27.13	26.93	26.43	26.7	0.92	0.77	0.23
Fat, %	3.69	3.61	3.70	3.68	0.10	0.58	0.48	0.75
Protein, %	3.53	3.52	3.49	3.50	0.07	0.34	0.94	0.73
Casein, %	2.70	2.67	2.66	2.66	0.06	0.45	0.60	0.71
Lactose, %	4.42	4.41	4.38	4.37	0.05	0.22	0.56	0.99
Urea N, mg/dL	12.96	12.99	12.59	11.97	0.53	0.11	0.49	0.45

[1] C: control (without addition of sodium benzoate onto the TMR), Benz: sodium benzoate (1.5 g/kg TMR as fed).
[2] Effects of S (sealing strategy), B (dietary benzoate) and interaction S×B.
[3] DMI: dry matter intake; FCM: 3.5% fat corrected milk yield.

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Animal performance and fermentation characteristics of maize silage treated with an inoculant containing *L. kefiri*, *L. brevis* and *L. plantarum*

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Keywords inoculant, dairy, milk yield, mini-silo, *L. kefiri*, *L. brevis*, *L. plantarum*, maize silage

Introduction In 2013, *L. kefiri* DSM 19455 obtained approval from the European commission for commercial use in the European Union (commission implementing regulation, 2013). The novel strain was added to the formulation of a commercial silage inoculant with the aim to improve aerobic stability. We tested the combination of three strains, two hetero-fermenters *(L. kefiri* DSM 19455 and *L. brevis* DSM 23231) and one homo-fermenter (*L. plantarum* DSM 19457), for their influence on the fermentation of maize silage and their effect on the performance of lactating dairy cows.

Materials and methods Whole crop maize with 35.5% dry matter (*Zea mays* L.; Baxxos FAO 200 variety) was inoculated with a silage inoculant (Biomin® BioStabil Mays, Biomin, Austria) containing *L. plantarum* DSM 19457 (2×10^4 cfu/g forage), *L. brevis* DSM 23231 (3×10^4 cfu/g forage) and *L. kefiri* DSM 19455 (5×10^4 cfu/g forage). A negative control was sprayed with an equal amount of water. Silage was prepared in mini-silos with 5 replications and in 2 bunker silos. Silage from the bunker silos was fed to 36 dairy cows (18 per treatment) for 92 days. The animals were selected from a 160 cow herd allowing selection of matched pairs for parity, days in milk, milk yield, weight and body condition score.

Results and discussion The silage inoculant significantly improved the silage quality in the mini-silos as shown in Table 1. When comparing the pH after 2 and after 90 days it can be concluded that the inoculant lowered the final pH, and more rapidly reduced the pH at the onset of fermentation. This could be the main factor in reducing the number of spoilage organisms and resulting in lower butyric acid, ethanol and ammonia levels as described in Table 1. Butyric acid levels were comparable to previously published results (Mari et al. 2009). As the production of these spoilage products consumes energy, the dry matter losses and NEL (GfE 2009) value would be improved in the inoculated silage. Even though the acetic acid level did not significantly increase, the inoculant doubled the aerobic stability. The average feed intake and milk yield over the entire trial period did not significantly differ. However when analyzing the data per week, the feed intake and milk yield were significantly improved in the final weeks of the trial.

Conclusions The novel formulation of biological silage inoculant containing the recently EU-authorized *L. kefiri* DSM 19455 significantly improved the fermentation quality of whole plant maize silage, lowered the fermentation losses and prolonged the aerobic stability.

Table 1 Maize silage quality with and without biological inoculant as assessed in mini-silos and in large scale bunker silos

Parameter	Unit	Mini-silo		Bunker silo	
		Control	Inoculant	Control	Inoculant
pH after 2 days		4.32[a]	4.02[b]		
pH after 90 days		3.94[a]	3.85[b]	3.86[a]	3.74[b]
Lactic acid	g/kg DM	43.4[a]	58.4[b]	45.8[a]	63.3[b]
Acetic acid	g/kg DM	17.0	18.7	19.5	21.1[b]
Butyric acid	g/kg DM	0.39[a]	0.05[b]	0.09[a]	0.02[b]
Propionic acid	g/kg DM			0.14[a]	0.25[b]
Ethanol	g/kg DM	10.6[a]	6.0[b]	9.52[a]	5.26[b]
Ammonia-N	% N	4.7[a]	3.1[b]	5.5[a]	4.0[b]
NEL	MJ/kg DM	6.88	6.93	6.88	6.95
DM losses	%	6.89[a]	4.63[b]	8.74[a]	5.14[b]
Aerobic stability	hours	90[a]	228[b]	90[a]	198[b]
Yeast	log cfu/g FM			3.54[a]	2.45[b]
Molds	log cfu/g FM			3.22[a]	2.18[b]

Means within a row with different superscripts differ significantly ($P<0.01$).
NEL means net energy for lactation and is calculated according to GfE 2009.

Table 2 Animal trial results for feed and milk parameters

Feed parameter	Control	Inoculant	Milk parameter	Control	Inoculant
Dry matter intake (kg /d)	12.00	12.21	Milk (kg/d)	24.4	25.1
NEL intake (MJ/d)	124	127	ECM (kg/d)	24.8	25.9
FCR (NEL MJ/kg ECM)	4.99	4.90	Fat (%)	4.12[a]	4.20[b]
Feed efficiency (ECM/DMI)	1.43[a]	1.47[b]	Protein (%)	3.16	3.18

Means within a row with different superscripts differ significantly ($P<0.01$).
NEL, net energy for lactation (GfE 2009); FCR, feed conversion ratio; ECM, energy-corrected milk; DMI, dry matter intake

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Performance of lambs fed inoculated corn silage associated with amylolytic enzymes at feeding

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Keywords alpha-amylase, average daily gain, dry matter intake, microbial inoculant

Introduction The starch digestion in the rumen is considered one of the most important factors that determine ruminant performance fed diets with high grain concentration (Britton & Stock, 1986; Huntington, 1997). The processing characteristics, starch source, chemical and heat treatments, in addition to interactions between rumen bacteria, type of diet, rate of passage and addition of additives that modify the ruminal microbiota composition may improve the rate degradation (Corona et al, 2006). Thus the use of amylases as well as the association with inoculated silages has been studied with the aim of improving the conversion efficiency of the diet, decrease production costs and increase the economic returns (Beauchemin et al., 1995; Beauchemin et al., 2003). The aim this research was to evaluate the dry matter intake, average daily gain, feed conversion ratio and final weight of lambs fed corn silage inoculated and associated with amylolytic enzymes added at feeding.

Materials and methods The maize studied was hybrid 2B710 Power Core (Dow AgroSciences), harvested with dry matter content between 33 to 35%. Treatments evaluated were: Control (Untreated); Enzyme (corn silage with enzymes at feeding); Inoculant (corn silage inoculated with 1×10^5CFU of *Lactobacillus plantarum* (MA 18/5U) and 1×10^5CFU of *Bacillus subtilis* (AT553098)); Enzyme and Inoculant (corn silage inoculated with 1×10^5CFU of *Lactobacillus plantarum* (MA 18/5U) and 1×10^5CFU of *Bacillus subtilis* (AT553098) associated with enzymes at feeding). Both inoculants were diluted in distilled water and sprayed on the forage during filling of silos. Alpha amylase from *Aspergillus oryzae* (Amaize, Alltech Inc.) was added at feeding applied at a rate of 2 g/kg of DM total diet. Forty lambs non-castrated males (Texel x Dorper), with average initial body weight of 23.9 kg were used. Animals remained in adaptation for 14 days. Dry matter intake was measured subtracting the orts from the offered. The diets consisted of 40% of the corn silage and 60% of concentrate (ground corn, soybean meal, urea and mineral supplement). Diet was offered twice a day (8am and 4pm hours) to allow *ad libitum* intake (over 10% of the quantity supplied). Animals were weighed after fasting (16 hours) at the beginning and end of the experimental period to obtain the average daily gain. The diet was balanced to daily gain of 250g/ day (NRC, 2007). The data were analyzed according a randomized block design in factorial 2x2 (silagem x enzyme) with ten replicates. All data were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., 237 Cary, NC). Differences between means were determined using the PDIFF, which differentiates means based on Fisher´s F-protected least significant difference test. Significance was declared at $P < 0.05$.

Results and discussion There was no significantly different and no interaction (P>0.05) among treatments in the parameters evaluated (Table 1). Rojo et al (2005) had similar results when lambs were fed diets with α-amylases (*Bacillus licheniformis*) or glucoamylase (*Aspergillus niger*) at concentrations of 0, 1.45 or 2.9 g of enzyme per kg/ DM. Another study also evaluated the effectiveness of feeding glucoamylase to lambs and no difference was observed in feed intake, weight gain or feed conversion (Lee-Rangel et al., 2006). Probably, this is due to insufficient amount of amylase enzyme to promote the breakdown of a large number of starch molecules, not occurring, therefore, changes in rumen fermentation and increased voluntary intake of the diet of animals, such verified by Tricarico et al. (2008). Furthermore, the optimum conditions for activity of certain enzymes, in most cases, are not found in the rumen. Thus, enzymes that act in different pH and temperature that ruminal conditions may be disabled or show low activity when submitted to these conditions.

Conclusions Inoculation of corn silage and association with amylases at feeding of lambs did not promote improvements on dry matter intake, average daily gain, feed conversion and final weight.

Acknowledgement National Council for Scientific and Technological Development (CNPq).

Table 1 Dry matter intake (DMI), average daily gain (ADG), feed conversion (FC) and final weight (FW) of lambs fed corn silage without inoculant (untreated), inoculated with *L. buchneri* and *Bacillus subtilis* associated or not with amylases (Amaise)

	Silages		Enzyme		*P value*			SEM[1]
	Untreated	Inoculated	None	Amaise	Silages	Enzyme	interaction	
DMI (g/day)	1120	1160	1130	1140	0.2620	0.7949	0.5707	0.022
ADG (g/day)	210	230	220	220	0.0842	0.6709	0.8994	0.005
FC (g intake/ADG)	5.27	5.09	5.08	5.29	0.2981	0.2109	0.3970	0.077
FW (kg)	35.5	34.8	34.9	35.4	0.2149	0.4284	0.3567	0.263

*Means followed by different letters in the row differ to 5% of significance. [1]Standard error of the means (%).

Carcass characteristics and meat quality of lambs fed inoculated corn silage associated with amylolytic enzymes at feeding

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Keywords alpha-amylase, carcass yield, microbial inoculant

Introduction The feedlot has been a strategy used to intensify the livestock, especially in dry season, when there is low forage on offer in the pastures. This system is characterized by the use of large amounts of starch in diet of animals, representing up to 80% of the energy cost depending of the level of inclusion. Their quantity as well as the structure is variable in each food, in addition, the starch grain is protected by a protein matrix with structural function, which prevent that solubilize and protect the granule of enzymes and microorganisms attack, limiting digestion. Thus, research has evaluated the use of exogenous amylases as well as their combination with other additives to improve the food utilization and increase the production and quality of the final product. The aim this research was to evaluate the carcass yield and qualitative aspects of meat from lambs fed corn silage inoculated with microbial additives and associated with amylolytic enzymes added at feeding.

Materials and methods The maize studied was hybrid 2B710 Power Core (Dow Agroceres), harvested with dry matter content between 33 to 35%. Treatments evaluated were: Control (Untreated); Enzyme (corn silage with enzymes at feeding); Inoculant (corn silage inoculated with 1×10^5CFU of *Lactobacillus plantarum* (MA 18/5U) and 1×10^5CFU of *Bacillus subtilis* (AT553098)); Enzyme and Inoculant (corn silage inoculated with 1×10^5CFU of *Lactobacillus plantarum* (MA 18/5U) and 1×10^5CFU of *Bacillus subtilis* (AT553098) associated with enzymes at feeding). Alpha amylase from *Aspergillus oryzae* (Amaize, Alltech Inc.) was added at feeding applied at a rate of 2 g/kg of DM total diet. Forty lambs non-castrated males (Texel x Dorper), with average initial body weight of 23.9 kg were used. When the target weight approximately of 35 kg BW was reached, lambs were slaughtered after a 16-hour solid food fasting. The carcasses were weight to calculate the hot carcass yield (HCY). After 24 hours of refrigeration the carcasses were weight to calculate cold carcass yield (CCY). Losses due to cooling (LC) were also calculated. Color and pH were determined in the *Longissimus lumborum* muscle, using a CR-200 Minolta colorimeter (illuminant D65), calibrated to standard white digital and a TESTO 205 pH meter coupled to a penetration electrode, respectively. Meat color parameters L* (lightness), a* (redness) and b* (yellowness) were determined after the meat cut was exposed to the environment for five minutes, according to Cañeque & Sañudo (2000). The data were analyzed according a randomized block design in factorial 2x2 (silages x enzyme) with ten replicates. All data were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., 237 Cary, NC). Differences between means were determined using the PDIFF, which differentiates means based on Fisher´s F-protected least significant difference test. Significance was declared at $P < 0.05$.

Results and discussion There was no significantly different and no interaction (P>0.05) among treatments in the parameters evaluated, less in the b* (Table 1). The pH values observed in this study are in agreement with the literature, about 5.8-5.5 (Osorio et al., 1998). Basso et al. (2014) observed no effect in HCY, CCY and LC in lambs fed with corn silage inoculated. In other study, Brito (2010) evaluated different doses of amylases in diets for lambs and observed quadratic effect for HCY and CCY, but the treated animals didn´t have higher yield that untreated animals. Meat from lambs fed untreated silage and added of amylase at feeding had greater redness, but L and b parameters had no effect. Inconsistent results are found in the literature about carcass characteristics and meat quality of lambs. This is due to the lack of studies involving amylase in diets for lambs and differences among enzyme products and application methods in the diets.

Conclusions Inoculation of corn silage and association with amylase at feeding of lambs did not affect the carcass characteristics and meat quality.

Acknowledgement National Council for Scientific and Technological Development (CNPq).

Table 1 Hot carcass yield (HCY), cold carcass yield (CCY), cooling loss (CL), temperature (ºC), pH and color (L*, a* and b* parameters) of *Longissimus lumborum* muscle of lambs fed corn silage without inoculant (untreated), inoculated with *L. buchneri* and *B. subtilis* associated or not with amylases (Amaise)

	Silages		Enzyme		*P-value*			SEM[1]
	Untreated	Inoculated	None	Amaise	Silages	Enzyme	interaction	
HCY (%)	48.5	47.2	48.2	48.0	0.3151	0.8459	0.7212	0.332
CCY (%)	46.7	46.5	46.9	46.4	0.7406	0.4475	0.8958	0.252
CL (%)	3.6	2.9	2.7	3.8	0.3527	0.1759	0.9269	0.074
T(ºC)	9.5	10.2	10.3	9.5	0.2148	0.1654	0.4700	0.230
pH	5.6	5.7	5.7	5.6	0.0855	0.2773	0.7030	0.039
L*	38.4	37.5	38.2	37.7	0.1506	0.3456	0.5076	0.225
a*	16.9	16.2	16.2[B]	17.0[A]	0.0633	0.0221	0.4151	0.181
b*	1.2	0.8	1.0	1.0	0.0605	0.8391	0.7890	0.086

*Means followed by different letters in the row differ to 5% of significance. [1] Standard error of the means (%).

Performance of lambs fed corn silage inoculated with *Lactobacillus plantarum* and *Bacillus subitilis* in two roughage: concentrate ratios

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Keywords average daily gain, dry matter intake, inoculates.

Introduction The production of lambs in feedlot has been gaining importance in recent years, enabling the use of conserved forages, mainly in the form of silage. Plants with high levels of fermentable sugars like corn, presents adequate fermentation when ensiled, but with significant losses during the fermentation process and silo emptying phase. Thus, the use of additives such as microbial inoculants, has been investigated to reduce such losses and provide highest silage quality. While *Lactobacillus plantarum* produces acids (mainly lactic acid) and decreases the loss of dry matter during fermentation, *Bacillus subtilis* enhances the stability of silages after silo opening, and these important aspects can produce a quality product, once the nutritional value of the diet is directly linked to animal performance.

Materials and methods The maize hybrid 2B710 power core (Dow AgroSciences) was harvested when the dry matter content reaches between 33-35%, chopped 1 cm, and silage inoculated or not (control) with *Lactobacillusplantarum* (LP) associated with *Bacillus subtilis* (BS) ($1x10^5$cfu / g forage) (remained closed for 90 days) and provided the animals associated with two forage: concentrate ratio (60:40 and 40:60). The inoculum was diluted in distilled water and sprayed on the forage 20 tons during silo filling. Forty males non castrated (DorperxTexel), with initial body weight of 23.9 kg were used in this study. The animals remained in the adaptation during 15 days, beginning the experiment thereafter. The dry matter intake was measured by subtracting the remains of the offered. The diets were offered composed of corn silage inoculated with *Lactobacillus plantarum* and *Bacillus subtilis* or not, and concentrate (soybean meal, corn, urea and mineral salt) in two silage forage: concentrate ratio (60:40 and 40:60). The diets will be provided twice daily, at 8 (50% supply) and 16 hours (50% of the supply), to allow *ad libitum* feeding and 10% in relation to the leftovers provided. Animals were weighed after fasting (16 hours) at the beginning and end of the experiment to obtain the average daily weight gain. The diet was formulated to daily gain of 250 g/d (NRC, 2007). Feed conversion was calculated. Animals were slaughtered with approximately 35 kg. Data were analyzed according to a randomized block design, in a 2×2 factorial design (two silages and two forage: concentrate), with ten repetitions. All data were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., 237 Cary, NC). Differences between means were determined using the PDIFF, which differentiates means based on Fisher´s F-protected least significant difference test. Significance was declared at $P<0.05$.

Results and discussion The dry matter intake was not affected by silage and concentrate level (Table 1). The average daily gain (ADG) was affected ($P <0.05$) by inoculation of

corn silage with LPBS (ADG untreated = 210 g / day; LPBS = 230 g / day) and forage: concentrate ratio (60: = 40 230 g / day; 40: 60 = 230 g / day) (Table 1). The higher weight gain in lambs fed corn silage with LPBS, maybe explained by increase in fiber digestibility of silage. Kung and Muck (1997) concluded that the homofermentative bacteria reduces the loss of DM to the minimum level (2-3%), while low pH, decreasing the proteolysis and formation of ammonia, lactic acid and increase the digestibility also.

Conclusion The inoculation of corn silage with *Lactobacillus plantarum* and *Bacillus subtilis* improved the animal performance.

Table 1 Feed conversion (FC), dry matter intake (DMI), avarage daily gain (ADG) and final body weight (FBW) of lambs fed maize silage without inoculant (untreated) and inoculated with Lactobacillus plantarum and Bacillus subtillis and two concentrate levels (40 and 60%)

	Silages		RO:CO		P-value			
Variables	Untreated	LPBS	40:60	60:40	Silage	VO:CO	Interaction	SEM
FC (g intake/ADG)	5.34	4.96	5.21	8.09	0.09	0.59	0.39	0.111
DMI (kg/day)	1.13	1.15	1.14	1.13	0.58	0.53	0.85	0.025
ADG (kg/day)	0.21[b]	0.23[a]	0.22[b]	0.23[a]	0.04	0.74	0.67	0.006
FBW (kg)	34.85	35.87	35.18	34.74	0.44	0.14	0.98	0.221

Meat quality of lambs fed corn silage inoculated with *Lactobacillus plantarum* and *Bacillus subtilis*

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Keywords meat quality, inoculants

Introduction In a meat production system, the quantitative and qualitative characteristics of carcass are fundamental importance because are directly related to the final product (Silva Sobrinho et al., 2008). As the consumer market requires homogenous production throughout the year, the strategic use of conserved forage and termination of animals in feedlot system are increasingly common. The use of inoculants on silage has the primary function the preservation of nutrients, and improves animal performance. The feed interferes with the carcass characteristics and meat composition, mainly in adipose tissue ratios relative to muscle. Studies with alternative foods may allow obtaining meat quality and reduce the slaughter age, thereby improving yields and carcass finish.

Materials and methods The 2B710 power core (Dow AgroSciences) hybrid was collected in 33-35% of DM, chopped, and ensiled inoculated or not (control) *with Lactobacillus plantarum* (LP) associated with *Bacillus subtilis* (BS) (1×10^5cfu/g forage) (remaining closed for 90 days) and provided the animals associated with two forage: concentrate ratio (60:40 and 40:60). Forty males non castrated (DorperxTexel), with initial body weight of 23.9 kg were used in the study. The animals remained in the adaptation during 15 days, beginning the experiment after this period. The diet contained 60% silage and 40% concentrate (ingredients: soybean meal, corn, urea and mineral salt) and has been formulated for daily weight gain of 250g / d. The lambs were housed in individual pens and fed *ad libitum* twice daily (08:00 and 16:00). The animals were slaughtered when they reached the target weight of approximately 35 kg, after 16 hours of fasting of solid foods. After 24 hours in the refrigerator, the carcasses were split lengthwise and the loins removed from socks half carcasses. Color, pH and temperature were determined using a colorimeter Minolta CR-200 (illuminant D65), calibrated with digital white pattern and a pH meter and temperature TESTO 205. The color parameters of meat L * (lightness), a * (redness) and b * (yellowness) were determined after cutting the meat was exposed to the environment for five minutes, according to Cañeque and Sañudo (2000). To obtain the hot carcass yield, divided the hot carcass weight to body weight at slaughter and multiplied by 100 to obtain and cooling loss, if subtracted from the carcass value cooled for 24 hours of carcass weight hot. Data were analyzed according to a randomized block design, in a 2x2 factorial design (two silages and two forage: concentrate), with ten repetitions. All data were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., 237 Cary, NC). Differences between means were determined using the PDIFF, which differentiates means based on Fisher´s F-protected least significant difference test. Significance was declared at $P<0.05$.

Results and discussion There was no significant difference for the evaluated qualitative

parameters, except for the pH (P<0.05), which remained lower after 24 hours for the animals fed untreated silage (pH 5.52 to untreated silage and 5.61 for silage inoculated with LPBS) (Table 1). The animals fed the control treatment showed higher values for b *, but according Sañudo et al. (2000), b * values are below the expected values (3.38 to 11.10 for b *). The b * value typically determines the amount of yellow which is influenced by the presence of fat beta-carotene. As the quantitative parameters, the hot carcass yield was significantly higher (P <0.05) in animals receiving more concentrated (40-60 was 48.14% and 46.72% 60:40). Yamamoto (2006), states that the carcass weight is influenced by the growth rate, by age at slaughter and the nutritional management. Gonzaga Neto et al. (2006) found that the yields of hot and cold carcass in Morada Nova lambs fed with different forage: concentrate and slaughtered at 17, 21 and 25kg were larger as the amount of concentrate in the diet increased, with average values of 46.9 and 44.9%, respectively. The loss was lower for cooling in animals receiving silage treated with LPBS (LC untreat = 310g and 275g LC = LBPs) (Table 1), this can be related to fat content, which acts as an insulator reducing the speed of cooling housing and chilling loss.

Conclusion The corn silage inoculated with microbial additives in relation 40:60 can be added in the diet sheep can improve yields of hot carcass and provide less cooling losses.

Table 1 Hot carcass yield (HCY), Cooling loss (CL), pH, temperature (T°C) and colors a*, b* and L*of lambs fed maize silage without inoculant (untreated) and inoculated with Lactobacillus plantarum and Bacillus subtillis and two concentrate levels (40 and 60%)

	Silages		RO:CO		P-value			
Variables	Untreat	LPBS	40:60	60:40	Silage	VO:CO	Interaction	SEM
HCY (%)	47.58	47.28	48.14[a]	46.72[b]	0.67	0.05	0.70	0.369
CL (%)	3.10[a]	2.75[b]	2.87	2.98	0.05	0.49	0.81	0.086
pH (24 Hours)	5.52[b]	5.61[a]	5.63	5.51	0.03	0.14	0.14	0.030
T°C	10.02	10.35	10.29	10.09	0.43	0.64	0.06	0.217
a*	16.85	16.37	16.3	16.92	0.25	0.14	0.89	0.204
b*	1.36	0.95	1.02	1.3	0.18	0.35	0.73	0.160
L*	38.64	38.54	38.49	39.69	0.86	0.74	0.27	0.330

Changes in fermentation products of guinea-grass silage exposed to aerobic conditions and subsequent intake by goats

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Keywords silage, aerobic stability, feed intake

Introduction Ensiling of tropical grasses typically results in fermentations characterized by low lactic acid accumulation, high acetic acid production and a high pH. However, limited information is available on aerobic stability of forages fermented in tropical climates and the effect of the length of aerobic exposure (LAE) on their subsequent intake. The objective of this research was to evaluate such effects on the fermentation profile of guinea-grass ensiled at two re-growth stages and intake by meat-type goats

Materials and methods Two identical experiments utilizing guinea-grass (GG) ensiled at 45 d (27.24% dry matter, DM) or 90 d (37.45% DM) of re-growth were conducted to study changes occurring during the course of aerobic exposure on fermentation products and voluntary intake by meat-type goats. At each re-growth stage, harvested GG was ensiled in 16 minisilos of 5 kg each and stored for 30 d. At opening, GG silage samples were exposed to air for 0, 24, 48, and 72h in a 4-compartment wooden feeder. Silage samples for each LAE were analyzed for pH, and fermentation products. To determine the effect of LAE on intake, silage resulting for each LAE was offered during a single day to four goats that previously had not consumed silage. Dry matter intake (DMI) was monitored at the four intake time point of 2, 5, 8, and 24 h (ITP) after first access to the silages (0 h time point). Each experiment included replicated observations. Within experiment, fermentation end-products and pH were analyzed as a single completely randomized design with 4 replicates, while intake was analyzed as a completely randomized design with a 4 (LAE) x 4 (ITP) factorial arrangement of treatments. Tukey-test was used for mean separation.

Results and discussion In GG ensiled at 45 d of re-growth, pH was the highest at 72 h ($P<0.02$) and higher ($P<0.05$) at 48 h than at 24 and 0 h exposure to air (Table 1). Acetic acid content decreased ($P<0.05$) at each successive LAE. Longer aerobic exposure periods also tended to decrease lactic acid ($P<0.7$) and butyric acid ($P<0.07$) contents but, to increase the ratio N-NH_3/Total N as compared to shorter LEA. In GG ensiled al 90 d of re-growth, pH ascended as LAE increased (Table 1). Silage exposed to air during 72 h had lower ($P<0.04$) acetic and butyric acids contents than silage exposed during 0, 24 or 48 h. No effect of the LAE was observed on lactic acid content or on NH_3-N/Total-N ratio. Silage intake expressed as a percentage of silage offered at each stages of GG, no significant interaction LAE*ITP was observed, and averaged 37.31% and 42.87% for 45 and 90 d silage, respectively. In both stages of GG, proportional silage intake was the

lowest (P<0.05) for fresh silage, and the highest in fermented material exposed to air during 72 h and intermediate for the silage exposed to air for 24 or 48 h (Figure 1). In this research, typical fermentation characteristics were observed for GG ensiled at 45 or 90 d of re-growth. The high acetic acid contents typical of tropical silages have been associated with decreased voluntary feed intake. The present results are consistent with this, silage made from GG at both re-growth stages, shower high acetic acid content early in the aerobic exposure period and thereafter a progressive decline, while silage consumption followed the opposite trend. The lower feed intake of GG fermented at 45 d of re-growth might be associated with the higher acetic acid and butyric acid content as compared to GG ensiled at 90 d. Additional research is needed to explore the relationships between LAE and nutritive value of tropical silages including voluntary intake, silage digestibility, and utilization of absorbed nutrients for production purposes.

Table 1 Effects of length of aerobic exposure on pH and fermentation products of guinea grass ensiled at two 45 and 90 d re-growth stages

Component	Length of Aerobic Exposure (h)	Re-growth stage 45 d	*P*	Re-growth stage 90 d	*P*
pH	0	4.62^{c}	0.02	4.98^{d}	0.01
	24	4.69^{c}		5.24^{c}	
	48	5.25^{b}		5.43^{b}	
	72	5.53^{a}		5.95^{a}	
Lactic Acid[1]	0	0.22^{z}	0.07	0.54	0.39
	24	0.35^{z}		0.57	
	48	0.11^{y}		0.46	
	72	0.11^{y}		0.47	
Acetic Acid[1]	0	2.66^{a}	0.03	1.18^{a}	0.04
	24	2.01^{b}		0.85^{b}	
	48	1.11^{c}		0.58^{c}	
	72	0.89^{d}		0.31^{d}	
Butyric Acid[1]	0	1.08^{a}	0.07	0.38^{a}	0.04
	24	1.16^{a}		0.24^{b}	
	48	1.15^{a}		0.20^{b}	
	72	0.82^{b}		0.09^{c}	
N-NH_3/N-Total	0	0.42^{y}	0.09	0.55	0.18
	24	0.69^{z}		0.67	
	48	0.67^{z}		0.57	
	72	0.60^{z}		0.55	

[1]*Dry matter basis.*
a, b Means with unlike superscripts in the same column differ P<0.05.
y,z Means with unlike superscripts in the same column differ P<0.15.

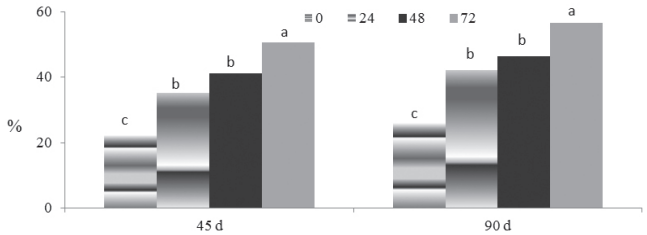

Figure 1 Effect of length of aerobic exposure in guinea-grass ensiled at 45 or 90 days of re-growth on intake as proportion of total silage offered to meat-type goats .

Conclusion The LAE affected the fermentation end-products of GG ensiled at 45 or 90 d of re-growth. For both re-growth stages of GG, silage intake was higher as LAE increased.

Effects of ensiling total mixed ration containing chopped dry corn stover on the fermentation quality and digestibility in sheep

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Keywords corn stover, digestibilty, fermentation, silage, TMR

Introduction Dry corn stover is an abundant by-product of corn grain harvesting, although there is some used as roughage for ruminants by general methods, such as grazing in the field; some is used to burn for cooking or warming by baling or stacking after drying and some used as compost; but unsuitable disposal leads to wasted resources, and possible environmental problems. Demand is increasing for deficient use of crop residue including corn stover due to economic and environmental concerns. Cao et al. (2009) reported that total mixed ration (TMR) silage had high lactic acid content and be of good quality, and unpalatable by-products or crop residues can be incorporated into the TMR if their low intake is altered by fermentation. The objective of this study was to determine effects of fermented or fresh TMR containing chopped dry corn stover and Chinese wildrye on digestion, rumen fermentation, and nitrogen retention in sheep.

Materials and methods Corn stover was cut at 10, 30 or 50 mm length to prepare TMR. The proportions of ground corn grain, soybean meal, concentrate and corn stover were fixed at 200, 80, 120 and 600 g/kg TMR dry mater, respectively. Experimental treatments were non-fermented TMR and fermented TMR. Non-fermented TMR was produced by separating all ingredients, and mixing them before feeding; while fermented TMR was adjusted with water to a moisture content of 550g/kg, and ensiled in a frecon bag (900 mm × 900 mm × 1200 mm) of 972L volume. Air was removed by a vacuum pump and the top of the bag was tied with nylon strings. These fermented TMR were stored outdoors from 15°C to 26°C for 60 days of fermentation. Fermented TMR samples were taken from the top, centre and bottom parts of the frecon bag. These samples were collected in sterilized bags; water extracts were prepared to determine microbial composition and fermentation products. Data on the fermentative characteristics of fermented TMR were evaluated by one-way ANOVA. Data on the chemical composition and digestion were analyzed using a completely randomized design with 2 × 4 (Fermentation × Length) factorial treatment structures. The GLM procedure of SAS ver. 9.0 (SAS Institute, Cary, NC, USA) was used for the analysis, and the model included the main effects of fermentation and length, and any effects of their interactions. Tukey's test was used to identify differences ($P < 0.05$) between the means.

Results and discussion Fermentation procession increased contents of ether extract (EE), aNDFom, and organic acid including lactica acid, acetic acid and ammonia-N but decreased organic matter (OM) and non-fibrous carbohydrate (NFC), and inhibited the

growth of harmful bacteria, such as coliform and aerobic bacteria, molds and yeasts in TMR silage. Moreover, fermentation procession also increased digestibility of EE and aNDFom and digestible crude protein (DCP) of TMR silage, and tended to increase ADG. The TMR with 10-mm corn stover improved the fermentation quality of silage with higher lactic acid, and reduced the cost of NFC during fermentation compared with 50-mm one.

Conclusion The fermentation process can inhibit the growth of molds in TMR and improve the digestion of dry corn stover as forage for sheep. TMR with 10-mm corn stover increased the digestibility of CP and EE, and also improved the fermentation quality of the silage.

Table 1 Fermentation products, pH and viable numbers of microorganisms (cfu/g of fresh matter) of fresh or fermented total mixed ration

	Non-fermented TMR			Fermented TMR		
	10-mm	30-mm	50-mm	10-mm	30-mm	50-mm
LAB	1.3 × 10	1.1 × 10	1.5 × 10	4.7 × 105	3.2 × 105	5.1 × 105
Coliform bacteria	2.4 × 106	3.2 × 106	2.8 × 106	8.7 × 10	4.3 × 102	7.7 × 102
Aerobic bacteria	3.6 × 105	3.3 × 105	2.9 × 105	3.7 × 104	4.1 × 104	5.5 × 103
Molds	7.3 × 103	6.8 × 103	7.1 × 103	ND	ND	ND
Yeasts	8.9 × 106	7.8 × 106	8.1 × 106	4.3 × 105	6.4 × 106	6.3 × 106

LAB, lactic acid bacteria; ND, not detected.

Table 2 Nutrient digestibility and nutrient content in non-fermented or fermented total mixed ration fed to sheep

	Apparent digestibility (%)						
	DM	OM	CP	EE	aNDFom	ADFom	GE
Contrast of non-fermented and fermented TMR							
Fresh TMR	71.9	73.9	66.1	72.4‡	73.2‡	69.5	73.7
Fermented TMR	75.7	78.2	67.1	83.3†	78.5†	75.7	74.8
Contrast of length							
10-mm	77.8	79.9	70.0†	89.6†	80.0	76.6	76.7
30-mm	74.5	76.9	65.9†‡	81.4†	74.8	73.6	74.3
50-mm	73.1	75.9	63.1‡	76.3†‡	73.8	72.3	74.7
P-value							
Fermentation	0.147	0.086	0.174	0.015	0.044	0.050	0.127
Length	0.183	0.143	0.039	0.003	0.324	0.243	0.141
Fermentation × Length	0.570	0.687	0.457	0.375	0.254	0.691	0.572

ADFom, Acid detergent fiber; CP, Crude protein; DM, Dry matter; EE, Ether extract; GE, Gross energy; aNDFom, Neutral detergent fiber; NFC, Nonfibrous carbohydrate = OM − CP − EE − aNDFom; OM, Organic matter.

Effects of aerobic exposure on fermentation end-products of corn and sugarcane silages and intake by goats

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Keywords aerobic stability, tropical silage, fermentation, feed intake

Introduction Aerobic exposure of silage leads to a decrease in its nutritional value and animal voluntary intake. Fermented forages exposed to air during the feeding phase in tropical environments at high temperature might undergo enhanced molding and yeast activities, fostering instability. The objective of this experiment was to determine the effect of length of aerobic exposure (LAE) of corn and sugarcane silages on pH, fermentation end-products, and intake by meat-type goats.

Materials and methods Two identical experiments utilizing fermented corn forage (*Zea mays*; 38.02% DM) and sugarcane (*Saccharum officinarum* 28.75 % DM) were conducted to evaluate the effect of LAE on fermentation end-products and voluntary intake by meat type goats. Each forage species was ensiled in 16-5 kg sealed plastic silos. After 45 d of fermentation, silages were aerobically exposed for 0, 24, 48 and 72 h in a 4-compartment wooden feeder prior to measurements. Silage samples for each LAE were analyzed for pH, and fermentation products. At the 0 h time point, dry matter intake (DMI) was determined during one day using four goats that previously had not consumed silage. Silage consumption for all LAE was monitored at 2, 5, 8, and 24 h hours after feeding (HAF). Each experiment includes 4 replicates (a total of four 4-compartment wooden feeder. Within experiment, fermentation end-products and pH data were analyzed as a simple completely randomized design, while intake was analyzed as a completely randomized design with a 4 (LAE) x 4 (AAF) factorial arrangement of treatments. Tukey-test was used for mean separation.

Results and discussion In corn forage silage, total acidity (inverse of pH), lactic acid content, and ratio of lactic acid:acetic acid values tended to be lower ($P<0.13$) in silages exposed to air for 72 h than in fresh silage, but similar to those in silages exposed for 48 and 24 h (Table 1). Acetic acid content was highest in fresh CS ($P<0.01$) and higher in 24 and 48 h LAE than in 72 h. Corn silage exposed during 48 h had ($P<0.05$) NH_3-N/Total-N ratio similar to the 24 LAE silage, but higher than fresh or 72 h LAE silages. Corn silage intake as a proportion of total silage offered was similar among treatments with regard to LAE, HAF, and their interaction, and averaged 68.3%. Sugarcane, silage exposed to air after 72 h tended to be higher in pH ($P<0.11$) and lower in lactic acid content ($P<.09$), and higher in N-NH_3/N-total ratio than 0, 24 and 48 h LAE silages (Table1). The longest LAE resulted in silages with the lowest acetic acid content ($P<.03$) and highest lactic acid/acetic acid ratio ($P<.03$) relative to 0, 24 and 48 h LAE silages. No significant interaction LAE*HAF on SC silage intake as proportion of silage offered was observed, and averaged 26.96% (Table 2). However, intake of sugarcane silage exposed to air for 72 (41.03%) as percentage of silage offered was higher ($P<0.05$) than fresh (19.75%) sugarcane silage and silages exposed for 24 (21.74%) and 48 (25.82%) h. These two experiments tested the hypothesis that longer aerobic exposure should worsen the silage characteristics and reduce consumption by meat-type goats. As expected, in both silages fermentation products content were influenced by length of aerobic exposure. However, longer aerobic exposure did not affect corn silage intake and even had a positive effect in the case of sugarcane

silage. Voluntary intake of silage by animals is a complex phenomenon under the influence of multiple factors. In the present case varying odor, taste, texture and other sensorial characteristics of the silages might have influence intake by the goats. Additionally, animals were not consuming silage prior to the test. Further research is needed to elucidate the relationships between LAE and animal preference, acceptability, and intake.

Table 1 Effects of aerobic exposure on pH and fermentation end-products of corn and sugarcane silages fermented in a tropical environment

Component	Length of Aerobic Exposure (h)	Corn	*P*	Sugarcane	*P*
pH	0	4.23^{y}	0.13	3.40^{y}	0.11
	24	$4.28^{y,z}$		3.42^{y}	
	48	$4.34^{y,z}$		3.52^{y}	
	72	4.55^{z}		3.71z	
Lactic Acid (%)[1]	0	4.09^{z}	0.12	3.66^{z}	0.09
	24	3.97^{y}		3.73^{z}	
	48	3.75^{y}		3.95^{z}	
	72	2.99^{y}		3.02^{y}	
Acetic Acid (%)[1]	0	0.79^{a}	0.01	3.99^{a}	0.03
	24	0.57^{b}		$3.32^{a,b}$	
	48	0.65^{b}		2.98^{b}	
	72	0.42^{c}		2.52^{c}	
Lactic/Acetic ratio	0	5.20^{y}	0.12	0.91^{b}	0.02
	24	6.97^{z}		1.12^{b}	
	48	5.90^{y}		1.32^{b}	
	72	7.07^{z}		1.59^{a}	
N-NH_3/N-Total	0	5.41^{b}	0.02	4.30^{y}	0.11
	24	$6.25^{a,b}$		4.52^{y}	
	48	8.73^{a}		4.51^{y}	
	72	5.77^{b}		5.82^{z}	

[a,b] Means with unlike superscripts in the same column differ P<0.05.
[y,z] Means with unlike superscripts in the same column differ P<0.15.

Table 2 Interaction between of length of aerobic exposure and hours after feeding on corn and sugarcane silages on intake by goats

	Silage intake/Silage offered, %							
LAE (h)	Corn				Sugarcane			
	Hours after feeding				Hours after feeding			
	2	5	8	24	2	5	8	24
0	15.40	34.2	45.8	63.9	13.9	18.0	20.7	26.2
24	19.6	36.4	43.2	72.6	15.8	16.9	26.4	28.3
48	23.2	35.3	440.1	62.5	119.6	23.6	26.7	33.2
72	38.5	58.3	58.3	75.2	35.0	36.8	43.8	46.5

Conclusion The LAE affected the content of the fermentation end-products of both silages but did not influence corn silage intake by goats. Intake of sugarcane silage exposed to air during 72 h was surprisingly higher than that of fresh silage and of silages exposed for 1 or 2 days probably due to lower acetic acid content.

Performance of dairy cows fed sugarcane silages inoculated with epiphytic strain of *Lactobacillus hilgardii* CCMA 0170

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Key words *Lactobacillus hilgardii*, *L. buchneri*, milk yield, silage inoculant

Introduction Sugarcane silages has great concentration of ethanol because sucrose fermentation by yeast (Kung and Stanley, 1982). The use of microbial inoculants at ensiling increases the population of desirable bacteria which resulted in quickly production of organic acids, drop the pH, and control undesirable microbes. The potential responses of the inoculants use in silages are modulated by interaction between strains and forage type (Muck, 1996). Based on that knowledge Ávila et al. (2014) isolated strains of lactic acid bacteria from sugarcane silage. Carvalho et al. (2014) evaluated the fermentation profile and aerobic stability of sugarcane silages with these strains. They concluded that a strain of *Lactobacillus hilgardii* CCMA 0170 has the best results. The improvement in milk production of dairy cows fed silage inoculated is not consistent. Some works shows positive responses (Kung et al., 2003; Taylor et al., 2002) while others did not (Kung et al., 2002; Arriola et al., 2011). To our knowledge any study was made evaluating the performance of dairy cows fed sugarcane silages inoculated with epiphytic strain isolated from the sugarcane crop. The objective of this trial was to evaluate the performance of dairy cows fed sugarcane silages inoculated with the novel strain of *L. hilgardii* CCMA 0170 compared to commercial strain *L. buchneri* NCIMB40788 or without inoculums.

Materials and methods Sugarcane ratton, 12 months old, was mechanically harvester and fine chopped (JF C120, Pinheiro, Itapira, SP). Approximately 9 t of fresh forage were ensiled for each one of the three treatments: silage without inoculums (**Ctrl**) silage treated with *Lactobacillus hilgardii* CCMA 0170 (**LHI**) or silage treated with *L. buchneri* NCIMB 40788 (**LBU**). The final inoculation rates were 5 log ufc/g of fresh forage. Fifteen Holstein cows (336 ± 175 DIM; 632 ± 96 kg BW at trial initiation) were randomly assigned to replicated 3×3 Latin squares. There were 3 periods of 21 day with 14 day of adaptation and 7 day of sample collection. The diets were formulated to contain 30% of forage coming from sugarcane silage and 70 % from corn silage without inoculums. Data were analyzed with the PROC MIXED procedure of SAS 9.3 with the model containing random effect of cow and fixed effect of period and treatment. Treatments were compared with two contrasts Ctrl *vs.* LHI and LHI *vs.* LBU. Significant difference were considered when $P \leq 0.05$, trends when $P < 0.10$ to $P > 0.05$ and weak trends when $P < 0.15$ to $P > 0.10$.

Results and discussion The sugarcane silages had similar compositions along the experiment between inoculated silages, but different composition compared with control silage. The neutral detergent fiber (**NDF**) concentration for Ctrl, LHI and LBU were respectively 68.6 ± 5.2, 64.8 ± 2.5 and 64.4 ± 2.9 % of DM (mean ± standard deviation), water soluble carbohydrates were 81.8 ± 23.4; 101.0 ±17.9 and 93.5 ± 8.6 g/kg of DM

and ethanol were 96.7 ± 22.8; 66.8 ± 17.2 and 62.3 ± 13.9 g/kg of DM. Milk yield tended to increase 0.8 kg/d in the LHI treatment compared to Crtl without increase in dry matter intake (Table 1). No statistically difference for milk yield was observed between LHI and LBU. The milk composition was similar across treatments ($P > 0.15$) except for lactose (Table 1). Milk production is dependent of lactose syntheses in the mammary gland, probably because their osmotic regulation. The blood glucose concentration had a weak tendency to increase in the LHI treatment compared to Crtl (57.9 *vs*. 55.3 mg/dL, $P = 0.13$) which may explain the response in lactose syntheses and milk production. Yield of milk components were higher for LHI treatment (Table 1) compared to Ctrl probably because the higher milk production, since, the milk composition were similar. Digestibility of DM (67.0 %), organic matter (71.5 %) and NDF (51.7 %) were similar across treatments. Cows fed LHI treatment had higher proportion of rumen propionate (22.8 *vs*.21.5 % of total volatile fatty acids) and lower acetate: propionate ratio (3.0 *vs*. 3.3) compared to Ctrl treatment.

Table 1 Performance of dairy cows fed sugarcane silages without inoculum (Ctrl) or inoculated with *L. hilgardii* CCMA 0170 (LHI) or *L. buchneri* NCIMB40788 (LBU)

Item	Ctrl	LHI	LBU	SEM	Treat[1]	Ctrl vs. LHI	LHI vs. LBU
DMI[2], kg/d	16.2	16.0	16.1	0.54	0.92	0.70	0.89
Milk yield, kg/d	18.0	18.8	18.1	1.04	0.15	0.07	0.14
Fat yield, kg/d	0.635	0.685	0.650	0.0401	0.09	0.03	0.14
Protein yield, kg/d	0.600	0.637	0.616	0.0346	0.16	0.05	0.27
Lactose yield, kg/d	0.781	0.845	0.812	0.0525	0.05	0.02	0.19
Solids, kg/d	2.179	2.341	2.239	0.1321	0.05	0.02	0.14
Milk Fat, %	3.62	3.66	3.61	0.125	0.68	0.52	0.41
Milk Protein, %	3.42	3.41	3.40	0.06	0.92	0.74	0.96
Milk Lactose, %	4.42	4.47	4.45	0.069	0.29	0.12	0.56
Milk Solids, %	12.37	12.46	12.39	0.182	0.41	0.22	0.29
ECM[3], kg/d	17.9	18.8	18.0	1.03	0.11	0.06	0.08
Milk yield/DMI	1.12	1.17	1.13	0.064	0.41	0.22	0.29
ECM/DMI	1.05	1.12	1.07	0.064	0.30	0.15	0.26
MUN[4], mg/dL	17.2	17.0	17.6	0.48	0.49	0.59	0.23
BCS[5], 1 to 5	3.3	3.1	3.3	0.11	0.33	0.21	0.19
BW[6], kg	692	691	660	37.3	0.46	0.96	0.29

[1]Probability value for the effect of treatment and contrasts, [2]Dry matter intake, [3]Energy corrected milk, [4]Milk urea nitrogen, [5]Body condition score, [6]Body weigh.

Conclusion Sugarcane silages inoculated with *L. hilgardii* CCMA 0170 improved milk yield without increase dry matter intake and reduced the acetate:propionate ratio in the rumen fluid of dairy cows.

Sorting index and ingestive behavior of beef cattle fed diets containing sugarcane silage with different particle sizes

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Keywords dry matter intake, chewing, eating, physically effective fiber, ruminating

Introduction As strategies to control possible rumen disturbances and increase weight gain rates, the inclusion of forages as physically effective fiber source has been recommended. This concept was applied to diets in order to increase the chewing time, the saliva secretion and its buffering agents. The chewing activity and rumination are not only affected by dietary neutral detergent fiber (NDF) content, but also by the fiber characteristics, such as the particle size. The fiber that promotes chewing is considered physically effective (peNDF). Levels of peNDF to maintain the productive performance are known, however, feed sorting may alter the actual proportion of peNDF intake. The aim of this research was to evaluate the sorting index and ingestive behavior of beef cattle fed diets containing sugarcane silage with different particle sizes.

Materials and methods The sugarcane studied was the RB86-7515 variety, harvested with dry matter level of 395 $g.kg^{-1}$. The treatments evaluated were: sugarcane silage with 5 mm of particle size (T5), sugarcane silage with 10 mm of particle size (T10), sugarcane silage with 15 mm of particle size (T15) and sugarcane silage with 20 mm of particle size (T20). All diets contained roughage: concentrate ratio of 33:67. Diets were offered twice a day (8 am and 4 pm) to *allow ad libitum* intake (over 5% of the supplied quantity). Forty non-castrated male beef cattle (Nellore), with average initial body weight of 395±32 kg were used. The experimental period lasted 99 days, divided into three periods of 28 days, plus 15 days to initial adaption and intake stabilization. The intake of dry matter (DM) and neutral detergent fiber (NDF) were measured by difference from the orts and the offered. Animal behavior was evaluated during 3 days at intervals of 10 minutes between observations, considering eating, ruminating and idle time. The sorting index was evaluated once by period, according Leonardi and Armentano (2003). The data were analyzed using randomized block design with 4 treatments and 10 repetitions. The results were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., Cary, NC) and, if significant, the orthogonal contrasts was used to determine the type of behavior (linear and/or quadratic). Significance was declared at $P < 0.10$.

Results and discussion There was effect ($P<0.10$) to DM intake (Table 1). However, the NDF intake was not affected by the particle size, with average of 3.82 $kg.day^{-1}$. The quadratic effect to DM intake probably was due to the high sorting index of short particles in the diet T20 (Table 2), and the low fiber digestibility of sugarcane silage was probably responsible by maintaining the NDF intake similar between diets. The ingestive behavior was affected by particle size (Table 1). Diets with larger particle sizes increased the eating

time when it was calculated in minutes per kg of DM (P=0.079), but the eating time, in minutes per day, was similar between the diets. It occurred probably due the time used for sorting diet components rather than feed intake. The ruminating time was affected by the diets and follows to the quadratic model. The animals fed with T10 and T15 diets remained greater ruminating time compared to the animals that received the diets T5 and T20 (P<0.10). This result indicates that animals fed the diet T20 ingested similar diet compounds when compared those animals fed the diet T5, because the diet particle size ingested is not the same that was offered. The idle time was affected by particle size (P=0.044). There was a linear effect for all sorting index variables (Table 2). Results of sieves of 19 mm (S1) and 8 mm (S2) showed that diets T15 and T20 led to greater rejection of this portion of the total feed. However, in the sieve of 1.18 mm (S3) the behavior was the opposite, where diets with larger particle sizes (T15 and T20) led to preferential intake of smaller particles sizes.

Conclusions Diets with sugarcane silage with different particle size affected dry matter intake, but did not alter neutral detergent fiber intake. Diets with larger silage particle size increased the ruminating time and preferential intake of smaller particles sizes.

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Table 1 Intake and ingestive behavior of beef cattle fed sugarcane silage with different particle sizes

	Particle size				Effects[1]		SEM[2]
	T5	T10	T15	T20	L	Q	
DM intake (kg.day^{-1})	10.8	9.91	10.2	10.4	0.220	0.025	0.28
NDF intake (kg.day^{-1})	3.87	3.78	3.81	3.83	0.803	0.545	0.11
Eating (minutes.day^{-1})	173	194	182	197	0.258	0.776	11.6
Eating (minutes.kg^{-1} DM)	15.6	18.6	17.5	18.9	0.056	0.511	1.14
Ruminating (minutes.day^{-1})	370	421	424	406	0.056	0.049	16.6
Ruminating (minutes.kg^{-1} DM)	33.3	40.5	40.7	38.8	0.011	0.020	1.69
Idle (minutes.day^{-1})	897	825	834	837	0.025	0.080	19.5

[1]Linear (L) and quadratic (Q) effects. [2]SEM: Standard error of the mean.

Table 2 Sorting index of beef cattle fed sugarcane silage with different particle sizes

	Particle size				Effects[1]		SEM[2]
	T5	T10	T15	T20	L	Q	
Sieve 1 (19 mm)	99.0	102	94.2	92.9	0.011	0.106	2.14
Sieve 2 (8 mm)	101	99.3	91.9	92.2	<0.01	0.528	1.41
Sieve 3 (1.18 mm)	99.3	101	103	103	<0.01	0.361	0.51
Pan	101	99.5	103	103	<0.01	0.104	0.76

[1]Linear (L) and quadratic (Q) effects. [2]SEM: Standard error of the means.

Dry matter intake and ruminal parameters of beef cattle fed diets containing sugarcane silages with different particles sizes

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Keywords acetate to propionate ratio, ammonia nitrogen, neutral detergent fiber intake, pH

Introduction Some sources of fiber could be included into the diet to control rumen acidosis, keeping the rumen pH greater than 5.8 (Van Soest, 1994). The addition of NDF from forage has the effect of raising the pH due to the saliva production (buffering) during rumination. Additionally, the NDF stimulates the MS intake due to the dilution of the energy concentration of the diet consumed per kilogram. On the other hand, the effect of repletion with increased ruminal fiber digestibility to control the intake makes this method disadvantageous. The objective of this trial was to evaluate the dry matter intake and ruminal parameters of beef cattle fed diets containing sugarcane silages with different particle sizes.

Materials and methods The sugarcane studied was the RB86-7515 variety, harvested with dry matter content of 390 g.kg^{-1}. The treatments evaluated were: sugarcane silage with 5 mm of particle size (T5), sugarcane silage with 10 mm of particle size (T10), sugarcane silage with 15 mm of particle size (T15) and sugarcane silage with 20 mm of particle size (T20). All diets contained roughage: concentrate ratio of 33:67. Diets were offered twice a day (8 am and 4 pm) to allow *ad libitum* intake (over 5% of the quantity supplied). Eight non-castrated male beef cattle (Nellore), fistulated in the rumen, with average initial body weight of 595±42 kg, distributed in double 4×4 Latin Square design were used. The experimental period lasted 80 days, divided into four periods of 20 days, with 14 days to initial adaptation, 5 days to measure dry matter (DM) and neutral detergent fiber (NDF) intakes and 1 day to collect rumen fluid. The intakes were measured by difference from the orts and the offered. The rumen fluid was collected with 0, 4, 8, 12 and 24 hours after the morning feeding and it had measured the pH, ammonia nitrogen and volatile fatty acids. The intake data were analyzed as double 4×4 Latin square design. The model for analyzing ruminal parameters data included a repeated measures statement. All data were analyzed using the MIXED procedure of SAS (v. 9.0 SAS Institute Inc., Cary, NC) and, if significant, the orthogonal contrasts was used to determine the type of behavior (linear and/or quadratic). Significance was declared at $P<0.10$.

Results and discussion The DM intake was affected by the silages and decreased linearly with increase of particle size (Table 1). However, the NDF intake had not been affected by the particle size of sugarcane silage, with average of 4.15 kg.day^{-1}. The effect of the dry matter intake was due to the larger particles have stayed longer into the rumen until to be degraded, resulting in lower solids passage rate by rumen (Van Soest, 1994). The average NDF intake between diets, in % of body weight, was 0.70%. This value is lower

than 1.1% recommended by Mertens (1987) to observe ruminal repletion. This low intake occurred due the low quality of sugarcane fiber, which digestibility is around 37% (in DM basis). The ammonia nitrogen and acetic acid concentrations were similar between the diets (P>0.10) (Table 2). There was effect of particle size to propionic acid with lowest concentration at T10 and T15 diets. The butyric acid and total VFA concentrations decreased linearly with increase of particle size. All variables were significantly influenced by sampling time, reaching a peak concentration at 8 hours after the morning feeding. This data shows accumulation of acids in the rumen over the day. There was interaction between treatment and sampling time to acetate to propionate ratio (A:P). Initial A:P values (0 hour) were similar between diets, but during the 4 hours of sampling time, T15 diet had shown higher concentration than T0 and T20, and similar concentration to the T10. The values were not significantly different between treatments with 24 hours of sampling time.

Conclusions Diets with sugarcane silage with different particle size affected dry matter intake, but did not alter neutral detergent fiber intake. Diets with different particle size alter ruminal parameters, but keep the pH value always greater than 5.8.

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Table 1 Dry matter intake (DMI) and neutral detergent fiber intake (NDFI) of beef cattle fed sugarcane silage with different particle sizes

Intake	Particle size				*P*-value	Effects[1]		SEM[2]
	T5	T10	T15	T20		L	Q	
DMI (kg.day^{-1})	12.2	12.0	11.4	11.5	0.045	0.01	0.47	1.32
NDFI (kg.day^{-1})	4.17	4.33	4.00	4.10	0.293	0.32	0.83	0.58

[1]Linear (L) and quadratic (Q) effects. [2]SEM: Standard error of the mean.

Table 2 Ruminal parameters of beef cattle fed sugarcane silage with different particle sizes

Item[1]	Particle Size –T (mm)				Sampling time –H (hours)					SEM[2]	*P*-values[3]		
	T5	T10	T15	T20	0	4	8	12	24		T	H	T×H
pH	6.38	6.46	6.53	6.59	6.90	6.43	6.21	5.99	6.92	0.07	<0.01	<0.01	0.12
NH_3	10.1	10.0	10.3	10.4	8.0	10.4	16.0	9.1	7.3	0.39	0.71	<0.01	0.51
AA	70.3	70.1	67.1	65.7	56.9	75.4	85.0	79.1	45.1	2.42	0.31	<0.01	0.43
PA	21.8	20.3	18.3	19.7	17.3	21.1	24.8	23.0	13.8	1.37	<0.01	<0.01	0.47
BA	16.9	13.3	13.1	13.1	10.8	15.3	19.7	16.5	8.40	0.98	<0,01	<0.01	0.28
VFA_T	113	108	102	102	89.0	116	134	122	70.6	5.60	0.03	<0.01	0.59
A:P	3.3	3.5	3.7	3.3	3.3	3.7	3.5	3.5	3.3	0.16	0.05	<0.01	0.03

[1]NH_3: ammonia nitrogen; AA: acetic acid; PA: propionic acid; BA: butyric acid; VFA_T: total volatile fatty acids; A:P: acetate to propionate ratio. [2]SEM: Standard error of the mean. [3]*P*-value: Particle size (T); Sampling time (H); Interaction between particle size and sampling time (T×H).

Intake and apparent digestibility of nutrients from high moisture triticale grain silage with different additives in sheep

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Keywords *Lactobacillus spp.*, lamb, sodium benzoate, urea

Introduction Among the forms of forage conservation, high moisture cereals grains silage can be an interesting alternative to reduce production costs. Many grains can be used for this purpose but triticale (*X. Triticosecale Wittimack*) has emerged as a good option for winter crop, being an energy substitute for animal feeding as whole plant silage or wet grain. Because these crops are rich in nutrients, it is necessary to be careful on account of the potential instability of these feedstuffs, due to its composition in carbohydrates that are vulnerable to microbial degradation under aerobic conditions. Therefore chemical and microbial additives are used in an attempt to reduce losses during making and use of silage. Due to feed intake and digestibility determine the nutritive value of a given feed, the aim of the study was to evaluate the intake and apparent digestibility of high moisture triticale grains silage ensiled with different additives in sheep.

Materials and methods Triticale (IPR-111) crop was sown and cultivated in Farm School of the University State of Londrina (UEL) in May 2012 for the purpose of harvesting crops in appropriate stadium for ensiling (pasty-dough). The silages were made in 16 shackles of concrete with a capacity of approximately 250 kg (four shackles per treatment). Coast-cross hay was used as forage source for all treatments. The treatments were: 1) high-moisture triticale grain silage - control treatment (HMTG); 2) high-moisture triticale grain silage with enzymatic-bacterial additive (HMTGE); 3) high-moisture triticale grain silage with 0.5% urea (HMTGU); and 4) high-moisture triticale grains silage with 1.5% (as fed basis) sodium benzoate (HMTGB). The enzymatic-bacterial additive was applied as an aqueous solution (8.6 g of product diluted in 2 L/t FM) and contained *Lactobacillus curvatus, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus buchneri, Pediococcus acidilactici, Enterococcus faecium, Lactobacillus lactis* (total count 10^9 cfu/g) plus 4% cellulase enzyme complex base (Lactosilo Gold®). Urea and sodium benzoate were added as powder and homogenized manually with triticale grains. Feed intake nutrient digestibility was measured by total feces collection. The animals had an initial adaptation phase of 14 days, followed by collecting a period of five days. After the first period, the other periods were proceeded adaptation of 10 days, in voluntary intake. Four castrated male lambs with an average weight of 25 kg were housed in appropriate metabolic cages equipped with individually feed troughs, mineral mixture and water. A Latin square 4×4 was used for the experiment, four lambs, four collection periods and four treatments. Samples of food provided and the leftovers were also collected daily for later analysis combined into composite samples animal / treatment / period. The digestibility coefficients were obtained using the system of equations quoted by Coelho da Silva and Leão (1979). Data were subjected to analysis of variance in SAS (2001).

Results and discussion As presented in Table 1 there were no differences (P>0.05) across DM intake and apparent digestibility of high moisture triticale grains silage ensiled with different additives. The best nutrients digestibility of inoculated silages is commonly related to the ability of such additives hydrolyze structural carbohydrates, allowing better use of these carbohydrates (Bumbieris et al., 2009). As the present work deals with a feed grain cereal with low levels of these structural carbohydrates, the possible role of these additives in the fiber was not significant to improve the digestibility and DM intake.

Table 1 Intake and apparent digestibility of nutrients from high moisture triticale grain silage with different additives in sheep

	GRAIN SILAGE + BERMUDA HAY (50%:50%)						
	HMTG	HMTGE	HMTGU	HMTGB	Mean	P-value	CV
DMI/kg BW $^{0.75}$(g)	74.43	72.86	76.73	80.97	76.25	0.55	10.55
DDM %	67.96	69.96	68.34	68.74	68.75	0.28	2.01
DCP %	70.75	70.36	68.97	71.52	71.15	0.08	2.67
DEE %	77.09	76.86	76.90	78.25	77.27	0.90	3.97
DNDF %	54.83	54.93	55.19	56.84	55.45	0.86	6.78
DADF %	57.84	60.74	60.22	59.75	59.64	0.80	7.32
DOM %	70.68	72.10	71.06	71.04	71.22	0.55	1.95

HMTG: High-moisture triticale grains silage - control treatment; HMTGE: High-moisture triticale grains silage with enzymatic-bacterial additive; HMTGU: High-moisture triticale grains silage with 0.5% urea; HMTGB: High-moisture triticale grains silage with 1.5% sodium benzoate; DDM: Digestibility Dry matter; DCP: Digestibility Crude Protein; DEE: Digestibility Ether extract; DNDF: Digestibility neutral detergent fiber; DADF: Digestibility acid detergent fiber; DOM: Digestibility organic matter; DMI: Dry matter intake; BW = body weight; CV= Coefficient of variation.

Conclusions None of evaluated additives were able to improve DM intake or nutrient digestibility of triticale grain silages.

Contribution of feed ingredients to nitrogen in milk, urine and feces, using stable ^{15}N isotope

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Keywords nitrogen partitioning, nitrogen use efficiency, dairy ration

Introduction Of the total nitrogen (N) consumed by dairy cows, 20 to 35% is generally secreted in milk, and the remaining N is excreted in manure (feces and urine). Nitrogen use efficiency (NUE, the percentage of total N consumed that is secreted as milk N) is highly influenced by the amount of N consumed and the sources of dietary protein. Earlier research has focused on impacts of levels of dietary protein, rumen degraded protein (RDP) and rumen undegraded protein (RUP) on milk production, N excretion in manure and environmental N losses. However, little is known about the contribution of specific feed ingredients to milk protein secretion, excretion of N in manure fractions, plant N uptake after manure application to soils, and environmental N losses. Studying the incorporation of ^{15}N from specific feed ingredients into milk, urine and feces should result in useful information on NUE of that specific feed ingredient. In the context of understanding the integrated nature of N use and loss in dairy systems, the objective of the study was to quantify N partitioning and relative amounts of ^{15}N secreted in milk, and excreted in urine and feces after feeding ^{15}N-enriched feed ingredients.

Material and methods Alfalfa silage (AS), corn silage (CS), corn grain (CG) and soybeans (SB) were grown with applications of ^{15}N-enriched fertilizer in the form of ammonium sulfate, ammonium nitrate and urea. After harvest, CG was processed through a hammer mill. The SB grain was de-hulled, flaked, and defatted with hexane to fabricate soybean meal (SBM). The resultant meal was cooked and passed through a hammer mill to simulate commercial heat-processing. Twelve pregnant (mean±SD; 143±37 days carrying calf) multiparous Holstein cows (264±18 days in milk) producing 35 ± 3 kg of milk/d were housed in a tie-stall barn. Cows were fed a pretreatment TMR once a day for 11 days containing (DM basis) 32.5, 31.2, 20.1, 13.5 and 2.7% of AS, CS, CG, SBM, and a mineral and vitamin premix, respectively. On day 12, cows were blocked by milk yield and randomly assigned within block to one of four dietary treatments, which were constructed by replacing AS, CS, CG and SBM at natural abundance of ^{15}N from the pretreatment diet with its homologue ingredient enriched with ^{15}N. As-fed replacement rates were 100, 75, 100 and 50% for AS, CS, CG and SBM treatments, respectively. After 4 days feeding the ^{15}N-enriched TMR, cows were fed the pretreatment non-enriched TMR during days 16 to 19. Total fecal and urine collection was conducted on each cow every 6 hours during days 12 to 19. Feed intake and lactation performance were also measured from day 12 to 19. Total N from feces, urine and milk N was measured by combustion assay (Elementar Vario MAX CN analyzer, Elementar, Hanan, Germany) and ^{15}N using a mass spectrometer (DeltaplusXP Mass spectrometer, Bremen, Germany). Cumulative ^{15}N recovery was modelled using the NLIN procedure of SAS (2012) with a single exponential model, , where y(t) (% of ^{15}N intake) is the cumulative recovery at time t; *a*, *b* and *k* are model parameters representing the intercept (%), the increment over *a* (%) and the fractional

constant rate (1/h), respectively. The conversion of the N in the labeled feed ingredient in milk, urine and feces was determined as the asymptotic recovery of ^{15}N in each of pool. For five out of the twelve cows the exponential model for feces did not converge because after four days of sampling ^{15}N recovery in feces had hardly begun to show signs of plateauing (See Fig 1a). For the seven cows in which the fecal model converged adequately, the sum of the three asymptotes (milk, urine and feces) was approximately 100%. Thus the parameter b for feces was calculated as 100 minus the asymptotic values of the models for urine and milk from the same cow. Data was analyzed using the MIXED procedure of SAS. Treatment was considered a fixed effect and block was considered random effect. Least square means are reported. Significance was declared at $P \leq 0.15$ and tendency for $0.15 < P < 0.20$.

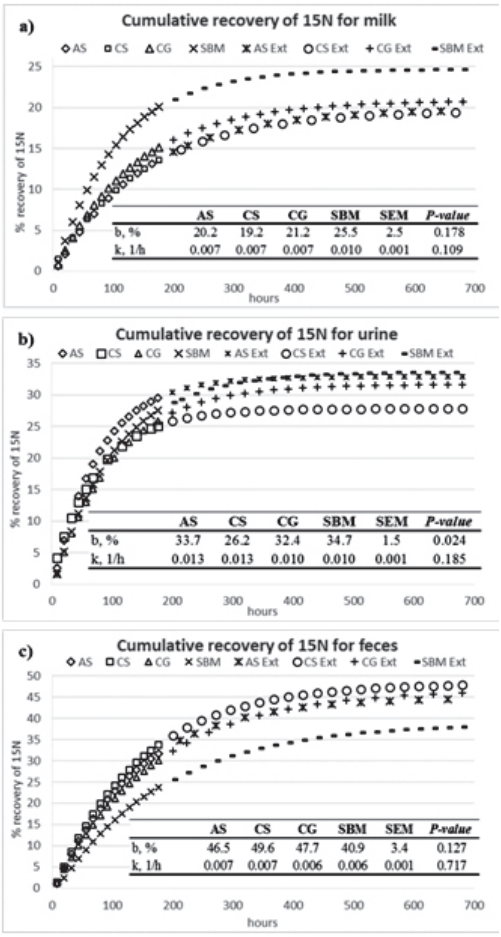

Figure 1 Model estimations of cumulative recovery of 15N from AS, CS, CG and SBM in milk, urine and feces (Ext = extrapolation over the 8-day data collection period).

Results and discussion Corn silage and CG had the highest ^{15}N enrichment (atom % ^{15}N of 1.857 and 2.040, respectively) whereas AS and SBM had the lowest (atom % ^{15}N of 0.730 and 1.385, respectively) due to ^{15}N dilution by the atmospherically-fixed N by these legumes. There was no difference in performance and total N partitioning among treatments (DMI [23.2±2.4 kg/d], milk yield [26.1±5.2 kg/d], N intake [601±61 g/d], protein yield [0.89±18 kg/d], NUE [0.23±0.05] and ^{15}N recovery [66.5±1.4%]), suggesting that the enriched feed ingredients did not differ from their homologues. The model parameter *a* was not different from zero, therefore *b* was considered to be the asymptotic recovery of ^{15}N. Nitrogen in SBM tended to have a greater fraction going into milk and faster rate of appearance when compared with the other ingredients (Figure 1a), which might be due to greater RUP, to a more favorable amino acid profile of the RUP fraction, and/or a greater digestibility shown by a lower excretion in feces (Figure 1c) when compared with the AS, CS and CG. Corn silage-N had lower urinary excretion, even though its rate of appearance tended to be higher, compared to others feeds (Figure 1b). Forages-N appearance rate in urine tended to be higher than for concentrates (Figure 1b).

Conclusions The partitioning of N from different feeds exhibited distinct kinetic behavior. The N fractions of each feed (and amino acid profile of the digested protein) likely played a role in N appearance in either milk, feces or urine.

Use of internal markers to estimate digestibility in sheep

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Keywords indigestible ash, indigestible fiber, indigestible dry matter, silage

Introduction The use of markers is an alternative method to the direct method (total collection of feces), the evaluation of digestibility, which has some advantages its simplicity and convenience as the animals need not be in metabolic cages and does not require the handling of large amounts of material. Internal markers provide the advantage of being indigestible components present in the feed, without therefore the necessity of previous preparation. However, the results are highly variable, probably due to the diversity of diets, animals and the methodologies used. In this context, the objective was to evaluate the internal markers iADF, iNDF, DM and ADIA in two times of incubation *in situ* (144 and 288 hours) to estimate the dry matter digestibility of corn silage in sheep.

Materials and methods The silage made with two hybrids were evaluated: the conventional technology and the technology YieldGard® (by inserting genes from *Bacillus thuringiensis* - Bt), with or without the addition of the inoculant (*Lactobacillus plantarum* and *Propionibacterium acidipropionici*). The diets were composed by roughage:concentrate of 70:30, that was provided to allow 5% remains. The diets were prepared: 1: Conventional Corn Silage without inoculants and concentrate, 2: Conventional Corn Silage with inoculants and concentrate, 3: BT corn silage without inoculants and concentrate, 4: BT corn silage with inoculants and concentrate. To quantify the internal markers present in the diets, indigestible dry matter (iDM), indigestible neutral detergent fiber (iNDF), indigestible acid detergent fiber (iADF), and acid detergent insoluble ash (ADIA) was incubated *in situ* in two periods of time (144 and 288 hours) in F57 bags (Ankom®). Statistical analysis was performed using Bayesian Inference.

Results and discussion The indigestible dry matter (iDM) did not presente as an efficient marker to estimate the digestibility of DM (Tables 1) for both times. The same way, the acid detergent insoluble ash (ADIA) did not provide the real estimate of the digestibility of DM. The presence of residues adhered to the tissues was observed in this study. These contaminants adhered to the fabric of post-incubation tissues, even after washing until the total whitening water, indicates that caution should be exercised in applying the MSi as internal marker. Results of laboratory tests to obtain the residue acid detergent are less variable compared to iNDF due to the absence of hemicellulose, a component that may be the most responsible for the variations found in several experiments with internal indicators. However, this difference can be eliminated with longer times of incubation, as found in this study in which the iNDF shows up as a more accurate indicator that the iADF, as the credibility interval (P2.5%; P97.5%) is lower (Table 1).

Table 1 Bayesian estimates digestibility by diet, time of incubation of 144 and 288 hours

		144 hours					288 hours				
Diet	Marker	Average	SD	$^{1}P_{2,5\%}$	$P_{97,5\%}$	σ	Average	SD	$^{1}P_{2,5\%}$	$P_{97,5\%}$	σ
1	TC	73.22	1.66	69.93	76.42	2.76	73.22	1.66	69.93	76.42	2.76
	iDM	65.03*	3.89	57.51	72.61	6.54	67.27*	1.86	63.68	70.90	3.13
	iNDF	72.57*	4.48	63.68	81.35	7.40	72.02	0.86	70.31	73.70	1.42
	iADF	72.23	1.17	62.42	91.16	2.01	71.89	1.61	68.71	75.02	2.77
	ADIA	77.09*	8.02	62.42	91.16	12.60	70.23*	5.60	59.99	80.06	8.80
2	TC	73.51	0.69	72.14	74.84	1.15	73.51	0.69	72.14	74.84	1.15
	iDM	58.64*	3.62	51.63	65.69	6.09	67.60*	0.50	66.64	68.57	0.84
	iNDF	69.42	4.68	60.13	78.59	7.73	71.87	0.78	70.33	73.39	1.28
	iADF	73.27	0.75	71.81	74.73	1.28	72.10	2.37	67.48	76.74	4.06
	ADIA	79.54	3.50	73.14	85.69	5.50	76.49*	7.12	63.47	88.99	11.19
3	TC	70.65	1.37	67.92	73.29	2.28	70.65	1.37	67.92	73.29	2.28
	iDM	59.20*	2.96	53.47	64.98	4.99	65.90	3.50	59.12	72.73	5.90
	iNDF	65.06	3.15	58.81	71.23	5.20	69.16	1.72	65.75	72.52	2.84
	iADF	68.44	2.68	63.21	73.70	4.60	69.70	2.44	64.94	74.48	4.18
	ADIA	71.25	11.64	49.90	91.69	18.30	61.94*	11.39	41.09	81.94	17.91
4	TC	71.95	0.97	70.01	73.82	1.62	71.95	0.97	70.01	73.82	1.62
	iDM	63.83*	0.80	62.27	65.39	1.35	60.90*	1.81	57.40	64.42	3.04
	iNDF	69.11	1.46	66.21	71.97	2.41	71.11	1.53	68.08	74.11	2.53
	iADF	70.37	1.04	68.34	72.42	1.79	71.04	1.42	68.27	73.82	2.43
	ADIA	72.43	9.53	54.97	89.17	14.99	69.63*	10.39	50.60	87.87	16.33

TC = total collection; SD = standard deviation;
* Statistically different from control by the Bayesian comparisons (P <0.05).
[1]Interval with 95% reliability (P2.5%; P97.5%).

Conclusions Rumen incubation time of 288 hours both iADF as iNDF presented themselves as efficient markers in estimating the digestibility of dry matter. In 144 hours of incubation, only iADF was efficient as a marker.

The impact of ensiling potato hash with Lalsil Fresh on the performance of growing pigs

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Keywords daily gain, feed intake, pigs, silage, inoculation

Introduction Small-scale pig production in South Africa is characterised by high inclusion of mixed cereals, for animal ration which are expensive. Feeding by-products to animals can be an alternative to reduce feed cost. Potato hash (PH), a potato by-product that derived from production of chips and snacks, can be used as an alternative feed resource (Nkosi, 2009). Feeding this by-product to pigs in its fresh form is possible, however if it is not consumed in a short period of time, it gets mouldy and quickly becomes useless for animal feeding. Therefore, PH should be ensiled to be fed to animals. Literature on the feeding of ensiled PH to pigs is scarce. Therefore, the present study aimed to evaluate the feeding of LAB treated silage from PH to pigs.

Materials and methods An amount of 700 g/kg PH was mixed with 300 g/kg of wheat bran to produce silage (PHS). The mixtures were treated with Lalsil Fresh or without LAB (Untreated PHS).The mixtures were compacted in 210 L drums. After 90 d of ensiling, samples were collected and analysed for fermentation characteristics. Diets that contained 200 g/kg of either LFPHS or UPHS were formulated and fed to32 crossbred pigs (Large White × Landrace). Pigs were housed individually in a grower house and randomly allocated to each diet. Pigs were fed until they reached 60 kg live weight. Data on the effects of inoculants on the growth performance of pigs were analysed using SAS (2012).

Results and discussion Inoculation reduced ($P<0.05$) the pH and residual WSC, whereas increased the contents of LA and AA compared to untreated PHS (Table 1). Also, inoculation improved ($P<0.05$) DMI, ADG and FCR compared with untreated PHS (Table 2), as reported by Nkosi and Meeske (2010). Peters et al. (2005) obtained a growth rate of 454 g/d in pigs fed diets containing 30% sweet potato root silage, which is comparable to the results obtained in this study.

Conclusion Inoculation of Lalsil Fresh at ensiling of potato hash improved the fermentation characteristics and the growth performance of pigs.

Table 1 Fermentation characteristics and chemical composition of diets (n=3)

	Treatments		SEM	P-Value
	UPHS	LFPHS		
Fermentation profile				
pH	4.58[a]	4.25[b]	0.03	0.003
WSC g/kg	39.30[a]	36.32[b]	3.65	0.260
LA g/kg DM	40.87[b]	52.95[a]	1.40	0.050
AA g/kg DM	30.35[a]	36.16[b]	0.18	0.040
Chemical composition*				
Dry matter	363.8[b]	386.3[a]	0.352	0.050
Organic matter	918.4[b]	931.5[a]	0.125	0.001
Crude Protein	67.7[b]	83.8[a]	0.006	0.001
GE, MJ/kg DM	15.4	15.4	0.025	0.089
Ether extract	35.9	35.9	0.231	0.067
ADF	132.8[a]	111.7[b]	0.068	0.001
aNDF	184.6[a]	150.5[b]	0.154	0.001

GE - gross energy; ADF - acid detergent fibre; NDF - neutral detergent fibre, WSC- Water-soluble carbohydrates; LA- Lactic acid; AA- Acetic acid, UPHS- Untreated potato hash silage, LFPHS- Lalsil Fresh potato hash silage
*After diet formulation

Table 2 Effects of dietary treatments on the growth performance of pigs (n = 16)

	Treatments			P-value		
Parameters	UPHS	LFPHS	SE	Trt	G	Trt×G
IBW (kg)	30.44	29.98	4.47	0.032	0.077	0.064
FBW (kg)	50.48[b]	57.99[a]	5.82	0.020	0.083	0.090
ADG (g/d)	201.6[b]	249.2[a]	14.23	0.010	0.069	0.089
DMI (g/d)	57.20[b]	69.80a	0.06	0.004	0.041	0.043
FCR (g/d)	5.50[b]	3.20[b]	0.62	0.001	0.082	0.062

[a,b] Means with different letters in a row differ significantly (P <0.05). IBW - initial body weight; FBW - final body weight; DMI - dry matter intake; ADG - average daily gain; FCR - feed conversion rate., Trt = Treatment, G = Gender, UPHS- Untreated potato hash silage, LFPHS- Lalsil Fresh potato hash silage

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Performance and feed intake of piglets fed with a liquid fermented diet

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Keywords sow milk, lactic acid bacteria, intake, weaning, piglets, fermented feed

Introduction The conventional pig industry in Colombia uses as routine antibiotics e.g. tetracycline and sulfonamide to prevent diarrhea mostly related to *Escherichia coli* and promote growth. However, safety issues are the major concern about the general use of antibiotics, because they might produce antibacterial resistance in animals and later may reproduce the same effects in humans. The objective of the study was to evaluate a fermented food using simple and low cost processes to offer probiotics to piglets from 21 to 35 days of life to observe possible health promoting effects on the growing piglets. The lactic acid bacteria (LAB) strain CIAT L605 was isolated from sow milk and it was selected by its potential intestinal adherence and fermentation capacity (Martens and Heinritz, 2012). It was used as inoculum in the fermented diet for weaning piglets.

Materials and methods A pelleted and extruded diet was prepared using 32% corn, 22.5% soybean meal, 10% rice fine dust, 10% lactose source, 5% corn gluten, 4% dextrose, 3% malt dextrin, 2.5% extruded soybean, 2.5% fishmeal, mineral and vitamin supplies. An artificial flavor was added to improve consumption. The chemical composition determined in laboratory was 22.2% crude protein (CP), 6% fat, 2.3% ADF, 7.9% NDF. Calculated contents were 3360 Kcal ME, 0.6% methionine, 1.5% lysine and 61.9 Meq/kg electrolytic balances. The treatments were: a) Positive control with growth promoter chlortetracycline (CON+), b) Negative control without growth promoter (CON-) and c) Liquid diet fermented for 24 h inoculated with LAB CIAT L605 (*Lactobacillus plantarum*), the proportion consisted of 1 kg of dry diet, 2 liters of water and one milliliter of LAB (10^{12} colony forming units/mL) (FER). Animals received half in a fermented food and the rest as flour. Sixty piglets weaned just the previous day, were divided into five groups of 12 animals each for the experimental period from day 22 to day 42 of life. Each group of 12 piglets was distributed in six cages equipped with heating and each one of three treatments was given two pairs of animals and replicated five times (block), for a total of 10 experimental units per treatment. Animals were weighed weekly (days 21, 28, 35 and 42) during experiment and consumption was measured daily. After the end of the experimental period pigs consumed a commercial diet with antibiotics (from day 43 to 70). Weighing control was taken at 70 days of age to assess the long-term effects of the treatments. The variables were analyzed using SAS, 1991, according to the model: $Yij = M + Bi + Tj + (Bi * Tj) + Eij$, where M is the average, the effect of the block B, T the effect of treatment, and E the experimental error. Analysis of variance was performed to detect differences between treatments. When detected they were analyzed by Duncan's test.

Results and discussion Fermented diet tended to improve feed conversion while the daily live weight gains and consumptions were close to the antibiotic treatment (Table 1). Fermented and Control positive treatments were superior to Control in terms of daily live weight and conversion. In the post experimental phase those who had been fed FER showed higher live weight gain (Table 1). However, no statistical difference was found among treatments in this respect. The use of a strain such as LAB 605 isolated from sow's milk can promote a positive effect on the replication of beneficial microorganisms for maintaining the health of piglets weaned early (Rebolledo 2014).

Table 1 Effect of a probiotic inoculum isolated from sow milk used in a liquid fermented diet for weaning piglets

	FER	CON-	CON+	SEM	P-value (treatment)
Experimental units	10	10	10		
Daily weight gain (22-42 d), g/piglet	137.4	99.4	142.7	36.2	0.08
Daily intake, g DM (22-42 d) /piglet	294.7	301.8	363.3	56.2	0.06
Feed conversion, g DM intake (22-42 d)/daily gain weight	2.2	3.26	2.76	0.72	0.09

FER: fermented; CON-: negative control; CON+: positive control;

Conclusions The LAB strain L605 isolated from sow milk showed interesting probiotic characteristics in fermented diets for piglets. The use of LAB 605 should be further examined on its effect on health and performance of piglets.

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Intake, growth performance, and carcass characteristics of meat-type rabbits fed with different levels of corn stalk silage

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Keywords rabbits, silage, performance, carcass characteristics

Introduction The last agricultural census in Puerto Rico reports decreases in the number of commercial rabbit farms and animal inventory. In 2007, there were 119 commercial farms and 39,519 rabbits on the island, numbers that decreased to 98 farms and 33,982 animals in 2012 (USDA/NASS, 2014). Among the most important problems facing local rabbit producers is the high cost of commercial feeds (CF); therefore, alternatives to its use need to be evaluated. Rabbits are non-ruminant herbivores capable of degrading fiber, thus inclusion of fermented forages might represent a useful option. An experiment was conducted to evaluate the inclusion of corn silage (CS) in diets for growing rabbits based on intake, growth performance, carcass yield and weight of body organs.

Materials and methods Thirty New Zealand white rabbits (averaging 5 weeks of age and 700 g live weight) were distributed by initial weight into 30 elevated cages and randomly assigned to one of three treatments; control (100% CF), and inclusion of CS at 25% or 50% of the total dry matter (DM) offered. Corn stalk was ensiled in 5 kg sealed plastic silos for a minimum of 21 days. Diets were offered at 5% of rabbit body weigh daily on a DM basis during 59 days. Nutrient composition of both feedstuffs was determined using standard procedures (AOAC, 1990; Van Soest et al, 1991), while pH and concentrations of fermentation products of CS were determined in a commercial laboratory. Commercial feed, CS offered and orts were weighted daily and total diet intake was calculated. Rabbit body weight was observed weekly to determine total and average daily gain and feed to gain conversion ratio. At slaughter hot carcass weight was recorded to determine carcass dressing percentage. Liver, kidney, and stomach weighs were also recorded to determined weighs of these organs as a percentage of final weight. Data collected on feed intake, weight gain, feed conversion, carcass dressing percentage, and organ weights were statistically analyzed using a completely randomized design with 10 replicates per treatment and Bonferroni test for mean separation.

Results and discussion The nutrient profile of the CF utilized in this experiment is in agreement with the manufacture's label while the CS had a chemical composition and ensiling characteristics typical of those observed for corn fermented in a tropical environment (Table 1). Total intake was higher ($P<0.05$) in rabbits fed the 100% CF diet (59.0g/d), than in those offered 50% CS (40.7g/d), but not significantly different from those fed 25% CS (49.53g/d). Control rabbits showed higher ($P<0.05$) daily gain (Control=17.5 g, 25%CS=12.4 g, 50%CS=6.60 g) and were more efficient in feed conversion than animals receiving either level of CS in the diet (Control=3.38, 25%CS=4.01, 50%CS=6.1). Carcass yield percentage was lower ($P<0.05$) in animals fed diets containing 50% CS than in those fed 0 and 25% CS. Similar organ weights as a percentage of final rabbit weight were observed in the three treatments. In this experiment inclusion of both levels of CS resulted in lower intake and productive performance than that obtained by rabbits fed 100% CF. The CS containing diets had lower crude protein and starch contents than the 100% CF, which might have affected animal performance. Therefore, to accomplish desirable growing rates in rabbit fed CS, ensiling of vegetative material with higher starch

content in combination with the use of protein supplementation seems to be necessary. Under the conditions of the present experiment, inclusion of CS in the diet at the 25% level did not significantly affect rabbit carcass yield, but lower yield was observed when CS was included at 50%, which might be a reflection of nutrient density of the diet.

Table 1 Chemical composition and fermentation profile of commercial feed and corn silage

Chemical Composition (%)								
Item	DM	OM[1]	Ash[1]	CP[1]	Starch[1]	NDF[1]	ADF[1]	Lignin[1]
CF	93.1	93.5	6.5	17.6	17.8	37.1	22.1	6.0
CS	26.4	97.6	2.4	9.5	0.4	63.5	41.1	5.9
Ensiling Characteristics (%)								
CS	pH	Lactic Acid	Acetic Acid	L/A ratio	Butyric Acid	Propionic Acid	N-NH_3/ N-Total	
	3.92	6.61[1]	1.96[1]	3.37[1,2]	.17[1]	.04[1]	10.1[1]	

[1] Dry matter basis, [2] Lactic acid/ acetic acid ratio.

Table 2 Intake, performance, and carcass yield of meat-type rabbits fed with different proportions of commercial feed and corn stalk silage

	Proportion CF:CS			
Component	100:0	75:25	50:50	P
Intake (g/d)				
CF	59.03[a]	38.9[b]	22.4[c]	.01
CS	-----	10.5[b]	18.3[a]	.01
Total	59.03[a]	48.5[ab]	40.7[b]	.01
Body Weight (g)				
Initial	695.0	699.4	688.1	.97
Final	1732.1[a]	1434.7[b]	1077.6[c]	.01
Gain (g)				
Daily	17.5[a]	12.4[b]	6.6[c]	.01
Total	1037.3[a]	735.27[b]	389.4[c]	.01
Feed Conversion Ratio	3.38[b]	4.01[b]	6.17[a]	.01
Hot Carcass Yield 9%)	49.2[a]	47.7[a]	43.8[b]	.01
Organ weight as % final weight				
Liver	5.91	5.93	5.02	.12
Stomach	14.0	13.2	12.4	.39
Kidney	1.3	1.3	1.3	.93

[a,b] Means with unlike superscripts in the same row differ $p<0.05$.

Conclusion Corn silage at 25 and 50% inclusion rates can be use in growing rabbit diets but with lower productive performance than that obtained by animals fed 100% CF. Inclusion of CS in the diet at the 25% level did not affect rabbit carcass yield.

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