XVI
ISC
2012

XVI International Silage Conference

Hämeenlinna, Finland, 2-4 July 2012

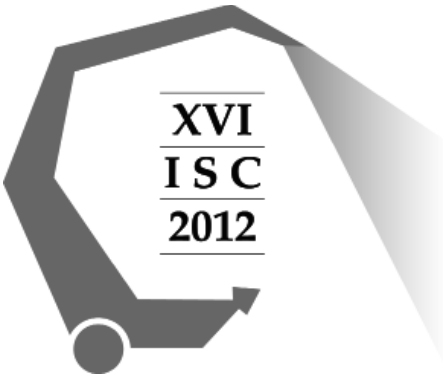
UNIVERSITY OF HELSINKI

MTT
Agrifood Research
Finland

Proceedings of the

XVI International Silage Conference

Hämeenlinna, Finland, 2-4 July 2012



Edited by

K. Kuoppala, M. Rinne and A. Vanhatalo

Published by

MTT Agrifood Research Finland

University of Helsinki

Cover design
Heini Kauppinen
Cover photos
Eeva Saarisalo, Jarmo Juga and Ville-Matti Vuollet
Printed in
Unigrafia, Helsinki
Printing year
2012

ISBN 978-952-487-384-0 (printed)
ISBN 978-952-487-385-7 (PDF)

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Today, we are part of a value chain that needs to increase productivity to ensure food safety and security for 7 billion people – a number that is expected to touch 9 billion by 2050.

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Foreword

We warmly welcome you to the XVI International Silage Conference, 2-4 July 2012, in Hämeenlinna. MTT Agrifood Research Finland and the University of Helsinki are jointly organising this conference. It is our great pleasure to host this event – now for the first time in Finland. We hope that it will be a memorable experience for delegates arriving from all over the world.

We have long traditions in silage research in Finland. With a grazing period of only three to four months, it has been essential for us to know how to conserve forage to get livestock over the long, cold winter period. This may have been the prime mover for our most famous scientist, A.I. Virtanen, in his silage research that led to his Nobel Prize for his forage preservation method in 1945. However, conditions are hardly optimal for year-round grazing anywhere in the world. In addition to cold, conditions may be challenging in terms of heat or drought or any other circumstance where feed ensiling is of high priority. Although Nordic perspectives are highlighted in the present Conference programme, we are very pleased that it includes high-quality papers focusing on research topics of importance to various parts of the world.

As organisers, we feel honoured that more than 300 participants from over 30 countries have registered to the conference. This Proceedings volume contains eight invited papers and 204 contributed 2-page papers, which will be presented as either oral or poster presentations. It begins with oral contributions organised into seven sessions according to the topics of the invited papers, which form the outline of the conference programme. This is followed by poster contributions categorised according to session themes. The sessions cover core areas of silage research from microbiology of ensiling and feed safety to ensiling technology and management. Feed characteristics of silage and challenges of silage feeding are naturally reviewed in terms of ruminant production animals. However, feeding silage to monogastrics, such as horses and pigs, is in focus as well. Although most of the volunteered papers deal with the biology and technology of ensiling, complexities of environmental issues related to silage and dairy production are also raised in this conference.

We hope that the XVI International Silage Conference will serve as a real multidisciplinary rendezvous for scientists, experts and other contributors interested in developments in silage science and technology. We wish you an inspiring and fruitful conference – and a good and enjoyable time in Finland.

On behalf of the organisers

Aila Vanhatalo
Chair of the Scientific Committee

Table of contents

Posters

Session 3. Biology of ensiling and food safety

An overview of silage research in Finland: from ensiling innovation to advances in dairy cow feeding

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Keywords: grass silage, ensiling, feed evaluation, nutrient intake, milk production

Introduction

Milk production systems in different climatic zones have developed to utilize local feed resources. Due to the short grazing period (100-120 days) in Finland grazed grass cannot contribute more than 20–25% of total feed energy intake for dairy cows. This has increased the importance of conserved forages in dairy cow rations. Relative competiveness of grass in Finland is high, since in the main milk production regions grass dry matter (DM) yields are more than two-fold compared with cereal grains (Kangas et al. 2010). Grasses can utilize efficiently the long days in early summer, and daily DM growths exceeding 200 kg are common (e.g. Kuoppala et al. 2008). The nutritive value of forages in terms of digestibility is high due to the relatively cool climate and long day length which delay the lignification of cell walls (Van Soest et al. 1978, Deinum et. al. 1981). Earlier high concentrate costs and a shortage of protein supplements favoured forage-based feeding systems, but since Finland joined EU in 1995 subsidised grain and protein prices have reduced the competiveness of grassland production.

Because of the climatic conditions, the Finnish milk production research has focused to improve the utilisation of grassland, mainly as conserved forages. The main research areas have been ensiling, evaluation of the forage feeding value, predicting nutrient supply from grass silage-based diet and the effects of forage quality and concentrate supplementation on milk production responses. More recently, environmental aspects of milk production and product quality, mainly milk fatty acid composition, have been important research subjects. The Finnish silage research was earlier reviewed by Lampila et al. (1988) and Huhtanen (1998). The objectives of this paper are to review the achievements of the Finnish silage research relative to international literature with the special emphasis on ensiling, feed evaluation, feed intake and milk production.

Ensiling

The control of major preservative factors of silage (e.g. pH, water activity, epiphytic flora), and their interactions, is the basis for biologically and economically efficient silage production. Virtanen (1933) was first to show systematically the importance of low pH and inhibition of plant and microbial enzymes in silage preservation. By using hydrochloric and sulphuric acids he introduced the A.I.V.-method and established the principle of rapid achievement of pH 4 to suppress respiration of plant cells, to prevent degradation of proteins and vitamins and to avoid clostridial fermentation. He also showed that different crops, e.g. leguminous plants vs. grasses, require different amounts of acids to achieve target pH.

Ensilability of silage crops

Ever since the innovation of A.I. Virtanen, the control of silage fermentation by silage additives has been the core of ensiling in Finland. In the late 1960's, combinations of inorganic acids and organic acids, mainly formic acid (FA), and additives containing formaldehyde were in the focus of research (Ettala et al. 1975). Corrosive nature of inorganic acids and other hazardous effects of formaldehyde were reasons to abandon these products later. The research done in Norway (Saue and Breirem 1969) demonstrated the effectiveness of FA which became the most commonly used silage additive also in Finland. Direct acidification using relatively high application rate of FA (approximately 4 l/t, expressed as 100% w/w) has facilitated that relatively wet and low sugar crops, predominantly timothy, meadow fescue and some legumes, can be ensiled successfully. The climatic conditions in Finland exclude the more easily ensiled crops like perennial ryegrass and fodder maize. This highlights the importance of adjusting harvesting and ensiling management according to crop characteristics and local conditions (Lampila et al. 1988). The most important ensilability factors of crops are soundly presented by Weissbach et al. (1974) in an equation predicting anaerobic stability and clostridial development from crop dry matter (DM), buffering capacity (BC) and water soluble carbohydrates (WSC).

Increasing size of Finnish farms and demand for high labour efficiency in the ensiling systems have been the major reasons for the technological development, like pre-wilting and harvesting techniques related to it. Although some of the techniques, e.g. chopping with harvesters and additive applicators, have had some important positive effects on silage quality the biological efficiency has not necessarily increased. Gordon (1989) concluded in Northern Ireland that a harvesting system based on

wilting decreased the output of animal product per hectare by 13% as compared to a direct-cut system. The increasing popularity of wilting, a concomitant decrease of application rate of FA and a shift to using biological additives have all changed the challenges of ensiling. Effluent losses and the risk of clostridial fermentation decreases with increasing DM content but at the same time wilting may increase nutrient losses during drying, impair the microbiological quality of crop and expose the silage to aerobic deterioration. Wilting grass to DM content of 300 g/kg did not alone prevent clostridia (Ettala et al. 1982) but in favourable harvesting conditions it supports achievement of good fermentation quality and feeding value without additives (Heikkilä et al. 2010). However, ensiling system based on baling of high DM grass without additive is more susceptible to unfavourable harvesting conditions and to lower feeding value of silage as compared to ensiling in bunker silo with lower DM content and FA-based additive (Jaakkola et al. 2008). In spite of low butyric acid and ammonia N content of untreated bale silage (380 g DM/kg), the use of inoculants or FA improved milk production and sensory quality of milk (Heikkilä et al. 1997). This demonstrates that fermentation parameters of high DM silage insufficiently describe the value of silage in animal production. The unpredictability of weather conditions and variation in crop DM and WSC concentration and epiphytic flora are important factors to be considered in the risk management of ensiling and when making decision on the use of additives. Currently 50-60% of the Finnish farm samples analysed in the laboratory of Valio Ltd are from silages treated with acid based additives, 25-30% from silages treated with biological additives and 10-15% from untreated silages (J. Nousiainen, personal communication).

A risk of undesirable fermentation is higher when forage and grain legumes with high BC are ensiled as compared to grass species. Slight wilting of lucerne, galega, red clover and lotus to 250 g DM/kg alone was not sufficient to avoid poor fermentation in research made in Germany, Sweden and Finland (Pahlow et al. 2002). Wilting to 400 g DM/kg prevented the production of butyric acid, but silage quality was further improved by the use of additives. The challenging ensiling characteristics of forage legumes are alleviated in a mixture with grass species having lower BC. Similarly, when whole-crop field bean and field pea were ensiled without an additive, inclusion of 0.25 to 0.50 of wheat ensured a good fermentation (Pursiainen and Tuori 2008). However, common vetch with a high BC and a low WSC concentration was best ensiled using FA to prevent extensive protein degradation.

Preservation of small grain cereal crops has been successful in our conditions when harvested at the dough stage (300 – 400 g DM/kg) and when ensiling is based on a low pH generated by fermentation and/or acid based additives (Vanhatalo et al. 1999b, Jaakkola et al. 2009). Ensiling of cereal crops either untreated or treated with urea resulted in clostridial fermentation (Alaspää 1986). Low DM content of whole crop cereals even at a late maturity in our conditions does not support alkaline preservation. Extensive research in 1970's in Finland demonstrated that ensiling of high moisture grain is an efficient storage method as an alternative to grain drying. Early harvest, crimping and treatment with an additive diminishes the challenges of short growing season, increases the grain yield and reduces the use of fossil fuels. In the later studies the use of dry barley and ensiled barley resulted in the same animal performance in growing cattle (Huhtanen 1984) and dairy cows (Jaakkola et al. 2005).

Restriction of fermentation

The variation in crop characteristics and application rate of FA in different experiments explains the inconsistent results obtained in the fermentation quality of FA-treated silage and consequently in animal responses (Harrison et al. 2003, Kung et al. 2003). A high application rate of FA restricts fermentation resulting in lower content of total acids [TA; lactic acid plus volatile fatty acids (VFA)] and ammonia N, and higher content of residual WSC in silage as compared with extensively fermented untreated or inoculated silage (Chamberlain et al. 1992, Heikkilä et al. 1998, Shingfield et al. 2002a). With lower FA application rates the differences in fermentation profiles are smaller. The low ammonia N content in silage reveals that FA treatment inhibits the conversion of herbage protein to non-protein-nitrogen (NPN) and increases the proportion of peptide N in silage NPN as compared with untreated silage (Nagel and Broderick 1992, Nsereko and Rooke 1999). The extent of silage fermentation thus dictates the amount and type of nutrients available for animals. Consequently, the nutritive value of restrictively fermented silages is equal compared to that of respective barn dried forages (Jaakkola and Huhtanen 1993).

The effects of increasing level of FA on silage fermentation pattern have been linear (Jaakkola et al. 2006a) or curvilinear (Chamberlain and Quig 1987, Jaakkola et al. 2006b). This indicates that the balance and survival of desirable and undesirable microorganisms may differ with the characteristics of ensiled material and the additive. Due to the corrosive nature and handling problems of pure FA the commercial additives generally contain salts of FA like ammonium and sodium formate. Ammonium tetraformiate maintains good silage quality if applied according to the molar concentration of acid (Randby 2000). Replacing FA (5,1 kg/t) with increasing proportion of ammonium formate up to 45% delayed the drop of pH in unwilted (210 g DM/kg) and wilted (406 g DM/kg) grass silage while the quality of silage was not compromised (Saarisalo and Jaakkola 2005).

Even a low application rate of FA disrupts cell membranes and releases soluble cell contents

(Kennedy 1990, Jaakkola et al. 2006a). As a result, in wet material increased effluent losses partly pay off the advantages of reduced fermentation losses. As a positive effect, cell wall degradation leads to efficient consolidation and increased storage density as compared with untreated silage. This partly explains why the use of high rate of FA may result in good aerobic stability and low yeast count despite restricted fermentation and high residual WSC content in silage (Saarisalo et al. 2006) which often have been considered risk factors for aerobic stability. Formic acid has a selective bactericidal effect but it is not specifically effective against yeasts (McDonald et al. 1991). More antifungal alternatives applied in a combination with FA have sometimes improved (Heikkilä et al. 2010) but sometimes not (Lorenzo and O'Kiely 2008) the aerobic stability as compared to untreated silage. The increased risk of aerobic deterioration concerns mainly wilted FA silages since low-DM or minimum wilted FA-treated grass silages have been shown to be more stable than untreated and inoculated silages (Pessi and Nousiainen 1999). As underlined already in the studies of Ettala et al. (1982) the feeding rate and good silo management are the key issues in preventing aerobic deterioration. However, even a small amount of oxygen may start the growth of yeasts and moulds responsible for aerobic deterioration. The use of combinations of hexamethylene-tetraamine, sodium nitrite, sodium benzoate and sodium propionate has improved the quality and storage stability of silage made from wilted grass (Lingvall and Lättemäe 1999, Knicky and Spörndly 2009).

Stimulation of fermentation

The interest on enzymes and inoculants as silage additives increased in Finland in the late 1970's (Vaisto et al. 1978, Poutiainen and Ojala 1982). Compared to early products, the improvements in inoculants and better understanding of the conditions in which inoculants are effective have generally improved the results (Kung et al. 2003). Inoculants alone are unable to produce enough lactic acid to lower the pH to an acceptable level if the WSC content of the original crop is a limiting factor (Seale et al. 1986). A content of 25-30 g/kg in fresh material has been suggested to ensure sufficient production of fermentation acids in untreated silage (Wilkinson et al. 1983, Pettersson 1988). Accordingly, high WSC content of grass (32 g/kg) resulted in minor differences in the fermentation of untreated silage and silages treated with inoculants or enzymes (Rauramaa et al. 1987). The amount of fermentable substrate can be increased by using efficient enzymes as an additive or the ensilability can be increased by wilting which increases the content of WSC in fresh weight of crop. The decreased rate of N fertilization has also enhanced ensilability by increasing WSC content of grass. However, the concomitant lower nitrate content may have an opposite effect since nitrite and nitric oxide, the reduction products of nitrate, effectively inhibit clostridia (Spoelstra 1985, McDonald et al. 1991).

Another purpose of using cell-wall degrading enzymes as an additive was to increase the rate and/or extent of digestion of cell wall carbohydrates in the rumen. The degradation of fibre in the silo was shown to increase with increasing cellulase level (Vaisto et al. 1978, Huhtanen et al. 1985). However, enzyme treatment had no consistent effect on organic matter digestibility but it decreased fibre digestibility in cattle (Jaakkola and Huhtanen 1990, Jaakkola et al. 1990) and in sheep (Jaakkola 1990). Enzymes clearly affected the most easily degradable fraction of fibre which is also completely degraded in the rumen. On the other hand, with a successful combination of cell-wall degrading enzymes even a high-moisture (172 g/kg) and low-WSC (16 g/kg) grass was well preserved (Jaakkola et al. 1991). Generally the ensiling results with enzymes have been inconsistent. Kung et al. (2003) suggested that e.g. the lack of synergistic activities of enzyme complexes or environmental factors (pH, temperature) may be the potential reasons for failures in improving silage fermentation with enzymes.

Selection of effective bacteria strains for the use as inoculants is crucial for successful ensiling. A screening method using grass extract proved to be useful in strain selection (Saarisalo et al. 2007). *Lactobacillus plantarum* strain (VTT E-78076) having a broad-spectrum antimicrobial activity against gram positive and gram negative bacteria, and Fusarium moulds, was originally isolated from beer (Niku-Paavola et al. 1999, Laitila et al. 2002) but was shown to be also efficient in producing lactic acid, lowering pH rapidly and especially decreasing the ammonia-N production in grass silage (Saarisalo et al. 2006, Saarisalo et al. 2007). However, the antimicrobial properties were not efficient enough to improve aerobic stability (Saarisalo et al. 2006). One possibility to overcome the inability of lactic acid to prevent yeast and mould growth is to use chemical additives in combination with the inoculants (Weissbach et al. 1991). Skyttä et al. (2002) showed that a combination of a selected inoculant, potassium sorbate and sodium benzoate inhibited *in vitro* the growth of four spoilage yeast strains isolated from grass silage. In two ensiling trials the combination of lactic acid bacteria and sodium benzoate (0.3 g/kg) had variable effect on the aerobic stability of wilted grass silage showing that the minimum effective application rate of sodium benzoate varies (Saarisalo et al. 2006). As shown in the meta-analysis of Kleinschmit and Kung (2006) improved aerobic stability has been observed in different types of forages when acetic and propionic acid production in silage fermentation is increased with *L.buchneri* inoculation. In our experiment, buffered propionic acid and a combination of *L. plantarum* and sodium benzoate were more efficient than a combination of *L. plantarum* and *L. buchneri* to prevent heating of high DM silage (Jaakkola et al. 2010).

Feed evaluation

Silage fermentation quality

Practical on-farm silages show a wide variation in the fermentation quality due to e.g. crop characteristics, additives used and ensiling technologies. In-silo fermentation can influence the profile of absorbed nutrients and especially intake potential compared with fresh herbage (Huhtanen et al. 2007). Silage quality assessment with traditional wet chemistry for on-farm feeds is too expensive. For the analysis of farm samples Moisio and Heikonen (1989) developed a rapid electrometric titration method (ET). From the titration curve the concentrations of lactic acid, VFA, WSC, amino acid carboxyl groups and the protein degradation products (ammonia, amines) can be predicted (Moisio and Heikonen 1989). Later work revealed that ET over-predicted WSC, especially for extensively fermented or very dry samples. The system has been used for on-farm silage assessment for more than 20 years, with the exception that WSC are currently determined with the NIRS from dried samples. A comparable ET system has been also studied in UK (Porter et al. 1995) as an alternative or an additional silage measurement to either wet or dry NIRS (Park et al. 1998). However, direct comparisons between dry or wet NIRS and ET have shown that ET can be more accurate especially for VFA and ammonia-N (M. Hellämäki personal communication).

Silage composition with reference to nutrient availability

The main aim of feed chemistry is to divide forage DM into (1) cell contents that can be digested by mammalian enzymes and (2) a cell wall fraction that can only be digested by anaerobic microbial fermentation. The proximate feed analysis (Weende system) has been available for over 100 years, and it divides feed OM into crude protein (CP; 6.25 × N), crude fat (EE), crude fibre (CF) and nitrogen free extracts (NFE). Within the system, CF should represent the least available and NFE readily available feed components with a high true digestibility. The primary problems associated with NFE and CF fractions (Van Soest 1994, Huhtanen et al. 2006b) were realised by Paloheimo (1953), who initiated research to develop improved analytical methods for plant cell wall. In the pioneering work, Paloheimo and coworkers (Paloheimo and Paloheimo 1949, Paloheimo and Vainio 1965) used a weak hydrochloric acid and a two-stage ethanol extraction to remove cellular contents to describe vegetable fibre. Despite the correct criticism against fractionating feed carbohydrates into CF and NFE, these methods were too laborious, not applicable to faecal samples and the fibre residue was contaminated with protein. Based on these ideas, Van Soest (Van Soest 1967, Van Soest and Wine 1967) introduced the neutral detergent (ND) fractionation, which mainly resolved these drawbacks. The evaluation based on a wide dataset of silages (Huhtanen et al. 2006b) clearly demonstrated the biological weaknesses of the proximate feed analysis.

Neutral detergent (ND) fractionation (Van Soest 1967) divides forage DM into neutral detergent fibre (NDF) and neutral detergent solubles (NDS). Originally NDS was calculated as DM – NDF, but because ash does not provide energy, expressing NDS as organic matter (OM – NDF) may be preferable. True digestibility of the NDS fraction is close to unity (Van Soest 1994, Weisbjerg et al. 2004) when estimated by the Lucas test. The Lucas test allows estimation of ideal nutritional entities that have a uniform digestibility across a wide range of feedstuffs by plotting the digestible nutrient concentration in DM against the nutrient concentration in DM. The slope of regression provides an estimate of the true digestibility and the intercept is an estimate of the metabolic and endogenous faecal matter (M). Huhtanen et al. (2006b) reported a value of 0.963 for true NDS digestibility for different forages. Regrowth silages had a lower true NDS digestibility (0.925), the reasons for which are not known. Based on the Lucas principles the concentration of digestible OM (DOM; g/kg DM) can be expressed as:

$$\text{DOM (g/kg DM)} = \text{NDS} + \text{dNDF} - \text{M} \quad [1]$$

Given that digestible NDF (dNDF) = NDF × NDF digestibility coefficient (NDFD), NDS = OM – NDF, M = 100 and digestibility of NDS = 1.00, the equation [1] can be written as:

$$\text{DOM (g/kg DM)} = 1.00 \times (\text{OM} - \text{NDF}) + \text{NDF} \times \text{NDFD} - 100 \quad [2]$$

The equation [2] indicates that variation in DOM and OMD (OM digestibility) of forages is primarily a function of the concentration and digestibility of NDF, implying that the main emphasis in the evaluation of forage feeding value should be focused to the NDF fraction.

A fraction of NDF in forages is completely indigestible even if it is subjected to digestion for an infinite time. This fraction can be defined as indigestible NDF (iNDF), and it can be determined e.g. by extended incubations *in situ* (Huhtanen et al. 1994) or *in vitro* (Van Soest et al. 2005). We have used a 12-d *in situ* incubation using bags with a small pore size (6 – 17 µm) to avoid particle losses. Potentially digestible NDF (pdNDF) is then calculated as:

$$\text{pdNDF (g/kg DM)} = \text{NDF} - \text{iNDF} \quad [3]$$

Since iNDF is by definition a uniform nutritional entity with constant zero digestibility, equation [2] can be rewritten as:

$$\text{DOM (g/kg DM)} = (\text{OM} - \text{NDF}) + \text{pdNDF} \times \text{pdNDFD} - 100 \quad [4]$$

where pdNDFD is pdNDF digestibility. This equation indicates that variation in DOM is a function of iNDF

concentration and pdNDFD. The smaller coefficient of variation (4.1 vs. 11.4%) and range (0.79 - 0.94 vs. 0.48 - 0.87) in pdNDF digestibility compared with total NDF digestibility for the wide range of silages (Huhtanen et al. 2006b) indicates that pdNDF is a more ideal nutritional entity than total NDF. Digestibility of pdNDF was on average 0.85 with a mean faecal pdNDF output of 60 (sd 23; range 13 – 105) g/kg DM intake (Huhtanen et al. 2006b). Faecal pdNDF can be defined as updNDF (= faecal NDF - iNDF) that represents the loss of potentially digestible OM in addition to obligatory losses of M.

Prediction of silage digestibility

Digestibility measured in sheep fed at maintenance still forms the basis of many feed evaluation systems. However, this method is not applicable for on-farm silages, and even not often for research samples. Hence, much research has been conducted to develop OMD prediction systems that are suitable for extension purposes i.e. that are rapid, accurate, precise and inexpensive. For this purpose, empirical models based on silage composition, in vitro methods using either rumen fluid or commercial fibrolytic enzymes and several in situ incubation procedures have been studied. In Finland, a database (n = 86) including grass and legume silages harvested at different maturity with detailed chemical analysis and in vivo digestibility in sheep has been collected (see Huhtanen et al. 2006b) to standardize in vitro or in situ OMD prediction models. In carefully conducted in vivo trials measurements of OMD are associated with a SD of 0.02 units (Van Soest 1994). For studies conducted according to Latin square designs the residual SD (RSD) was 0.014 units (Nousiainen 2004); i.e. determination of forage in vivo OMD in 4 × 4 Latin squares would be associated with a minimum inherent error of 0.007 units. However, the development of any prediction model for silage OMD should take in account inter- and intra-laboratory variation in both in vivo and in vitro OMD measurements and laboratory analyses. To tackle this problem in Finland, we adopted a strategy that in vivo and in vitro determinations as well as laboratory analyses and NIRS calibration are conducted only in one or two forage laboratories with standardized methods. Supporting this strategy, Hall and Mertens (2012) reported relatively high 95% probability limits for within-lab repeatability and between-lab reproducibility (0.102 and 0.134, respectively) for in vitro forage NDFD as determined according to the method by Goering and Van Soest (1970).

Many attempts have been made in developing regression equations that relate various chemical components to forage OMD, but without success owing to large interspecies and environmental variation (Van Soest 1994). In the Finnish silage dataset statistically significant relationships between chemical components and OMD were identified, but prediction error using CP, NDF and ADF as independent variables was not markedly lower than SD of *in vivo* OMD (Huhtanen et al. 2006b). Lignin was the best single predictor of OMD, but this entity could only account for proportionately 0.43 of observed variation, whilst the prediction error (0.042) is too high for practical feed evaluation. Van Soest et al. (2005) suggested a universal and constant relationship between lignin and iNDF over several types of forages (iNDF = 2.4 × Lignin). However, evidence from the Finnish forage dataset does not support this, suggesting that biological methods are required in predicting forage iNDF and OMD (Huhtanen et al. 2006b).

Several *in vitro* laboratory methods have been used for estimating forage OMD. The two-stage rumen fluid *in vitro* technique by Tilley and Terry (1963) and Goering and Van Soest (1970) are the most widely used methods. Tilley and Terry (1963) demonstrated a close correlation between DMD determined *in vivo* and *in vitro* and reported that the values determined *in vitro* were almost the same as those determined in sheep. However, even with a good lab practice it is important to calibrate any *in vitro* method using *in vivo* data to derive reliable prediction equations (Weiss 1994, Nousiainen 2004).

Due to several practical difficulties in conducting rumen fluid in vitro method enzymatic *in vitro* procedures for the determination of forage digestibility have been studied (Jones and Theodorou 2000, Nousiainen et al. 2003a and 2003b). In principle, these methods include removing cell solubles with HCl-pepsin or ND followed by incubation in buffered enzyme solution. Determined OM solubility (OMS) differs from *in vivo* OMD in at least two key respects; no metabolic and endogenous matter is produced and the capacity of commercial enzymes to degrade NDF is substantially less than that of rumen microbes (McQueen and Van Soest 1975, Nousiainen 2004a). In predicting *in vivo* OMD from OMS the coefficient of determination (R^2) was 0.804 and RSD 0.025 digestibility units (n = 86, Huhtanen et al. 2006b). Because the relationship was highly dependent on forage type, using a forage specific correction equation increased R^2 to 0.925 and decreased RSD to 0.015. With a mixed model regression analysis, RSD was further decreased to 0.010 units, indicating that OMS predicted OMD within a study very accurately. The reduction in RSD can be attributed to differences between sheep used in digestibility trials and/or the contribution of between-year variation in the relationship between OMS and OMD. Using the general OMS correction underestimated the OMD of primary growth grass silages but overestimated OMD in regrowth grass and whole-crop cereal silages (Huhtanen et al. 2006b). The OMS method was also successfully used in predicting OMD for herbage samples taken before ensiling, provided that silages are well-preserved (Huhtanen et al. 2005). Owing to the problems in standardizing OMS method in different laboratories (Nousiainen 2004a), it is recommended that each

laboratory should develop their own forage specific correction equations. In conclusion, OMS method provides a reliable basis for OMD prediction, but caution should be directed to forage specificity. A recent comparison (Jančík et al. 2011) of different laboratory methods in predicting OMD revealed that OMS gave substantially higher OMD estimates than empirical iNDF equation or mechanistic model using gas *in vitro* production kinetics, especially for *Lolium perenne*. This suggests that specific OMS correction equations may be needed even for different grass species.

The equation [4] suggests that iNDF should correlate closely to forage OMD. Indeed, the evaluation of Finnish dataset showed that iNDF correlated with in vivo OMD for silages made from 1st cut and regrowth grass (Nousiainen et al. 2003b), and over a wider range of silage types (Huhtanen et al. 2006b). The relationship between iNDF and *in vivo* OMD was more uniform compared with OMD equation based on OMS. Mean square prediction error of OMD was 0.010 for mixed regression model (within study) and 0.019 for fixed regression model. A reliable prediction of OMD can be attributed to a more consistent digestibility of pdNDF compared with total NDF and the inverse relationship between iNDF content and the rate of pdNDF digestion. However, iNDF seems to underestimate the digestibility of legume silages, mainly lucerne, probably because of their higher rate of pdNDF digestion relative to iNDF concentration (Rinne et al. 2006). Precision of OMD estimates was slightly improved when the concentrations (g/kg DM) of iNDF and NDF were used:

$$OMD = 0.882 - 0.00121 \times iNDF - 0.00011 \times NDF \quad [5]$$

Prediction error for this fixed model regression was 0.0174 and 0.0090 for the mixed model regression and the respective parameter estimates were biologically sound. The more recent work with a wider range of forage types (Krizan et al. 2012) confirmed that empirical OMD equation based on forage iNDF forms a relatively universal basis for NIRS, especially for a more heterogeneous sample population. Under-prediction of OMD for lucerne silages by iNDF (Rinne et al. 2006, Krizsan et al. 2012) suggests that this assumption is not always true. An additional advantage of iNDF in forage evaluation is that it can be predicted with a relatively good accuracy by NIRS either on scans from dried feed (Nousiainen et al. 2004) or faeces (Nyholm et al. 2009). In our digestibility dataset *in vivo* OMD could be predicted as accurately from iNDF determined by NIRS as with iNDF determined by 12-d *in situ* incubation. However, it must be highlighted that both feed and faecal iNDF calibrations are based on reference values obtained from two laboratories that have standardized *in situ* procedure with no substantial inter-lab bias in the iNDF values and scans from only one NIRS lab. Evidence from the iNDF ring-test (Lund et al. 2004) suggests that a reliable reference database for NIRS cannot be established by simply compiling data from several labs.

NIRS applications in forage evaluation

Since Norris et al. (1976) first introduced NIRS equations for predicting forage quality, considerable progress has been made to implement NIRS applications for silage analysis. The development of computers, optical devices and calibration soft wares has facilitated this process (Deaville and Flinn 2000). Although any wave length in NIR spectrum lacks specificity to important feed parameters, especially being non-specific for functional properties of feeds (e.g. NDF, digestibility, intake potential), quantitative analysis of forage quality by NIRS is possible by calibrating the reflectance spectrum against biologically sound reference methods (Deaville and Flinn 2000, Nousiainen 2004a). NIRS applications for forage evaluation include quantitative analysis of both cell wall (NDF, iNDF) and cell content (CP, WSC, silage fermentation products) characteristics (Deaville and Flinn 2000, Nousiainen 2004a). The scans may be obtained from dried and finely ground or coarse wet samples, although the latter may be less accurate. Interpretation of published NIRS equations reveal that OM digestion and cell wall lignin bonding of forages is associated to spectral regions near to 1650-1670 and 2260-2280 nm (Deaville and Flinn 2000). In agreement with this, Nousiainen et al. (2004a) demonstrated that the absorbance in these regions was negatively correlated with iNDF content of grass silages.

The precision and repeatability of NIRS are known to be much better than any feed chemistry method (Deaville and Flinn 2000). Consequently, within a single lab NIRS calibration statistics often suggests very accurate prediction of any feed trait. When several chemical, *in vitro* and *in situ* reference methods in calibrating silage OMD were compared (Nousiainen 2004a), the calibration statistics for all of them showed high R^2 and a low standard error of calibration (SEC) and cross validation (SECV). However, the total error of prediction (*in vivo* vs. NIRS) was highly dependent on the biological validity of the reference method used. Therefore caution should be used in the choice of calibration method for NIRS. A high correlation (R^2 0.23) between the residuals of OMD estimates based on iNDF or OMS in the Finnish dataset (Huhtanen et al. 2006b) suggests that *in vivo* reference values include some random error. Therefore it is likely that with NIRS the true errors may be smaller than apparently estimated. For commercial laboratories OMS method may be the most practical choice for calibrating the NIRS in the prediction of OMD (Nousiainen 2004a, Huhtanen et al. 2006b). By using forage specific corrections for OMS and a sufficiently diverse range of reference samples total prediction performance can be considered satisfactory. The standard error of prediction (SEP) for D-value using OMS based

calibrations was circa 17-20 g/kg DM (Huhtanen et al. 2006b), consistent with a RSD of 14 g/kg DM for measurements of OMD in digestion trials (Nousiainen 2004a). Alternatively iNDF can be used for OMD or D-value calibration for NIRS in one of two ways; (1) predict digestibility with a direct regression equation (Nousiainen 2004a) or (2) use a summative method of uniform feed fractions (Huhtanen et al. 2006b). In the future, NIRS may be used to predict forage traits for use in dynamic digestion models. Digestion rate of pdNDF can be calculated from OMD, NDF and iNDF using the Lucas principle for NDS fraction and constant passage kinetic parameters at maintenance intake (Huhtanen et al. 2006a). Incubation of isolated NDF in automated *in vitro* gas production system resulted in similar digestion rate of pdNDF as estimated from the *in vivo* data (Huhtanen et al. 2008c).

Digestibility at production intake

Digestibility determined in sheep fed at maintenance describe the intrinsic digestibility of the diet, i.e. *in vivo* digestibility under optimal conditions (Mertens 1993). Feed values for cattle diets are traditionally computed using these digestibility coefficients by summing up individual dietary components. In general, the digestibility coefficients for a given feed are similar in sheep and cattle (Yan et al. 2002). Because diet digestibility decreases with increased feed intake, energy values are adjusted for the level of feeding in many feed evaluation systems. In a recent meta-analysis based on the evaluation of 497 diets in lactating cows, OMD was on average 0.038 units lower in dairy cows fed at production level of intake compared with OMD estimated at maintenance intake (Huhtanen et al. 2009). Digestibility in cows was shown to decrease with DM intake, the extent of depression being greater for highly digestible diets (Huhtanen et al. 2009). Dietary CP concentration had a positive effect on OM and NDF digestibility, while OMD decreased in a quadratic manner with increases in the proportion of whole-crop silage in the diet and linearly with concentrate fat intake. The RSD of a multivariate mixed regression model was 0.007 indicating that the differences in OMD between the diets of lactating cows could be predicted accurately from digestibility at maintenance, feed intake and diet composition (Huhtanen et al. 2009). Interestingly, there was no difference in the accuracy of OMD prediction in cows when OMD at maintenance were determined either *in vivo* with sheep or based on predictions from various *in vitro* measurements. The variation in OMD in dairy cows was almost completely related to the concentration and digestibility of NDF (Huhtanen et al. 2009). This indicates that the negative associative effects of feeding level and diet composition on OMD at the production level of intake are mainly associated with decreased NDF digestibility. It is therefore important to distinguish between iNDF and uNDF. Indigestible NDF is not digested by ruminants, whereas uNDF represents faecal output of pdNDF per kg DM intake. Total faecal NDF also includes a proportion of pdNDF that is not digested because the retention time in the fermentation compartments is not long enough for complete pdNDF digestion. In dairy cows fed at production level of intake pdNDFD was substantially lower than in sheep fed at maintenance (0.75 vs. 0.85) resulting to a greater loss of potentially digestible NDF in faeces.

Nutrient supply

Feed intake

Accurate prediction of DM intake (DMI) is a prerequisite for the formulation of economical dairy cow diets. Despite intensive research, no generally accepted intake model has been developed. Limited success is at least partly due to complicated interactions between the animal and feed factors, and difficulties in distinguishing and quantifying these factors. Many intake models include observed milk yield as a predictor of intake. However, these models are primarily useful in predicting intake required to sustain a given level of milk production, as stated by Keady et al. (2004a). It should also be remembered that the yield can only be known retrospectively after the diet has been fed (Ingvartsen 1994). Several attempts have been made to develop prediction equations for practical ration formulation using multiple regression equations for individual animal data. However, these models have usually large residual errors, and consequently the effects of e.g. silage fermentation characteristic were non-significant in these models. This is probably due to large between animal variations in intake within a diet and study, and large between study variations both in the intake and composition of diets. Mixed model regression analysis with random study effects allows estimating quantitative relationship between dietary variables and DMI and the relative intake potential of diets.

The first relative silage DMI index (SDMI-index) model included D-value (g digestible OM in DM), quadratic negative effect of TA concentration and logarithmic of ammonia N (Huhtanen et al. 2002a). Volatile fatty acids, especially propionic acid, had a stronger negative effect on intake than lactic acid. Digestibility was a much better predictor of SDMI than CP and NDF. The effects of D-value and fermentation quality were combined into a single index by defining standard silage (SDMI-index = 100) and that 0.10 kg DM is one index point. Root mean squared prediction error (RMSE) adjusted for the random study effect was 0.41 kg/d, i.e. the model predicted precisely the differences in the intake potential of silages within studies. The model was revised to include other variables that significantly influence

SDMI (Huhtanen et al. 2007). In addition to D-value and fermentation characteristics, the revised model includes the concentrations of silage DM and NDF, harvest of grass silage (primary vs. regrowth) and forage type (grass, legume and whole-crop). Silage DM concentration influenced quadratically SDMI with maximum intake at DM concentration of 350-400 g/kg. Intake of regrowth silages was 0.4 kg DM/d smaller than that of primary growth silages when the differences in other variables were taken into account. Both legume and whole-crop silages displayed positive associative effects on SDMI, i.e. the intake of silage mixtures was greater than the mean of the two silages when fed alone. Maximum NDF intake was observed at a D-value of 640 g/kg DM suggesting that the cows do not use the full rumen capacity when fed high D silages. Indeed, rumen NDF pool has reduced with increased silage digestibility (Bosch et al. 1992, Rinne et al. 2002) despite increased SDMI. These observations do not support the bi-phasic intake regulation theory (e.g. Mertens 1994); it rather suggests that DMI is regulated by interplay between physical and metabolic factors. In the revised model fermentation variables were simplified to the linear negative effect of TA concentration. However, with silages displaying secondary fermentation the intake predictions can be improved by including acetic acid or VFA in the model (Eisner et al. 2006). Adjusted RMSE of the revised model was 0.34 kg/d and it explained 0.85 of the variation in SDMI within a study. D-value, fermentation quality and DM concentration were the three most important variables.

It is well-known that both the amount and composition of the concentrate supplements influence SDMI. Therefore the next step in developing the intake prediction model was to include concentrate factors in the model (Huhtanen et al. 2008a). Total DMI increases with increased concentrate DMI (CDMI) but the increases diminished at high levels of supplementation; i.e. substitution rate increased. Substitution rate also increased with increased intake potential (SDMI-index) of silages. Interestingly, SDMI explained the variation in substitution rate better than any single component of it. The interaction between forage intake potential and concentrate supplementation is also included in the Feed into Milk model presented by Keady et al. (2004b). In their model silage intake potential is determined by NIRS calibrated against standardized intake data by cattle. In addition to CDMI, the model of Huhtanen et al. (2008) includes the quadratic effect of supplementary protein intake, negative linear effect of fat and positive linear effect of concentrate NDF. Adjusted RSME of the CDMI model in studies in which different concentrate treatments were used with the same silage was 0.27 kg. The two indexes were combined to a single total DMI index (TDMI-index) that describe quantitative differences in DMI within a study by assuming the effects are additive. In the model evaluation the observed DMI response at 0.095 kg/index point was close to default value of 0.100 and the adjusted RMSE of the TDMI-index model was 0.37 kg DM/d.

Evaluation of the TDMI-index model indicated that quantitative differences in the intake potential of the diets can be estimated accurately. The modelling was based on an assumption that within a study the animal factors [e.g. yield, live weight (LW)] are similar for all diets. However, in practical ration formulation in addition to relative intake potential related to diet characteristics, accurate predictions of actual intake including animal factors is required. Most intake prediction models use milk yield and live weight as animal variables. Because milk yield is a function of both cow's genetic potential and diet characteristics, it is important that animal and diet variables are modelled independently of each other to avoid double-counting. It is important to note that cow's genetic intake potential does not increase when she is fed a better diet; the intake response is entirely due to the diet effect. To avoid this double-counting and to have unbiased estimates of diet effects in the model, we used standardised energy corrected milk (sECM) rather than observed yield to describe production potential of the cow (Huhtanen et al. 2011b). Observed ECM was adjusted for days in milk, TDMI-index and dietary metabolizable protein (MP) concentration, i.e. to predict how much the cow would produce at a given stage of lactation when fed a standard diet. An advantage of this approach is that all data is available at the time of prediction, in contrast to observed ECM yield. The final model comprised sECM, LW, days in milk as animal factors and TDMI-index to describe the dietary intake potential. The regression coefficient of TDMI-index (0.088) remained close to the default value suggesting that the true animal and diet effects were separated properly.

Rumen fermentation

Typically the molar proportion of propionate is low in cattle fed diets based on restrictively fermented grass silages with moderate levels of concentrate supplementation; for example in the review of 34 diets fed to growing or lactating cattle the molar proportion of propionate was only 165 mmol/mol (Huhtanen 1998). Water soluble carbohydrates are fermented to lactic acid and VFA during ensilage with the extent and type depending on ensiling characteristics of forages and additives used. These changes have a strong influence on ruminal fermentation pattern. Increased concentration of silage lactic acid increases propionate in rumen VFA. Intraruminal infusions of lactic acid demonstrated that propionate is the main end-product of lactate fermentation (Jaakkola and Huhtanen 1992, Chamberlain et al. 1993). Jaakkola and Huhtanen (1992) calculated that propionate comprised about 50% of the end-product of lactate fermentation in the rumen. Consistently, increased lactic acid concentration in silage has increased pro-

pionate in rumen VFA (van Vuuren et al. 1995, Harrison et al. 2003). In contrast to lactic acid, the effects of silage WSC on rumen fermentation pattern have been inconsistent: sometimes butyrate (Jaakkola et al. 1991, 2006a) and sometimes acetate (Cushanan et al. 1995, Huhtanen et al. 1997) has increased.

Rumen fermentation pattern in cattle fed grass silage-based diets appears to be rather resistant to increased concentrate supplementation. The effect of dietary starch concentration on the proportion of propionate in rumen VFA was not significant in multiple regression models derived from the Nordic data (107 diets in 29 studies) (Sveinbjörnsson et al. 2006). In this dataset dietary lactic acid concentration had the strongest effect on rumen propionate suggesting that silage lactic acid is a more important factor influencing rumen fermentation pattern than starch. Mixed model analysis of an unpublished Finnish dataset (106 diets) indicated that dietary starch concentration influenced rumen propionate in a quadratic manner with a minimum at 200 g/kg DM. In the same dataset molar proportion of acetate decreased quadratically and that of butyrate increased linearly with increased starch concentration. The results suggest that at low levels of concentrate (starch) supplementation silage lactate dominates the rumen fermentation pattern, whereas at moderate levels of dietary starch concentrations the role of rumen protozoa becomes more important. The number of rumen protozoa increases with increased starch supplementation (Rooke et al. 1992, Jaakkola and Huhtanen 1993) that can explain the changes in rumen fermentation pattern with increased concentrate supplementation in cattle fed grass silage-based diets.

As for increased starch supplementation, the effects of fat supplementation on rumen fermentation pattern are rather small in cattle fed grass silage-based diets. In the analysis of the Finnish dataset there was a quadratic positive response in rumen propionate to increased dietary concentration of concentrate fat. The model predicts 10-15 mmol/mol increases in rumen propionate for dairy cows fed 500 g/d of supplementary fat as plant oils. Only at high inclusion rates of plant oils quantitatively important changes in rumen fermentation pattern can be expected in animals fed grass silage-based diets (Tesfa 1993, Shingfield et al. 2008).

Protein supply

Microbial protein synthesised in the rumen comprise the major part of the supply of amino acids (AA) absorbed from the small intestine. Regression coefficients of bivariate regression model predicting milk protein yield were five times greater for bacterial MP compared with feed MP both in North American and North European dairy cow trials (in total >1 700 diets) emphasizing the importance of microbial protein (Huhtanen and Hristov 2009). It has generally been believed that the efficiency of microbial protein synthesis (MPS) is lower in animals fed grass silage-based diets than in those fed dried or fresh forages, but there is little experimental evidence to support this. Three reasons have been suggested for the lower efficiency of MPS: silage fermentation products provide less ATP for microbial growth than WSC (Chamberlain 1987), the nature of N constituents (more ammonia and NPN) and asynchronous energy and N release from the silage (Thomas and Thomas 1985). Microbial protein production in the rumen increased when silage fermentation was restricted using formic acid based additives (e.g. Jaakkola et al. 1991, 2006a, Huhtanen et al. 1997). In addition to increases in measured MPS, increased plasma concentrations of AA, particularly branched-chain AA, (Nagel and Broderick 1992, Huhtanen et al. 1997) indicated greater amount of absorbed AA in response to restricting in-silo fermentation. There were no differences in the total or microbial protein flow at the duodenum between diets based on dried hay or restrictively fermented silage harvested simultaneously from the same sward (Jaakkola and Huhtanen 1993). All these results suggest that the preservation method *per se* does not influence MPS and that the extent, and possibly type, of the in-silo fermentation are more important factors influencing the protein value of forages than preservation method.

The asynchrony, often assumed to be a main reason for the low efficiency of MPS, has attempted to be minimized by feeding soluble carbohydrates. Feeding sugar supplements has decreased rumen ammonia N concentration (Syrjälä 1972, Chamberlain et al. 1985). However, the marginal increases in MPS with sugar supplements have not been greater than those predicted from the increased supply of fermentable energy (Chamberlain and Choung 1995), i.e. no extra benefits from a better synchrony. In line with this, Khalili and Huhtanen (1991) reported significant increases in microbial protein flow with different sucrose supplements in cattle fed a grass silage-based diet. However, the continuous infusion of sucrose decreased rumen ammonia N and increased microbial N flow numerically more than feeding sucrose twice daily despite a better synchrony of energy and N release with the latter. Similar conclusions can be drawn from the studies of Henning et al. (1993) and Kim et al. (1999); continuous supply of energy stimulated MPS more than attempts to catch high post-prandial ammonia concentrations by pulse doses of rapidly fermentable carbohydrates.

Despite rather small contribution to the total MP supply, forage factors influencing the supply of rumen undegraded protein (RUP) have been investigated more intensively than factors influencing MPS. Studies conducted with the *in situ* method have suggested large differences in ruminal degradability of

forage protein, but very seldom these differences have been realized as production responses. Two reasons can be suggested for this discrepancy: the differences in RUP supply are overestimated by the current methods and/or that the value of forage RUP is low. In the analysis of omasal flow data the slope between the predicted (NRC 2001) and measured feed N flow was 0.76 (Broderick et al. 2010) suggesting that the differences in ruminal degradability of dietary CP are smaller than the model predictions based on the tabulated *in situ* data. The models computing ruminal degradability from the kinetic data assume that immediately disappearing fraction (buffer/water soluble N) is degraded at infinite rate. However, there is plenty of evidence that soluble non-ammonia N (SNAN) fractions can escape from the rumen in the liquid phase (e.g. Choi et al. 2002, Reynal et al. 2007). Ahvenjärvi et al. (2007) reported using ^{15}N labeled silage buffer soluble N that approximately 15% of SNAN fraction escaped ruminal degradation in dairy cows. Consistently with these results, a meta-analysis based on 253 diets did not indicate any negative influence of the proportion of SNAN in silage on milk protein yield when silage MP values were calculated using a constant CP degradability irrespective of the proportion of soluble N (Huhtanen et al. 2008b). The meta-analysis of milk production data (Huhtanen et al. 2010) showed that silage D-value and especially intake potential were more important determinants of milk protein yield than silage CP or ammonia concentrations.

Production responses

Silage digestibility

The effects of silage quality on feed intake and production responses can be attributed to intrinsic nutritive value of grass at the time of harvest and changes in the composition of grass during ensilage. In the northern latitudes the digestibility of primary growth grasses decreases very rapidly (0.65 %-units/d; in the dataset of Huhtanen et al. 2006b) with concomitant rapid increases in grass DM yield. Therefore the timing of the harvest of primary growth of grass is one of the most important management decisions in a dairy farm. Improved silage digestibility, expressed as D-value, clearly increases intake and ECM yield (Figure 1). The average increases in silage DMI and ECM yield were 0.027 and 0.045 kg per one gram in D-value. In the studies of Kuoppala et al. (2008) and Randby et al. (2012) intake of grass (mixtures of timothy and meadow fescue) silage was 17 kg DM/d when fed with 8 kg/d of concentrates. These results indicate a high intake potential of restrictively fermented grass silages harvested at early stages of maturity and wilted to DM concentration of approximately 300 g/kg. The effects of silage digestibility on milk fat concentration have been variable and usually small, whereas milk protein concentration has increased with improved digestibility (Rinne et al. 1999a, Kuoppala et al. 2008), probably reflecting an increased energy supply. In all studies (Figure 1) the silages were supplemented with different levels of concentrate allowing calculating concentrate sparing effects of improved silage digestibility. The average ECM yield response was 0.48 (SE = 0.04) kg ECM per kg increase in concentrate DMI. The average "concentrate sparing effect" was 0.81 (SE = 0.12) kg DM per 10 g/kg DM increase in silage D-value. Assuming that silage D-value decreases 5 g/kg DM per day, one day delay in harvest corresponds to 0.22 kg decrease in ECM yield or 0.45 kg DM greater concentrate requirement to maintain ECM yield.

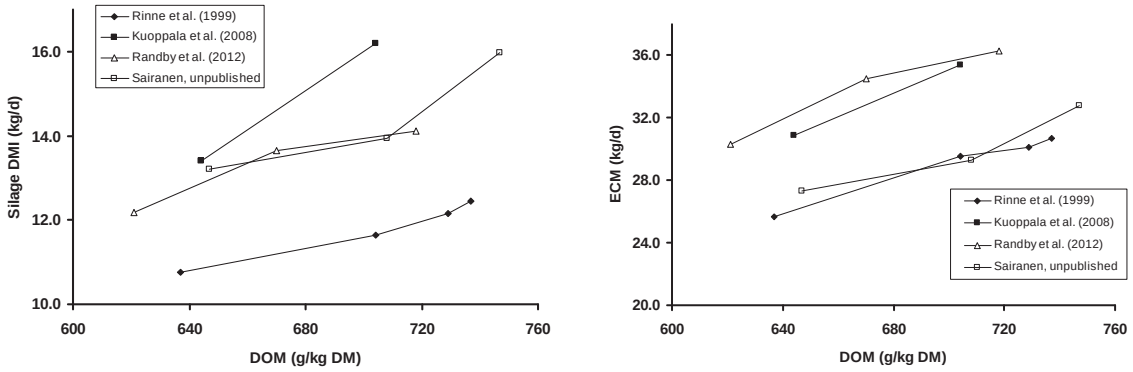

Figure 1. The effects of the concentration of digestible organic matter (DOM) on silage DM intake and ECM yield. The values are means over 2 or 3 concentrate levels in each study.

Silage fermentation

In a meta-analysis of data from silage fermentation studies (47 studies, 234 diets) both the extent and type of in-silo fermentation influenced milk production variables (Huhtanen et al. 2003). In the dataset the silages were harvested at the same stage of maturity and ensiled with different additive treatments.

The yields of milk, ECM and milk components decreased with increased concentrations of lactic acid and VFA in silage. Numerically the effects of VFA were stronger than those of lactic acid. Proportional decreases in the yield of milk components with increasing extent of in-silo fermentation were the smallest for lactose and the highest for milk fat. When silage DMI was included in the prediction models, the effect of TA concentration on milk yield was not significant. However, increased silage TA concentration influenced negatively on the ECM yield even when silage DMI was included in the model, but the regression coefficient was much smaller (-5.8 vs. -18.6 g per 1 g TA/kg DM). It can be concluded that the effects of in-silo fermentation on the production of milk and milk components are mainly derived from the changes in feed intake.

Milk fat and protein concentrations decreased with increased in-silo fermentation (Huhtanen et al. 2003). Reduced milk protein concentration can be attributed to decreased feed intake and the lower efficiency of MPS, whereas the lower fat concentration is most likely related to the reduced proportion of lipogenic VFA in the rumen. The effects of silage TA concentration remained negative even at fixed DMI indicating that the changes in the composition of absorbed nutrients influenced milk composition beyond the responses related to DMI. Decreases in milk protein yield with increased in-silo fermentation were not greater than those predicted from reduced intake, even though negative effects of high TA concentration in silage on the efficiency of MPS are well-documented (Harrison et al. 2003). It is possible that increased propionate production from silage lactate increases hepatic gluconeogenis thereby sparing AA from being used for glucose production. Higher plasma glucose concentration in cows fed extensively fermented silages compared with those fed restrictively fermented silages (Heikkilä et al. 1998, Shingfield et al. 2002b) support this hypothesis. However, the lack of responses to dietary supplementation of propylene glycol of cows given restrictively fermented silages (Shingfield et al. 2002a, Jaakkola et al. 2006b) do not support the hypothesis that the diets based on restrictively fermented silages are specifically limited by the glucose supply.

Improved silage fermentation can be realized as increased yield or as “concentrate sparing effect”. Compared with formic acid-treated silage the cows given untreated silage had required an additional 2·9 kg concentrate per cow per day to produce the same amount of milk fat plus protein (Shingfield et al. 2002a). The “concentrate sparing effect” of formic acid treatment was greater than reported by Mayne (1992) and Keady and Murphy (1996). The greater value in the study of Shingfield et al. (2002a) may be related to the higher levels of concentrate feeding, and therefore smaller marginal responses to supplements attained.

Concentrate supplementation

It is well-known that concentrate supplementation decreases silage DMI but increases total DMI. Silage DMI decreased by 0.45 kg and total DMI increased by 0.55 kg per 1 kg increase in concentrate DMI in our data-set from milk production trials (233 treatment means from concentrate supplementation studies, Huhtanen et al. 2008a). The effects of concentrate DMI on total DMI were strongly curvilinear with decreasing responses at high levels of supplementation. When the data was divided according to the relative silage DMI index into two groups [<100 (mean 91) and >100 (mean 107)] the total DMI increased less (0.51 vs. 0.61 kg per kg increase in concentrate DMI) for silage of high compared with low intake potential, respectively. As a result of interactions between the forage quality and the level of concentrate supplementation substitution rates can be high, even close to 1.0, in cows fed high quality grass silages with moderate to high amounts of concentrates (Kuoppala et al. 2008, Randby et al. 2012).

The mean linear ECM yield response to increased concentrate allocation was 0.71 kg/kg concentrate DM, but it decreased with the increasing supplementation level (Huhtanen et al. 2008a). With high quality silages marginal production responses to increased concentrate allocation were small (Kuoppala et al. 2008) or even negative (Randby et al. 2012). Small production responses are related to the high substitution rate, negative associative effects in digestion and possibly repartitioning nutrients towards body tissues with high concentrate levels. Although the digestibility of concentrates at maintenance level is greater than that of forages, diet digestibility in dairy cows at production level was not related to the concentrate intake (Nousiainen et al. 2009). Interestingly, when the data-set from concentrate supplementation studies were divided according to mean milk yield (<27 kg/d and >27 kg/d) the linear ECM responses were greater (0.76 vs. 0.63 kg/kg concentrate DM) at low (mean 23 kg/d) compared with high (31 kg/d) production level. This is mainly because total DMI responses were greater (0.65 vs. 0.47) at low production level. In the analysis of a larger dataset ECM yield responses to increased ME intake did not depend on the production level of the cows (Huhtanen and Nousiainen 2012).

Protein supplementation

Proper determination of animal protein requirements is critically important for maximizing production and minimizing N input in dairy production systems. Efficiency of N utilization in milk production is relatively low at 25-28% (Huhtanen and Hristov 2009). Although increasing N input usually increases milk pro-

tein yield, conversion of dietary N to milk N will decrease. Earlier when the feed protein evaluation was based on digestible CP the strategy in Finland (Green Line) was to increase CP concentration in grass silage by high levels of N fertilization and early harvest (Hiltunen 1979). As discussed before, maturity stage at harvest has a strong influence in intake and milk production. However, when CP concentration in grass silage was increased from 120 to 150 g/kg DM by greater application rate of N fertilizer feed intake or output of milk and milk protein were not influenced, while provision of additional N in concentrate supplements improved all of these parameters (Shingfield et al. 2001).

Inclusion of protein supplements such as soybean and rapeseed meals in grass silage-based diets increased milk protein yield, but at the same time reduced the efficiency of N utilization (Huhtanen and Hristov 2009). The increases in milk protein yield ranged from 98 (soybean meal) to 136 g/kg increase in CP intake (untreated rapeseed meal) in recent meta-analysis by Huhtanen et al. (2011a). Similar differences were reported in a single study by Shingfield et al. (2003), who compared soybean mean and rapeseed expeller at four graded isonitrogenous levels. Plasma AA profile suggested that rapeseed increased the supply of histidine and branched-chain AA compared with soybean meal (Shingfield et al. 2003). Positive production responses to supplementary protein in cows fed grass silage-based diets are partly associated with increased ME intake resulting from a greater silage DMI (Huhtanen et al. 2008) and improved diet digestibility (Nousiainen et al. 2009). Marginal responses to incremental ME (0.16 – 0.18 kg ECM/MJ ME) in protein studies (Huhtanen et al. 2011a) were greater than usually obtained with inclusions of concentrate feeds (about 0.10). This may indicate that a greater AA/ME ratio in absorbed nutrients can improve the efficiency of ME utilization for milk production. The data from a whole lactation study (Law et al. 2010) indicated that calculated ME balance was greater for cows fed low vs. medium and high protein diets, but the differences in blood metabolites, body condition score or live weight change did not indicate any true differences in energy balance.

Two main strategies, reducing ruminal CP degradability of supplementary protein and balancing profile by absorbed AA by using AA supplements or balancing dietary ingredients, to improve milk N efficiency have widely been investigated. In the meta-analysis (Huhtanen et al. 2011a) untreated and heat-treated rapeseed meal elicited similar milk protein yield responses. This is consistent with the meta-analysis by Ipharraguerre and Clark (2005), who did not find any differences in milk production between soybean meal and different RUP sources. According to the meta-analysis of Huhtanen and Hristov (2009) ruminal CP degradability had a significant effect on milk protein yield, but calculated marginal responses to MP derived from reduced degradability was only 6-8%. It has been suggested that the protein supplements treated to reduce ruminal protein degradability have not increased milk yield as the untreated supplements already met the cow's MP requirements. To test this hypothesis Rinne et al. (1999b) fed untreated and heat-treated rapeseed meal at four different levels. Both supplements increased milk and protein yields linearly, but no differences between untreated and treated rapeseed feeds were observed.

Methionine and lysine are often considered as limiting and/or co-limiting AA in dairy cows, but there is no evidence that these AA limit milk protein production in cows fed grass silage-based diets (Choung and Chamberlain 1992 and 1995, Varvikko et al. 1999). Vanhatalo et al. (1999a) infused post-ruminally histidine alone or in combinations with methionine, lysine or both. Histidine increased significantly milk protein yield, whereas lysine, methionine or lysine + methionine did not produce any further response. Attempts to identify methionine, lysine and branched-chain AA as the second limiting AA were not successful (Vanhatalo et al. 1999a, Huhtanen et al. 2002b, Korhonen et al. 2002). It is possible that after the first-limiting AA the differences between the next limiting AAs are small in cows fed grass silage–based diets, and that the ranking of these AA can vary between experiments.

Analysis of data from milk production trials clearly indicated that dietary CP concentration was the best single variable predicting milk N efficiency (Huhtanen and Hristov 2009). Intake of N has often been used as a predictor of milk N efficiency (e.g. Castillo et al. 2000), but the adverse effect of increased N intake is much stronger when derived from increased dietary CP concentration rather than from increased DMI. As could be expected from relatively small effects on milk production, ruminal protein degradability had relatively small influence on milk N efficiency (Huhtanen and Hristov 2009). Milk urea concentration is closely related to dietary CP concentration and it predicted the differences between diets in milk N efficiency and calculated urinary N output accurately (Nousiainen et al. 2004b) suggesting that it can be used as a farm diagnostic tool.

Conclusions

The silage research in Finland during the latest 30 years has systematically focused on the production and ensiling of grass and legume silages with special reference to the utilization and supplementation of silages in cattle production. This work has facilitated the development of ration formulation systems based on meta-analyses of large and comprehensive datasets that has been compiled mainly from Finnish and North European studies. Successful economical dairy cattle ration optimization requires (1) well-performing feed evaluation system, (2) accurate and cheap feed analyses for on-farm produced

silages, (3) DM intake prediction model integrating independently dietary and animal constraints and (4) equations to estimate true nutrient supply and marginal production responses to changes in nutrient intake. Based on these principles Huhtanen and Nousiainen (2012) presented milk production response models that are currently used in practical feed ration planning in Finland.

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Can histidine be limiting milk production in dairy cows fed corn silage and alfalfa haylage-based diets?

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Keywords: dairy cow, corn silage, histidine, metabolizable protein

Introduction Nitrogen losses from livestock operations represent a significant water and air pollutant. It has been repeatedly demonstrated that reduction in dietary N input can be a successful strategy for improving N utilization efficiency and reducing N losses with manure in dairy cows and beef cattle (Hristov et al. 2011). If animal requirements for metabolizable protein (MP) are not met, however, production cannot be sustained (Lee et al. 2011). Supplementation with rumen-protected (RP) amino acids (AA) limiting milk production and milk protein synthesis may compensate for the lack of MP in dairy cow diets. Methionine (Met) and lysine (Lys) have commonly been considered most limiting AA in typical North American dairy diets based on corn silage and alfalfa haylage with complementary protein feeds (NRC, 2001). Histidine (His) has also been shown to limit milk production in diets based on grass silage, low in ruminally-undegraded protein (RUP), or supplemented with protein feeds low in His, such as feather meal (Vanhatalo et al. 1999, Kim et al. 1999). The basis for His being potentially an AA limiting milk and milk protein yields is the relatively lower His than Met concentration in microbial protein synthesized in the rumen (Schwab et al. 2005). Our data for cows fed diets based on corn silage (typically 40% of dietary DM) and alfalfa haylage indicated an average His:Met ratio in ruminal bacteria of about 1:1.4. Thus, His may become a limiting AA in North American dairy diets, if microbial protein is the primary source of MP. In a study with high-producing dairy cows fed MP- and RUP-deficient diets based on corn silage, alfalfa haylage, corn grain, and whole roasted soybeans, Lee et al. (2012) observed a sharp drop (about 42%) in blood plasma His concentrations. Based on these data, we developed the hypothesis that His may be a limiting AA in dairy cows fed MP-deficient diets, when microbial protein synthesized in the rumen is the main source of MP for the cow.

Materials and methods To test the above hypothesis, a randomized complete block design experiment with 48 Holstein cows was conducted. The duration of the experiment was 12 wk. Following a 2-wk covariate period, cows were blocked by days in milk (average of 54 to 95 d) and milk yield and randomly assigned to one of 4 treatments (12 cows per treatment): control, MP-adequate diet (AMP; MP balance: +9 g/d; NRC, 2001); MP-deficient diet (DMP; MP balance: -317 g/d); DMP supplemented with RPLys (AminoShure®-L) and RPMet (Mepron®; DMPLM); and DMPLM supplemented with an experimental RPHis product (DMPLMH). The RPLys and RPHis products were from Balchem Corp. (New Hampton, NY, U.S.A.) and the RPMet was from Evonik Industries AG (Hanau, Germany). The diets contained (approximate %, DM basis): corn silage, 40; alfalfa haylage, 16; grass hay, 6; ground corn grain, 6 to 12; bakery byproduct, 7; whole, roasted soybeans, 5 to 6; canola meal, 3 to 5; SoyPLUS® (West Central®, Ralston, IA, U.S.A.), 0 to 5; molasses, 4; and mineral/vitamin premix. Analyzed crude protein content of the AMP and DMP diets was 15.7 and 13.5 to 13.6%, respectively. Data for dry matter intake (DMI), milk yield and composition, fecal and urinary N losses, and blood plasma AA concentration were analyzed using the MIXED procedure of SAS (2003; SAS Inst. Inc., Cary, NC, U.S.A.).

Results Compared with AMP, DMI tended to be lower (P = 0.06) for DMP, but was similar for DMPLM and DMPLMH (Table 1). Milk yield was decreased by DMP, but was not different from AMP for DMPLM and DMPLMH, paralleling the trend in DMI. Milk fat and true protein content did not differ among treatments, but milk protein yield was increased by DMPLM and DMPLMH compared with DMP and was not different from AMP. Milk urea-N and urinary-N excretion were decreased by all DMP diets compared with AMP. Milk N secretion as a proportion of N intake was greater for the DMP diets. Plasma essential AA, Lys, Met, and His were lower for DMP compared with AMP. Supplementation of the DMP diets with RPAA increased plasma Lys, Met, and His.

Conclusions A diet approximately 15% deficient in MP (NRC, 2001) decreased DMI and milk yield in dairy cows. Supplementation of the MP-deficient diet with RPLys and RPMet diminished the difference in DMI and milk yield compared with AMP and additional supplementation of RPHis eliminated it. As DMI tended to increase with RPAA supplementation, we propose that, similar to monogastric species, AA play a role in DMI regulation in dairy cows. Our data implicate His as a limiting AA in high-producing dairy cows fed corn silage, alfalfa haylage, and corn grain-based diets, deficient in MP, for which microbial

protein represents a large proportion of MP. The MP-deficient diets clearly increased milk N efficiency and decreased dramatically urinary N losses.

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Table 1. Effect of metabolizable protein supply and rumen-protected amino acid supplementation on dry matter intake (DMI), milk production and composition, urinary N losses, and blood plasma amino acid concentration in dairy cows.

	Diet					
Item	AMP	DMP	DMPLM	DMPLMH	SEM	*P*-value
DMI, kg/d	24.5	23.0	23.7	24.3	0.43	0.06
Milk yield, kg/d	38.8^{a}	35.2^{b}	36.9^{ab}	38.5^{a}	0.74	0.004
Milk ÷ DMI	1.59	1.56	1.57	1.59	0.032	0.89
Milk fat, %	3.50	3.51	3.32	3.30	0.117	0.44
Yield, kg	1.34	1.20	1.21	1.23	0.045	0.10
Milk true protein, %	2.98	2.94	2.99	3.03	0.030	0.23
Yield, kg/d	1.13^{a}	1.01^{b}	1.10^{a}	1.14^{a}	0.025	0.002
Milk protein-N, % of N intake	29.4^{b}	34.2^{a}	34.4^{a}	33.6^{a}	0.99	0.003
Milk urea N, mg/dL	13.0^{a}	10.3^{bc}	10.1^{c}	11.1^{b}	0.37	< 0.001
Urinary N excretion, g/d	143^{a}	92^{b}	87^{b}	97^{b}	5.7	<0.001
Urinary urea N, g/d	104^{a}	47^{b}	41^{b}	49^{b}	5.8	<0.001
Blood plasma AA, mg/100 mL						
Histidine	0.75^{a}	0.40^{b}	0.40^{b}	0.64^{a}	0.042	<0.001
Lysine	1.03^{a}	0.86^{b}	0.92^{ab}	0.99^{a}	0.039	0.032
Methionine	0.25^{b}	0.27^{b}	0.39^{a}	0.36^{a}	0.013	<0.001

^{a,b,c} Within a row, means without a common superscript letter differ ($P < 0.05$). AMP = MP balanced diet; DMP = MP-deficient diet; DMPLM = DMP supplemented with RPLys (AminoShure®-L) and RPMet (Mepron®); DMPLMH = DMPLM supplemented with an experimental RPHis product.

Comparison of methods for estimating feed N flow in cows fed grass or red clover silage based diets

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Keywords: Degradability, feed protein, grass silage, rumen, red clover silage

Introduction Proper determination of animal protein requirements is critically important for maximizing production and minimizing N input in dairy production systems. When the requirements of ruminally degradable N are met, the supply of absorbed amino acids can only be increased by ruminally undegraded protein (RUP). The meta-analysis of large datasets of milk production trials in dairy cows indicated that reduced ruminal degradability of crude protein (CP) had positive effects on milk protein yield and milk N efficiency (Huhtanen & Hristov 2009). However, quantitatively the effects were relatively small. Smaller than expected responses to increased RUP can be related to methodological restraints of the *in situ* method that may overestimate the true differences in the supply of RUP (Broderick et al. 2010). The objective of this study was to compare feed N flow in lactating cows fed grass or red clover silage-based diets estimated by *in situ*, particle kinetic technique or omasal sampling (*in vivo*).

Material and methods Five ruminally cannulated Finnish Ayrshire dairy cows were used in a 5 × 5 Latin square with 21 d periods. Experimental silages were prepared from grass and red clover harvested at two stages of maturity. The four pure silages and a mixture of late harvest grass and early harvest red clover were fed to cows at 0.95 of *ad libitum* in addition to 9.0 kg/d concentrate during d 16-21 of each period. Ruminal contents were evacuated before the morning feeding and 6 h after feeding as described previously for the current experiment (Kuoppala et al. 2009). The ruminal and faecal samples were divided into seven particle size fractions (38-80, 80-160, 160-315, 315-630, 630-1250, 1250-2500 and >2500 µm) by wet sieving. The particle fractions were analyzed for the concentrations of N and indigestible NDF (iNDF). The concentration of iNDF was determined by incubating the particle fractions for 12 d in the rumen using nylon bags with 17 µm pore size. Particle N flow (PNF, g/d) was calculated using the following equation:

$$PNF = 0.16 \times \sum_{i=1}^{n} DMPool \times CP \times (FiNDF \div RiNDF)$$

where DM-Pool = rumen DM pool (kg) of the i^{th} particle size fraction, CP = CP concentration of the i^{th} particle size fraction (g/kg DM), FiNDF = faecal output of i^{th} particle size fraction (kg/d) and RiNDF = rumen iNDF pool of the i^{th} particle size fraction (kg). The ruminal disappearance of CP in concentrate and forage samples was determined by the *in situ* method. Samples of 2.0 g (10 mg/cm^2 surface area) were weighed into nylon bags with a pore size 38 µm and a pore area equal to 31% of the total surface. All bags were pre-soaked in tap water before placing into the rumen. The nylon bags were introduced in reverse sequence into the rumen of each cow starting at 0700 h and incubated for 3, 6, 12, 24, 36, 48, 72 and 96 h. Ruminal *in situ* degradability of CP (CPD_{IS}) was calculated using the equation: $CPD_{IS} = a + b \times (c / (c + k))$, where a = rapidly degradable fraction, b = in time degradable fraction, c = degradation rate of b (1/h) and k = fractional passage rate from the rumen (1/h). The passage rate of forages and concentrates were calculated according to Krizsan et al. (2010).

In vivo feed protein flow was determined by omasal sampling technique (Ahvenjärvi et al. 2000) using a triple-marker method with iNDF, Yb-acetate and Cr-EDTA as digesta flow markers and ^{15}N as a marker to determine microbial N flow. Feed N flow was calculated as non-ammonia N minus microbial N assuming no endogenous N.

Results and discussion Feed N flow determined by *in situ* or from particle kinetics were strongly correlated ($R^2 = 0.71$, n = 24), but the values derived from particle kinetics were on average 38% greater. The difference may be associated with a greater microbial contamination of rumen particles than the undegraded *in situ* residues. Particle losses were not expected to be different, since the smallest particle size fraction was collected using the same cloth as used for ruminal *in situ* bags.

Feed N flow determined by either *in situ* or particle kinetics was poorly correlated with *in vivo* feed N flow. This can partly be related to the greater random variation in the *in vivo* flow data, but large differences in the mean values (177, 107 and 148 g/d for *in vivo, in situ* and particle kinetics, respectively) also suggest that at least some of the methods resulted in biased estimates of feed N flow (Table 1). The results suggest that a considerable amount of feed N flow occurred either as soluble NAN fraction

and/or as particles below 38 µm. In both cases the difference can be attributed to the false assumption of the *in situ* method; no escape of soluble NAN fractions and particle loss that can take place after the immediate loss. In addition, significant interactions between the methods and diets indicate that not all three methods ranked the diets correctly. For example, *in vivo* feed N flow was 67 g/d greater for the red clover diets compared with the grass diets, whereas the differences were smaller for estimates based on *in situ* (23 g/d) or particle kinetics data (16 g/d). *In vivo* feed N flow decreased with advancing maturity (-21 g/d), whereas it was on average 19 and 35 g/d greater for late compared with early harvested silages flow when determined by *in situ* or particle kinetic methods, respectively. The differences could be attributed to a greater escape of soluble NAN fractions with early harvested silages and to a larger microbial contamination of the residues with late harvested silages.

Conclusions The results suggest that the *in situ* method mainly describes the feed N flow in the particle phase, but not total feed N as it ignores substantial escape of feed N either in soluble form or in particles <38 µm. Mean differences between the methods and interactions between the methods and diets may be attributed to the methodological problems of the *in situ* technique.

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Table 1. Feed protein flow estimated by three methods in cows fed grass or red clover silages.

	Grass		Red Clover				P-value[1]			
	Early	Late	Early	Late	Mix	SEM	S	M	S x M	Mix
Flow[2]										
IV	145	130	219	191	202	12.0	<0.01	0.11	0.59	0.13
IS	90	99	103	132	115	3.4	<0.01	<0.01	0.01	0.06
PNF	130	144	125	180	161	6.0	0.02	<0.01	<0.01	0.06
Difference										
IV-IS	55	32	116	60	87	10.5	<0.01	<0.01	0.09	0.74
IV-PNF	15	-13	94	11	41	11.4	<0.01	<0.01	0.04	0.36
IS-PNF	-40	-45	-22	-49	-46	3.9	0.09	<0.01	0.02	0.19
Degradability										
IV	0.703	0.688	0.627	0.658	0.641	0.0191	0.02	0.69	0.24	0.56
IS	0.816	0.765	0.823	0.767	0.793	0.0011	<0.01	<0.01	0.02	0.64
PNF	0.735	0.657	0.784	0.681	0.711	0.0069	<0.01	<0.01	0.09	0.74

[1] S = species, M = maturity, S × M = Interaction, Mix = mixture vs. early grass + late red clover
[2] IV = *in vivo*, IS = *in situ*, PNF = particle N flow

Changes in maize silage fermentation products during aerobic deterioration and its impact on feed intake by goats

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Keywords: aerobic deterioration, feed intake, fermentation products, goat, maize silage

Introduction Silages made of maize and grass have become the most important forage in the feeding of ruminants. However, most silages, especially well preserved, energy-rich silages are prone to aerobic deterioration after opening. There is evidence of an impact of aerobic deterioration on feed intake, which is, for example, caused by an accumulation of degradation products or changes in volatiles. These fermentation end products have long been believed to impact silage intake through an effect on palatability (Krizsan et al. 2007). Weiß et al. (2011) mentioned that, for example, volatiles like ethyl ester or alcohols could affect feed intake negatively. Wichert et al. (1998) fed to dairy cows a silage of poor hygienic quality that had undergone deterioration after being exposed to air, and observed a decrease in forage intake of about 10 to 20% in comparison to fresh silage. However, no indication was given which substances or properties of the silages were the major determinants for preference of the fresh or avoidance of the poor-quality silage.

The aim of the following study was to describe the changes in chemical composition and fermentation products during aerobic deterioration of maize silages and to evaluate their impact on feed intake and preference by goats.

Material and methods Eight whole-crop maize silages were produced differing in dry matter (DM) content (34% and 40%), chopping length (10 mm and 21 mm) and packing density in the silo (high and low). Each factor combination, i.e. treatment, was ensiled in six 110-L plastic barrels for at least three months. At the day of silo opening (day 0) and then at two-day intervals (day 2, 4, 6, and 8) the following measurements were conducted: temperature, sensory evaluation, chemical composition and microbiological testing. Chemical analysis consisted of proximate constituents, fibre fractions, starch and *in vitro* 24 h gas production. Furthermore, pH, lactic acid (HPLC), volatile fatty acids and alcohols (GC) as well as esters (ethyl lactate (EL) and ethyl acetate (EA)) and water-soluble carbohydrates were determined after coldwater extraction. Dry matter was corrected for the loss of volatiles during drying according to Weißbach and Strubelt (2008).

For use in preference trials, samples of silages (1.7 - 2.0 kg fresh matter per portion) were taken and stored anaerobically in evacuated and vacuum-sealed polyethylene bags. Afterwards, eight preference trials (Buntinx et al. 1997) with goats (n = 6) were carried out, each one lasting 21 days. All trials were conducted with castrated male Saanen type goats (German Improved White Goat breed, mean body weight 85.8 kg ± 13.9 kg). During the experimental phase, each possible two-way combination of the five silages (days 0, 2, 4, 6, and 8) and one standard lucerne hay (n = 15), was offered for free choice for 3 h in the morning.

All data were analyzed using SAS 9.1. The experimental design allowed statistical analysis by multidimensional scaling (PROC MDS), which is used to develop a spatial arrangement representing the differences expressed as selective forage intake by the animals. Each experiment was also tested by analysis of variance after averaging dry matter intake (DMI) of each forage (averaged across each combination, n = 6). The analysis of variance only included variables for animal and forage. Within the forage treatments, means were separated using the minimum significant difference (MSD) from the Waller-Duncan k-ratio t-test. Furthermore, correlation coefficients between silage composition and DMI were calculated.

Results All silages were well fermented with a pH ranging between 3.9 and 4.0 and an absence of butyric acid at the day of opening. The concentrations of proximate constituents were located within orientation values for good quality maize silage. Lactic acid (LA) and acetic acid (AA) were strongly negatively correlated with DM content (-0.82 and -0.65, $p<0.001$), which is in coincidence with literature. EL and EA were detected in all fresh silages, with mean concentrations of 159 and 284 mg/kg DM.

Concerning the contents of proximate constituents, no changes were measured within the eight days of aerobic storage. Concentration of LA and AA decreased, resulting in an increase of the mean pH of all eight experimental silages from 3.9 (day 0) to 5.8 (day 8). Counts of yeasts (germ group 7) rose drastically during the first four days of aerobic storage and in all treatments target values for

maize silages (10^6 cfu/g) given by VDLUFA (2011) were clearly exceeded. Consequently, mean silage temperature reached 29°C above ambient temperature. Silages stored in vacuum-sealed bags for use in preference trials contained higher amounts of ethanol, EL and EA ($p<0.01$), possibly due to anaerobic yeast activity.

In the preference trials, DMI significantly decreased after 4 days of aerobic storage ($p<0.05$) for most treatments (Table 1). When using only the fresh silages (day 0) that had not undergone aerobic deterioration, DMI was negatively correlated with AA ($r = -0.72$, $p<0.05$). With an average of 12.9 g/kg DM, concentrations of AA were generally quite low.

Table 1. Intake (g dry matter) of silages with different length of aerobic storage (0 to 8 days) and lucerne hay by goats (n = 6) in eight preference trials.

	Length of aerobic storage (days)							
Silage	0	2	4	6	8	Lucerne hay	Mean	MSD
S-34-2	650[a]	610[a]	633[a]	380[b]	136[c]	680[a]	515	128
S-40-2	644[a]	620[a]	607[a]	518[b]	334[c]	684[a]	568	97
S-34-1	651[a]	657[a]	650[a]	625[a]	464[b]	575[a, b]	604	118
S-40-1	723[a]	779[a]	752[a]	490[b]	294[c]	588[b]	605	121
L-34-2	609[b, c]	700[a, b]	720[a]	585[c]	284[d]	732[a]	605	104
L-40-2	715[b]	657[b]	467[c]	444[c]	256[d]	816[a]	559	101
L-34-1	580[a]	597[a]	641[a]	373[b]	223[c]	602[a]	503	92
L-40-1	598[a, b]	569[a, b]	542[b]	349[c]	247[d]	635[a]	490	82

MSD = Minimum significant difference (Waller Duncan k-ratio t-test), n = 40, S = Short chopping length, L = Long chopping length, 40 = 40% DM, 34 = 34% DM, 2 = high packing density, 1 = low packing density

Correlation analysis between preference when expressed as DMI and silage composition showed a weak negative relationship with ethanol ($r = -0.33$, $p<0.05$) as well as with EL ($r = -0.33$, $p<0.05$). *In vitro* 24 h gas production was positively associated with DMI ($r = 0.51$, $p<0.001$).

Conclusions This study demonstrated that strong changes in the fermentation products of maize silage occurred during eight days of aerobic storage. Counts of spoilage organisms, especially yeasts rose above target values within four days. There was a strong impact of deterioration on feed intake and preference by goats, marked by a decrease of DMI after four days of storage. Some fermentation products, especially ethanol, AA and EL, affected short-term feed intake, i.e., preference, of goats negatively.

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Effect of replacing grass silage with red clover silage on rumen lipid metabolism and milk fatty acid composition

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Keywords: red clover silage, biohydrogenation, milk, polyphenol oxidase

Introduction Red clover has a polyphenol oxidase (PPO) activity different to grasses that has been implicated in a higher ruminal escape of forage lipids accounting for the greater enrichment of polyunsaturated fatty acids (PUFA) in milk of cows fed red clover silage (RC) than grass silage (G) (Dewhurst et al. 2006, Kim et al. 2009). Our objective was to evaluate the effect of replacing G with RC on ruminal lipid metabolism, milk production and milk fatty acid composition.

Material and methods Four multiparous rumen fistulated Finnish Ayrshire cows in mid-lactation were used in a 4 x 4 Latin Square with 21 d experimental periods. Experimental treatments consisted of total mixed rations (TMR) containing 600 g forage/kg diet DM with RC replacing G in the diet in the ratio of 0:100, 33:67, 67:33, and 100:0. Forages were supplemented with a standard concentrate comprised (g/kg TMR DM) rolled barley (180), molassed sugar beet pulp (90; Raisio Ltd, Raisio, Finland), solvent extracted rapeseed meal (115; Raisio Ltd) and a low phosphorus vitamin and mineral premix (15; Onni, Rehumelica Ltd, Vaasa, Finland). All diets were fed ad libitum. Omasal sampling and triple marker techniques were used to assess nutrient flow entering the omasal canal of lactating cows (Ahvenjärvi et al. 2000). After adjusting the pH to 2.0 by hydrochloric acid, lipids in feeds and omasal digesta were extracted in duplicate using ultrasound and a 3:2 (v:v) mixture of hexane and isopropanol. Extracts were separated into several lipid classes by thin layer chromatography using silica plates (1.13895.0001, Merck, Darmstadt, Germany) and a 70:30:2 (v:v) mixture of hexane, diethylether, and acetic acid. Esterified lipids were methylated by methanolic sodium methoxide and free fatty acids (FFA) by methanolic sulphuric acid. The preparation of milk samples and the analysis of fatty acid methyl esters were made as described previously (Halmemies-Beauchet-Filleau et al. 2011).

Results and discussion Despite the inherently higher activity of PPO in red clover, there were no differences in the proportion of total lipid in RC and G silages in the form of FFA. Due to extensive lipolysis, FFA accounted on average for 74% of total lipid in ensiled forages. Intake of DM and milk yield tended ($P \leq 0.06$) to be higher when RC and G were fed together rather than when offered separately. Treatments had no effect ($P > 0.05$) on milk fat or crude protein secretion. Increases in the proportion of RC in the diet decreased linearly ($P < 0.05$) whole tract DM, OM, NDF and nitrogen digestibility. Forage species had no effect ($P > 0.05$) on rumen pH or VFA concentration, but increases in the proportion of RC in the diet decreased linearly ($P < 0.05$) the molar ratio of lipogenic to glucogenic VFA. Ingestion of total fatty acids was similar ($P > 0.05$) among treatments, but the intake of specific fatty acids varied due to differences in the lipid composition of G and RC. Replacing G with RC decreased linearly ($P < 0.05$) 18:3n-3 intake but increased ($P < 0.05$) 18:2n-6 consumption.

Forage species had a major impact on ruminal lipid metabolism. Replacing G with RC decreased linearly ($P < 0.05$) the lipolysis of dietary esterified lipids in the rumen from 85 to 70%. Furthermore, these changes were associated with linear decreases ($P < 0.05$) in ruminal 18:3n-3 biohydrogenation (93 to 85%) and tended ($P \leq 0.08$) to lower the extent of *cis*-9 18:1 and 18:2n-6 biohydrogenation. The proportion of RC in the diet had no effect ($P > 0.05$) on the amounts or on the relative proportions of major lipid classes at the omasum. On average, FFA, polar lipids, triacylglycerols, diacylglycerols, and monoacylglycerols accounted for 79.9, 12.4, 4.4, 2.4, and 0.8% of total fatty acids in omasal digesta, respectively. Replacing G with RC increased linearly ($P < 0.05$) the flow of 18:3n-3 in all lipid fractions at the omasum (Figure 1). Omasal flow of *cis*-9 18:1 (24.2 to 26.9 g/d) and 18:2n-6 (Figure 1) also increased linearly ($P < 0.05$) in response to replacing G with RC in the diet. Treatments had no effect ($P > 0.05$) on the flow of bound phenols as a measure of PPO oxidation at the omasum (mean 41 mg/d).

Milk fat 18:2n-6, 18:3n-3, and total PUFA content increased linearly ($P < 0.05$) from 1.2 to 1.8, from 0.5 to 1.2, and from 3.8 to 5.4 g/100g fatty acids, respectively, in response to higher proportions of RC in the diet. Treatments had no effect ($P > 0.05$) on milk fat *cis*-9, *trans*-11 or total conjugated linoleic acid content. Concentrations of 4- to 14- carbon fatty acids and 16:0 content decreased ($P < 0.05$) in a linear manner to RC in the diet.

Conclusions The proportion of RC in the diet had no effect on the relative distribution of fatty acids in lipid fractions in the rumen, but modified substantially the flows of 18:2n-6 and 18:3n-3 at the omasum. Increases in the flow of PUFA available for absorption accounted for the higher enrichment of PUFA in milk on RC containing diets. Effects of forage type on milk fat composition could, in the most part, be explained by differences in lipolysis and biohydrogenation of dietary unsaturated fatty acids in the rumen. Furthermore, data suggest that these changes in rumen lipid metabolism for RC compared with G are not related to PPO activity lowering lipolysis in the rumen, but are most probably related to a combination of lipid entrapment within protected protein matrices as a result of PPO induced protein binding and the role of forages on rumen digestion kinetics influencing ruminal escape of certain forage particle sizes.

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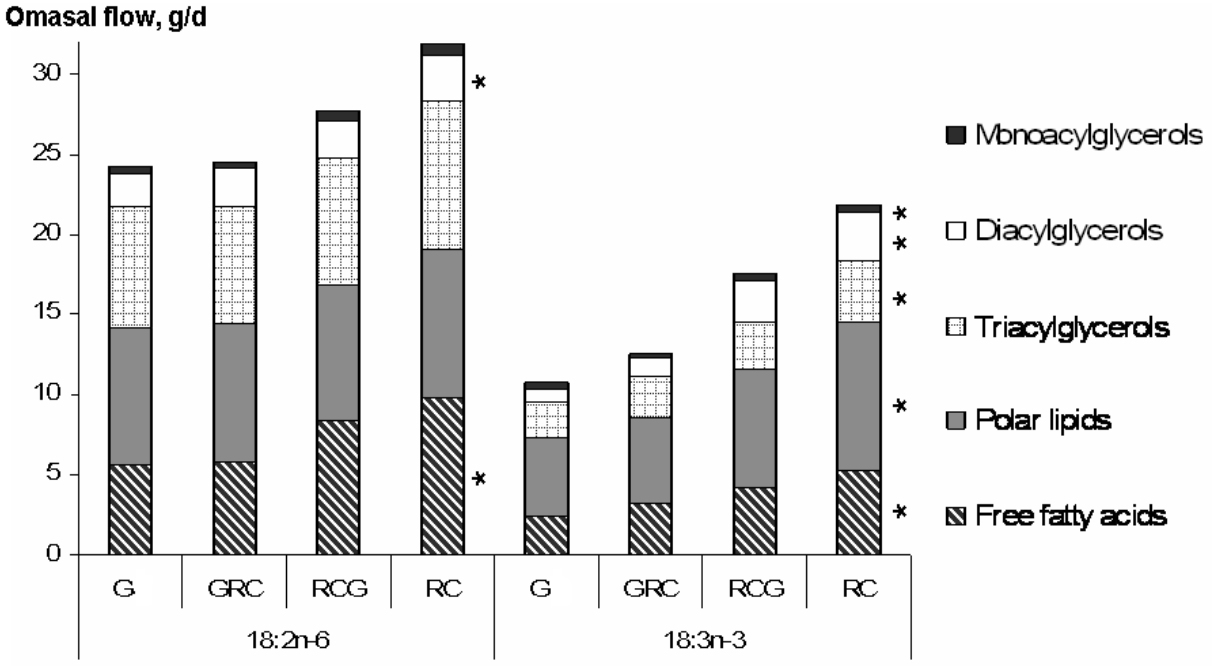

Figure 1. Effect of replacing grass silage with red clover silage in the diet on the flow of 18:2n-6 and 18:3n-3 at the omasum of lactating cows. Refers to total mixed rations comprised (600 g/kg DM) grass silage (G), mixtures (on a DM basis) of grass and red clover silage of 2:1 (GRC) or 1:2 (RCG) or red clover silage (RC).* Indicates linear changes (P<0.05) within lipid classes across treatments.

Feeding silage and haylage to horses

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Keywords: digestibility, equine, forage, grass, moulds, plant maturity

Introduction

During the latest 10-15 years, wrapped forages in bales such as silage and haylage have partially or totally replaced hay in Scandinavian equine diets (Holmquist and Müller 2002, Enhäll et al. 2012). The underlying reasons for this shift are several, including the difficulties of producing and storing hay under dry conditions to avoid mould growth and subsequent respiratory problems in horses (Robinson et al. 1996, Vandenput et al. 1997). The idea of feeding ensiled forage to equine species is however older; already in 1897, Nourse concluded from experiments that maize silage was a suitable feed for horses and mules if the animals were given time to adapt to the feed, and in 1913, Rommel provided feeding recommendations for silage to horses and mules. The amount and depth of scientific knowledge on the use of different forages for horses have been scarce for a long time period, but during recent years, research and scientific publication within the area has increased. As the equine industry and management is quite different from *e.g.* dairy or beef production, research questions naturally differ and require attention from an equine feeding perspective. The main objective of this paper is to review recent knowledge within the area of feeding wrapped forages to horses, and to identify some of the current knowledge gaps that may be important for future research.

The horse is a grass and forage eater

First of all, it is important to recognize that horses are grass eaters and have developed during millions of years to survive and thrive on steppe grass through microbial fermentation in the hindgut (Janis 1976). As with other herbivore species, the digestive system works best with the feed it is adapted to (Hummel et al. 2006), and therefore horses should be fed rations which are composed of as much forage as possible. No horse should be fed less roughage dry matter (DM) than an amount corresponding to 1 % of the body weight daily (NRC 2007), irrespective of the type of roughage used. However, horses are used for a number of different purposes, from the strenuous performance during endurance riding or racing to being a pet animal, and nutrient requirements differ vastly. Overfeeding resulting in obesity and metabolic diseases such as Equine Metabolic Syndrome (EMS) and laminitis are not uncommon problems in Western Europe and USA (Asplin et al. 2007, Wyse et al. 2008, Pratt-Phillips et al. 2010). The nutritive content in forages for horses should therefore not be the highest possible, but adapted to the horse category in question. In practice, this often results in that forages should be harvested in a comparably late plant maturity, in order to provide the horse with sufficient amounts of forage without risking overfeeding of energy from a forage-only diet. A late harvest may however also affect fermentation, conservation and microbial composition of silage and haylage during harvest, storage and feedout.

Silage and haylage – a dry matter definition

The most common type of conserved forages fed to horses in Sweden is hay, haylage and silage (Holmquist and Müller 2002, Enhäll et al. 2012). Haylage is defined as forage containing 400-600 g DM/kg, and stored anaerobically (Gordon et al. 1961, Finner 1966). A sufficiently high water content and water activity (a_w) is required for lactic acid bacteria (LAB) to be able to ferment glucose/fructose to lactate via Embden-Meyerhof-Parnas glycolytic pathway (Gibbs et al. 1950). Fermentation therefore becomes restricted as DM content increase, and haylage is primarily preserved through a combination of drying and airtight storage, and not by ensiling. Generally, haylage contains higher levels of residual water-soluble carbohydrates (WSC), higher pH and lower concentration of volatile fatty acids (VFA), lactic acid, ammonia-N and alcohols (ethanol and 2,3-butanediol) compared to silage (Gordon et al. 1961, Greenhill 1964, Finner 1966, Jackson and Forbes 1970, Nicholson et al. 1991, Pahlow and Weissbach 1996, Dawson et al. 1999, Driehuis and van Wikselaar 2000, Han et al. 2006, Müller and Udén 2007, Müller et al. 2008). As haylage is not conserved by a fermentative process, but by air-tight storage, it is crucial that an anaerobic seal is used and kept intact during the entire storage period, to avoid impaired hygienic quality and heating of the forage.

Aerobic storage stability of opened silage and haylage bales

The aerobic storage stability of bales may be an issue for equine operations, although it may not be an issue in other livestock production systems. The majority (3/4) of Swedish horses are kept in stables housing only 1-4 individuals (Persson 2005), which means that the daily forage consumption is com-

parably low and that big bales of silage or haylage often contains too much forage (ca 400 to 600 kg) in order to be consumed before onset of deterioration. In addition, haylage may contain residual WSC in amounts of up to >120 g/DM (Müller et al. 2008), which may provide a good source of energy for deteriorating microorganisms which further shortens the aerobic stability of opened bales. When oxygen diffuses into silage, lactate-assimilating yeasts grow and a gradient of pH, lactic acid as well as other acids, and oxygen is created from the silage surface, producing heat and niches with micro-environments suitable for different deteriorating micro-organisms (Jonsson 1991). Once heating has started in haylage, the warm-up process may be rapid, as there is little water to buffer the heat produced. However, research within this area have shown that aerobic storage stability of haylage may vary between 12 to over 120 hours, and that additives may restrict mould but not yeast growth once the bale has been opened (Müller 2005). Further research on the effect of different types of additives on aerobic storage stability of opened bales is warranted. Another factor that has been reported to have a major impact on aerobic storage stability of silage is compaction. Less dense silage have higher oxygen diffusion rates (Rees et al. 1983), as oxygen penetration depends mainly on diffusion and volumetric flow (McGechan and Williams 1994). For high dry matter silages, compaction has been reported to reduce oxygen penetration and subsequent aerobic deterioration (Hoxey and Billington 1987). If actions taken to prolong the aerobic storage stability of opened big bales are not successful, or if handling and use of big bales is not possible, small bale silage and haylage is an alternative, however more expensive. Small bale silage and haylage can be produced by the use of a conventional high-density hay baler and a mini-wrapper intended for this type of bales (Müller 2005).

Factors influencing equine digestion and digestibility of silage and haylage

The single most important factor that has the largest influence on equine digestion and digestibility of all forages in horses is the plant maturity at harvest (McDonald et al. 2002, Edouard et al. 2008, Ragnarsson and Lindberg 2008, Ragnarsson and Lindberg 2010a). Although different forage preservation methods results in differences in (bio)chemical and microbial composition of hay, haylage and silage, digestion of these does not seem to differ to any large extent when the plant material used is of the same origin (Müller et al. 2008, Miyaji et al. 2008, Muhonen et al. 2009a and b, Bergero and Peiretti 2011). The effect of feeding the same grass crops conserved as silage, haylage and hay on biochemical and microbial composition in right ventral colon (RVC) content and faeces of cannulated horses have been studied (Müller et al. 2008, Muhonen et al. 2009b). Counts of *Streptococci* were higher in RVC and in faeces when hay was fed (log 5.9 and 6.9 CFU/g) compared to haylage and silage diets (log 5.3 and 5.9 CFU/g for haylage and log 6.3 and 5.6 CFU/g for silage, respectively). The biochemical composition in RVC and faeces was however not different between silage, haylage and hay diets. Fermentation kinetics in RVC was also followed for all diets through VFA composition, pH and lactic acid concentration at different timepoints after feeding, and was found to be similar for silage, haylage and hay (Müller et al. 2008). Abrupt feed changes from hay to silage and from hay to haylage (using the same forages as reported by Müller et al. 2008) did not result in any major changes in biochemistry or microbiology in the RVC during the first 28 hours after the feed change (Muhonen et al. 2009b). Results pointing in the same direction were also reported by Miyaji et al. (2008), who studied fibre digestibility and fermentation variables in different segments of the equine hindgut when horses were fed hay or silage produced from the same timothy sward. No differences were found among hay or silage diets in total VFA concentration in any segment of the hindgut, and apparent digestibility of DM, organic matter and fibre (NDF, ADF, cellulose, hemicelluloses) was similar among hay and silage in all segments (Miyaji et al. 2008). Furthermore, a German study comprising 34 equine establishments compared the use of grass hay and grass silage in equine feeding, and, among other factors investigated the effect of forage type on faecal pH (Müller 2002). Average pH in faeces from horses being fed grass silage was 6.78 and from horses being fed grass hay 6.64, and the author concluded that the VFA present in silage did not influence pH in faeces. Calculations of the average apparent organic matter digestibility coefficients were similar; 0.66 for silage and 0.67 for hay rations (Müller 2002). Also, Bergero and Peiretti (2011) found that ponies fed haylage and hay produced from the same permanent meadow had similar voluntary intakes of both forage types, and no difference in digestibility coefficients was found between haylage and hay.

However, contrasting results regarding the influence of forage conservation methods on digestibility have also been reported. Austbø (1990) fed bunker silage, round bale haylage and hay (harvested from the same meadow) to horses and compared apparent digestibility, water intake and pulse frequency of the horses as they were exercised on a treadmill. The apparent digestibility of organic matter was higher when horses were fed bunker silage (0.678, $P<0.03$) compared to round bale haylage and hay (0.645 vs. 0.635, NS). Total water intake (from feed and drinking water) was highest when horses were fed bunker silage (23.3 l/day), intermediate for round bale haylage (21.7 l/day) and lowest when horses were fed hay (19.4 l/day), but water intake from drinking water was lowest for silage and highest for hay. However, there were no differences between the diets in pulse frequency during the treadmill exercise (Austbø 1990). The reason for the higher apparent digestibility of bunker silage and higher total water intake

was not discussed by Austbø (1990), but the explanation provided by Muhonen et al. (2009a) may be valid also for the results reported by Austbø (1990). Muhonen et al. (2009a) compared the digestibility of hay and silage produced from the same crop when fed to exercised Standardbred horses. Silage had a slightly higher apparent digestibility than hay one to two days after an abrupt feed change between the forages, and this difference persisted for ADF digestibility at day 18-20 after the change of diet. The total water intake was higher on the silage diet, but water output did not differ between diets, meaning that evaporation losses were higher on the silage diet, probably due to a larger heat increment of feeding (Blaxter 1989, Muhonen et al. 2009a*).* The reason for the higher digestibility of silage compared to haylage and hay may have an easy explanation; loss of highly digestible leaves in the field due to mechanical handling of dry crops during haylage and hay production may decrease overall digestibility of the forage (Honig 1980, McGechan 1988). Another explanation may be the previously known loss of DM during ensiling without loss of energy. This "increase" in energy content during ensiling is attributed to the microbial fermentation products containing a higher gross energy value than the substrates (McDonald et al. 1991). However, the silage used in the study reported by Muhonen et al. (2009a) was not fermented to any large extent, and the apparent digestibility of dry matter, organic matter and crude protein did not differ between hay and silage 18-20 days after the abrupt change of forage, and apparent digestibility of NDF did not differ among the forages at all (Muhonen et al. 2009a).

Other studies that have investigated equine digestibility of forages conserved with different methods, or different chop lengths of silage and hay, are difficult to evaluate, as silage, haylage and hay used within the same experiment have had very different nutritive values, and clearly did not originate from the same sward or contained the same plant material (*e.g*. McLean et al. 1995, Morrow et al. 1999, Moore-Colyer and Longland 2000). Another factor that may make comparisons of forage digestibilties among or within different experiments difficult is feeding level. Increased feeding level has been reported decrease the digestibility of forages in horses (Ragnarsson and Lindberg 2010b), and thus needs to be taken into account in such comparisons.

The plant species included in silage and haylage may also affect digestibility. The most common plant material used in forages for horses are grasses of different species. In a recently published study, Särkijärvi et al. (2012) studied the effect of grass species and cutting time on digestibility of silage in equines. Silage from a timothy (*Phleum pratense*)/meadow fescue (*Festuca pratensis*) sward was compared with silage from a tall fescue (*Festuca arundinacea*) sward. Both swards were harvested at three different cutting times of the primary harvest. The digestibility was higher for timothy/meadow fescue silages compared to tall fescue silages, and this difference was attributed to the higher fibre digestibility of timothy/meadow fescue (Särkijärvi et al. 2012). Advancing plant maturity produces higher lignin content and a higher stem:leaf ratio, which both contributes to decreased digestibility (Van Soest 1994). However, Särkijärvi et al. (2012) reported that timothy/meadow fescue silage contained fewer leaves than tall fescue silage at all cutting times, but still had a higher digestibility. This means that fibre concentration or stem:leaf ratio alone cannot explain the different digestibilities of the forages, but fibre composition should also be regarded in feed evaluation for horses (Särkijärvi et al. 2012). The digestibility of whole-crop silage of oats has also been studied when fed to horses (Särkijärvi and Saastamoinen 2001). In comparison with grass silage, oat silage had higher apparent digestibilities of crude protein and crude fat, lower digestibility of nitrogen-free extract and similar digestibilities of crude fibre and NDF (Särkijärvi and Saastamoinen 2001).

Factors influencing equine intake and preference of silage and haylage

Factors influencing equine intake and preference of forages are important from different perspectives: a perfect nutritive value and hygienic quality of forage is of no value if the horse refuses to eat it. Also, as many horses may consume more feed than needed to meet their energy requirements, it is of interest to know which factors control forage intake, and how different forage types may influence intake-related factors such as eating time and preference. Eating time is an important equine welfare issue (*e.g.* McGreevy et al. 1995, Redbo et al. 1998), but if horses with low nutrient requirements and/or known to be "easy-keepers" are allowed to consume forage *ad libitum,* overconsumption of energy and nutrients, and development of obesity and metabolic diseases is a great risk (*e.g.* Asplin et al. 2007, Pratt-Phillips et al. 2010).

One factor that may influence eating time is plant maturity at harvest. Plants harvested in late plant maturity does not only have lower digestibility (Darlington and Hershberger 1968, Deinum 1984, Woodward et al. 2011), but consists largely of fibrous tissue and may take longer time to chew and ingest. This was confirmed in a recently published study, where haylage harvested in June, July and August, all from the same sward and from the primary growth, was fed to horses and different intake variables were measured (Müller 2011). It was found that horses ingested June haylage faster (29 min/kg DM) than July (37 min/kg DM) and August (36 min/kg DM) haylages, and that the number of chews before swallowing was highest for August haylage and lowest for June haylage (81 vs. 50, $P<0.0001$). In addition, in order to fulfil nutrient requirements using a close to forage-only diet, August haylage was

fed in larger amounts (1.8 kg DM/100 kg BW) compared to June haylage (1.4 kg DM/kg BW). This also resulted in a prolonging of the total eating time for haylage harvested in August (Müller 2011).

Many opinions about horse preferences for different forages exist, but the scientific knowledge in the area is scarce. Studies have mainly dealt with voluntary intake of forage in combination with digestibility studies (Austbø 1990, Smolders et al. 1990, Istasse et al. 1996, Moore-Colyer and Longland 2000, Bergero et al. 2002, Bergero and Peiretti 2011), effects of forage availability on behaviour (Goodwin et al. 2002, Benhajali et al. 2009) or effect of plant species on voluntary intake (Darlington and Hershberger 1968, LaCasha et al. 1999, Särkijärvi et al. 2012). Few studies have dealt with preference *per se*. Results from a Swedish study where silage (309 g DM/kg), haylages (577 and 684 g DM/kg) and hay (884 g DM/kg) was produced from the same sward and offered simultaneously to horses, showed that silage was the preferred forage (Müller and Udén 2007). Silage had the highest rate of consumption (0.90 kg DM/day, s.d. 0.14) and longest eating time (28.4 min/day, s.d. 5.16), while hay had the lowest rate of consumption (0.23 kg DM/day, s.d. 0.14) and shortest eating time (6.8 min/day, s.d. 4.08), and haylages were intermediate between silage and hay in both rate of consumption and eating time. Silage was the first choice 72 of 84 times (85 %), and was never left in favour of another forage after smelling or tasting it. The reason for this preference is not fully understood, but the resemblance of silage to fresh grass in physical structure and water content may play a role, as preference, eating time and rate of consumption inversely followed DM content of the forages. All of the forages in the preference study were successfully conserved, but when conservation is not dominated by lactic acid fermentation and the forage contains other fermentation products, horses may refuse to eat the forage. Austbø (1990) reported that horses rejected clamp silage when it had a noticeable smell of butyric acid, but not when the same silage was free of butyric acid odour. Concentration of acetic acid may also be a factor influencing preference and intake of silage (acetic acid concentration may be high in some silages), as foals have been reported to reject drinking water containing more than 0.16 ml acetic acid/100 ml water (Randall et al. 1978).

Intake and preference of silage and/or haylage may also be influenced by physical structure of the forage. Wrapped forages used in equine nutrition are often conserved long-stemmed, although cutting or chopping the herbage prior to conservation is commonly used in other ensiling systems, as it has been shown to be beneficial in terms of achieving a good fermentation and to prolong aerobic storage stability (Charmley et al. 1999). An experiment comparing conservation and feeding of cut and long-stemmed grass haylage in bales was therefore performed (Müller 2009a). Eating time was however similar among cut and long-stemmed haylage (28 vs. 30 min/kg DM, $P>0.05$), but chewing rate was slightly higher for cut (84 chews/min) compared to long-stemmed haylage (82 chews/min, $P = 0.01$) and number of chews/kg DM was lower when horses were fed cut haylage (2368) compared to long-stemmed (2441) ($P<0.0001$). The differences were small, but the implications of them over longer periods is not known (Müller 2009a).

Hygienic quality of silage and haylage, and horse health

There have been, and still are, many prejudices on the use of silage as a feed for horses. These were noted already in 1913 by Rommel: “In many cases horses have been killed by eating moldy silage, and the careless person who fed it at once blamed the silage itself, rather than his own carelessness and the mould which really was the cause of the trouble”. Regardless of the type of forage fed, the hygienic quality is always the most important issue for all animals. Toxins of microbial origin may be found in badly preserved forage, irrespective of the conservation method used. Irish studies have pointed out that there may be large differences between farms in the extent of mould growth in wrapped forages, and that these differences were correlated to different management practices of bales on the farms (McEniry et al. 2007, O’Brien et al. 2008). Hence, it is important to follow good management practices during the production, storage and feeding of wrapped forages.

Moulds, spores and mycotoxins

One of the major reasons for the shift from hay to wrapped forages in equine diets are the difficulties in producing and storing hay dry and keep mould growth to a minimum. Mould spores are detrimental to horses and humans and may cause respiratory diseases such as Recurrent Airway Obstruction, formerly known as COPD (Chronic Obstructive Pulmonary Disease) or more commonly as ”heaves” or “broken wind” (Robinson et al. 1996). This condition was described and recognized as a man-made disease already by Aristotle (384 to 322 BC). Several studies have reported that feeding silage instead of hay may improve the condition of horses already affected by RAO, and that affected horses kept free of symptoms on a silage diet showed clinical symptoms of RAO when silage was replaced with hay (Vandenput et al. 1998, Franchini et al. 2000). Also, studies where the amount of respirable particles in forages have been measured have reported lower values in silage and haylage compared to hay, even when the hay was judged to be of “good hygienic quality and low in dust” by subjective analysis (Raymond et al. 1997, Vandenput et al. 1997, McGorum et al. 1998). Using silage and haylage as forages

for horses can therefore be preventive in avoiding development of respiratory diseases (Peiretti and Bergero 2004).

However, in order for silage and haylage to be low in mould spores, it is important that they are produced and stored properly. If the surrounding stretch film layers can not manage to keep an airtight seal around the forage, fungal growth will take place. As plant maturity increases, the risk of punctures in the stretch film probably also increase, and it is not uncommon that forage producers specialized in haylage production for horses in Sweden apply more than 10-12 layers of stretch film (unpublished data, Schenck and Müller 2011). A reason often given for the high number of stretch film layers is that a higher DM content requires a thicker plastic layer to keep anaerobiosis during storage. This is supported by O'Brien et al. (2008), who found a positive correlation between increasing DM content and presence of moulds in Irish bale silage. Mould species belonging to the genera *Aspergillus* spp. and *Penicillium* spp. may be present in wrapped forages used for horses (Schenck et al. 2010, Müller et al. 2011), and as species within these genera have the capability to produce mycotoxins, care should be taken to prevent their growth. Different mycotoxins have been reported to cause mycotoxicosis and immunological reactions (Pitt and Leistner 1991, Scudamore and Livesey 1998), skin problems and gastrointestinal disorders (Smith and Girish 2008) as well as reproductive failure (Minervini et al. 2010) in horses. Studies on the presence and abundance of mycotoxins in wrapped forages are scarce, but O'Brien et al. (2006) found that based on the mycological flora, a cocktail of different mycotoxins may be present in silage.

Most of the knowledge about moulds and mycotoxins in wrapped forages has been achieved by two studies; a survey of bale silage in Norway reported by Skaar (1996), and an extensive study of Irish baled silage, reported by O'Brien (2007). The predominant fungal species found in the largest number of bales by O'Brien et al. (2008) was *Penicillium roqueforti*. In Norway, *Aspergillus fumigatus* was found to be the most frequently isolated fungus from bale silage (Skaar 1996). Both species have also been reported to be present in Swedish haylage fed to horses (Müller et al. 2011), and Buckley et al. (2007) estimated 0.37 of Irish haylage fed to horses to contain pathogenic fungi belonging to *Aspergillus* spp. Both *Aspergillus* spp. and *Penicillium* spp. have the capability to produce different mycotoxins (Samson et al. 2000), and more information is needed on the frequency of occurrence of these (and other) fungal species in haylage, as well as which production factors and management practices that are related to presence of moulds and/or mycotoxins in wrapped forages. At present, a screening study of the presence of moulds and mycotoxins in wrapped forages for horses in Sweden is running, also including method development for identification of fungal species in forage samples, using molecular tools (Schenck et al. 2010).

Bacterial problems

Hygienic problems in wrapped forages are not only concerning moulds, but also bacteria. Bacterial problems is mainly an issue in forages of lower DM content (i.e. silages), as water content and water activity in haylages are often too low for bacterial growth (Adams and Moss 1995). Harmful bacteria belonging to the genera *Clostridia spp.* (Roberts 1988) and enterobacteria (van Duijkeren et al. 2000) may be present in forage due to contamination with soil, manure, cadavers or decaying plant material, and can cause gastrointestinal disorders and/or toxicosis in horses as well as in other animals (Wilkinson 1999). These bacterial genera are primarily found in poorly fermented silage, which is characterized by high concentrations of butyric acid and/or ammonia, and a pH-value between 5 and 7 or higher (McDonald et al. 1991).

The clostridial species found in silage usually belong to one of three phenotypically different groups, described by Pahlow et al. (2003) as; 1) proteolytic clostridia producing ammonia, amines, acetic acid and butyric acid from peptides and amino acids, including *e.g. Clostridium sporogenes* and *C. bifermentans*; 2) saccharolytic clostridia including the *C. butyricum*-group, which ferments monosaccharides to mainly butyric acid and acetic acid; and 3) the *C. tyrobutyricum* group, which mainly ferments lactic acid to butyric acid even when the pH-value is low. The latter group is the most studied clostridia in silage due to its economic impact in the dairy industry, and *Clostridium tyrobutyricum* has been found to be the most common clostridial species present in Swedish big bale silage (Jonsson 1990, Jonsson 1991). As most of the studies of clostridial species isolated from silage have been based on classic microbial culturing rather than modern DNA-based techniques, the species reported should be regarded as phenotypic groups, rather than strict taxonomic species (Pahlow et al. 2003). Clostridial growth in silage has been shown to depend on a_w and DM content (Hengeveld 1983), the amount of WSC in relation to DM level, buffering capacity of the crop, degree of laceration, NO_3-concentration of the crop, epiphytic microflora, ensiling technology and use of additives (Wieringa 1958, Weissbach et al. 1974, Leibensperger and Pitt 1987, Spoelstra 1990, Pauly 1999), but clostridial spores are seldom found in haylage (*e.g*. Müller et al. 2008, Müller 2009b, Müller et al. 2011). Silage and haylage in bales are however heterogenous materials, meaning that clostridial growth may be present in micro-niches (10 µm distance), although these may not affect the general chemical quality of the silage (Spoelstra

1990, Pahlow et al. 2003). Niches of clostridial growth with high levels of spores, butyric acid and ammonia have been found in bales where the general DM level would indicate a restricted clostridial growth (Pauly 1999).

The major concern of the presence of clostridia in silage for horses is the species *C. botulinum*. This species can produce the lethal neurotoxin botulin under certain conditions (Roberts 1988, Hatheway 1989), and very small amounts of the toxin causes equine death (Gill 1982). However, *C. botulinum* is rarely found in silage (Notermans et al. 1979, Spoelstra 1981). Notermans et al. (1979) also showed that toxin production by *C. botulinum* in grass took place only at a_w ≥0.94 at pH 6.5 and 5.8. At pH 5.3, toxin production (with grass as a substrate) was demonstrated only at a_w ≥0.985. This means that botulin production in well-preserved silage or haylage is highly unlikely, as pH is too low in silage and a_w is too low in haylage. Case reports of feed-related botulism in horses often fail to show the presence of botulinum neurotoxin in affected animals or in suspected feed or water, due to difficulties with sampling and analytical methods (a mouse inoculation test is the standard for detection of botulin, Szabo et al. 1994, Johnson et al. 2010). Also, the clinical signs usually appear three to seven days after ingestion of the toxin, at which time there may be no detectable toxin in the serum or gut content of the horse (Blood et al. 1979) and most often no feed left to sample. A number of published case reports have therefore based the diagnosis of botulism on clinical symptoms. A clear connection between feeding silage and incidents of botulism does not seem to be present in the literature. Rather, inclusion of cadavers or a generally poor feed hygiene in feedstuffs of different types seems to be causative, along with grazing in geographic areas where spores of *C. botulinum* are abundant and may infect foals causing toxico-infectious botulism (Szabo et al. 1994, Johnson et al. 2010). In cases of botulism where silage has been fed, the forage was reported as being badly fermented with a high pH (Haagsma et al. 1990), with inclusion of cadavers (Gudmundsson 1997) and with a high pH and a strong smell of ammonia (Ricketts et al. 1984). In cases were silage was not fed, inclusion of cadavers in oat chaff (Kelly et al. 1984) or alfalfa hay cubes (Kinde et al. 1991), grass clippings subjected to heating (Switzer et al. 1984), feed-through dirt from a rack where only green oat hay had been fed (Heath et al. 1990), mouldy lucerne-hay (Wichtel and Whitlock 1991), feed and water contaminated with carcasses *via* birds as vectors (Schoenbaum et al. 2000), round bale hay stored outdoors resulting in rotten and mouldy material in the bale centre (Hunter et al. 2002) and mouldy hay owing to moist storage conditions or fed outdoors during unusually warm winters (Johnson et al. 2010) were all reported as sources of the neurotoxin.

The genera *Enterobacteriacae* is large and comprise several species, both harmless and potentially harmful. Enterobacteria found in silages are Gram-negative bacteria which are facultatively anaerobic and have catalase activity as well as NO_3-reducing ability (Spoelstra 1987; Heron et al. 1993). Enterobacteria can ferment glucose to acetic and formic acid, ethanol and butanediol and can also produce ammonia in anaerobic environments, depending on the species (Pahlow et al. 2003). As ethanol, butanediol and ammonia do not contribute to a decrease in pH, they are not desired fermentation products. Although the species of enterobacteria most frequently found in silages are considered to be non-pathogenic, they contain endotoxins in the outer cell membrane, which may be associated with health problems in dairy cows (Lindgren 1991) and potentially with digestive disorders in horses (van Duijkeren et al. 2000). In ensiling experiments where *E. coli* have been added and survival during ensiling was followed, the fate of the added *E.coli*-organisms has been death as long as the silages have been well-fermented (Byrne et al. 2002). This is also in accordance with the results reported by Heron et al. (1993) and Östling and Lindgren (1995), where enterobacteria were no longer present after nine days of ensiling as pH decreased. However, as pH does not drop in haylage, questions remain whether enterobacteria survive in drier plant material, and more knowledge within the area is clearly needed.

Plant maturity influencing conservation and microbial composition of forages

Apart from presence of directly detrimental and/or harmful microbes in silage and haylage, the epiphytic microbial composition may also influence conservation quality of silage and/or haylage. Silage fermentation is influenced by both number of epiphytic lactic acid bacteria (LAB) as well as the composition and metabolic activity of the LAB-population on the material to be ensiled (Müller et al. 1991). As the epiphytic microflora changes over the harvest season and with increasing plant maturity (Fehrmann and Müller 1990, Pahlow 1991, Adler and Lew 1995, Behrendt et al. 1997), herbage harvested late may have less of a chance of being successfully preserved. Podkówka and Potkanski (1991) showed a negative influence of increased plant maturity on the ensilability of grass due to a reduction in the concentration of water-soluble carbohydrates and increase in the buffering capacity in the herbage, as well as difficulties in obtaining sufficient compaction of the herbage followed by mould growth in the silage. Furthermore, increased plant maturity at harvest resulted in a decreased lactic acid concentration, and increased pH and butyric acid concentration in the silage (Podkówka and Potkanski 1991). These results were obtained studying silage, but the effect of plant maturity at harvest on the conservation of haylage has not been investigated to the same extent. As the DM content is higher in haylage, the com-

position of the epiphytic microflora (specifically LAB) may be less important for a successful preservation of haylage, compared to ensiling of forages with lower DM contents. In a laboratory silo experiment with three harvest dates of haylage (May, June, and August) of the same primary growth, August harvest date resulted in higher yeast and LAB counts in haylage compared to May- and June-harvest dates, but counts of moulds, enterobacteria and clostridia were not affected by harvest date (Müller, 2009b). Also, Ragnarsson and Lindberg (2008) did not report microbial values, but concentrations of acetic acid, 2,3-butanediol and ethanol seemed to be higher in the two latest of four cuts of the primary growth of a Timothy sward. Särkijärvi et al. (2012) compared three different harvest dates of the primary growth of a timothy/meadow fescue sward and of a tall fescue sward, and reported low contents of lactic acid and higher pH-values in the third and last cuts for both crops.

Evaluation of hygienic quality

In order to be able to evaluate the influence of different factors on hygienic quality of silage and haylage, both sampling and analytical methods must be accurate and precise. Classical culturing methods are presently in use for routine microbial analysis of feeds, although they have well-known limitations. Further development of analytical methods for this purpose should include molecular tools (Schenck et al. 2010). In a classical microbial analysis of fungi, it is important to use the appropriate culturing conditions (including *e.g.* culturing substrate, incubation temperature and incubation atmosphere) at the laboratory, as identification of fungal species is done on typical growth, macro- and microstructures and sporulation, which may differ with different culturing conditions (Samson et al. 2000). By using at least two substrates (malt extract agar and dichloran 18 % glycerol agar) and two incubation temperatures (25° and 37°C), the chance of identifying mould species/genera present in a haylage sample was larger compared to using only one substrate and/or one incubation temperature, but using more than two substrates and incubation temperatures did not increase the chance of detecting more mould species (Müller et al. 2011).

Other horse health issues (un)related to feeding wrapped forages?

During the last ca 10 years, a condition known as “Scandinavian knuckling horse syndrome” or polyneuropathy have been detected in horses in Norway, Sweden and Finland (Hanche-Olsen et al. 2008, Hahn et al. 2008). The disease involves weakness in the hindlimb digital extensors and polyneuropathy involving sciatic nerves, and may have a lethal outcome. The cause of the disease is still unidentified, but mycotoxins and/or moulds are suspected as causative agents. One report stated that feeding wrapped forages may be a risk factor (Hanche-Olsen et al. 2008), although cases were included in the same report where only hay had been fed. Also, Hahn et al. (2008) reported the condition in horses that were fed freshly cut ryegrass. Further investigations are currently on-going in Sweden (Gröndahl, personal communication, 2012).

A recent report on the presence of peripheral dental caries in Swedish horses mentioned the increased use of silage in equine feeding as a cause for the disease (Gere and Dixon 2010). However, the study comprised horses with no known feeding history, and the conclusion was based on several assumptions including the statement that silage fed to horses contains high amounts of acids, both added and naturally produced, as well as a low pH (Gere and Dixon 2010). However, this conclusion could be questioned, as most horses are fed haylage which have pH-values similar to hay and a very low (if any) content of acid fermentation products (Gordon et al. 1961, Greenhill 1964, Finner 1966, Jackson and Forbes 1970, Nicholson et al. 1991, Field and Wilman 1996, Pahlow and Weissbach 1996, Dawson et al. 1999, Driehuis and van Wikselaar 2000, Han et al. 2006, Müller and Udén 2007, Müller et al. 2008). The use of additives in bales is not very common. Furthermore, data from a small experiment where the effect of forage type on pH-value in the oral cavity of horses was studied reported no differences in oral pH that were attributed to the type of forage fed (Ellevik 2006). The forages used in the experiment were frozen fresh grass, silage, haylage and hay produced from the same grass crop, and were offered one at a time to the horses, and resulted in an oral pH of 8.7-8.8 (Ellevik 2006).

Conclusions

Irrespective of the type of forage that is used for equine feeding, it is important that harvest, preservation and storage is performed correctly in order to achieve and sustain a high hygienic quality of the forage. Further development is needed for methods to describe the hygienic quality of forages accurately. Different preservation methods result in different biochemical and microbial composition of the forage, but this does not seem to influence digestion of the forage to any large extent. Digestibility of forages is mainly attributed to plant maturity at harvest irrespective of preservation method, although silage may sometimes show a higher apparent digestibility compared to haylage and hay. More research is needed on fibre composition and digestibility of forages of different plant maturity and botanical composition. Also, factors influencing content of total WSC and its’ components in forages are of interest, especially for horses prone to laminitis and/or decreased insulin sensitivity. Preference of horses may be affected

by preservation method, to the favour of silage over hay, if the silage is well-preserved. Furthermore, different agronomic factors that influence the forage chemistry, biochemistry, microbiology and physical structure needs to be considered from an equine feeding point of view in further research within the area.

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Screening exogenous fibrolytic enzyme products for improved in vitro ruminal fiber digestibility of bermudagrass haylage

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Keywords: digestibility, enzyme, ferulic acid, forage, NDF, screening, sugars

Introduction Tropical/substropical grasses are the basis of livestock production in many parts of the world but their quality is usually lower than that of temperate grasses. Exogenous fibrolytic enzymes (EFE) can be used to improve forage quality but the results of recent research with EFE have been equivocal because many factors influence their efficacy (Beauchemin et al. 2003). This experiment is the first of a series aimed at maximizing the efficacy of EFE to hydrolyze bermudagrass silage. The objective was to evaluate effects of 18 EFE products on nutritive value of bermudagrass haylage and select the top 5 EFE products that give the greatest fiber digestibility for further studies.

Materials and methods Eighteen EFE from 5 companies were evaluated for their effect on digestibility of a 4–week regrowth of Tifton 85 bermudagrass haylage (68.1, 34.2, 3.7 and 18.7% NDF, ADF, ADL, and CP, respectively). All EFE were evaluated with a 24 h in vitro ruminal digestibility assay using bermudagrass as substrate. EFE were diluted in citrate–phosphate buffer (pH 6) and applied in quadruplicate to the substrate at the manufacturer–recommended rates. The suspensions were incubated at 25°C for 24 h before addition of buffered rumen fluid (39°C) and further incubation for 24 h. The run was repeated once (Experiment 1). In Experiment 2, the 12 EFE with the greatest NDFD improvement from Experiment 1 were tested using similar procedures for in vitro digestibility. In addition, preingestive hydrolytic effects of the 12 EFE were evaluated in a similar manner except that sodium azide was added as an antimicrobial agent (0.02% w/v) in the EFE solution and that after the 24 h incubation at 25˚C, 30 mL of water was added, then tubes were shaken for 1 h and then filtered. In Experiment 1, the model included enzyme and run effects and in Experiment 2 it only included enzyme effects. Data was analyzed with the GLM procedure of SAS v9.1 and Fisher's test was used to differentiate means.

Results and discussion In Experiment 1 (Table 1), when compared to the Control (EFE range vs. control mean; $P < 0.05$), 6 EFE-treated substrates had greater DMD (%, 53.8 to 54.9 vs. 52.4), 6 had greater NDFD (%, 40.4 to 41.8 vs. 38), 8 had greater hemicellulose digestibility (%, 37.4 to 40.2 vs. 35.1), 12 had greater total VFA concentration (TVFA, mM, 55 to 58.9 vs. 52.3) and 7 had lower acetate : propionate ratio (A:P, 2.97 to 3.15 vs. 3.24). The lower A:P ratio following treatment indicates that EFE could potentially increase the pool of gluconeogenic substrates in the rumen. In Experiment 2 (Table 2), when compared to the Control ($P < 0.05$), 5 EFE-treated substrates had greater DMD (%, 54.3 to 56.0 vs. 51.2), 10 had greater NDFD (34.0 to 39.7 vs. 30.7), 10 had greater hemicellulose digestibility (32.6 to 38.5 vs. 28.9), and one had lower A:P ratio (2.92 vs. 3.24). Differences in the extent of EFE effects on fiber digestibility and rumen fermentation measures between experiment 1 and 2 may be due to differences in rumen fluid inoculum activity. The following preingestive hydrolytic EFE effects were evident: Compared to the control, 10 EFE released more water-soluble carbohydrates (WSC, %, 2.68 to 5.85 vs. 2.28), 10 EFE released more ferulic acid release (µg/g; 207 - 391 vs. 198) and 4 had lower NDF concenctrations (%; 62.8 – 65.3 vs. 67.3). Preingestive incubation results showed that several EFE hydrolyzed the NDF of the bermudagrass and thereby released WSC and ferulic acid. Such reductions in NDF could potentially increase intake in ruminants. Also, WSC released could stimulate microbial action in the rumen and the release of ferulic and p-coumaric acids from the cell wall could enhance the digestion of the cell walls.

Conclusions Several promising EFE candidates that reduced bermudagrass fiber concentration and increased its digestibility were identified in this experiment. These results suggest that certain EFE can be used to improve the nutritive value of tropical/subtropical forages. Future work is aimed at optimizing the enzyme treatment conditions in order to use such EFE to improve the performance of ruminants fed tropical/subtropical forages.

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Table1. Experiment 1: Effects of exogenous fibrolytic enzymes on dry matter (DMD), neutral detergent fiber (NDFD) and hemicellulose digestibility (HEMD), concentrations of total volatile fatty acids (TVFA), acetate and propionate and acetate to propionate ratio (A:P).

Treatment	DMD (%)	NDFD (%)	HEMD (%)	TVFA (mM)	Acetate (mM)	Propionate (mM)	A : P
Control	52.4	38.0	35.1	52.3	31.7	9.8	3.24
1A	54.1*	40.5*	37.4*	57.2*	34.7*	10.9*	3.17
2A	54.2*	40.7*	37.4*	57.8*	35.4*	11.3*	3.11*
3A	52.9	39.3	36.7	53.9	33.1	10.4	3.19
4A	52.9	38.0	36.6	57.7*	34.5*	11.8*	2.97*
5A	52.3	37.9	35.9	56.7*	34.9*	10.8*	3.21
6A	52.2	37.7	34.1	57.4*	35.8*	10.9*	3.23
7B	52.6	38.3	35.5	52.3	31.7	9.9	3.21
8B	52.2	37.6	34.4	56.3*	34.7*	10.5*	3.30
9C	53.1	38.3	35.4	54.5	33.0	10.6*	3.13*
10C	53.0	38.8	36.9*	58.9*	34.5*	11.4*	3.08*
11C	54.9*	41.8*	40.2*	55.9*	33.5*	10.8*	3.09*
12C	53.8*	40.1*	37.7*	54.4	32.9	10.3	3.19
13D	54.6*	41.3*	39.8*	54.5	33.4*	10.1	3.30
14D	52.9	38.7	36.9*	58.0*	35.0*	10.9*	3.21
15D	53.8*	40.4*	38.6*	55.3*	33.7*	10.6*	3.15*
16D	52.2	37.7	35.8	55.0*	32.9	10.7*	3.09*
17D	53.1	39.1	35.9	55.1*	32.5	10.5*	3.17
18E	50.1	34.4*	31.0*	54.0	32.3	9.9	3.28
S.E.	0.5	0.7	0.7	1.0	0.7	0.2	0.03

* Significantly different to control ($P < 0.05$)

Table 2. Experiment 2: Effects of exogenous fibrolytic enzymes on DMD, NDFD, HEMD, TVFA, A:P, and concentrations of NDF, and release of water soluble carbohydrates (WSC) and ferulic acid (µg/g).

Treatment	DMD (%)	NDFD (%)	HEMD (%)	TVFA (mM)	A:P	NDF (%)	WSC (%)	Ferulic Acid (µg/g)
Control	51.2	30.7	28.9	61.7	3.24	67.3	2.28	198
1A	55.1*	38.3*	36.2*	69.2*	3.11	66.0	3.39*	225*
2A	56.0*	39.7*	38.1*	60.7	3.26	62.8*	5.18*	391*
3A	52.2	35.3*	33.6*	61.8	3.30	65.3*	3.56*	241*
4A	55.8*	39.4*	38.5*	67.6*	3.13	66.1	2.94*	210*
5A	52.5	35.6*	34.0*	66.6	3.15	65.9	2.72*	221*
9C	51.3	32.0	29.3	63.6	3.28	66.8	2.68*	207*
11C	55.0*	36.4*	33.5*	62.6	3.27	64.4*	4.54*	285*
12C	54.3*	35.8*	34.8*	67.2	3.21	66.7	2.33	203
13D	53.0	34.0*	32.6*	70.7*	3.19	66.3	2.27	200
14D	51.9	31.6	30.0	59.4	3.23	66.9	2.89*	207*
15D	52.8	34.6*	33.6*	58.1	3.30	66.6	3.13*	340*
17D	53.2	35.2*	32.6*	70.3*	2.92*	63.7*	5.85*	298*
S.E.	0.8	0.9	1.0	2.1	0.05	0.6	0.04	2

* Significantly different to control ($P < 0.05$)

Protein quality dynamics during wilting and preservation of grass-legume forage

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Keywords: additive, preservation, protein quality, silage, wilting

I**ntroduction** Solubility and degradability of forage protein change during wilting and preservation (Muck et al. 2003; Richardt & Steinhöfel 2008). Furthermore, only limited information is available on the effects of additives on silage protein quality (Slottner & Bertilsson 2006; Richardt & Steinhöfel 2008). It is imperative to evaluate changes in protein quality during storage of silage as differences in silage protein quality can affect intake and protein utilisation by ruminants (Broderick et al. 2007; Huhtanen et al. 2008). The objective of this study was to evaluate the effects of wilting, ensiling and silage additive on the protein quality of a grass-legume forage.

Material and methods A sward (77% grass, 18% clover, 5% lucerne) was mowed on 3 June, 2010 and wilted for ca 23 hours from 150 g/kg of dry matter (DM) to 350 g/kg of DM by wide spreading. Wilted forage was precision chopped and ensiled in 1.7-L silos at Lantmännen Dairy Research Farm Nötcenter Viken, Falköping, Sweden. The forage was either untreated or treated with KOFASIL® LIFE, containing *Lactobacillus plantarum* DSM 3676 and 3677 at an application rate of 400 000 cfu/g of forage or with KOFASIL® ULTRA K, containing sodium nitrite, hexamethylene tetramine, potassium sorbate, sodium benzoate and sodium propionate, at 2 L/ton forage (ADDCON EUROPE GmbH). The treated silages were compared to untreated silage. Forage was ensiled for 5, 10, 30 and 125 days (d) and was analysed for crude protein (CP) fractions according to Licitra et al. (1996) at LKS mbH, Lichtenwalde, Germany. Models by Kirchhof et al. (2006) were used to calculate rumen undegraded dietary protein (UDP). Data were analysed as a completely randomized design in PROC GLM of SAS 9.2, with treatment and storage length as fixed factors, using three replicates per treatment. For silages, data were analysed as treatment comparisons for each storage length separately and as main effect of storage length as no interactions between treatment and storage length were found. To correct for differences in DM content of the silages, silage DM content was used as a covariate in the model comparing untreated and treated silages. When the overall *P* – value was significant at 5% level, pair wise comparisons between MEANS and LSMEANS of forage treatments and storage lengths were done using Tukey´s test.

Results and discussion The concentration of water soluble carbohydrates in unwilted and wilted forage was 215 g/kg DM. The mean CP content of forage and silage was 149 g/kg DM. Concentrations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash of wilted forage were 375, 245 and 88 g/kg DM, respectively. *In vitro* organic matter digestibility of wilted forage was 917 g/kg (Lindgren, 1979). Concentrations of non-protein nitrogen (NPN), neutral detergent soluble protein (NDSP) and acid detergent soluble protein (ADSP) increased, while the buffer soluble protein (BSP) decreased during wilting, resulting in an increase in UDP at 8%/h ruminal passage rate (UDP8; Table 1). However, when the wilted forage was ensiled without additive for 125 d the NPN content increased, while the contents of BSP and NDSP decreased, resulting in a decreased UDP8 during ensiling (Table 1). Addition of KOFASIL LIFE and KOFASIL ULTRA K tended to increase the UDP8 of untreated silage after 125 d of storage (Table 2). The 11% increase in UDP8 by the additives was mostly achieved by their abilities to decrease the NPN but also by their tendency to increase the NDSP compared to untreated silage, with no differences between the additives (Table 2). No large differences in CP fractions were found between silage treatments at 5, 10 and 30 d of storage (data not shown).

As a mean over silage treatments, the NPN increased from 455 to 557 g/kg of CP ($P < 0.0001$) while the BSP decreased from 47 to 33 g/kg of CP ($P < 0.05$) from 5 to 30 d of storage. The NDSP decreased from 431 to 283 g/kg of CP from 5 to 125 d of storage ($P < 0.0001$). This decreased UDP8 from 264 to 218 g/kg of CP from 5 to 30 d of storage, when averaged over treatments ($P < 0.0001$). Furthermore, the ADSP and the acid detergent insoluble protein (ADIP) increased from 40 to 83 g/kg of CP and from 22 to 37 g/kg of CP, respectively, from 30 to 125 d of storage ($P < 0.0001$).

Conclusions Wilting increased protein quality of fresh forage by increasing UDP8, followed by a decrease during preservation. Use of additives tended to increase the UDP8 of the silage, with KOFASIL LIFE and KOFASIL ULTRA K being equally effective. None of the treatments affected the ADIP concentration.

Acknowledgements This project was funded by Agroväst, ADDCON EUROPE GmbH, VL-foundation, Lantmännen R & D, AIC and SLU.

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Table 1. Crude protein (CP), true protein (TP), CP fractions and rumen undegraded protein of forage as affected by wilting and ensiling (125 d of storage).

	Unwilted forage	Wilted forage	Untreated silage	SEM	*P* - value
CP, g/kg DM	150[a,b]	143[b]	152[a]	2.1	< 0.05
TP, g/kg DM	132[a]	118[b]	62[c]	1.8	< 0.0001
----g/kg CP[1]----					
NPN	115[c]	175[b]	593[a]	6.2	< 0.0001
BSP	352[a]	180[b]	33[c]	6.9	< 0.0001
NDSP	475[b]	550[a]	259[c]	8.9	< 0.0001
ADSP	17[b]	61[a]	79[a]	5.9	< 0.001
ADIP	40	35	35	4.2	0.692
UDP8	292[b]	350[a]	210[c]	7.4	< 0.0001

[1]NPN = non-protein nitrogen, BSP = buffer soluble protein, NDSP = neutral detergent soluble protein, ADSP = acid detergent soluble protein, ADIP = acid detergent insoluble protein, UDP8 = rumen undegraded protein at a ruminal passage rate of 8%/h. [a,b,c]MEANS with different superscripts within a row differ significantly at $P < 0.05$.

Table 2. Crude protein (CP), true protein (TP), CP fractions and rumen undegraded protein of untreated and treated silages after 125 d of storage.

	Untreated	KOFASIL LIFE	KOFASIL ULTRA K	SEM	*P* - value
CP, g/kg DM	150	153	148	2.1	0.224
TP, g/kg DM	61	71	68	2.2	0.097
----g/kg CP[1]----					
NPN	597[a]	540[b]	535[b]	10.4	< 0.05
BSP	31	49	39	5.2	0.192
NDSP	260	289	298	8.3	0.080
ADSP	77	84	87	12.1	0.869
ADIP	36	38	39	2.0	0.640
UDP8	210	233	232	4.6	0.060

[1]See footnotes to Table 1. [a,b]LSMEANS with different superscripts within a row differ significantly at $P < 0.05$.

Contribution of endo- and exopeptidases to formation of non-protein nitrogen during ensiling of alfalfa

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Keywords: alfalfa silage, endopeptidase, exopeptidase, proteolysis, non-protein nitrogen

Introduction Proteolysis in the ensiled forage mainly results from plant proteinases. Basically, plant proteinases are divided into two classes, namely, the exopeptidase and endopeptidase based on their actions on substrates and their active sites, respectively. Few studies have been addressed on contributions of different endo- and exopeptidases to proteolysis in ensiled alfalfa. The aims of this study were to clarify the classes of endo- and exopeptidases that are involved in proteolysis within ensiled alfalfa, and to determine the contribution of these proteinases to formation of non-protein N (NPN) during ensiling.

Materials and methods Five g of fresh alfalfa leaves were ground in liquid N (1:4, w/v) into powder with a mortar and pestle. The powder was then suspended in 20 ml of 25 mM TRIS-HCl, pH 7.5, containing 1% (w/v) insoluble polyvinylpolypyrrolidone and 0.1% (v/v) β-mercaptoethanol. Samples were centrifuged (10 min, 20, 000 × g), and the supernatants were used directly for peptidase activity assays. To determine the classes of endo- and exopeptidases in the crude enzyme extracts, inhibitor treatments (triplicate for each treatment) included a specific inhibitor within each of the four defined endopeptidases and the six defined exopeptidases as described by Tao et al. (2011) and Guo et al. (2011).

After determination of the classes of endo- and exopeptidases in alfalfa leaves and the concentration of each inhibitor required for different endo- and exopeptidases, samples of fresh and chopped alfalfa forage were homogenized in a blender with a volume of water that was equal to four times the weight of the fresh forage. The aqueous extracts were dispensed into 20 ml tubes with screw caps. Triplicate extract samples were either untreated (control) or treated with each of the endo- and exopeptidase inhibitors. Thereafter, all of the tubes were sealed tightly and fermented at 30 °C; fermentation of the extracts prepared from alfalfa relied on the action of epiphytic bacteria and lasted for 14 d. Total N, NPN, peptide-N and free amino acid N in the fermented alfalfa extract were analyzed after 14 d of fermentation. Details regarding the experimental procedures and analytical methods see Tao et al. (2011) and Guo et al. (2011). Data were subjected to analysis of variance using the one-way ANOVA procedure of the Statistical Package for the Social Science (2011), and multiple comparison was conducted using a least significant difference test to separate means (P<0.05).

Results and discussion Four classes of endopeptidases (*i.e.*, serine, metallo, aspartic and cysteine peptidase) and five classes of exopeptidases (*i.e.*, aminopeptidase, carboxypeptidase, dipeptidase, dipeptidyl-peptidase and tripeptidyl-peptidase) were shown to be present in alfalfa leaves, each playing a different role in alfalfa protein degradation (Table 1). Among the four classes of endopeptidases, the metallopeptidase was the principal peptidase in formation of free amino acid N (AA-N). Serine and metallo peptidase contributed to degradation of peptides into free AA, and degradation of protein into oligopeptides was mainly due to aspartic and cysteine peptidases. Metallo and cysteine peptidases were the principle peptidases for hydrolyzing forage protein into NPN during ensiling. Among the five exopeptidases, tripeptidyl-peptidase appeared to be the principle exopeptidase in hydrolyzing forage protein into peptide, while carboxypeptidase, dipeptidase appeared to be more important in contributing to the formation of AA-N. Dipeptidase, carboxypeptidase and tripeptidyl-peptidase were the principle exopeptidases for hydrolyzing forage protein into NPN during ensilage. Previous research has suggested that the exopeptidase enzymes might be the principal enzymes by which the proteins of the ensiled forage are hydrolysed (McKersie and Buchanan-Smith 1982). Our results show that inhibition of endo and exopeptidase activities could reduce the NPN content in the fermented alfalfa extract to about 44% and 42% of that in the control, respectively, after 14 d of fermentation.

Conclusions Endopeptidase and exopeptidase were comparable in contributing to formation of NPN during ensiling of alfalfa.

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Table 1. Non-protein N (NPN), Peptide-N, free amino acid-N (AA-N) and ammonia-N (NH3-N) contents (mg/g total N) in alfalfa extract fermented with different specific enzyme inhibitors for periods of up to 14 days.

Inhibitor	Inhibitor classes	NH3-N	AA-N	Peptide-N	NPN
Endopeptidase					
Control	-	9.8^{b}	172^{b}	236^{c}	416^{a}
Phenylmethanesulfonyl fluoride	Serine peptidase	4.6^{c}	127^{c}	252^{b}	383^{c}
Pepstatin A	Aspartate peptidase	11.4^{a}	192^{a}	188^{e}	392^{b}
1,10-phenanthroline	Metallo peptidase	<0.01^{d}	94^{e}	247^{b}	341^{e}
E-64	Cysteine peptidase	9.5^{b}	106^{d}	228^{d}	344^{d}
Combined inhibitors	Four of the peptidases	<0.01^{d}	43^{f}	259^{a}	302^{f}
SEM		1.1	12.0	15.1	9.3
Exopeptidase					
Control	-	26.0^{a}	321^{c}	273^{cd}	620^{a}
Bestatin	Aminopeptidase	8.4^{b}	274^{d}	317^{b}	589^{a}
Potato carboxypeptidase inhibitor	Carboxypeptidase	12.2^{b}	236^{e}	292^{bc}	540^{b}
1,10-phenanthroline	Dipeptidase	0.0^{c}	219^{e}	258^{d}	473^{c}
DriprotinA	Dipeptidyl-peptidase	28.6^{a}	347^{b}	212^{e}	587^{a}
Butabindide	Tripeptidyl-peptidase	24.3^{a}	382^{a}	133^{f}	539^{b}
Combined inhibitors	Five of the peptidases	0.0^{c}	110^{f}	351^{a}	460^{c}
SEM		2.4	77.2	37.4	62.4

Effect of forage type on silage fermentation characteristics assessed by vacuum bag ensiling

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Keywords: festulolium, lucerne, perennial ryegrass, red clover, white clover, vacuum bag ensiling

Introduction It is well known that legumes compared to grasses are more difficult to ensile due to lower sugar and higher protein concentrations and due to a higher buffer capacity. Silages in Denmark are mainly maize whole crop and grass-clover, whereas pure legume silages are rare, and the experience with pure legume silages is therefore limited and mainly with pea whole crop. However, there is a growing interest to increase homegrown protein, which could be obtained with pure red clover or lucerne crops for silage. Therefore information is required on how to assure high quality silage from these crops, e.g what dry matter (DM) concentrations are required by the prewilting process when ensiling without additives. The aim of this study was to examine the effect of forage type, season and harvest time on silage quality measured as fermentation products and protein degradation products, with main focus on the effect of forage type and prewilting on protein degradation products.

Material and methods Perennial ryegrass (Mikado), festulolium (Perun), white clover (Milo), red clover (Rajah) and lucerne (Pondus) grown at AU Foulum were harvested in 2006 at 3 harvest times (one week between each harvest time) in both primary growth and second regrowth. First and third regrowth were not sampled. N fertilization was zero for legumes and 126, 90, 72, 72 kg/ha for primary growth and first to third regrowth, respectively, for ryegrass and festulolium.

Samples for chemical analysis and digestibility measurements before ensiling were dried at 60°C. Samples were analysed for chemical composition and digestibility using conventional wet chemistry methods. Subsamples were ensiled without additives in duplicate in lab scale (vacuum bags). In total 120 silages were produced. For each bag 1 kg fresh crop was used, predried in drying oven at 30°C to either 25 or 31 % DM before it was vacuum packed and left to ensile in dark at 19°C for 91 days. For extraction 100 g silage was blended with 1 l water. VFA's in extract were determined with gas chromatography, glucose and L-lactate with YSI 7100 MBS Biochemistry Analyser, alcohols with headspace GC-MS, ammonia enzymatically, and amines with GC-MS as carbamates after derivatization with isobutyl chloroformate.

Results and discussion Legumes showed higher buffer capacity than grasses, and buffer capacity was also considerable higher in primary growth than in second regrowth. Digestibility of grasses and lucerne decreased with later harvest time. Digestibility of white and red clover was less affected by harvest time, except for red clover in second regrowth. A trend for higher solubility of protein in buffer with increased protein concentration in silage DM was seen, but adjusted for protein concentration buffer solubility was lower for red clover and second regrowth white clover compared to others, both for fresh and ensiled samples. Compared to un-ensiled crops, ensiling process increased buffer solubility with on average 27 percentage units for samples prewilted to 31 % DM. The lower solubility for red clover was even more pronounced for silage, indicating that red clover protein is more protected against degradation during ensiling than protein form other crops.

Legume silages had higher pH than grasses, although clover, especially white clover, showed a high lactic acid concentration (Table 1). Acetic acid concentrations were higher, and ethanol concentrations were lower in legumes than in grasses. Prewilting to 31 compared to 25 % DM reduced fermentation products in a similar way for all species, with propanol as only exception, as propanol was fully eliminated in ryegrass, festulolium and red clover, but only halved in white clover and lucerne with higher dry matter concentration. Despite a lower sugar concentration in second regrowth compared to primary growth, pH was lower in second regrowth probably due to a lower buffer capacity.

Large variation was seen between species in resulting protein degradation products (Table 1). The amines putrecine, tyramine, caverdine, histamine and tryptamine were measured, and especially lucerne silage had a high concentration of amine N. Amine N as proportion of total N varied (as species means) from 1.5% in red clover to 4.5% in lucerne. Amine N concentrations were between 1/3 and 1/2 of ammonia N concentrations. In % of total silage N, ammonium and amine N together made up from 6.4% in red clover to 11.5% in lucerne. Prewilting to higher DM concentrations reduced amine N, and when prewilted to 31 % DM amine N as proportion of total N was only 56 % of the proportion when only prewilted to 25 % DM, whereas ammonium N was only reduced to 93% by the extra prewilting. Prewilting seemed to affect all forage types similar, significant interactions between forage type and

prewilting were only seen for histamine, however several of the amines and total amine tended to show interactions. Prewilting to 31 instead of 25 % DM reduced amine N as proportion of total N with 27, 37, 41, 50 and 46 % for perennial ryegrass, festulolium, white clover, red clover and lucerne, respectively, although the interaction was not statistically significant (P=0.2). This indicates that protein degradation in legumes is more affected by prewilting than grasses, which especially could be important for lucerne silage due to its high amine concentration. Further, it seems that ammonium N is not a good predictor of how different ensiling technologies affect amine production, as prewilting affected amine N much more than ammonium N.

Conclusions In conclusion, species, growth and harvest time all highly affected chemical composition and digestibility. Grasses were easier ensiled than legumes, probably due to higher sugar concentration, lower buffer capacity and lower protein concentration. Vacuum bag ensiling resulted in very high quality silage, and high repeatability of duplicate bags. This indicate, that conditions in vacuum bags were better than normal conditions when ensiling in praxis, however, the effects of the experimental factors found are believed to be qualitatively applicable for praxis, although not necessarily quantitatively. Increased prewilting heavily reduced amine production in the silages, especially for legumes.

Table 1. Effect of forage type and prewilting on some silage quality parameters.

	Buffer capacity (meq/100 g DM)	pH	Ethanol (g/kg DM)	L-lactate[1] (g/kg DM)	NH_3N (% of total N)	Amine N (% of total N)
Forage type (F)[2]						
P. ryegrass	64.7^{a}	4.20^{a}	11.45^{a}	28.9^{a}	6.24^{a,b}	2.05^{a}
Festulolium	64.5^{a}	4.07^{b}	14.59^{b}	30.7^{a}	5.36^{c}	2.03^{a}
White clover	91.9^{b}	4.36^{c}	4.36^{c}	41.5^{b}	5.89^{b}	1.74^{a}
Red clover	88.3^{b}	4.43^{c}	4.93^{c,d}	42.0^{b}	4.99^{c}	1.53^{a}
Lucerne	89.2^{b}	4.65^{d}	7.93^{d}	30.6^{a}	8.07^{d}	3.54^{b}
SEM	1.1	0.03	1.1	1.1	0.18	0.23
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Wilting (W)						
25 % DM	84.5	4.32	9.60	36.9	6.33	2.79
31 % DM	75.0	4.37	7.70	32.5	5.88	1.57
SEM	0.7	0.02	0.7	0.7	0.1	0.15
P	<0.0001	0.04	0.06	<0.0001	0.007	<0.0001
F x W						
P	0.8	0.7	1	1	0.9	0.2

[1]Total lactate concentration approx. twice L-lactate; [2]Different letters indicate forage types differ (P<0.05)

Forage harvesting scheduling

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Keywords: decision support, weather forecasts, moisture content prediction

Abstract

In Denmark, it is assessed that one-third of forage grass on farms is harvested too dry and that another third is harvested too wet when compared with the optimal moisture content in terms of quality. This indicates that the production of quality forage requires a close monitoring of the crop conditions for optimized handling efforts. Farmers are searching targeted decision support to help with the harvesting and the treatment of the cut biomass before collection and chopping and prior to the transportation to the storage location. This paper has evaluated the feasibility and perspectives of implementing a modelling suite for scheduling multiple operations related to the harvesting and handling of grass forage. The scheduling models have included moisture content predictions and have been applied to a number of usage scenarios. Preliminary results and testing results showed a correct prediction rate of 80%.

Introduction

The production of quality forage requires a close monitoring of the crop conditions for optimized handling efforts (Foulds and Wilson 2005; Sørensen and Bochtis 2010) The grass must be cut, raked, and collected at the right time in order to achieve maximum digestibility (Kuoppala et al. 2008). In Denmark, it is assessed that one-third of forage grass on farms is harvested too dry and that another third is harvested too wet when compared with the optimal moisture content in terms of quality (Danish Advisory Centre 2001). If, for example, the harvested grass is too dry the storage compaction is compromised and will to lead to deteriorating of the stored silage through infusion of oxygen.

Considering the above mentioned conditions and parameters, farmers are searching targeted decision support to help with the harvesting and the treatment of the cut biomass before collection and chopping and prior to the transportation to the storage location. Such envisioned decision support will have to involve predicting the moisture content of the crop in the field as a function of weather forecasts. In this way, it will be possible to estimate and predict the optimal drying time in terms of dry matter content as a determinant of the actual operations scheduling. Also, such an approach must be capable of continuous optimisation for each individual operation, where the moisture content and biomass amount is measured and estimated during execution and subsequently used for the scheduling of the next operation in line. Finally, the measurements made during the various in-field operations may be used as input for optimizing the storage quality by controlling the degree of compaction in the silage stack or by the adding of conservation additives.

Sensors for biomass mapping and moisture content determination are currently available and marketed but such sensors are not yet used in decision making context as described above (Prado 2004; Amoodeh 2006). Dry matter sensors give a yield map for kg dry matter per hectare as opposed to a yield map for kg biomass per hectare. However, these sensors and systems are not integrated as part of the optimisation of the forage operations.

This paper has evaluated the feasibility and perspectives of implementing a modelling suite for scheduling multiple operations related to the harvesting and handling of grass forage. The scheduling models have included moisture content predictions and have been applied to a number of usage scenarios.

Materials and methods

At the time of collection in the field, three different quality levels were considered as derived from recommended moisture content threshold values. These levels included 1: high quality, 2: reduced but acceptable quality, and 3: poor quality. Furthermore, the depicted quality levels were associated with corresponding operational decisions so that level 1 translated into the decision *harvesting* and level 3 to the decision *not harvesting*. In contrast, level 2 corresponded to a situation, where the decision can implicitly be considered as *harvesting under uncertainty*. As a consequence, two threshold values was be considered, $\vartheta_{\max}, \vartheta_{\min}$, the max value give the upper value below which the moisture content is considered "acceptable", and the min value give the lower value below which the moisture content is considered corresponding the "high"quality. By scaling the different moisture content where the output will

be one of the three predicted levels: 1) high quality, 2): reduced but acceptable quality, and 3): poor quality, the minimum predicted moisture content useful for the selection of the optimal machinery system, and the time window when the minimum moisture content is expected to occur.

In-field drying and wetting of crops involves complex biophysical processes (Bosma and Gabriels 1992). In terms of using drying models in decision support systems, the moisture content of, for example, grass must be modelled as a function of time and weather conditions. Jenkins et al. (1984) mention multiple empirical approaches involving diffusion equations, or energy balance approaches and mass and energy flows. Often, energy balance models include the Penman–Monteith equation (Monteith and Unsworth 1990) for taking into account the evapotranspiration and the effects of net radiation, vapour pressure deficit (VPD) and wind speed. (*i.e.*, Smith et al. 1988; Atzema 1992). In this case, the moisture prediction model described by Atzema (1992) was used based on the notion that the model input include easily available data from meterological services. These input include air temperature, due point, precipitation, wind speed, cloud cover, and global radiation. The moisture prediction was validated according to the same weather conditions as the developed decision support was validated and a maximum prediction error of 3% was found.

The weather data for the simulations were extracted from a climate database developed and run by the Faculty of Science and Technology at Aarhus University, Denmark (Foulum weather station: [N 56° 29´ 21.55, E 009° 34´ 59.40]). This facility consists of a fully equipped meteorological station giving also the recorded values of soil heat flux and net radiation.

The architectural setup of the scheduling model involved a number of components as depicted in Fig. 1. These components included, the weather forecast data, the coverage ratio of the cuts grass distributed over the field area, the expected yield as influencing the height of the cut biomass layer on the field which subsequently influence the drying process, the predefined threshold values as determined by the type of the grass, the usage of the forage, etc., the time period and availability of the required machinery or labour for different operations.

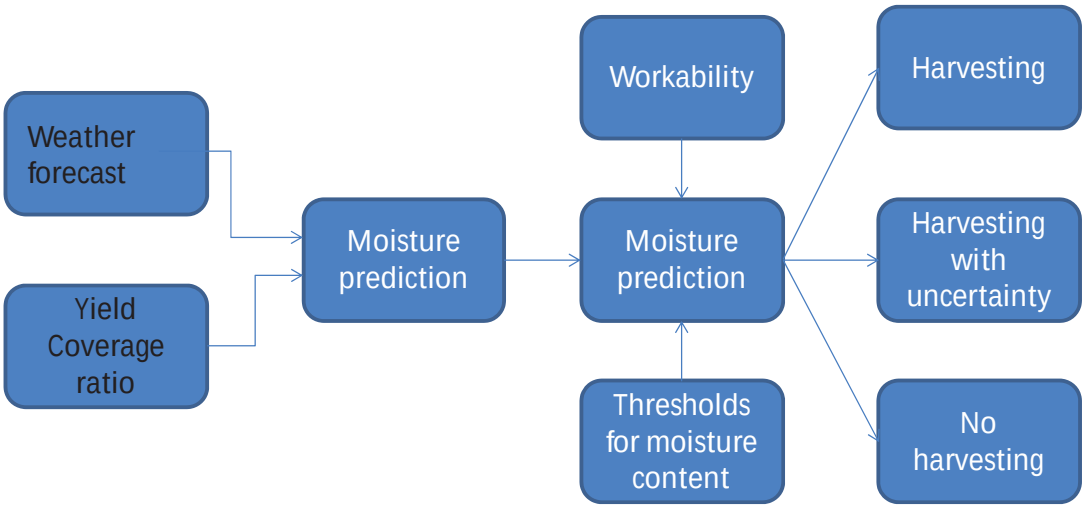


Figure 1. The architecture of the one stage decision making.

Results

The moisture prediction model was validated based on prediction error and based on the forecasted weather data as compared with measured weather data. In Fig 2, the mean absolute errors in the prediction of the moisture content are given. It can be seen that the prediction error is closely related to the precipitation at the specific time interval.

For the harvesting period (May to September, 2009), the model was run on a daily basis giving decision support as to the scheduling of operations based on the forecasted weather data of the upcoming 48 h. Normally, a dry matter content of 30-35% is adequate for silage storage in silos (equals 70-65% moisture content), whereas in the case of silage making using bales, the dry matter content must be in the in the range of 45-50% (Danish advisory centre, 2002). In this specific case, and based on general recommendations, the threshold values was set at $\vartheta_{\min} = 65\%$, and $\vartheta_{\max} = 70\%$.

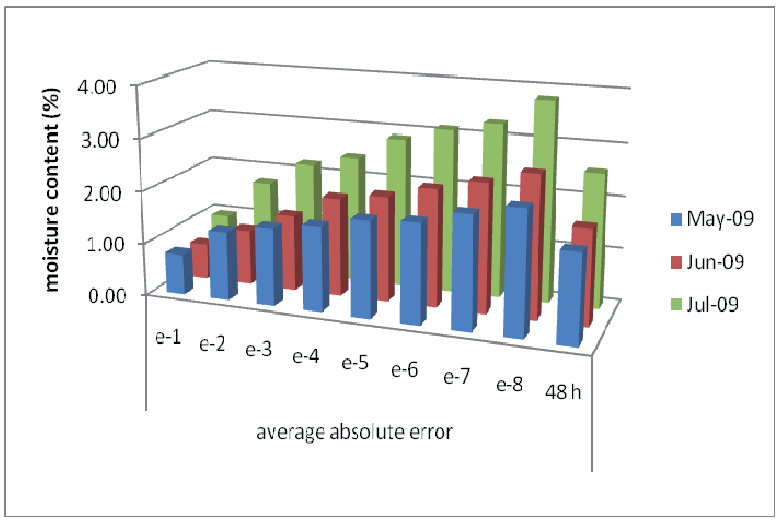
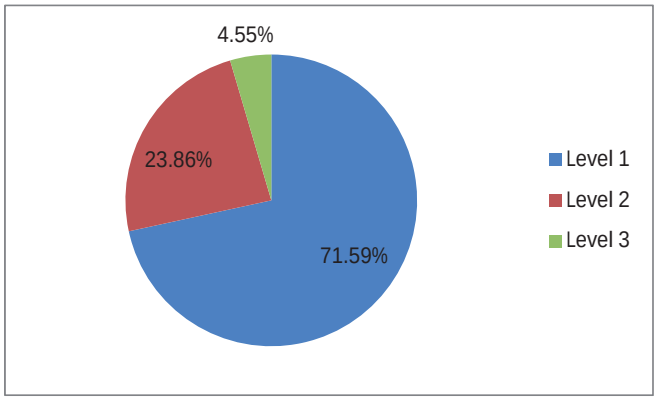


Figure 2. The mean absolute error in the forecasted data based moisture prediction (adapted from Bochtis et al. 2012).

Figure 3a gives the distribution percentage of the prediction levels for all simulations of the decision support system within the designated period. The fraction of the correct predictions (after the reveal of the real weather data) is given in Figure 3b.

The distribution of the different decision outcomes will also depend on the uncertainty levels in the moisture prediction models. Fig 4 gives the distribution for different uncertainty levels. As a way to evaluate the accuracy of a suggested decision, the same period was run using the historical or the revealed weather data. The ratio of correct decision as compared with the historical data is given in Fig 5.

a.



b.

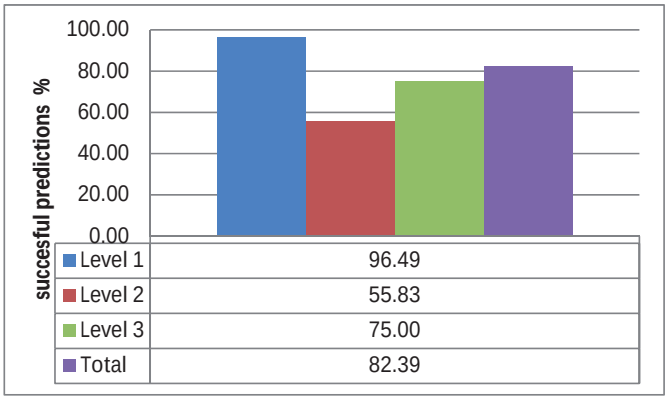


Figure 3. The distribution of the predicted levels of moisture content by the 1sDM (a), and the precedence of successful predictions (b), during the examined period.

Conclusions

A decision support model to support scheduling in grass harvesting has been shown to be feasible to implement. The developed is a prototype providing recommendations on the optimal scheduling and execution of grass handling operations. The set up of the model includes components for moisture content prediction as a function of weather data (forecasted or historical). Validations of these models on independent data have revealed reliable predictions.

Preliminary results from model simulation show a 80% correct prediction rate in terms of decision alternatives presented to the decision maker. The perspectives of further implementing the model would involve the transformation into a system directly usable by farmers. However, this step will require comprehensive validation of the model including the insertion of a user-interface.

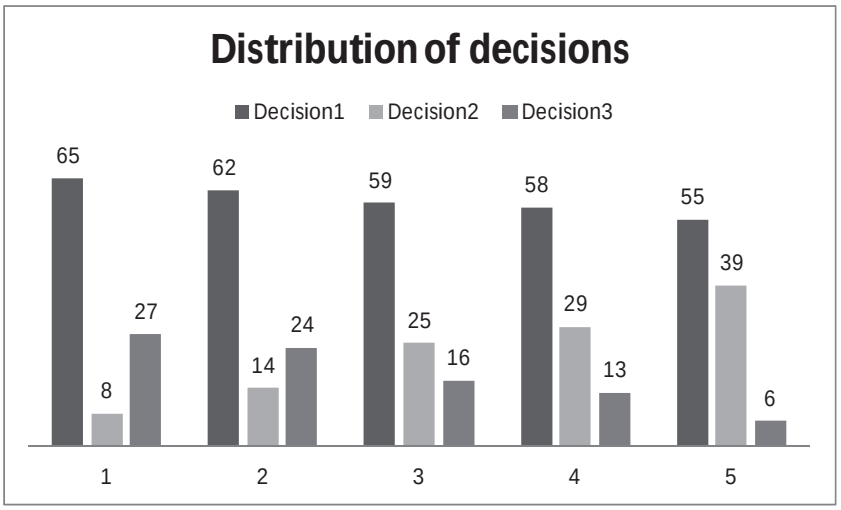


Figure 4. Distribution of the decision outcomes as derived from the model (Sørensen et al. 2010).

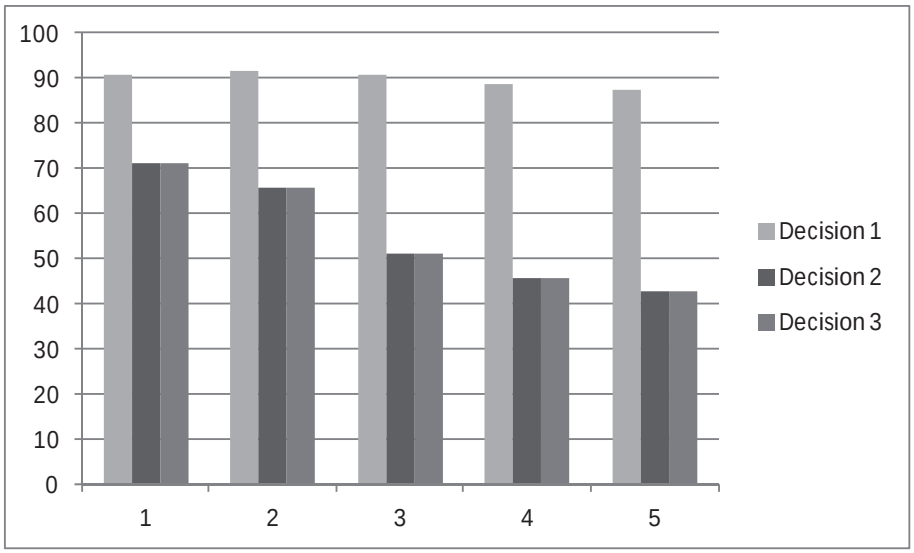


Figure 5. Percentage of correct decisions for the five predefined uncertainty levels.

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Targets for the aerobic stability of silage

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Keywords: additives, aerobic stability, composition, losses, microbiology, silage

Introduction When silage is exposed to air either on opening the silo, or subsequently after removal from the silo, fermentation acids are oxidised by aerobic bacteria, yeasts and moulds which can develop rapidly in well-preserved silages. The consequential loss of dry matter (DM) represents a nutritional and a financial loss to the farmer.

The physical, microbiological, and biochemical factors affecting the aerobic stability of silage are reviewed in this paper with the objective of defining targets which should be achievable with good management of the entire ensiling making and feeding process. Research targets are outlined and a standard protocol for assessing silage aerobic stability is proposed.

Definition of aerobic stability Aerobic deterioration is normally determined under laboratory conditions at constant ambient temperature by mixing several sub-samples of fresh silage and then placing the composite sample loosely in a polystyrene box and leaving the silage exposed to air for several days. During this period the temperature of the silage is monitored along with the ambient temperature. Aerobic stability is defined as the time which elapses before the silage shows clear evidence of heating i.e. when temperature of the silage exceeds ambient by 2°C (Ranjit and Kung 2000). Some workers (e.g. Weinberg et al. 2008) measure carbon dioxide production directly at 5 to 10 cm depth at the exposed silo face. Silages producing less than 10 g CO_2/kg DM and a change in pH of less than 0.5 units over a 5-day period are deemed to be stable.

Key factors affecting aerobic stability The over-arching factor affecting the aerobic stability of silage is exposure to oxygen during the storage period and after the silo is opened for feed-out (Pahlow and Muck 2009). The most important crop factor is probably the count of epiphytic yeasts and moulds at the time of harvest, which should ideally be less than 10^5 colony forming units/g fresh matter. Key physical factors affecting rate of ingress of air into the silage mass during the feed-out period are silage density and porosity (Holmes and Bolsen 2009) together with the extent of permeation of oxygen through the sealant film (Borreani et al. 2007). The concentration of undissociated acetic acid in silage is probably the most significant biochemical factor affecting aerobic stability (Wolthusen et al. 1989).

Practical targets A realistic practical target for silage aerobic stability is 168 hours (7 days) exposure to air without significant temperature rise or visible mould development, inclusive of time in the feed trough. To reach this target four key objectives should be achieved: i) minimal pre-harvest contamination of the crop with epiphytic yeasts and moulds; ii) sufficient consolidation of the silage mass; iii) effective sealing of the silo, preferably with an oxygen barrier film; iv) a rate of removal of silage from the exposed feed-out face which exceeds that of air ingress.

For the control of aerobic deterioration in crops of 250 to 350 g DM/kg fresh weight ensiled in bunker and clamp silos, targets include close coordination of speed of harvest with total packing tractor weight to achieve a minimum density of 210 kg DM/m^3, maximum proportional porosity of 0.4, removal of at least 1 metre depth of exposed silage feed-out face per week in winter and 2 metres per week in summer.

The use of additives designed to increase aerobic stability is recommended when there is a significant risk of the above objectives not being met. However, current microbial approaches such as the use of heterofermentative lactic acid bacteria (e.g. *Lactobacillus buchneri*) produce silages with increased losses of dry matter compared to additives containing homofermentative lactic acid bacteria such as *L. plantarum*. Additives comprising combinations of homofermentative bacterial inoculation and chemical suppression of yeasts and moulds are promising recent developments.

Research targets A standard protocol is proposed for research on silage aerobic stability comprising i) definition of the crop epiphytic yeast and mould count at the time of harvest; ii) exposure of mixed silage to air for at least 240 hours (10 days) at a constant temperature relevant to the climatic region; iii) assessment of either CO_2 production or silage temperature during the entire period of exposure to air.

Research is needed to define the factors affecting populations of epiphytic yeasts and moulds on crops for silage and on chemical components in legume silages which might be of significance in contributing to their enhanced aerobic stability compared with grass silages (Pahlow et al. 2001). Rapid methods are required for assessing the microbial, physical and biochemical status of crops and silages to aid prediction of aerobic stability.

Novel microbial approaches to solving the problem of silage aerobic deterioration are needed, which could lead to the development of improved additives capable of increasing aerobic stability without increasing loss of dry matter during the fermentation process.

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Comparison of methods for determining the density of grass silage

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Keywords: density, grass silage, measurement methods

Introduction Fodder compaction is essential for the production of high quality silage. It minimises heating and the energy loss accompanying the opening of a silo. High bulk density reduces oxygen diffusion in the forage pile. To minimise microbial activity it should not exceed 20 l/m^2*h (Honig 1987).
In order to check the actual bulk density, it is necessary to deploy a suitable sampling procedure. There exists no generally recognized standard procedure for this. The most popular methods are based on silage block extraction and core sampling. Silage blocks are often used to determine bulk density as they are easy to extract, weigh and measure. This type of sampling does not include problem zones at ramps, silage edges and surfaces (Kleinmans et al. 2005, Thaysen et al. 2006). Drilling cylinders can be used to determine the density of maize silage (Bundesarbeitskreis Futterkonservierung 2006). Due to the fibrous structures of grass silage, however, this method produces mechanical disturbance in the samples. The aim of this study is to identify the strengths and weaknesses of different sampling procedures and the method with the best fit to reality. Thereby manageability and accuracy were evaluated.

Material and methods All samples were taken from two different horizontal silos. The theoretical cutting length of the silage trailer involved was 40 mm. The stored green material originated from both natural grassland and temporary ley. Compaction was carried out by a standard ballasted tractor with a laden weight of 10230 kg and an internal tyre pressure of 2.5 bar.

The average overall bulk density of each “silo” investigated was recorded on the basis of the harvested product introduced and the measured overall volume of the forage pile. One silage “big block” was taken from each horizontal silo. The comparison was made between the “silo”, “big block”, “small block” and “Pioneer™ drilling jig” variants and a “drilling cylinder” developed in-house, which was used in an inclined and vertical drilling direction (Table 1).

Table 1. Technical data of samplers used.

Variant	Sampler	Producer	Type	Sample dimensions (w × d × h)
“silo”	sensor bridge	ART (Ettenhausen, CH)	ultrasonics	6 m × 25.6 m × 1.4 m
“big block”	block cutter	Trioliet (Oldenzaal, NL)	TU 145	1.75 m × 0.75 m × 1.2 m
“small block”	electric silage cutter	OMC (Correggio, IT)	AS/85	0.2 m × 0.15 m × 0.2 m
“Pioneer drilling jig”	core drill	Pioneer (Buxtehude, DE)	Hi-Bred	Ø 45 mm × depth
“drilling cylinder”	core drill	ART (Ettenhausen, CH)		Ø 56 mm × 0.1 m

As silage blocks can expand vertically when extracted, the layers for testing (each 0.2 m) were premarked in the undisturbed silage. The height of the silage block was limited to 1.2 m for the trial. The precise measurements and weight of the silage blocks were determined following extraction. Samples were subsequently taken from these “big blocks” with the hand-held devices. The volume and weight of all the samples were calculated to determine density. The trial was supplemented by pairwise comparisons of each of the sampling devices affected in the same manner directly in the silage pile.

Results and Discussion The densities determined from the two silage blocks and the small blocks and core drillings taken from them, as well as the extra samples, are summarised in Table 2.

Compared with the target values put forward by Honig (1991) (800 kg FM/m^3 at 20 % DM content; 560 kg FM/m^3 at 40 % DM content) the densities of both “big blocks” (27 and 31% DM content), and hence of the overall silos, may be considered high. By comparison, the density of the “big block” from Silo 1 was 24 % higher, the “big block” from Silo 2 16 % higher than that of the respective overall silo. The pairwise comparison of “big blocks” and “small blocks” demonstrates the enormous heterogeneity of silage blocks.

An explanation for the not inconsiderable difference between “silo” and “big blocks” may be that the “big blocks” were taken from well compacted positions in the silage pile and not from problematic silo zones like the beginning and end, the wall areas or silage surfaces. Evaluation of the measurements confirms observations whereby density decreases as distance from the base plate increases (Amours and Savoie 2005, Craig and Roth 2005). Whole silage blocks are therefore only suitable for a quick assessment of the average overall density of horizontal silos. The “small block” method was subsequently used as a reference for the comparability of selectively drawn samples.

Figure 1 shows the values for the three drilling variants with reference to the “small block” variant. The residual standard error (Res. SE), as a measure of the dispersion of the data points around

Table 2. Data to measured bulk density [kg fresh matter/m³].

	Big block						Extra samples		
	Silo 1			Silo 2			Silo 1		
Variant	Ø	SD	n	Ø	SD	n	Ø	SD	n
"silo"	690		1	756		1			
"big block"	857		1	880		1			
"small block"	800	123	15	824	82	15	791	143	11
"Pioneer drilling jig"	694	95	15	811	88	15			
"drilling cylinder inclined"	769	117	15	768	101	15			
"drilling cylinder vertical"	807	116	15	816	81	15	737	129	11

(Ø – average; SD – standard deviation; n – sample size)

the regression line, is comparatively close together in the three drilling variants. Here the "inclined drilling cylinder" variant compares favourably with the other two variants due to lower dispersion, expressed by a lower standard error. But if, for example, the difference in the prediction accuracy of both drilling cylinder variants is calculated, these only differ by between 1 and 2 %. Both the gradient and the displacement of the regression lines to the x=y line were calculated for x = 869 kg FM/m³ (average bulk density of both "big blocks"), but played a subordinate role in the given dispersion range of the values. All three variants underestimated the density of the reference "small block".

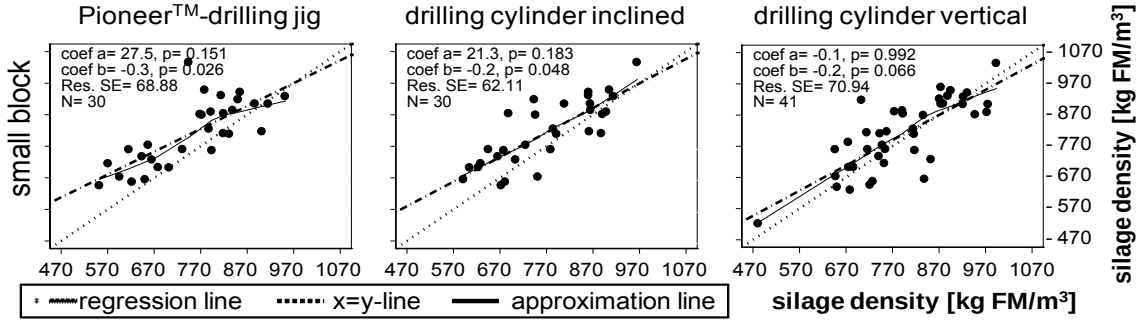

Figure 1. Silage density of the drilling variants with reference to the small block. (coef a – Shift of regression line from x=y-line in point x=869; coef b – Difference in inclination between regression line and x=y-line; Res. SE – Residual Standard Error; N – number of samples)

Horizontal drilling is recommended by Kleinmans et al. (2005) for the extraction of maize with the Pioneer™ drilling jig. By comparison, the fibrous structure of grass silage results in the silage being pulled out of the drilling jig again during horizontal sampling and leads to an underestimated bulk density. Drilling carried out at an angle to the horizontal bedding layers of the silage generally effects better cutting of the grass silage fibres. As a result, the individual layers are less pulled out of the drilling jig by still connected fibres. Using an electric driven drill represents a huge saving in labour, particularly when extracting a sizeable number of samples. An inclined drilling direction is preferable to a vertical one, as in this way samples can be taken at the cutting front of the silage.

Conclusions Samples taken with hand-held devices testified to the enormous heterogeneity of density conditions within the silage blocks. A sizeable number of small silage samples represent the heterogeneous density conditions within horizontal silos whereas large-volume samples only express one single average. Drilling should be carried out obliquely or vertically in relation to the bedding direction of the fibres in order to cut the fibrous structure of the grass silage and to obtain good filling of the drilling cylinder. In statistical analysis the drilling variants tested showed only slight differences of between 1 and 2 % in density prediction accuracy, the tendency being to underestimate the density compared to "small blocks". Rather better statistical consistency with the reference "small block" and comparatively easier handling made the "inclined drilling cylinder" variant the preferred variant in this trial.

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Effect of silo management factors on aerobic stability and extent of spoilage in farm maize silages

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Keywords: aerobic stability, maize silage, silo management, yeast and mould count.

Introduction Whole-plant corn silage, stored in horizontal silos, is the main source of forage for lactating dairy cows throughout the world. Aerobic deterioration can cause large losses of dry matter (DM) and quality because of the large surface area prone to oxygen penetration, non perfect sealing, and the great dependence on management practices during filling and feed-out. The main factors to prevent aerobic deterioration are: correct removal rate from the silo; packing down fresh forage when filling the silo; weigh down shoulder and top sheets; additives and inoculum types; types of plastic films to cover silage. The results that will be presented in this paper were obtained in two projects carried out to quantify the extent of aerobic deterioration of maize silages on commercial farms in Northern Italy, to determine the effects of silage aerobic deterioration on cheese making and to define good management practices that should be applied as the basis for safe silage production.

Materials and methods The surveys involved over 100 dairy farms (breeding Italian Friesian cows) that give milk to different cheese factories in six years (2005-2010). Half of the silages were surveyed during winter and half during summer consumption. Temperatures were measured within the stored silages during feed-out at various depths into the working face (following Borreani and Tabacco 2010). During the feed-out, area of the working face with visible moulds or spoilage was also determined. Core samples were taken from each silo to determine fermentation characteristics, pH, DM content, and yeast and mould counts in different parts of the silo (silage core, peripheral areas and, when present visible moulded spots). The core depth and weight were measured to determine the silage density. Linear feed-out rate of the working face (m week^{-1}) was measured by marking successive locations of the face over two weeks period (Ruppel et al. 1995). A detailed questionnaire was filled in on each farm reporting information about silo sizes, amount of silage consumed each day, filling and packing methods, number and thickness of plastic sheets used to cover the silos, material used to weight down the silo surfaces, and the presence of silo walls. These management factors were related by regression analysis (SPSS 16.5) to the extent of aerobic deterioration and to the aerobic stability measured in each silo.

Results and discussion All surveyed maize silages were covered with at least one polyethylene film and most of them were ensiled in bunker with concrete walls (85%). The presence of severely spoiled silage occurs for 29% and 46% in winter and summer seasons, respectively (Figure 1). From the surveys resulted that the driving factor to prevent aerobic spoilage is the feed-out removal rate of silage from the silo face (Figure 2). It is noteworthy that, over winter, the silages that were consumed with a feed-out rate greater than 1.1 m week^{-1} had a moulded surface lower than 2% irrespectively of other silo management practices. Over summer the feed out rate should be higher than 1.75 m week^{-1} to have moulded surface lower than 2%. Conversely, all silages that were consumed at a feed-out rate lower than 0.50 m week^{-1} were severely deteriorated. The silage grouped in the dotted circle were well conserved silages and this was due to other management practices such as paying great care in silo compaction, using more than one plastic cover or barrier films, weighing down firmly silage cover with soil, gravel or sand bags. Over winter also freezing temperatures could contribute to slow down or stop spoiling microrganisms. When recommended silage feed-out rates to reduce to a minimum aerobic deterioration were retrieved from international literature, a great range of values was observed (Table 1). These recommendations come from experience and from observations made on farm and are typically rates at which little heating of silage occurs during consumption. These values ranged from a minimum of 1.0 m week^{-1} for winter season in the Netherlands to a maximum of 2.15 m week^{-1} for summer season in Kansas. When the suggested feed-out rates were related to average seasonal temperatures of the country, whose these values are referred to, a high coefficient of determination was found (Figure 3). The data from our survey (empty circles in Figure 3) are close to the regression line of the data set.

Conclusions These farm-scale studies on maize silages, underlines the importance of coupling high feed-out rates with careful silo management in order to control aerobic deterioration. For the Northern-Italy environment, a feed out rate below 0.5 and 0.8 m week^{-1}, for winter and summer consumption, respectively, cannot prevent aerobic deterioration even if very good silo management practices were applied. Furthermore, to ensure a correct feed out rate of the silo the amount of silage consumed by the herd each day and the average temperature of the unloading period should be taken into account before silo sizing and filling.

Table 1. Seasonal recommended silage removal rates from bunker silos during feed-out in different countries.

Country	Latitude	Mean temperature (°C)			Feed-out rate (m week^{-1})	
		Annual	Winter[1]	Summer[2]	Winter	Summer
The Netherlands (Visser et al. 2007)	51-53°N	9.5	4.4	14.8	1.00	1.50
North Dakota (Schroeder 2004)	47°N	5.7	-7.2	18.0	1.05	2.10
Italy (Tabacco and Borreani 2002)	45°N	11.6	3.7	19.5	1.05	1.60
Wisconsin (Pitt and Muck 1993)	44°N	8.3	-2.4	18.8	1.10	2.10
Kansas (Berger and Bolsen 2006)	38°N	13.8	3.6	23.8	1.35	2.15
Israel (Weinberg 2003, pers. com.)	31°N	19.1	14.2	23.7	1.40	2.10

[1] average monthly temperature November- March period; [2] average monthly temperature May-September.

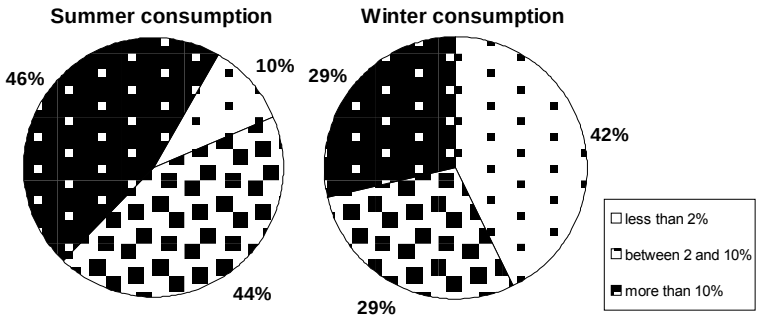

Figure 1. Extent of spoilage at the silo face of farm maize silages in Northern Italy, quantified by silo face temperatures and moulded areas following Borreani and Tabacco (2010).

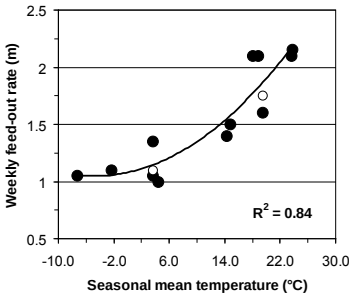

Figure 3. Recommended silage feed-out rates from bunker silos in relation with temperature (Table 1).

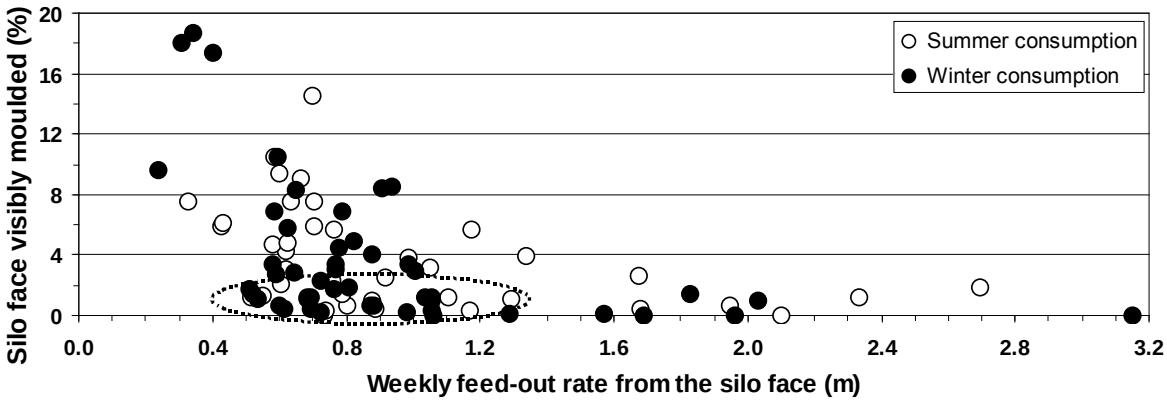

Figure 2. Percentage of the moulded surface of the silo face of farm maize silages in relation to the weekly feed-out rate in Northern Italy. The silages grouped in the dotted circle were well conserved silages due to other management practices as explained in the text.

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Optimising the application technique for silage additive in harvesting machinery

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Keywords: application technique, evenness, loader wagons, loss, precision choppers, silage additive

Introduction Additives generally improve silage quality. For a good result, the additive has to be distributed evenly and dosed with the correct quantity in the forage. The objective of this study was to identify the best application methods of formic acid based additive in loader wagons and in towed (tractor-driven) and self-propelled precision choppers, regarding evenness and losses of application. Losses typically arise from evaporation and wind drift of additive droplets. In this text, loader wagon means a self-loading wagon with a rotor that pushes the herbage through a row of *stationary* knives, rather than a self-loading wagon with an integrated precision chopper with *rotating* knives.

Materials and methods In trial 1, application methods were compared in a loader wagon and a towed precision chopper (Table 1). In trial 2, other application methods were compared in the same machines as in trial 1 (Table 2). However, methods C and D were included in both trials. In trial 3, application methods were compared in a self-propelled precision chopper (Table 3). Methods A and B (Table 1) represent the current practise in loader wagons: additive is sprayed on the windrow in front of the pickup, or on the forage flow on the pickup. C is a new method that we developed with the aim to get more even distribution: half of the additive was sprayed from above on the pickup, and half underneath the grass flow on the pickup. For this, a plastic pipe with 20 mm outer diameter and 1.1 mm holes at an interval of 100 mm was fastened to the pickup surface, so that the grass slid over it. In A, B, C and D, the application from above was done through flat-fan nozzles. In methods 4 and 5, the perforated plastic pipe had holes with 1.1 mm diameter at an interval of 60 mm. The pipe was perpendicular to the direction of the grass flow in the inlet channel between the pickup and the knife rotor. In method 5, the normally open top side of the inlet channel was covered with a plastic film. In the self-propelled chopper (Table 3), the first method was a plastic pipe with 1.5 mm holes at an interval of 33 mm placed above the front opening of the inlet channel. Injection in the curved chute was on the outer (grass) side/arc or the inner (air) side/arc. In trials 1 and 2, four loads per method were tested, and eight grass samples per load analysed for formic acid. In trial 3, five loads per method were tested, and ten samples per load analysed for formic acid. The evenness of application was calculated as the loadwise coefficients of variation (CV) for the formic acid concentration of the samples from the load. The mean CV for each application method was calculated, and the differences in CV between the methods were statistically tested with analysis of variance. The loss of additive was determined as the difference between the amount of additive consumed from the additive vessel per load and the amount of additive in the grass of each load, calculated from the load weight and the formic acid concentration in the samples. In trial 1 the additive was AIV Prima (620 g/kg formic acid, 240 g/kg ammonium formate). In trials 2–3 the additive was AIV 2 Plus (760 g/kg formic acid, 55 g/kg ammonium formate). Wind speed and temperature in trial 1 were 0–2.5 m/s, 15–25 °C, in trial 2 0–6.0 m/s, 12–21 °C and in trial 3 0–0.6 m/s, 15–21 °C. The trials had a randomised complete block design. The statistical analyses were based on the following mixed model: $y_{ij} = \mu+\rho_i+\tau_j+\varepsilon_{ij}$ where μ is the overall mean, τ_j is the fixed effect of the application method, ρ_i is the random effect of block and ε_{ij} is the random error term of the model.

Results and discussion The new method C in the loader wagon distributed the additive with significantly better evenness (CV 50%) than the traditional methods (CV 79–84%, Table 1). In trial 2, however, both varieties of the new method (C and 9, Table 2) resulted in significantly worse evenness (CV 89%) than the precision chopper (CV 23–46%). This does not necessarily contradict the result from the first trial that the new method seems better in evenness than the traditional ones in loader wagons, because the second trial did not include any such comparison. It is logical that the current practise in loader wagons, to spray the additive only on top of the ingoing forage, results in uneven distribution. The additive remains on the surface of the windrow or forage flow. The feeding rotor has a limited mixing effect and does not even out the uneven distribution. Precision choppers have a fast rotating knife cylinder which mixes forage and additive. The new method improves the preconditions to get good silage quality with loader wagons. In trial 2, replacing flat-fans (C) with narrow jets from a perforated pipe (9) reduced the losses from 47 to 28%. Water is less sensitive to evaporation than acid. Therefore the reduction of loss might be smaller with biological additives in water solution. In the towed chopper, methods 1, D, 3 and 4 are not worth recommending because they involve a risk of bigger losses in the open space in windier conditions. Methods 5 and 6 can be considered the best ones regarding evenness and loss. Method 6

can be recommended in practise, since it is easier to install than 5 where the user himself has to make the perforated pipe.

In the self-propelled chopper, the distribution was significantly better with application at the inlet channel than in the chute (Table 3). Application in the inlet channel utilises the mixing effect of the knife rotor and the accelerator. In the chute, the stream of herbage is thick, and additive applied at the surface of the thick stream is not mixed but remains at the surface of the stream. However, application in front of the inlet channel brought acid odour into the cabin, which is unpleasant for the driver. This could probably be avoided with application further back in the channel, but still before the accelerator to ensure good mixing. Application in the chute resulted in good evenness in the towed chopper but not in the self-propelled one. The reason is probably that the forage stream in the chute of self-propelled choppers is thicker and denser than in towed ones, so the injected additive penetrates the forage stream worse in the chute of self-propelled choppers. Acidic additives corrode machinery to some extent, which can make some users unwilling to apply them in the inlet channel. Application in the top deflector saves the machine from corrosion. However, fodder quality should be considered more important than corrosion.

Table 1. Evenness and loss of additive in trial 1. Treatments with the same letter were not significantly different.

Machine	Application method	Evenness CV, %	Loss, %	P
Loader wagon	A. From above, in front of pickup	79.3[A]	48.3	[A] versus [B]: 0.01<*P*<0.05
	B. From above, at pickup	83.6[A]	33.9	
	C. From above + jets under, at pickup	49.7[B]	32.9	
Towed precision chopper	D. From above in open inlet channel	46.2[B]	42,0	

CV = coefficient of variation (standard deviation divided by mean). The smaller CV, the better evenness.

Table 2. Evenness and loss of additive in trial 2. The percentage given for flat-fan nozzles is the ratio between the real flow (l/min) and their nominal flow at 1 bar (nominal size). The smaller ratio, the coarser spray. The P values were calculated for methods 5, 7, C and 9 versus the other methods, and for C versus 9. The most recommendable method in each machine is bolded. lw = loader wagon; prec. ch.= precision chopper.

	Place of application	Nozzle and spray type (n) = number of nozzles abreadth	Evenness of application CV, %	P	Loss Loss,%	P
Towed prec. ch.	1. Pickup	flat-fan nozzles, coarse spray 33% (5)	27.9		16.9	
	D. Open inlet channel	flat-fan nozzles, fine spray 150% (5)	31.4		22.4	
	3. Open inlet channel	flat-fan nozzles, coarse spray 29% (3)	45.5		7.55	
	4. Open inlet channel	perforated pipe; solid jets	29.3		13.5	
	5. Covered inlet channel	perforated pipe; solid jets	22.8	* vs. 3, 7	9.22	
	6. **Chute, lower part**	**solid-jet nozzles (3)**	26.0		6.78	
	7. Top deflector	solid-jet nozzles (2)	45.5		1.19	* vs. D
lw	C. Flat fans from above + solid jets under, at pickup		89.5	*** vs. 1, D, 3, 4, 5, 6, 7, 9	47.2	*** vs. 1, D, 3, 4, 5, 6, 7
	9. Solid jets both above and under, at pickup		89.0	*** vs. 1, D, 3, 4, 5, 6, 7, 9	28.2	* vs. C. ** vs. 3, 5, 6 *** vs. 7

Table 3. Additive evenness and amount of grass which got too little additive in self-propelled chopper.

Location of application	Evenness of application		% of the herbage which got less additive than		Applied dose
	CV, %	Significance A-B	1.5 l/t	3.0 l/t	l/t
Front of inlet channel	20 [A]		0	0	5.1
Chute base, outer (grass) side	61 [B]	** P = 0.0017	2	22	5.8
Chute base, inner (air) side	49 [B]	* P = 0.0154	10	24	4.4
Chute, top deflector	64 [B]	*** P = 0.0009	14	36	4.9

Conclusions The current practice of spraying additive only on top of the ingoing forage in loader wagons results in uneven distribution of additive in the forage. Spraying half of the additive on the forage from below improves the evenness, and reduces the risk of uneven silage quality. For the application from above, narrow jets from a perforated pipe cause less loss of acid-based additive than flat-fan nozzles. Smaller losses of additive can be achieved in precision choppers than in loader wagons. In towed precision choppers, the best places of application are the lower part of the chute, and the inlet channel if it is covered. In self-propelled choppers, the best place is in the inlet channel before the accelerator.

Microbiology of ensiling

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Keywords: lactic acid bacteria, PCR, aerobic stability, inoculants

Introduction

Over the last 10 years, dramatic changes have occurred in how we study the microbiology of ensiling. The Eighth Silage Conference, held in 1987 in Hurley, England, was the first International Silage Conference that I attended. One notable part of that conference for me was an unofficial gathering of people doing silage microbiology. What did we discuss? The agars and incubation conditions we used to enumerate lactic acid bacteria (LAB), yeasts, moulds, clostridia and enterobacteria. As someone new to silage microbiology, I took detailed notes that I used for years. Twenty-five years ago, our knowledge of what occurred in the silo was limited by what would grow on various selective media, and identification of species from those agar plates was tedious work. API 50 strips allowed us to grow strains on 50 different substrates simultaneously. That did speed the identification of lactic acid bacteria, but the strips were not infallible. We struggled to know cause and effect in the silo. Were the acids and other products that we measured during fermentation due to this strain or that strain?

Today, we still have issues with understanding cause and effect in the silo. However, we have much better tools to know which microorganisms have been involved in the ensiling process. We also have a better understanding regarding how microorganisms affect silage quality over beyond production of lactic acid, volatile fatty acids, alcohols and carbon dioxide. In this paper, I will discuss recent developments in the measurements of microbial dynamics during ensiling, our current knowledge of the species that contribute to ensiling as well as the species that spoil silage, the extent to which microbial additives modify fermentation and the utilization of silages by livestock, and efforts to find new microbial additives.

Recent microbial techniques

The ability to extract microbial DNA from silages, amplify portions of DNA, and then separate those portions by the strains of microorganisms that have produced them has been at the core of the changes that have occurred recently in silage microbiology. These developments have allowed us to enumerate strains that do not grow on agar and reveal new species in silages. These techniques can be divided into two groups: 1) identification and quantification of specific species/strains and 2) community analysis.

Identification and quantification of specific species/strains

Most of these techniques use the polymerase chain reaction (PCR) to make many copies of a portion of the DNA in microorganisms. The most commonly amplified portion of the DNA from bacteria is the 16S ribosomal RNA gene, which is the primary basis today for classifying bacteria instead of what substrates they use, products they secrete, etc. The PCR primer binding sites for this gene are highly conserved across bacteria while other portions of the 16S sequence generally are more variable between species, which permits classification. Some techniques use other portions of the DNA as will be discussed.

For identification, DNA can be extracted, for example starting from a colony on an agar plate where there is one strain of microorganism present. The 16S rRNA gene can be amplified by PCR and then sequenced. Once the sequence is known, a program such as BLAST may be used to compare the sequence of the unknown strain with those of known species. If there is a good match with a known species, then it can be presumptively classified as that species. If there is not a good match, the colony may be that of a new species subject to other tests. Consequently, one can enumerate lactic acid bacteria or other types by standard plating techniques and then use PCR to identify the species on those plates.

A related technique is real-time PCR (RT-PCR) or quantitative real-time PCR. This analytical technique allows one to quantitate specific species present in a sample. Primers (set sequences of nucleotides) are selected from the region of a gene that is specific to the species of interest. A portion of the 16S rRNA gene has been most commonly used (e.g., Schmidt et al. 2008), but other genes such as the *recA* gene have been used with lactic acid bacteria (e.g., Stevenson et al. 2006) because it was easier to find sequences to separate species that have very similar 16S rRNA genes. This method is very useful for following species that you expect to be in the environment such as comparing the level of *Lactobacillus plantarum* in silages that are untreated vs. those inoculated with *L. plantarum*. The key to success is finding sequences that do not react with other species that may be present in your silages. Initial studies using RT-PCR in silages have compared their primers against other known species to be sure that all known strains of the species of interest react with the primers whereas known strains of

other species do not. That has provided some measure of confidence in their results, but there still may be species not tested, particularly unknown species, that provide false positives or strains of the species of interest with slight differences in the sequence in the region of the primers causing a false negative. The latter is not a problem if you are following an inoculant strain that reacts with the primers.

Quantification in RT-PCR is based on how many cycles of amplification are needed to reach a target number of copies of the sequence that is specific to the species being measured. So if the species of interest is the dominant species in the silage, it will take only a few cycles to reach the target. If it is at a low level, it may take many cycles. The advantage of this technique is that you can enumerate a species that is present at a low level. For example, by standard techniques we may pick 100 colonies from an MRS plate and then identify the species of each colony by standard methods like API 50 strips or by PCR. Such methods make it unlikely to detect a species that is present at less than 1% of the total population. With RT-PCR, we may be able to enumerate a species that is at 100 cfu/g silage even though the total LAB population is 10^9 cfu/g. Thus RT-PCR allows us to follow known species more rapidly and at a much lower detection limit than previously possible.

Community analysis

Even with PCR and RT-PCR, investigating the effects of various factors on the microbial community in the silo is laborious. Fortunately, a variety of techniques have been developed that allow us to get a snapshot of the bacterial community and then using statistical techniques like principal component analysis determine if there are significant differences in communities. If there are, then we can use PCR and RT-PCR to document the differences. At least four techniques have been used to study microbial communities in silages: length heterogeneity PCR (LH-PCR), terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) and automated ribosomal intergenic spacer analysis (ARISA). All four techniques use PCR to amplify a portion of the microbial DNA and then use various methods to separate the amplified DNA. It is beyond the scope of this paper to discuss these methods in detail. It is more important to know general differences between the techniques.

Length heterogeneity PCR uses the variation in the length of a gene between different microbial species to determine how many species may be active in an environment. The specific gene is amplified by PCR and then a sample of that DNA is run by capillary electrophoresis to separate the copies of that gene by their length. Brusetti et al. (2006) investigated this technique using the differences in the length of a region of the 16S rRNA gene to follow the development of various LAB species during the ensiling of maize. The technique was successful in following most of the species identified in the silages. However, two species identified, *Weissella confusa* and *W. kimchii*, had identical fragment lengths of 379 base pairs and thus could not be differentiated by LH-PCR. Similarly the *Enterobacter* species identified had the same fragment lengths. A substantial number of peaks (29-58%) were not identified.

Terminal restriction fragment length polymorphism operates on a similar principal to LH-PCR. A gene or other portion of DNA may be amplified with a fluorescent marker placed on one end of the copy. A restriction enzyme that attaches to a specific recognition site on the DNA is added to the amplified DNA cutting it in two. The DNA is separated by either gel or capillary electrophoresis according to the length of the fluorescent fragments. McEniry et al. (2008) investigated bacterial community dynamics in wilted grass silage using T-RFLP. The 16S rRNA genes were amplified, then digested with the restriction endonuclease *MspI*, and separated by electrophoresis. The technique did show that there were shifts in the species with time (0, 2, 6, 14, 35 and 98 d) and ensiling method (baled vs. precision-chop silage). However, identification of species based on the length of a fragment using a database and simulated digest of the 16S rRNA gene by the restriction enzyme was often not possible because multiple species had the same fragment length.

Denaturing gradient gel electrophoresis refers to the method of separating DNA. As in the other methods a portion of DNA is amplified such as the 16S rRNA gene or a portion of that gene. The DNA is loaded onto a gel with a gradient of DNA denaturant. Each DNA segment moves through the gel until it encounters the right amount of denaturant that causes the DNA to uncouple into two strands and then moves much slower forming a band. Thus the composition of the nucleotides in the DNA rather than just the length of the DNA sequence determines the distance moved. Normally this is used where the amplified DNA is relatively similar in length. For example, Li et al. (2011) amplified the V3 region of the bacterial 16S rRNA gene and the fungal 18S rRNA gene and analysed these by DGGE. An advantage of this procedure was that they could excise prominent bands in the gel and then use PCR techniques/BLAST to identify the species that produced each band.

Automated ribosomal intergenic spacer analysis: the name highlights the region of DNA that is amplified. With bacteria, the region between the 16S rRNA gene and the 23S rRNA gene is highly variable in both sequence and length across species. It is this region that is amplified by PCR. Analysis of the amplified DNA can be by gel electrophoresis (i.e., utilizing variation in sequence and length), but ARISA uses fluorescent primers and separates by fragment length like T-RFLP. Brusetti et al. (2008) used both ARISA and LH-PCR to assess bacterial communities in maize silage. ARISA gave 12 peaks

on average compared with 9 peaks with LH-PCR, and the range of base pairs was much larger for ARISA. These suggest more sensitivity in the ARISA measurements because the ARISA is amplifying a much more variable region. They used PCR to identify 388 isolates taken from MRS agar plates, falling into 11 known species. However, no attempt was made to match these isolates with the peaks from either ARISA or LH-PCR. So the authors were not able to determine which community method was more accurate in describing differences between silages.

All of the community techniques allow a relatively rapid comparison of communities between treatments. Of the three techniques that separate by length of DNA (LH-PCR, T-RFLP and ARISA), it would appear that ARISA is the least likely to have multiple species with fragments of the same length due to the heterogeneity of the intergenic spacer region in bacteria. However, it is possible for a strain to produce more than one peak in ARISA so that community diversity may appear greater than it is. Because DGGE is performed by gel electrophoresis, the results are more qualitative and variable from one gel to the next compared with using capillary electrophoresis in the other three techniques, where their results are easily imported into statistical software for principal component analyses, etc. On the other hand, DGGE has the advantage mentioned earlier that bands can be excised and cloned by PCR for species identification.

New microbial species in silage

These new techniques have made it possible to more easily find new species and understand the dynamics of the microbial populations with time in the silo. However, these new techniques have often verified that the traditional species associated with ensiling are the predominant species. Brusetti et al. (2006) using LH-PCR reported the presence of *Bacillus megaterium* early in the ensiling of maize (day 0 and 1), *Weissella kimchii* (d 6) and *Enterococcus flavescens* (d 13). While these species had not been reported in silage, other more common species dominated the silages. Rossi and Dellaglio (2007) surveyed farm silages, primarily lucerne, maize and Italian ryegrass as well as mixtures of maize silage and maize grain. The LAB isolates matched known silage species with the exception of *Lactobacillus zeae*. Anaerobic spore formers found were largely known silage clostridial species with the exception of *Clostridium baratii* and *Paenibacillus macerans*. Three yeasts species were identified: *Candida mesenterica*, *Candida apicola* and *Pichia fermentans*. This was the first report of the two *Candida* species in silages even though *Candida* species are commonly found in silage.

Several new species have been isolated from silage and had names proposed. These include *Lactobacillus taiwanensis* (Wang et al. 2009) and *Pediococcus lolii* (Doi et al. 2009). Parvin et al. (2010) analysed laboratory silages made with Italian ryegrass, maize, guinea grass and rhodes grass ensiled with and without *L. plantarum* or *Lactobacillus brevis* inoculants. DGGE analysis followed by cloning and sequencing of prominent bands found mostly well known silage species. However, the untreated Italian ryegrass had 6 prominent bands, all but one (*L. plantarum*) was unusual: *Pediococcus dextrinicus*, *Paralactobacillus selangorensis*, *Burkholderia* spp., *Serratia* spp. and an uncultured bacterium.

Pang et al. (2011b) identified strains isolated from maize stover, and 86% of the LAB consisted of *L. plantarum*, *Lactobacillus pentosus* and *L. brevis*, three species commonly reported in silages. On the other hand they reported the presence of *Leuconostoc lactis*, *Enterococcus mundtii* and *Weissella cibaria*, the latter a recently described species. The same group isolated LAB strains from maize, rice, sorghum and lucerne silages (Pang et al. 2011a) and found that *W. cibaria* and *Weissella confusa* were the dominant species observed in maize silage. In the other silages, *L. plantarum* was the dominant species.

Li et al. (2011) using DGGE identified the dominant bacterial and fungal species in maize silage. Pre-ensiling and later in the silage, *L. brevis*, *Pediococcus parvulus*, *W. confusa* and *Klebsiella pneumoniae* were reported. Additionally in the silage, *Weissella paramesenteriodes*, *L. plantarum* and *Lactobacillus lactis* were observed. Yeasts in pre-ensiled maize and maize silage were predominantly *Candida* species and *Cryptococcus flavus*. *Candida* species have been reported in silages but not the species that they reported (*magnolia*, *intermedia*, *glabrata* and *quercitrusa*). When the maize silages were subjected to aerobic exposure, *Saccharomyces* and *Pichia* species appeared, genuses commonly found in silage, but some of the species were newly reported as being in silages (*S. martiniae*, *P. deserticola*, *P. kudriavzevii*).

Li and Nishino (2011b) sampled maize silage from bunker silos. They detected a number of uncommon silage LAB species, *Lactobacillus acetotolerans*, *Lactobacillus panis* and *Lactobacillus reuteri*, as well as other new species in silages: *Acetobacter pasteurianus*, *Stenotrophomonas maltophilia*, an *Acinetobacter* species and a *Rahnella* species. Li and Nishino (2011a) studied wilted Italian ryegrass silages in mini-silos, finding enterobacteria such as *Erwinia persicina*, *Pantoea agglomerans* and *Rahnella aquatilis* in untreated silages. Known LAB species accounted for the majority of the other species detected.

Overall, these studies have indicated that the new PCR based techniques are uncovering some new species. In most cases, traditional silage LAB species have been the dominant bacterial species

present. However, we still have a difficult time knowing how significant the new species are to silage preservation because these PCR methods do not indicate the contribution of the various species to the overall fermentation.

Effects of various factors on silage microbial populations

The new techniques have also been useful to study how various factors affect fermentation. The most common factor is the use of a bacterial inoculant, which will be discussed later. However, there have been a few recent studies that have investigated the effects of other factors on the course of fermentation.

McEniry et al. (2010) compared the microbial community in perennial ryegrass in baled vs. precision chop ensiling at two DM concentrations (185 and 406 g DM/kg) over 0 to 14 d using T-RFLP. Using a T-RFLP database, they assigned fragment lengths they observed to general groups, e.g., LAB, enterobacteria, etc. The most abundant fragment was associated with enterobacteria and was most prevalent early in ensiling and in the drier silages whereas the most prevalent fragments associated with LAB behaved in an opposite direction with those two factors. Only three minor species were affected by the ensiling system. In a second experiment, unchopped, unwilted perennial ryegrass was ensiled with or without compaction and with and without air infiltration for 100 d. For the top 20 fragments, most were not affected by either compaction or air infiltration. Six fragments were affected by compaction, having a negative effect on LAB and enterobacteria and a positive effect on clostridia. Only 4 fragments were affected by air infiltration: two clostridia negatively and one clostridia and bacillus species each positively.

Naoki and Yuji (2008) compared the microbial community in vacuum-packed bag Italian ryegrass silage with wrapped bale silage using DGGE. Specific bands were not excised and identified, but the band pattern in the outer portions of one bale was similar to the pattern in the vacuum-bag silage. However, there were differences in microbial community between bales and locations within a bale, suggesting field variability was affecting, in part, the dominant species.

Brusetti et al. (2006) investigated the usefulness of LH-PCR by following the progression of fermentation in maize silage up to 30 d. *Pediococcus pentosaceus* and *W. confusa* were the most prevalent species present at ensiling. Both species were present throughout the 30 d. *Lactococcus lactis* subsp. lactis was also present at ensiling, reaching its highest level at 6 d. *Lactobacillus* species were reported at various times during the course of the 30 d: *L. plantarum*, minor levels throughout; *L. brevis*, d 6-30; *L. paraplantarum*, d 13, d 20.

Parvin and Nishino (2009) used DGGE to study microbial changes with storage time (15 to 180 d) from the ensiling of guinea grass at two DM concentrations (286, 443 g/kg). In the wetter silage, *Lactococcus lactis* and *L. brevis* were the dominant species at 15 d but by 180 d, *Lacto. lactis* was a faint band and *L. pentosus* was more prevalent. This shift coincided with a reduction in lactate to acetate ratio in the silage with time. In the drier silage, two strains of *L. plantarum* were observed in addition to *Lacto. lactis* and *L. brevis*. The *Lacto. lactis* band did not diminish with time, but an *L. pentosus* band did appear at later time points. In the drier silage, lactate to acetate ratio did not change with time, but both acids increased with time.

Parvin and Nishino (2010) measured the changes in microbial community with storage time (15 to 180 d) in rhodes grass silage using DGGE, and prominent bands were sequenced. *L. brevis*, *L. plantarum* and *L. pentosus* were present at all time points although the *L. plantarum* strain in the early time points appeared to be a different strain than that at later times. *Lactococcus lactis* had a strong band at 15 d that became fainter with each succeeding time point. There was a faint band of *Escherichia coli* through 90 d.

Ávila et al. (2010) investigated microbial population changes in 5 cultivars of sugarcane silage at 10, 20, 30 and 40 d. Of particular interest were the yeast populations that varied by cultivar and time. For 3 cultivars, the highest yeast counts occurred at 10 d with yeast counts being significantly lower at 40 d. For the other 2 cultivars, the highest yeast counts occurred at 30 d with counts dropping significantly at 40 d. Colonies were picked, and identified by PCR techniques. Of the cultivatable yeasts, only 4 species were present in the 10 d silages (*Torulaspora delbrueckii*, *Pichia anomala*, *Saccharomyces cerevisiae* and *Candida glabrata*), which were the dominant species across all time points. Five other species were identified. The sugarcane cultivars varied in the yeast species observed: one cultivar with just two species (*Torulaspora delbrueckii*, *Pichia anomala*), three with five species of yeast, and one with seven species of yeast.

Villa et al. (2010) compared the ensiling of two maize varieties, one grown in a warm climate and one grown in a cool climate in Colombia. The maize from the warm climate had a higher initial count of LAB, leading to a more rapid decline in pH. The fermentation of the cool climate variety was dominated by *Lactobacillus* and *Pediococcus* species with populations of both genuses peaking upon reaching pH 3.8 at 7 d. In the warm climate variety, both genuses peaked at 2 d when the pH was below 4.0, but *Leuconostoc* species also contributed, peaking at 5 d when pH had reached 3.7.

The dynamics of microbial groups in Danish stack silos of maize silages were observed every other month beginning in January and continuing until September (Storm et al. 2010). Yeasts and lactic acid bacteria declined with time over this period whereas moulds were highest in the March and May sample periods. The filamentous fungi were identified and the primary species found were *Penicillium roqueforti*, Zygomycetes (primarily *Mucor* spp.), *Penicillium paneum* and *Aspergillus fumigatus*. The percentage of stack silos with these species was highest either in March or May. By September, there was a greater diversity across the 20 silos.

Several recent studies have investigated the effects of low temperature on ensiling. They have not used PCR or other sophisticated techniques, but their results are of interest. Wang et al. (2011) ensiled reed grass at 0 and 4°C and sampled at weekly intervals from 3 to 8 weeks. By week 5, pH had decreased significantly at both temperatures. At week 5, the pH was 4.20 in the silage at 4°C, significantly lower than the silage at 0°C. Also acetic acid was lower and lactic acid higher at 4°C. Pauly and Spörndly (2011) investigated maize silage made at 6, 12 and 18°C in the first year and 2.6, 6, 12 and 20°C in the second year. The pH was below 4.0 by 20 d in the two warmest temperatures. Fermentation at 6°C appeared to have stabilized by 60 d at a pH of 4.1 in both years with a fermentation that was lower in lactic and acetic acids and higher in ethanol than the fermentations at warmer temperatures. When some of the silages stored at 6°C were raised to 18°C after 45 d and stored for an additional 61 d, more fermentation occurred reducing pH similar to that of the 18°C silages but with higher concentrations of lactic acid, acetic acid and ethanol than the 18°C treatments.

Drawing general conclusions across these studies is difficult. It would appear that type of silo (even field-scale vs. laboratory) or density has only small effects on the dominant species during ensiling. There are considerable differences in the dominant species from one trial to the next, but in most studies, common *Lactobacillus*, *Pediococcus* and *Lactococcus* species were the most prevalent. The biggest deviation appeared to be in maize silage in warm climates where *Weissella* and *Leuconostoc* species contributed to early stages of fermentation. Finally, silage fermentation can occur at near freezing temperatures, but active fermentation continues over weeks, not days.

Aerobic deterioration of silage

When oxygen is introduced to silage, aerobic microorganisms begin to grow, initially respiring soluble substrates and then more complex compounds. This reduces the digestibility and feeding value of silage. The general pattern of spoilage has been known for approximately two decades. Yeasts are generally the initiators of aerobic deterioration, consuming sugars and fermentation acids and raising silage temperature and pH (Pahlow et al. 2003). With increased pH, bacilli and other aerobic bacteria grow, increasing temperature further. Finally, moulds complete the deterioration of the silage. In maize silage, acetic acid bacteria have been found to be initiators of aerobic deterioration in some cases.

Much of the recent work on aerobic deterioration has been in the study of inoculants to inhibit the rate of spoilage. However, there are a number of recent studies that contribute to our understanding of microbial dynamics in spoiling silages. Dolci et al. (2011) studied microbial dynamics during aerobic exposure of inoculated (*L. buchneri*, *L. plantarum*, *E. faecium*) maize silages stored in polyethylene or oxygen barrier film bags for 110 d. The latter had a permeability to oxygen that was 5% of that of the polyethylene film. The silages were sampled 6 times between opening and 14 d of aerobic exposure. DGGE was used to study both the bacterial and fungal communities, and prominent bands were excised and cloned for identification of species. At opening, bands identified as *L. buchneri* were dominant in both treatments. With aerobic exposure, the *L. buchneri* band diminished within one week in the polyethylene treatment where the silage began to heat in 3 d. At day 5 and continuing through day 14, the dominant band in that treatment belonged to *Acetobacter pasteurianus*, and a fainter band identified as *Bacillus subtilis* was present. In the silage made with the oxygen barrier film, these species did not appear until day 9 and 14, respectively, where heating did not begin until 9 days. The two fungal species present at opening (*Kazachstania exigua*, *Aureobasidium pullulans*) were unusual species for silages. Both bands disappeared after 5 days in the polyethylene treatment. Later *Pichia kudriavzevii* and *Aspergillus fumigatus* appeared in the polyethylene treatment whereas *A. fumigatus* and an unknown species appeared in the oxygen barrier film treatment. The unknown species appeared tied to the beginning of heating, being the dominant band at 9 d. Overall, it is interesting that in the film with higher oxygen permeability it appeared that *Acetobacter* was the initiator of aerobic deterioration whereas an unknown fungal species initiated spoilage in the other treatment. *Bacillus* and mould species became more prevalent after heating and increases pH had occurred, as expected.

Borreani and Tabacco (2010) cored the faces of 54 maize silage bunker silos measuring fermentation products, yeasts, moulds, clostridial spores and also taking temperatures at 200 mm behind the feed out face. At least 18 core samples were taken from each silo spanning the width and height of each bunker. They compared these temperatures with the temperature in the middle of the face at 400 mm depth, where temperature is relatively constant, similar to temperatures deeper in the

bunker. The 200 mm temperature at a specific location minus the temperature at 400 mm in the middle of the bunker was positively correlated with pH, yeast and mould counts. This suggests that temperature measurements at the farm could be used to rapidly estimate fungal counts and assess the aerobic stability of silage. This survey was done in northern Italy. It would be interesting to know if differences in silage temperature will be a good predictor of fungal counts in more severe climates.

Tabacco et al. (2011) surveyed 42 farm silos with maize silage, half of them treated with an *L. buchneri* inoculant. Aerobic stability was negatively related to yeast count, and it appeared that improved aerobic stability in the inoculated silages was due to the reduction in yeast count. Pearson correlation coefficients between yeast count and various chemical and management variables indicated the strongest relationship was a negative correlation with feed out rate (-0.579). Also highly correlated ($P < 0.01$) with yeast count were lactic acid (0.549), pH (-0.456), silo DM density (-0.451) and lactic-to-acetic acid ratio (0.437). Acetic acid was correlated at $P < 0.05$ (-0.331). These results confirm that in the real world susceptibility to aerobic losses is a function of both the fermentation in the silo and the management factors (density and feed out rate) that influence the exposure of the silage to oxygen prior to removal from the silo.

Perhaps one of the more puzzling observations in the real world has been the appearance of clostridia and butyric acid in or near spoiled layers in silos. Given that clostridia are anaerobic bacteria, such observations seem to contradict logic. A recent study by Tabacco et al. (2009) adds some new information to help explain what may be happening. Maize and sorghum silages were made with (*L. plantarum* or *L. buchneri*) and without inoculant in 30 l silos. After 90 d ensiling, the silages were tested for aerobic stability and analysed for chemical and microbial changes after 0, 5, 7, 9 and 14 d aerobic exposure. In the maize silage, heating began after approximately 2 d in the untreated and *L. plantarum* treatments. At 5 d, the pH was above 5.0 in these two treatments, but clostridial spore counts were approximately 2 $\log_{10}$ cfu/g silage. At 7 d, the pH was above 6.5, temperatures were more than 20°C above ambient, and clostridial counts had risen to >6 $\log_{10}$ cfu/g silage. Coincidentally nitrates in those treatments, which were at approximately 1000 mg/kg herbage at opening, had fallen linearly to undetectable levels by day 7. Nitrate content is indirectly related to inhibition of clostridia when reduced in anaerobic environments to nitrite (Pahlow et al. 2003). These results suggest that a combination of factors together are allowing clostridia to grow near spoiling layers: an increase in pH and temperature due to aerobic microorganisms, a return to anaerobic conditions due to spoilage microorganisms exhausting the oxygen supply closer oxygen source, and the utilization of nitrates that would normally inhibit clostridial growth under anaerobic conditions. This loss of nitrate suggests that enterobacteria (or possibly some LAB) may be proliferating prior to the clostridia because these bacteria are associated with nitrate reduction early in ensiling.

Inoculants

Microbial inoculants have become the dominant silage additive type in most parts of the world and have been available in many countries for decades. Until recently, most of these products were strains of facultative heterofermentative LAB (commonly called homofermenters) such as *L. plantarum*, *L. casei*, various *Pediococcus* species and *Enterococcus faecium*. The goal was to have a rapid and efficient fermentation that produced mostly lactic acid, minimizing DM losses and attempting to keep nutritive value similar to that of the crop at ensiling. The best of these products have not only enhanced silage fermentation and DM recovery but also improved animal performance: milk production, gain, feed efficiency (Weinberg and Muck, 1996). However, these products have had a negative effect on aerobic stability in whole-crop maize and small grain silages, presumably because of the reduction in acetic acid.

In the late 1990's, a new class of inoculants, based on obligate heterofermenters such as *L. buchneri*, entered the market. These strains grow slowly even after the active fermentation period is finished, producing acetic acid from sugars or lactic acid. The primary goal is to increase acetic acid so that yeast and mould growth is inhibited and aerobic stability is improved. However, these products appear to have limited effects on animal performance other than by keeping silage cool. Today we have a third class of inoculants that combine *L. buchneri* with more traditional strains attempting to get the DM recovery and animal performance of the facultative heterofermentative strains along with the aerobic stability improvements provided by *L. buchneri*.

There are many papers that report on the testing of various inoculants across a wide variety of forages. To review all of those studies properly is a manuscript by itself. Certainly this work is important in allowing scientists in various parts of the world to provide good recommendations to producers. However, from a more broad scientific perspective it is more interesting to understand why these products work and to look at current innovative efforts to find a new crop of inoculants.

How inoculants dominate silage fermentation

Recent studies are helping us understand why inoculants are often successful in the silo and how they may alter silage quality in a way that affects livestock response. The facultative heterofermentative LAB inoculant strains have been selected for rapid, homofermentative growth under a wide range of temperatures and DM concentrations. We expect that these strains will be highly competitive and produce largely lactic acid, reducing pH compared to an untreated silage with its mixture of obligate and facultative heterofermenters. However, there are a substantial number of incidences (e.g., Muck 1989) where the inoculant was applied at less than 10% of the epiphytic LAB population and still affected silage fermentation. Such instances suggest that at least some inoculant strains are not just faster but also have other competitive advantages over their fellow LAB as well as other epiphytic bacteria.

Some inoculant LAB strains produce anti-microbial compounds. Gollop et al. (2005) investigated whether 10 inoculants/commercial strains produced antibacterial activity. Nine of 10 inoculants when grown on MRS broth did produce compounds in the broth that inhibited the growth of *Micrococcus luteus*, a bacterial species susceptible to bacteriocins and other antibacterial compounds. Extracts of inoculated silages were also tested for inhibition of *M. luteus*. In 15 of 27 cases, the silage extracts from crop inoculated with one of the nine positive strains showed inhibition of *M. luteus* whereas none of the silage extracts from untreated control or the negative strain inhibited *M. luteus* (0 of 6 cases). Others (e.g., Marcinakova et al. 2005, Ratanapibulsawat et al. 2005) have isolated LAB strains from silage that produce inhibitory activity against a variety of bacterial species: *Staphylococcus aureus*, *Salmonella* sp., *Bacillus* sp., *Listeria* sp. and *Escherichia coli*.

Vazquez et al. (2005) studied the effects of bacteriocins from 6 strains of LAB (*L. brevis*, *L. casei*, *L. helveticus*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *P. acidilactici*) on the growth of those strains. The bacteriocin from a particular strain generally promoted the growth of that strain when added to the culture as well as increased bacteriocin production. When that bacteriocin was added to cultures of the other LAB strains, one would expect reduced growth. That was true particularly with the bacteriocins from *L. brevis* and *Lactococcus lactis*. However, the *L. casei* and *L. helveticus* bacteriocins enhanced growth in the other five strains. Similarly bacteriocin production in one strain was most often reduced, but in some cases increased, by the presence of bacteriocin from another strain. The largest increase in bacteriocin production across species (50%) was in *L. casei* when bacteriocins from *P. acidilactici* were added.

There is also evidence that some LAB produce antifungal compounds. Broberg et al. (2007) inoculated grass silage with two *L. plantarum* strains, one isolated from silage, that produce antifungal compounds in MRS broth. The antifungal compounds identified in laboratory culture, 3-phenyllactic acid and 3-hydroxydecanoic acid, were found at higher concentrations in the inoculated silages compared with those in the untreated silage. Other antifungal compounds, largely acids associated with lignin synthesis, were also elevated in the inoculated silages. It was not known what caused the increased concentrations of these compounds. Recently, Prema et al. (2010) also isolated a *L. plantarum* strain from grass silage that produced 3-phenyllactic acid and demonstrated that the acid inhibited a wide range of mould species common to silage.

These studies show that some LAB strains are capable of inhibiting a considerable spectrum of bacteria and fungi. The results of Gollop et al. (2005) indicate that it is relatively common to find antibacterial activity in inoculant strains. However, it is likely that much more could be done to select inoculant strains that are not only capable of dominating silage fermentation but also inhibiting undesirable anaerobic and aerobic microorganisms and so potentially reducing losses in quality and DM beyond that attained from an efficient fermentation.

How inoculants alter animal performance

The effects of inoculants on gain or milk production in livestock have been greater than expected (Weinberg and Muck, 1996). In fact, there are a significant number of reported cases where animal performance has been increased even though there was either no or only minor changes in pH or silage fermentation products. This is certainly intriguing. However, beyond scientific curiosity, improvements in animal performance provide a bigger return to the farmer than improvements in DM recovery. So there is incentive both scientifically and in helping farmers choose effective inoculants to understand how LAB silage inoculants affect livestock.

In some cases, there is an apparent linkage between changes in silage quality and animal performance. For example, Ando et al. (2006) found that guinea grass silage treated with *L. rhamnosus* had higher DM and organic matter digestibility and higher voluntary intake in wethers than untreated silage. When digestibility is improved, livestock should eat more if intake of the diet is limited by rumen fill. But we are still left wondering why an inoculant that consumes soluble portions of the crop should affect DM digestibility, which is primarily a function of the digestibility of insoluble structural polysaccharides.

In the past 10 years, we have begun to get important clues as to what may be happening at

least with some inoculants. These clues point to changes occurring in the rumen of ruminant livestock. Weinberg et al. (2003) found that inoculant LAB could survive in rumen fluid, and some of the strains appeared to buffer pH, keeping it from dropping as much as pH in unamended rumen fluid. Given that cellulolytic activity decreases at low rumen pH, perhaps this may be a key to improved digestibility. Following up on these results, Muck et al. (2007) made silages using a wide range of inoculants. In vitro analysis was performed in serum bottles, measuring gas pressure. Surprisingly, some of the inoculated silages had reduced gas production compared with the untreated silages. Because digestibility has not been depressed by inoculants, these results suggested that in vitro fermentation was being shifted from gas production to another product – volatile fatty acids or rumen microorganisms.

Recent research has indicated that in vitro fermentation is altered by some inoculant strains. Cao et al. (2010) investigated the effect of a *L. plantarum* strain on an ensiled total mixed ration (TMR) based on whole crop rice. In vitro analysis of the inoculated TMR silage showed reduced methane production (P=0.065) at 6 h of incubation compared with that of untreated TMR silage. Dry matter digestibility was not affected nor was the production of volatile fatty acids with the exception of butyrate being higher in the in vitro fermentation of the untreated silage. Cao et al. (2011) found similar results with the same inoculant strain in vegetable residue silage with the inoculated silage having the highest in vitro DM digestibility and lowest methane production. Contreras-Govea et al. (2011) performed in vitro analysis of maize and lucerne silages inoculated without or with one of four inoculants. While the inoculants produced only minor changes in silage fermentation, in vitro results were affected by treatment. At 9 h incubation, three of the four inoculated silages produced more microbial biomass yield as estimated by true minus apparent digestibility as compared with the untreated silages. At 48 h, two of the inoculated silages had higher microbial biomass yield than the untreated silages. At both times, gas and volatile fatty acid production were not affected by treatment, and there were no inoculant by crop interactions. These results together suggest that some, but not all, inoculants are altering in vitro ruminal fermentation, whether by reduced methane production or increased microbial biomass production, in ways that should lead to increased animal performance.

One of the inoculants that increased microbial biomass yield in the Contreras-Govea et al. (2011) study was one with considerable published animal data (*L. plantarum* MTD/1), showing positive effects even in some cases where silage fermentation was not affected (Weinberg and Muck, 1996). An animal trial has been performed to investigate whether this inoculant can improve rumen microbial biomass production (Muck et al., 2011). Milk production on inoculated lucerne silage was increased compared to the untreated silage. This was accompanied by a significant reduction in milk urea nitrogen that suggests better nitrogen utilisation and most likely more rumen microbial protein production. However, we are awaiting the results of the omasal samples to confirm that. Using ARISA to look at the rumen microbial community, we did not observe significant differences due to treatment, but real-time PCR did find elevated levels of *L. plantarum* in the rumens of cows on the treated silage (Mohamed et al. 2012).

Certainly there is considerably more research to be done in this area. Even if in the case of *L. plantarum* MTD/1 we can confirm that there is improved rumen microbial protein production that in turn explains increases in milk production, we still do not understand why that may be happening. Fortunately, it appears that in vitro analyses and our new PCR-based tools may be helpful in uncovering the secrets of how inoculation of silages by particular LAB strains affects silage utilisation by ruminants.

Strides to find new inoculants

Most of the published efforts to develop new inoculant strains have been in Asia, South America and Africa. This might be expected. The major international companies producing inoculants are based in Europe and North America. So more of the inoculant research from these parts of the world is involved in testing commercial products. In addition, these products have been developed for cool-season grasses, whole-crop maize and lucerne, the dominant crops ensiled on those continents. These inoculants may or may not be as effective when used on warm-season grasses and tropical legumes.

One of the most complete, recently published screening procedures focused on identifying potential homofermentative strains with antimicrobial properties for ensiling cool-season grasses in Finland (Saarisalo et al. 2007). They began by selecting LAB strains from various sources, not just silages, based on antimicrobial activity against a wide range of microorganisms (coliforms, clostridia, *Bacillus* spp., *Listeria* spp., yeasts and moulds). Second, they grew the candidate strains on a grass extract medium, measuring fermentation products, growth rate, ammonia N and pH. They also grew the strains on an API 50 CHL test kit to determine the range of substrates each strain could ferment. They selected four strains that grew rapidly on a wide range of sugars, producing high levels of lactic acid, low pH and low ammonia N. These four strains were then tested in a mini-silo trial with a timothy-meadow fescue mixture and with silos being opened at 1, 3, 5, 7, 14, 21, 63 and 84 d. Fermentation characteristics, gas production, and microbiological changes were measured. The ensiling trial confirmed the results of

the grass extract screening. While they did not proceed further, additional winnowing of strains could be carried out, investigating the range of DM concentrations and temperatures under which the candidate strains could grow.

The approach that one takes in selecting strains depends upon the goals. In the Finnish research, the goal was to find a strain that could actively suppress non-LAB microbial species while producing an efficient fermentation largely of lactic acid and little breakdown of amino acids to ammonia. Similarly, Marcinakova et al. (2008) studied an *E. faecium* isolate that produces bacteriocins as a potential inoculant. More commonly the goal is to find species that rapidly and efficiently ferment sugars to lactic acid with little or no ammonia production. Recent such research includes for example: Penteado et al. (2007) in *Panicum maximum* silage, Kim et al. (2008) in whole-crop rice silage, Kim et al. (2009) in whole-crop barley silage, Yan et al. (2011) in maize silage.

Other goals depend upon the particular issues with a crop or its use. With sugar cane silage, the two major concerns are high ethanol concentrations and aerobic instability. Ávila et al. (2009, 2010) identified an *L. buchneri* strain that was better than commercial strains in reducing ethanol concentration and yeast counts while improving aerobic stability. In guinea grass silage, the targets for Pasebani et al. (2011) beyond low pH and ammonia were high crude protein and low fibre concentrations. Today, there can be alternate uses for silage as seen in the utilisation of silage to produce methane via anaerobic digestion. Banemann et al. (2010) investigated the potential of an inoculant to increase methane yield from maize silage. Consequently the selection goal will change the strain that will be most effective in the preservation or utilisation of a silage.

The most difficult target today is improvement in animal performance. Animal trials are expensive and time-consuming. This means that one has to narrow the field of candidates to two or three strains without having an effective measure to know how livestock will respond. Hopefully as we understand how inoculants affect animal performance we will be able to develop in vitro techniques that will allow us to more easily find strains that will be beneficial to livestock.

Conclusions and future directions

Over the past decade we have seen a marked increase in the use of PCR-based techniques in silage research. These techniques are greatly enhancing our ability to detect and monitor the species involved in ensiling. Often, common species like *L. plantarum* have been found across diverse crops and different continents, making it appear that perhaps we do not need to spend more time looking at the species in silages. In other cases such as with maize in warm climates, it appears that obligate heterofermenters such as *Weissella* and *Leuconostoc* species may play more significant roles than in temperate climates. This may or may not be significant to the utilisation of maize silage by livestock or the value of an inoculant. At this time, we do not know.

More studies of the microbial ecology of ensiling using these new PCR-based techniques are needed. Currently we have a limited number of snapshots of microbial dynamics over a wide variety of crops and locations. In some studies, not enough snapshots have been taken in the first week of ensiling to capture the dynamics when the major changes in fermentation products and pH are occurring. So we may be missing species that are keys to silage quality. We need to have more pictures of major silage crops in various locations so that we can build a global picture of whether those populations vary substantially by location or climate or not, and thus what inoculant characteristics may be most important.

The studies so far have primarily focused on ensiling under good conditions and on the spoilage of silages after silo opening. What would be of particular value is to better understand the microbial ecology of silage fermentations that go wrong. For example in the U.S., farmers occasionally get high acetic acid silages that have reduced intake, but other high acetic acid silages like those inoculated with *L. buchneri* are consumed at a level expected based on standard nutritive characteristics. The difference is likely the microbial species that produced the acetic acid, and the factor affecting intake is probably not acetic acid but some other product not measured. As we better understand the species that are causing such problems, we will be able to devise management strategies or additives to prevent those problems.

In concert with our ability to identify species in silage, we need to strive to do more than analyse for the major fermentation products. Some of the recent inoculant research discussed above has found various antimicrobial compounds at low levels, suggesting there are many minor compounds that may influence the course of fermentation in the silo and possibly rumen fermentation in cattle and other ruminants. There are concerns about volatile organic compounds coming from silages and their effect on the environment, and we need to understand whether those compounds are directly caused by microorganisms or indirectly by chemical interactions during storage. Metabolomics is just entering agricultural research (Ametaj et al. 2010) allowing us to identify many more minor compounds. It along with the PCR-based techniques may be keys to bringing us to a new level of understanding of what

occurs in the silo and how these processes affect livestock and the environment. Armed with this new understanding, we will hopefully be able to improve the quality of the silage that we deliver to our livestock.

Acknowledgements

The author wishes to express his appreciation to F.E. Contreras-Govea and D.M. Stevenson in reviewing the manuscript.

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Silage and the safety and quality of dairy foods: a review

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Keywords: bacterial spores, milk quality, mycotoxins, pathogens, silage quality

Introduction

Food producers are responsible for the safety and quality of their products for consumers (European Commission 2000). Quality assurance of food products requires an integrated approach that assures safety and quality at all stages of the production chain. The safety and quality of milk and dairy products depend on the quality of raw milk produced at dairy farms, the quality of any other ingredients, processing conditions and distribution and storage conditions. As suppliers of raw milk, dairy farms have an important role in the dairy production chain. Therefore, milk production at dairy farms needs to meet the demands and criteria with respect to animal health, feed quality and milking hygiene. The objective of dairy farm quality assurance is to prevent contamination of raw milk by residues of veterinary medicines and agricultural chemicals, environmental contaminants from for instance feed or soil and by harmful micro-organisms arising from feed, the housing system or the animals themselves. Feed is an important source of chemical and microbiological contaminants of milk. The diet of high-yielding dairy cattle consists of two main classes of feedstuffs: forages and concentrates. Fresh, dried or ensiled forages generally constitute the largest fraction of the diet, usually 50 to 75%. Forage preserved as silage is the most popular form of forage in many countries. For example, grass and maize silage represented on average 67% of dry matter dietary intake of Dutch dairy cows in autumn and winter (Driehuis et al. 2008b).

This paper summarizes the present scientific knowledge about silage as a source of microbiological and chemical contaminants in the dairy chain. The paper focuses on three groups of safety or quality hazards of milk and dairy products: (1) spores of endospore-forming bacteria, such as *Clostridium* and *Bacillus* species, (2) the zoonotic pathogenic bacteria *Listeria monocytogenes* and *Escherichia coli*, and (3) silage-associated mycotoxins.

Spores of endospore-forming bacteria in silage: contamination pathway of raw milk

Endospore-forming bacteria are an important group of contaminants of raw milk because of the resistance of the spores to heat and other adverse environmental conditions. Spores of many species survive pasteurization of milk and some even survive sterilization conditions. Important sources of bacterial spores are soil, silage and bedding materials. The main contamination pathway of spores from these sources to milk is shown in Figure 1. The original source of spores occurring in silage is often soil. Contamination of a crop by soil occurs during growth in the field or during harvesting. In crops that are ensiled this soil contamination usually determines the initial spore concentration. Whether a spore population will increase in concentration during ensilage depends on the properties of the micro-organism and the conditions prevailing in the silage. This will be discussed later in this paper. Spores occurring in silage or other feeds that are consumed by a cow pass the gastrointestinal tract of the animal unaffected and are excreted with the faeces. There is evidence that spore concentrations increase during passage through the intestinal tract (Ali-Yrkkö and Antila1975, Vissers et al. 2007b), which can be explained by digestion of feed components. Vissers et al. (2007b) measured concentrations of spores of butyric acid bacteria in mixed grass and maize silage, faeces, bedding materials and raw milk from 24 Dutch dairy farms and observed that the concentration in faeces was on average about three times higher than the concentration in silage. Bedding materials that are used in barns where cows are housed usually become contaminated by excreted faeces. Under normal dairy farming practices, for instance when cows

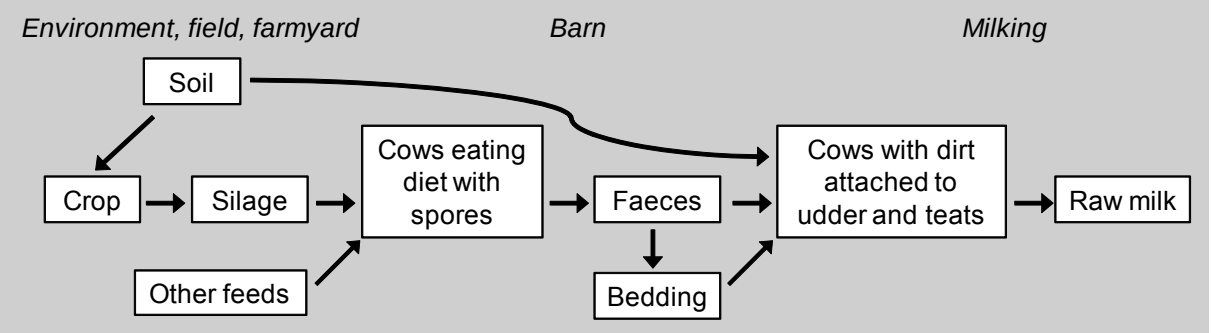

Figure 1. Contamination pathway of bacterial spores from silage and other feeds to raw milk.

are lying in the barn, it is inevitable that bedding and faeces attach to the surface of the cow's udder and teats.

Good dairy farming practice requires that teats are cleaned before milking (FAO and IDF 2011). However, since teat-cleaning methods are rather relatively inefficient from a microbiological perspective, a fraction of bacteria and spores from dirt and faecal matter remains attached to the teat surfaces and is rinsed off during milking operations. An evaluation of different manual teat-cleaning methods revealed that spore concentrations in milk were reduced by 45% to 96% when compared to milking without teat-cleaning (Magnusson et al. 2006). Some teat-cleaning methods involve treatment with solutions, foams or wetted towels containing disinfectants. These methods to some extent inactivate vegetative bacteria, but no evidence is currently available that spores are inactivated. Other theoretical contamination pathways, such as aerial contamination of raw milk by spores from silage and direct contamination of milk by silage are insignificant under normal production conditions (Vissers et al. 2006, Vissers 2007).

Vissers et al. (2006) developed a predictive model of the contamination of raw milk by spores of butyric acid bacteria from feed or other sources in the farm environment, for instance soil. The model was based on a mathematical translation of the contamination pathway described above and was used to evaluated the most important variables and to identify effective and non-effective strategies to control levels of spores of butyric acid bacteria in farm tank milk. It was concluded that the variation of the concentration of spores in silage is, by far, the most important variable, and significantly more important than, for instance, teat-cleaning efficiency and barn hygiene (see also Vissers 2007).

Spores of endospore-forming bacteria in silage: *Clostridium* species

Clostridium species occurring in silage have been summarized by Pahlow et al. (2003). These authors divided the most common species in three groups, based on their protein and carbohydrate fermentation properties (Table 1). The first group consists of so-called proteolytic clostridia, of which *Clostridium sporogenes* is the predominant species in silage. Species of this group derive their energy from fermentation of both proteins and carbohydrates. The second group was named the *Clostridium butyricum* group. Species of this group typically ferment a wide range of carbohydrates but are unable to ferment proteins. Klijn et al. (1995) showed that many strains originating from silage, farm environment and milk and originally identified as *C. butyricum* on the basis of phenotypic characteristics, genetically belonged to the species *Clostridium beijerinckii*). The third 'group' is formed by *Clostridium tyrobutyricum*, which ferments a limited number of carbohydrates but in addition has the ability to ferment lactic acid to acetic and butyric acid at low pH. This type of fermentation is known as butyric acid fermentation and the bacteria conducting it are referred to as butyric acid bacteria. In addition to the above-mentioned species, Rossi and Dellaglio (2007) detected *Clostridium saccharolyticum* and *Clostridium baratii* in silages with high counts of clostridial spores. Using a cultivation-independent DNA-based method (PCR-denaturing gradient gel electrophoresis; DGGE), Julien et al. (2008) identified *Clostridium disporicum* as another predominant member of clostridial populations in silage.

For two reasons *C. tyrobutyricum* is the species most studied in relation to silage quality. Firstly, because *C. tyrobutyricum* due to its tolerance to low pH condition in combination with its ability to use lactic acid as a substrate for growth is the main cause of butyric acid fermentation in silage. This can have large negative effects on the preservation quality, nutritive value and palatability of silage. The second reason is that spores of *C. tyrobutyricum* occurring in silage and transferred to milk can lead to a defect called

Table 1. The predominant *Clostridium* species occurring in silage and of their characteristics. Adapted from Pahlow et al. 2003. Based on data from Ali-Yrkkö and Antila 1978, Bühler 1985, Klijn et al. 1995, Rossi and Dellaglio 2007, Julien et al. 2008.

Characteristic	Proteolytic group	*C. butyricum* group	*C. tyrobutyricum*
Species	*C. sporogenes* *C. bifermentans* *C. baratii*	*C. butyricum* *C. beijerinckii* *C. acetobutyricum* *C. saccharolyticum* *C. disporicum*	*C. tyrobutyricum*
Minimum pH allowing growth	>5	>4.5	>4.2
Substrates fermented:			
Proteins	+	-	-
Carbohydrates	+	+	+
Monosaccharides	variable	many	few
Lactate	weak	-	+

late-blowing in semi-hard and hard cheese types, such as Gouda, Emmental and Gruyère. Late-blowing is caused by butyric acid fermentation taking place during cheese ripening and resulting in off-flavours and excessive gas formation leading to texture defects. Late-blowing may cause significant loss of product. Interestingly, the factors that are important for growth of *C. tyrobutyricum* in silage and cheese are the same: low pH, water activity, use of lactic acid as a substrate and the concentration of nitrate. Both in silage and cheese the concentration of nitrate is a determinative factor in the germination of spores and outgrowth of *C. tyrobutyricum*. Studies by Klijn et al. (1995) showed that late-blowing in Gouda cheese is exclusively associated with growth of *C. tyrobutyricum*. Cheese made from milk to which spores of other silage-associated *Clostridium* species, such as *C. beijerinckii* and *C. sporogenes*, was added showed no signs of late-blowing. Moreover, in all experimental and commercial cheeses showing obvious signs of late-blowing, growth of *C. tyrobutyricum* was detected. Since *C. tyrobutyricum* is unharmful to man and animals, its occurrence in silage and cheese is only of economical importance.

The pathogen *Clostridium botulinum*, the causative agent of botulism, is rarely found in silage. Botulism is caused by highly potent neurotoxins produced by *C. botulinum* (botulinum toxins). Occurrence of *C. botulinum* and botulinum toxins in silage can be associated with the presence of carcasses of birds or small mammals, for instance due to killing of the animals during harvesting of the crop (Cobb et al. 2002). Poultry manure is a notorious source of spores of *C. botulinum*. Silage crops may become contaminated with *C. botulinum* spores when contaminated poultry manure is used as a fertilizer (Livesey et al. 2004). However, under normal ensiling conditions, *C. botulinum* does not develop in silage. Occurrence of *C. botulinum* in silage and its relevance in cattle have been reviewed previously (Kehler and Scholz 1996, Lindström et al. 2010). Foodborne botulism occurs when foods are consumed in which botulinum toxins have been formed. Foods associated with foodborne botulism include canned vegetables and low acid (pH > 4.6) foods (in particular home-canned foods), sausages, meat products and seafood products (Sobel et al. 2004). Because of their occasional occurrence in silage, transfer of *C. botulinum* spores to raw milk cannot be excluded. However, taking into consideration the processing conditions, product properties and storage conditions of milk and dairy products, germination and outgrowth and toxin production by *C. botulinum* in dairy products is an unlikely event. Up to now, dairy products have not been associated with outbreaks of foodborne botulism (Shapiro et al. 1998, Sobel et al. 2004).

The concentration of spores of butyric acid bacteria are traditionally determined by most probable number (MPN) methods, using (1) a medium containing lactic acid and incubation conditions that are selective for anaerobic, gas-forming bacteria, and (2) pasteurization of sample dilutions before inoculation of the medium to inactivate vegetative bacteria (Bergère and Sivelä 1990). These methods are useful for enumeration of *C. tyrobutyricum* spores, but they are not specific for this species. Other species, for instance *C. beijerinckii*, are sometimes detected as well. Detection of butyric acid bacteria spores in raw milk is part of the milk quality systems of a number of dairy companies, for instance Dutch dairy companies. Apart from not exclusively detecting *C. tyrobutyricum*, MPN methods for enumeration of butyric acid bacteria spores have several other disadvantages: the analysis time is long (4 to 7 days) and the results have a high uncertainty (which is inherent in most MPN procedures). Alternative methods for detection of *C. tyrobutyricum* are available, for instance methods based on qPCR (Herman et al. 1995, Lopez-Enriquez et al. 2007) or immunological techniques (Nedellec et al. 1992, Lavilla et al. 2010). However, these methods are currently not used for routine analyses in the dairy sector, presumably because they are relatively laborious and costly.

Concentrations of butyric acid bacteria spores in silage varies from 10 to 100 spores/g fresh matter, which equals to initial contamination level of fresh crops arising from soil contamination at harvest, to 10^6 to 10^7 spores/g in silages with extensive butyric acid fermentation (Stadhouders and Spoelstra 1990, Pahlow et al. 2003, Vissers et al. 2007b). Information about concentrations of butyric acid bacteria spores in farm-scale silages is rather scarce. Studies in France conducted in the 1970s showed that about 20% of grass silages contained more than 10^5 butyric acid bacteria spores/g, a level that can be described as 'poor quality' (ITEG/ITB 1980). In a survey conducted in the Netherlands in 1982, 44% of grass silages exceded the level of 10^5 butyric acid bacteria spores/g and the average concentration was 4.9 $\log_{10}$/g (Spoelstra 1984, 1990). More recent data from the Netherlands show that the quality of grass silage with regard to butyric acid bacteria spores has significantly improved since the 1980s: in 5% of grass silages produced between 2002 and 2004 the concentration of butyric acid bacteria spores exceeded 10^5/g and the average concentration was 3.2 $\log_{10}$/g (Table 2). These results were based on samples taken from unopened silages sample and, on average, eleven weeks after ensiling. The improvement of silage quality with regard to butyric acid bacteria spores in the Netherlands since the 1980s is in agreement with information from Dutch dairy companies, who experienced a decreasing trend in the level of butyric acid bacteria spores in raw milk delivered by Dutch farmers between 1980 and 2000. The survey conducted in the Netherlands between 2002 and 2004 also included samples from unopened maize silages. The average concentration of butyric acid bacteria spores in maize silage were approximately 0.5 $\log_{10}$ unit lower than in grass silage and maize silages with a high level of butyric acid bacteria spores almost absent: none of 197 tested maize silages exceeded 10^5 spores/g and only

0.5% exceeded 10^4 spores/g (Table 2). The findings were in accordance with the knowledge that, due to the low buffer capacity of the maize, lactic acid fermentation in maize silage is generally fast and the final pH low (pH 3.8 to 4.0), conditions that do not favour outgrowth of butyric acid bacteria.

Table 2. Concentration of butyric acid bacteria spores in unopened grass and maize silages and in mixed grass and maize silage offered to cows in the barn of commercial dairy farms in the Netherlands (Vissers et al. 2007b, Driehuis, unpublished results). The data were collected between 2002 and 2005.

Sample type	Number of samples	Average concentration (log_{10} spores/g)	Percentage of samples containing (spores/g):			
			$<10^3$	10^3-10^4	10^4-10^5	$>10^5$
Grass silage, unopened	460	3.2	48%	32%	15%	5%
Maize silage, unopened	197	2.7	79%	21%	0.5%	0%
Mixed silage in barn[a]	122	4.2	17%	24%	41%	18%

[a] Mixed grass and maize silage offered to dairy cows in the barn

Until recently, the generally accepted view was that high concentrations of butyric acid bacteria spores are associated with anaerobic instability of silage due to insufficient pH decline during the primary fermentation phase and that growth of clostridia in silage depends on the initial crop composition with respect to contents of dry matter, water soluble carbohydrates and nitrate and buffer capacity (McDonald et al. 1991, Kaiser et al. 2002, Pahlow et al. 2003). A different view on the issue of butyric acid bacteria spores in silages came from a study by Vissers et al. (2007a), who showed that on Dutch dairy farms increased concentrations of butyric acid bacteria spores were often related to aerobic instability problems rather than to anaerobic instability problems. In this study, samples were taken at 21 commercial dairy farms from various locations in clamp silos of grass and maize silage and from mixed grass and maize silage that was offered to dairy cows in the barn. It was found that the samples of mixed silage in the barn had an average concentration of butyric acid bacteria spores that was more than 10-fold higher than samples taken from the core of the grass and maize silage clamps, which represented the major fraction of the silage in offered to cows. In addition, it was found that a high percentage of samples of mixed silage (18%) contained butyric acid bacteria spores in a concentration exceeding 10^5 spores/g (Table 2). The study showed that the total quantity of butyric acid bacteria spores consumed by cows was determined by a small fraction of silage with a high concentration of above 10^5 spores/g. Further analysis of the silages that were used at the farms revealed that high spore concentrations were detected particularly in samples from areas showing signs of aerobic deterioration, i.e. areas with a high concentration of yeasts and moulds and increased temperature and pH. High concentrations of butyric acid bacteria spores were found most often in surface layers and in particular in areas with visible moulds (up to 10^7 spores/g). Unexpectedly, high concentrations of butyric acid bacteria spores were detected more often in maize silage than in grass silage (Table 3). The data showed that at the surveyed farms, which were representative for dairy farming in the Netherlands, maize silage contributed more to the total intake of butyric acid bacteria spores by dairy cows than grass silage. The results by Vissers et al. (2007a) confirmed earlier observations by Jonsson (1989, 1991), who showed that *C. tyrobutyricum* has the ability to grow and produce spores in silage exposed to air. Also recent studies from Italy indicated that high clostridia spores levels in maize silage are associated with air penetration and aerobic deterioration processes (Borreani and Tabacco 2008, 2010).

The growth of strictly anaerobic *C. tyrobutyricum* in aerobically deteriorated areas of silage may seem contradictory. However, microbial ecosystems with aerobic and anaerobic zones are found in many environments, for example in sediments and intestines (Brune et al. 1995, Fourcans et al. 2004). The occurrence of anaerobic niches in aerobically deteriorating silage was postulated for the first time by Jonsson (1989). The occurrence of these niches may be explained as follows. Aerobic deterioration in silage areas that are exposed to air is usually initiated by growth of acid-tolerant, lactate-assimilating yeasts that oxidize residual sugars and organic acids, leading to an increase in pH. Since the concentration of oxidizing yeasts is relatively low during the early phases of aerobic deterioration, the consumption rate of oxygen is low too and oxygen penetrates relatively deep into the silage. But, as the concentration of the yeasts increases, the consumption rate of oxygen increases too. As a result, oxygen penetrates less deeply into the silage and deeper parts of the silage return to anaerobic conditions (Muck and Pitt, 1994). Consequently, anaerobic niches with an increased pH may develop close to air-exposed areas. Due to the increased pH in these niches, growth of *C. tyrobutyricum* and possibly other clostridia is no longer inhibited.

Table 3. Concentration of butyric acid bacteria spores in samples from the core, surface layer and areas with visibles moulds of opened grass and maize silages that were used for feeding to cattle at 22 commercial dairy farms in the Netherlands. Data from Vissers et al. (2007a) and Vissers and Driehuis, unpublished results.

Sample type	Number of samples	Average concentration (log_{10} spores/g)	Percentage of samples containing (spores/g):			
			$<10^3$	10^3-10^4	10^4-10^5	$>10^5$
Grass silage						
Core	22	3.0	50%	41%	9%	0%
Surface layer	22	3.1	64%	23%	9%	5%
Area with visible moulds	14	3.9	21%	29%	29%	21%
Maize silage						
Core	21	3.0	62%	19%	14%	5%
Surface layer	21	3.6	43%	24%	14%	19%
Area with visible moulds	15	5.5	7%	13%	13%	67%

Spores of endospore-forming bacteria in silage: *Bacillus cereus* and other aerobic spore-forming bacteria

Spores of aerobic spore-forming bacteria are ubiquitous and can be isolated from a wide variety of sources in the dairy farm environment, including soil, silage, concentrate feeds, bedding and faeces. Contamination of raw milk by spores from these sources occurs during milking via contaminated udders and teats, as described earlier in this paper. After the initial contamination the concentration of spores may increase further during storage of the milk at the farm, for instance when the storage temperature is not low enough and spores of psychrotrophic spore-forming bacteria germinate and start growing. Another possible route is contamination via insufficiently cleaned milking equipment. Certain spore-formers are known to be capable of forming biofilms attached to stainless steel and release high numbers of spores in surpassing milk.

Aerobic spore-formers with particular relevance for dairy products are *Bacillus cereus* and the highly heat-resistant spore-formers *Bacillus sporothermodurans* and *Geobacillus stearothermophilus*. *B. cereus* is a major spoilage organism of pasteurized milk and milk products stored at refrigeration temperature (Griffiths 1992, Te Giffel 1997, Heyndrickx and Scheldeman 2002). Psychrotrophic strains of *B. cereus* are capable of germination and growth in pasteurized milk and milk products at temperatures as low as 5°C and high levels may cause off-flavours and curdling. The content of spores of psychrotrophic *B. cereus* often limits the shelf life of these products. *B. cereus* is also of concern with respect to food safety because it can produce different types of toxins and is a potential food poisoning agent (Stenfors Arnesen et al. 2008). Therefore, the organism is generally regarded as a pathogen. Dairy products are only sporadically involved in outbreaks of foodborne illness caused by *B. cereus*. *B. sporothermodurans* and *G. stearothermophilus* are thermophilic bacteria producing highly heat-resistant spores and can cause non-sterility problems in ultrahigh-temperature (UHT) processed or sterilized milk products (Huemer et al. 1998, Scheldeman et al. 2006). This paper focuses on the role silage has as a source spores of aerobic spore-formers in raw milk.

Aerobic spore-formers isolated from silage belong to the taxonomic families *Bacillaceae* and *Paenibacillaceae* (Table 4). Species frequently isolated include *B. cereus*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus pumilus*, *Bacillus sphaericus* and *Paenibacillus polymyxa*. Also populations of heat-resistant spore-formers isolated from silage show high diversity and includes *B. sporothermodurans*, the species associated with spoilage of UHT-products. The primary habitat of most aerobic spore-formers is soil. Concentrations of spores of aerobic spore-formers and spores of *B. cereus* in soil vary from 10^5 to 10^7 spores/g and 10^1 to 10^6 spores/g, respectively, depending on soil type, sampling site and season (Rammer et al. 1994, Slaghuis et al. 1997, Christiansson et al. 1999, Vissers et al. 2007c, Vissers and Driehuis, unpublished data). Levels of spores of aerobic spore-formers and spores of *B. cereus* on crops prior to ensiling depend on the amount of soil that contaminates the crop during growth in the field and during harvesting. It also depends on whether the soil or crop has been fertilized with cattle manure, since spore concentrations can be high in cattle faeces. Slaghuis et al. (1997) detected a concentration of spores of aerobic spore-formers in grass and maize prior to ensiling of 10^2 to 10^4 spores/g. Several studies, summarized by Pahlow et al. (2003), reported on concentrations of spores of aerobic spore-formers occurring in farm-scale silages. The reported spore concentrations vary considerably between the silos. For wilted grass silages these concentrations ranged from 10^3 to 10^8 spores/g, for whole crop maize silages from 10^2 to 10^9 spores/g and for sugar beet pulp and brewers' grain silages from 10^3 to

Table 4. Species of aerobic spore-forming bacteria isolated from silage. Data from Lindgren et al. 1985, Jonsson 1989, McDonald et al. 1991, De Silva et al. 1998, Inglis et al. 1999, Pettersson et al. 2000, Te Giffel et al. 2002, Driehuis et al. 2009.

	Species
Heat-resistance not specified	*Bacillus cereus*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus pumilus*, *Bacillus sphaericus*, *Bacillus firmus*, *Bacillus lentus*, *Bacillus circulans*, *Paenibacillus polymyxa*, *Paenibacillus validus*, *Paenibacillus pabuli*, *Brevibacillus chosinensis*
Highly heat-resistant species	*Bacillus cereus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus sporothermodurans*, *Bacillus oleronius*, *Bacillus siralis*, *Brevibacillus borstelenis*, *Aneurinibacillus* ssp.

10^7-10^8 spores/g. Concentrations in core samples of silages were generally on the lower side of these concentration ranges: 10^4 to 10^5 spores/g in grass silages and 10^3 to 10^4 spores/g in maize silages. These data are in line with the view that in well-fermented silage no germination of spores and outgrowth of vegetative bacteria occurs. Growth of aerobic spore-formers probably occurs during the later phases of aerobic deterioration, i.e. after aerobic deterioration has been initiated by yeasts or acetic acid bacteria (Pahlow et al. 2003). High levels of spores of aerobic spore-formers have been detected in the surface layers of grass and maize silage (Slaghuis et al. 1997, Driehuis et al. 2009). Table 5 summarizes results from studies in the Netherlands on concentrations of spores of aerobic spore-formers in unopened and opened farm-scale grass and maize silages and on the distribution of spores in opened silages. The highest levels were detected in surface layers and areas with visible moulds of opened silages and in mixed grass and maize silage offered to cows in the barn. These data confirm that high concentrations of spores of aerobic spore-formers relate to aerobic deterioration problems.

As described previously, spores of *B. cereus* occur in silages. However, this species probably does not increase in numbers to the extent that other aerobic spore-formers do. Vissers et al. (2007c) monitored *B. cereus* spore concentrations in different feeds, faeces, bedding, soil and raw milk at 24 commercial Dutch dairy farms and detected average concentrations of *B. cereus* spores in mixed silage offered to cows of 2.2 to 2.8 $\log_{10}$ spores/g and maximum concentrations of 4.0 $\log_{10}$ spores/g. Although feed in general and silages in particular were found to be important sources of *B. cereus* spores in raw milk, it was concluded that the concentrations detected in silage were not critical with respect to the quality and safety of dairy products. The authors indicated that soil and insufficiently cleaned milking equipment are more critical potential sources of *B. cereus* spores at dairy farms. In studies conducted in Sweden soil was identified as the major source of contamination of raw milk by *B. cereus* during grazing of cows, whereas used sawdust bedding material, in particular in free-stalls with deep sawdust beds, was a major source when cows were kept indoors (Christiansson et al. 1999, Magnusson et al. 2007).

Table 5. Average pH and concentrations of spores of aerobic spore-formers and yeasts and moulds of unopened and opened grass and maize silages and mixed grass and maize silage offered to cows in the barn of commercial dairy farms in the Netherlands (Driehuis et al. 2009). The data were collected between 2002 and 2005.

Sample type	Number of samples	Spores of aerobic spore-formers ($\log_{10}$ spores/g)	Yeasts & moulds ($\log_{10}$ cfu/g)	pH
Grass silage				
Unopened, core	460	4.8	3.8	4.8
Opened, core	22	5.1	3.3	4.8
Opened, surface	22	5,7	4.9	5.3
Opened, area with visible moulds	14	8.0	6.8	7.1
Maize silage				
Unopened, core	197	3.3	6.0	3.8
Opened, core	21	4.5	5.8	3.9
Opened, surface	21	5.1	7.0	4.2
Opened, area with visible moulds	15	7.7	7.8	6.6
Mixed silage in barn[a]	122	6.2	6.4	4.8

[a] Mixed grass and maize silage offered to dairy cows in the barn

Listeria monocytogenes

The facultatively anaerobic Gram-positive bacterium *Listeria monocytogenes* is an important food-borne pathogen because it is the causative agent of listeriosis. Due to the severity of this disease, the high mortality rate and the increasing incidence, *L. monocytogenes* is of great concern to public health (European Food Safety Authority 2011). The bacterium is widely distributed in the environment and has been isolated from a variety of sources, including soil, surface water and vegetative materials. It is occurring at low numbers in many raw and ready-to-eat foods. Consequently, humans are commonly exposed to low numbers of *L. monocytogenes* from various types of food. Generally, this is not considered a serious health hazard (Food and Drug Administration 2001). However, ingestion of food contaminated with high numbers of *L. monocytogenes* may result in disease, in particular in populations with an increased risk for listeriosis, such as immunocompromised patients, elderly and neonates. High numbers of *L. monocytogenes* in foods usually arise from growth during storage of contaminated food products that are suitable as a medium for growth of the bacterium. Foods particularly linked to *L. monocytogenes* contamination include raw and smoked fish, raw and cooked meat, and soft and semi-soft cheeses.

An important feature of *L. monocytogenes* is its psychrotolerance. The bacterium has the ability to grow at temperatures as low as 0°C, which implies that it is able to grow during refrigerated storage of foods. The bacterium also has considerable osmotolerance and acid tolerance, although it is unable to grow at pH lower than 4.4. Probably due to its high tolerance to stress conditions, *L. monocytogenes* is capable of survival for extended periods in environments in which it is unable to grow. However, *L. monocytogenes* is quite sensitive to heat. For instance, *L. monocytogenes* is effectively killed by pasteurization of milk used in the dairy industry. For that reason heat treatment is an effective processing tool in the control of the bacterium in foods. Contamination of processed food products by *L. monocytogenes* often results from recontamination during the manufacturing process or packaging and environments inside food processing plants have been recognized as important potential sources of *L. monocytogenes* (Wiedmann 2003).

Outbreaks and sporadic cases of listeriosis in cattle, sheep and goats have been associated with feeding of silage contaminated with *L. monocytogenes* (Fenlon, 1988, Ho et al. 2007). Different studies have shown a high diversity of *L. monocytogenes* strains in silages and in faeces shed by cows that were fed silage (Nightingale et al. 2004, Borucki et al. 2005). Not only animals with clinical signs of listeriosis shed *L. monocytogenes* in their faeces, but also asymptomatic animals from farms with a outbreak of listeriosis and healthy animals from farms without a record of listeriosis cases (Unnerstad et al. 2000, Nightingale et al. 2004, Vilar et al. 2007). Contamination of raw milk by *L. monocytogenes* has been linked to occurrence of high levels of *L. monocytogenes* in silage (Sanaa et al. 1993, Tasci et al. 2010). Transmission of *L. monocytogenes* to raw milk is most likely to take place via faeces and bedding contaminated by faeces, as described previously for bacterial spores.

The degree of anaerobiosis and the pH are important factors determining survival and growth of *Listeria* spp. in silage. *L. monocytogenes* added to grass at ensiling rapidly disappeared under strictly anaerobic conditions and at a pH lower than 4.4. However, at an oxygen tension of 0.5% (v/v) survival was prolonged, and growth was observed even at a pH as low as 4.2. Higher oxygen tensions strongly encouraged *L. monocytogenes* growth (Donald et al. 1995). High numbers of *L. monocytogenes* and other *Listeria* species have been detected in different types of silage. For instance, *L. monocytogenes* levels in excess of $1x10^6$ cfu/g were detected in surface layers of big bale grass silages that were visibly infested by moulds (Fenlon 1986). Different studies have shown that the incidence of *Listeria* species in silage increases with increasing pH (Ryser et al. 1997, Vilar et al. 2007, Tasci et al. 2010). For instance, Vilar et al. (2007) detected *Listeria* spp. in 30% of silage samples with a pH ≥4.5 and in 6% of samples with a pH <4.5. These data are in line with the view that the occurrence of *Listeria* species in silage is associated with aerobic deterioration problems. The relatively high pH values that are generally occurring in aerobically deteriorated areas in combination with presence of oxygen create conditions that favour growth of *Listeria*. Silages with a greater likelihood of aerobic surface spoilage, such as silage with low packing density, silage that is inadequately sealed and big bale silage (Fenlon et al. 1989), are more susceptible to contamination by *Listeria*.

In conclusion, *L. monocytogenes* has frequently been detected in silages and is associated with occurrence of aerobic spoilage. Its presence in silage has been linked to contamination of raw milk. However, because *L. monocytogenes* is effectively inactivated by pasteurization used in milk processing, the food processing plant environment appears to be the major source of finished product contamination.

Enterobacteriaceae and Escherichia coli

Several species of the facultatively anaerobic *Enterobacteriaceae* belong to the epiphytic microflora of most forage crops. *Erwinia herbicola* and *Rahnella aquitilis* often dominate the fresh crop, but after ensiling these species are rapidly superseded by other species, such as *Hafnia alvei*, *Escherichia coli* and *Serratia fonticola* (Heron et al. 1993). The most important species in this group from the viewpoint

of human health risks is *E. coli*. Most *E. coli* are harmless and are part of the normal intestinal microbiota of humans and many animals. However, some types of *E. coli* cause severe gastrointestinal diseases. Among the pathogenic *E. coli*, the group of Shiga toxin-producing *E. coli* (STEC), also called vero-cytotoxin-producing *E. coli* (VTEC) or enterohaemorrhagic *E. coli* (EHEC), is of serious public health concern. STEC may cause anything from uncomplicated diarrhoea to haemorrhagic colitis, which can progress into haemolytic uremic syndrome (HUS), a disease that is characterised by acute renal failure. Of this group strains with the serotype O157:H7 (*E. coli* O157:H7) are the most serious pathogens.

The gastrointestinal tract of healthy ruminants, including cattle, is recognised as the main natural reservoir of STEC, in particular for *E. coli* O157:H7. Major sources of *E. coli* O157:H7 and other STEC strains for human infection are (raw) meat products, faecally contaminated vegetables and drinking water, and direct contact with animals. In addition, raw milk and unpasteurized dairy products have been implicated in outbreaks caused by infection with *E. coli* O157:H7 (Hussein and Sakuma 2005). The presumed route of transmission to raw milk is faecal contamination during milking, as described previously for bacterial spores and *L. monocytogenes*. Fortunately, *E. coli* O157:H7, like other *E. coli*, is sensitive to heat and is effectively killed by pasteurization of milk used in the dairy industry. Therefore, as described previously for *L. monocytogenes,* heat treatment is an effective processing tool in the control of *E. coli* O157:H7 and other STEC strains in dairy products.

During the early stages of silage fermentation, *Enterobacteriaceae* compete with the lactic acid bacteria and other bacterial groups for nutrients. Most *Enterobacteriaceae* do not grow and loose viability at pH values lower than 4.5 to 5.0. A fast pH decline therefore decreases growth and survival of *Enterobacteriaceae* in silage (Heron et al. 1993). However, the presence of oxygen prolongs their survival in silage and some enterobacteria that survive the storage phase may start growing again and reach numbers in excess of 10^8 cfu/g when silage pH increases during aerobic deterioration (Lindgren et al. 1985, Donald et al. 1995). No studies are known to the author that have shown the presence of *E. coli* O157:H7 and other STEC strains in silage. In a number of studies the growth and survival of *E. coli* O157:H7 in grass, maize and barley silage, inoculated with this bacterium prior to ensiling, was investigated. These studies showed that *E. coli* O157:H7 does not survive in well-fermented silage with a fast pH decline and low pH (Byrne et al. 2002, Bach et al. 2002, Pedroso et al. 2010). The same result was achieved for STEC serotype O26 in maize silage (Duniere et al. 2011). However, other studies showed that *E. coli* O157:H7 potentially can survive and grow in poorly fermented silage and in aerobically deteriorated silage (Fenlon and Wilson 2000, Pedroso et al. 2010).

In conclusion, *E. coli* O157:H7 and other STEC strains do not survive normal ensiling conditions and no data showing occurrence of these bacteria in silages are currently available. However, the bacteria may survive in poor quality silage, particularly in aerobically spoiled material. Pasteurization of raw milk effectively inactivates *E. coli*.

Mycotoxins

This chapter summarizes scientific knowledge about the major mycotoxins occurring in silages, under which conditions they are formed and how they can be prevented. Mycotoxins in silage are of dual concern. Firstly, they can have adverse effects on animal health and cause production losses. Secondly, they may jeopardize the safety of food products of animal origin. Of the major mycotoxins in silage crops, the second concern holds true for aflatoxin only, as is described later in this paper. The metabolism of mycotoxins in ruminants and their carry-over into milk are briefly described. Toxic effects of mycotoxins in animals and man, analytical methods for detection of mycotoxins and legislative aspects are not described in this paper. Information about these topics can be found elsewhere (Council for Agricultural Science and Technology 2003, Krska et al. 2008, Driehuis et al. 2010).

Mycotoxins are a large, diverse group of naturally occurring toxic metabolites of fungi. Currently, more than 300 mycotoxins have been identified. Mycotoxins can be found in a wide variety of crops all around the world, including crops that are commonly fed as silage, such as maize, wheat and grasses (Council for Agricultural Science and Technology 2003, Driehuis et al. 2010). Moulds and mycotoxins that are of relevance for silage produced from these crops are listed in Table 6. A distinction is made between mycotoxins that are formed before ensiling and those that are formed after ensiling. 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. Mycotoxins that are formed before ensiling are associated with moulds that infect a crop during its growth in the field or by endophytic moulds that live as symbionts in for instance grasses or cereals (field-derived mycotoxins). Field-derived mycotoxins include trichothecenes, zearalenone, fumonisins, aflatoxins and alkaloid mycotoxins. Mycotoxins that are formed after ensiling are associated with moulds that develop in silage during storage or feeding-out (ensilage-derived mycotoxins), usually as a result of poor silage management practices. These mycotoxins include mycotoxins formed by *Penicillium roqueforti* and *Penicillium paneum* and a diverse group of mycotoxins formed by *Aspergillus fumigatus*.

Table 6. Major mycotoxigenic moulds and mycotoxins in silage crops and silages.

Mycotoxin group	Major toxin(s)	Mould species	Crop(s)	Field- or ensilage-derived
Aflatoxins	Aflatoxin B_1 (M_1), B_2, G_1, G_2	*Aspergillus flavus, A. parasiticus*	Maize	Field
Trichothecenes	Type A: T_2, diacetoxyscirpenol	*Fusarium langsethiae, F. poae, F. sporotrichioides*	Maize, Sg cereals[1]	Field
	Type B: DON, nivalenol	*F. graminearum, F. culmorum*	Maize, Sg cereals, grass	Field
Fumonisins	Fumonisin B_1, B_2	*F. verticillioides, F. proliferatum*	Maize	Field
Resorcylic acid lactones	Zearalenone	*F. graminearum, F. culmorum*	Maize, Sg cereals, grass	Field
Ochratoxins	Ochratoxin A	*A. ochraceus, Penicillium verrucosum*	Sg cereals	Field
Alkaloids	Clavines, lysergic acid amide, ergotamine	*Claviceps purpurea*	Sg cereals	Field
	Lolitrem B, ergovaline	*Neotyphodium lolii, N. coenophialum*	Grass	Field
P. roqueforti toxins	Roquefortine C, mycophenolic acid	*P. roqueforti, P. paneum*	All types of silages	Ensilage
A. fumigatus toxins	Gliotoxin, fumigaclavines	*A. fumigatus*	All types of silages	Ensilage
M. ruber toxins	Monacolin K, citrinin	*Monascus ruber*	All types of silages	Ensilage

[1] Sg cereals: Small grain cereals (wheat, triticale, rye, barley).

Field-derived mycotoxins

The major toxinogenic moulds capable of producing field-derived mycotoxins are *Fusarium* species, *A. flavus* and *Aspergillus parasiticus* and endophytic *Claviceps* and *Neotyphodium* species. The most frequently occurring mycotoxins produced by *Fusarium* species are trichothecenes, zearalenone and fumonisins. These moulds are occurring world-wide, but seem to be particularly prevalent in temperate climates. Weather conditions strongly influence development of *Fusarium* mycotoxins. Infection of plants by *Fusarium* can take place via kernels, leafs, the stalk or infected seeds. Soil and decaying plant residues in the field are the main sources of *Fusarium* spores and conidia. A high level of mechanical or insect damage of the plant increases the risk of infection and is often associated with higher mycotoxin levels.

Examples of plant diseases associated with *Fusarium* infection include ear rot and stalk rot in maize and ear blight in wheat. The predominant species causing these diseases are *Fusarium graminearum* and *Fusarium culmorum*. These species are capable of producing zearalenone and different types of trichothecenes, including deoxynivalenol (DON; synonym for vomitoxin), nivalenol, diacetoxyscirpenol and T-2 and HT-2 toxin. DON is the most commonly occurring trichothecene. DON and zearalenone are often co-occurring in contaminated crops. An important and often unrecognized feature of DON and zearalenone contamination of maize and wheat is that these mycotoxins occur not only in the grains and kernels but also in the green parts of the plant, *i.e.* the leafs and stalk. This is of significance because these crops often are fed as whole crop silage. The limited information that is available on this topic indicates that DON and zearalenone levels in leafs and stalk of maize can be even higher than in the cob (Oldenburg et al. 2005). Fumonisins are formed by *Fusarium verticillioides* (syn., *Fusarium moniliforme*) and *Fusarium proliferatum*, species associated with pink or white ear rot disease in maize. Fumonisins are found exclusively in maize. It is generally assumed that DON, zearalenone and other *Fusarium* mycotoxins are not produced in silage. *Fusarium* species do not survive the acidic and anaerobic conditions of silage and usually have a lower prevalence in silage than for instance *Aspergillus*, *Penicillium* and *Monascus* species. However, few studies report development of *Fusarium* mycotoxins in silage. An example is a study conducted in Italy in which zearalenone concentrations in highly aerobically deteriorated peripheral areas of maize silage were detected that were up to 40 times higher than in non-deteriorated central areas of the silage. The concentration in the central areas was similar to the concentration of the forage at ensiling (Cavallarin et al. 2004).

Aflatoxins are produced by *A. flavus* and, to a lesser extent, *A. parasiticus*. Aflatoxins are highly toxic and carcinogenic to man and animals. Aflatoxin B_1 is the most prevalent and most toxic form. Aflatoxin B_1 is transformed in the liver of cattle into aflatoxin M_1, the form in which it is (partially) excreted into milk. With respect of the risks of mycotoxins in feed in relation to safety of dairy products to consumers aflatoxin M_1 is the only mycotoxin of concern. This relates to its significant feed-to-milk carry-over rate and high toxicity. Though *Aspergillus* is generally 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, sunflower, peanut and several tree nuts. 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. If conditions are favourable the mould colonizes the cobs and penetrates into the kernels. Aflatoxin development in the kernels occurs within narrow ranges of moisture content and temperature. Drought stress generally increases aflatoxin development in maize.

Alkaloid mycotoxins are produced by *Claviceps purpurea* in rye and barley and some grasses and by endophytic *Neotyphodium* moulds in perennial grasses. *C. purpurea* infects the plant when flowering. It produces a resting structure about the size of grain kernels, called sclerotia or ergots, which allow the mould to survive adverse conditions. These ergots contain high concentrations of alkaloids (e.g. clavines and lysergic acid amide). Several grasses, such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*), can harbour endophytic *Neotyphodium* species capable of producing alkaloid mycotoxins (e.g. lolitrem B and ergovaline). Benefits for the plant from this symbiosis are increased drought tolerance and resistance to insects. Endophytic *Neotyphodium* are highly prevalent in 'wild' grass populations in natural or extensively managed pastures in the North America, Australia, New Zealand and Europe. The prevalence of mycotoxin-producing endophytes in intensively managed pastures is generally low. Grass cultivars selected for grazing or silage production often do not contain these types of endophytes.

Ensilage-derived mycotoxins

Since the majority of mould species are obligate aerobic micro-organisms they do not develop in well-preserved, anaerobic silage. However, in practice silages are not completely anaerobic. Firstly, because silage covering materials are generally not fully airtight. Secondly, because of unintended damages to the silage covering during storage (for instance caused by rodents, birds). Moreover, exposure to air

becomes inevitable after the silo is opened for feeding. Growth of moulds and development of mycotoxins in silage are associated with the duration and extent of air infiltration. The extent of infiltration of air into the silage mass is mainly dependent on the porosity and density of the silage and the rate of silage removal after opening. The occurrence of moulds in silage is usually highest in surface layers.

Commonly detected moulds in silage are *P. roqueforti* and *P. paneum*, *Monascus ruber*, *A. fumigatus*, *Byssochlamys nivea*, *Mucoraceae* (in particular *Rhizopus nigricans*) and *Chrysonilia sitophila* (Scudamore and Livesey 1998, Pahlow et al. 2003). No mycotoxins from *Mucoraceae* and *C. sitophila* are documented. The predominant mould species in silages is *P. roqueforti*, which is tolerant to acidic conditions and able to grow at oxygen levels as low as 0.1% (v/v). At silage surfaces it usually forms white to grey coloured spots or layers. Occasionally, particularly in maize silage, the species forms typical green to blue coloured balls or lumps of mouldy silage approximately 50 to 100 cm below the top surface. *P. paneum* is closely related to *P. roqueforti* and the occurrence of these species in silage and cannot be differentiated visually. *P. roqueforti* and *P. paneum* are capable of producing a wide range of mycotoxins *in vitro* under laboratory conditions, including for instance different roquefortines, mycophenolic acid, PR-toxin, festuclavine and agroclavine (Nielsen et al. 2006, O'Brien et al. 2006). *P. paneum* additionally produces patulin. However, a number of these mycotoxins are probably not formed in silage or may not be stable under conditions prevailing in silage (as discussed later in this chapter). *A. fumigatus* is a mould species that is particularly detected in heavily moulded parts of silage and capable of producing a large number of different toxic metabolites, including gliotoxin, verruculogen, fumitremorgens, fumigaclavines and trypacidin (Richter et al. 2009). Apart from production of mycotoxins, the occurrence of *A. fumigatus* in silage is considered a health risk because inhalation of spores of this mould can cause lung disease (aspergillosis) in animals and man. *M. ruber* forms red-purple spots on silage surfaces is a producer of monacolin K and citrinin. *B. nivea* is a producer of patulin.

Stability of mycotoxins in silage

Information about the stability of mycotoxins in silage is not fully conclusive and for some mycotoxins contradictory. There are data indicating that certain field-derived and ensilage-derived mycotoxins are degraded in silage. However, reports in the literature about this subject are contradictory. This possibly relates to the heterogeneity of silage and the fact that the conditions change over time.

Zearalenone is generally regarded as being stable in silage. No effect of ensiling on the zearalenone concentration was detected in studies in which the level of zearalenone was monitored during up to nine months of ensilage (Lepom et al. 1988, Garon et al. 2006). This finding is consistent with data showing that the average and range of zearalenone concentrations in maize silage and unfermented maize products used as feed ingredient are similar (Dänicke et al. 2000). With respect to the stability of DON in silage there is contradictory information. In a study investigating DON stability in wheat and maize silage it was concluded that ensiling induced a strong reduction of DON (Richter et al. 2002). However, in other studies no effect of ensiling on DON concentration was detected (Lepom et al. 1990, Garon et al. 2006). Furthermore, the average and range of DON concentrations in maize silage and unfermented maize products used as feed ingredient are similar (Dänicke et al. 2000). It can be concluded that DON is stable in silage under most conditions or may be degraded to a limited extent. Aflatoxin B_1 produced in maize in the field has been found to be degraded slowly in maize silage (Kalac and Woolford 1982). This observation was confirmed in a recent French study, in which a 3-fold decline of aflatoxin B_1 was detected during nine months storage of maize silage (Garon et al. 2006). Likewise, partial degradation of ochratoxin A, a mycotoxin that is associated with small grain cereals, has been observed in ensiled barley (Rotter et al. 1990). No information is available about the fate of fumonisins in silage, but probably these mycotoxins are stable.

Contradictory information is available about the fate of ergot alkaloids produced by *Claviceps* and *Neotyphodium* species in silage. Health problems of cattle have been associated with high concentrations of ergovaline in silage from endophyte infected perennial ryegrass (Lean 2001) and with high concentrations of ergocryptine in silage from maize that was contaminated with a weed containing *Claviceps* ergots in the field (Naude et al. 2005), indicating that these substances were at least partially stable in silage. On the other hand, the concentration of *C. purpurea* ergot alkaloids (ergometrine, ergotamine and ergocryptine) in extensively managed grasslands strongly reduced when the grass was ensiled (Wyss et al. 1997). The *Penicillium* mycotoxins roquefortine C and mycophenolic acid are stable in silage, whereas PR-toxin and patulin are presumably unstable. In contrast to roquefortine C and mycophenolic acid, PR-toxin and patulin are rarely detected in silage. Experiments with blue-veined cheeses manufactured with *P. roqueforti* strains showed that PR-toxin was degraded and detoxified as a result of a chemical reaction with ammonia and free amino acids (Scott and Kanhere 1979). Many types of silage contain relatively high concentrations of ammonia and free amino acids, so reaction with these compounds may be the reason that PR-toxin is often undetectable. For patulin a similar mechanism may apply. Patulin is known to react with SH-groups of cysteine and other sulphur containing amino acids in protein rich environments and to be inactivated in fermented foods, such as wine, beer and cheese (Ciegler et al. 1979, Scott 1984).

Occurrence of mycotoxins in silage

Information about the incidence and concentrations of mycotoxins in silages is relatively scarce, in particular for silages other than maize silage. Table 7 gives an overview of results from surveys in Europe and the United States of America for DON and zearalenone in silage, conducted between 1989 and 2007. The data show that the incidence of DON in maize silage was high: in six out of seven surveys the incidence was 72 to 100%, in one it was 42%. The average DON concentration of positive samples in these surveys varied between 0.60 and 1.85 mg/kg. The incidence of zearalenone in maize silage was high too, but generally lower than that of DON: in five out of six surveys the incidence was 32 to 59%, in one it was 96%. The average zearalenone concentration of positive samples varied between 0.05 and 0.45 mg/kg. Information about the occurrence of DON and zearalenone in other silages than maize silage are scarce. In a survey in the Netherlands between 2002 and 2004, DON was not detected in 120 grass silage samples and in 3 of 30 (10%) wheat silages, whereas zearalenone was detected in 7 of the grass silages (6%) and none of the wheat silages (Driehuis et al. 2008a). Fumonisin contamination of maize is widespread, as indicated by the high incidence of fumonisins in maize and maize by-products intended for use in animal feed (Binder et al. 2007). Incidence of fumonisins in maize silage is likely to be high too, since evidence indicating degradation of fumonisins in silage is lacking. This is confirmed by the results of a survey in Midwestern USA in 2001 and 2002, in which fumonisin B_1, fumonisin B_2, and fumonisin B_3 were detected in, respectively, 97%, 72% and 57% of maize silages and average concentrations in positive silages were, respectively, 0.615, 0.093, and 0.051 mg/kg (Kim et al. 2004). In contrast, in the survey in the Netherlands described earlier fumonisin B_1 and B_2 were detected only in 1.4% of the maize silages (Driehuis et al. 2008a). This low incidence probably reflects that the environmental conditions of forage maize growth in the Netherlands are not favourable for infection by fumonisin producing moulds (*F. verticillioides*). Aflatoxin B_1 has been detected in maize silages in some surveys, but in most surveys this mycotoxin was undetectable in silages (Scudamore and Livesey 1998, Whitlow and Hagler 2005, Storm et al. 2008, Driehuis et al. 2008a). The occurrence of aflatoxins in silage is associated with geographical regions with a tropical or sub-tropical climate and is generally field-derived. However, there are reports indicating development of aflatoxins in poorly preserved silage with extensive mould infestation (Gonzalez Pereyra et al. 2008, Gonzalez Pereyra et al. 2011).

As described earlier, the occurrence of ensilage-derived mycotoxins produced by *P. roqueforti* and *P. paneum*, *A. fumigatus* and *M. ruber* relates to the preservation quality of silage and is dependent

Table 7. Incidence and average and maximum concentrations of the *Fusarium* mycotoxins DON and zearalenone (ZEA) in silage in different surveys.

Mycotoxin	Silage crop	Location	Year(s)	Percentage positive (total number)[1]	Concentration (mg/kg)[2]		Reference
					Average (of positive samples)	Maximum	
DON	Maize	North Carolina, USA	1989-1993	76% (106)	1.85	-	Whitlow and Hagler 2005
DON	Maize	Austria	1995-1999	91% (418)	0.75	2.8	Hochsteiner and Schuh 2001
DON	Maize	Germany	1998	79% (24)	1.61	9.86	Dänicke et al. 2000
DON	Maize	Pennsylvania, USA	2001-2002	42% (62)	0.6	3.7	Mansfield et al. 2005
DON	Maize	Netherlands	2002-2004	72% (140)	0.85	3.14	Driehuis et al. 2008a
DON	Maize	Netherlands	2005	100% (16)	0.93	2.39	Driehuis et al. 2008b
DON	Maize	Denmark	2007	100% (20)	1.06	5.09	Storm et al. 2010
DON	Wheat	Netherlands	2002-2004	10% (30)	0.62	1.17	Driehuis et al. 2008a
ZEA	Maize	North Carolina, USA	1989-1993	32% (93)	0.45	-	Whitlow and Hagler 2005
ZEA	Maize	Germany	1993-1995	38% (44)	0.05	0.17	Dänicke et al. 2000
ZEA	Maize	Austria	1995-1999	59% (149)	0.07	0.6	Hochsteiner and Schuh 2001
ZEA	Maize	Germany	1998	96% (24)	0.13	1.07	Dänicke et al. 2000
ZEA	Maize	Netherlands	2002-2004	49% (140)	0.17	0.94	Driehuis et al. 2008a
ZEA	Maize	Netherlands	2005	50% (16)	0.15	0.48	Driehuis et al. 2008b
ZEA	Grass	Netherlands	2002-2004	6% (120)	0.09	0.31	Driehuis et al. 2008a
ZEA	Grass	Netherlands	2005	13% (16)	0.13	0.21	Driehuis et al. 2008b

[1] The percentage of positive samples and total number of samples analysed.
[2] Concentration in dry matter.

on infiltration of oxygen during storage or during feeding-out. Very low incidences of roquefortine C and mycophenolic acid in maize and grass silages were found in a survey in the Netherlands, in which samples were analyzed that were taken relatively shortly after ensiling (3 to 6 weeks) from completely sealed silages that were not yet in use for feeding purposes. Roquefortine C was detected in none of 140 maize silages and in one of 120 grass silages, and mycophenolic acid was detected neither in maize nor in grass silages (Driehuis et al. 2008a). In contrast, samples taken from opened silages at 16 Dutch dairy farms showed high incidences of roquefortine C and mycophenolic acid in surface layers of maize silages (50%) and grass silages (19%). Concentrations of both mycotoxins were highest in surface areas with visible moulds. For example, the average roquefortine C concentration in samples of visibly moulded maize silage was 16 times higher than that in silage surface samples and 270-fold higher than that in silage centre samples (Driehuis et al. 2008b). Similar observations were made in studies conducted in Germany, not only with respect to the incidence and distribution in silages of roquefortine C and mycophenolic acid but also with respect to the incidence and distribution of the *M. ruber* mycotoxins monacolin K and citrinin and the *A. fumigatus* mycotoxins gliotoxin, verruculogen and fumigaclavin C (Richter et al. 2009). Remarkably, low levels of roquefortine C, mycophenolic acid and patulin were detected in freshly harvested maize prior to ensiling in a recent study conducted in Pennsylvania USA, suggesting that *Penicillium* mycotoxins may also be formed in maize in the field, at least under the environmental conditions of maize growth in that particular geographical area (Mansfield et al. 2008).

In a survey of mycotoxins occurring in the total diet of high-yielding dairy cows at 24 dairy farms in the Netherlands DON, zearalenone, roquefortine C and mycophenolic acid were identified as the mycotoxins with the highest incidence (Driehuis et al. 2008b). As expected, roquefortine C and mycophenolic acid were detected in ensiled feeds only. DON and zearalenone were detected in compound feed, feed commodities and ensiled feeds. Maize silage was found to be the most important source of all of these four mycotoxins in the diet. Maize silage represented on average 30% of the total daily feed intake of the animals, but contributed about 80% of the total dietary intake of DON and zearalenone and more than 95% of that of roquefortine C and mycophenolic acid.

Metabolism of mycotoxins in ruminants and impact on food safety

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 (Driehuis et al. 2010). 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 B_1 are not metabolized in the rumen. Aflatoxin B_1 is transformed into aflatoxin M_1 in the liver of ruminants. Aflatoxin M_1 is less mutagenic and genotoxic than aflatoxin B_1, but the cytotoxicity of aflatoxin M_1 and B_1 is similar. Information concerning the metabolism of *Claviceps* and *Neotyphodium* alkaloid mycotoxins and *A. fumigatus* mycotoxins is lacking. Research on the metabolism of roquefortine C and mycophenolic acid in cattle is currently in progress.

Aflatoxin B_1 is the only mycotoxin with significant carry-over into milk: between 1 and 6 percent is excreted in milk (as aflatoxin M_1). Carry-over rates of DON, zearalenone, fumonisin B_1, ochratoxin A and the alkaloid ergovaline appear to be at least about 100-fold lower (Driehuis et al. 2010). Carry-over rates of other mycotoxins frequently occurring in silage are not experimentally assessed. However, there are no indications that significant transfer of these mycotoxins into milk occurs.

Prevention of mycotoxins in silage

Regarding prevention strategies, a distinction is made between field-derived and ensilage-derived mycotoxins. Prevention of field-derived mycotoxins focuses on two areas: reduction of the infection pressure of moulds and reduction of the susceptibility of the plant to fungal infections (Council for Agricultural Science and Technology 2003). Codex Alimentarius issued codes of practice for the reduction of mycotoxins in cereal crops (Codex Alimentarius Commission 2003). Recommendations in these codes of practice are summarized in Table 8.

The most important factor prevention strategy for ensilage-derived mycotoxins is to restrict exposure of silage to oxygen. At ensiling, oxygen is entrapped in the ensiled mass, but this is rapidly consumed (within hours) by respiratory activity of the plant and (facultative) aerobic microorganisms.

Once the silo is filled, the material should be protected from oxygen as quickly as possible, for instance by sealing with sheets of plastic or foil. Where appropriate, measures should be taken to prevent damages of the seal. However, since in practice the sealing of silos is never completely airtight, it is inevitable that surface layers will be exposed to air and some air will penetrate the silage during storage. A high packing density of the silage is important because it restricts air ingress during storage and after opening of the silo for feeding, when exposure to air becomes inevitable. Another factor of importance is the silage removal rate during feeding. Maintaining a high silage removal rate minimizes ingress of air into the material behind the silage face. Finally, when preventive measures have not been successful, visibly moulded silage should be discarded before feeding, since these areas are hot-spots of ensilage-derived mycotoxins, as discussed previously.

In conclusion, silage can be contaminated with a variety of mycotoxins, originating from infection of the crop by moulds in the field or from growth of moulds in silage during storage or feeding-out. Prevention of mycotoxin contamination of silage requires different strategies. Field-derived mycotoxins can be reduced by application of recommended agricultural practices in crop production, whereas ensilage-derived mycotoxins can be reduced by application of adequate silage management, with emphasis on prevention of aerobic spoilage. DON and zearalenone are the mycotoxins with the highest incidence in silages and maize silage is the most important source of these mycotoxins. Based on current knowledge, aflatoxin B_1 is considered the only silage-associated mycotoxin of potential concern for the safety of milk and dairy products, due its high carry-over rate into milk as aflatoxin M_1 and the high toxicity of this toxin. However, the incidence of aflatoxin B_1 in silages is very low in most parts of the world and national and international surveys of aflatoxin M_1 in milk indicate a high degree of compliance with existing legislation (World Health Organisation 2001, European Food Safety Authority 2004). Relatively little information is available about the effects that the ensilage-derived mycotoxins produced by *Penicillium* species and *A. fumigatus* can have on animal health and productivity. This subject should be investigated in more depth in future research.

Table 8. Recommended agricultural practices for the prevention of development of field-derived mycotoxins (Codex Alimentarius Commission 2003).

Apply crop rotation, to reduce infection pressure
Remove crop residues from field, for instance by deep ploughing, to reduce infection pressure
Use seed varieties developed for resistance to fungal infections
Apply fertilization in conformity to crop demand, to avoid plant stress
Apply good agronomic practices (irrigation, weed control, plant spacing) and avoid plant stress from high temperatures and drought
Apply proper phytosanitary measures on seeds and crops, to avoid insect damage and fungal infections
Minimize mechanical damage, to avoid plant stress and fungal infections

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Characterisation of different lactic acid bacteria in terms of their oxygen consuming capacity, aerobic stability and pathogen inhibition

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Keywords: aerobic stability, lactic acid bacteria, oxygen scavenging

Introduction Silage inoculants are used to increase the rate of pH reduction with the aim to reduce growth of spoilage microorganisms and preserving nutrients. However, silage inoculants do much more than that, both in terms of mode of action and effect on the ruminant. Therefore, there is a need to understand the mechanisms of individual bacterial strains not only with respect to the preservation of nutrients and production of organic acids in the silage, but also to a much higher degree beneficial micronutrients and detoxification of various substances in the silage. The current studies focus on the characterisation of five different single bacterial strains and their mode of action.

Materials and methods The single strains of lactic acid bacteria (LAB) were tested in artificial silage medium (ENS) as described by Woolford and Wilkins (1975) and inoculated with 150000 CFU/ml. Changes in optical density were read every 30 minutes in a 30 °C set Bio-Tek-reader using 96-well-microtiterplate and KC4 software program. The pH and organic acid (Dionex-ion-chromatograph) were measured manually at 11 time points over 48 hours (n=1). Oxygen reduction was measured manually over 9 hours with an oxygen electrode (SG6 Mettler Toledo) (n=3). For the investigation of the ability to inhibit spoilage strains a streak method was used for the pathogens (BHI agar, 37°C), double-layered well method used for yeast inhibition (MRS and Malt agar, 30°C) and overlay method for mold (MRS and PDA agar, 25 °C). A 3-L-mini silo experiment was conducted and arranged in a randomized complete block design (n=5) at the Animal Nutrition and Feed Department, Institute of Animal Science of LHSU, Lithuania. Red clover-ryegrass (70:30) from the first cut and initial flower stage of maturity in red clover (*Trifolium pratense* L.), wilted to 31.7 % DM was chopped to 2 to 3 cm by a forage harvester under farm conditions. Forage was inoculated at 100000 CFU/g and aerobic stability was determined after 90 days of preservation.

Results The antagonistic assay testing of the effect of antimicrobial compound of five LAB´s against four clostridia strains showed that *Lactococcus lactis* NCIMB 30117 was superior in inhibiting all four clostridia strains (inhibition 2,3) while *Lactobacillus plantarum* DSM 16568 and *Lactobacillus buchneri* DSM22501 were superior in inhibiting both yeast and molds (Table 1). The production of lactic acid after 48 h was highest for *L. plantarum* DSM 16568 with 8.8 mg/ml of ENS, while *Lactococcus lactis* DSM11037 produced as little as 1.1 mg/ml of ENS. *L. buchneri* DSM 22501, a heterofermentative strain, produced 1.9 mg/ml of acetic acid. *L. lactis* NCIMB 30117 had the shortest lag time (figure A), when measuring the growth, and the fastest pH reduction below 4.0 (Figure 1 B). *L. plantarum* DSM 16568 had the highest growth and the lowest final pH close to 3.0. *L. buchneri* DSM22501 had a lagtime of close to 24 hours, but reduced the pH to 3.5 measured at 48 h. *L. lactis* DSM 11037 was superior in reducing oxygen, while *L. lactis* NCIMB 30117 and *E. faecium* NCIMB 111181 were intermediate. *L. buchneri* DSM 22501 and *L. plantarum* DSM 16568 did not reduce oxygen during the 9 hours of measurement. All strains improved aerobic stability in the mini-silo experiment, but *L. buchneri* Lb1819 was superior in improving aerobic stability (Figure 1 D). All strains improved the silage quality parameters (DM loss, pH, increased lactic acid and decreased butyrate and alcohol concentration) during 90 days of preservation. Differences in the reported parameters were both related to differences in bacterial species and *L. lactis s*trains.

Discussion The batch culture experiments and the mini-silo experiment illustrated clearly that the characterisation of single lactic acid bacteria is an important tool for combining inoculation products for ensiling and screening and selecting strains with specific abilities. The inhibition of clostridia by *L. lactis* NCIMB30117 is subscribed to its production of nisin Z (Swedish patent 511828), a bacteriocin, with a wide pathogen inhibiting property e.g. against *S. aureus* and *L. monocytogenes*. Yeasts and molds were inhibited most effectively by *L. plantarum* DSM16568 and *L. buchneri* DSM 22501. In 2002 Ström et al. showed the ability of a *L. plantarum* strain to inhibit or change the morphology of selected fungi, due to antifungal cyclic dipeptides. The antifungal inhibition of *L. buchneri* Lb1819 is largely related to the acetate production, but other antifungal compounds could also be produced by this strain. *L. lactis* DSM 11037 has been explicitly selected for its ability to scavenge oxygen. The removal of oxygen is a critical factor in reducing fungal growth at the start of the fermentation process. In traditional silage experiments the oxygen scavenging ability of silage inoculants has not yet been in focus and should be investigated

in more detailed studies. *L. buchneri* DSM 22501 has consistently been superior in reducing fungal growth and keeping temperatures low in silage.

Table 1. Inhibition of spoilage microorganisms inhibited by the five lactic acid bacteria.

		E. faecium NCIMB 111181	*L. buchneri* DSM 22501	*L. lactis* NCIMB 30117	*L. lactis* DSM 11037	*L. plantarum* DSM 16568
Pathogens	*Clostridia perfringens*	1	0	3	1	0
	Clostridia tyrobutyricum	0	0	2	0	0
	Clostridia sporogens	0	0	3	0	0
	Clostridia bifermentas	0	0	3	0	0
Yeast	*Hansenula anomala*	0	3	0	0	2
	Pichia canadensis	0	3	1	0	3
	Pichia fermentans	0	2	0	0	1
	Candida humilis	0	0	0	0	2
Molds	*Aspergillus flavus*	0	1	0	0	2
	Aspergillus fumigatus	0	1	0	0	3
	Aspergillus niger	0	2	0	0	3
	Monascus ruber	1	1	1	1	1

Change in yeast morphology around well; 0= no inhibiton zone, 1=low inhibition zone, 2=intermediate inhibition zone; 3=large inhibition zone

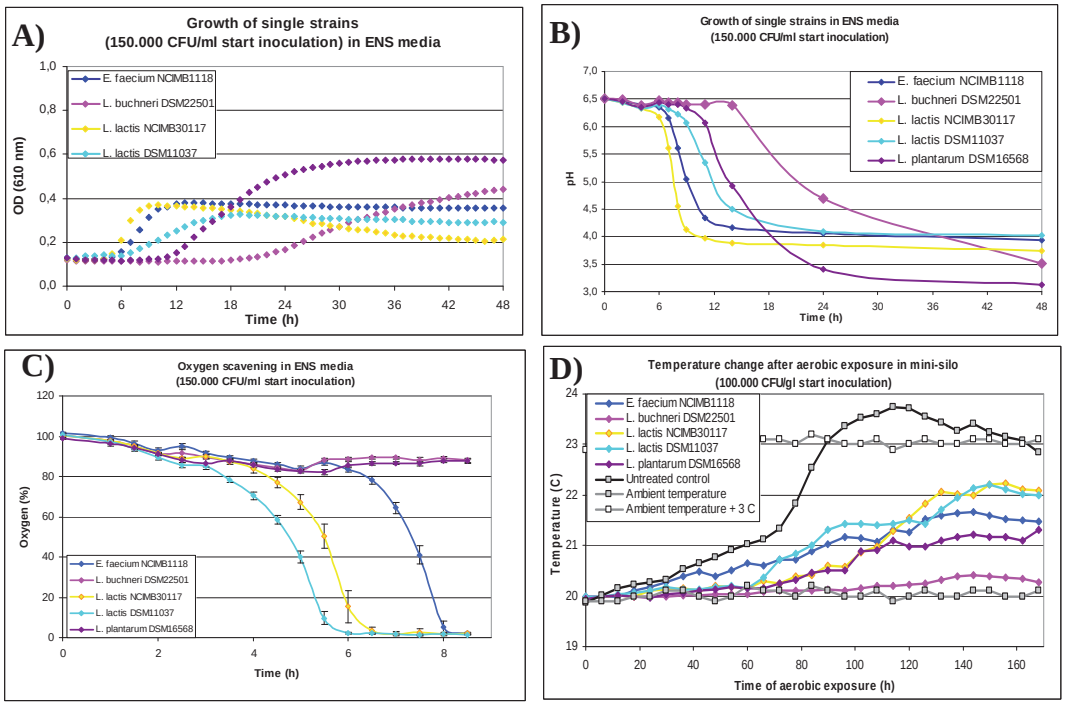

Figure 1. The figures show how the optical density (A), pH (B) and oxygen (C) changes over time, when inoculating in silage simulating media (ENS) with 150.000 CFU/ml, as well as the temperature change in mini-silos, when exposed to air.

Conclusions Oxygen scavenging, pH reduction and clostridia inhibition differed significantly among the LAB species and strains. *L. lactis* DSM 11037 was superior in removing oxygen, while *L. lactis* NCIMB 30117 was the only strain inhibiting all of the tested clostridia. *L. buchneri* DSM 22501 reduced pH much slower, but improved aerobic stability compared with other LAB. All silage inoculants improved silage quality parameters, but the magnitude differed depending on species and strains and future assays will test different combinations of LAB with different features and further validation in mini-silo experiments, silage bunkers and feeding trials.

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Effect of microbial inoculants on the quality and stability of bermudagrass haylage

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Keywords: Bermudagrass, haylage, inoculants.

Introduction Ensiling is an alternative method of forage preservation to making hay that requires relatively lower dry matter concentrations in the forage for successful preservation. Tifton 85 bermudagrass is an improved tropical grass cultivar that is widely used in southern US dairy systems because it has greater NDF digestibility compared with other grasses adapted to the region (Hill et al. 1993). However, ensiling bermudagrass is challenging due to its low concentration of readily fermentable carbohydrates (Kunkle et al. 1988). Little information exists on inoculant effects on bermudagrass silage. The objective was to compare the efficacy of four bacterial inoculants at improving the fermentation, and aerobic stability of bermudagrass haylage.

Material and methods Tifton 85 bermudagrass was grown at the Santa Fe Beef Unit, University of Florida in May, and harvested as a four week regrowth with a mower on July 7. The grass was wilted for 2.5 h in the windrow and then treated with or without inoculants prior to baling. Inoculants were dissolved in 12 L of deionized water and sprayed onto the forage in the windrow using a sprayer mounted in front of the baler while it was pulled by a tractor. This ensured that the forage was sprayed with the inoculant immediately prior to baling. The following treatments were applied: 1) Water (CON); 2) Buchneri 500 inoculant (B500), applied at 9.9 mg/kg of fresh forage to supply 1×10^5 cfu/g of *P. pentosaceus* and 4×10^5 cfu/g of *L. buchneri* 40788, 3) Biotal Plus II inoculant (BPII), applied at 4.0 mg/kg of fresh forage to supply 1×10^5 cfu/g of *Pediococcus pentosaceus* and *Propionibacteria freudenreichii*, 4) Silage Inoculant II (SI) applied at 4.0 mg/kg of fresh forage to supply 1.2×10^5 cfu/g of *L. plantarum and P. pentosaceus*; 5) Silo K inoculant (SK), applied at 7.9 mg/kg of fresh forage to supply 1×10^5 of *L. plantarum, Enterococcus faecium*, and *P. pentosaceus*, respectively.

Four replicate round bales (441 ± 26 kg) per treatment were wrapped with 7 layers of 6 mm plastic and stored for 112 d. Four additional bales per treatment were prepared and analyzed for pH after 3, 7 and 30 d of ensiling.

After 3, 7 and 30 days, four replicate bales from each treatment were sampled to analyze DM, pH, and VFAs. Fifty grams of each subsample was mixed with 450 ml of distilled water and blended using a Stomacher Lab-blender 400 for one minute and the juice was used to measure pH. After 112 days, the remaining four bales per treatments were subsampled for further analysis. Samples were analyzed for aerobic stability by placing temperature sensors (Onset Computer Corporation, Bourne, MA) at the center of a bag containing 1 kg of silage, within a 20 L bucket. The silages were covered with 2 layers of cheesecloth to prevent drying. The temperature was recorded every 30 min for 14 d. Aerobic stability was denoted by the time (h) that elapsed before a 2ºC rise in silage temperature above ambient temperature (23ºC). Subsamples from each bale were analyzed of yeast and mold counts, VFA, pH, IVDMD, NDFD, and chemical composition. The DM concentrations at d 0 were used as a covariate for the statistical analysis of the rest of the results. Data was analyzed with the Mixed procedure of SAS and Fisher's F-protected least significant different test was used to compare least square means. Significance was declared at $P < 0.05$.

Results and discussion The DM concentration of Control samples was lower than those of other silages on d 0 because Control samples were harvested earlier in the day than other samples.
The pH of Control and inoculated d 3 silages were similar ($P > 0.05$; Table 1) but B500 and BPII had lower pH (5.77 vs. 6.17; 5.06 vs. 5.66; respectively; $P < 0.001$) than other treatments after 7 and 30 d of ensiling. By 112 d of ensiling, all inoculated silages had lower pH than the Control silage ($P = 0.003$; 4.77 vs. 5.37), indicating that inoculation improved the fermentation of the silages. Nevertheless, the pH of all treatments was within the range 4.57 to 5.37, which is similar to that found in other studies with ensiled bermudagrass in Florida (Umana et al., 1991).

Silages treated with SI tended to have lower CP concentration than the Control silage for unknown reasons. No difference ($P > 0.05$; Table 2) was found among treatments in other measures of chemical composition or digestibility.

Treatments B500, BPII, and SK had lower ammonia concentration than Control and SI silages (Table 2; 167.1 vs. 234.1 mg/dl), which shows that these treatments reduced proteolysis and deamination

of the silage. Treatment SK had lower clostridia counts than the Control silage (P = 0.03; 1.19 vs. 1.98 cfu/g).

Aerobic stability was improved by all inoculant treatments and the magnitude of the improvement was greatest for B500 and least for SK. Treatments B500, BPII, SI, and SK improved (P < 0.001) aerobic stability by 236%, 197%, 188%, and 95%, respectively compared to the Control (276 vs. 99 h).

Conclusions All treatments improved the fermentation and aerobic stability of bermudagrass haylage to varying extents. All inoculants except SI reduced ammonia-N concentration. The rate of pH decline was greatest for B500 and BPII, but Clostridial counts were only reduced by SK.

Acknowledgements We gratefully acknowledge Southeast Dairy Check-Off, Lallemand Animal Nutrition Inc., WI, USA for funding this study.

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Table 1. Effect of microbial inoculant on pH and dry matter concentration of ensiled bermudagrass after different days (d) of ensiling.

	CON	B500	BPII	SI	SK	SEM	P-value
pH d 3	6.36	6.24	6.29	6.37	6.45	0.06	0.10
pH d 7	6.21[a]	5.71[b]	5.83[b]	6.09[a]	6.21[a]	0.04	<.0001
pH d 30	5.52[a]	5.08[b]	5.04[b]	5.55[a]	5.90[a]	0.13	0.0016
pH d 112	5.37[a]	4.57[b]	4.77[b]	4.83[b]	4.89[b]	0.07	0.0002
DM d 0, %	46.6	56.5	55.7	50.5	53.4	3.6	0.008
DM d 112, %	51.3	54	54.8	48.4	55.5	2.2	0.2

Table 2. Effect of microbial inoculants on chemical composition, fermentation parameters, microbial counts and aerobic stability at silo opening (day 112).

	CON	B500	BPII	SI	SK	SEM	P-value
CP, % of DM	18.8	18.3	18.2	16.7	18.2	0.52	0.063
ADF, % of DM	32.1	32.9	32.1	31.5	31.6	0.57	0.357
NDF, % of DM	69.1	65.3	65.3	65.9	65.3	0.96	0.149
NDF digestibility, %	50.9	57.5	55.5	54.2	56.2	1.75	0.324
Ammonia, mg/dl	223.7[a]	179.6[b]	172.9[b]	227.8[a]	168.6[b]	6.82	0.001
Lactic acid, % of DM	1.04	3.2	2.6	2.9	1.8	0.35	0.077
Acetic acid, % of DM	0.28	0.50	0.41	0.45	0.41	0.04	0.054
Yeasts, log cfu/g	1.03	0.98	1.22	1.01	1.00	0.12	0.552
Molds, log cfu/g	4.47	2.45	2.89	1.97	3.62	0.58	0.057
Clostridia, log cfu/g	2.13[a]	2.09[ab]	1.97[ab]	1.75[ab]	1.19[b]	0.23	0.034
Aerobic stability, h	99.0[c]	332.8[a]	294.1[a]	285.1[a]	193.4[b]	22.4	0.0005

Means with different superscripts in the same row differed, P < 0.05

[1]CON = control, no inoculant; B500 = *Pediococcus pentosaceus* and *Lactobacillus buchneri;* BPII =*P.* and *Propionibacteria freudenreichii*; SI = *L. plantarum and P. pentosaceus;* SK = *L. plantarum, Enterococcus faecium, and P. pentosaceus.*

Bacteria associated with ensiling fermentation and aerobic stability of total mixed ration silage

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Keywords: aerobic stability, fermentation, silage, total mixed ration

Introduction In Japan, high-moisture by-products are often mixed with dry feeds to prepare total mixed rations (TMR) which are then preserved as silage. This may minimize the risk of effluent production and omit the time required for mixing prior to feeding. Unpalatable by-products can be incorporated into TMR silage because their odours and flavours may be altered by fermentation in a silo. An interesting property of TMR silage is its high stability on exposure to air. Heating does not occur even in summer, whereas non-ensiled TMR deteriorates rapidly. We have investigated the factors involved in resistance to deterioration and demonstrated the presence of sourdough lactic acid bacteria (LAB) in commercial silages produced in warm seasons (Wang and Nishino, 2010). Because sourdough is also known to have a long shelf life, we speculated that the LAB species may confer spoilage resistance on TMR silage. However, the aerobic stability of commercial silages was not determined in our previous study, and seasonal changes in the bacterial community might not be attributable to differences in storage temperature. In this study, TMR silages were stored at 5, 15, 25 and 35°C, and live counts, fermentation products, aerobic stability and bacterial communities were examined.

Materials and methods A pre-ensiled TMR mixture, containing wet brewer's grains and soybean curd residue as major ingredients, was obtained from a feed company that produces TMR silage. The mixture (300 g) was packed in a plastic pouch and stored at 5 (refrigerator), 15 (incubator), 25 (air-conditioned room) and 35°C (incubator) for 10, 30 and 90 days. After the silage was opened, one-third of the content (100 g) was placed in a polyethylene bottle (500 mL) without compaction and exposed to air for 7 days. The aerobic deterioration test was carried out at 25°C and at the same temperature as that during storage. Live counts, fermentation products and bacterial community were determined according to Wang and Nishino (2010). Samples of commercial TMR silage were also examined.

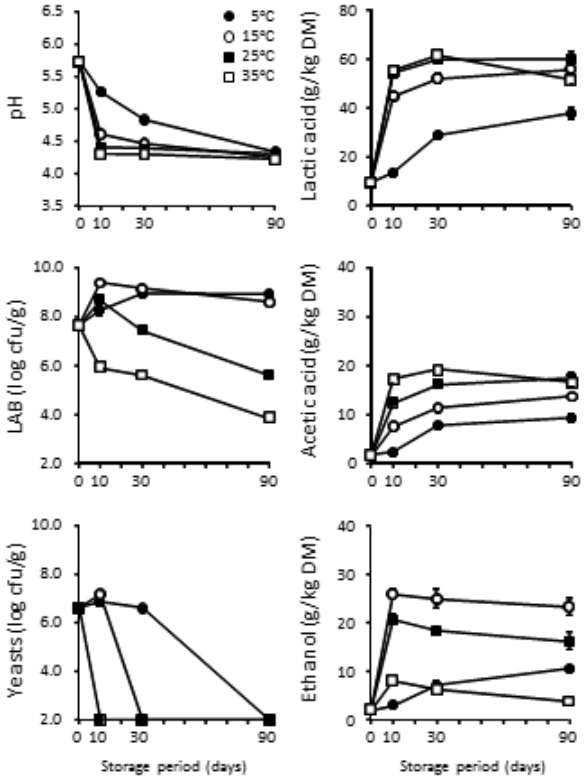

Figure 1. Microbial counts and fermentation products of laboratory-scale total mixed ration silage stored at 5, 15, 25 and 35°C for 10, 30 and 90 days.

Results and discussion A marked difference due to storage temperature was seen in fermentation products on day 10. Fermentation occurred marginally in TMR silage stored at 5°C, whereas acceptable levels of lactic acid production took place in TMR silage stored at greater than 15°C. The lactic and acetic acid contents increased as the storage temperature increased, whereas the ethanol content was higher than the acetic acid content if stored at 15 and 25°C. Ethanol content was reduced by storage at 35°C compared with storage at 25°C. Large numbers (>106 cfu/g) of yeast were found in TMR silages stored at 5 and 15°C.

Storage temperature affected fermentation products on day 30. Weak fermentation appeared to have continued and the pH remained at 4.84 in TMR silage stored at 5°C. Similar to the findings on day 10, the lactic and acetic acid contents increased as the storage temperature increased, whereas ethanol content was greater than the acetic acid content if stored at 15 and 25°C. Unlike day 10, yeasts were detected only in TMR silage stored at 5°C. If storage was prolonged to 90 days, the pH decreased to 4.31 even in TMR silage stored at 5°C. The contents of lactic acid, acetic acid and ethanol were 43.4, 10.1 and 13.9 g/kg DM, respectively, indicating that acceptable fermentation can be achieved under prolonged cold storage. In TMR silage stored at 25°C, more acetic acid than ethanol was observed on day 90, although the ethanol content remained higher than the acetic acid content in TMR silage stored at 15°C. No yeasts or enterobacteria were detected on day 90 in any TMR silages, regardless of storage temperature.

Aerobic deterioration did not occur when TMR silage was exposed to air at the same temperature at which it was stored. When the spoilage test was conducted at 25°C, however, 10-day TMR silages stored at 5 and 15°C and 30-day TMR silage stored at 5°C deteriorated with significant heating observed within 2 days after silo opening. When storage was extended to 90 days, no heating was observed even if the spoilage test was performed at 25°C.

Bands indicative of *Lactobacillus helveticus*, *Lactobacillus brevis*, *Tetragenococcus halophilus* and *Lactobacillus buchneri* were detected in 10-day TMR silage stored at 5°C. In addition to these bacteria, *Lactobacillus plantarum* appeared in TMR silage stored at 15°C from day 10 onward and on day 90 in silage stored at 5°C. When TMR silage was stored at 25°C, *Lactobacillus panis* was detected from the initial ensiling period. When the storage temperature was increased to 35°C, bands indicative of *L. plantarum* and *Lactobacillus delbrueckii* became faint and another band of *L. panis* appeared from the beginning of ensiling. The denaturing gradient gel elecrophoresis pattern of large-scale TMR silage resembled those of laboratory-scale TMR silage stored at 35°C; bands indicative of *L. panis* were common in these TMR silages.

Conclusions High ambient temperature enhances the acetic acid production in TMR silage. Sourdough LAB, particularly *L. panis*, may be associated with changes in the fermentation products owing to the storage temperatures.

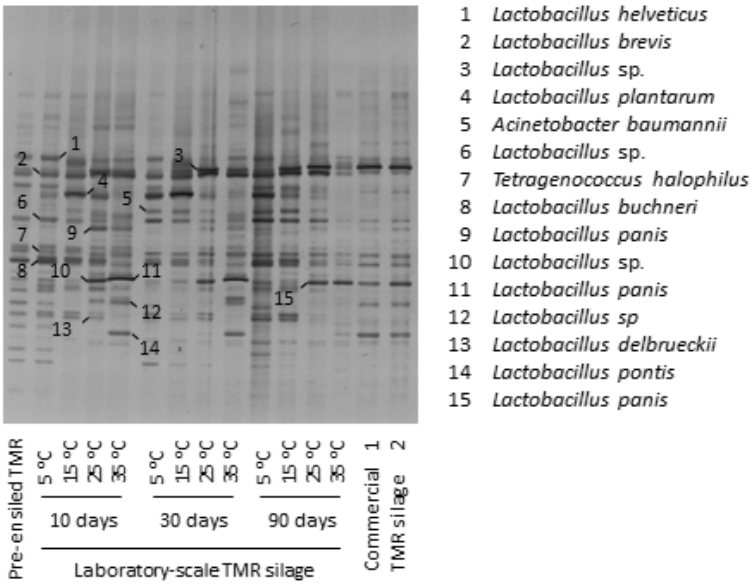

Figure 2. Bacterial community of laboratory scale and practical scale total mixed ration silages. Laboratory silos were stored at 5, 15, 25 and 35°C for 10, 30 and 90 days, and commercial silos were stored outside in summer for about 60 days.

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A chemosensor system for assessment of silage quality

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Keywords: electronic nose, forage quality, grass, maize, method

Introduction Usually the assessment of silage quality is based on the nutrient contents, the presence of the fermentation products butyric acid and acetic acid and the targeted pH value (DLG 2006). The DLG (2004) recommends complementing such assessments with sensory evaluation. Especially the comparative sensory evaluation is difficult because it is strongly dependent on subjective sensory perceptions. Therefore, a chemosensor system (electronic nose) was improved and evaluated in measuring silage volatile components. In this study, the following objectives were targeted: 1) Evaluating silage quality objectively, 2) Developing an alternative to microbiological and chemical analysis and to animal experiments, 3) Reducing quality losses and 4) Increasing reliability of process and product safety.

Material and methods The volatile components of silages were prepared and measured in a chemosensor system. The actual measurement of molecules in the silage gas was achieved using quartz microbalance sensor technology. For this, the inverse piezoelectric effect is used to stimulate the oscillation of a small quartz plate. The molecules are absorbed in the sensor layer and the resulting change in mass of the quartz leads to a difference in the frequency of the quartz oscillation. Hereby a number of sensors with different coatings were applied using the same measurement principles in the form of sensor arrays that differed in regard to sensitivity to various substances. To increase measurement sensitivity, the analyte molecules are first enriched in an adsorbent before being thermally desorbed and channelled to the quartz sensors in concentrated form (Haas et al. 2008).

Results and discussion Maize and grass silages with different dry matter contents, cutting lengths, bulk densities and at different stages of aerobic spoilage were produced for the experiments. All silage samples that were measured by chemosensor system were also studied by animal experiments and chemical and microbiological analyses. This allows a comparison of the chemosensor system data with the results of common analytical methods. Figure 1 presents data of good quality maize silage (aromatic odour) and aerobically deteriorated maize silage (alcoholic odour). The data show two characteristic effects of chemosensor system measurements. The relative relationship of the sensor signal (the order) represents a pattern dependent on the composition of the silage gases. Different patterns indicate different compositions. Additionally, the intensity of the sensor signal reflects the concentration of the measured gases. Both characteristics can be used for differentiation and evaluation of silages. In Figure 1 the signals for silage with alcoholic odour are more intensive than those for the fresh silage. Additionally, the relationship between the sensors or the ranking of the sensors (sensor pattern), is important. In the good quality silage, sensor 4 had the highest strength of signal and in the deteriorated silage sensor 4 was in the middle area.

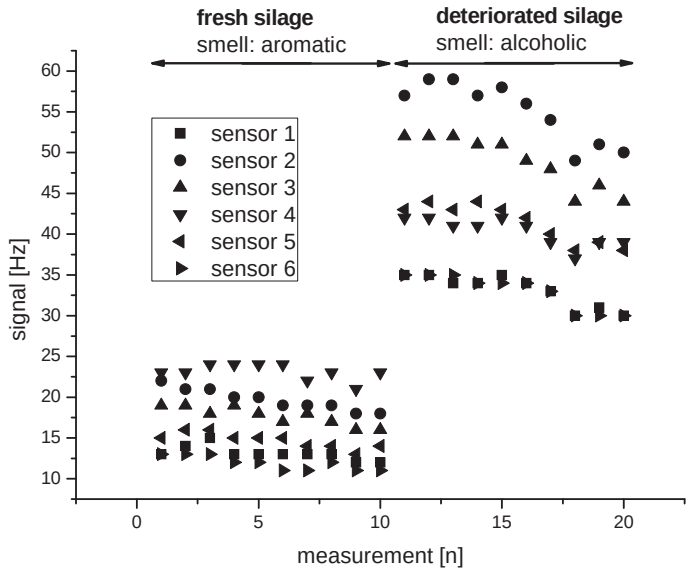

Figure 1. Signal patterns of fresh and deteriotrated silages.

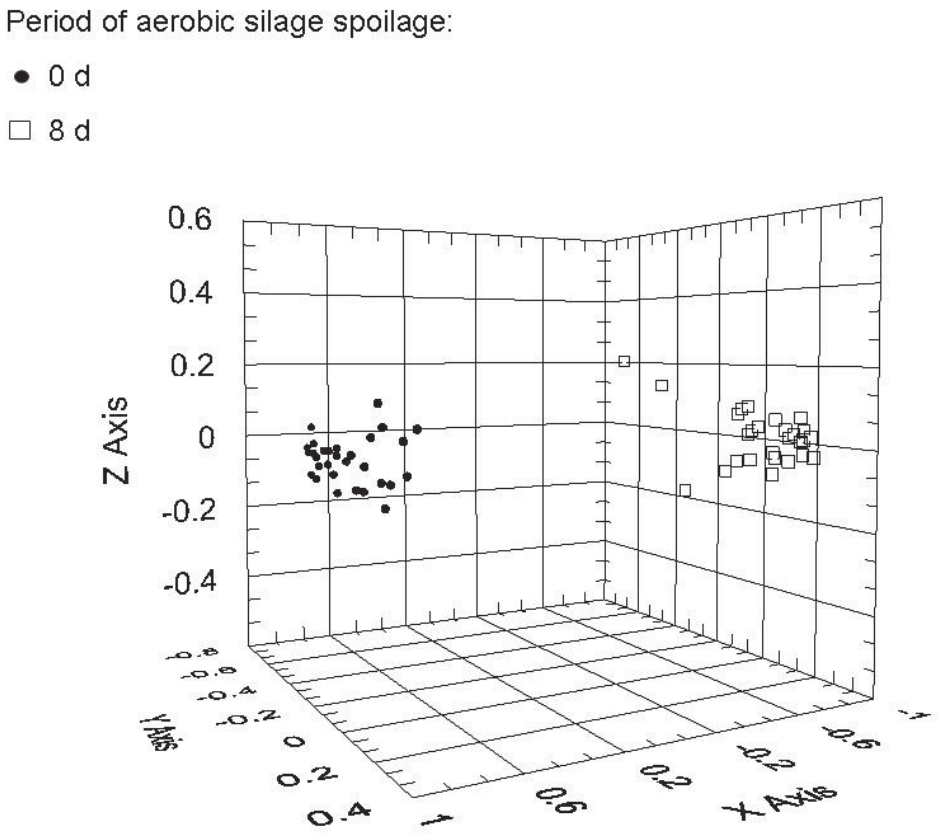

Figure. 2. 3D-illustration of silage with different time durations of aerobic storage.

Figure 2 illustrates the results in three dimensions. The tested silage was a short-cut maize silage at 40% dry matter content and prodcued with 0.2 MPa compacting pressure. High quality of silage (0 days of air influence) is represented by the points and low quality of silage (8 days of air influence) is represented by the quadrates. The 3D-illustration shows a clear separation between low silage quality and high silage quality.

Conclusions The experimental design allowed an efficient gas sampling method. The results of these studies show a significant discrimination of the silage quality. The measurement system succeeded in detecting two different deterioration stages of silage under laboratory conditions and a subsequent data evaluation. To determine the reproducibility, the consistency of the measurement and the analysing method further investigations should be done.

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Opportunities for reducing environmental emissions from forage-based dairy farms

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Keywords: ammonia, diffuse water pollution, farm-scale model, greenhouse gas, mitigation

Introduction

The dairy sector, in common with other agricultural sectors, currently faces a great challenge to meet rising global food demands, particularly for livestock-derived food products, in a sustainable way (Godfray et al. 2010). There are important interactions between food production, the environment and other ecosystem services, as discussed by Pilgrim et al. (2010) for temperate grassland systems, and the sustainable intensification of production relies on a good understanding of these interactions and our ability to identify potential 'win-win' strategies.

The assessment of such interactions for given management or mitigation scenarios on forage-based dairy farms was the primary aim of the development of the farm-scale $SIMS_{DAIRY}$ model (Del Prado et al. 2011). Specifically, $SIMS_{DAIRY}$ simulates the effect of interactions between farm management, climate and soil characteristics on losses of nitrogen (N), phosphorus (P) and carbon (C), including effects on farm profitability and giving a more qualitative indication of effects on biodiversity, milk quality, soil quality and animal welfare. While developed for UK dairy systems, and noting that outputs can vary depending on the model-scenario farm characteristics (particularly soil and climate), this can be used more generically as a useful tool in providing an assessment at the whole farm system level of the introduction of single or multiple mitigation methods, showing trade-offs between production and environmental effect, or between different environmental effects and identifying win-win scenarios. It is important that environmental effects are expressed per unit of production (e.g. litre of milk), i.e. an emission intensity metric, such that strategies leading to sustainable intensification of production can be identified as distinct from those which may reduce environmental impact at the expense of production.

The aims of this paper are to give an overview of the potential environmental impacts to air and to water of predominantly forage-based dairy systems, to discuss some of the most promising potential mitigation strategies and to assess the impacts of a number of these using the farm-scale model $SIMS_{DAIRY}$.

Environmental impacts of dairy farms

Emissions to the atmosphere

The key emissions to the atmosphere of environmental concern from dairy farms are ammonia (NH_3) and the greenhouse gases methane (CH_4) and nitrous oxide (N_2O). Other potential emissions of environmental concern include non-methane volatile organic compounds, fine particulates and heavy metals (Misselbrook et al. 2011), and while these may be of local importance in some instances such as around very intensive feedlots (e.g. Shaw et al. 2007), agriculture is generally not considered to be a major source for these species and they are not discussed further here.

Agriculture is the major source of NH_3 emissions to the atmosphere, accounting for >80% of total anthropogenic emissions in the UK (Passant et al. 2011), with the dairy sector accounting for approximately one third of total agricultural emissions. In a dairy farm context, NH_3 emissions arise predominantly from the urea content of urine excreted by dairy cows, the urea being readily hydrolysed to ammonium in the presence of the ubiquitous enzyme urease. Emissions will therefore occur from wherever cattle urine is deposited, at grazing, in housing and yards, and from manure storage and spreading. In addition, emissions occur from urea- and ammonia-based inorganic fertilisers applied to land. Ammonia is of concern because of potential damage to sensitive ecosystems through acidification and eutrophication, and also because of its role in the formation of secondary particulates in the atmosphere (ammonium nitrate and ammonium sulphate) and their implications regarding human health (Erisman et al. 2007).

The NH_3 flux from an emitting surface depends on a number of factors, including the concentration at the emitting surface, pH, total exposed surface area (and surface area to volume ratio), temperature and the air flow above the emitting surface. Management, in addition to environmental conditions, can therefore have a great influence on emissions from livestock housing and manure storage (Sommer et al. 2006), from manure application to land (Sommer et al. 2003) and from fertiliser applications (Sommer et al. 2004). Mitigation strategies are therefore generally aimed at reducing the overall emitting surface area, reducing the concentration at the emitting surface or reducing air flow at the emitting surface.

Agriculture is a significant source of anthropogenic CH_4 emissions to the atmosphere, accounting for c. 40% of emissions in the UK (MacCarthy et al. 2011), with the dairy sector estimated to account for approximately one third of total agricultural emissions. Methane is a greenhouse gas, with a global warming potential of 25 times that of CO_2, over a 100 year lifetime (Forster et al. 2007). The major source of CH_4 emissions from the dairy sector is enteric fermentation in the rumen of cattle, whereby CH_4 is a by-product of microbial carbohydrate degradation. Enteric emissions are influenced by the gross energy intake of the animal and the digestibility of that energy, with the energy intake in turn being dependant on the energy requirements of the animal for maintenance, production (milk and/or growth), pregnancy and activity. Mitigation strategies are aimed at directly inhibiting the methanogenic bacteria in the rumen, manipulating the microbial breakdown pathways in the rumen, manipulating the digestibility of the diet or maximising the proportion of energy intake over the lifetime of an animal ultimately being used for milk production.

Methane emissions also arise from manure management, deriving from the microbial breakdown of excreted volatile solids under anaerobic conditions. Key driving factors are temperature, manure composition and degree of anaerobicity, which will be influenced by management (Chadwick et al. 2011). Mitigation strategies are aimed at reducing storage duration and/or temperature, minimising anaerobic conditions or through capturing and utilising produced CH_4.

Agriculture is also a major source of N_2O emissions, accounting for c. 80% of emissions in the UK (MacCarthy et al. 2011), with the dairy sector estimated to account for approximately one fifth of total agricultural emissions. Nitrous oxide is a potent greenhouse gas, with a global warming potential of 297 times that of CO_2, over a 100 year lifetime (IPCC 2007). Nitrous oxide emissions arise as products, or partial products, of the microbial processes of nitrification (conversion of ammonium to nitrate (NO_3^-)) and denitrification (conversion of NO_3^- to dinitrogen gas (N_2), with intermediary products as nitrite (NO_2^-), nitric oxide (NO) and N_2O). Nitrification is essentially an aerobic process, while denitrification occurs under anaerobic conditions. The key direct sources of N_2O emission from dairy farming are from N amendments to the soil, either as inorganic fertiliser, manure applications, grazing returns or crop residues, and the management of livestock manure during housing and storage. Major influencing factors are the availability of N and C, anaerobicity and, to a lesser extent, temperature. A proportion of NO_3^- leached and N deposited to land is re-emitted as N_2O. These indirect losses of N_2O are significant. Mitigation strategies for direct N_2O emissions are aimed at reducing the availability of N, particularly under anaerobic conditions (e.g. wet soils), and at impeding the microbial processes through the use of inhibitors. Mitigation of indirect losses of N_2O, for example via NO_3^- leaching, are aimed at optimising N supply for crop demand and minimising the risk of excess N in the soil.

Emissions to ground and surface waters

Sources of diffuse water pollution on dairy farms include the farm steading (uncollected seepage from buildings, manure stores, yards frequented by dirty equipment and livestock), tracks, and the land itself (via fertiliser and manure applications, livestock grazing on the grassland, and nutrient applications to and cultivation of maize or cereal land). The principal diffuse water pollutants are NO_3^-, ammonium, P, sediment, pathogens and organic matter (which generates an oxygen demand in the water course) (Chadwick and Chen 2003).

As much as 60% of the NO_3^- found in UK watercourses is thought to come from agriculture. It arises from excess N input from fertiliser, manures and grazing livestock that is not utilised by the grass or crop. Rainfall then leaches the NO_3^- through the soil profile to drains and into watercourses.

Ammonium is a cation and hence can by immobilised in the soil profile. It is also readily nitrified to NO_3^-, so is generally only found in low concentrations below grasslands. However, it can be lost following rainfall events that result in rapid overland flow, or movement of slurry through cracks in the soil to drains. The effect of excess N in watercourses is to provide nutrients to algae and other aquatic plant life (eutrophication), resulting in excessive growth and potential algal blooms. Nitrite and NH_3 are also found in drainage water and are toxic to freshwater fish.

Phosphorus is another nutrient that contributes to eutrophication of watercourses. It is immobilised on soil surfaces and complexes with organic matter and metals such as iron, and is held strongly within the soil profile. Most of the P applied to grasslands is found in the top soil layer, so it's main pathway to watercourses is via detachment of soil particles and colloids in storms followed by overland flow (or again via cracks in the soil to drains following a slurry application). In general, temperate grassland may lose 1-3 kg total P ha^{-1} $year^{-1}$ (depending on inputs), but an individual rainfall event following a slurry application could result in 'incidental' losses as high as this in just one storm (Preedy et al. 2001).

Phosphorus losses are associated with sediment transfers from agricultural land. Sediment is a pollutant per se, as it affects the spawning grounds of salmonids. Although arable land is known to be a large source of agricultural sediment, grasslands are also a source (Granger et al. 2010), which is exacerbated by grazing livestock under wet soil conditions. On dairy farms, land used for forage maize is a potential critical source for sediment (and P) erosion and transfer to watercourses, especially if late

harvests coincide with wet soil conditions.

The organic matter in dairy slurry and dirty water can generate an oxygen demand if it finds its way into a watercourse. This biological oxygen demand (BOD) results in rapid proliferation of micro-organisms in the watercourse which respire rapidly, removing oxygen from the water – resulting in asphyxiation of aquatic life. The BOD of a typical dairy slurry is ca. 10,000 mg l^{-1} (Chadwick and Chen 2003), and that of dirty water ranges from 200-1000 mg l^{-1} (Cumby et al. 1999). The recommendation for treated effluent entering a watercourse is 20 mg l^{-1} (HMSO 1980), so any significant loss of slurry or dirty water into a river will have an environmental impact.

Livestock manures and faeces deposited during grazing are sources of a range of pathogenic organisms. For dairy farming, the key pathogens include Cryptosporidium and Campylobacter. Whilst specific pathogens are of key interest in terms of human health, it is the indicator species of E coli and Intestinal Enterococci, known as faecal indicator organisms (FIOs) on which legislation is based (CEC 2006). The risks of FIO losses from livestock farms to watercourses has been explored (Chadwick et al. 2008b), and are greater from farms with a greater number of livestock, steeply sloping land, and limited slurry storage capacity (Oliver et al. 2009). However, risks are also affected by farmer attitudes. In some instances, the topography of a farm can act as a 'safety net', e.g. where flat land reduces the risk of transfers, even if a farmer has limited slurry storage capacity or is ignorant about the consequences of injudicious management of livestock and their manures.

Haygarth et al. (2005) introduced the concept of pollutant movement from source to the watercourse, via the *source-mobilisation-delivery-impact* model. This model lends itself to addressing mitigation of diffuse water pollutants at each stage. Thus the *source* can be reduced, either through e.g. application of less fertiliser nitrogen, or by applying it in frequent doses and not all at once – thus reducing the risk of excess nitrogen in the soil at risk of loss. *Mobilisation* is the process by which a pollutant starts its journey towards the watercourse, and can occur via detachment or solubilisation. So mitigation methods suitable to reduce mobilisation would include the use of a nitrification inhibitor with and ammonium-based fertiliser, or the incorporation of slurry into a maize field, rather than leaving it on the soil surface. Finally, *delivery* can be reduced by intercepting pollutant rich drainage or overland flow via e.g. a constructed wetland.

Guidance is supplied to farmers to protect watercourses from diffuse water pollution, e.g. the UK Joint Code of Practice (Anon 2009). In some countries legislation is in place to reduce the impact of agriculture on water quality, e.g. the EU Nitrates Directive (EC 1991) has resulted in individual member states developing action plans to reduce the NO_3^- concentrations of vulnerable watercourses. The action plans include closed periods for the spreading of high available N content manures, e.g. dairy slurry, and set a maximum N loading for a farm, thus introducing a stocking rate limit. The EU has also introduced the Water Framework Directive (CEC 2000) to protect the ecological status of watercourses. This covers a wider range of pollutants than just NO_3^-, and governments are putting in place guidance to farmers to help them comply with strict targets on future ecological status of watercourses.

Through a greater knowledge of the behaviour of different water pollutants, mitigation methods can be developed that are method-centric and can tackle multiple pollutants, rather than addressing just one individual pollutant (Granger et al. 2010). It is also essential that guidance on choice of mitigation methods takes account of any secondary impacts, e.g. pollution swapping. Cuttle et al. (2006) produced a Mitigation Manual for Diffuse Water Pollutants. The 44 methods included management of land use, soil, fertilisers, manures, livestock and farm infrastructure, e.g. provision of bridges to allow livestock to ford streams to reduce sediment and pathogen transfers to water. This Mitigation Manual was provided to Catchment Sensitive Farming Officers to provide advice on practical methods which could be introduced on farms, and at what cost. It has recently been updated to include mitigation methods for greenhouse gas and NH_3 emissions. Both guidance documents highlight the relevance of each method to different farming systems, expresses the potential effectiveness in reducing the target pollutant(s) and the secondary impacts on other pollutants, the indicative cost of introducing the method, its practicality and likely uptake.

Potential mitigation methods

Considerable research effort in recent years has been aimed at developing mitigation methods and strategies to reduce the environmental impact of agricultural production practices. Specifically for dairy farms, these include animal health, diet, crop nutrient management, grazing management and genetic improvement in both livestock and crops. These are discussed in more detail below, with some specific scenarios assessed using the $SIMS_{DAIRY}$ model.

Livestock health

Production losses as a consequence of animal ill health and/or poor fertility result in an increase in the environmental emissions per litre of milk produced. In particular, the proportion of replacement animals required in a herd (related to the average number of lactations per dairy cow) can have a significant ef-

fect on emission intensity. Garnsworthy (2004) showed that significant reductions in CH_4 and NH_3 emissions could be made through improvements to dairy cow fertility.

Dietary strategies

Dairy diet manipulation can lead to reductions in enteric CH_4 emissions, and in nitrogen and phosphorus excretion, while having no detrimental effect on productivity. Potential dietary manipulations include the use of dietary additives with specific inhibitory effects on rumen CH_4 production, manipulation of the in-house diet composition, particularly with respect to protein content and form, and manipulation of the grazed sward composition.

A number of dietary additives have been assessed for their effectiveness in reducing enteric CH_4 emissions, either by direct inhibition or depopulation of rumen methanogens or through encouraging alternative microbial pathways of removing rumen hydrogen (e.g. Martin et al. 2010, Cottle et al. 2011), but *in vitro* -effects are often difficult to replicate *in vivo* (van Zijderveld et al. 2011) or are short-lived (Guan et al. 2006).

Dairy cow dietary P intake is often in excess of requirements (e.g. Powell et al. 2002, O'Rourke et al. 2010) and improved matching of requirement in the diet can result in significant reductions in P excretion (Dou et al. 2002) without compromising production or fertility (Wu and Satter 2000). The subsequent reduction in environmental impact of excreted P was reported by O'Rourke et al. (2010), who observed a 63% reduction in manure total P content from a 43% reduction in dietary P, and a significant reduction in the P concentration in overland flow following manure application for manure form the low dietary P treatment. However, the same authors also concluded that the time interval between manure application and the generation of overland flow has a greater impact on P losses than does varying the dietary P content.

Manipulating the protein content of the diet, both in terms of the total amount and the form of the protein has been shown to have significant effects of the amount of N excreted by cattle. Many studies have shown the potential that reducing the crude protein (CP) content of the diet can have on N excretion (e.g. Kulling et al. 2001, Broderick 2003) and therefore subsequent losses of N to the environment. Misselbrook et al. (2005b) showed this for a lactating dairy cow diet with a CP content of 14% compared with one of 19% (with the same proportion and type of forage), but also showed the influence of including condensed tannins in the diet (through manipulation of forage type), with significant reductions in NH_3 emissions from the cattle excreta. Both dietary strategies had the effect of reducing total N excretion by the cattle, predominantly by reducing urinary N excretion (which is more susceptible to environmental losses, at least in the shorter term, than faecal N).

Dietary manipulation at grazing relies on management of the sward composition, The use of grass varieties with a high content of water soluble carbohydrate, so called high sugar grasses (HSG) can reduce N excretion by cattle and enhance productivity (Miller et al. 2001, Moorby et al. 2006). In a recent trial, CH_4 emissions from growing lambs grazing HSG were also shown to be reduced, by an absolute value of 20%, when compared with lambs grazing a conventional ryegrass sward, and also showed increased intake values and live weight gain (Anon 2010). Inclusion of red clover in the sward, with the protein-binding action of the polyphenol content (Jones et al. 1995), has been hypothesized to reduce N excretion, but empirical evidence is not supportive (e.g. van Dorland et al. 2007, Powell et al. 2009).

Model scenarios for diet manipulation

Farm scale modelling enables the impact of dietary (and other) strategies on a number of potential production and pollutant outputs to be assessed and, in particular, highlight where trade-offs in impacts may have to be made. The farm scale model, $SIMS_{DAIRY}$ (Del Prado et al. 2011) was used to assess the impact of two dietary strategies: i) growing and feeding HSG (i.e. replacing conventional grass cultivars); and ii) restricting CP intake either through the increased use of forage maize produced on-farm or by just reducing N concentration in the concentrates diet (depending on intensity of dairy system). Herd typologies were defined for a set of locations and intensity production systems (intensive/fully-housed, medium, extended), with full details given in Anon (2010). New (from associated experimental work in Anon 2010) and existing (Miller et al. 2001) experimental information at the animal level were incorporated on the effect of different methods on enteric CH_4 output, milk production and N excretion.

For the HSG scenarios (Fig 1), overall greenhouse gas emissions were reduced by up to 19% per litre of milk, through reductions in both CH_4 and N_2O. Ammonia emissions per litre of milk were reduced by up to 22%, mainly due to the combination of fewer hectares required to produce 1 litre of milk and also due to reductions in excreted N (particularly urine N). Reductions in N excretion were also associated with reductions in NO_x emissions, because of the smaller pool of inorganic N subject to nitrification. Nitrate leaching was not significantly affected. Despite the potential beneficial effect of HSG on greenhouse gas emissions, if reseeding is required more frequently than for conventional grass varieties (to ensure persistence of effect), then the reduction in emissions described above could be offset by an increase in soil N_2O emissions and a decrease in potential soil C storage.

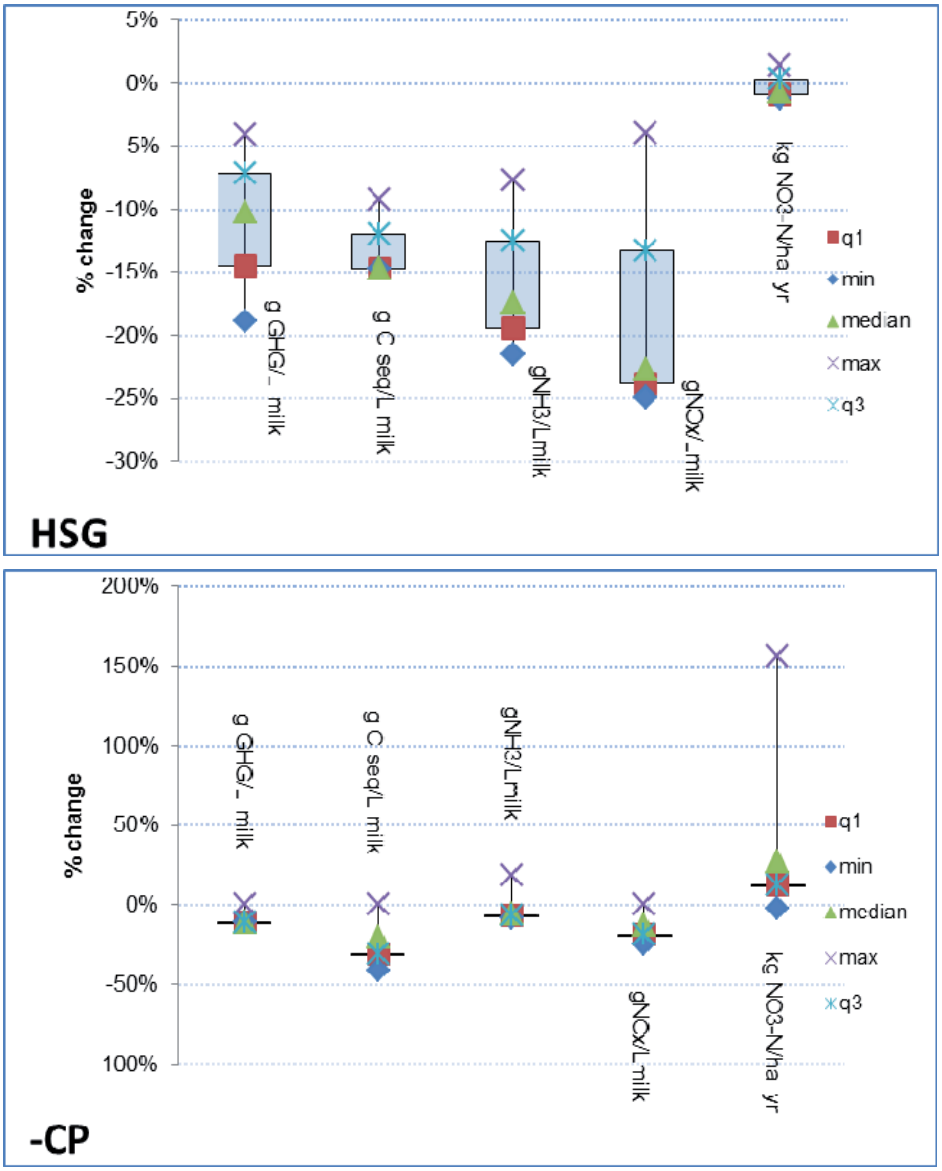

Figure 1. Change (%) in greenhouse gases (GHG), soil C storage, NH_3, NO_x and NO_3^- leaching for high sugar grasses (HSG) and reduced crude protein (CP) mitigation measures compared with baseline scenarios. Range reflects the model outputs across the range of locations and intensities of production.

For lower CP intake scenarios (Fig 1), overall greenhouse gas emissions were reduced by up to 11% per litre of milk. The details of the reduction depended on the intensity of the dairy system. For example, for the intensive-fully housed system there was no reduction in enteric CH_4 as the starch to fibre ratio was not altered in the diet, whereas N_2O emissions were reduced because of reductions in N excretion, particularly in urine. For medium and extensive systems, enteric CH_4 was reduced through a higher starch to fibre concentration in the diet, but soil N_2O emissions were increased mainly caused by the replacement of grass with forage maize and the changes in manure application rates and timing. The proportion of land use change from grassland to maize determined the extent of the potential soil C loss. Results for NH_3 and NO_x emissions very much depended on the intensity of the system, with a balance between reductions in N losses associated with lower N excretion and increases in N losses through indirect management changes after grassland conversion to maize. The main effect on NO_3 leaching losses was the conversion of grassland to maize and the associated changes in manure application timing.

Crop nutrient management

The soil nitrogen cycle is complex and potential crop uptake and losses to water and the atmosphere are very dependent on the form, rate and timing of the nitrogen inputs to the soil, soil texture and water status, and subsequent environmental conditions.

For inorganic nitrogen fertilisers, much can be achieved by attention to the type, timing and rate of application, ensuring that nitrogen supply matches crop requirements and is not applied in excess.

Urea fertiliser, in particular, can be associated with large NH_3 emissions (Misselbrook et al. 2004), particularly if used under hot, dry conditions. Use under cooler conditions, at low application rates will be associated with much lower emissions (Misselbrook et al. 2004), and the incidence of rainfall soon after application will also reduce emissions by ensuring rapid dissolution and transport of the urea into the soil matrix (Sanz-Cobena et al. 2011). There is some evidence that direct N_2O emissions are less from urea fertiliser applications than for other fertiliser types (Smith et al. 2012), but indirect emissions associated with the greater NH_3 emissions from urea would have been greater, so on balance there was no overall difference between fertiliser types. Emissions of N_2O may increase disproportionately with fertiliser application rate, as shown for fertiliser applications to grassland at three sites in England by Cardenas et al. (2010) where the annual emission factor (proportion of total fertiliser N applied during the year lost as N_2O) was greater for higher cumulative annual application rates.

The use of forage legumes, such as clover in grass leys, offers the potential to offset applied inorganic N with biologically fixed N. Perceived disadvantages with the use of white clover are year to year variation in sward content and persistence (Frame et al. 1986). With greatly increasing fertiliser prices in recent years, there is a growing resurgence of interest in forage legumes, and a combination of improved traits through breeding and improved management practices may overcome some of these main perceived disadvantages (Parsons et al. 2011). Whilst the clover is growing, soil N_2O emissions are generally smaller than those from inorganic fertilised soils as N originating from biological fixation is generally less available for nitrification and subsequent denitrification. Bacteria fix the N_2 gas from the air into the NH_4^+ ion that is largely used by the clover to form protein compounds. Once the legume crops are harvested, however, the protein compounds in residues are susceptible to decomposition and mineralisation to NH_4^+, which can then be nitrified and denitrified, leading to N_2O emissions (Snyder et al., 2009).Nitrate leaching losses have been shown to be lower from grass-clover pastures than from fertilized grass (e.g. Hooda et al. 1998, Stopes et al. 2002), although may be similar for equivalent levels of N input (Sprosen et al. 1997, Scholefield et al. 2002).

Urease and nitrification inhibitors offer potential to reduce nitrogen emissions from fertiliser applications. Urease inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), delay the hydrolysis of urea to ammonium (Gill et al. 1999), thus delaying the opportunity for NH_3 emissions to occur. Significant reductions (40 – 70%) in NH_3 emissions from urea fertiliser have been demonstrated using NBPT (e.g. Sanz-Cobena et al. 2008, Zaman et al. 2008, Chambers and Dampney 2009).

Nitrification inhibitors block the conversion of ammonium to NO_3^- (Amberger 1989), thus the N is retained in the soil for longer in the ammonium form, thereby being less susceptible to losses via NO_3^- leaching and denitrification. A recent meta-analysis of literature research results by Akiyama et al. (2010) suggested a mean reduction in N_2O emissions of c. 40% through the use of nitrification inhibitors over a range of soil types and climatic conditions. A significant body of research has been conducted in New Zealand over the past 7-8 years assessing the use of nitrification inhibitors to reduce NO_3^- leaching and N_2O emissions from pasture systems, assessing reductions in emissions from urea fertiliser applications and urine returns by grazing livestock through the use of dicyandiamide (DCD). Reductions in N_2O emission of up to 90% have been reported (de Klein and Eckard 2008), although Clough et al. (2007) proposed a more conservative 50% reduction to be applied to the emission factors used within the New Zealand inventory. Pasture yield increases are also reported from some studies, but not consistently (de Klein and Eckard 2008).

When using nitrification inhibitors with urea fertiliser or urine, there is the potential to reduce N_2O emissions and NO_3^- leaching at the expense of increased NH_3 emissions, as the N is being retained in the ammonium form for longer. The use of a double inhibitor (urease and nitrification) may prevent such trade-offs, but this has not been shown consistently (Zaman and Blennerhassett 2010).

There are opportunities to mitigate environmental impacts from manure management throughout the management continuum of housing, storage and spreading (Sommer and Hutchings 2001, Sommer et al. 2006, Chadwick et al. 2011). Opportunities are limited during the cattle housing phase, and depend also on choice of system. In general, a slurry-based system is associated with greater NH_3 emissions throughout the management continuum than a straw-bedded deep litter system (Thorman et al. 2003) For a slurry-based housing system, there may be some potential in the rapid removal of excreta from fouled concrete areas to storage and in the use of urease inhibitors to reduce NH_3 emissions (e.g. Varel et al. 1997, Misselbrook et al. 2006). For straw-bedded deep litter systems, NH_3 emissions can be reduced through the targeted use of straw bedding to ensure adequate supply particularly in key locations (Gilhespy et al. 2009).

Options for reducing gaseous emissions during slurry storage include covering the store, the effectiveness of which will depend on the nature of the cover (e.g. Sommer et al. 1993, Blanes-Vidal et al. 2009, van der Zaag et al. 2010b) with natural crust formation providing some mitigation (Misselbrook et al. 2005a, Petersen et al. 2005). Anaerobic digestion of slurries can reduce CH_4 emissions if the gas is properly captured and utilised, but increased availability of N in the digestate may increase losses of NH_3, N_2O and NO_3^- leaching during subsequent storage and application to land if not properly

managed. Minimising slurry storage during warmer months will reduce CH_4 emissions (van der Zaag et al. 2010a) and NH_3 emissions (Sommer et al. 2006). Covering and compaction of farmyard manure heaps can decrease gaseous emissions (Chadwick 2005), although may not be widely viewed as a practical measure.

As with inorganic fertilisers, rate and timing of application are important in managing the environmental impact of manure applications to land. Smith et al. (2002) showed a very clear relationship between NO_3^- leaching, crop N uptake and timing of application for slurry applications to freely draining soils in England, with up to 50% of applied N being lost via leaching and largest losses from applications in the September to November period. Application technique has a large effect on NH_3 losses following slurry application, and significant reductions can be achieved through using slurry application techniques designed to minimise the emitting surface area and/or encourage slurry transfer to the soil matrix. Compared with surface broadcast application, reduction in emission of the order of 50-80% can be achieved using shallow injection, 40–60% using trailing shoe (designed for applications to grassland) and 10-40% using band spreading (more suitable for use in growing crops) (e.g. Misselbrook et al. 2002). Emissions may be further reduced by applying slurry beneath a more developed crop canopy, using band spreading (to arable crops) or trailing shoe (to grassland) application, where the combined effects of reduced air speed and temperature at the ground surface and the direct uptake of emitted NH_3 by the crop canopy reduce emissions significantly compared to slurry applied to a bare surface (Thorman et al. 2008). Slurry application by trailing shoe to grassland can increase the window of opportunity for applications to be made; Laws and Pain (2002) and Laws et al. (2002) showed that grazing or silage harvesting could be made sooner after slurry application with this technique, compared with surface broadcast application, with no detrimental effects. The effect of slurry application technique on N_2O emissions is less clear, with some reports of increasing emissions (e.g. Flessa and Beese 2000, Wulf et al. 2002, Velthof et al. 2003), which might be expected in particular for slurry injection where the anaerobic conditions in the injection slots with high available nitrogen and carbon concentrations would favour denitrification, and other reports of no net increase when compared with surface broadcast application (e.g. Sommer et al. 1996, Vallejo et al. 2005).

Model scenarios for nutrient management

Nutrient management scenarios, specifically aimed at mitigating greenhouse gas emissions through improvements in fertilization management, were evaluated in a modelling study using $SIMS_{DAIRY}$ (del Prado et al. 2010). The scenarios consisted of firstly, optimization of mineral fertilizer N application rates and timing, and secondly, the use of nitrification inhibitors. Mineral fertilizer N use (rate and timing) was optimized using the in-built routine within $SIMS_{DAIRY}$ according to one of three criteria: (i) to maximize the efficiency ratio (defined as kg N in herbage per kg N loss (Brown et al. 2005); (ii) to maximize annual herbage N production; or (iii) to meet a field-specific target for annual herbage N production equal to that of the baseline farm. Values were averaged for a range of farms differing in site conditions and nutrient use intensity.

Tactically matching the plant N requirements to the rate and temporal distribution of mineral N fertilizer through $SIMS_{DAIRY}$´s optimization led to a reduction in overall N losses. For example, NH_3 emissions were reduced by about 10%, NO_x by 97% and NO_3 leaching by 6-14%. Denitrification losses were also decreased but site conditions greatly influenced the form of N loss (i.e. as N_2O or N_2). Nitrogen optimisation for the drier site with light soils was carried out favouring fertilization applications at weather conditions that promoted smaller N_2 losses but large N_2O:N_2 ratios. As observed in a previous study by del Prado and Scholefield (2008), the optimized fertilizer distributions were achieved by lower annual rates of inorganic N fertilizers and higher relative rates in early spring. Lowering the total annual fertilizer rate also reduced the indirect pre-farm CO_2 emission due to fertilizer manufacture.

Use of white clover in grass leys as a substitute for inorganic fertilizer N was one of the main differences between a conventional and organic dairy farm in a simulation by del Prado et al. (2011) using $SIMS_{DAIRY}$. Greenhouse gas emissions per litre of milk were lower by 11-25%, although differences in C sequestration, with the organic system assumed to be ploughed and reseeded every 5 years to ensure persistence of clover in the sward, were not taken into account. Ammonia emissions and concentration of NO_3^- in leachate were also lower for the organic system.

Nitrification inhibitors (e.g. DCD) added to both mineral N and manures applied to land reduced most forms of soil N losses. Whereas N_2O and NO_3 leaching were reduced up to 55 and 40%, respectively, emissions of NO_x and NH_3 were not substantially affected. Nitrous oxide, for example, was greatly reduced as a consequence of a simulated increase in plant N use efficiency and a reduction in the rate of nitrification (and, therefore, subsequent denitrification). Greater reductions in emissions were achieved for drier soil conditions. The mitigation of N_2O emissions was also greater in light-textured soils than in heavy-textured soils, which reflects, at least indirectly, the more effective nitrification inhibition found by experimental evidence in lighter soils with low organic matter content (e.g. Sahrawat and Keeney 1985).

Grazing management

Dairy farms demonstrate a number of different strategies in terms of grazing management, ranging from year round grazing (where climate and soil conditions allow) to year round housing for all or part of the herd. Webb et al. (2005) discussed the trade-off between grazing strategies in terms of NO_3^- leaching losses, expected to be greater from grazing livestock from the high N intensity urine patches, and NH_3 emissions, expected to be greater from housed livestock through the manure management continuum. They concluded that for a conventional UK system of approximately 6 months housing, extending the grazing season by one month in each of the spring and autumn periods reductions in NH_3 emissions would be more than offset by increases in NO_3^- leaching in terms of total N loss. Recent research has indicated that increasing the housing period can reduce N_2O emissions at the farm level, both from indirect and direct emissions by about 10% (e.g. de Klein et al. 2006, Luo et al. 2008). However, pre-farm CO_2 emissions from mineral fertilizer manufacture increased substantially due to a shift towards more forage area needed for grass for conservation and hence more total mineral fertilizer needed. Using $SIMS_{DAIRY}$, del Prado et al. (2010) suggested that reducing grazing during the wetter parts of the season (by c. two months) reduced GHG emissions per litre of milk. Increasing the housing period can reduce N_2O emissions, especially through a more uniform return of excreta via managed manure compared with very localized urine returns deposited by grazing (Oenema et al. 2006). There is also more potential for improved ration formulation when animals are housed and there is greater control over diet (Chadwick et al. 2008a).

Model outputs are very dependent on system conditions (production system, soil and climatic conditions). For example del Prado et al. (unpublished data) showed that simulations of UK dairy farms under projections of future climate change scenarios resulted in more productive farms for most future time-slices and for most regions of the UK, mainly caused by a longer grass growing season. One proposed potential adaptation measure is to increase the grazing season by this extra growing season time (e.g. one month). The implications on other pollution N and C losses were not consistent across all regions. For example, for the South West UK region in the 2020s time-slice this adaptation measure implied pollution swapping between N emissions to water and to air (Fig 2). There were much larger NO_3 leaching losses than in the un-adapted scenarios and slightly larger N_2O emissions and enteric CH_4 emissions. Methane from manure management would be greatly reduced by requiring smaller storage volumes of manures. Overall net greenhouse gas emissions (as kg CO_2eq l^{-1} milk) were reduced by increasing the grazing season, as were NH_3 and NO_x emissions. The net farm income and the other socio-economic scores all improved. Milk quality, for example, improved because of the shift to a larger proportion of fresh grass (grazed) over silage in the forage diet, associated with a better profile of polyunsaturated fatty acids in the milk. Animal welfare scores improved because of implied improvements in lameness and on the social structure of the cattle. Feeding cows mainly on fermented herbage (silage) also poses increased risks, which are principally generated by undesirable microorganisms (e.g. Listeria), undesirable chemicals (mycotoxins), and excess acidity (Wilkinson 1999).

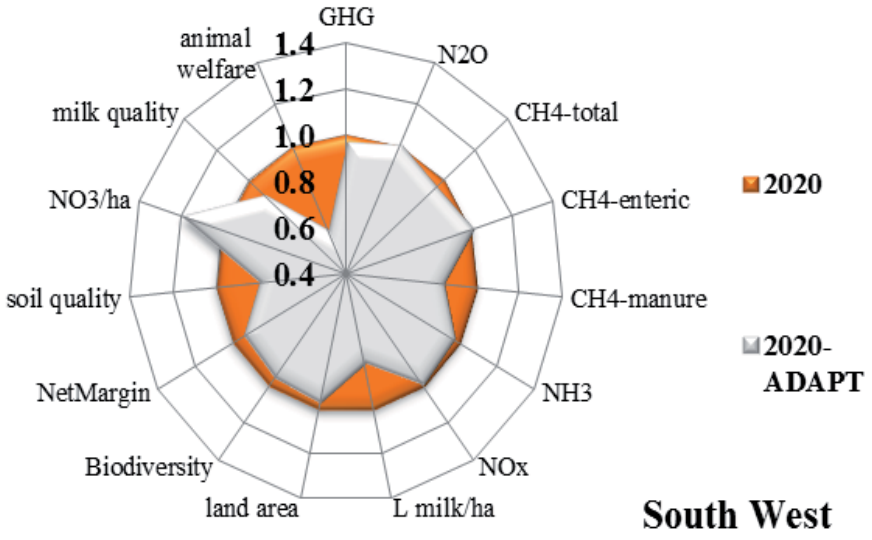

Figure 2. Comparison between simulated results in terms of N, C pollution and other socio-economic parameters (e.g. biodiversity, animal welfare, soil quality, economics) between un-adapted and adapted (one extra grazing month) dairy farm in the South West UK (2020). Values for the adapted scenario <1 indicate an improvement over the baseline scenario.

Genetic potential

Genetic improvement of livestock is a particularly effective technology, producing permanent and cumulative changes in performance. Wall et al. (2010) discuss the use of genetic selection tools for breeding schemes with the aims of improving productivity and efficiency and, potentially, selecting for inherently low CH_4 emitting animals, although it is important that this selection is on the basis of multiple traits including feed efficiency and yield to ensure that gains are realised as reduced emissions per unit product. Improvements in fertility would lead to a reduction in the required number of replacement animals, as discussed previously. However, it should be noted that dairy cows must breed to lactate and a reduction in total livestock numbers can only be achieved with improved fertility in dairy cows if a greater proportion of the dairy-bred calves can replace beef-cow calves, i.e. through the use of a beef bull.

Improved N use efficiency by grass varieties is an on-going aim of breeding programmes. However, while this may result in lower N losses through reduction in fertiliser requirement, an enhanced grass CP content could increase N excretion by cattle, thereby leading to increased losses from grazing returns and manure management (del Prado et al. 2010). Breeding for increased polyunsaturated fatty acid content, potentially decreasing enteric CH_4 emissions may be another aim, although Dewhurst et al. (2001) noted that genetic variation in this trait is small compared with variation through the growing season. Other plant changes may involve traits in the shoot to root biomass ratio or plants with exudates capable of altering the mineralization rate from decaying biomass remaining after harvest or grazing. Both measures have potential trade-offs between N forms lost (Del Prado and Scholefield 2008).

Combinations of measures

Del Prado et al. (2010), using $SIMS_{DAIRY}$, investigated the potential errors incurred if we estimate the effectiveness of GHG mitigation measures in combination compared with studies where the effectiveness of each method applied singly is simply added together. This latter, linear approach obviously ignores many of the potential synergies that may occur when applying different methods affecting soil, plant and/or animal components of the farm system. It also ignores the fact that some mitigation options may be mutually exclusive. The extent to which mitigation methods target processes that are interrelated is key to estimating the effectiveness of combined mitigation methods. The results from the del Prado et al. (2010) study indicated that for the measures considered in the scenario, the overall impact of applying a combination of measures was less than the simple addition of the effect of the measures applied singly.

Conclusions

Dairy production undoubtedly impacts upon the environment, particularly through emissions of NH_3 and greenhouse gases to the atmosphere and transfers of pollutants to water. Research has improved our knowledge of the transfer processes and enabled the (on-going) development of a range of mitigation measures. However, it must be accepted that within the complex biological systems involved in dairy production, the complete elimination of environmental impacts is impossible.

Most of the mitigation measures discussed in this paper are associated with systemic improvements in the efficiency of production in dairy systems, rather than specific end-of-pipe technological fixes (although these may also have a place). Much can be achieved through attention to livestock health, matching dietary requirements with supply, attention to the quantity, timing and method of application of nutrients to forage crops and utilising advances made through genetic improvements. The relative impact of many of the mitigation measures is specific to the soil, climate and management system of a particular dairy farm and therefore the use of decision support tools to explore alternative scenarios, and identify site-specific optimum practices are recommended.

Areas where further research and development are required include on-going genetic improvements in livestock and plant traits, development of diets or additives which have a consistent and persistent inhibitory effect on CH_4 production in the rumen, assessment of alternative plant species and varieties for inclusion in grazed and ensiled forages, cost-effective delivery mechanisms for using urease and nitrification inhibitors, and a more complete accounting for the effects of silage production and management on forage quality in existing farm-scale models.

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Grass silage management affecting greenhouse gas emissions and farm economics

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Keywords: grass silage, management, greenhouse gas emissions, economics

Introduction Limiting the losses at harvesting, storage and feed out period have a positive effect on dry matter (DM) production, nutritional value and intake of grass silage. A higher feeding value results in a higher intake by dairy cows and in a reduction of methane production per kg milk (Tamminga et al. 2007). A higher DM production and nutritional value reduce feed purchases which has a positive effect on farm economics and energy-related CO_2 emissions. The objective of this study was to evaluate the effect of grass silage management on greenhouse gas (GHG) emissions and farm economics under Dutch conditions.

Material and methods A survey was made on DM and Net Energy for lactation ($NE_{L)}$ losses during harvesting, conservation, storage and feed out period of grass silage. Together with a whole farm dairy model (Dairy Wise) (Schils et al., 2007) the results of the survey were used to calculate the effects of extra losses of grass silage due to aerobic deterioration and poor fermentation on GHG emissions and farm economics. A case study was performed for a farm with 100 dairy cows, 43 ha of grassland and 11 ha of silage maize land.

Results and discussion Field losses varied from 5.3 to 18.8% DM, conservation and storage losses from 4.2 to 14.4% DM and total feed out losses from 3.0 to 13.5% DM (Table 1). During each stage for losses besides DM losses also decrease in nutritive value can occur which results in higher total losses of NE_L than of DM. Based on information of silage analyses in practice it was hypothesized that under common practical conditions, there is a considerable risk of suboptimal utilization of energy and nutrients from grass silage due to aerobic deterioration and moderate conservation. In a relative dry year grass is often ensiled at a DM content higher than 50% which involves a considerable risk for losses due to aerobic deterioration, while in a relative wet year grass is often ensiled at a suboptimal DM content which increases the risk for decreased fermentation quality. Therefore the effects of extra losses due to aerobic deterioration and moderate conservation where calculated under conditions of a dry and wet year, respectively. In a dry year the extra losses due to aerobic deterioration of grass silage (6.5% DM and 8.8% NE_L) causes also extra feed out losses (4% DM) and a lower intake (8% DM) (Table 2). The GHG emissions were 1.3% higher than in a situation with no extra losses. This was mainly caused by CO_2 emissions related to a higher purchase of roughage and concentrates. Net return to labour and management decreased almost by € 4,000. In a wet year with moderately preserved grass silage, GHG emissions were 1.4% higher than in a normal year with well-preserved grass silage (Best practice). This increase was mainly caused by CO_2 emissions related to a higher purchase of concentrates. The net return to labour and management was almost € 3,200 lower in this scenario. By using an adequate silage additive, GHG emissions were 1.0% higher than in a normal year and net return to labour and management was still € 2,300 lower than the benchmark.

Although the results are not shown in table 2, protein losses have also been taken into account in the calculations of GHG emissions and farm economics.

Conclusions It was concluded that aerobic deterioration and decreased fermentation quality of grass silage increase GHG emissions to a limited extent and furthermore have a considerable negative effect on net return to labour and management.

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Table 1. Typical losses of DM and NE_L during the conservation of grass as silage.

		DM-losses (%)	NE_L decrease (%)	NE_L losses (%)	Risk of extra losses[1]
Field losses	Mowing	1.2 – 2.0	-	1.2 – 2.0	I
	Tedding	2.4 – 6.4	-	2.4 – 6.4	II
	Rowing and loading	1.7 - 3,4	-	1.7 - 3.4	I
	Respiration and leaching	0.0 - 7.0	0.0 - 3.0	0.0 - 9.8	II
Total		*5.3 - 18.8*	*0.0 - 3.0*	*5.3 - 21.6*	
Conservation and storage losses	Conservation	3.0 - 10.0	2.0 - 8.0	4.9 - 17.2	III
	Effluent	0.0 - 2.0	0.0 - 1.0	0.0 - 3.0	II
	Storage	1.2 - 2.4	1.2 - 2.4	2.4 - 4.7	III
Total		*4.2 - 14.4*	*3.2 - 11.4*	*7.3 - 24.9*	
Feed out losses	Removal, residues	3.0 - 7.0	-	3.0 - 7.0	IIII
	aerobic deterioration	0.0 - 6.5	0.0 - 2.5	0.0 - 8.8	IIII
Total		*3.0 - 13.5*	*0.0 - 2.5*	*3.0 - 15.8*	

[1] I = low risk IIII = high risk

Table 2. Greenhouse gas emissions and economics on farm level under conditions of good management, a relative dry year and a relative wet year.

	Best	Dry year		Wet year	
	practice	No AD[1]	With AD[1]	Good conservation	Moderate conservation
Grass silage					
Cutting yield (kg DM/cut)	3,309	3,149	3,098	3,445	3,461
DM content (%)	40	40	50	30	30
NH3-fraction (NH3-N of total N,%)	8	8	6	10	13
NEL (MJ/kg DM)	6.20	+0.04	-0.13	-0.08	-0.17
Feed purchase					
Silage maize (kg DM)	16,745	+4,665	+12,230	-11,777	-39
Concentrates (kg)	224,636	+717	+16,704	+13,032	+17,364
Harvest, storage and feeding					
Field losses-DM (%)	5.51	+0.18	+0.21	-0.06	+1.11
Conservation losses- DM (%)	3.06	0	-0.51	+2.06	+4.63
- NE_L (%)	7.07	0	-0.79	+3.2	+7.1
Aerobic deterioration losses- DM (%)	0	0	+6.5	0	0
- NE_L (%)	0	0	+8.8	0	0
Feed out losses- DM (%)	3	0	+4	0	2
Relative intake (%)	100	+1	-8	-5	-6
Greenhouse gas emissions (kg/kg milk)					
Energy related CO_2	0.287	+0.002	+0.016	+0.002	+0.009
Nitrous oxide in CO_2 equivalents	0.193	+0.001	+0.001	+0.004	+0.005
Methane in CO_2 equivalents	0.516	-0.001	-0.002	+0.003	+0.000
Total CO_2 equivalents	0.996	+0.002	+0.015	+0.009	+0.014
Economics (€/farm)					
Feed costs – Roughage	2,138	+428	+1,123	-1,082	-4
- Concentrates	37,275	+90	+2,189	+2,545	+3,197
Silage additive	0	0	0	+1,465	0
Net return to labour and management	8,906	-1,008	-4,785	-2,302	-3,172

[1]AD=Aerobic deterioration

Occurrence of volatile organic compounds and ethanol in different types of silages

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Keywords: ethanol, ethyl acetate, ethyl lactate, volatile organic compounds (VOC)

Introduction Based on observations from commercial farms that well preserved, but odd smelling maize silages may cause problems regarding feed intake and milk yield by dairy cows, volatile organic compounds (VOC) were analyzed in recent studies (Weiss et al. 2009a, b). Strong correlations were found between ensiling conditions, type of silage additive and ethanol content and the concentrations of the ethyl esters – ethyl acetate (EA) and ethyl lactate (EL). In addition, those substances have been discussed in relation to climate-damaging ozone formation, and it was reported that silages on dairy farms may be a significant source of VOC emission (Mitloehner et al. 2009). The aim of the current study was to determine the incidence and concentrations of VOC in silages, particularly those of ethanol and esters, and to describe the correlations between individual VOC.

Material and methods Data on VOC from numerous laboratory ensiling experiments as well as from farm silages was used (table 1). Lactic acid was analyzed by HPLC, whereas volatile fatty acids, alcohols and VOC were determined by GC after cold water extraction. Based on the available data set, a regression model was developed to predict ester concentrations as a function of ethanol content. This model excluded the effects of the experiment.

Table 1. Characterization of the data set.

Type of Silage	DM g/ kg	n	Storage length (days)	Silage additives
Lab-scale ensiling experiments				
Whole-crop maize 1	310	60	60, 90	biological, chemical (Weiss et al. 2009a)
Whole-crop maize 2	316	30	2,14, 28, 49, 90	biological (Weiss et al., 2009b)
Whole-crop maize 3	349	180	2,14, 28, 49, 90	biological, chemical *)
Whole-crop maize 4	332	12	90	chemical (Weiss and Auerbach, 2012)
Whole-crop maize 5	315 – 513	79	112 anaerobic, 0-8 erobic	without (Gerlach et al. 2011)
Whole-crop wheat	276	34	60, 90	biological *)
Sorghum 1 (*Goliath*)	216	42	56, 105	biological, chemical (Auerbach and Weiss 2012)
Sorghum 2 (*Maya*)	261	42	56, 105	
High-moisture corn	635	30	97	biological, chemical *)
Natural grassland	406	33	86	biological *)
Commercial farm silages				
Whole-crop maize 6	254 – 322	3	approx. 90	without (Weiss et al. 2009a)
Whole-crop maize 7	299 - 403	11	approx. 90 - 180	biological *)

* (Weiss and Auerbach, unpublished)

Results and discussion Regardless of silage type, silage additive and ensiling conditions, in the most cases there was found a strong correlation between ethanol and ester concentrations, thereby confirming the pure chemical nature of the reaction of ester formation. Elevated levels of ethanol and EA and EL were not only detected in maize silages, but also in silages from whole-crop cereals and sorghum (table 2). Ester and ethanol levels were highest in silages stored under strict anaerobic conditions. It was also shown that esters remain detectable in silages for a few days after opening of the silos under aerobic conditions (Weiss et al. 2011). Data from farm silages presented in Table 2 also showed high ethanol and ester concentrations. These silages were well fermented and highly compacted. In 7 out of 14 silages biological additives were used.

Results from ensiling experiments on the effects of silage additives on ester formation clearly indicated that only chemical products containing active ingredients with specific antifungal effects (sodium benzoate and potassium sorbate) can significantly reduce ester concentration, whereas buffered formic acid-containing products, which were always applied at 4 l/t, stimulated it due to an increase in ethanol content (Weiss and Auerbach 2012; Auerbach and Weiss 2012).

Silages were grouped based on their ethanol content. Figure 1 gives the average total ester

concentration for each ethanol class. It can be derived from the regression equation that for each increase in ethanol by 5 g/kg DM, total ester concentration increased by 100 mg/kg DM. Thus, the ratio between ethanol and total esters was determined to be approximately 50:1.

Conclusions Ethyl esters of lactate and acetate can be frequently found in silages from different crops. Their concentrations are strongly positively correlated with the ethanol content.

Table 2. Contents of volatile organic compounds, especially esters and their correlation, in different types of silages.

Type of Silage	Lactic acid	Acetic acid	Ethanol	Ethyl acetate (EA)	Ethyl lactate (EL)	Regression EA+EL(y), Ethanol (x)	
	g/ kg DM	g/ kg DM	g/ kg DM	mg/ kg DM	mg/ kg DM	y = ax + b	R^2
Whole-crop maize 1	6.9 – 74.8	5.8 – 79.4	0.9 – 51.7	12 – 284	16 – 379	12.50x+ 91.2	0.70
Whole-crop maize 2	32.5 – 119.8	8.6 – 25.8	3.2 – 28.3	55 – 343	30 – 683	26.47x+121.5	0.65
Whole-crop maize 3	13.7 – 67.4	0.5 – 26.7	3.3 – 20,1	38 – 639	0 – 224	18.10x+ 91.7	0.20
Whole-crop maize 4	73.8 – 124.6	5.3 – 29.2	6.2 – 50.8	116 – 262	156 – 661	11.55x+266.0	0.93
Whole-crop maize 5	0 – 75.5	0 – 36.6	0 – 36.9	0 –1109	0 – 986	52.51x+ 0.2	0.88
Whole-crop wheat	20.7 – 99.9	9.1 – 42.4	21.9–121.8	84 – 951	309 - 1277	6.76x+684.0	0.24
Sorghum 1 (*Goliath*)	17.1 – 148.4	4.7 – 58.0	4.7 – 43.1	0 – 154	81 –1137	17.33x+ 49.5	0.45
Sorghum 2 (*Maya*)	16.0 – 101.3	21.3 – 54.3	6.5 – 43.7	39 – 223	172 – 911	17.32x+150.5	0.53
High-moisture corn	6.1 – 20.7	1.0 – 14.5	0.2 – 7.6	0 – 107	0 – 47	17.62x+ 0.3	0.78
Natural grassland	38.5 – 61.3	4.3 – 32.7	4.9 – 15.1	0 – 76	104 – 246	6.98x+115.1	0.22
Whole-crop maize 6	11.3 – 70.8	25.8 – 48.7	21.0 – 64.0	357 – 789	118 -1263		
Whole-crop maize 7	37.2 – 86.9	10.4 – 28.3	1.1 – 24.1	12 – 64	47 -1305		

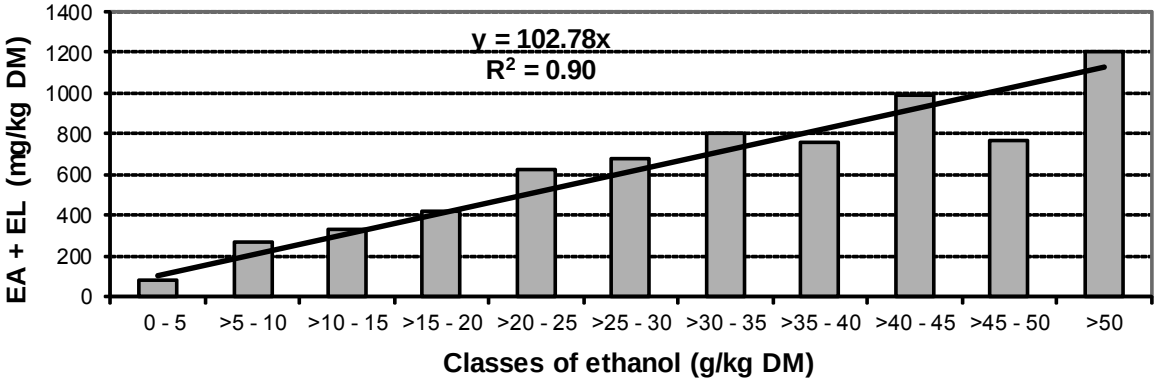

Figure 1. Average total content of esters (ethyl acetate and ethyl lactate) in classes of ethanol

Acknowledgements We wish to thank Katrin Gerlach and Karl-Heinz Südekum, University of Bonn, Germany, very much for provision of selected data.

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Nutrient use efficiency in different harvesting strategies of silage swards based on timothy and two fescue species

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Keywords: cutting time, grass, harvesting strategy, nutrient use efficiency, silage

Introduction Mineral nutrient composition of grasses is usually used to evaluate the effect of mineral intake in feeding. The concentration of nitrogen (N) is integrally related to the protein concentration of the dry matter (DM). Along with calcium, phosphorus (P) has an important role in the mineral needs of any animal. It is known that grass swards can uptake so great amounts of potassium (K), that it addresses some problems concerning the balance of mineral intake of the cattle, since a surplus of K can affect the balance between the univalent and bivalent minerals.

In addition to implications on feeding, the composition of mineral nutrients in forage also reflects the removal of nutrients from the soil and thus the nutrient use efficiency (NUE) of the crop. By changing the cutting time of silage swards, both DM yield and the content of mineral nutrients of the yield change, which may have evident implications for NUE. The choice of harvesting strategy of silage, i.e. the combination of different cutting times and the number of cuts, can be a farm-specific solution. At the moment, there is no advisory framework for the timing of the cuttings in regard to NUE or environmental effects of forage production. For environmental and economical reasons, the aspect of NUE of different harvesting strategies should be recognized along with the effects to animal performance.

Materials and methods The DM yield accumulation and composition of mineral nutrients in different harvesting strategies of grass silage swards were studied in Maaninka and Ruukki, Finland, in three different field experiments with 6–12 m^2 plots in randomized block designs. During 2009-2011, Experiment I (Maaninka) had three replicates of mixture with timothy (TIM) and meadow fescue (MF) and Experiment II (Ruukki) four replicates of pure TIM, both with four harvesting strategies: one three-cut strategy with the first cut at booting stage and consecutive cuts after approximately 6 weeks and again after 10 weeks, and three two-cut strategies with differing cutting times in the first cut (flag-leaf stage, booting stage and full flowering) with the regrowth being cut on average 7-8 weeks after the first cut, usually in mid-August. Experiment III consisted of TIM and tall fescue (TF) in pure stands with three replicates in Maaninka, which were cut at booting during the first cut and after 4, 6 and 8 weeks for the regrowth in 2006–2007.

The amount of applied mineral nutrients N, P and K (kg ha^{-1}) were recorded. Inside each experiment, the number of applications and nutrient levels were the same with the exception of plots for three-cut strategy in Experiments I and II, which were given a third application for the second regrowth. Nutrient levels were different between experiments, as they were based on soil type and the availability of nutrients according to soil samples. No chemical plant protection or irrigation was used. During each cut, the DM yields (kg DM ha^{-1}) were analyzed by harvesting the plots with Haldrup 1500 plot harvester and by determining for fresh matter yield, DM content and consequently DM yields. Concentration of N was analyzed with Kjeldahl method and mineral nutrients P and K (g kg^{-1} DM) were analyzed with ICP at MTT Laboratories, Jokioinen. Using these data, the annual NUE (nutrient yield divided by nutrients applied) for N, P and K were calculated. The effect of harvesting strategy (along with effects of species and year and their interactions) on DM yield, NUE_N, NUE_P and NUE_K were analyzed by SAS 9.2 Mixed procedure.

Results and discussion The effect of timing of the cut in first cut to annual DM yield and NUE_N, NUE_P and NUE_K was studied with Experiments I and II, while the timing of regrowth cut was examined with Experiment III (Table 1).

Over the three years, the delay in the timing of the first cut clearly increased the average annual DM accumulation in the two-cut harvesting strategies with the TIM-MF mixture (Experiment I) and the pure TIM (Experiment II) (Table 1). In TIM-MF there were no differences in annual NUE_N, NUE_P or NUE_K between two-cut harvesting strategies, which shows that the annual efficiency of N, P or K uptake into the sward is not increased even if the grass is let to mature until flowering stages. In Maaninka, were no K fertilization was given for the regrowth, the efficiency of K use in TIM-MF sward was high: the herbage was able to uptake over 8 times of K compared to the amount of K given in fertilizers. This demonstrates well the potential of grass swards in mobilization of nutrients from the soil.

In TIM sward with two-cut strategy, annual NUE_N and NUE_K were consistently but mildly increased by delaying the first cut (Table 1). This might be a result of a high content of available organic N in the soil in Ruukki and the potential of ample N reserves to maintain the uptake of K into the herbage (Alfaro

et al. 2003). The effect of organic N in soil can be seen also in the constantly high levels of annual NUE_N in Ruukki (typically over 1.30) when compared to annual NUE_N from sandy soil in Maaninka (below 1.00 in all harvesting strategies). With TIM in Ruukki, the rate of uptaking additional P was reduced after booting, as the level of annual NUE_P did not increase along with NUE_N and NUE_K.

In the three-cut strategy, the average annual DM yield over three years was intermediate in pure TIM, but the lowest in TIM-MF mixture when compared to all strategies (Table 1). In pure TIM annual NUE_N and NUE_K were the lowest of all harvesting strategies, while in TIM-MF the levels of annual NUE_N or NUE_K were no different from the two-cut strategies. With both sward types, annual NUE_P was the highest when taking three cuts.

When prolonging the time for regrowth, annual NUE_N showed no significant differences between harvesting strategies, even though DM yields increased as the cutting time of regrowth was delayed (Table 1). This points out that the uptake of N for regrowth takes place early after first cut, and the total amount of N is simply diluted into the accumulating DM (Eckersten et al. 2007). Annual NUE_K and NUE_P increased only until the regrowth week 6 and remained at the same level later on, which reveals that no new P and K was uptaken after 6 weeks of regrowth time.

When considering the effects of altered timings of harvest for the whole growing season, the two experiments in Maaninka can be used as a template. During both of the cuts, the NUE_N was not affected by the timing of harvest. Since the levels of NUE_N were the same in Experiments I and III but different from Experiment II in Ruukki, the availability of N in the soil might have been the restricting factor in N uptake. The differences in NUE_P between harvesting strategies were statistically significant, but minor in practice. Majority of the difference in the levels of NUE_K between experiments in Maaninka was due to different fertilization practices and availability of K in the soil.

Table 1. Annual dry matter (DM) yields and nutrient use efficiencies (NUE) of nitrogen (N), phosphorus (P) and potassium (K) in three different harvesting strategies for grass silage (timothy, TIM; meadow fescue, MF; tall fescue, TF). Differences between harvesting strategies inside each experiment are statistically significant (p <0.05) when marked with different letters (a, b, c, d).

			Maaninka				Ruukki			
Harvest strategy	First cut timing	Regrowth weeks	Annual DM yield	NUE_N	NUE_P	NUE_K	DM yield	NUE_N	NUE_P	NUE_K
			Experiment I: TIM + MF, 2009-2011				*Experiment II: pure TIM, 2009-2011*			
3 cuts	Flag leaf	+6; +10	8708 a	0.87	1.67 b	8.55	11239 b	1.30 a	3.52 c	2.04 a
2 cuts	Flag leaf	+8	8901 a	0.85	1.55 a	8.18	8919 a	1.29 a	2.69 a	2.08 a
2 cuts	Booting	+7	9513 b	0.86	1.57 a	8.45	10904 b	1.35 b	2.90 b	2.24 b
2 cuts	Flowering	+7	10216 c	0.85	1.55 a	8.38	12268 c	1.42 c	2.97 b	2.37 c
			Experiment III: pure TIM, pure TF, 2006-2007							
2 cuts	Booting	+4	7119 a	0.90	1.34 a	1.97 a	.	.	.	.
2 cuts	Booting	+6	8426 b	0.92	1.46 b	2.16 b	.	.	.	.
2 cuts	Booting	+8	9380 c	0.89	1.47 b	2.28 b	.	.	.	.

Conclusions Grass swards with timothy and fescue species show great capacity of nutrient uptake, since the efficiencies for P and K use are easily positive and in organic soils this is possible for N, too. Although the choice of harvesting strategy of silage did affect the nutrient use efficiency of the sward in some cases, the differences were not dramatic or remarkable in practice. Majority of N, P and K may be uptaken into the herbage well before the range of reasonable cutting times. Greater changes on NUE might be expected on different soil types and when the fertilization level of the sward is adjusted.

Acknowledgements:This study was partially financed by the EU Rural Development Programme for Mainland Finland, www.rural.fi.

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Co-ensiling temperate grasses to improve protein use efficiency in ruminants

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Keywords: Grass silage, protein, polyphenol oxidase, lambs, nitrogen use efficiency

Introduction Preserving high quality forage in cool humid regions of agricultural production remains a challenge due to potentially high levels of protein degradation during ensiling. Some forages such as red clover contain high levels of polyphenol oxidase (PPO) and *o*-diphenols to effectively inhibit proteolysis during the ensiling process (Jones et al. 1995, Sullivan and Hatfield 2006). Previous work has shown that some temperate grasses (i.e., orchardgrass and smooth bromegrass) contain high levels of polyphenol oxidase, but not sufficient levels of *o*-diphenol substrates to inhibit proteolytic activity during ensiling (Marita et al. 2010). Other grasses such as tall fescue and timothy contain high levels of *o*-diphenols but little to no PPO activity.

Materials and methods Experiments were carried out to evaluate the feasibility of co-ensiling a PPO grass with one that contains high levels of *o*-diphenol substrates. Forages chosen for this work included Orchardgrass (*Dactylis glomerata* L.; OG) and Smooth Bromegrass (*Bromus inermis* Leyss.; BG) as forage sources with PPO activity. Tall fescue (*Festuca arundinasea* Schreb.; TF) and Timothy (*Phelum pratense* L.; Tim) were used as forage sources of *o*-diphenols. Treatments were designed to test 1) individual forage performance and 2) combination forage performance with a PPO grass and an *o*-diphenol grass co-ensiled. Table 1 shows the seven treatments for each ensiling trial/feeding trial.

Table. 1 Forage combinations.

o-diphenol grass	PPO grass	Mixing conditions
None	OG	None
None	BG	None
TF	None	None
Tim	None	None
TF	OG	Field mix
TF	BG	Field mix
Tim	OG	Field mix
Tim	BG	Field mix
TF	OG	Mechanical mix
TF	BG	Mechanical mix
Tim	OG	Mechanical mix
Tim	BG	Mechanical mix

Forages were cut, wilted to the appropriate moisture level, chopped and macerated through a hammer mill before ensiling in plastic wrapped bales. Bales ranged form 500-800 kg at ensiling. After a minimum of thirty days of ensiling, silage bales were opened and fed to young lambs. Samples were removed when the individual silage bales were opened for feeding to the lambs. Total feed intakes were measured as well as total feces and urine excretion by individual lambs. Protein use efficiency (crude protein metabolizability, CPM, % of protein consumed and retained by the lambs) was calculated for each feed treatment.

Results and discussion Field plot yields were variable depending upon the grass type; TF =3760 kg/ha, Tim=2880 kg/ha, SB=5480 kg/ha, OG=3760 kg/ha, SB+TF= 4200 kg/ha 70:30, SB+Tim=3640 kg/ha 66:34, OG+TF=2840 kg/ha 54:46, and OG+Tim=4000kg/ha 70:30. For the mechanical mixes equal weights of the two forages were mixed to form the composite forage before maceration. Characteristics of the silage bales are summarized in Table 2. Dry matters ranged from approximately 25% to 30%. Conductivity measurements were made indicating the degree of maceration that was imposed upon the forages going in the treatment silage bales. All forages were macerated in order to help ensure an optimal level of mixing between the PPO enzyme containing grasses and the *o*-diphenol substrates containing grasses in the co-ensiled treatment bales. Conductivities for most grasses were greater than 70% indicative of a high level of cell breakage allowing cell contents (e.g. PPO enzyme and *o*-diphenol substrates) to mix during the ensiling process.

Feeding trials consisted of seven individual treatments per trial with two lambs per treatment. Each feeding trial was repeated for each treatment silage type using a second silage bale. This resulted in four individual feeding trials, two using the OG combinations and two with the BG combinations with four lambs for each treatment silage. After feeding all lambs a reference hay, lambs were provided treatment silages ad libidum and at restricted levels. All lambs gained weight over the course of all trials indicating that there were no nutritional limitations imposed upon the lambs with respect to diets

consisting solely of the ensiled grasses. Total nitrogen (N) inputs and outputs were measured on an individual lamb basis to determine if the total N use efficiency increased when PPO enzyme activity and *o*-diphenols were present in the co-ensiled treatment bales compared to the control bales. Differences between the intake of total protein and the excretion of N can be used to calculate the crude protein metabolizability (CPM, % of N consumed and retained within the lamb). The preliminary analysis on CPM is summarized in Table 3. For most of the silages N use efficiency (CPM) increased in lambs fed the co-ensiled treatment forages compared to the monoculture controls. This would indicate there is an advantage to feeding animals co-ensiled forages with one containing a source of PPO enzyme activity and the other a good source of *o*-diphenol substrates in order to prevent excessive protein degradation during the ensiling process.

Table 2. Dry matter content of individual silages and level of maceration by conductivity.

	Dry Matter		Conductivity			Dry Matter		Conductivity	
Bale type	Bale 1	Bale 2	Bale 1	Bale 2	Bale type	Bale 1	Bale 2	Bale 1	Bale 2
BG	26.3	26.1	57.5	83.9	OG	28.9	27.9	83.8	87.2
BG+TF (F)	24.7	23.8	82.2	90.5	OG+TF (F)	24.8	26.4	85.4	74.8
BG+Tim (F)	24.1	24.2	78.0	71.7	OG+Tim (F)	27.8	26.6	81.0	67.0
BG+TF (M)	25.4	25.3	77.2	85.0	OG+TF (M)	26.9	26.7	91.1	93.9
BG+Tim (M)	24.2	25.5	81.5	80.9	OG+Tim (M)	30.7	25.3	80.7	79.9
TF	28.1	30.1	61.6	65.1	TF	25.7	26.5	80.0	79.3
Tim	24.5	25.9	74.1	85.2	Tim	25.6	22.9	73.5	83.8

Table 3. Summary of N use efficiency for the different silages.

Grass silage type	Crude protein metabolizability	Grass silage type	Crude protein metabolizability
BG	0.1561	OG	0.2144
BG+TF (Field)	0.3043	OG+TF (Field)	0.3848
BG+TIM (Field)	0.1293	OG+Tim (Field)	0.2985
BG+TF (Mech)	0.1963	OG+TF (Mech)	0.2901
BG+Tim (mech)	0.1317	OG+Tim (Mech)	0.1673
TF	0.0549	TF	0.0950
Tim	0.118	Tim	0.2188

Conclusions Co-ensiling mixtures of PPO grass (BG or OG) with substrate grass (TF) has a positive effect on nitrogen use efficiency in sheep. Timothy also showed a positive impact when mixed with BG or OG, but the results were a little more variable.

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Milk production from silage: comparison of grass, legume and maize silages and their mixtures

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Keywords: clover silage, feed intake, grass silage, maize silage, milk production, milk quality

Introduction

This paper focuses on silages used for milk production in the maritime region of North-West Europe. Following the transition from hay to silage over the last half century, the main forage on many dairy farms is often grass silage, typically based on timothy and meadow fescue in cooler areas, and ryegrasses in more temperate areas. There are areas where red clover and maize are important, and experience in these areas has prompted renewed interest in alternative forages in other areas. Maize is a tropical crop and so has not always achieved an adequate level of maturity in cooler parts of the region; the use of other whole-crop cereals has expanded in some of these areas. Plant breeders have made considerable advances in achieving earlier maturing maize varieties that are more reliable in areas such as Northern Britain and Ireland.

The main focus of the paper is on feed intake, milk production and milk composition when cows are offered diets based on silages prepared from grass, red clover, white clover or maize. The high production potential of legume silages has long been recognised – both for white clover (Castle et al. 1983), red clover (Thomas et al. 1985), and lucerne (Hoffman et al. 1998). Some early studies evaluated milk production potential with silage as sole feed. Castle (1982) obtained milk yields of between 13.3 and 16.0 kg/day from high-digestibility grass silage as sole feed in mid-lactation. Rae et al. (1987) conducted a multi-year study with Spring-calving cows offered just grass silage and grazed grass. At one location, with high-quality silage, they obtained lactation yields of 4700 kg without feeding concentrates. Mean silage dry matter (DM) intakes were 13.2 kg/day and mean milk yields were 21.1 kg/day for cows and 16.1 kg/day for heifers in the 3-month period prior to turnout. Particularly impressive were the DM intakes (19.3 and 17.7 kg/day) and milk yields (26.8 and 19.6 kg/day) achieved by Castle et al. (1984) and Cohen et al. (2006) respectively with white clover silage as sole feed. Steinshamn and Thuen (2008) recorded milk yields of 22 kg/day when they offered diets based on grass silage with either white clover silage (28% of DM) or red clover silage (42% of DM) as sole-feed to cows in early lactation (average 74 days in milk). In addition to considering the effects of different silages on productivity and milk composition, this paper also considers effects on nutrient efficiency and emissions.

Chemical composition and digestibility

Table 1 uses mean feed values taken from INRA (2007) to illustrate the mean and range for grass, legume and maize silages. These relate to conditions in France, but the pattern of greater variation in grasses in comparison with legumes is evident in other areas.

Legumes generally contain more protein and less fibre than grasses, whilst maize contains less protein and less fibre than grass. Early work suggested that the rate of decline in digestibility is less for legumes than grasses (Ulyatt 1970, Thomas et al. 1981). Rinne and Nykänen (2000) showed a more rapid decline in the digestibility of timothy than red clover during primary growth. Hetta et al. (2004) showed that the rate of decline in digestibility was greater for timothy than for red clover during spring growth, but not during summer growth. Phipps et al. (2000) evaluated maize silages of widely divergent maturity, varying from 23 to 38% DM content. Fibre replaced starch as the crop matured from 23 to 33% DM (NDF: 57% vs. 43% of DM; starch: 11 vs. 31 % of DM).

Digestibility of silages is affected both by rates of fermentation in the rumen, and residence time within the digestive tract. The higher rates of fermentation for legumes in comparison with grasses (Smith et al. 1972), as well as higher rates of physical breakdown and passage from the rumen (Wilson and Kennedy 1996) have long been recognised. The higher rates of fermentation of clover silages have been confirmed in more recent work (Dewhurst et al. 2003a). White clover has relatively low fibre content and an inherently high rate of fermentation, so that despite a much lower retention time than ryegrass (and consequent higher intake), it remains more digestible than ryegrass (Dewhurst et al. 2003a). Some studies showed higher total tract apparent digestibility of N for red clover silage (Vanhatalo et al. 2009), whilst others showed higher values for grass silage (Dewhurst et al. 2003a).

Table 1. Range of composition of different silages in feed tables from France (INRA 2007).

		Unité Fourragère Lait (per kg DM)	Crude protein (% of DM)
Perennial ryegrass	1st cut, prior to 10% ear emergence	1.01	15.1
	1st cut, 10% ear emergence	0.97	14.1
	1st cut, end of heading	0.83	11.2
	2nd cut, stemmy, heading	0.83	13.1
	Range (% of highest value)	**17.8**	**13.2**
Lucerne	1st cut, 10% budding	0.82	19.0
	1st cut, 50% budding	0.77	18.2
	2nd cut, stemmy, budding	0.76	18.7
	Range (% of highest value)	**7.3**	**4.2**
Red clover	1st cut, 10% budding	0.90	17.8
	1st cut, 50% budding	0.86	17.1
	2nd cut, stemmy, budding	0.81	18.1
	Range (% of highest value)	**10.0**	**5.5**
Maize	Milk-dough (25% DM)	0.90	8.6
	Dough-flint (30% DM)	0.90	8.4
	Flint (35% DM)	0.90	8.2
	Range (% of highest value)	**0**	**4.6**

Rumen function

Despite the differences in rumen fermentation rates, Dewhurst et al. (2003a) found no effect of legume silages on rumen pH or VFA concentrations, though rumen ammonia-N concentrations were significantly higher than for diets based on grass silage. Whilst Vanhatalo et al. (2009) confirmed the increased rumen ammonia-N when feeding red clover silage (in comparison with grass silage), they also observed higher VFA concentrations and a higher molar proportion of acetic acid.

Whilst legumes may be attractive diet components for other reasons, their high protein content can lead to wastage of nitrogen, so there has been interest in reducing the rumen degradation of N. Table 3 summarises some data from nylon bag studies of N degradation with comparing grasses and legumes at different growth stages. Overall, estimates for legumes were higher than for perennial ryegrass, though Hoffman et al. (1993) found higher values for perennial ryegrass when comparisons were made with immature herbage. The values in Table 2 were calculated using an estimated rumen outflow rate of 5% per hour, though as has been noted this may be higher for the legumes, which would reduce the differences.

Table 2. Estimates of Nitrogen degradability (g/g) of forage legumes, assuming a rumen outflow rate of 5%/hour.

	Perennial ryegrass	White clover	Red clover	Lucerne
Fresh herbage: early season[1]	0.70	0.83	---	---
Fresh herbage: mid season[1]	0.67	0.79	---	---
Fresh herbage: late season[1]	0.67	0.75	---	---
Dried herbage: vegetative[2]	0.89	---	0.88	0.85
Dried herbage: bud/boot[2]	0.87	---	0.82	0.79
Dried herbage: flowering[2]	0.69	--	0.73	0.73
Dried silage: mixed cuts[3]	0.76	0.83	0.77	---

[1]Beever et al. 1986, [2]Hoffman et al. 1993, [3]Dewhurst et al. 2003a

Dewhurst et al. (2003a) showed similar soluble ('a') and insoluble but potentially degradable ('b') fractions for N in grass silage and white clover silage, but a much higher degradation rate for white clover silage (6.3 vs. 3.1 %/hour). For red clover silage, the 'a' fraction was reduced ('b' fraction increased) and the degradation rate intermediate (4.6%/hour). Broderick et al. (2004) evaluated a wide range of accessions of red clover and lucerne, using an in vitro system, in order to obtain a comprehensive description of the range of N degradability for these forages. They confirmed that N degradability was lower for red clover than for lucerne. This appears to be related to the action of PPO in producing protein-quinone complexes that are resistant to rumen degradation (Lee et al. 2008).

Results based on urinary excretion of purine derivatives provide little evidence for the effects of different silages on N-use efficiency (NUE) being mediated via effects on microbial protein synthesis. The low NUE with diets based on legume silages, particularly lucerne silage (Dewhurst et al. 2003b), were associated with increased microbial efficiency (g microbial N per kg digested organic matter; Dewhurst et al. 2003a). Further, there were no consistent effects of microbial efficiency associated with increased NUE for legume/cereal silage mixtures (Dewhurst et al. 2010, Cheng et al. 2011).

Rumen methanogenesis

High-forage diets have long been known to lead to production of more methane per unit of energy intake than high-concentrate diets (Blaxter and Clapperton 1965) and this is a particular challenge to production systems based on high levels of forage, including silage. The high fibre, high rumen pH and low rumen passage rates all favour rumen methanogenic Archaea (Beauchemin et al. 2008).

There are a number of reasons to predict that methane production should be less from legumes than from grass, including lower fibre content, higher DM intakes and an increased passage rate from the rumen (Beauchemin et al. 2008). A number of early studies with fresh herbage showed reduced methane from legumes (Waghorn et al. 2006, McCaughey et al. 1999). We have used a methanogen marker (archaeol) to show a reduced archaeal population after a meal of white clover (McCartney et al. 2012), presumably reflecting the difficulty that archaea have in surviving when intake and rumen passage rates are high. However, evidence from feeding studies with silages does not confirm this effect. Van Dorland et al. (2007) found no difference between grass silage, red clover silage and white clover silage in their effects on methane output, whether expressed per day, per kg of milk, or per kg digested organic matter, though clover silages only made up 40% of forage mixtures which also contained 60% ryegrass silage.

Extensive fermentation in the silo leaves a reduced proportion of dietary energy available for rumen fermentation, so it would be expected that methane production is reduced. Cushnahan et al. (1995) demonstrated this effect with significantly less methane production from extensively-fermented grass silage, prepared with a bacterial inoculant, in comparison with grass silage prepared from the same herbage, with a high rate of application of an acid-based additive. Unfortunately, the overall reduction in methane production was counteracted by an increase in urine N, so there may be no net effect on greenhouse-gas emissions when methane and potential nitrous oxide are taken into account.

Whilst it is envisaged that the higher starch content and lower fibre content of maize silage will lead to reduced methane production in comparison with diets based on grass silage, this has not been verified experimentally (Beauchemin et al. 2008). Further, Vellinga and Hoving (2011) caution that reductions in methane production from feeding maize silage may be offset by the loss of soil carbon associated with ploughing permanent pasture to grow maize.

Feed Intake

The lower intakes of grass silages are the most remarkable feature when comparisons are made across silage types. Legume silages generally lead to higher intakes than grass silages of comparable digestibility. Huhtanen et al. (2007) in a Meta analysis showed a curvilinear effect with increasing intakes as legume silages replaced grass silages up to 80% inclusion. The same situation applied when Cheng et al. (2011) compared grass silages with mixtures of legume and cereal silages – despite lower digestibilities, the latter led to higher intakes. This effect is not confined to legume silages – total intakes and short-term intakes of total-mixed rations based on maize silage where higher than those based on grass silage (Abrahamse et al. 2008).

Rinne et al. (2002) investigated feed intake and rumen function for a series of timothy/meadow fescue silages of increasing maturity. The increased intakes of early-cut grass silage were associated with a reduction in rumen fill, suggesting that rumen fill is not solely responsible for control of feed intake. The effects of the level and composition of nutrients derived from silages, and their interaction with animal potential, are also important in regulation of silage intake. There have been few studies of the effects of stage of maturity on intake and milk production from red clover silage. Hoffman et al. (1997) showed reductions in intakes of red clover silage in two separate years (milk yields were also reduced in one of the studies), whilst Vanhatalo et al. (2009) showed a significant increase in intake with a more mature red clover silage. Vanhatalo et al. (2008) showed reductions in intake with more mature red clover silages, whether from primary growth or regrowths. They found significantly higher intakes of red clover silages prepared from regrowths as opposed to primary growths.

With the exception of diets that contain less than 25% NDF and are likely to be associated with rumen acidosis, there is a general negative relationship between diet NDF and DM intake (Allen et al. 2000). The fibre content of white clover silage is much lower than in other silages (Dewhurst et al. 2003b) and this explains the high intake characteristics, both as sole forage (Castle et al. 1984, Cohen et al. 2006), and with concentrate feeding (Dewhurst et al. 2003b). However, the fibre content of silages from grass, lucerne, red clover and maize are more similar and other mechanisms must explain

differences in feed intake. The higher intake of these silages relative to grass silage relates to their more rapid fermentation and physical breakdown within the rumen.

Differences in intake have been attributed to both faster rates of fermentation (Beever and Thorp 1996) and more rapid particle breakdown and clearance from the rumen (Moseley and Jones 1984, Waghorn et al. 1989, Jamot and Grenet 1991). Dewhurst et al. (2003a) suggested that fermentation rate may be more important for white clover silage, whilst rapid particle breakdown may be more important for lucerne silage. Bosch and Bruining (1995) compared grass silages at differing maturities and suggested that the control of rumen fill is related to the disappearance rate of small particles (0.07 to 1.25 mm) from the rumen. In comparison with the elongated vein structure of grasses, the reticular vein structure of legumes breaks down into small particles more readily (Wilman et al. 1996, Wilson and Kennedy 1996). Since fermentation may contribute to the buoyancy of rumen particles, which limits the ability to leave the rumen (Baumont and Deswysen 1991), more rapid fermentation would also increase passage rates from the rumen. Some aspects of intake regulation of legume silages remain to be elucidated; Kuoppala et al. (2009) could not explain the low intakes of early-cut red clover silage in terms of silage digestibility, fermentation quality or rumen fill and concluded that it must be related to some other aspect of nutrient composition.

Intakes of mixtures of grass and legume silages were usually intermediate to intakes of the grass and legume silages separately (Dewhurst et al. 2003b). High intakes were also achieved with mixtures of red clover silage and maize silage (Dewhurst et al. 2010). Intakes of maize silage increase with increasing maturity, up to the optimal 30-35% DM (Phipps 1990).

There has been a lot of work in North America over the last decade evaluating effects of varying chop length in a range of forages, including lucerne, maize and oats silages. Part of the reason for this interest is the better compaction and ensiling that can be achieved with shorter chop material. At the same time, the short forage particles will increase problems with sub-acute rumen acidosis because of increased fermentation rates and reduced chewing, rumination and saliva production. Whilst a number of studies showed effects on rumen fermentation measures, there were no consistent effects on feed intake, milk production or milk composition (Bhandari et al. 2007, 2008).

The basis for differences in DM intake between well preserved grass silages is less clear. Kuoppala et al. (2009, 2010) compared silages prepared from primary growth and regrowth of timothy/meadow fescue at two growth stages. They found no explanation for the higher intake and milk production from first-cut silages, despite extensive studies of feed chemistry, kinetics of rumen digestion and passage, and the protein/energy ratio of absorbed nutrients. The lack of explanation for differences in intake characteristics between silages from different herbage species and managements is an important area for further research because these effects drive the differences in milk production and nutrient utilisation described below. Whilst we understand some of the mechanisms involved in regulating silage intake, and have some analytical tools, new insights are needed. For example, Huhtanen et al. (2007) speculated that microbiology of herbage and the proportion of dead material may explain some of the difference between first-cut and later silages.

Milk production

Diets based on legume silages, maize silage, or mixtures of the two often lead to higher milk production than diets based on grass silage (e.g. Thomas et al. 1985, Phipps et al. 1988, 1992, RJ Dewhurst, unpublished observations). However, in most situations diets often involve mixtures of these silages with grass silage. With average-quality grass silage, Phipps et al. (1992) found that a 50/50 mixture of grass silage and maize silage maximised intakes and milk production, whilst with a poorer grass silage, the optimal mixture was 75/25 maize silage/grass silage. Comparisons of milk production from lucerne silage and red clover silage are equivocal (Hoffman et al. 1997, Broderick et al. 2000, 2001, Dewhurst et al. 2003b).

Grasses and legumes tend to contain adequate levels of protein, so that production is limited by their intake characteristics and energy content. Red clover and lucerne silage can often be of lower digestibility than grass silage, but performance can be higher as a result of higher DM intakes (lucerne: Hoffman et al. 1998; red clover silage: Dewhurst et al. 2003b, Moorby et al. 2009).

Although legume silages generally lead to higher intakes and milk production than grass silages, there remains considerable variation due to weather conditions and the success of ensilage, so there are examples of legume silages with lower intake and production characteristics (Bertilsson and Murphy, 2003).

Milk yield were maximised using maize silage of 33% DM (31% starch on a DM basis) in the study of Phipps et al. (2000), which compared a range from 23 to 38% DM. There was no further increase in production with higher maturity (38% DM; 35% starch on a DM basis). These authors suggest that milk production will be optimised across a relatively wide range of maize silage maturity (30-35% DM).

Milk fat and protein content

Phipps et al. (1992) found no effect of varying the ratio of grass silage to maize silage from 100/0 to 75/25 in the diet on fat % or protein %. Similarly, there appears to be little difference between grass silage and lucerne silage in their effects on milk fat % and protein %. Legume silages have often led to reductions in milk fat % and/or milk protein % and this is summarised in Table 3. The reduction in milk fat % with clover silages is most consistent, with reductions in milk protein % often small and occasional significant increases in milk protein % when clover silages with exceptionally high intake characteristics were fed.

Table 3. Effects of clover silages on milk fat % and milk protein %.

Reference	Comparison	Effect of clover silage on milk fat %	Effect of clover silage on milk protein %
Al-Mabruk et al. 2004	Red clover vs. grass	No effect	Small increase
Bertilsson & Murphy 2003 (1)	Red clover vs. grass	No effect	No effect
Bertilsson & Murphy 2003 (2)	Red clover vs. grass	No effect	Reduction
Dewhurst et al. 2003a (1)	Red clover vs. grass	No effect	No effect
Dewhurst et al. 2003a (2)	Red clover vs. grass	No effect	Small reduction
Moorby et al. 2009	Red clover vs. grass	Reduction	Reduction
Thomas et al. 1985	Red clover vs. grass	Reduction	No effect
Vanhatalo et al. 2009	Red clover vs. grass	Reduction	Reduction
Bertilsson & Murphy 2003 (1)	White clover vs. grass	No effect	No effect
Bertilsson & Murphy 2003 (2)	White clover vs. grass	Reduction	Reduction
Dewhurst et al. 2003a (1)	White clover vs. grass	No effect	No effect
Dewhurst et al. 2003a (2)	White clover vs. grass	Reduction	Increase
Broderick et al. 2000	Red clover vs. lucerne	Reduction	Small reduction
Broderick et al. 2001 (1)	Red clover vs. lucerne	No effect	No effect
Broderick et al. 2000 (2)	Red clover vs. lucerne	Reduction	No effect
Hoffman et al. 1997 (1)	Red clover vs. lucerne	No effect	No effect
Hoffman et al. 1997 (2)	Red clover vs. lucerne	No effect	Reduction
Steinshamn & Thuen (2008)	Red clover vs. white clover	Tendency for reduction	Small reduction

Dewhurst et al. (2010) and Cheng et al. (2011) found no differences in milk fat % and milk protein % when comparing diets based on grass silages and diets based on a series of mixtures of red clover silage with either maize silage or whole-crop oat silage. Phipps et al. (2000) found no consistent effect of maize silage maturity on milk fat % or protein %.

Milk fatty acids

In comparison with milk from cows fed grass silages, clover silages had only small and inconsistent effects on proportions of the various saturated fatty acids, as well as conjugated linoleic acid, in milk (Dewhurst et al. 2006). In contrast, both red clover and white clover silages led to highly significant increases in the proportion of the n-3 fatty acid α-linolenic acid (18:3 *n-3*) in milk. There was a three-fold increase in this fatty acid when red clover silage made up a high proportion of the diet (concentrate level was 4 kg/day) in the studies reported by Dewhurst et al. (2003b) and Moorby et al. (2009). Other studies have confirmed the increase in 18:3 content of milk from cows fed red clover silage (Al-Mabruk et al. 2004, Vanhatalo et al. 2007, Van Dorland et al. 2008) and white clover silage (Van Dorland et al. 2008). Vanhatalo et al. (2007) showed a greater increase in 18:3 content of milk fat for early-cut red clover silage in comparison with late-cut red clover silage. The effect of clover silages is probably the basis for increased levels of 18:3 in organic milk, at least in the UK where red and white clover an important components of organic systems (Dewhurst, 2003, Ellis et al. 2006, Butler et al. 2011). Steinshamn and Thuen (2008) compared diets based on grass silage with either white clover silage (28% of DM) or red clover silage (42% of DM), either as sole-feed or with 10 kg/day concentrates, and showed higher 18:3 content milk from cows fed red clover silage (0.87 vs. 0.73% of milk fatty acids).

The mechanism for these effects relates to a reduction in rumen biohydrogenation of 18:3 from red clover silage and white clover silage (Dewhurst et al. 2003a). In the case of red and white clover silages, this may be related to the higher rate of passage from the rumen (Dewhurst et al. 2003a), whilst in the case of red clover silage, it appears also related to the action of PPO (Lee et al. 2008).

Other milk attributes

The increase in polyunsaturated fatty acids, both 18:2 and 18:3, in milk from cows fed red clover silage increases the likelihood of problems with oxidation. Al-Mabruk et al. (2004) demonstrated a reduction in the shelf-life of milk from cows fed diets based on red clover silage.

Bertilsson and Murphy (2003) observed an increased deviation from good organoleptic characteristics of milk when grass silage was replaced with silage made from white, and particularly, red clover silage. It seems likely that higher levels of dietary protein are one cause of off-flavours since compounds such as methyl sulphide and skatole are derived from degradation of amino acids. Ethanol is another silage component that can lead to off-flavour in milk (Randby et al. 1999).

Moorby et al. (2009) showed changes in the physical appearance of milk produced from red clover silage in comparison with grass silage. Milk from cows fed red clover silage had a reduced whiteness score and a 'thinner' texture; it was suggested that this relates to lower levels of β-carotene in the milk. Feeding cows diets based on red clover silage increased levels of flavonoids (Steinshamn et al. 2010), which may affect human health.

Utilisation of dietary N and urinary N output

Kebreab et al. (2001) analysed a series of five Nitrogen Balance studies conducted at the Centre for Dairy Research in Reading, with 30 diets based on grass silage and concentrates and obtained the following relationship (equation 1) for urine N output:

Urine N (g/day) = 0.003 N intake (g/day)$^{1.8}$ (r^2=0.67 based on individual values) [1]

Huhtanen et al. (2008) obtained a relationship (equation 2) that produces very similar predictions within the range of N intake encountered in practice. This relationship was based on a large number of treatment means taken from the literature and was based largely on grass silage-based diets:

Urine N (g/day) = -126 +0.676 N intake (g/day) (n=515; RMSE=12.3) [2]

As might be predicted from equations (1) and (2), the high N content of legume silages can lead to low efficiencies of conversion into milk N (NUE) and particularly high urinary N output (Cohen et al. 2006, Dewhurst et al. 2003b, 2009). Urine N has been reduced by offering low protein supplements, such as barley (Cohen et al. 2006) or maize silage (Margan et al. 1994, Auldist et al. 1999, Dewhurst et al. 2010) alongside legume silages. There is no evidence that the inefficiency is driven by asynchronous supply of energy and N to rumen micro-organisms since meal patterns had no effect on NUE (Cohen et al. 2006).

Whilst the high urine N from diets based on legume silages as sole forage are as expected, it is important not to miss two attributes of legume silages that may improve NUE and reduce urine N relative to expectations. The first mechanism relates to the action of the enzyme polyphenol oxidase (PPO) in red clover. PPO produces quinones that bind to proteins and reduce N degradability in the rumen (Broderick and Albrecht 1997, Cassida et al. 1997). Red clover silage contains approximately 30-40% less non-protein-N than lucerne silage (Owens et al. 1999, Albrecht and Muck 1991). This may explain observations of reduced rumen ammonia, reduced milk urea N, reduced urine N, and increased NUE with diets based on red clover silage in comparison with those based on lucerne silage (Broderick et al. 2000, 2001, 2007).

In two studies comparing grass silage-based diets with diets based on mixtures of legume silages and maize silage or other cereal silages, we noticed that urine N was 50 to 100 g/day less than predicted using equations (1) or (2) (Dewhurst et al. 2010, Cheng et al. 2011). Further analysis revealed that this effect was related to effects on DM intake. Further statistical analysis was conducted using treatment means from our studies (21 treatment means from Dewhurst et al. 2010; Cheng et al. 2011; and two unpublished studies with diets based on legume silages, grass silages and mixtures with maize silage). There was no significant relationship between DM intake and N intake for these treatment means (r^2=0.07; P>0.1), so in addition to a simple regression (equation 3), we were able to conduct a multiple regression analysis (equation 4).

Urine N (g/day) = -122 + 0.614 N intake (g/day) (r^2=0.60; P<0.001) [3]

Urine N (g/day) = 105.5 + 0.769 N intake (g/day) – 16.86 DM intake (kg/day)
(r^2=0.91; P<0.001) [4]

The interpretation of equation 4 is that diets affect urine N both through the supply of N, and through effects on DM intake – presumably affecting the ability of the animal to utilise feed N in the rumen or tissues. In fact, the same effect was noted in the analysis by Huhtanen et al. (2008; equation 5), so there is a general principle that achieving higher intakes without increasing N intakes will increase NUE and reduce urine N.

Urine N (g/day) = 27 +0.844 N intake (g/day) – 13.0 DM intake (kg/day)
(n=515; RMSE=9.28) [5]

The effect described in equations (4) and (5) is the basis for the success of diets based on mixtures of legume and cereal silages. However, there are examples of this effect operating with other forages. Cushnahan et al. (1995) demonstrated a significantly higher proportion of N intake going to urine N with extensively-fermented grass silage of low intake characteristics, despite its having a lower N concentration. Cammell et al. (2000), comparing diets based on maize silages of varying digestibility, showed a reduction in urine N, relative to N intake, with diets based on the maize silage (33% DM) that maximised intake of digested organic matter.

Future research

The effects of legume and maize silages in increasing feed intake and milk production are well-recognised, as is the effects of legume silages on milk fatty acids. It is likely that the use of legumes for silage will increase and there is a need to follow up on the increasing evidence of effects on the physical and organoleptic properties of milk. We need to properly describe the range and frequency of effects, as well as identifying the mechanisms involved, as a basis for amelioration strategies.

Many of the benefits of legume silages in comparison with grass silages – increased intakes and milk production, increased polyunsaturated fatty acids in milk, and a reduction in urine N at a given N intake – relate to the increased rumen passage rates. In addition to seeking to change the chemical composition of grasses and silages, further work should investigate potential to achieve differences in physical and chemical breakdown characteristics of grasses (e.g. cell structure and lignification).

Whilst differences in rumen fermentation rate, particle breakdown, and passage from the rumen explain some of the higher intake with legume silages, there are clearly other facets of the control of silage intake that remain to be understood. Ammonia-N has long been recognized as merely a proxy for some aspects of poor fermentation quality in silages, which are not fully characterized. Ammonia and amines directly added to the diet have not reduced silage intake. Further studies are required to understand the complex balance between fermentation quality, effects on rumen kinetics and fill, as well as the balance between nutrient supply and animal requirements, in their effects on silage intake.

The observation that diets based on high intake characteristic forages leads to an increased NUE and a reduction in urine N should be investigated further. In particular, there is a need to understand the basis for the independent effects of N intake and DM intake since this offers potential to increase productivity without increasing problems linked to urine N output. Many aspects of good grassland management result in high levels of herbage N, and the search for low N/high intake forages may be limited by the requirement to have protein present in the plant photosynthetic machinery. Differences in rumen microbial efficiency appear to explain only a small proportion of the effect, which appears to be more based at the tissue level.

The lack of data on methane production from alternative silages is an important gap to fill, particularly in the case of legume silages that have the additional benefit of reducing carbon intensity of production by replacing inorganic N fertilizer.

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The influence of physical structure of silage on rumen metabolism, feed intake and milk production in dairy cows

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Keywords: chewing index, forage, grass, particle size, rumination

Introduction Forage preserved as silage for dairy cows can vary in physical form depending on the harvesting system used. The physical structure is further influenced by the equipment used for feeding, such as the effect of an auger with knives in a mixer wagon. It has been proposed that when the feed is too finely cut and macerated, negative effects on rumination could occur with subsequent health problems for the cow (Young and Beauchemin 2006). Our objective was to compare two commonly used types of grass silages which differ widely in structure, the round bales with a limited number of knives installed and chopped silage macerated and packed in tubes. The aim was to compare the effect on dairy cows with regard to eating behaviour, intake, rumen metabolism and milk production.

Material and methods A first cut of a grass dominated crop (timothy, meadow fescue and red clover) was cut with a mower conditioner and wilted to 45 % dry matter (DM). One part was ensiled in round bales, which implies gentle mechanical treatment giving a chop length of 14 cm. Another part was ensiled in tubes (bags) which implies intensive treatment, precision chopped and macerated, producing a chop length of 1.6 cm (Table 1).

The silages were fed *ad libitum* to 8 lactating dairy cows (Swedish Red) in a 2x2 changeover model. Chewing and rumination measurements with IGER behavior recorders (Rutter et al. 1997) were performed on 6 of the cows and two rumen fistulated cows were used to measure pH, ammonia and volatile fatty acids in the rumen fluid. Feed intake and milk production was recorded on all 8 cows and fecal samples were taken to estimate fiber digestibility. The cows were fed concentrate to a fixed amount during the experiment, set according to their individual milk production at start.

Results and discussion Silage quality was good in both systems and equal in terms of DM, NDF, CP, soluble CP, ammonia N and NDF. Silage pH differed being 5.3 in round bales and 4.6 in tube silage, respectively. The silage intake was the same in the two treatments but the cows spent 43 % longer time consuming the round baled silage. There was no difference in the rumination time but the number of boli differed significantly although not to a great magnitude (Table 1). No differences could be detected between the silages in digestibility of fiber and organic matter or in the passage rate. Average rumen pH did not differ but there was a tendency for slightly lower pH and lower ammonia nitrogen in the rumen of cows fed tube silage during the first 6 hours after morning feeding (Table 2).

The length and width of particles followed the same pattern. As can be seen in Table 3 the drastic difference in the feed was reduced to no significant differences whatsoever in particle size in the rumen or feces.

Feed intake is presented during different periods in Tables 1 and 2 with no difference between treatments. Also in Table 4, where the intake over the whole experiment is presented together with milk production data, the two types of silage treatments resulted in equal intakes. As a logical consequence the milk yield and milk constituents were unaffected by silage treatment.

The animals were high producing with some cows yielding over 40 kg ECM per day. They were neck tied with free access to the feed in individual feeding troughs. Transferring the results to a free stall situation where cows compete for the same eating place should be made with caution. It is possible that the extended eating time of the round baled silage could result in lower intake, at least for low ranked cows or heifers.

Conclusions The experiment suggests that the drastic difference in the particle size of round baled silage and precision chopped silage macerated in a tube packer results in an almost 50 % longer eating time when fed to dairy cows. But when the silage is consumed, no further differences could be detected. The equal silage intake surprises and so does the absence of difference in rumination time. The fast reduction in particle size after intake explains the absence of negative effects on intake, rumination, rumen pH and production response.

Table 1. Eating, rumination, and chewing time and intake of silage. Six cows during three days on each feed (N=12). Ei= eating index, Ri=rumination index, Ci=chewing index in minutes per kg DM.

	Eating time, min/24 h	Rumination time, min/24 h	Chewing time, min/24 h	Intake[1)] silage, kg DM /24 h	Ei, minutes / kg DM	Ri, minutes / kgDM	Ci, minutes / kg DM	Boli, number /24 h
Round bale	309	474	783	13.9	23.2	35.2	58.3	533
Tube silage	216	463	678	14.1	16.1	33.7	49.8	510
SEM	18.2	3.7	18.2	0.5	1.7	1.2	2.8	5.9
Sign.p<	0.02	n.s.	0.01	n.s.	0.04	n.s.	0.09	0.05

[1)]Intake during the three days of chewing measurement.

Table 2. Digestibility of fiber and organic matter, particle passage rate (Kp), rumen pH and ammonia content. The digestibility determined with 8 cows in a changeover design (N=16). Other parameters determined on two rumen fistulated cows (N=4). n.s. = not significant

	Intake [1)] silage, kg DM	Dig.NDF, %	Dig.OM, %	K_p	Rumen pH	Rumen NH_3-N, mg/100 ml	Rumen pH, 1-6 h after feeding
Round bale	14.0	59.3	67.5	0.030	5.82	10.5	6.00
Tube silage	13.5	60.3	68.4	0.030	5.74	8.3	5.86
SEM	0.69	5.30	4.76	0.008	0.019	0.51	0.033
Sign.p<	n.s.	n.s.	n.s.	n.s.	n.s.	0.06	0.10

[1)]Intake during the 4 days when the digestibility was measured.

Table 3. Geometric mean of particle length (PL) and particle width (PW) in feed, rumen content and feces measured according to Nørgaard (2006). One analysis in feed (N=1). Rumen content and feces were analyzed on two cows eating each feed (N=4).

	Feed, PL,mm	Feed, PW,mm	Rumen content, PL,mm	Rumen content, PW,mm	Feces, PL,mm	Feces, PW,mm
Round bale	140.3	6.3	2.95	0.42	0.86	0.19
Tube silage	15.6	3.2	2.48	0.32	0.89	0.19
SEM			0.54	0.02	0.11	0.00
Sign.p<			n.s.	n.s.	n.s.	n.s.

Table 4. Feed intake[1)] and milk yield. Eight cows in a changeover design during 2 weeks per feed (N=16).

	Intake[1)] Concentrate	Intake Silage	Intake, Total DM	Yield, kg milk	Milk fat, %	Milk protein, %	Yield, kg ECM
Round bale	9.9	14.8	24.7	29.7	4.26	3.54	31.0
Tube silage	9.9	14.7	24.6	29.7	4.35	3.51	30.9
SEM	0.1	0.40	0.37	0.87	0.294	0.018	0.59
Sign.p<	n.s	n.s	n.s	n.s	n.s	n.s	n.s

[1)] Refers to intake during the entire experiment.

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Energetic value of ethanol for lactating dairy cows: how should it be considered?

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Keywords: intake, milk yield, volatile organic compound

Introduction Ethanol is a volatile organic compound found in fully fermented silages. In sugarcane silages, concentrations up to 10% of dry matter (DM) are common, although higher levels as 22% of DM have been found (Zopollatto et al. 2009, Freitas et al.,2006). Conventionally lab processed silage samples are virtually free of ethanol and acetic acid because of the oven drying (Weissbach 2009), but when silages are used as ration ingredients, these fermentation end-products are consumed by the animals (Randby et al. 1999; Hutchinson and Wilkins 1971). Heat of combustion from ethanol (kcal/g) is higher than that of either acetic acid or glucose, thus ethanol fed animals could be more efficient. The objective of this study was to determine whether ethanol and acetic acid affect performance and energy efficiency of high producing dairy cows.

Material and methods Thirty lactating Holstein cows were grouped in ten blocks and fed either: Control (33% Bermuda hay + 67% concentrates); Ethanol (control diet + 5% ethanol); or Acetic acid (control diet + 5% acetic acid, DM basis) diets, during seven weeks. Ethanol and acetic acid were diluted in water (1:2) and sprayed onto total mixed ration twice daily before feeding. The same amount of solution was replaced with water in the control diet. During the 1st week, cows received half-dose of these chemical compounds. Dry matter intake (DMI) and milk yield were recorded every day and milk composition was determined once weekly. The actual DMI was corrected for disappearance of ethanol and acetic acid at feed bunk. Milk energy content (Mcal/kg) was calculated as (NRC 2001): milk NE_L = 0.0929 * fat % + 0.0547 * protein % + 0.0395 * lactose %. Daily excretion of milk energy (Mcal/d) was calculated as milk NE_L * milk yield. Energy efficiency of diet was estimated by dividing the excretion of milk NE_L by DMI. Cows were weighted and body scored at the beginning of week one and at the end of the week seven. Diet digestibility was measured by total collection of feces on 6th week. Diet NE_L was calculated by NRC (2001) equations with nutrient digestibility. Ethanol and acetic acid NE_L was estimated through NE_L of control and supplemented diets. Blood samples were obtained from coccygeal vessels 1 h before and 6 h post morning feeding on days 7, 14, and 42. Plasma was separated by centrifugation and submitted to a commercial laboratory (Plimorlabor, Piracicaba, Brazil) for analysis of glucose, insulin, nonesterified fatty acids, ethanol, and gamma-glutamyl transferase activity. Data were analyzed using the MIXED procedure of SAS.

Results and discussion Cows fed ethanol yielded more milk than those fed control or the acetic acid diets (Table 1), due to the higher DMI (23.7, 22.2, 21.6 kg/d, respectively). The significant diet*week interaction for DMI (P = 0.02), mainly during the 2nd and 3rd weeks (when the 5% acetic acid achieved the full dose) was related to the decrease in DMI of the acetic acid diet. Milk fat yield, milk urea nitrogen and somatic cells counts were unaffected by diets, however protein and lactose yields were higher for ethanol diet, which agrees with the higher milk yield. Energy efficiency also showed diet*week interaction (P = 0.06) and again, during 2nd and 3rd weeks the acetic acid diet increased NE_L milk/DMI ratio due to the lower DMI and body weight loss. Otherwise, energy efficiency was similar across diets (1.1 Mcal NE_L milk/kg DMI). The net energy for lactation (NE_L) of ethanol was estimated as 2.6 Mcal/kg, which is much lower than its heat of combustion (7.1 Mcal/kg) and in agreement with the plasmatic concentration of ethanol which was lower than the detection threshold in all cows. Other arterial blood metabolites were similar across treatments. Up to the studied dose, dietary ethanol showed no risks to animal health. Rumen conversion of ethanol to acetate and concomitant increase of methane production (Yoshii et al. 2005) is a plausible explanation to these findings.

Conclusions Ethanol supplemented diet led to improved DMI and milk yield. However, ethanol did not achieve the expected energy value based on heat of combustion. Rumen conversion of ethanol to acetate might be a plausible explanation to the deviation on the predicted energetic value.

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Table 1. Responses of dairy cows fed ethanol or acetic acid.

Item	Control	5% Ethanol	5% Acetic acid	SE[1]	*P*
DM intake[2], kg/d	22.16[ab]	22.72[a]	21.42[b]	0.58	<0.01
Milk yield, kg/d	35.50[b]	37.82[a]	35.70[b]	1.38	<0.01
BW change, kg/d	0.11	0.08	0.00	0.13	0.82
BCS change, /7 weeks	0.13	0.08	0.03	0.07	0.63
Milk NE_L, Mcal/d	24.70	25.52	25.15	0.94	0.75
Energy efficiency[3]	1.12[b]	1.12[b]	1.19[a]	0.02	<0.01
Apparent digestibility of DM, %	71.38	69.23	70.21	1.86	0.74
Diet NE_L, Mcal/kg	1.55	1.58	1.59	0.04	0.76
Supplement NE_L, Mcal/kg	-	2.57	2.59	-	-

[a, b] Means within a row with different superscripts differ ($P < 0.05$).
[1] SE = standard error of the mean.
[2] $P = 0.02$ for diet*week interaction.
[3] Energy efficiency (Mcal/kg) = Milk NE_L / DMI.

Feed intake and milk yield responses during early lactation of cows offered grass silages harvested at early maturity stages

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Keywords: concentrate level, early harvest, grass silage, milk production

Introduction Increasing demand for human food in the future may favour sustainable grassland-based ruminant production systems, saving arable land for crops for direct human consumption and for production of concentrate feeds for nonruminant species. Grass or grass-legume swards harvested at young maturity stages are rich in energy, protein and minerals, and may support a reasonable milk yield even without concentrate supplements (Steinshamn and Thuen 2008). The main objective of this experiment was to evaluate the potential of very high quality grass silages to support and sustain high milk yield with low or moderate, or even without, supplements of concentrates.

Material and methods The effect of grass silages harvested at three stages of maturity in the primary growth and supplemented with various concentrate levels was evaluated in a study involving 66 Norwegian Red dairy cows. Roundbale silage was produced from a timothy dominated sward at a very early (H1), early (H2), and normal (H3) stage of crop maturity. Crops were rapidly wilted (< 24 h) and 4.3 L/t of a formic acid-based additive applied. Dietary treatments in a 3 × 3 factorial arrangement consisted of the three silages supplemented with three concentrate levels, 4, 8, and 12 kg/day, and, additionally, H1 was offered without concentrates and H3 with 16 kg/d, giving a total of 11 diets. Two concentrates, one mixture based mainly on grain ingredients (165 g crude protein (CP)/kg dry matter (DM)) and one with a higher proportion of protein ingredients (240 g CP/kg DM) were used. All cows, except those not fed concentrates, were offered 4 kg of the protein concentrate plus grain concentrate to the total level. Cows blocked according to parity and calving date were introduced to the experiment 2-3 weeks before calving and kept in the experiment until lactation week 16. Silage was offered *ad libitum* in loose housing and concentrate was available in feed stations. Effect of diet on intake and yield was analysed using the Proc Mixed procedure in SAS. The model included fixed effect of diet (11 classes), block (6 classes), and week in milk (WIM) (16 classes), and the diet × WIM interaction. For treatment comparisons, the sum of squares for experimental diets were further divided into single degree of freedom comparisons of linear and quadratic effects of harvesting time and concentrate level (4, 8 and 12 kg) and their interactions.

Results and discussion Due to successful wilting and application of a formic acid based additive, all silages were restrictedly fermented with 62, 75 and 41 g lactic acid/kg DM and 6, 7 and 5 g acetic acid/kg DM for H1, H2 and H3, respectively. The silages contained 299, 271 and 322 g of DM/kg, 166, 145 and 113 g of CP/kg DM, 477, 533 and 601 g of NDF/kg DM and 94, 128 and 209 g of iNDF/kg NDF for H1, H2 and H3, respectively. Digestible organic matter in DM (DOMD) determined with sheep were 747, 708 and 647 g /kg DM, pH was 4.43, 4.18 and 4.31 and NH_3-N per kg total N was 82, 94 and 101 g, for H1, H2 and H3, respectively.

Daily intake of grass silage (H1) when fed as the sole feed was 16.9 kg DM in average for lactation week 1 to 16 (Table 1). When H1 was supplemented with 4 or 8 kg concentrates, silage intake did not change, but total DM intake increased to 20.6 and 23.7 kg/day, respectively. Daily energy corrected milk yield increased from 23.4 kg when H1 was offered without concentrate supplement to 29.1 and 32.8 kg when supplemented with 4 or 8 kg concentrate, respectively (Table 2). None of the other diets equaled the obtained yield with H1 plus 8 kg concentrate. Feed intake and yield of cows offered H3 plus 4 kg concentrates were strongly constrained by high dietary fiber concentration. They consumed 16.5 g NDF per kg body weight and spent more time eating silage (257 min/d) than cows offered other diets (163 – 235 min/d). The obtained milk yield on the highest concentrate level within each silage quality was similar to (H2) or lower (H1 and H3) than the yield obtained with 4 kg less concentrates. Those diets seemed to be constrained by reduced fiber digestion due to high dietary starch + water soluble carbohydrate concentration although the intake of physically effective fiber (NDF) was above the critical minimum.

Table 1. Effect of harvesting time for grass silage (H) and level of concentrate supplement (C) on daily intake of dry matter (DM), neutral detergent fibre (NDF), net energy lactation (NE_L), amino acids absorbed in the intestine (AAT) and protein balance in the rumen (PBV) (mean values for lactation week 1-16).

		DM, kg										
H	C	Concentrate				DM, g/kg BW		NDF,g/kg BW		NE_L[4]	AAT	PBV
	kg	Prot	Grain	Silage	Total	Silage	Total	Silage	Total	MJ	g	g
H1	0	0	0	16.9	16.9	30.1	30.1	14.3	14.3	114	1303	499
	4	3.62	0	17.0	20.6	29.7	36.1	14.2	15.4	144	1741	699
	8	3.61	3.42	16.7	23.7	27.6	39.3	13.2	15.5	165	2098	686
	12	3.31	6.14	14.2	23.6	24.4	40.8	11.7	14.9	165	2171	593
H2	4	3.65	0	14.5	18.2	24.9	31.3	13.3	14.5	120	1529	318
	8	3.61	3.13	14.4	21.1	23.9	35.2	12.7	15.0	140	1860	311
	12	3.52	6.37	12.9	22.8	20.5	36.2	10.9	14.1	153	2102	290
H3	4	3.65	0	14.4	18.0	25.4	31.9	15.2	16.5	108	1486	-113
	8	3.62	3.37	13.3	20.3	21.3	32.5	12.8	15.0	126	1783	-95
	12	3.54	6.31	11.9	21.7	20.0	36.7	12.0	15.4	138	1994	-71
	16	3.30	8.74	9.3	21.4	16.6	38.1	10.0	14.3	139	2050	-31
SEM				0.49	0.55	0.91	1.10	0.48	0.52	3.5	43.8	15.8
P	Diet[1]			<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
P	H[2]			<0.001	<0.001	<0.001	<0.001	NS	NS	<0.001	<0.001	<0.001
P	C[3]			<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	0.02

[1]All 11 diets [2]Linear effect of H within diets with 4-12 kg concentrates [3]Linear effect of C within diets with 4-12 kg concentrates. No significant H × C interactions were found within diets with 4-12 kg concentrate.[4]Calculated according to Van Es (1978)

Table 2. Effect of harvesting time for grass silage (H) and level of concentrate supplement (C) on daily milk production, BW gain and N use efficiency (mean values for lactation week 1-16).

		Milk yield		Milk composition					BW[5]	
H	C	Milk	ECM	Fat	Protein	Lactose	Urea	FFA[4]	gain	Milk N/
	kg	kg	kg	g/kg	g/kg	g/kg	m*M*	mEq/L	g/d	N intake
H1	0	23.7	23.4	41.4	31.5	44.4	4.11	0.63	-134	0.275
	4	29.1	29.1	41.3	32.2	45.2	4.83	0.39	152	0.256
	8	32.8	32.8	40.9	32.8	45.6	4.35	0.31	88	0.258
	12	31.6	31.0	39.7	33.2	45.1	4.62	0.26	264	0.249
H2	4	26.7	27.1	42.6	32.0	44.6	4.53	0.55	-204	0.295
	8	29.4	29.3	41.2	31.8	45.5	4.13	0.40	166	0.275
	12	29.2	28.8	39.6	33.6	45.6	4.02	0.35	330	0.260
H3	4	25.8	24.9	38.8	32.2	45.3	4.06	0.61	-185	0.333
	8	27.6	27.3	39.5	32.8	45.6	4.01	0.72	16	0.311
	12	30.8	30.1	38.9	32.2	46.5	4.38	0.37	153	0.309
	16	29.3	28.7	39.5	32.3	45.4	3.79	0.46	58	0.295
SEM		1.70	1.60	0.97	0.69	0.54	0.213	0.110	103.0	0.0143
P	Diet[1]	0.02	0.008	NS	NS	NS	0.04	0.1	0.008	0.001
P	H[2]	0.03	0.009	0.06	NS	NS	0.01	0.01	0.04	<0.001
P	C[3]	0.02	0.03	0.07	NS	NS	NS	0.05	<0.001	0.08

[1] [2] [3] See Table 1 [4] Free fatty acids [5] Body weight

Conclusions The obtained milk yield responses suggest that increasing amounts from 8 to 12 kg/d of concentrate were optimal to maximise milk yield when maturity of grass silages increased from very early harvesting, represented by H1, to a commonly used harvesting time, represented by H3. However, H1 may successfully be used with less concentrates, or even without, if future conditions should limit the amount of concentrates available for ruminant production.

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Effects of replacing dietary lucerne silage with birdsfoot trefoil silage containing different levels of condensed tannin on production of lactating dairy cattle

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Keywords: lucerne, birdsfoot trefoil, condensed tannins, milk production

Introduction Extensive degradation of crude protein (CP) in ensiled legumes impairs N utilization when these silages are fed to lactating dairy cattle. There is an extensive literature on the effects of dietary condensed tannins (CT) on ruminal N metabolism and utilization in ruminants; however, the influence of CT on N efficiency in lactating cows has been inconsistent. Miller and Ehlke (1997) selected germplasm for low and high levels of CT, starting from a cultivar of birdsfoot trefoil (BFT; *Lotus corniculatus*) with medium concentrations of CT. Previously, we reported that silages made from these three BFT materials had reduced levels of nonprotein N and, relative to diets containing similar silage dry matter (DM) from lucerne (*Medicago sativa*) or red clover (*Trifolium pratense*), feeding these forages gave rise to improved yield of milk and milk components (Hymes-Fecht et al., 2005). In this paper we report on results of a follow-up trial comparing milk production on silages prepared from subsequent harvests of the same BFT germplasm relative to lucerne silage (LS).

Materials and methods Second-cutting forage material from BFT germplasm previously selected for low (LTBFT), medium (MTBFT) and high (HTBFT) concentrations of CT, and lucerne, were field-wilted to 43 to 48% DM and ensiled in plastic bag silos. Forages fermented about 130 days before silos were opened for the feeding trial. Thirty-two multiparous lactating Holstein cows, with means (SD) 2.8 (0.9) parity, 128 (67) days-in-milk, 48.6 (6.3) kg/d of milk yield, and 659 (59) kg body weight, were blocked by days-in-milk into 8 squares and, within block, randomly assigned to treatment sequences in balanced 4x4 Latin squares. Cows were fed total mixed rations containing 51% of dietary DM as LS or one of the three BFT. Diets also averaged (DM basis): 10.4% maize silage, 29 to 32% rolled high moisture maize, plus minerals and vitamins; differences in forage crude protein (CP) were adjusted weekly by varying dietary soybean meal content. Over the trial, the LS diet averaged 5.1% soybean meal and the three BFT diets averaged 6.9 to 8.9% soybean meal. All diets contained about 17% CP and 27% NDF. Periods were 4-wk long (16 wks total); data were collected over the last 2-wk of each period. Cows were weighed on 3 consecutive days at the start of the trial and at the end of each period. Results were analyzed using the mixed procedures of SAS; square, period, treatment (forage source), and their interactions, were considered fixed and cow-within-square was considered random. The PDIFF option of SAS was used to test treatment differences among least squares means.

Results and discussion All four silages were similar in fibre content (mean 36% NDF) but LS averaged 25% CP while the three BTF silages averaged 21% CP over the trial. Intake of DM was lowest on LS and greatest on MTBFT; low DMI resulted in 0.2 kg/d of body weight loss on LS, while cows fed one of the three BFT diets gained about 0.5 kg/d (Table 1). There were no differences in yield of milk, but trends were detected for treatment effects on yield of energy-corrected milk (ECM) and fat, which appeared to be related to numerically lower fat secretion on HTBFT. The mean range in weight change of 0.67 kg/d between cows fed LS and BFT confounded results and mobilization of tissue nutrients probably explain the greater efficiencies (milk/DMI and ECM/DMI) observed on LS. Body weight changes measured in changeover studies likely are somewhat inaccurate. Huhtanen and Hetta (2012) estimated SD for body weight change of 0.29 and 0.10 kg when determined, respectively, in a changeover trial with 28-day periods and a 12-week continuous trial. That the magnitude of differences among treatments was substantially greater than these SD estimates indicated that there was a clear difference in body weight change between the LS diet and the three BFT diets. Greater yield of true protein on BFT silages with low and medium CT concentrations, and reduced MUN on all BFT diets, indicated improved N utilization relative to LS. Although these effects were confounded by lower DM intake on the LS diet, these results suggest that presence of CT in the BFT silages improved N efficiency.

Conclusions Greater true protein secretion and reduced MUN concentrations indicated that N utilization was improved when BFT silages replaced LS in the diets of lactating cows. Based on true protein yield and numerically higher milk and ECM yield, the results suggested that MTBFT was the optimal forage.

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Table 1. Production of dairy cows fed silage from lucerne or birdsfoot trefoil with varying levels of condensed tannins.

	Silage[1]					
Trait	LS	LTBFT	MTBFT	HTBFT	SE	P > F[2]
DM intake, kg/d	25.3[c]	27.6[ab]	28.1[a]	26.7[b]	0.58	< 0.01
Weight change, kg/d	-0.19[b]	0.50[a]	0.47[a]	0.47[a]	0.097	< 0.01
Milk, kg/d	41.8	42.4	43.3	41.8	0.90	0.19
Milk/DMI	1.66[a]	1.53[b]	1.54[b]	1.57[b]	0.025	< 0.01
ECM, kg/d	40.7	41.3	42.2	39.5	1.04	0.06
ECM/DMI	1.61[a]	1.49[b]	1.50[b]	1.48[b]	0.031	< 0.01
Fat, kg/d	1.68	1.65	1.72	1.57	0.054	0.08
True protein, kg/d	1.26[b]	1.33[a]	1.33[a]	1.27[b]	0.034	0.02
SNF, kg/d	3.61	3.74	3.79	3.62	0.091	0.07
MUN, mg/dl	14.8[a]	12.8[b]	12.4[bc]	12.0[c]	0.43	< 0.01

[1]Forage sources: LS = Lucerne silage, LTBFT = low tannin birdsfoot trefoil, MTBFT = medium tannin birdsfoot trefoil, HTBFT = high tannin birdsfoot trefoil.
[2]Probability of a significant effect of silage source.
[abc]Means in the same row with different superscripts are different (P < 0.05).

Grass and alternative forage silages for beef cattle and sheep: effects on animal performance

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Keywords: beef cattle, sheep, grass silage, maize silage, whole crop wheat silage, red clover, lucerne, kale, intake growth

Introduction

Traditionally grass silage is the basic component of beef and sheep production systems, in many parts of Europe, Scandinavia, New Zealand, Australia and North America. Levels of animal performance achieved from grass silage are variable and often characterised by low intakes and growth rates. Conversely high quality grass silage can deliver good levels of production but in practice the preparation of high feed value ryegrass silage is often difficult due to a wide variety of factors, including weather.

Given the increase in costs of concentrates inputs and availability and costs of major protein sources such as soya there is much renewed emphasis on maximising production from both grazed and ensiled forages. As silage differs in feed value beef cattle and sheep are normally supplemented with concentrates to achieve commercially optimum production levels. In recent years other ensiled forages including maize, whole crop wheat and legumes such as red clover (Trifolium pratense), lucerne (Medicago sativa) and kale (Brassica oleracea) have partially replaced grass silage in the diet. The objective of this paper is to investigate factors influencing the feed value of grass silage and effects on animal performance. The effect of including maize and whole crop wheat silages in grass silage-based diets on performance of beef and lamb is discussed along with recent progress in the application of high protein forage legumes.

Effects of ensiling on forage intake

Traditionally, it has been considered that ensiling results in a reduction in forage intake and animal performance, as in practice cattle and sheep grazing outdoors have higher intakes than those indoors receiving silage. However this is not a valid comparison as the animals are usually at different stages in their production cycle, the grazing animals can select the forage, whilst those offered silage (particularly if precision chopped) cannot select, and other management, animal and feed factors differ. Keady and Murphy (1993) reviewed data from 75 and 14 comparisons undertaken with sheep and beef cattle and showed a mean decrease in silage dry matter (DM) intake of 37% and 6% relative to the parent herbage respectively. However the fermentation characteristics of the silages offered in these studies differed dramatically. Silage intake characteristics are different for the ovine and bovine (Cushnahan et al. 1994). Keady and Murphy (1993) reviewed 7 comparisons of the effects of ensiling on forage intake of heifers and sheep and reported that whilst offered the same forages, ensiling reduced forage intake by sheep whilst having no effect when offered to heifers. More recently Keady et al. (1995) and Keady and Murphy (1998) reported that when silage is produced using good ensiling management that ensiling per-se had no effect on forage intake (Table 1), but decreased animal performance due to changes in the nitrogenous components and reduced energy value of volatile fatty acids as energy sources to the rumen microbes.

During the ensiling process, major changes occur in the chemical composition of herbage. Two major changes are the conversion of water-soluble carbohydrate primarily to lactic and volatile fatty acids and secondly an increase in the rapidly soluble component of crude protein due to proteolysis and deamination process (McDonald et al. 1991). Supplementation of silage with sucrose to replenish loss of carbohydrate, which occurred during the ensiling process, doesn't compensate for the reduced animal performance due to ensiling per-se (Keady and Murphy 1998). However, supplementation with fishmeal, a known source of undegraded dietary protein, increased animal performance probably due to improved efficiency of rumen microbial protein synthesis as protein in silage is extensively degraded in rumen (Keady and Murphy 1998).

Grass silage feed value

To obtain the optimum level of performance from beef cattle, finishing lambs and pregnant ewes grass silages are normally supplemented with concentrates. The level of concentrate supplementation is dependant on the feed value of the silage and the stage of the production cycle of the animals being offered the silage. The feed value of grass silage is a combination of its intake potential and nutritive value, which is determined primarily by digestibility.

Silage digestibility

Digestibility is the most important factor influencing feed value and consequently the performance of animals offered grass silage based diets (Keady 2000). The effects of digestibility on animal performance are well documented (Steen 1987, Gordon 1989c, Keady et al. 1999, 2008a, 2008b, Steen et al. 2002).

Table 1. The effects of ensiling on herbage composition and animal performance.

	Treatment	
	Fresh grass	Silage (70 days ensiled)
Forage composition		
Dry matter (g/kg)	172	184
pH	6.41	3.89
Crude protein (g/kg DM)	176	173
Water soluble carbohydrate (g/kg DM)	130	33
Dry matter digestibility (g/kg DM)	739	752
Animal performance		
Forage dry matter intake (kg/day)	13.6	13.0
Milk yield (kg/d)	14.1	12.3
Fat plus protein yield (kg/d)	0.98	0.84

(Keady et al. 1995, Keady and Murphy 1998)

Gordon (1989c) concluded that a 10g/kg increase in digestible organic matter in silage DM (DOMD, D-value) resulted in a daily increase in silage DM intake and milk yield of lactating dairy cattle of 0.16 and 0.37 kg/cow respectively. The effect of silage digestibility on animal performance declines as the concentrate proportion of the diet increases. Keady et al. (2008a) reported a milk yield response of 0.38 and 0.31 kg per 10 g increase in DOMD when cows were supplemented with 7 and 11 kg concentrate daily, respectively. Keady and Mayne (1998) from a review of the literature and Keady et al. (2008a) reported that each 10g/kg increase in silage DOMD increased milk protein concentration by 0.14 and 0.16 g protein/kg milk respectively.

Similarly silage digestibility impacts on the performance of beef cattle. Steen (1987) concluded from a review of the literature that a 10g/kg increase in D-value resulted in a daily increase in carcass gain of beef cattle of 33 g when silage was offered as the sole diet and 28 g when concentrate constituted between 20% and 37% of total DM intake. As concentrate feed level increases the effect of silage digestibility on the performance of beef cattle declines. Steen et al. (2002) reported that a 10g/kg increase in DOMD resulted in a daily increase in carcass gain of beef cattle of 29, 30 and 13g when concentrates contributed 20%, 40% and 60% of total DM intake, respectively. Keady et al. (2008b) observed that when concentrate constituted 52% of total DM intake that each 10 g/kg increased in DOMD resulted in an increase in daily carcass gain of 23 g similar to the response reported by Steen et al. (2002) when concentrate constituted similar proportions of the diet.

Steen et al. (2002) and Keady and Kilpatrick (2006) concluded that high feed value grass silages can sustain high levels of beef cattle performance. Steen et al. (2002) using finishing steers offered high feed value grass silage (DOMD 750 g/kg DM) reported no increase in carcass gain (0.78 kg/day) when concentrate accounted for greater than 40% of the diet. Keady and Kilpatrick (2006) showed that bulls offered high feed value grass silage (DOMD 775 g/kg DM) as 50% of the diet sustained the same liveweight gain (1.6 kg/day) as bulls offered *ad-libitum* concentrate.

Increasing silage digestibility has been shown to increase the performance of ewes in mid and late pregnancy (Keady and Hanrahan 2009a, 2010, 2012a) (Table 2) and finishing lambs (Keady and Hanrahan 2012b, 2012c) (Table 3). Furthermore, as concentrate feed level increases, the effect of silage digestibility on finishing lamb performance declines (Table 4). Keady and Hanrahan (2012b) reported that each 10 g/kg increase in DOMD resulted in a daily increase in carcass gain of finishing lambs at 14, 6 and 6 g when concentrate constituted 33%, 51% and 65% of total DM intake, respectively. More recently Keady and Hanrahan (2012c) reported that each 10 g/kg increase in DOMD resulted in a daily increase in carcass gain of finishing lambs of 19, 7 and 10 g when concentrate constituted 19, 43 and 63% of total DM intake.

Lamb birth weight is positively correlated within weaning weight. Keady et al. (2007) and Keady and Hanrahan (2009b and c) concluded that each 1 kg increase in lamb birth weight increased weaning weight by 3.35, 3.16 and 3.17 kg respectively. For the mean of 3 recent studies (Keady and Hanrahan 2009a, 2010 and 2012a) in which ewes in mid and late pregnancy were offered grass silages, differing in feed value, and supplemented with a range of concentrate feed levels, each 10 g/kg increase in DOMD increased lamb birth weight by 0.06 kg and ewe weight post lambing by 1.45 kg respectively.

Table 2. The effects of silage feed value on performance of pregnant ewes and of their lambs.

	Silage feed value	
	Medium	High
Chemical composition		
Dry matter (g/kg)	230	259
Dry matter digestibility (g/kg DM)	702	765
Ewe weight post lambing (kg)	58.7	66.7
Lamb weight (kg) - birth	4.35	4.67
- weaning	30.5	31.7

(Keady and Hanrahan 2009a, 2010, 2012a)

Table 3. The effect of silage feed value on the performance of finishing lambs (offered 0.5 kg concentrate/ lamb daily).

	Silage feed value	
	Medium	High
Chemical composition		
Dry matter (g/kg)	240	266
Dry matter digestibility (g/kg DM)	740	775
Lamb performance		
Live weight gain (g/d)	81	147
Carcass gain (g/d)	39	68
Carcass weight (kg)	19.2	21.1

(Keady and Hanrahan 2012b, 2012c)

Table 4. Effect of silage feed value and concentrate feed level on carcass gain of finishing lambs.

	Concentrate (kg/day)		
	0.30	0.65	1.0
Silage DMD (g/kg DM)			
740	15	72	98
775	62	91	119
Additional carcass weight after 100 day finishing period	4.7	1.9	2.1

(Keady and Hanrahan 2012b, 2012c)

In summary, silage digestibility is positively correlated with carcass gain of beef cattle and finishing lambs, milk yield and composition of dairy cows, and lamb birth weight and ewe weight post lambing.

Major factors affecting digestibility of grass silage

Most of the factors which effect silage digestibility can be controlled by the producer.

Harvest date

Harvest date is the most important factor affecting digestibility. Silage digestibility declines as harvest date is delayed. Digestibility (DOMD) of herbage harvested between 10 May and 7 June declines linearly by 4.2 g per day delay in harvest (Keady et al. 2000). Similarly, the rate of decline in herbage digestibility from the primary regrowth is similar to that of the primary growth. Gordon (1980) and Keady et al. (1999) reported declines in digestibility of 4.9 and 5.0 g/day delay in harvesting primary regrowths of predominantly perennial ryegrass swards. Consequently, silage digestibility declines by 3 to 3.5% units for each one week delay in harvest. Therefore, for each one week delay in harvesting grass silage, to sustain milk yield of dairy cows, carcass gain of beef cattle and finishing lambs and lamb birth weight from pregnant ewes, an additional 1.5 kg/day, 1.2 kg/day, 0.3 kg/day and 8 kg during late pregnancy of concentrate must be fed to lactating dairy cows, finishing beef cattle, finishing lambs, and pregnant ewes respectively.

Crop lodging

Lodging, or flattening, of the grass crop prior to harvest accelerates the rate of decline in herbage digestibility. The accelerated decline in digestibility is due to the accumulation of dead leaf and stem at the base of the sward. Digestibility may decline by as much as 9 percentage units per week in severely lodged crops (O'Kiely et al. 1987).

Sward type

Normally, silage produced from old permanent pastures has a lower digestibility than silage produced from a perennial ryegrass sward. However, the negative impact of old permanent pasture on silage digestibility is dependent on botanical composition. However, if old permanent pastures are harvested at the correct stage of growth, they are capable of consistently producing high feed value silage.

A two year study was undertaken by Keating and O'Kiely (2000), using 4 harvests per year, to evaluate the effects of sward type on grass silage feed value. In the first year of the study, beef carcass output (kg/ha) was similar for silage produced from old permanent pasture (45% meadow grass, 26% bent grass, 10% perennial ryegrass, 6.5% meadow foxtail, 2% docks, 10.5% other) and a perennial ryegrass sward. However, in the second year of the study beef carcass output was lower for the silage produced from the first harvest of the old permanent pasture due primarily to the lower digestibility (Keating and O'Kiely 2000).

Table 5. Effect of sward type on silage composition, digestibility and intake.

	Sward Type			
	Old permanent pasture	Perennial ryegrass	sem	sig.
Silage Composition				
pH	4.1	4.0	0.025	NS
Ammonia nitrogen (g/kg N)	75	74	2.4	NS
Metabolisable energy (MJ/kg DM)	12.0	11.7	0.08	*
Silage DM intake (kg/day)	3.66	3.56	0.17	NS

(Keady et al. 1994)

The effects of sward type on feed value of silage harvested from the second re-growth (third harvest) (Keady et al. 1994) are presented in Table 5. Silage produced from an old permanent pasture (52% perennial ryegrass, 28% creeping bent, 10% meadow grass, 10% yorkshire fog) and that from a perennial ryegrass pasture resulted in silages that had similar (high) feed values as determined by ME concentrations (determined in-vivo) and intake when offered to growing cattle. Consequently, high feed value silage can be produced from old permanent pasture provided it has a moderate level of perennial ryegrass and is ensiled at the correct stage of maturity using good ensiling management.

Perennial ryegrass varieties are classified according to heading date. Whilst the general recommendation is to harvest swards at approximately 50% ear emergence, the actual date of emergence depends on the varieties of grass in the sward and thus on their heading date. The effect of heading date (intermediate or late) of perennial ryegrass varieties and date of harvest on the performance of beef cattle was evaluated in two studies by Steen (1992), and is presented in Table 6. The intermediate and late heading swards each consisted of 3 different varieties of perennial ryegrass. Whilst the mean heading date of the intermediate and late heading swards differed by 24 days (19 May and 12 June) herbage from the late heading swards had to be ensiled within 8 days of that from the intermediate varieties to give the same silage digestibility and daily carcass gain of finishing beef cattle. If the harvest of the late heading sward was delayed until 50% ear emergence the silage DOMD would be 51 g/kg lower than the silage from intermediate heading sward, consequently reducing silage intake and carcass gain (from 0.63 to 0.40 kg/day).

Similarly results from studies using small scale silos show that herbage from late heading varieties (heading date 10 June) must be ensiled on 31 May to produce similar silage digestibility as that for intermediate varieties (heading date 22 May) (Humphreys and O'Kiely 2007). However these authors also noted that the rate of decline in digestibility with harvest date was not as rapid for late-heading varieties as for intermediate-heading varieties.

Table 6. Effect of grass variety heading date at harvest date on annual performance.

	Variety heading date					
	Intermediate (19 May)			Late (12 June)		
Harvest date	20 May	28 May	5 June	28 May	5 June	13 June
Silage DOMD(g/kgDM)	727	702	665	719	684	676
Silage DM intake (kg/d)	6.8	6.2	6.3	6.6	6.4	5.9
Carcass gain (kg/d)	0.63	0.51	0.46	0.61	0.55	0.40

(Steen 1992)

Silage fermentation

Relative to well-preserved silage, poorly preserved untreated silage with low lactic acid concentrations and high concentrations of ammonia nitrogen normally has lower digestibility. The decline in digestibility due to deterioration in silage fermentation may be as high as 50 to 60 g/kg of DOMD.

Fertilizer nitrogen (N) application

Excess fertilizer N application alters silage digestibility. Increasing fertilizer N rate from 72 to 168 kg/ha for the primary growth of predominantly perennial ryegrass swards reduced silage DOMD by 13 g/kg (Keady et al. 2000).

Wilting

Wilting reduces silage digestibility. From reviews of the literature Wilkins (1984) and Rohr and Thomas (1984) reported average proportional decrease in silage DM digestibility, as assessed through sheep, of 0.031 and 0.041 respectively. More recently Steen (1984) and Gordon et al. (1999) using beef cattle, and Yan et al. (1996) and Keady et al. (1999) using dairy cows, reported proportional decreases in total diet digestibility of 0.03, 0.045, 0.02 and 0.01 respectively. The decline in digestibility due to wilting is due to a loss of available nutrients and an increase in ash concentration. The rate of decline in digestibility due to wilting depends on the length of time between mowing and ensiling the herbage, and soil contamination due to mechanical treatment. Rates of loss in digestibility vary from 2.3 to 9.0 g/kg per 10 hour wilting period. Thus each day (24 hours) of wilting will reduce silage DOMD by between 6 and 22 g/kg DM.

Wilting

Wilting herbage prior to ensiling has many advantages including reducing effluent production, improved ensilibility characteristics, reduced quantities of silage for transport during feed out and reduced straw requirement for bedding livestock. When wilting, a rapid wilt is desirable to minimize the decline in digestibility. The rate of water loss during wilting is primarily related to solar radiation and the weight of herbage per unit area in a swath (Wright 1997). Furthermore the lower the initial DM content of the herbage at mowing the more water that has to be removed to increase the DM concentration by 100 g/kg e.g. if herbage is mowed at a DM concentration of 150 g/kg and dried to 250 g/kg 1 kg more water per kg of DM is lost than herbage with an initial DM concentration of 200 g/kg which is dried to 300 g/kg (Wright et al. 2000). Reducing the density of the cut herbage involves covering the total ground area with herbage which results in a higher drying rate. Herbage mown in auto-swaths (two swaths placed into one) has a higher density than when the herbage is tedded out: thus management practices have a big impact on herbage drying rate (Table 7). The data in Table 7 show that to increase herbage DM from 160g/kg to 250g/kg required 65, 30 and 14 hours respectively for herbage which was mown in auto-swaths (6 meters of herbage in one swath), single swaths (3 meters of herbage in one swath) or tedded out to cover the total ground area immediately post mowing, respectively

Table 7. Effects of swath treatment and wilting period on herbage dry matter concentration (g/kg) (Yield = 29.4 t/ha)

	Wilting period (hours)		
	0	24	48
Swath treatment			
Auto-swathed	160	192	228
Single swath	160	229	317
Tedded out	160	304	500

(Wright, 1997)

Many studies have been undertaken which evaluated the effects of wilting on animal performance. Steen (1984) from a review of 40 comparisons in the literature, Steen (1984) from the mean of four studies and O'Kiely (1994) from one study reported that wilting herbage prior to ensiling resulted in an 18%, 5% and 13% increase in silage DM intake, 41, -30 and -56 g change in daily liveweight gain and -30, -40 and -31 g reduction in daily carcass gain of beef cattle, respectively. Using pregnant ewes Chestnutt (1989) reported that wilting herbage at ensiling increased silage DM intake by 7.4% whilst having no beneficial effect (- 0.05 kg) on lamb birth weight. Similarly using finishing lambs Fitzgerald (1986) reported that wilting herbage at ensiling increased silage DM intake by 26% but had not effect on daily carcass gain. More recently, using data from dairy cows, from the mean of 11 comparisons (Patterson et al. 1996 and 1998), summarised by Keady (2000), show that rapid wilting of herbage from a DM concentration of 160g/kg to 320g/kg increased silage intake by 17% and milk solid output by 3% but reduced cow feeding days per hectare by 174 and milk output by 3074 liters.

Many producers delay harvesting in showery weather conditions, with the intention of getting dry weather for wilting. However, in a prolonged period of showery weather crop digestibility is declining, whilst there may be opportunities to harvest and ensile as direct cut (unwilted). The effects of direct cutting, ensiling following water application (equivalent to rainfall) and wilting on animal performance were evaluated (Keady et al. 2002) and presented in Table 8. The wilted herbage was ensiled at a DM

Table 8. Effect of herbage dry matter at ensiling on dairy cow performance.

	Herbage dry matter at ensiling (g/kg)				
	131	187	277	sem	sig.
Silage dry matter intake (kg/day)	9.7	9.6	13.6	0.026	***
Milk yield (kg/day)	20.1	20.0	20.0	0.14	NS
Fat (g/kg)	39.9	40.1	41.3	0.53	NS
Protein (g/kg)	33.2[b]	32.8[a]	34.2[c]	0.13	***

(Keady et al., 2002)

concentration of 277 g/kg following a 30 hour wilting period. Whilst wilting increased silage intake it had no effect on the yield of milk or fat plus protein. Application of water at ensiling reduced herbage DM concentration at ensiling from 187g/kg to 131g/kg but had no effect on silage intake or on milk yield or composition of lactating dairy cows, illustrating that herbage ensiled direct cut (unwilted) during showery conditions has no negative impact on animal performance.

The data clearly show that whilst wilting reduces effluent production, it increases daily silage DM intake and reduces the number of animal feeding days and animal product output per hectare.

Fertilizer management

Nitrogen (N)

To achieve the maximum response to fertilizer N, soil P (phosphorous), K (potassium) and pH need to be at the optimum levels. The response in herbage yield to inputs of fertilizer N is presented in Table 9. The response varied form 5.2 to 10.2 kg herbage DM per 1 kg N. The response varies depending on the base level of nitrogen applied, prevailing weather conditions and harvest date. Fertilizer N also affects herbage composition. Increasing the rate of fertilizer N applied increases herbage crude protein concentration and reduces herbage DM concentrations (Keady and O'Kiely 1996, 1998; Keady et al. 2000) thus producing a greater challenge for silage preservation. Furthermore increasing nitrogen fertilizer application can reduce water soluble carbohydrate (WSC) concentrations (Wilson and Flynn, 1979, Keady et al. 2000) probably associated with increased herbage yield causing a reduction in the capture of solar radiation per unit of plant area. Therefore applying excess fertilizer N can have a negative impact on herbage ensilibility. However, an inadequate level of fertilizer N reduces herbage yield and the crude protein concentration of the subsequent silage. The optimum level of N for the first, second and third harvests of predominantly perennial ryegrass swards are 120, 100 and 80 kg/ha, respectively. If closing paddocks after grazing, assume that between 20 and 30% of the N applied for the most recent grazing is available for the silage crop.

Table 9. Effect of fertilizer (N) application on herbage dry matter (DM) yield in the primary growth.

Source	Range in N application (kg/ha)	kg DM per kg nitrogen
Long et al. (1991)	100 - 150	10.2
Keady and O'Kiely (1998)	120 - 168	5.2
Keady et al. (2000)	72 - 168	7.9

Table 10. Effect of potassium (applied on 2 March) on herbage yield at the first and second harvest (K soil index = 3) and composition of the herbage from the first harvest.

	Potassium applied (kg/ha)						
	0	60	120	180	240	sem	sig.
Herbage dry matter yield (t/ha)							
- first harvest	6.31	6.57	6.74	6.93	6.93	0.091	***
- second harvest	2.56	2.73	2.83	2.94	2.99	0.056	***
Dry matter (g/kg)	179	170	169	171	169	3.1	NS
Buffering capacity (mEq/kg DM)	430	442	454	445	442	9.7	NS
Water soluble carbohydrate (g/kg DM)	101	93	94	100	96	2.7	NS
Nitrate (mg/kg DM)	35	18	18	13	15	9.1	NS

(Keady & O'Kiely, 1998)

Potassium

Large quantities of potassium are required, and removed, by silage crops. Each tonne of herbage dry matter removes up to 26 kg of potassium (Keady and O'Kiely 1998). It had been suggested previously that there is a strong correlation between soil K concentration and the subsequent buffering capacity of

alfalfa (Muck and Walgenback 1985) and that this may have a negative impact on the composition and feed value of the resultant silage. A study was undertaken by Keady and O'Kiely (1998) to evaluate the effects of fertilizer potassium on herbage yield, composition and feed value (Table 10). Increasing the level of potassium fertilizer increased herbage yield at both the first and second harvests (Table 10). The mean response, between the two harvests, was 4.5 kg herbage DM per 1 kg potassium applied. Excess potassium fertilizer application can result in luxury uptake of potassium by the crop. However excess potassium application has no effect on herbage composition or ensilability (Table 10). The quantity of potassium fertilizer which should be applied for silage production depends on the soil potassium index and expected herbage yield (thus crop requirements).

Chop length

Whilst chop length has no effect on silage intake or the performance of beef cattle (Steen 1984) or dairy cows (Gordon 1982), chop length affects the intake characteristics of silage when offered to pregnant ewes (Chestnutt 1989) and finishing lambs (Fitzgerald 1996). Reducing silage chop length, as influenced by harvester type, increased silage intake and liveweight gain of finishing lambs by up to 34% and 242%, respectively (Fitzgerald 1996). When offered to pregnant ewes reducing silage chop length, by use of a precision chop harvester relative to single chopping, increased silage intake and lamb birth weight by 0.25 kg and reduced weight loss by ewes during pregnancy by 4.9 kg (Chestnutt 1989).

In a recent study (Keady and Hanrahan 2008) big-bale and precision-chop silage systems were compared in both the first and second harvests using herbage that had been ensiled at a mean dry matter concentration of 249 g/kg (Table 11). Results showed that system of ensiling had little impact on silage intake or on lamb birth weight. However weaning weight was 1.8 kg higher for lambs from ewes which were offered the precision chopped silage during pregnancy and was due to higher daily live weight gain during weeks 0 to 5 and 5 to 10.

Table 11. Effect of silage harvester system on ewe performance.

	System	
	Big Bale	Precision
Silage dry matter intake (kg/d)	0.95	0.97
Lamb weight - birth (kg)	4.7	4.8
- weaning (kg)	32.5	34.3
Lamb weigh gain (g/day) - 0 to 5 weeks	314	338
- 5 to 10 weeks	314	332

(Keady and Hanrahan 2008)

Additive management

Previously, the principal objective in applying a silage additive was to improve silage fermentation under difficult ensiling conditions. This was achieved by applying acid or sugar-based additives. However, more recent research has shown that the use of effective inoculants can substantially improve animal performance without necessarily altering the fermentation quality of the silage at the time of feeding (Gordon 1980a, b; Keady and Steen 1994, 1995).

Animal performance is the most important measure of the efficacy of a silage additive, as producers are paid for animal product and not for the preservation quality of silage as measured by conventional laboratory analysis. When applying additives it is important to apply them at the correct rate, taking account of changes in the moisture content of the grass being ensiled. For example, if the DM of the herbage is increased from 180 to 250 g/kg, the fresh weight of grass will be reduced from 29.5 to 21 t/ha consequently reducing additive requirement by 40% per ha.

Many studies have been undertaken to evaluate different classes of additives on the performance of beef cattle and lactating dairy cattle. From a review of eleven published studies in which molasses (mean application rate of 15.8 l/t of herbage) and formic acid treated silages were compared with untreated silages Keady (1996) concluded that whilst molasses treatment improved silage fermentation it did not increase animal performance. In the same review Keady (1996) concluded that formic acid treatment increased animal performance by 17%. From a review of 95 comparisons in which different types of additives were compared to untreated silages Keady (1998) concluded that use of proven effective inoculants under a wide range of ensiling conditions or formic acid under difficult conditions increased animal performance. Whilst use of molasses, sulphuric acid and enzyme based additives improved silage fermentation, they had no significant effect on animal performance.

Alternative forages

Traditionally in many parts of Europe, Scandinavia, Australia, New Zealand and North America, grass silage was offered to cattle and sheep during the indoor feeding period. However in recent times other ensiled forages, such as maize and whole crop wheat have increased in popularity and have partially replaced grass silage in the diet.

Maize silage

Major developments, both in plant breeding and in the use of agronomic practices, have enabled the consistent production of high yields of maize forage in areas in which it was not possible to grow the crop 20 – 30 years ago. For example, the DM yield of maize crops produced in Northern Ireland have increased by 300%, to 12.2 t/ha, primarily due to plant breeding (Keady 2005). Development in agronomic practices, particularly the complete cover plastic mulch system (CCPM) has further increased yield potential and maturity of crop grown in more temperate climates. The CCPM system involves covering the crop with a thin clear film (6 to 9 microns), through which the plant emerges at approximately the 6 leaf stage. Use of this system increases forage yield by up to 50% as it enables later maturing varieties to be planted at earlier sowing dates (Keady 2005, Keady and Hanrahan 2012c). Keady (2005) concluded that silage produced from maize sown under the CCPM system, because of its higher yield potential, could be produced at the same cost as grazed grass, but if sown in the open the cost of silage from maize was 30% and 20% more expensive than grazed grass and 3-cut grass silage, respectively.

Effects of maize on beef cattle performance

Keady (2005) concluded from the mean of nine comparisons that partially or totally replacing grass silage, with maize silage significantly increased forage DM intake (1.5 kg/day) and animal performance of beef cattle as determined by liveweight gain (0.23 kg/day), carcass gain (0.11 kg/day) and carcass weight (12 kg). In the same review Keady (2005) concluded, based on dairy cow studies, in order to achieve optimum levels of performance from dairy cattle that maize silage should be harvested and ensiled at a DM concentration of approximately 300 g/kg. More recently using beef cattle, Keady et al. (2012) concluded that the response to maize silage inclusion in the forage component of the diet in terms of daily carcass gain, was dependant on stage of maturity at harvest and level of inclusion in the diet. Keady et al. (2012) concluded that maize silage with a DM of 304 g/kg, when offered *ad-libitum* increased daily carcass gain by 31% due to a combination of increased ME intake and improved efficiency of utilization of ME, and produced carcasses of whiter fat. However when offered as 50% of the forage component of the diet, stage of maturity (maize silage with DM concentrations of 217 and 304 g/kg, respectively) had no significant effect on beef cattle performance.

As outlined by Keady (2005) and Keady et al. (2007, 2008a and 2012) when the cost of concentrate is high relative to the price of animal product, one of the potential benefits of including an alternative forage in grass silage based diets is the potential to maintain animal performance whilst reducing concentrate feed level. Keady (2005) and Keady et al. (2007, 2012) reported potential concentrate sparing effects of maize silage inclusion in the diet of beef cattle of up to 2.4 kg/animal daily, depending on level of maize inclusion in the diet and maturity at harvest.

Effects of maize on pregnant ewe performance

In many parts of Ireland, the UK and other sheep producing regions ewes are normally housed during the winter feeding period and offered ensiled forages. Whilst many studies have shown that including maize silage in the forage component of diets offered to beef cattle and dairy cows increases animal performance (Keady 2005, Keady et al. 2007, 2008a, 2012) few studies have been undertaken to evaluate the effects on the performance of pregnant ewes or finishing lambs. One of the characteristics of maize silage is it's low crude protein concentration (consistently less than 100 g/kg DM) which decreases as maturity increases (Keady et al. 2003, 2008a, 2012) and may impact on its ability to meet the protein requirements of ewes in early and mid pregnancy. Robinson (1983) concluded that forages when offered during early and mid pregnancy should contain a minimum crude protein concentration of 10 g per MJ of ME, otherwise the forage needs to be supplemented with protein to meet the ewe's requirements. Keady and Hanrahan (2008) evaluated the effects of replacing grass silage with maize silages differing in maturity at harvest and supplemented with either 0 or 200 g soyabean during mid and late pregnancy. Replacing grass silage with maize had no effect on ewe performance at lambing or on lamb birth or weaning weights. Supplementation of maize silage with soyabean meal during mid and late pregnancy increased ewe condition at lambing but did not alter lamb performance. More recently Keady and Hanrahan (2009) evaluated the effects maturity of maize at harvest, grass silage feed value, soyabean supplementation during mid and late pregnancy and concentrate supplementation during late pregnancy on ewe and lamb performance (Table 12). Increasing maturity of maize significantly increased ewe weight at lambing. Whilst soyabean supplementation increased ewe weight at lambing, and lamb birth weight, there was no effect on lamb weight at weaning at 14 weeks. Both studies (Keady and Hanrahan 2008, 2009) indicated that increasing maturity of maize at harvest tended to increase lamb weaning weight by 1 kg. Keady and Hanrahan (2009d) offered pregnant ewes maize silage as the based forage and concluded that there is no benefit to supplementing ewes with protein in mid pregnancy and that total concentrate supplementation during late pregnancy for twin bearing ewes could be reduced to 10 kg soyabean without having any negative impact on ewe or subsequent lamb performance.

Table 12. The effects of maturity of maize silage, grass silage feed value and concentrate level on animal performance.

	Maize silage (MS) dry matter				Grass silage feed value (GS)								
	Low		High		Low		High				Significance[3]		
Soya (S)[1]/conc (C)[2]	0/15	200/15	0/15	200/15	0/15	0/25	0/5	0/15	0/25	sem	MS	S	GS
Ewe weight (kg)[4]	63.0	68.6	68.2	76.6	61.2	61.6	70.4	73.6	73.6	2.15	**	**	***
Litter size (lambs/ewe)	2.09	1.74	2.04	2.03	1.95	1.80	1.62	1.65	1.81	0.171	NS	NS	NS
Lamb weight (kg)													
- birth	4.62	4.92	4.65	5.29	4.60	4.56	4.85	5.13	5.13	0.168	NS	**	***
- weaning	33.4	32.8	34.1	34.3	33.6	32.1	34.0	35.0	34.3	1.05	NS	NS	*
Lamb weight gain (g/d)	296	289	303	299	300	284	301	309	302	10.17	NS	NS	NS
Age at slaughter (days)	162	160	159	141	170	175	170	154	164	9.0	NS	NS	P=0.08
Dressing proportion	0.43	0.43	0.42	0.43	0.43	0.43	0.42	0.44	0.43	0.009	NS	NS	NS
Carcass weight (kg)	18.9	18.2	19.1	18.7	18.8	19.1	19.4	19.6	19.2	0.43	NS	NS	NS
Fat classification [5]	3.1	2.8	3.0	2.9	2.8	3.0	2.9	3.0	2.9	0.12	NS	P=0.07	NS

[1]Soya = g/d for duration of the study. [2]Concentrate = kg in late pregnancy. [3]There were no significant concentrate (C) effects or maize silage x soyabean meal or grass silage x C feed level interactions. [4]Weight post lambing. [5] Fat classification (scale 1 – 5; where 1 = thin, 5 = fat)

These studies show that maize silage can replace high feed value grass silage in the diet of ewes in mid and late pregnancy. Maize silage, whilst low in crude protein can be offered as the sole forage without protein supplementation until late (last 6-7 weeks) pregnancy. Increasing the maturity of maize at harvest tended to increase lamb weaning weight by 1 kg.

Effects of maize on finishing lamb performance

Prime lamb production is seasonal and grass-based with lambing normally targeted to coincide with the start of grass growth in spring. However, in Ireland for example, 20% of the lamb kill occurs during the first 3 months of the year which helps to maintain seasonality of supply of lamb to the market. As grass growth rates decline to as low as 0 kg/day a large proportion of these lambs are finished on high concentrate diets, or diets containing conserved forages and/or concentrates.

Keady and Hanrahan (2012c) reported that increasing the maturity of maize silage, as determined by starch concentration from 33 to 277 g/kg DM, improved carcass gain of finishing lambs. The effects of mature maize silage, grass silage feed value and concentrate feed level on the performance of finishing lambs from two studies (Keady and Hanrahan 2012b, 2012c) are presented in Table 13. Maize silage replaced high feed value grass silage and was better than medium feed value grass silage in the diet of finishing lambs as determined by daily carcass gain, regardless of concentrate feed level. As concentrate feed level increased the response to forage feed value declined, but was still evident when concentrates accounted for up to 70% of total DM intake. Keady and Hanrahan (2012b and 2012c) reported that replacing medium feed value grass silage with maize silage resulted in a potential concentrate sparing effect of up to 0.48 kg per lamb daily.

Table 13. Effects of forage type and concentrate feed level on lamb performance.

		Forage		
		Grass silage		Maize
	Conc (kg/d)	Low	High	
Total DM intake (kg/d)	0.3	0.9	1.1	1.1
	0.7	1.1	1.2	1.1
	1.0	1.2	1.3	1.3
	ad-lib		1.4	
Carcass weight (kg)	0.3	17.3	20.6	20.2
	0.7	21.1	22.4	22.1
	1.0	22.8	24.2	23.3
	ad-lib		26.6	
Carcass gain (g/d)	0.3	14	62	59
	0.7	72	91	87
	1.0	98	119	107
	ad-lib		150	

(Keady and Hanrahan 2012b, 2012c)

Effect of whole crop wheat inclusion on animal performance

There has been an increased interest in the production of whole crop cereal silage for feeding to beef and dairy cattle in recent years. The increased interest in this crop is due primarily to the similar cost of production relative to grass silage and the perceived potential benefits in forage intake and subsequently animal performance. Whole crop wheat is predominantly ensiled and fermented at DM concentrations ranging from 250 to 450 g/kg. However, whole crop wheat can also be ensiled at high DM concentrations ranging from 550 to 800 g/kg and treated with either urea or a urea-based additive to encourage an alkaline environment. Recent developments in the ensiling of whole crop cereals involves the ensiling of crops at high DM concentrations (700-800 g/kg), harvested through a forage harvester fitted with a grain processor and ensiled with a urea-based additive.

From a review of 20 comparisons involving dairy cows and 7 comparisons involving finishing beef cattle Keady (2005) concluded that whilst partially or totally replacing grass silage with whole crop wheat, either fermented or urea treated, increased forage intake it had no beneficial effect on the yield of milk or fat plus protein of dairy cows or carcass gain of beef cattle. However, more recently, Walsh et al. (2008) noted that replacing a poorly preserved low feed value grass silage (pH and concentration of DM, ammonia nitrogen and DOMD of 4.3, 174 g/kg 155 g/kg nitrogen and 634 kg DM, respectively) with whole crop wheat increased performance of finishing beef cattle.

Forage legumes

Advances in silage technology have opened up the possibility for alternative forages to be ensiled as high-protein winter forage for livestock. For example, legume forages, grown for two or three years, such as red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*), were previously regarded as being unsuitable for ensiling due to their low water-soluble carbohydrate concentrations and high buffering capacity. Recent studies have shown that these legumes, offered fresh or ensiled, can increase growth rates in lambs due to higher nitrogen (N)-utilisation efficiency and voluntary intake of legumes (Fraser *et al*., 2000; Speijers *et al*., 2004, 2005a; 2005b) than grasses when compared at similar levels of digestibility (Thomas et al. 1985; Wu et al., 2001). Secondly, the introduction of big bales for silage production provided the opportunity to conserve kale (*Brassica oleracea*) more effectively than had been previously achieved in clamps, despite its low dry matter (DM) content. Kale can be grown as an early-sown catch crop or a late-sown main crop with potential for a high crude protein (CP) concentration, with reports of 250 g CP kg^{-1} DM (Martyn et al. 1997). Other advantages of kale are its high DM yield compared to other brassica crops (Drew et al. 1974; Poole, 1990) and high digestibility (greater than 0.88) in the whole plant (Young, 1997 a; Young, 1997b; Young et al. 1997c). Finally, forage pea (*Pisum sativum*) is a high-yielding forage legume with a high CP concentration that, although traditionally harvested for seed, may be harvested as a short-term forage crop for ensiling (Entec, 1997; Potts, 1980; Fraser et al. 2001b).

Despite the potential for these ensiled alternative forages to contribute to livestock systems, there has been relatively little research into their effects on voluntary intake and productivity in growing lambs. Some studies have focused on the effects of different ensiling approaches on the nutritive value of individual forages (Merchen et al. 1986; Narasimhalu and Sanderson, 1994; Fraser et al. 2001a). Other studies have investigated the voluntary intake and productivity in finishing lambs or twin-bearing ewes and their progeny when fed ensiled red clover or lucerne compared with ryegrass (Speijers et al. 2005a; 2005b). Fraser et al. (2001b) compared the effects of harvest date and inoculation on the yield, fermentation characteristics and feeding value of forage pea and field bean silages and Vipond et al. (1998) studied the effects of feeding ensiled kale on the performance of finishing lambs.

The effects of offering ensiled red clover (*Trifolium pratense*), lucerne (*Medicago sativa*), kale (*Brassica oleracea*) and hybrid ryegrass (*Lolium hybridicum*) on the productivity and nutrient use efficiency of lambs were investigated by Marley *et al*. (2007). Live weight gain, food conversion and nitrogen use efficiency was higher in lambs offered the red clover, lucerne and kale silages compared with those offered ensiled ryegrass.

Conclusions

1. Grass silage

a. Digestibility is the most important factor influencing feed value and consequently the performance of animals offered grass silage based diets.

b. Each 10 g/kg increase in DOMD increases

i. Daily milk yield of lactating dairy cows by 0.37 kg.

ii. Daily carcass gain of beef cattle by 28 g/head.

iii. Daily carcass gain of finishing lambs by 10 g/head.

iv. Lamb birth weight by 0.06 kg.

v.Ewe weight post lambing by 1.45 kg.

c. Harvest date is the main factor effecting silage digestibility. Each one week delay in harvest reduces digestibility by 3 to 3.5 % units.

d. To sustain animal performance due to delay of harvest by one week requires an additional

i. 8 kg concentrate per ewe in late pregnancy.

ii. 0.3 kg concentrate daily per finishing lamb.

iii. 1.2 kg concentrate daily per finishing beef animal.

iv. 1.5 kg concentrate daily per lactating dairy cow.

e. Solar radiation and swath density are the major factors effecting rate of water loss from herbage.

f. Wilting results in increased silage DM intake and reduced animal output per hectare.

g. Potassium fertilizer impacts on herbage yield, but does not effect herbage ensilability or silage feed value.

h. Chop length has no effect on silage intake by dairy and beef cattle. However reducing chop length increases silage intake by pregnant ewes and finishing lambs.

i. Use of bacterial inoculants across a wide range of ensiling conditions and formic acid under difficult ensiling conditions increase animal performance.

2. Alternative forages

a. Maize when produced using the CCPM system, can be produced at a similar cost as grazed grass.

b. Optimum stage to harvest maize is at approximately 300 g/kg.

c. Maize silage increases the performance of finishing beef cattle, finishing lambs and preweaned lambs sucking their dams (which had been offered maize silage during pregnancy).

d. Whole crop wheat silage increases forage intake without any beneficial effect on animal performance.

e. Advances in silage technology have opened up opportunity for ensiling high protein legume-based forages such as red clover, lucerne and kale.

f. Studies have demonstrated improvements in live weight gain, food conversion and nitrogen use efficiency in lambs offered red clover, lucerne and kale silages compared with those offered ensiled ryegrass.

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Growth, feed efficiency, carcass quality and consumer perception of beef cattle fed PM vs AM cut grass or a red clover-grass mixture

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Keywords: beef, carcass, consumer perception, red clover

Introduction The practice of finishing steers on forage-based diets is increasingly popular in Canada. However, steer performance on grass silage alone is often poor unless the silage is of high quality (Berthiaume et al. 2006). Conversely, dry matter intake (DMI) of forage legumes, such as red clover (RC), often exceeds that of grass (Steen and McIlmoyle 1982). Unfortunately RC is perceived as being difficult to ensile due to a high buffering capacity and low concentration of fermentable carbohydrates (Owens et al. 1999). Nonstructural carbohydrates (NSC) are the main source of fermentable substrates during ensiling. Recently, Pelletier et al. (2010) reported more NSC in RC cut in the afternoon versus RC cut in the morning. Delaying forage harvesting until the afternoon may be a practical means to improve the ensiling process for RC and other forages. Therefore, the objective of this study was to examine effects of forage type (RC-grass versus grass silage) and cutting time (morning versus afternoon) on silage composition, steer performance (DMI, gain, feed efficiency), carcass quality and consumer perception of beef quality.

Material and methods The experimental work was conducted at the Kapuskasing Beef Research Farm (Canada) in 2009. A total of 32 angus (AN) crossbred steer calves, weaned at 240 d of age, were used. Four silages with contrasting levels of NSC were prepared. Two fields, one of cocksfoot and one of RC-grass, were divided in 2 to study the effect of cutting time. Forages were either cut at 16:00 (PM) or the following day at 06:00 (AM), left to wilt, and ensiled in wrapped round bales. Pure grass (GS) and RC-grass (RCS) silages were harvested on June 24 (primary growth) and August 19 (regrowth) respectively. Due to different climatic conditions in June and August, RCS bales were ensiled between 31 and 36 % DM whereas GS bales were ensiled between 49 and 50% DM. Using botanical separation RCS contained a mixture of RC, grass and weeds (55:35:10) whereas GS contained a mixture of cocksfoot and weeds (77: 23). Standard feed intake and growth data were collected for approximately 125 d. At 365 d of age, steers were slaughtered with collection of appropriate carcass measures. Sensory properties of the *longissimus* (tenderness, juiciness and flavour) were determined by a 12-person taste panel whereas a 60-person panel of meat consumers was asked to visually appraise the intensity of the meat's attributes, assigning rates on a scale reflecting perceived intensity. Presentation of steaks mimicked the conditions found in a commercial meat counter. Data were statistically analyzed as a 2 x 2 factorial arrangement within a randomized complete block design to compare the main effects of forage type (RCS vs GS) and cutting time (AM vs PM) and the interaction between forage type and cutting time.

Results and discussion Red Clover-grass silages (Table 1) had a lower pH, contained less DM, WSC, NDF, N, soluble N and more ash, ADF, ADL, and VFA than GS. Afternoon cut silages had a higher pH, contained more DM, WSC and less ash, NDF, ADF, N and VFA than AM cut silages. The interaction between forage type and cutting time for several quality traits was likely due to the larger effect of PM cutting on RCS DM content. Contrary to published findings (Steen and McIlmoyle 1982) (Table 2), RCS and PM cutting did not affect DMI (20.1 g kg^{-1} live weight), average daily gains (ADG; 830 g d^{-1}) and gain to feed ratios (0.117 kg kg^{-1}). Fat deposition was lower in carcasses from steers fed RCS s versus GS (Table 3) with less fat cover (2.04 vs. 2.77 mm; P = 0.04) and lower amounts of intramuscular fat (IMF; 1.90 vs. 2.41%; P = 0.03). Forage type and cutting time had no effect on Warner Bratzler shear force (WBSF) and on the tenderness score given by the taste panel (Table 4). Nevertheless, beef from RCS carcasses affected (P = 0.0001) consumer perception of IMF colour as assessed using visual appraisal of steaks. Using a scale from 1 (extremely dislike) to 5 (extremely like), consumers found the colour of IMF from RCS fed animals less intense (2.58 vs. 2.95, P = 0.0004) and more acceptable (4.02 vs. 3.77, P = 0.02). Using visual appraisal, consumers also perceived the meat from RCS fed animals as more tender (P = 0.05) and more appealing (P = 0.06).

Conclusions Although it had no effect on animal performance, RCS as opposed to GS produced leaner meat, with similar sensory properties, that was visually more tender and appealing to the consumer.

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Table 1. Effect of AM or PM cutting of red clover(RC)-grass or grass on silage composition.

	RC-grass		Grass			P		
	AM[1]	PM	AM	PM	SEM	Forage type	Cutting time	Interaction
Dry matter, %	31.3	36.3	49.4	50.3	0.63	<0.0001	0.0002	0.006
pH	5.19	5.59	5.53	5.64	0.061	0.005	0.0007	0.03
		% Dry matter basis						
Ash	10.5	9.6	7.6	6.9	0.15	<0.0001	<0.0001	0.62
WSC	1.5	3.2	3.2	5.8	0.30	<0.0001	<0.0001	0.14
NDF	52.4	52.2	57.0	54.3	0.62	<0.0001	0.03	0.06
ADF	36.7	36.3	35.2	32.9	0.38	<0.0001	0.002	0.02
ADL	5.7	5.9	4.5	4.5	0.12	<0.0001	0.53	0.28
N	2.2	2.2	2.5	2.4	0.03	<0.0001	0.01	0.58
Total VFA	2.26	1.88	1.38	1.03	0.087	<0.0001	0.0007	0.90
		% of Total N						
Soluble N	43.7	42.9	50.7	55.1	1.58	<0.0001	0.27	0.12

[1]AM = morning, PM = afternoon.

Table 2. Feed intake and growth of Angus cross yearlings fed AM or PM cut red clover(RC)–grass or grass silage.

	RC-grass		Grass			P	
	AM[1]	PM	AM	PM	SEM	Forage type	Cutting time
Final weight, kg	401	408	406	394	10.9	0.72	0.82
DMI, g kg^{-1} body weight	20.7	19.7	19.5	20.6	0.85	0.84	0.97
ADG, kg d^{-1}	0.82	0.80	0.80	0.90	0.043	0.31	0.36
Gain : feed, kg kg^{-1}	0.113	0.113	0.114	0.128	0.006	0.14	0.19

[1]AM = morning, PM = afternoon.

Table 3. Carcass quality traits of Angus cross yearlings fed AM or PM cut red clover(RC)-grass or grass silage

	RC-grass		Grass			*P*	
	AM[1]	PM	AM	PM	SEM	Forage type	Cutting time
Hot carcass weight, kg	198	196	195	193	6.1	0.55	0.69
Grade Fat, mm	2.4	1.6	2.6	3.0	0.34	0.04	0.55
Intramuscular fat, %	2.1	1.7	2.4	2.4	0.23	0.03	0.56
Shear force, kg	6.5	5.3	6.1	5.6	0.76	0.93	0.25

[1]AM = morning, PM = afternoon.

Table 4. Taste panel and consumers perception of meat from Angus cross yearlings fed AM or PM cut red clover (RC)-grass or grass silage.

	RC-grass		Grass			*P*	
	AM[1]	PM	AM	PM	SEM	Forage type	Cutting time
Taste Panel assessment (scale 1-5)							
Tenderness	4.2	3.8	4.4	4.3	0.33	0.28	0.44
Juiciness	3.1	2.9	3.1	3.0	0.21	0.98	0.46
Flavour	3.9	3.8	3.7	3.5	0.14	0.10	0.65
Visual appraisal by consumers (scale 1-5)							
Intramuscular fat (IMF) colour	2.5	2.7	2.8	3.1	0.09	0.0004	0.02
Acceptability of IMF colour	4.1	3.9	3.8	3.8	0.08	0.02	0.45
Tenderness	3.9	3.8	3.5	3.6	0.08	0.05	0.90
Appeal	3.9	3.6	3.3	3.4	0.09	0.06	0.57

[1]AM = morning, PM = afternoon.

The effects of forage type and feed value, concentrate feed level and protein concentration, and shearing on lamb performance

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Keywords: grass silage, maize silage, concentrate feed level, protein, finishing lambs

Introduction Whilst prime lamb production in Ireland is grass based and seasonal, about 20% of lambs are not slaughtered until the first quarter of the following year. As daily grass growth rates during the winter months are as low as zero many of these lambs are finished indoors on conserved forages and/or concentrates. Grass silage is the main conserved forage produced. Previous studies have shown that increasing silage digestibility increases the performance of dairy cows (Keady et al. 2008b), finishing cattle (Keady et al. 2008a), pregnant ewes (Keady and Hanrahan 2009a, 2010) and finishing lambs (Keady and Hanrahan 2011). Results from previous studies have also shown that inclusion of maize silage in diets based on grass silage increases the performance of beef cattle (Keady 2005, Keady et al. 2007) and dairy cows (Keady 2005, Keady et al. 2008b). More recently it has been shown that maize silage can replace high feed-value grass silage in the diet of pregnant ewes (Keady and Hanrahan 2008 and 2009a) and finishing lambs (Keady and Hanrahan 2011).

Shearing pregnant ewes at housing increases forage intake and subsequent lamb birth and weaning weights (Keady and Hanrahan 2009b). The effect of shearing lambs prior to finishing may depend on the level of metabolizable energy (ME) intake. The aim of the current study was to evaluate the effects on the performance of finishing lambs of: (a) forage type and feed value, (b) concentrate feed level and crude protein (CP) concentration, (c) concentrate offered *ad libitum*, (d) shearing and (e) potential interactions among these factors.

Materials and methods Maize, cv. Surprise, was grown under the complete-cover plastic-mulch system (sown 23 April) and ensiled on 29 September, precision chopped and treated with an inoculant-based additive. High and low feed-value grass silages (FVGS) were produced from herbage ensiled precision chopped and treated with an inoculant on 24 May and 17 June, respectively. Iso-energetic concentrates were formulated to contain CP concentrations of 180 (HP) or 130 (LP) g/kg dry matter (DM) and offered as a course ration. The HP and LP concentrates contained (kg/t) 360 and 400; 100 and 120; 210 and 90; 300 and 360; and 30 and 30 of barley, maize, soya bean meal, citrus pulp and molasses, respectively. The 3 silages were offered *ad libitum* and supplemented with 0.4, 0.8 or 1.2 kg/lamb daily of HP concentrate. The maize silage was also offered with 0.4 kg/lamb daily of LP concentrate. A set of lambs offered concentrate *ad libitum* plus 0.5 kg/lamb daily of high FVGS was also included. Lambs offered diets based on grass silage, maize silage, and concentrates *ad libitum* received 20, 30 and 30 g/d of a mineral/vitamin mixture. Each of the 11 dietary treatments was offered to 24 castrate male Suffolk-X lambs (initial weight 39.0 kg), housed in slatted pens (6/pen) for a 54-day finishing period; lambs in 2 pens/treatment were shorn immediately prior to initiation of the study. All data analyses employed mixed model procedures of SAS with shearing x diet as a fixed effect and pen as a random term. Differences among treatments were partitioned using orthogonal contrasts.

Results The mean DM, ammonia N and ME concentrations of the high and low FVGS and maize silage were 239, 262 and 319 g/kg; 83, 84 and 58 g/kg N; and 11.7, 11.3 and 11.8 MJ/kg DM, respectively. Starch concentration of the maize silage was 324 g/kg DM. There were no interactions ($P>0.05$) between shearing and diet for forage intake or lamb performance. The effects of diet are shown in Table 1. Lambs offered maize silage had higher forage intake than those offered high FVGS, which was greater than the intake of the low FVGS. Lambs offered the maize silage had higher final live weight, carcass gain and dressing proportion, being significantly higher than those offered the low FVGS. Increasing concentrate level reduced forage intake and increased carcass gain, final live weight and dressing proportion. The response to concentrate was linear regardless of forage type or feed value. However, the response to concentrate increased as forage feed value declined. The CP concentration of the concentrate offered with the maize silage did not alter ($P>0.05$) animal performance. Shearing lambs at housing increased forage intake ($P<0.001$) but had no effect ($P>0.05$) on daily live-weight gain or carcass gain. Shearing increased dressing proportion (Table 2).

Conclusions Offering maize silage resulted in a higher forage intake and animal performance than either of the grass silages. Response to increasing concentrate feed level depended on forage feed value. Concentrate CP concentration did not affect the performance of lambs offered maize silage. Re-

gardless of diet, shearing lambs at housing increased forage intake but had no beneficial effect on lamb performance.

Table 1. Effect of dietary treatment on lamb performance.

	Conc (kg/d)	Forage[1]			s.e.	Linear response to 0.8 kg concentrate			
		LS	HS	MS		Forage	Response	Contrast	
Forage dry matter Intake (kg/d)	0.4	0.69	0.81	0.96		LS	-0.18 ± 0.04 ***	LSvHS	*
	0.4LP[2]			0.95		HS	-0.29 ± 0.04 ***	MSvGS	***
	0.8	0.60	0.65	0.77		MS	-0.35 ± 0.04 ***	MSHPvMSLP	NS
	1.2	0.49	0.48	0.56					
	Ad libitum		0.14		0.040				
	Mean	0.59[a]	0.64[b]	0.77[c]	0.015				
Final live weight (kg)	0.4	41.9	45.2	46.3		LS	8.65 ± 0.85	LSvHS	***
	0.4 LP			45.7		HS	5.35 ± 0.85	MSvGS	**
	0.8	47.6	48.4	48.7		MS	3.58 ± 0.86	MSHPvMSLP	NS
	1.2	49.9	50.5	49.6					
	Ad libitum		51.6		0.84				
	Mean	46.4[a]	48.0[b]	48.2[b]	0.53				
Carcass gain (g/d)	0.4	15	50	63		LS	94.0 ± 7.6 ***	LSvHS	***
	0.4LP[2]			73		HS	73.9 ± 7.6 ***	MSvGS	***
	0.8	82	97	106		MS	60.1 ± 7.7 ***	MSHPvMSLP	NS
	1.2	109	124	123					
	Ad libitum		144		5.4				
	Mean	68[a]	90[b]	98[b]	3.37				
Dressing proportion (g/kg)	0.4	438	448	459		LS	32.2 ± 5.8	LSvHS	**
	0.4LP			468		HS	38.1 ± 5.8	MSvGS	**
	0.8	465	468	478		MS	35.6 ± 5.9	MSHPvMSLP	**
	1.2	470	486	487					
	Ad libitum		501		4.4				
	Mean	458[a]	468[b]	475[b]	2.7				

[1]LS = Low FVGS, HS= High FVGS, MS = maize silage; 2LP = low protein concentrate

Table 2. Effect of shearing on lamb performance.

	Treatment		s.e.	Sig
	Unshorn	Shorn		
Forage dry matter intake (kg/d)	0.59	0.70	0.018	***
Live-weight gain (g/d)	161	170	6.6	NS
Final live weight (kg)	48.1	47.3	0.51	*
Carcass gain (g/d)	90	89	3.3	NS
Carcass weight (kg)	22.5	22.4	0.19	NS
Dressing proportion (g/kg)	465	473	2.5	**

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Performance of pigs fed with fresh and ensiled forage of *Vigna unguiculata* CIAT 4555, *Lablab purpureus* CIAT 22759 and *Cajanus cajan*

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Key words: Central America, forage legumes, live weight gain, pigs

Introduction In Nicaragua, monogastric farm animals (e.g., swine, poultry) are important for family nutrition and income of smallholder farmers. The main constraint to production and market competitiveness is scarcity of high-quality (protein) feed. Typically feed of low nutritional value in combination with costly cereal-based concentrates is used. By producing high-value feed on-farm, increased animal production and better product quality becomes a livelihood strategy that improves household nutrition and income. Potentially suitable high-quality forage legumes are identified as an option to improve productivity and decrease costs, and are therefore tested for integration within smallholder production systems.

The objective of this work was to evaluate the effect of the substitution of 250 g/kg of cereal-based concentrates with fresh forage and silage of *Vigna unguiculata* (VF and VS) and *Cajanus cajan* (CF and CS), and silage only of *Lablab purpureus* (LS), on the productive performance of growing pigs.

Material and methods From September to November 2011, 36 castrated male pigs (Yorkshire – Landrace and Yorkshire – Landrace – Duroc crosses) were offered 6 diets in a completely randomised design during two cycles of 35 days each. Feeding trials took place at an agricultural training centre in El Sauce, Department of León, Nicaragua, at 200 masl, with an annual average temperature of 27 °C and relative humidity of 75%. During the first cycle, average initial age and live weight (LW) of the pigs were 4 months and 28.5 (±2.9) kg respectively, and during the second cycle 3 months and 19.8 (±2.9) kg. The diets were as follows: Control (base ration: 500g/kg DM ricebran, 500 g/kg DM sorghum meal), and five treatments in which 250 g/kg DM of the base ration was substituted by respectively VF, VS, CF, CS and LS. Samples of the different components of the rations were analysed and the characteristics of the different treatments are presented in Table 1. Adverse climatic conditions caused some of the materials (especially *Cajanus*) being harvested and ensiled at a suboptimal stage, leading to relatively high fibre contents. Feed was offered based on an intake of 90 g DM/kg $LW^{0.75}$*d (approximately 40 g DM /kg LW), with the forage part at 30% excess of its estimated intake. Daily feed intake, live weight gain, feed conversion ratio and metabolic feed intake was measured.

Fresh forage of *Vigna unguiculata,* biweekly planted, was harvested at flowering stage on a daily base before feeding and leaves of *Cajanus cajan* were plucked from existing one-year-old shrubs. For silage production, *Vigna* and *Lablab* were harvested equally at flowering stage, and *Cajanus* leaves from shrubs. After wilting to achieve a dry matter (DM) content of 300 g/kg fresh matter (FM), the material was chopped, 40 g molasses per kg FM were added, inoculated with 10^5 cfu/g FM of a tropical *Lactobacillus* strain (CIAT S66.7) and compacted manually in 60 l plastic bags and 20 l plastic buckets, tightly closed and kept for at least 30 days in a storage room at ambient temperature before feeding. Organoleptic characteristics of silage were in general good. The smell was pleasant and free of butyric acid. The structure, texture and colour were well maintained. For statistical analysis the SPSS GLM procedure and multiple range test of Tukey were applied.

Results and discussion The overall performance in terms of live weight gain was poor. This was in the first place caused by the low (initial) live weight of the pigs, which affected negatively the digestive utilization of the legume supplements; parallel on-farm trials with larger animals showed far higher live weight gains. Secondly, adverse weather conditions (both drought and rainfall excess) reduced legume biomass production at the legume plots to such an extent that (more fibrous) legume material from other sites had to be used.

The rations had a significant effect on intake, live weight gain and feed conversion (Table 2). The pigs fed with Control, CS and VS showed highest total intake, but the relatively high fibre contents of some of the forage materials curbed total intake somewhat leading also to lower crude protein consumption. Highest live weight gain was achieved with VS and LS. LS showed also best feed conversion ratio. When compared to fresh forage, silage of especially *Vigna unguiculata* showed 20% higher live weight

gain and 25% better feed conversion ratio. When comparing only the materials of which both silage and fresh forage were available (i.e., *Cajanus* and *Vigna*), pigs tended to consume more silage than fresh material (P<0.1), but no significant effects on live weight gain were observed. No differences in performance of the pigs were found between the two cycles (P>0.05), but (initial) live weight affected all performance parameters significantly (P<0.001) except for feed conversion and was therefore included in the statistical model as a covariable.

Conclusions Silages of especially *Vigna unguiculata* and *Lablab purpureus* are promising options to improve smallholder pig production. When compared to fresh forage, silage tends to improve pig performance parameters, and on-farm experiences confirm that additional advantages like conservation for dry season use and easier transport and storage are important factors increasing the likelihood of adoption of this technology by smallholders.

Acknowledgements The financial support by the Federal Ministry for Economic Cooperation and Development, Germany (BMZ), is gratefully acknowledged.

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Table 1. Composition of the experimental diets for growing pigs fed with fresh and ensiled forage legumes as partial source of protein (g/kg).

	Control	Control + CF	Control + CS	Control + LS	Control + VF	Control + VS
Dry matter	868	724	781	726	698	711
Organic matter	876	871	872	859	855	847
Crude protein	129	144	146	139	134	147
Acid detergent fibre	62	197	195	163	149	142
Lignin	18	68	59	45	51	51

Table 2. Performance of growing pigs fed with fresh and ensiled forage legumes as partial source of protein.

Parameter	Control	Control + CF	Control + CS	Control + LS	Control + VF	Control + VS	SE	Sig.
DM intake (g/day)	797^{b}	555^{a}	763^{ab}	671^{ab}	713^{ab}	742^{ab}	24	***
Protein intake (g/day)	104^{ab}	81^{a}	113^{b}	94^{ab}	96^{ab}	110^{ab}	3.3	*
Digestible Energy intake (Kcal/day)[1]	2863^{c}	1357^{a}	1881^{ab}	1840^{ab}	2031^{b}	2160^{b}	95	***
Live weight gain (g/day)	127^{ab}	97^{a}	113^{ab}	159^{ab}	144^{ab}	176^{b}	8.1	***
Feed conversion (DM intake/weight gain)	7.46^{a}	5.96^{a}	6.90^{a}	4.24^{a}	5.60^{a}	4.44^{a}	0.38	*

CF: *Cajanus cajan* Fresh; CS: *Cajanus cajan* Silage; LS: *Lablab purpureus* Silage; VF: *Vigna unguiculata* Fresh; VS: *Vigna unguiculata* Silage
Different superscripts (a,b,c) within a row denote significant differences between treatments (Tukey, P<0.05)
[1]estimate based on ADF content of the ration (Drennan and Maguire 1970)

The determination of silage quality on maize and soybean grown on different cropping systems

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Keywords: crude protein, dry matter, fleig score, Glycine max L. Merr., pH, Zea mays L.

Introduction Ensiling is a common preservation method for moist forage crops. Whole crop maize is the major crop ensiled in Turkey. Maize silage is normally a high-energy forage with high dry matter (DM) yield in relation to other forage crops (Coors 1996). However, maize silage is poor in protein content at approximately 7.5 % of crude protein (CP) in DM when cut at the hard dough stage (Titterton and Maasdorp 1997). Protein supplementation is required for maintenance and production of low yielding dairy cows on a maize silage ration. Mustafa and Seguin (2005) reported that forage-type soybean cultivars were well preserved as silages. Soybean and their legumes are usually difficult to ensile because of low sugar content and high buffering capacity. No information is available regarding the feeding value of forage soybean silage for dairy cows. The objective of the present study was to evaluate silage quality of maize and soybean grown on different cropping systems.

Material and methods This study was conducted in Isparta province (37°45′N, 30°33′E, elevation 1035 m) located in the Mediterranean region of Turkey during 2009 and 2010 growing seasons. Cadiz as maize (*Zea mays* L.) cultivar and Yemsoy (*Glycine max* L. Merr.) as soybean cultivar were used in this research.

The experiment was established in a randomized complete block design with three replicates. Five different cropping systems (sole maize, sole soybean, one row of maize and one rows of soybean, two rows of maize and one rows of soybean, one row of maize and two rows of soybean) were used in this study. Sowing was done by hand. The experiment was repeated on an adjacent site in the second year. Plots were irrigated with drip irrigation systems. A fertilizer application (100 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5) was uniformly sprayed after sowing. Pesticides were not used for crop protection.

The plots were harvested for silage when the maize was on milk stage and plants were chopped and filled into jars (2 litres) removing all air. Preservative agents were not used in silages.

At the end of the fermentation period, jars were opened and chemical analyses were carried out to determine the feeding quality of the silage. Some physical parameters (colour, smell and structure) were observed according to Alçiçek and Özkan (1997). pH values of silage samples were measured according to Polan et al. (1998) using a pH-meter. DM and CP content analyses were carried out following the method reported in AOAC (1990). The ANKOM Fibre Analyzer was used for NDF and ADF analysis. In determining the quality of the silage samples, the following Fleig equation was used (Nauman and Bassler 1993):

Fleig score = [220 + (2×silage dry matter content (%) – 15)] - 40 × pH value

The data were analysed by GLM procedure using the SAS statistical software (SAS 1998). Means were separated by LSD at the 0.05 level of significance.

Results and discussion Cropping systems had significant effects on physical parameters such as colour, smell and structure. Sole maize and maize-soybean mixtures had significantly better colour and structure than sole soybean (Table 1). According to the physical parameters, sole soybean silage had medium quality, while sole maize, 1maize - 1 soybean and 2 maize – 1 soybean silages had excellent quality. These results were consistent with the findings of Altınok et al. (2005).

Table 1. The physical parameters of maize and soybean silages.

Cropping Systems	Colour	Smell	Structure	Total	Quality Class
Sole Maize	2.00 a	14.00 a	4.00 a	20	Excellent
1 Maize – 1 Soybean	2.00 a	13.33 a	3.67 a	19.00	Excellent
2 Maize – 1 Soybean	2.00 a	13.33 a	4.00 a	19.33	Excellent
1 Maize – 2 Soybean	1.67 a	8.33 b	3.67 a	13.67	Good
Sole Soybean	1.00 b	4.00 c	2.00 b	7.00	Medium
LSD (0.05 level)	0.41	1.42	0.43		

Means on each column followed by the same letter were not significantly different at 0.05 level using LSD test

Sole soybean silage had the highest DM content, pH and CP content, while having the lowest ADF and NDF contents and Fleig score (Table 2). Increased pH value may have been caused by relatively higher nitrogen content of soybean (Saricicek and Kılıc 2011). The CP content of sole soybean (15.2 %) was significantly higher than that of the other silages. Sole maize silage had poor CP content (6.15 %). The reduction in CP content of silage was attributed to the extensive proteolysis during the ensiling process. Although legume silage usually has a high protein content (Jacobs and McAllan 1991), legume material on its own is extremely difficult to ensile because of its high buffering capacity and low levels of fermentable carbohydrate (Harrison et al. 1994).

Table 2. The DM content, pH, CP, ADF and NDF contents and Fleig scores on maize and soybean silages.

Cropping Systems	DM (%)	pH	CP (%)	ADF (%)	NDF (%)	Fleig Score	Quality Class
Sole Maize	22.11 cd	3.58 c	6.15 d	26.05 a	46.15 a	106.0 a	Excellent
1 Maize – 1 Soybean	22.60 c	3.82 c	7.73 c	24.11 b	44.11 b	97.4 a	Excellent
2 Maize – 1 Soybean	23.50 c	3.71 c	7.82 c	24.44 b	44.40 b	103.6 a	Excellent
1 Maize – 2 Soybean	25.21 b	4.21 b	9.61 b	22.07 c	39.51 c	87.0 a	Excellent
Sole Soybean	30.05 a	5.18 a	15.2 a	16.13 d	29.11 d	57.9 b	Medium
LSD (0.05 level)	1.21	0.38	1.27	1.51	1.52	18.1	

DM: Dry Matter, CP: crude protein, ADF: acid detergent fibre, NDF: neutral detergent fibre

Maize silage is a major source of NDF for many dairy cows. Increasing the concentration of NDF of maize silage could mean that lesser amounts of other forages would have to be grown or purchased by the dairy farmer to meet NDF requirements (NRC 2001). According to Fleig scores, sole soybean silage had medium quality, while the other cropping systems had excellent quality.

Conclusions According to the average of a-two year growing season study, the quality of silages that included maize were found better than the silages originating from other cropping systems. The silages made by sole soybean were found as medium in quality. The crude protein contents of silages were increased by increasing of soybean rate in the mixtures. As a result, the seeding of soybean with maize in alternate rows as 1 row maize + 1 row soybean or 1 row maize + 2 row soybean had substantial effects on the increasing of silage quality and crude protein rate. In other words, crude protein contents of silage were increased by increasing of soybean rate in the mixtures.

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Productivity and quality of meadow fescue, tall fescue and festulolium in silage cutting regime in Finland

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Keywords: *Festulolium, Festuca pratensis, Festuca arundinacea*, grassland production quality, yield

Introduction Although timothy (*Phleum pratense*) is the main species in forage production on the most northern areas in Europe, several *Festuca* species are commonly used. The use of tall fescue has increased in Finland in consequence of high yields obtained in experiments (Niemeläinen et al. 2001, Kangas et al. 2011) and on encouraging experiences in commercial production. Recently also cultivars of festulolium have entered into the portfolio of species for forage production. Two festulolium cultivars are listed on the Finnish National list of plant varieties (Anon 2011). Meadow fescue, tall fescue and festulolium cultivars have been tested together in the same official cultivar trials in Finland and here we present results of the performance of these species during 2005–2011. Yield performance and quality characteristics as well as differences between meadow fescue, tall fescue and festulolium are presented and discussed.

Material and methods Data of official variety testing in Finland for the period 2005–2011 was used in this study. Fescue trials are principally cut three times per growing season (silage cutting regime). Mineral fertilizer is applied in the spring and after the first and the second cut. Nitrogen level is 100, 100 and 50 kg N ha^{-1} for the first, second and third cut, respectively. Trials are on mineral soils. Each trial lasts for three production years. The whole trial including all fescue species is cut at the same date.

From each species studied, four cultivars were chosen to this study. Currently all the chosen meadow fescue (MF) and tall fescue (TF) cultivars are on the national list of cultivars in Finland, but in contrast, two of the festulolium cultivars have not been approved on the list. Used MF cultivars were 'Ilmari', 'Inkeri', 'Kasper' and 'Valtteri', and TF cultivars 'Karolina', 'Kora', 'Retu' and 'Swaj'. Of the used festulolium (FL) cultivars, 'Felina' and 'Hykor' are on the national list of cultivars and 'Fojtan' and 'Paulita' are not on the list. 'Felina' and 'Hykor' represent quite similar genome constitution with each other and are distinctly different to 'Paulita' cultivar (Kopecky et al. 2006). Data comprised altogether of 63 trials from 11 locations. The trials were cut three times in 60 percent of the trial and twice a year in about 40 percent of the trials (mostly at locations in northern Finland). The set of tested cultivars changed from year to year. On average, each trial included 5.1 cultivars of the 12 cultivars used in this study, so the number of results used were different for each cultivar and consequently for each species. The highest number of results was for cultivars 'Kasper' (n = 63) and 'Retu' (n = 59) and the lowest for new cultivars 'Valtteri' (n = 12) and 'Swaj' (n = 13). Analyses of the quality characteristics (acid detergent fibre, ADF; neutral detergent fibre, NDF; lignin, D-value and the content of total nitrogen in DM) were carried out by NIRS methodology at MTT Agrifood Research Finland laboratory in Jokioinen. The content of protein (% in DM) was calculated as total nitrogen in DM × 6.25.

Mutually comparable means were needed for 12 cultivars to compare three species (4 cultivars per species). It was not possible to use the arithmetic mean because the tested set of cultivars changed from year to year. REML estimation method was used to estimate mutually comparable means for all cultivars by separating genotypic effects from environmental effects. First, second and third cut were analyzed separately. The MIXED procedure of SAS was used to analyze the data. Used model included environmental effects as random effects: year, site and trial. Cultivar was the only fixed effect in the model. Differences between species were made by contrasts (i.e. F-tests using MIXED/contrast-statements).

Results and discussion Estimated dry matter yield (DMY) and quality characteristics of each species are presented according to cuts in Table 1. Estimated total annual DMY was significantly higher in TF (10 784 kg DM ha^{-1}) than in MF (9 490 kg DM ha^{-1}) or FL (9 261 kg DM ha^{-1}), relative values are 100, 88 and 86 for TF, MF and FL, respectively. The difference was not statistically significant between MF and FL. DMY profile of the species differed. These results take into account that in 40 percent of the trials only two cuts were taken.

MF had significantly higher DMY in the first cut than TF and FL (Table 1). However, in the second and third cut the situation was the opposite. When the proportion of DMY in the first cut of the annual DMY was calculated, it was 45 % for MF, 36 % for TF and 32 % for FL. Good regrowth ability of TF is reported e.g. by Niemeläinen et al. 2001. TF also produces higher yields compared to MF when the stand grows older (see Niemeläinen et al. 2001; Kangas et al. 2011).

Harvest date could dramatically influence the quality characteristics of the yield in the first cut.

Whole trials were harvested at the same date and cutting was scheduled so that D-value would be close to 690. This is a general guideline in Finland to schedule first cut in order to obtain both adequately high digestibility and high DMY. Results for the fibre fractions and D-value in the first cut were in practice at the same level in all of the species, although some differences were statistically significant (Table 1). The species means, however, hide differences which occurred between the cultivars of the same species, particularly in FL. FL 'Paulita' had very high D-value in the first cut (746) in relation to 'Felina' (681) and 'Hykor' (684). The share of Lolium chromosomes is much higher in 'Paulita' than in 'Felina' and 'Hykor' (Kopecky et al. 2006), which may cause the difference. 'Paulita' had also much lower ADF, NDF and lignin values in the first cut than the other FL cultivars (data not shown). Different quality values of 'Paulita' in relation to other studied FL cultivars were observed also in the yield of the third cut (data not shown).

Content of protein differed between the species in all cuts (Table 1). Nitrogen fertilizer application was the same for all species and the differences in the protein concentration between the species at each cut seemed to be negatively correlated to the differences in DMY. In the second cut, the differences in D-value between the species were major (Table 1). MF had higher D-value than TF in all cuts.

Table 1. Quality characteristics and dry matter (DM) yield of meadow fescue (MF), tall fescue (TF) and festulolium (FL) in first, second and third cut. Data from Finnish official cultivar trials from years 2005-2011.

Cut / Species	ADF g kg DM^{-1}	NDF g kg DM^{-1}	Lignin g kg DM^{-1}	D-value g kg DM^{-1}	Protein %	DM yield kg DM ha^{-1}
First Cut						
MF	339 a*	583 a	33.5 a	699 a	13.5 b	4236 a
TF	330 b	568 b	32.3 b	692 b	13.9 ab	3926 b
FL	320 c	555 c	32.8 ab	696 ab	14.9 a	2918 c
s.e.m.	10	13	2.3	10	0.7	289
P-value	< 0.001	< 0.001	0.01	0.03	< 0.001	< 0.001
Second Cut						
MF	325 b	575 a	30.0 c	707 a	14.9 a	3478 c
TF	337 a	568 b	31.8 b	677 b	13.3 b	4617 a
FL	339 a	573 ab	34.5 a	670 c	13.2 b	4082 b
s.e.m.	6	6	2.5	5	0.7	268
P-value	< 0.001	0.03	< 0.001	< 0.001	< 0.001	< 0.001
Third Cut						
MF	328 a	589 a	32.2 a	707 a	13.1 a	2361 b
TF	334 a	570 b	31.6 a	689 b	12.0 b	3127 a
FL	330 a	561 b	32.0 a	693 b	11.8 b	3068 a
s.e.m.	10	18	2.4	9	0.8	177
P-value	0.29	< 0.01	0.64	< 0.01	< 0.01	< 0.001

Quality characteristics: ADF = acid detergent fibre; NDF = neutral detergent fibre; D-value = digestible organic matter in dry matter, Protein (%) = 6,25 x total nitrogen in DM.
* Means with the same letter in the column within each cut do not differ significantly from each other.

Conclusions Meadow fescue produced higher dry matter yield in the first cut than tall fescue or festulolium. However, tall fescue produced the highest annual dry matter yield. Quality characteristics of festulolium cultivars differed significantly from each other and the genetic constitution of the cultivar has to be known to schedule the cutting time in order to obtain good feeding value.

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Assessing the relative silage yield production potential of perennial ryegrass varieties in comparative trials

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Keywords: perennial ryegrass, silage timing, variety testing

Introduction The Agri-Food and Biosciences Institute (AFBI) annually produces perennial ryegrass *(Lolium perenne L.)* variety recommended lists (Gilliland & Meehan 2011). The varieties are tested in maturity groups defined by the mean date of ear emergence (MDEE) of two delineating varieties, Lilora and Barplus. This restricts the heading date span between varieties, but as all varieties in a group are cut together a heading date range remains, and so varieties are cut at different stages of reproductive development. The timing of the first cut influences the rate and composition of herbage produced after a fixed regrowth period to the second cut (Gilliland 1995), which is interpreted as being due to differences in reproductive stage at the first silage cut inducing a reversal or compensating response at the second cut. Furthermore, Hoppe et al. (2012) reported that delaying the first silage cut by up to four weeks progressively changes variety ranking. This raises concerns that variety maturity differences might induce the same bias in trials. This study examined the yield and quality of perennial ryegrass (PRG) varieties in AFBI recommended list trials to assess the extent of any effects between maturity and fixed cutting dates, on the ranking of varieties at the first and second silage cuts.

Material and methods A ten year variety performance data base for 22 intermediate and 27 late maturing PRG varieties were assessed under the AFBI variety list testing protocol (Grogan & Gilliland 2011). Dry matter (DM) yields and digestibility were derived from the 1st cut (at an estimated average of 50% MDEE across all varieties in trial) and 2nd cut (6 weeks regrowth) in the third harvest years (2001-2010) of the silage management (3 cuts per year), as described by Fera (2011). As this testing system annually includes new varieties and discards older poorer performers, the results comprised an incomplete 10 year data matrix of all 49 varieties. This was analysed by fitted constant statistic, whereby the confidence limits on any comparison is dependent on the number of data points (annual tests) present for that comparison and the root mean square and df of the data matrix.

Results and discussion The 10 year MDEE for Lilora was 16 May and for Barplus was 1 June, giving a possible heading date range of 16 days in the intermediate group, within which the earliest variety was Niagara (17 May) and the latest AstonEnergy (30 May). Similarly, although the late maturity group is theoretically open-ended, the latest heading variety was Twytop (14 June) and the earliest was Elgon (1 June), giving a 13 day span. In the intermediates, the silage yields (Table 1) ranged from 6.31 t/ha (AberStar) - 7.67 t/ha (AberGlyn) in the first cut and from 3.88 t/ha (Cashel) - 4.92 t/ha (Dunluce) at the second cut. Similarly, digestibility ranged from 64.8% (Boyne) - 76.1% (AberGreen) at Cut 1 and at Cut 2 between 68.2% (Boyne) - 74.7% (Eurostar). When correlations were performed between the MDEE and the performances of the 22 intermediate varieties, there was an R^2=0.463 for Cut 1 DM yield and R^2=0.115 for Cut 2. Digestibility relationships were much weaker at R^2=0.154 at Cut 1 and R^2=<0.01 at Cut 2. For the late maturing varieties there was again a wide range in variety performances, as Cut 1 DM yields ranged between 8.27 t/ha (Delphin) - 5.50 t/ha (Twytop) and at Cut 2 between 4.65 t/ha (AstonEnergy) - 3.29 t/ha (Tyrella) and were again loosely correlated with MDEE (Cut 1 R^2=0.322, Cut 2 R^2= 0.142). Digestibility ranges again showed even weaker relationships as the Cut 1 correlation (Tyrella 65.8% - AstonEnergy 74.7%) was only R^2=0.025 and at Cut 2 (Twytop 67.9% - Dunloy 75.0%) was only R^2=0.055.

These results suggest that the confounding effects between silage timing and yield or digestibility is not as great in these trials as found in the experimental studies of Hoppe *et al.* (2012) and Gilliland (1995). This may not, however, be an entirely accurate deduction as any interaction between heading dates and performance would be expected to be year specific. Although the testing system allows adjustment in cut timing for seasonal variation, heading date is highly compressed or extended by growing conditions and so annual interactions may be masked by 10-year fitted means.

It is notable that when Cut 1 and Cut 2 were compared there were very large changes in variety ranking. Table 1 shows only a subset of the examined varieties, giving the highest and lowest diploid and tetraploid variety for each parameter. Some varieties showed high consistency in ranking. AberGlyn was ranked highly for yield and lowly for digestibility at both cuts. In contrast, Twytop and Tyrella displayed very large and opposed ranking changes between Cut 1 and Cut 2 for yield and digestibility. Some

varieties also showed acute changes in only yield or only in digestibility between the cuts, with their rank switching significantly above or below the median values. As varieties differ in their seasonal yield distribution, this may cause differential responses to defoliation at different reproductive development stages. Such differential responses would explain the lack of strong relationships between MDEE and performance as well as the observed changes in variety ranking. Calculating Cut 1 and Cut 2 means, as used in some recommended lists, appears to offer an adjustment for some of these effects as the relationships between MDEE and performance were further weakened (correlation data not shown). This approach also changed the variety rankings (Table 1), with Magician and Delphin highest for both yield and digestibility. This is an inadequate solution as it only provides further masking of possible confounding effects.

Conclusions It was concluded that cultivar potential differs depending upon the cut. Further studies are needed to quantify annual interactions and ensure test results correctly reflect variety potential.

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Table 1. Summary of highest and lowest performing diploid and tetraploid varieties at the first and second silage cut.

		Silage Cut 1					Silage Cut 2					Silage Cut 1+2			
Variety	Maturity	test	rank	DM Yield	rank	DMD	test	rank	DM Yield	rank	DMD	rank	DM Yield	rank	DMD Yield
Intermediate Varieties			***(22)***	***t/ha***	***(22)***	***%***		***(22)***	***t/ha***	***(22)***	***%***	***(22)***	***t/ha***	***(22)***	***t/ha***
AberGreen	28 May	2	22	5.63	1	76.08	1	2	4.56	9	71.78	22	10.18	4	8.53
AberStar	24 May	4	19	6.31	3	72.16	5	15	3.99	7	72.58	18	10.30	13	7.65
Boyne	19 May	2	3	7.50	22	64.84	3	9	4.18	22	68.19	4	11.68	11	7.93
Cashel	18 May	5	17	6.41	16	69.58	5	22	3.88	13	71.24	19	10.28	22	7.18
AberGlyn(T)	18 May	5	1	7.67	19	67.09	4	18	3.97	14	70.94	5	11.63	9	7.95
Dunluce(T)	28 May	5	18	6.31	2	74.40	4	1	4.92	4	73.72	8	11.23	5	8.51
Eurostar(T)	24 May	5	7	7.21	5	71.94	4	11	4.11	1	74.66	7	11.32	12	7.88
Magician(T)	18 May	5	2	7.60	4	72.09	5	3	4.40	5	73.50	1	12.00	1	8.66
Niagara(T)	17 May	4	12	6.86	9	71.04	5	12	4.11	3	73.77	13	10.97	7	8.08
	Mean			6.81		70.23			4.17		71.54		10.98		7.87
	Median			6.65		70.46			4.40		71.43		11.01		7.89
Late Varieties			***(27)***	***t/ha***	***(27)***	***%***		***(27)***	***t/ha***	***(27)***	***%***	***(27)***	***t/ha***	***(27)***	***t/ha***
AberBite	4 Jun	4	9	7.45	15	70.69	4	4	4.00	24	68.29	5	11.45	6	8.31
AstonEnergy	30 May	5	26	6.11	1	74.67	5	1	4.65	2	74.29	16	10.75	13	8.10
AstonPrincess	5 Jun	3	11	7.34	19	69.42	4	18	3.62	18	70.17	12	10.96	3	8.50
Twytop	14 Jun	6	27	5.50	7	71.65	4	2	4.40	27	67.93	27	9.90	23	7.63
Tyrella	2 Jun	4	3	7.96	27	65.82	4	27	3.29	9	71.56	8	11.25	27	7.51
Delphin(T)	31 May	6	1	8.27	20	69.35	6	14	3.74	13	71.25	1	12.01	1	8.63
Dunloy(T)	7 Jun	4	22	6.87	5	71.72	5	8	3.89	1	74.98	15	10.76	9	8.23
Elgon(T)	1 Jun	5	12	7.24	14	70.97	6	24	3.32	15	70.87	21	10.56	18	7.86
Kintyre(T)	5 Jun	4	16	7.12	17	70.13	4	5	3.98	22	69.08	9	11.10	7	8.30
Millennium(T)	9 Jun	6	23	6.79	6	71.72	6	11	3.81	19	70.12	20	10.60	25	7.55
	Mean			7.18		70.59			3.73		70.90		10.91		8.01
	Median			6.88		71.67			3.97		71.46		10.95		8.01
Residual Mean Square				26.34		251.89			0.48		8.35				
Degrees of Freedom				598		598			630		630				

test = number of trial years in 10 data matrix; values in parenthesis are total number of varieties examined; single and double underline identify highest and lowest ranked respectively, for diploid and tetraploid (T) varieties

The effect of harvest timing on the amount and the quality of total yield of grass silage per growing season

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Keywords: D-value, harvest timing, NDF, non-fiber carbohydrates, silage

Introduction The harvest timing during the growing season affects notably the amount and the quality of grass silage both in the first and the second harvest. It is important to understand the sum influences of all cuts in order to succeed in cattle feeding rationing.

Material and methods The experiment was conducted at MTT Maaninka (63º08'N, 27º19'E) and MTT Ruukki (64º40'N, 25º00'E), Finland, during the growing seasons 2009–2011. The study included four grass silage (timothy–meadow fescue at Maaninka and pure timothy at Ruukki) harvesting strategies: A early (target D-value 690 g kg^{-1} DM), B delayed (650 g kg^{-1} DM), C late (620 g kg^{-1} DM) in the first harvest combined with one regrowth cut, and D three harvests (target D-value 690 g kg^{-1} DM in the first harvest) per growing season. In strategies A–C the second cut was harvested in August. In strategy D the second cut was harvested at the end of July and the third cut at the end of September or early in October. Neutral detergent fiber (NDF), organic matter solubility (*in vitro*), crude protein (CP) and ash were determined in the Animal Production laboratory at MTT. ME yield was calculated in GJ as DM yield * 0,016 * D-value in g kg DM^{-1}/1000 as presented in MTT 2012. Non-fiber carbohydrates (NFC) were calculated by 1000 – (NDF + CP +ash + crude fat) (NRC 2001). The fat content was estimated using Finnish feed tables (MTT 2012). NDF and NFC contents for the total yield were calculated by weighting the values by the DM yields of the cuts.

The analyses were performed using the *Mixed* procedure of the SAS 9.2. Maaninka and Ruukki were analyzed separately. Harvesting strategy, year and their interaction were the fixed effects while replicate and replicate × year interaction were the random effects. Year was used as a repeated measurement with Toepliz or Compound Symmetry-covariance structure.

Results and discussion Delaying the first cut increased both the dry matter (DM) and the metabolized energy (ME) yield (Table 1) but it decreased the grass digestibility. The changes in the first cut were partly reversed in the second cut. Delaying the first cut decreased the DM yield-weighted D-value of the total yield by 40 g kg^{-1} DM (682 → 646 g kg^{-1} DM at Maaninka, $p<0.001$; 686 → 643 g kg^{-1} DM at Ruukki, $p<0.001$). The low D-value of the silage requires an increasing amount of concentrate supplementation in the diet to maintain constant milk yield (Kuoppala et al. 2008). High proportion of concentrate increases the risk of feeding disorders which decreases the feasibility of low D–value silages in practise. The strategy D produced as high ME yield as the strategy C and as high DM yield as the strategy B at Ruukki. At Maaninka, though, the strategy D produced only as high ME and DM yield as the strategy A. The strategy D produced the highest D-value: 706 g kg^{-1} DM at Maaninka and 703 g kg^{-1} DM at Ruukki. Success of the strategy D varied a lot between experiment places and between years, which emphasizes the importance of the field and weather conditions when choosing the best harvest timing strategy. The proportion of NDF in the yield of the first cut increased while delaying the harvest (580 → 630 g kg^{-1} DM at Maaninka, $p<0.001$; 552 → 630 g kg^{-1} DM at Ruukki, $p<0.001$). Because there were no differences between the NDF contents in the second cut, the NDF content in the first cut dominated the average value of the total yield (Table 1). The proportion of NDF in the total yield was the lowest in the strategy D, because the NDF content in the third cut was low (about 500 g kg^{-1} DM).

According to the feed tables (MTT 2012), the NFC content decreases when delaying the first cut. However, in our experiments the change in the grass NFC content depended on the location of the experiment. Delaying the first cut decreased its NFC content at Ruukki (170 → 157 g kg^{-1} DM, $p<0.001$) but increased it at Maaninka (131 → 176 g kg^{-1} DM, $p<0.001$). The reason for the increase in NFC was the rapid decrease in CP at the same time. The moderate decrease in the grass CP content at Ruukki could be a consequence of organic soil type. However, the relatively small change in the NFC content in the silage does not have a large influence on the diet of dairy cattle, where the majority of the NFC originates from concentrated feed.

Table 1. Dry matter (DM) yield, metabolized energy (ME) yield and the amount of neutral detergent fiber (NDF) in the total yield of the growing season for different harvest timing strategies.

	Maaninka			Ruukki		
	DM yield	ME yield	NDF	DM yield	ME yield	NDF
	kg DM ha^{-1}	GJ ha^{-1}	g kg^{-1} DM	kg DM ha^{-1}	GJ ha^{-1}	g kg^{-1} DM
A Early	8901 a	97.2 a	577 a	8919 a	97.8 a	568 a
B Delayed	9513 b	101.7 b	601 b	10904 b	115.3 b	599 b
C Late	10216 c	105.4 c	606 b	12268 c	126.3 c	613 c
D Three harvests	8708 a	98.1 a	560 c	11239 b	126.0 c	545 d
SEM	92.0	1.00	2.7	134.5	1.50	2.1
p-values						
Harvest timing strategy	***	***	***	***	***	***
Year	***	**	*	**	o	***
Interaction	**	**	**	***	***	**

SEM = standard error mean
Values marked with the same letter do not differ (Tukey's test).

Conclusions Delaying the first harvest increased both the total DM and ME yield per hectare but simultaneously the average digestibility of the total yield decreased. The three harvests strategy produced the most digestible yield, but the success of the third cut depends on the soil type and the weather conditions. An average of 8 % increase in the ME yield between the early and the late harvest at Maaninka was not remarkable when taking into account the increased challenges in the feeding rationing for the dairy cows. In contrast, the 30 % increment in the grass yield at Ruukki was considerable. The NDF content increased while delaying the first cut, but changes of the grass NFC contents change were variable. The results show that it is important to take into account all the cuts when considering the timing of the first cut.

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Yield, feed value and fermentation quality of ryegrass (*Lolium perenne* L.) silages as affected by cutting frequency and genotype

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Keywords: cutting frequency, genotype, feed value, fermentation quality, ryegrass, digestibility trial

Introduction Production costs play an important role in profitable silage production. In comparison with maize silage, high energy grass silages are more expansive to produce, and nutrient concentrations often vary widely. The aim of the study was, therefore, to reduce costs of grass silage production (while maintaining high quality) by using grass genotypes (di- and tetraploid) having a wider harvest window, which can therefore be harvested at a lower cutting frequency.

Material and methods Two *Lolium perenne* L. (LP) leys of 7.5 ha each were established on the research center of the Chamber of Agriculture in Futterkamp, Schleswig-Holstein, Germany, in 2008. The used mixtures of LP differed in maturation type and ploidy. Diploid varieties *Respect* and *Fenemma* are diploid and moderately-late maturing, whereas *Gemma* and *Delphin* are tetraploid and late maturing. Beginning after the first cut, samples of each variety were taken on 10 days intervals to determine the optimal harvest date so that in each of the two main years of use, the moderately-late maturing genotypes were harvested at 4 cuts per year, whereas the late-maturing varieties had a frequency of 3 cuts per year.

Two to four weeks prior to the start of the use of the leys by cutting until one date after cutting, representative samples were taken by a plot harvester to evaluate parameters of feed value. The production of silages was made by commercial machinery. All forages were chopped to 40-60 mm theoretical particle length and treated with a blend of homo- and heterofermentative lactic acid bacteria of Pioneer GmbH, Buxtehude, Germany. Forages of the first cut were ensiled in separate silos, whereas all forages from the following cuts were ensiled separately in layers in one silo. All fresh forages were analyzed for DM, sugar, fermentability coefficient (FC) and epiphytic lactic acid bacteria (LAB) count.

Silages for digestibility trials in wethers, which were carried out according to the guidelines of GfE (1991), were made in 120 L drums. The calculation of metabolizable energy (ME) and net-energy-lactation (NEL) was performed by using the concentrations of digestible nutrients (GfE, 1995, Van Es, 1978).

Results and discussion The development of fiber (ADF) concentration as the main factor determining the optimal harvest date regarding energy concentration, was not different between di- and tetraploid grass varieties when the first and third cut of 2010 were considered (Figures 1 and 2). However, an obviously higher flexibility in use by a slower increase in ADF was seen in cuts 2 to 4 but this effect was alleviated if first cut material was also included in the evaluation. The differences in ADF accumulation between the first and the following cuts may be explained by the unusual weather pattern in 2010, which showed a severe shortage of rainfall in spring.

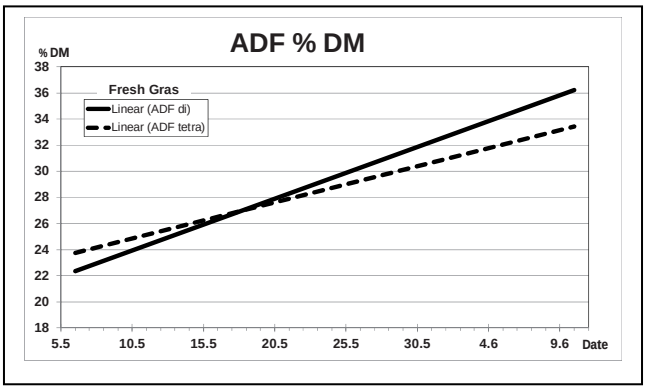

Figure 1. Development in ADF before 1 st cut 2010

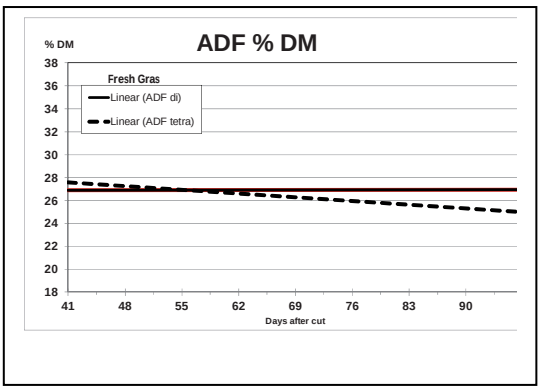

Figure 2. Development in ADF after 2nd cut in 2010

Fermentability parameters were not affected by cut and ploidy of LP (table 1), and FC of all forages was higher than 45. Only fourth cut material had too a low epiphytic LAB count.

Tetraploid LP used 3 times a year yielded 1 t DM per ha more but contained 0.44 MJ NEL/kg DM less than found in diploid LP at 4 cuts (table 2). Energy yield per ha was unaffected by ploidy level.

Results of silage digestibility measurements reflect well the differences between di- and tetraploid varieties that were found in fresh grass (table 3). Silages made from tetraploid varieties contained 0.4 to 0.5 MJ NEL/kg DM less energy than determined in silages from diploid grasses. However, energy concentration was generally higher by about 0.5 MJ NEL/kg DM than frequently observed under practical farming conditions.

Table 1. Fermentability parameters of crops harvested in 2010.

Parameter	Cut				Ploidy	
	1	2	3	4	diploid	tetraploid
DM (g/kg)	255	387	390	299	331	346
Sugar (g/kg DM)	182	179	173	267	199	180
FC[1)]	49	83	58	67	58	72
LAB[2)] (lg cfu/g FM)	5.30	5.92	5.32	3.41	5.27	5.28

[1)]Fermentability Coefficient=DM+(8*Sugar/Buffering capacity); [2)]lactic acid bacteria

Table 2. Yield and energy concentration of the crops harvested in 2010.

Parameter	Cut								Ploidy	
	1		2		3		4		d	t
	Ploidy									
	d 26.05.10	t 05.06.10	d 26.05.10	t 30.06.10	d 20.07.10	t 07.09.10	d 01.10.10	t		
Yield (t DM/ha)	5.9	7.2	3.4	3.0	2.0	2.8	0.7	-	12.0	13.0
Energy content[1)] (MJ NEL/kg DM)	6.4	5.7	6.3	6.3	6.1	6.1	7.2	-	6.36	5.92
Energy yield (GJ NEL/ha)	37.8	41.0	21.4	18.9	12.2	17.1	5.0	-	76.4	77.0

[1)] determined by NIRS according to GfE (2008); d diploid; t tetraploid

Table 3. Nutrient content, digestibility and energy concentration of the production silages.

Parameter	Dimension	1th cut			Mean of 2nd to 3th/4th cut		
		diploid	tetraploid	Sign.[1)]	diploid	tetraploid	Sign.[1)]
Dry matter	g/kg	273	228		372	345	
Crude protein (CP)	g/kg DM	175	127		146	123	
ADF_{OM} (ADF)	g/kg DM	297	331		255	286	
Digestibility[2)] OM	%	81.4	76.0	***	80.7	77.0	**
Digestibility[2)] CP	%	77.7	70.1	***	73.6	65.1	**
Digestibility[2)] ADF	%	82.3	74.4	***	80.8	78.9	*
Metabolizable energy	MJ/kg DM	11.4	10.5	***	10.9	10.4	**
Net-Energy-Lactation	MJ NEL/kg DM	6.9	6.3	***	6.7	6.3	**

[1)]statistical significance; differences among means were tested by t test; [2)]measured in wethers

Conclusions The hypothesis could not be confirmed that late maturing, tetraploid LP (3 cuts) would give similar annual yields and energy densities as moderately late, diploid LP (4 cuts). Differences in fermentability parameters were not detected. All crops were characterized by high sugar levels and fermentability coefficients (>35), and only fresh grass of the fourth cut had too a low epiphytic LAB count (<lg 5.0 cfu/g FM). Only production silages from fourth cut material were inferior in fermentation quality. *In vivo* silage digestibility of organic matter and of almost all individual nutrient fractions as well as energy content was significantly lower in tetraploid varieties than determined in diploid grasses.

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Chemical composition and nutritive value of different plant species used for forage production in South Karelia, Russia

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Keywords: grasses, legumes, nutritive value

Introduction Biodiversity plants of the grassland swards are very important components in a complex of forage-fed animals and sustainability of animal production. The quality of vegetation cover is greatly influenced by the abiotic, biotic and anthropic factors. The chemical composition and nutritive value of plant species used for forage production is affected by botanical composition (Misztal and Zarzycki 2008), growth stage (Gruber et al. 2011), plant structure (Pakarinen et al. 2008), harvest time (Weisbjerg and Soegaard 2008) and environmental conditions. The objective of this study was to assess changes in chemical composition and nutritive value of different plant species in the conditions of South Karelia in Russia.

Material and methods In the first experiment, the nutritive value of 12 species was investigated. The experiment included the following species: 1. *Alopecurus pratensis* L., 2. *Dactylis glomerata* L., 3. *Poa pratensis* L., 4. *Bromopsis inermis* (Leys.) Holub., 5. *Festuca pratensis* Huds., 6. *Phalaroides arundinacea* Rausch., 7. *Phleum pratense* L 8. *Trifolium pratense* L., 9. *Trifolium hybridum* L., 10. *Trifolium repens* L, 11. *Galega orientalis* Lam., 12. *Lathyrus pratensis* L.). In the second experiment, 4 herbs species from semi-natural grasslands (1.*Taraxacum officinale* Wigg., 2.*Leontodon autumnalis* L., 3.*Achillea millefolium* L., 4.*Achillea ptarmica* L.) were investigated.

The experiments were conducted in the conditions of South Karelia (61°47'N, 34°21'E), where average annual air temperature is about 3.0° C (12.1° C during in growing season) and average annual rainfall is 600 mm (365 mm during the growing season). The soil of the experimental site was a sod podzolic light loam with top soil pH 6.0, available phosphate of 235 g/kg and potash of 152 g/kg. Mineral fertilizers were applied at the following rates: annual amounts of nitrogen (60-90 N ha^{-1} y^{-1}), phosphorus (60 P ha^{-1} y^{-1}) and potassium (60 K ha^{-1} y^{-1}). The herbs species were grown in semi-natural grasslands on the mineral soil. Samples were collected at the first cut from 21 to 25 June (full bloom). The nutritive value was assessed by chemical composition, estimation of crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), the concentrations of P, K, Ca, Mg, Cu, Zn, Fe in different species. CP was measured by the Kjeldahl method. The macro and microelements content in biomass was determined using ICP technique. NDF, ADF and ADL contents were estimated by near infrared reflectance spectroscopy (NIRS). Hemicellulose was calculated as the difference between NDF and ADF.

Results and discussion The results are presented in the Tables 1 and 2. The nutritive value of plants varied depending on species. The data of variation quality characteristics of plants from different botanical groups are presented in Table 1.

The statistical parameters of quality from different botanical groups show considerable variation in the contents of CP, CF and a majority of macro and microelements, and revealed links between species and element content (standard deviation of error 0.4-26.2, coefficients of variation 19-64, coefficients of correlation 0.43-0.71). There were significant differences between species and elements content inside the botanical groups of plants also. The study of the nutritive value of the different plants showed significant differences existing among species and between botanical groups (Table 2).

The herbage quality in terms of fibre composition varied greatly: NDF ranged from 171 to 680 g/kg DM, ADF from 149 to 360 g/kg DM, hemicellulose from 22 to 328 g/kg DM, and ADL from 7 to 29 g/kg DM. In many samples, grass species increased the NDF, ADF, hemicellulose and ADL content of the forage. The results demonstrated high importance of biodiversity in improving the feed quality of grassland.

Conclusions Results showed significant differences in the quality characteristics of plants from different botanical groups and demonstrated the role of biodiversity on the feed quality of grassland. The most important factor influencing chemical composition of samples was species of grasses, legumes and herbs. The concentrations of selected organic and mineral components in the swards were sufficient to satisfy the nutritional requirements of farm animals.

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Table 1. Variation of quality characteristics of plants from different botanical groups.

Plants	Crude protein	Crude fibre	Ca	P	K	Cu	Zn	Fe	Mn
	g/kg DM	g/kg DM	g/kg DM	g/kgDM	g/kgDM	mg/kg DM	mg/kg DM	mg/kg DM	mg/kg DM
Grasses									
Minimum	109	228	3.0	1.8	19.1	4.3	25.1	82.4	37.4
Maximum	133	303	5.5	3.7	32.2	6.5	30.1	182.5	60.3
Legumes									
Minimum	147	202	11.0	2.5	18.2	1.3	14.2	55.6	31.1
Maximum	211	255	18.3	4.1	26.3	6.5	44.4	119.8	49.2
Herbs									
Minimum	-	-	11.6	2.7	38.4	5.1	30.7	70.4	34.3
Maximum	-	-	28.4	4.4	53.1	6.3	34.6	175.0	71.2

Table 2. Chemical composition of plants from different botanical groups (g/kg DM).

Species	Neutral detergent fibre (NDF)	Acid detergent fibre (ADF)	Hemicellulose	Acid detergent lignin (ADL)
Grasses				
Alopecurus pratensis	572	320	252	20
Dactylis glomerata	572	330	242	27
Poa pratensis	568	308	260	12
Bromopsis inermis	577	343	234	18
Festuca pratensis	630	360	270	21
Phalaroides arundinacea	583	288	295	29
Phleum pratense	680	352	328	16
StD	37	20	-	7
Legumes				
Trifolium pratense	344	262	82	25
Trifolium hybridum	249	199	50	15
Trifolium repens	313	230	83	7
Galega orientalis	423	292	131	24
Lathyrus pratensis	355	277	78	16
StD	41	26	-	4
Herbs				
Taraxacum officinale	171	149	22	6
Leontodon autumnalis	329	261	68	21
Achillea millefolium	348	267	81	17
Achillea ptarmica	380	263	117	22
StD	53	21	-	3

Biodiversity and zonal resistance to diseases and environment among grasses and fodder crops

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Keywords: fescue, fodder root crops quality

Introduction An increase in the mass development of phytopathogen micromycetes including the grass and wheat diseases has been noted in recent years. This is leading to systematic deterioration of phytosanitary conditions in the main regions growing grass and cereal crops in the Russian Federation.
The purpose of the present work was to analyze the effect of the principal agrobiological factors on the economic-valuable characteristics and pathogenesis of the fescue. In the article the data about influence of grasses, and fodder root crops quality samples provenances' biogeochemical variety on its quality and resistance to diseases was shown.

Material and methods The work was performed in the GNU LNIISH «Belogorka» and in the Department of the Phytopathologv and Entomology of St. Petersburg Agrarian University on an experiment field of the Pushkin laboratories, and in the quarantine nursery of the Vavilov All -Russian Research Institute of Plant industry (VIR).

In the research red fescue grass (*Festuca rubra L.*), meadow fescue grass (*Festuca pratensis Huds.*) from collection nurseries, wild material and breeds-populations from the VIR collection (St. Petersburg) and fodder root crops of various geographical origin was used.

Grass selection material was screened according to the complex of an economic-valuable characteristics: winter hardiness, regrowth rate in the spring and after hay crops, perennial, high quality of a forage, resistance to the basic diseases and high efficiency of fodder weight and seeds.

In red fescue grass and meadow fescue grass selection the big attention was given for a feature of tolerance to pathogenes, allowing to receive a perspective selection material with stable productivity of vegetative weight and seeds on years, and reducing a necessity of pesticides application on herbages of cereal grasses. An estimation of the selection material for affect by pathogens was realized with the technique of Khokhryakova et al. (1984).

Results and discussion Two accessions of perennial grasses were transferred to State commission by the Laboratory in 2010. The red fescue accession, Severnaya 32, was received by selection and directed intercross of samples from republic Komi (Russian Federation, northern areas of the European plain). The content of carotene (mg 100 g^{-1} of leaves) was 2.25 mg. As a standard grade the Baltic sample Shilis damaged in an average and strong degree by *Scolecotrichum graminis Fckl*. has been used. Type of new meadow fescue accession, Shwedskaya, is haying - pasturable. It grows well after cutting and pasturing; for the vegetative period 2 hay crops or 3-4 pasturing are possible, in herbage Shwedskaya is kept till 5 years and more. Productivity of foliage green mass - 30-56 tonne/hectares, hay - 8-14 tonne, seeds - 330-733 kg/hectares. The content of crude protein in dry matter was 17.9 %. It is tolerant to agents of net blotch, rust and powdery mildew in contrast to standard accession Suydinskaya, which was selected in conditions of Leningrad region and used as a grade-indicator for *Festuca pratensis L.* leaf pathogens. Provenance of the this new meadow fescue accession, handed over in State commission - areas with non-frosty winters and damp climate, caused by Gulf Stream warm current.

The screening of perspective samples for resistance to diseases (nine formulas or red fescue and 8 – meadow fescue) was carried out in 2011. High air humidity and warm weather in May-June have provoked appearance of diseases on grasses. In the second decade of June distinctions between samples for an affect degree by ordinary root rot (*Bipolaris sorokiniana* (Sacc.) Shoemaker (=*Helminthosporium sativum* Pam., King et Bakke) became appreciable. In the base of inferior merithallus under a leaf dark brown necrosis were generated. White stems and empty ears of generative bines have appeared.

To a lesser degree white stems was presented on new red fescue accession Severnaya 32 and samples of Kalinin provenance. Accession Severnaya 32, registered in the State register as lawn grass sample and describable by its decorative qualities, had low productivity of verdurous mass.
The meadow fescue grass samples were affected by decay to a lesser degree in comparison with red fescue grass. More tolerant to diseases were new accessions of meadow fescue grass Shvedskaya and selection samples from the Kirov accession.

Our experiments demonstrated German cultivars of Festuca pratense L. had lower Fe-contents (58-85 ±26,3mg/kg of dry matter yields) in leaves of the second cutting plants than the Russian cv.

Lubawa from the Leningrad area, with affected by local population of leaf spot plant pathogens. Hybrids of xFestulolium of our selection (1983-3 F_5, 1982-52 F_3) differencing on resistance to rust fungi and powdery mildew were remarkable for Fe-contents (78-104 ±26.3mg/kg). The same hybrids had also high contents of Cu (6.0-9.1 ± 3.8mg/kg). Festulolium hybrids have been synthesized by authors due to crosses of tetraploidy cv. Orlinsky plants (Lolium perenne L.) belonging to the Western-European variety-type group with plants of the cv. Baltica (Festuca arundinacea Schreb.) selected from the VIR collection of Sahalin island sample. It may suppose cell and tissues of the plants have more activity of Fe- and Cu-containing enzymes. Differences on Mn-content were not found . Both cv. Baltica and German cultivars had high content NO_3 was 144 mg/kg of green mass and 55-235 mg/kg, cv. Orlinsky and cv. Lubawa had more less was 60 mg/kg and 58-95 mg/kg (Pozdnyakov et al. 1998).

Fodder quality of swede, beetmangel and fodder cabbage varieties were tested. The frost-resistant (ten below zero) fodder cabbage var. Mozgovaja zelenaja from the Leningrad area the protein content in plant leaves was 18.5%, the digestion of foliage green mass was 70-80%, total content of chlorophyll was 2.93% (Volkova 1971).

The revealed level of grass and fodder root crops genetic diversity resistance to the leaf pathogen and foliage green mass quality with consideration of the effect of a set of agrobiological factors and biogeochemical variety of samples' provenances makes possible the use of the results of the investigation in breeding and utilization grasses and fodder root crops.

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Weed management of grassland and harmful effects of weeds in swards - on-farm experiences

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Keywords: grassland, harmfulness, silage, survey, toxicity, weed management

Introduction One argument to control weeds from grassland is the harmfulness or toxicity of weeds to the cattle. Animals can reject and leave some of these weeds uneaten at pasture, but precise selection is difficult when cattle are feeding on silage. The toxicity of some weed species has been proven and it is clear that the growth of these species in pastures must be restrained by management. However, it is unclear if the toxicity of e.g. *Ranunculus* ssp. or the harmful effects of numerous weeds to milk and meat quality are endured during the process of pre-wilting and ensiling the silage, or if these problems are connected to pastures only. Nevertheless, herbicide manufacturers do still use these arguments to encourage herbicide use on grassland.

Material and methods To evaluate the weed management practices and real-life harmful effects of weeds noticed by farmers, an enquiry survey was performed for milk and meat producers in North Savo and North Ostrobothnia, which are the two most important regions for cattle and grassland production in Finland. Contact information for the farms was given by ProAgria advisory centres of these regions. The survey was carried out with Webropol 1.0 Survey Software during growing season 2011 as a part of KARPE project (Profitable Field Management on Cattle Farms). Results were analyzed by SAS 9.2 with procedure Univariate and Freq for frequencies and with procedure Mixed for analysis of variance, depending on the best applicability of these methods.

Results and discussion A total of 287 answers were collected, which we distributed quite well to the area (56 % from North Savo and 44 % from North Ostrobothnia) and both conventional and organic farmers answered (Table 1). Two-thirds belonged to age class 40–59 years and virtually all (97 %) farmers were male. Nearly 80 % of them had some kind of agricultural education. Dairy farms (Table 1) produced on average 9360 kg milk year^{-1} cow^{-1}. Beef producers reported to have on average a 610 g day^{-1} animal^{-1} net weight gain. Around 10 % had suckler cow production. For fifteen answerers the main farming was something else than dairy or meat production (recently retired, only grassland production etc.) and these were excluded from the analyses concerning effects on cattle.

There were slight differences between the farm types in their grassland management practices. Farmers had on average 65 ha of field, of which, on average 73 % was grassland (Table 1). Most of the grassland was used for silage (approximately 78 % of the grassland), but nearly all farms (95 %) had notable acreage of pasture, too. Typically, farmers gave less information about their practices with pasture than with silage. The most typical ways to renew silage and pasture swards were having cereal as an cover crop or harvesting the stand for whole-crop silage during the renewal year. Permanent re-seeded silage swards were not very common, but with pasture this was more typical. Swards were most commonly renewed at 3–4 year intervals; milk producers were the most likely to have this practice (Table 1). Renewing interval was perhaps a bit longer for pastures, but there was an uncertainty because every third farmer did not report the renewal interval of their pastures.

Table 1. Farming practices, herd and farm sizes and the proportion and age of grassland of different farm types.

	Answers	*Practices*	*Herd size*	*Total field*	*Distribution of renewal interval: 1-2 yr / 3-4 yr / 5-6 yr*	
Farm type	*n (%)*	*conventional/ organic (%)*	*average (median)*	*(ha)*	*silage swards*	*pasture swards*
Dairy	200 (71)	94 / 6	35 (26)	63	1 / 86 / 13	4 / 75 / 21
Beef	44 (16)	91 / 9	189 (140)	90	0 / 79 / 21	17 / 33 / 50
Suckler cow	28 (10)	57 / 43	37 (35)	62	4 / 64 / 32	4 / 68 / 28

Farmers were asked to evaluate the occurrence of weeds in all swards and the most common weed species on their most weed-rich swards. There were seldom significant differences between farm types or between organic and conventional farms. Farmers reported to have a spectrum from clean swards (zero infestation with weeds; this comprised 15 % of the grassland area on conventional farms and 7 % on organic farms, P 0.03) to swards with heavy infestation (>20 % of the herbage).
The most common species in silage swards were couch grass (*Agropyron repens*) and dandelion

(*Taraxacum* spp.). The proportions of these two species in the most weed-rich fields were typically assumed to be 10–20% or even more. The probability of having high proportion of dandelion in swards increased ($P < 0.05$) as the renewal interval was prolonged to 5–6 years.

In the most weed-rich pastures, couch grass, dandelion and *Rumex* spp. were the most common species. In pastures, typical proportions of the most invasive weeds were slightly lower than in silage swards, usually 10–20 % of the herbage, and there was a tendency (P 0.06) for dandelion to increase if the pastures were older than 4 years. The proportion of poisonous species, such as *Ranunculus* spp. was on average estimated to be below 10 % in both silage and pasture swards. Less than 7 % of the farms had more than 20 % of *Ranunculus* spp. in the herbage of silage or pasture swards, but the occurrence of cattle poisonings inside this group did not differ from other farms.

The farmers were asked to specify their reasons for controlling weeds and to answer in survey claims about the effects of weeds on animal health and productivity. Only about 5 % of the farmers reported not to control weeds in their swards at all. Usually farmers specified many reasons and means to perform weed control on their farm. They agreed considering weeds to have harmful effects on animals and on yield potential of fields; conventional milk producers agreed most uniformly with these kind of survey claims. The most common reason for controlling weeds (chemically or otherwise) was the high amount of weeds in the swards (75 % of the farmers). More than half mentioned that the inferior feeding value of weeds is a reason for weed controlling. In agreement with this, farmers claimed that high proportion of weeds in pasture and silage decreased the intake of forage and that weed controlling practices have increased the feeding value (digestibility, palatability) of forage, although organic farmers and suckler cow farmers differed from others in being not so convinced with these changes. On average 40 % of the farmers considered weeds to impair yield potential so that controlling is necessary, but again the suckler cow farmers and in some cases organic farmers did not see this as harmful as others. 20–30% of the farmers also considered the economic reasons and better looks of the non-weedy sward to be reasons to start weed control practices.

Some 25 % of the farmers reported the toxicity or harmfulness to be the reason for weed control. Typically these were milk producers with conventional farming practices; beef and suckler cow farms and organic farmers rarely saw associations between health problems and weeds and seldom reported to suffer from fertility and calving problems. While more than 40 % of the farmers assume weeds being able to cause poisonings, only 3 % of the farms have experience of them and only one was blaming these problems to be caused by toxic weeds. More than 50 % of all farmers accuse weeds being the source of taste or colour defects in milk or meat, but none of the milk producers and only a couple of beef producers reported quality problems in end products that might be connected to the feeding of weed containing silage. Interestingly, organic farmers (especially in organic suckler cow farms) did not agree that weeds cause poisonings or taste and colour defects in end products.

Farmers claimed to control weeds from their rented fields as eagerly as from their owned fields. They all reported that farm advisory work and articles by agronomists or other experts have affected their weed controlling decisions, although organic farmers seemed to be more immune to these external influences. Nearly all farmers have observed advertising about weed controlling agents, but they refused to be influenced by it.

Conclusions Most farmers assume weeds disadvantageous for both yield potential and feeding value of the forage. Farmers are keen to control the weed proportion in their swards and they usually use several methods to do that. Milk producers seem to be the most enthusiastic in weed controlling and they sometimes take the potential risks more seriously than other producers. Organic and suckler cow farmers do not consider weeds as harmful as conventional farmers. This could be because they have more limited means to control the invasion of weeds in their swards, or they are convinced by their own experiences with slightly more weedy forages which have not caused serious harms. Many health and productivity problems do occur on milk, beef and suckler cow farms, but are only rarely connected to high proportion weeds or occurrence of toxic species in the forage. From the farmers' point of view, it seems that the main arguments to control weeds are better productivity of the field and enhanced palatability and feeding value of the forage.

Acknowledgements This study was financed by the EU Rural Development Programme for Mainland Finland, www.rural.fi

Importance of senescence and dead material on nutritive value of grass silage

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Keywords: digestibility, *Phleum pratense*, *Festuca pratensis*, *Festuca arundinacea*, nutritive value

Introduction In a grass sward, tillers and leaves die and new tillers and leaves are formed simultaneously. One reason for senescence and death is that leaves have an ontological life span and after this they die (Virkajärvi and Järvenranta 2001). The second reason for leaf and tiller death is competition for light and nutrients inside a canopy (Virkajärvi et al. 2012). In some cases, this senescence can be accelerated by fungi (Kuoppala et al. 2008). Senescent leaves and tillers form dead material which presumably has a low nutritive value. Under the Nordic climate, daylength and consequently incoming solar radiation decrease rapidly from July onwards. This may well lead to a substantial proportion of dead material in the sward, especially during the regrowth. Recent studies have raised the question if this increase in the proportion of dead material can reduce the nutritive value of silage. The aim of our study was to determine the importance of accumulation of dead material to silage production under Nordic climate. In addition, we examined the effect of cutting time on the accumulation of dead material and its nutritive value.

Material and methods The results are from four different field experiments (EXP I–IV) carried out during 2006–2011 at MTT Maaninka (63º08'N, 27º19'E) and MTT Ruukki (64º40'N, 25º00'E). The grass species used were timothy (*Phleum pratense* L., EXP I–IV), meadow fescue (*Festuca pratensis* Huds., EXP II, IV) and tall fescue (*F. arundinaceae* Schreb., EXP I). The plot size was 6 or 12 m^2 with three or four replications. The aim in EXP I–III was to quantify changes in DM yield and its nutritive value at different cutting times in each cut and the abundance of senescent material was recorded during this work. The EXP IV focused on the accumulation of senescent material in the regrowth (Cut 2) during decreasing daylength and on the effect of fungiside treatment (combination of Azoxystrobin, Fenpropidin and Propiconazole) in controlling common leaf diseases and the senescence process.

The plots were cut two or three times per year (Cuts 1–3). Cutting times in each cut ranged from early to late cutting time compared to typical silage stage. In each cutting time, DM yield was determined by Haldrup 1500 plot harvester. A subsample was taken prior to cutting to 0 cm (EXP I) or to 7 cm (EXP II–IV). The subsamples were separated into living material and dead material (in EXP I into loose and attached dead material), all fractions were dried at 60 °C for 40 h and in EXP I and IV analyzed for organic matter digestibility, ash and D value, N, NDF, indigestible NDF (EXP I), and lignin (EXP I). The amount of dead material was not always sufficient for the chemical analyses and therefore the sample number may vary between the fractions or the statistical analyses. Differences in chemical composition between living and dead fractions (EXP I) and between with or without fungiside treatment (EXP IV) were analyzed by ANOVA. The analyses were performed using the *Mixed* procedure of the SAS 9.2. Finally, regression equations were created between the growing degree days (GDD, base temperature 5 °C) and corresponding proportion of dead material in yield (> 7 cm) for each cut (Cut 1–3) using combined data from EXP II-IV.

Results and discussion The results show that the D value of dead material of timothy and tall fescue was significantly lower than that of living material, especially in the Cut 2 of EXP I (Table 1). The lowest D value was observed in loose senescent material, which is logical as it is most likely formed earlier than the attached dead material and thus the senescence has proceeded further. In EXP IV, the D value of dead material of timothy and meadow fescue was 40–96 g kg^{-1} DM lower than that of living material which was slightly less than in EXP I. This is most likely due to sampling height of 7 cm as it excluded the most of loose material from the sample. The one year results of the fungiside treatment show a slight decrease in the proportion of dead material (cutting time × fungiside; P=0.028) and a slight increasing effect on D value of the dead material (P=0.027) in timothy but not in meadow fescue (P >0.10).

The proportion of dead material was below 5 % of DM yield in the Cut 1, but in the Cuts 2 and 3 the highest observed proportions were up to 25 % of DM yield (Figure 1). The proportion increased when the cutting interval or thermal time was prolonged. Growing degree days explained 60–64 % of the variation in the proportion of dead material. The increase was much stronger in Cut 2 and Cut 3 than in Cut 1. The main reason for low proportion of dead material in the Cut 1 is that the harvest was carried out when accumulated thermal time is still relatively low compared to leaf life span (210°C GDD for timothy and 230 GDD °C for meadow fescue in spring growth; modified from Virkajärvi & Järvenranta 2001). In addition to this, both increasing irradiation before solstice and reproductive canopy may delay the accumulation of dead material (Parsons 1988). In Cut 2 and 3 the increase in accumulation of dead material

coincides well with the leaf life span (290°C GDD for timothy and 460 GDD °C for meadow fescue in regrowth; modified from Virkajärvi and Järvenranta 2001). As there was no exponential increase in the proportion of dead material and the effect of fungiside was minor, the results suggest that main reason for accumulation of the dead material is the ontological life span of leaves. As the D value of dead material is clearly lower than that of the living material, it is likely to be one of the reasons for the decrease in the nutritive value of silage in the Cuts 2 and 3.

Table 1. The D values of living and dead material in timothy and tall fescue EXP I at MTT Maaninka 2006–2007. Living = living stems, leaves or inflorescences; Attached dead = dead material attached to living material; loose dead = not attached to living material. Mean values over years, species and cutting times. Samples cut to ground level.

	Living		Attached dead		Loose dead			
Cut	n	D value	n	D value	n	D value	SEM	P
1	109	656 [a]	11	638 [a]	8	560 [b]	18.2	<0.001
2	80	669 [a]	9	574 [b]	8	469 [c]	14.0	<0.001

Values denoted with the same letter on each row do not differ significantly (Tukey's procedure).

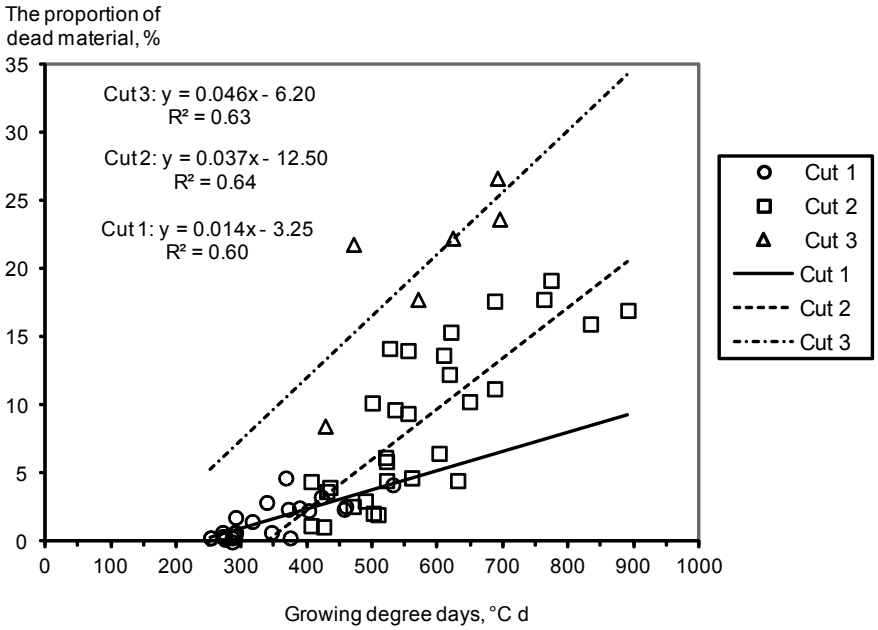

Figure 1. The proportion of dead material in relation to growing degree days in Cuts 1–3 in EXP II–IV. Mean values over years and species (timothy, meadow fescue). N = 60.

Conclusions Digestibility of the dead material, especially in the loose fraction in timothy and tall fescue is lower than that of the living material particularly in the second cut. The proportion of dead material is insignificant in the first cut, but substantial in the second and third cut and it increases clearly in relation to thermal time.

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Changes in the production of silage and ruminant concentrate feeds in the United Kingdom between 1990 and 2010

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Keywords: Silage, hay, concentrates, animal populations, statistics

Introduction The United Kingdom government has set a target of an 80% reduction in emissions of greenhouse gases (GHG) by the year 2050 compared to the baseline of 1990 (Office of Public Sector Information 2011). Currently GHG inventories are derived from statistics of livestock populations and emission factors (MacCarthy et al. 2011). There are uncertainties in determining emission factors and proxy estimates relating to efficiency of production, for example feed per unit of animal product, can indicate changes in GHG intensity i.e. GHG per unit of livestock product. Changes in diet composition to more silage and compounds may also indicate a change in the GHG intensity of ruminant livestock systems since silage and compounded feeds require more primary energy for their production than grazed pasture (Williams et al. 2006).

In this paper we assess changes in production of conserved forages and ruminant compounds in the UK in the 20-year period from 1990 to 2010.

Material and methods Hay, silage and the industrial production of compounds and blended concentrate fresh matter (FM) for cattle and sheep in the UK in 1990 and 2010 were obtained from national statistics (Anon 1991; DEFRA 2011abc).

Estimates of the production of dry matter (DM) were derived using the following concentrations (g DM kg^{-1} FM): Compounds, 860 (AFRC, 1993); hay, 850 (Thomas, 2004); grass and arable silage in 1990, 260 and 300, respectively (Wilkinson et al. 1996); grass and arable silage in 2010, 324 and 320, respectively (Pickering 2011ab).

Table 1. Estimated production of ruminant animal feeds in the UK: 1990 and 2010.

	1990		2010	
Million tonnes	Fresh matter	Dry matter	Fresh matter	Dry matter
Hay and artificially dried fodder	4.5	3.8	2.7	2.3
Grass silage	46.5	12.1	41.8	13.5
Arable silage	1.3	0.39	6.2	2.0
Total conserved forages	-	16.3	-	17.8
Hay and haylage for horses	-	2.0	-	2.0
Ruminant conserved forages	-	14.3	-	15.8
Ruminant compounds and blends	4.2	3.6	5.6	4.8

Results Estimated UK production of hay DM decreased between 1990 and 2010 whilst the production of grass and arable (mainly maize) silage and ruminant compound DM increased (Table 1). We estimate that around 2 million tonnes of hay and haylage DM were used for horses in the UK in 1990 and 2010. In the same period the UK populations of dairy cows and breeding sheep decreased by 36 and 29%, respectively, from 2.9 to 1.8 million dairy cows and from 21 to 15 million breeding sheep; whilst the number of beef breeding cows increased from 1.6 to 1.7 million (DEFRA 2011c). The decreased populations of dairy cows and breeding sheep accounted for most of the reduction in UK methane production over the period (MacCarthy et al., 2011).

Ruminant conserved forage per ruminant livestock unit increased by 39% between 1990 and 2010. Ruminant compound feed DM per ruminant livestock unit increased by 68% in the same period. There were also increases in average annual milk yield per cow, average carcase weight of beef cattle and average carcase weight of lambs, of 42%, 21% and 7%, respectively (DEFRA 2011d), which reflected the increased inputs of conserved forage and concentrate feed per livestock unit over the period. The average level of fertiliser nitrogen applied to grassland in England and Wales decreased from 132 kg N/ha in 1990 to 62 kg N/ha in 2010 (DEFRA 2011d), suggesting that grazed pasture yields were unlikely to have increased. The reduction in fertiliser N accounted for most of the decrease in estimated UK nitrous oxide emissions over this period (MacCarthy et al. 2011).

Conclusions Estimated UK production of conserved forage DM and ruminant concentrate feed DM increased between 1990 and 2010, indicating a probable increase in GHG emissions from ruminant feed production. There was also a reduction in the UK ruminant livestock population during the period, which was reflected in large increases in the production of silage and compound feeds per ruminant livestock unit. There may also have been less reliance on grazed pasture in 2010 than in 1990. However, there were also increases in average output per animal over the period which would have mitigated an increase in GHG emissions per unit of livestock product associated with increased production of silage and compound feeds.

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Ensilability characteristics of perennial ryegrasses in a national variety evaluation scheme

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Keywords: ensilability, maturity, perennial ryegrass, ploidy, variety evaluation scheme

Introduction National variety evaluation schemes for perennial ryegrass evaluate varieties primarily based on seasonal and annual dry matter (DM) yield, persistence and nutritional quality. Thus varieties can be selected that are particularly suited to the management practices and productivity goals of a farming enterprise. However, despite the widespread practice of ensiling occurring on Irish farms (Connolly et al. 2002), limited information is provided on the ensilability properties of varieties in national evaluation schemes (Conaghan et al. 2008). Grass ensilability at harvest is important as it influences the efficiency of silage conservation. Variety, maturity and ploidy are all known to influence the chemical composition and nutritive quality of swards (Burns et al. 2012), and thus are likely to also influence the ensilability of swards. The supplementation of productivity and nutritive quality data with ensilability indices in evaluation schemes could help improve the efficiency of the ensiling process on farms by enabling the selection of varieties particularly suited for this purpose. The aim of this study was to assess the effects of variety, maturity and ploidy on ensilability characteristics of perennial ryegrass varieties in a national variety evaluation scheme.

Material and methods Eight perennial ryegrass varieties (Table 1) selected from within recommended list trials for grass and clover varieties for Ireland were sown as monocultures in the year prior to first harvest at Backweston, Co. Kildare, Ireland. Plots (7.0 x 1.5 m) underwent a 6-cut simulated grazing and conservation management as described by Grogan and Gilliland (2011). From each of two silage cuts, taken in mid-May and late-June, a sub-sample (*c.* 300 g) taken from each of four replicated plots was oven dried, to determine dry matter (DM; g/kg) content, and milled prior to analysis by near infrared reflectance spectroscopy (Burns et al. 2010) for, water-soluble carbohydrate concentration (WSC; g/kg aqueous extract ($_{AE}$)) and buffering capacity (mEq/kg DM). Heading date was determined from separate trials by the mean date at which 10 individual spaced plants produced seed heads. The effect of variety was determined by ANOVA using a model that accounted for variety and block, while the effects of maturity and ploidy were determined using a model that accounted for maturity, ploidy, maturity*ploidy and block (Genstat v14.0).

Results and discussion The buffering capacity and WSC_{AE} can provide a reliable indication of herbage ensilability (O'Kiely and Muck 1998). At the 1st silage cut there were significant varietal effects on buffering capacity ($P<0.001$) and DM ($P<0.05$) but not for WSC_{AE}. Thus, varieties had similar ensilabilties with the exception of Portstewart whose low WSC_{AE} and high buffering capacity indicate that this variety would most likely incur a greater challenge to silage preservation. At the 2nd silage cut all varieties had lower WSC_{AE} and higher buffering capacities than at the 1st cut. This would have resulted in forage from the 1st silage cut being easier to preserve than from the 2nd silage cut.

The only significant effect of maturity occurred at the 1st silage cut where intermediate heading varieties had a lower buffering capacity ($P<0.001$) than late heading varieties. The 1st silage cut closely coincides with the heading date of varieties which is an important physiological stage in determining optimal sward productivity and quality. The higher buffering capacity of late heading varieties would suggest intermediate heading varieties would be better suited for silage preservation However, heading date had a significant positive correlation with buffering capacity ($R^2=0.73$) at the first silage cut. As all plots within a maturity groups are cut on a fixed date, this may indicate that the development stage of the variety is confounding this result. Targeting the cutting of individual varieties to a similar development stage, such as heading date, would allow for an unconfounded comparison of varieties (Gilliland 1995). A higher yield and increased WSC concentration in DM of tetraploid varieties mean they are often suited to ensiling (Burns et al. 2012). The current study found tetraploid varieties to be higher yielding than diploid varieties at both silage cuts (data not presented). However, neither WSC_{AE} nor buffering capacity of the two ploidy groups differed significantly at either silage cut. This contrasts with Conaghan et al. (2008) who found tetraploids to have a lower WSC_{AE} than diploids, driven by the lower DM of tetraploids. Results from the current study indicate that the effect of ploidy on silage fermentation is likely to be minimal, although the lower DM content of tetraploid varieties at the 1st silage cut may result in greater

losses through effluent. Conaghan et al. (2008) and Burns et al. (2012) have also shown diploids to have a lower yield and digestibility than tetraploids. These results are similar to the current study (data not presented), which provides a challenge for the end user to select a variety that will maximise animal productivity from the ensilaged forage.

Conclusions There were significant differences between perennial ryegrass varieties and among maturity groups in terms of ensilability characteristics. The expected improved ensilability of tetraploids was not shown in the current study, although productivity and nutritive quality traits should also be considered. However, this work has also shown evidence of a possible confounding effect of the grass harvest management system employed on the buffering capacity of varieties.

Acknowledgements Funding was provided by the DAFM Research Stimulus Fund RSF – 07 526. The input of field staff at DAFM Backweston, laboratory staff at Teagasc Grange and Eamonn Meehan is acknowledged.

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Table 1. Ensilability traits of eight perennial ryegrass varieties at 1st and 2nd silage cuts - effects of variety, maturity and ploidy.

		1st silage cut			2nd silage cut		
	Heading date	WSC_{AE}	BC	DM	WSC_{AE}	BC	DM
Variety							
Portstewart	04 June (L)	49	351^{d}	234^{ab}	42	443	167
Gilford	05 June (L)	68	318^{cd}	255^{bcd}	46	391	168
Spelga	20 May (I)	65	302^{bc}	269^{d}	32	399	162
Abercraigs (T)	06 June (L)	70	325^{cd}	250^{abcd}	40	454	157
Magician (T)	21 May (I)	63	272^{ab}	230^{a}	45	398	169
Fornax (T)	22 May (I)	59	267^{ab}	233^{ab}	57	391	195
Orion (T)	29 May (L)	75	305^{bc}	242^{abc}	46	388	170
Cashel	19 May (I)	69	259^{a}	260^{cd}	51	380	181
S.E.D.		8.4	19.2	10.6	11.2	39.2	19.2
Sig.		N.S.	***	*	N.S.	N.S.	N.S.
Maturity							
Intermediate		64	275	248	46	392	177
Late		66	325	245	43	419	165
S.E.D.		4.4	10.5	5.5	5.6	20.0	9.5
Sig.		N.S.	***	N.S.	N.S.	N.S.	N.S.
Ploidy							
Diploid		63	308	254	43	403	170
Tetraploid		67	292	239	47	408	173
S.E.D.		4.4	10.5	5.5	5.6	20.0	9.5
Sig.		N.S.	N.S.	**	N.S.	N.S	N.S.
Maturity x Ploidy		*	N.S.	**	N.S.	N.S.	N.S.

DMD – *in vitro* dry matter digestibility (g/kg). WSC – water-soluble carbohydrate (g/kg aqueous extract). BC – Buffering capacity (mEq/kgDM). DM – Dry matter (g/kg). (I); (L) – Intermediate-; Late- heading variety respectively (T) – Tetraploid. Mean values within varieties that have different superscripts differ at P<0.05.

Ensilage characteristics of perennial ryegrass grown under two nitrogen fertiliser inputs and red clover, each harvested at five dates in the primary growth

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Keywords: Perennial ryegrass, red clover, nitrogen fertiliser, harvest date, ensilability

Introduction Much reseeded temperate grassland in Europe is dominated by perennial ryegrass (*Lolium perenne* L.) due to its high digestibility when harvested at the appropriate growth stage, high yield in response to nitrogen fertiliser application and ease of preservation as silage as a result of its relatively high water soluble carbohydrate content. However, legumes such as red clover (*Trifolium pratense* L.) can also represent a prominent component of temperate grasslands and can be grown alone or in combination with compatible grasses, thereby reducing the requirement for fertiliser N, while also increasing herbage yield and quality (Peyraud et al. 2009). However, legumes generally have a lower concentration of water soluble carbohydrates and a higher buffering capacity than grasses, making them more difficult to preserve as silage. This study investigated the effects of N fertiliser application on the ensilage characteristics of perennial ryegrass, compared to red clover, harvested at five dates in the primary growth.

Material and methods Triplicate plots of perennial ryegrass (PRG; *Lolium perenne* L. var. Gandalf) grown under two inorganic N fertiliser inputs (low = 0 kg N/ha, high = 125 kg N/ha) and red clover (*Trifolium pratense* L. Merviot; no N fertiliser) were harvested at five dates (fortnightly from 12 May – 7 July; Harvests 1 to 5, respectively) in the primary growth. At each harvest date, appropriate plots were precision-chopped without wilting and representative 6 kg samples were ensiled in laboratory silos. After 100 days ensilage, representative silage samples were oven dried at 85°C for 16 h to estimate dry matter (DM) concentration (corrected for loss of volatiles as per Porter and Murray (2001)), while aqueous extracts were used to determine pH, total fermentation products (lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ethanol) and ammonia-N (NH_3-N) as previously described by McEniry et al. (2006). Data were analysed as a split plot design with harvest date as the main plot, and silage type as the sub plot, and accounting for replicate blocking.

Results At the later harvest dates (Harvest 3, 4 and 5) the DM concentration of the red clover silage was lower (P<0.001) than the PRG silages (Table 1). On average, silage pH was higher (P<0.001) for the red clover silage and this was particularly evident at Harvest 5. In addition, at Harvest 5, the LA concentration of the red clover silage was lower (P<0.001) and the AA (P<0.01) and PA (P<0.05) concentrations were higher than the two perennial ryegrass silages

On average, the ethanol concentration was lower (P<0.05) for the red clover silages and this was particularly evident at Harvests 1, 3 and 4, with little difference being observed between the two grass silages. Of the two grass silages investigated, on average the AA (P<0.001) and NH_3-N (P<0.05) concentrations were higher for the high N fertiliser treatment. On average, the proportion of DM recovered was greater (P<0.05) at the later harvest dates (Harvests 3, 4 and 5) than the early harvest dates (Harvests 1 and 2) and it was higher (P<0.05) for both grass silages than red clover silage.

Discussion With the exception of the Harvest 5 red clover, all herbages produced well preserved silages. This late harvest red clover had a high pH and low LA concentration (0.37 of total fermentation acids) indicating unsatisfactory silage preservation (Parker and Bastiman 1982). The low water soluble carbohydrate concentration (72 g/kg DM) and high buffering capacity (593 mEq/kg DM) of this herbage at harvest presented a greater challenge to preservation than at earlier harvest dates. In general, N fertiliser application had little apparent effect on the characteristics of silage made from PRG. However, on average, silage pH and NH_3-N were numerically higher for the high than the low N fertiliser treatment. The lower DM recovery for the early harvest dates and for red clover silage can be partly explained by a lower DM concentration and thus a higher production of effluent.

Conclusions Although applying fertiliser N at 125 kg/ha did not cause PRG silage to preserve poorly or reduce the rate of DM recovery, it did nevertheless present a greater challenge to preservation. In contrast, red clover was more difficult to preserve than either of the PRG treatments, and suffered greater losses during ensilage. Harvesting at an earlier date in the primary growth also resulted in greater losses during ensilage.

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Acknowledgements Funding was provided under the National Development Plan, through the Research Stimulus Fund (#RSF 07 557), administered by the Department of Agriculture, Food & Marine, Ireland.

Table 1. Fermentation characteristics (g/kg DM; unless indicated otherwise, and excluding pH) and dry matter (DM) recovery rate of silages made from perennial ryegrass (PRG) grown under two N fertiliser inputs and red clover.

			Variables[3]								
Harvest[1]	Silage type		DM	pH	LA	AA	PA	BA	Ethanol	NH_3-N	DM recovery
1	PRG	Low N[2]	197	3.73	127	20	3.6	4.4	27	57	0.87
1	PRG	High N[2]	163	4.07	93	37	2.2	0.0	36	58	0.86
1	Red clover	Low N	167	4.15	112	22	2.5	1.1	16	61	0.79
2	PRG	Low N	182	3.62	68	36	3.1	0.3	42	38	0.87
2	PRG	High N	161	4.20	99	51	3.8	0.6	36	71	0.86
2	Red clover	Low N	157	4.39	43	46	5.7	0.0	31	61	0.81
3	PRG	Low N	224	3.78	52	13	0.6	2.0	31	45	0.94
3	PRG	High N	217	3.81	66	16	0.0	0.0	32	49	0.98
3	Red clover	Low N	186	3.92	86	20	0.5	0.0	11	47	0.95
4	PRG	Low N	274	3.66	57	10	0.0	0.8	35	42	0.91
4	PRG	High N	270	3.68	70	11	0.0	0.4	29	55	0.93
4	Red clover	Low N	188	3.88	82	21	0.7	0.0	7	46	0.93
5	PRG	Low N	241	3.54	70	19	0.0	0.6	33	62	0.98
5	PRG	High N	234	3.52	76	23	0.5	0.0	27	62	0.96
5	Red clover	Low N	170	4.58	35	54	6.4	3.3	40	69	0.89
s.e.m[4]			7.2	0.129	13.4	5.5	1.01	1.05	6.9	8.4	0.036
Levels of significance											
Harvest			***	NS	NS	***	***	NS	*	NS	*
Silage type			***	***	NS	***	**	NS	**	*	*
Harvest x Silage type			***	***	***	**	*	NS	*	NS	NS

[1] Harvest 1 = 12 May, Harvest 2 = 26 May, Harvest 3 = 9 June, Harvest 4 = 23 June, Harvest 5 = 7 July; [2] Low N = 0 kg N/ha, High N = 125 kg N/ha; [3] DM = dry matter (g/kg), LA = lactic acid, AA = acetic acid, PA = propionic acid, BA = butyric acid, NH_3-N = ammonia-N (g/kg N); [4] s.e.m. relates to two-factor interaction

The chemical composition of silages made from five grass species grown under two nitrogen fertiliser inputs and harvested at five stages of the primary growth

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Keywords: grass species, nitrogen fertiliser, plant maturity, ensiling

Introduction Grasslands are usually managed to enhance the production of herbage and its quality. Three of the main management factors affecting herbage yield, chemical composition and ensilability are grass species, rate of nitrogen fertiliser application and stage of maturity at harvest. Environmental factors also play a significant role. The objective of this study was to investigate the effects of ensiling on the fermentation characteristics of a range of common grass species grown under two N fertiliser regimes and harvested at five stages of maturity.

Material and methods Triplicate plots of each of five common grass species [cocksfoot (*Dactylis glomerata* var. Pizza), tall fescue (*Festuca arundinacea* var. Fuego), Italian ryegrass (IRG; *Lolium multiflorum* var. Prospect), perennial ryegrass (PRG; *Lolium perenne* L. var. Gandalf) and timothy (*Phleum pratense* var. Erecta)] were grown in field plots (20 m^2; n = 150) under two inorganic N fertiliser inputs (low = 0 kg N/ha, high = 125 kg N/ha) at Grange, Dunsany, Ireland. The plots were harvested at five stages (fortnightly from 12 May – 7 July in 2009; Harvests 1 – 5) in the primary growth. At each harvest date, appropriate plots were precision-chopped and representative 6 kg samples were ensiled in laboratory silos. After 100 days ensilage, representative silage samples were oven dried at 85°C for 16 h to estimate dry matter (DM) concentration, while aqueous juice extracts were used to determine pH, total fermentation products (lactic acid (LA); acetic acid (AA); propionic acid (PA); butyric acid (BA) and ethanol) and ammonia-N (NH_3-N) as previously described by McEniry et al. (2006). Data were analysed as a split-split plot design with harvest date as the main plot, nitrogen as the sub-plot and grass species as the sub-sub plot, and accounting for replicate blocking.

Results In general, silage DM concentration was higher (P<0.001) at the later rather than the early harvest dates (Table 1). However, there was a decrease (P<0.001) in DM concentration for silages from all grass species between Harvests 4 and 5, but with the exception of timothy where there was no difference (P>0.05). On average, timothy had the lowest (P<0.001) DM concentration of the five grass species investigated. A similarly high (P<0.001) silage pH was observed for the cocksfoot and timothy silages at Harvests 1 and 2. At Harvest 5, but with the exception of the tall fescue, cocksfoot had a higher silage pH (P<0.001) than the other grass species.

The PRG silage had a higher concentration of LA than the timothy (P<0.001) and cocksfoot (P<0.01) silages at Harvest 1 and than the timothy silage (P<0.001) at Harvest 2, while little difference was observed between the other grass species. At each harvest date there was no difference (P>0.05) in the concentration of AA between species. The timothy silage had a higher (P<0.001) concentration of PA than all other grass species at Harvest 1, while the PA concentration was also higher (P<0.05) than the two ryegrasses at this harvest date. Of the five grass species, the timothy silage had the highest (P<0.001) BA and NH_3-N concentration at Harvest 1 and 2 (except NH_3-N), while these variables were highest (P<0.01 and P<0.05, respectively) in the cocksfoot silage at Harvest 5. On average, ethanol concentration was highest (P<0.001) for IRG than any other species.

Although numerically higher at each harvest date, concentrations of AA (P<0.001), PA (P<0.001) and NH_3-N (P<0.01) were significantly higher (data not shown) in silages made from the high N fertiliser treatment at Harvest 1 only.

Discussion With the exception of Harvest 1 and 5 cocksfoot silages, and Harvest 1 and 2 timothy silages, BA was < 10 g/kg DM for all silages, indicating successful ensilage (Haigh and Parker 1985). The NH_3-N concentration was relatively high (i.e. >100 g/kg N) for the aforementioned timothy and cocksfoot silages, and together with the higher silage pH and lower concentration of LA in total fermentation products (< 0.40) was indicative of undesirable clostridial activity. Increasing the rate of N fertiliser application generally increased the herbage buffering capacity and reduced the water soluble carbohydrate concentration, thereby making grass more difficult to preserve as silage. This explained the resultant increase in silage pH and NH_3-N concentration.

Conclusions All grass species produced well preserved silages with the exception of the early timothy (Harvests 1 and 2) and the early and late cocksfoot (Harvests 1 and 5) grasses. High N fertiliser input increased the challenge to silage preservation. Poor preservation will affect energy losses during storage and the undesirable end products of fermentation could have negative consequences for ruminant nutrition or where grass silage biomass is used for industrial purposes.

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Acknowledgements Funding was provided under the National Development Plan, through the Research Stimulus Fund (#RSF 07 557), administered by the Department of Agriculture, Food & Marine, Ireland.

Table 1. Harvest date and species effects on silage fermentation characteristics (g/kg DM; unless indicated otherwise, and excluding pH).

		Variables[3]							
Harvest[1]	Species[2]	DM	pH	LA	AA	PA	BA	Ethanol	NH_3-N
1	Cocksfoot	180	4.34	56	29	6.8	14.6	35	69
1	Tall fescue	172	4.09	70	29	4.3	4.2	40	58
1	IRG	220	3.85	80	28	2.7	2.9	44	50
1	PRG	272	3.90	110	28	2.9	2.2	31	57
1	Timothy	237	4.69	38	28	14.5	30.7	27	151
2	Cocksfoot	190	4.14	42	40	3.2	4.4	38	47
2	Tall fescue	185	3.79	50	48	4.1	1.0	45	56
2	IRG	240	3.76	73	37	2.5	1.4	52	57
2	PRG	254	3.91	84	43	3.5	0.4	39	54
2	Timothy	208	4.49	29	38	4.6	13.9	26	83
3	Cocksfoot	179	3.92	57	14	0.3	1.5	10	46
3	Tall fescue	179	3.89	65	16	0.4	0.5	22	52
3	IRG	223	3.73	60	15	0.0	0.0	73	40
3	PRG	261	3.79	59	15	0.3	1.0	32	47
3	Timothy	221	4.11	43	24	1.1	0.2	23	56
4	Cocksfoot	171	3.80	62	7	0.0	1.9	6	51
4	Tall fescue	174	3.73	74	11	0.0	0.0	16	55
4	IRG	225	3.60	67	13	0.0	0.3	41	52
4	PRG	294	3.67	63	10	0.0	0.6	32	49
4	Timothy	213	3.69	80	10	0.0	0.2	10	62
5	Cocksfoot	173	4.31	31	22	3.0	11.6	16	109
5	Tall fescue	161	3.67	66	24	0.5	0.0	22	60
5	IRG	201	3.53	69	31	0.4	0.0	32	68
5	PRG	237	3.53	73	21	0.2	0.3	30	62
5	Timothy	242	3.58	74	18	0.2	0.1	26	66
s.e.m[4]		5.9	0.112	8.3	3.4	0.77	1.95	5.3	10.0
Levels of significance									
Harvest date		***	**	NS	***	***	***	***	NS
Species		***	***	***	NS	***	***	***	***
Harvest date x Species		***	***	***	*	***	***	***	***

[1] Harvest 1 = 12 May, Harvest 2 = 26 May, Harvest 3 = 9 June, Harvest 4 = 23 June, Harvest 5 = 7 July; [2] IRG = Italian ryegrass, PRG = perennial ryegrass; [3] DM = dry matter (g/kg), LA = lactic acid, AA = acetic acid, PA = Propionic acid, BA = butyric acid, NH_3-N = ammonia-N (g/kg N); [4] s.e.m. relates to two-factor interaction

The relationship between crop composition and silage fermentation products under well-controlled ensiling condition

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Keywords: laboratory silage studies, regression model, silage database

Introduction Silage is an important feedstuff in modern animal production. Fermentation end products impact on the hygienic and nutritive quality of silages. Therefore, it is important to establish linkages between silage fermentation products and factors contributing to their production. This study was designed to investigate the potential relationship between crop composition and silage fermentation products.

Material and methods A silage database was constructed from laboratory studies where no additives had been used at the Department of Animal Nutrition and Management of the Swedish University of Agricultural Sciences between 1994 and 2010. The database contained 112 observations (29 grasses, 14 grasses/legumes, 9 legumes and 60 maize). The crops varied in stage of maturity and order of cuts. The ensiling length and silo type varied from 90 to 151 days and from 1.5 L glass silos to 25 L stainless steel silos, respectively. All silos were kept at room temperature and there was no access of air during the ensiling period. For statistical analysis, data from grasses, legumes and grasses/legumes silages (referred as lay silages) were treated separately from maize silages. Arithmetical mean and the range of crop composition are given in Table 1.

Table 1. Arithmetical mean and the range of crop composition (g/kg DM unless otherwise stated).

	Lay silages (grasses, legumes and grasses/legumes silages)			Maize silages		
	Mean	Range	n	Mean	Range	n
DM (g/kg)	346	131-623	52	297	200-406	59
CP	152	61-220	52	83	58-103	60
WSC	119	27-333	52	107	10-235	60
Starch	-	-	-	217	13-400	59
NDF	-	-	-	442	351-553	59
Ash	-	-	-	45	36-58	59
BC (g LA/100g DM)	5.6	3.8-10.7	34	-	-	-
LAB (log CFU/g FM)	3.4	1.2-5.5	39	4.8	2.5-7.6	44

DM: dry matter; CP: crude protein; WSC: water soluble carbohydrates; NDF: neutral detergent fibre; BC: buffering capacity; LA: lactic acid; LAB: lactic acid bacteria; CFU: colony forming units; FM: fresh matter.

Effects of crop composition on silage fermentation products were evaluated using the stepwise regression procedure of SAS (v. 9.2; SAS Institute Inc., Cary, NC). Variables were entered and retained in the regression model at $P<0.15$. Crop variables were tested for **multicollinearity** using the variance inflation factors procedure (VIF) and unacceptable variables (VIF>10) were removed from the regression models (Manson 1987).

Results and discussion According to multicollinearty diagnostic test, starch, water soluble carbohydrates (WSC) and NDF variables were removed from the regression analysis of the maize silage data base. These variables had both high inter-correlations and were highly correlated with most crop variables (data not shown) listed in Table 1. The regression models of silage fermentation products of lay and maize silages are presented in Table 2 and Table 3, respectively. For lay silages, increasing dry matter (DM) and WSC contents reduced ammonia-N and butyric acid concentrations with DM and WSC having similar weights in the corresponding regression models. The effect of DM on ammonia-N concentration was reversed in maize silages compared to lay silages probably as a result of decreasing WSC concentration with increasing DM content ($r=-0.776$). For maize silages, the regression model of butyric acid was not reported because the residual of the model severely violated normal distribution. There was a pronounced effect of crude protein (CP) on the concentration of acetic acid in both lay and maize silages. The best prediction equations were obtained for acetic acid in lay silages (Table 2) and lactic acid in maize silages (Table 3) with R^2 values of 0.63 and 0.74, respectively.

Table 2. Stepwise regression model for silage fermentation products of lay silages (grasses, legumes and grasses/legumes silages). Variables are in g/kg DM unless otherwise stated.

	Intercept/slope	SE	P value	R^2 cumulative
Ammonia-N (g/kg total N)				
Intercept	295.69	42.02	<0.001	
DM (g/kg)	– 0.45	0.12	0.001	0.342
WSC	– 0.42	0.21	0.057	0.426
Lactic acid				
Intercept	-1.60	1.64	0.340	
DM (g/kg)	0.01	0.003	0.054	0.124
WSC	0.01	0.004	0.046	0.201
BC (g LA/100g DM)	0.33	0.20	0.105	0.286
Acetic acid				
Intercept	8.52	15.50	0.587	
DM (g/kg)	-0.10	0.02	<0.0001	0.474
LAB (log CFU/g FM)	4.89	2.00	0.022	0.579
CP	0.13	0.07	0.083	0.626
Butyric acid				
Intercept	77.92	12.22	<0.0001	
DM (g/kg)	-0.15	0.04	<0.001	0.410
WSC	-0.12	0.06	0.061	0.483

DM: dry matter; WSC: water soluble carbohydrates; BC: buffering capacity; LA: lactic acid; LAB: lactic acid bacteria; CFU: colony forming units; FM: fresh matter; CP: crude protein.

Table 3. Stepwise regression model for silage fermentation products of maize silages. Variables are in g/kg DM unless otherwise stated.

	Intercept/slope	SE	P value	R^2 cumulative
Ammonia-N (g/kg total N)				
Intercept	53.48	17.07	0.003	
DM (g/kg)	0.16	0.05	0.003	0.233
LAB (log CFU/g FM)	-3.50	1.53	0.028	0.322
Lactic acid				
Intercept	-91.28	32.32	0.007	
CP	1.31	0.25	<0.0001	0.598
Ash	1.18	0.49	0.020	0.728
DM (g/kg)	-0.08	0.05	0.121	0.744
Acetic acid				
Intercept	-0.50	0.77	0.518	
CP	0.04	0.01	<0.0001	0.319

DM: dry matter; LAB: lactic acid bacteria; CFU: colony forming units; FM: fresh matter; CP: crude protein.

In this study, data were obtained from well-controlled laboratory silage studies to ensure uniform ensiling conditions (e.g. temperature, air penetration). However, the regression models indicate that the overall relationship between crop composition and silage fermentation products were relatively weak, similar to the study of Wilkinson et al. (1981).

Conclusions Overall, a relatively weak relationship was found between crop composition and silage fermentation products. However, there was a strong linear relationship between crop composition and acetic acid in lay silages and lactic acid in maize silages. Acetic acid concentration in both lay and maize silages was related to CP content of silage crops.

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Fermentation quality of *Medicago sativa* and *Bromus inermis leyss* mixed silage

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Keyword: *Bromus inermis leyss*, fermentation quality, *Medicago sativa*, mixed silage

Introduction Alfalfa (*Medicago sativa*) is now the most cultivated forage legume in the world for its extensive adaptability and high nutritive value. However, the lack of water soluble carbohydrates (WSC) greatly restricts the fermentation quality of sole alfalfa silage. This experiment was conducted to study the fermentation quality of mixed silage prepared from alfalfa (*Medicago sativa*) and increasing proportions of bromegrass (*Bromus inermis leyss*).

Material and methods Alfalfa (*Medicago sativa*) and bromegrass (*Bromus inermis leyss*) used for ensiling were harvested from experimental plots located in Beijing of China in June. They were firstly chopped into approximately 1 to 2 cm theoretical lengths, then mixed and ensiled in different proportions (100:0, 75:25, 50:50, 25:75, 0:100, respectively). According to the experimental design, 15 bag silos were prepared for each treatment and three bag silos per treatment were randomly opened after 3, 7, 14, 28 and 56 days of ensiling in a room maintained at 25°C and the contents were processed for quality assessment and laboratory analysis.

Silage samples were dried in a forced draught oven at 60°C for 48 h and ground to pass a 1 mm screen with a Wiley mill. Dry matter (DM) and crude protein (CP) were analyzed according to methods 934.01 and 976.05, respectively, of AOAC (1990). The acid detergent fiber and neutral detergent fiber were analyzed by the methods of Van Soest et al. (1991) with amylase and sodium sulphite, and the results were expressed inclusive of residual ash. The water soluble carbohydrates (WSC) were estimated by the method of McDonald and Henderson (1964). Silage pH and organic acids were determined using the procedure described by Xu et al. (2007). The numbers of LAB, yeast and mould in silage were counted by the method of Cai et al. (1998).

The data were analyzed by One-way ANOVA and the means were compared for significance by Duncan's multiple range test. All statistical procedures were performed using the statistical packages for the social sciences (SPSS 17.0 for Windows).

Results and discussion Chemical compositions of fresh alfalfa and bromegrass harvested for ensiling are presented in Table 1. The alfalfa had a lower DM and WSC content of approximately 232 g/kg and 32.6 g/kg DM, respectively, and a higher CP content of 205 g/kg DM when compared with those of bromegrass.

Changes of pH during ensiling are shown in Fig.1. Increasing proportion of bromegrass sustained lower ($P<0.05$) pH than the sole alfalfa silage (100:0) throughout the ensilng periods. There was a rapid decline in pH during the first 14 days for all treatments; then it kept relatively stable until day 56 except for the 100:0 and 75:25 treatments, where pH was risen up to above 5.5. Overall, compared with alfalfa silage (100:0), all other treatments improved fermentation quality and attained lower pH. The 0:100 treatment had the lowest ($P<0.05$) pH followed by the 25:75 and 50:50 treatments.

Lactic acid concentration of silages had similar tendency during ensiling for all the treatments (Fig. 2). It firstly increased quickly up to day 14, then became relatively stable, except for the treatments 50:50 and 100:0 decreasing rapidly from day 28 to day 56 post-ensiling. The 0:100 and 25:75 treatments accumulated quickly in lactic acid contents and kept the highest ($P<0.05$) level at all ensiling periods.

For all treatments, the number of lactic acid bacteria (log_{10} cfu/g fresh material) varied from 7.8 to 9.2., while that of the 0:100 and 25:75 treatments kept the highest ($P<0.05$) level during almost all ensiling periods. No yeast or mould was detected in any of the silages after day 3 post-ensiling.

We can conclude that the 0:100 and 25:75 treatments tended to have the best fermentation quality in this study. The fermentation quality of the silage was improved with the progressive increases in bromegrass proportions.

Conclusions Overall, increasing proportions of bromegrass in the mixed silage has potential to improve the fermentation quality of alfalfa silage.

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Table 1. Chemical composition of fresh alfalfa and bromegrass.

Items	Alfalfa	Bromegrass
DM (g/kg fresh material)	232 ± 0.3	317± 0.4
CP (g/kg DM)	205 ± 0.2	148 ± 0.2
Neutral detergent fiber (g/kg DM)	513± 0.2	479 ± 0.2
Acid detergent fiber (g/kg DM)	351 ± 0.1	272 ± 0.3
WSC (g/kg DM)	32.6 ± 1.13	54.6 ± 0.92

Means ± S.D., *n* = 3

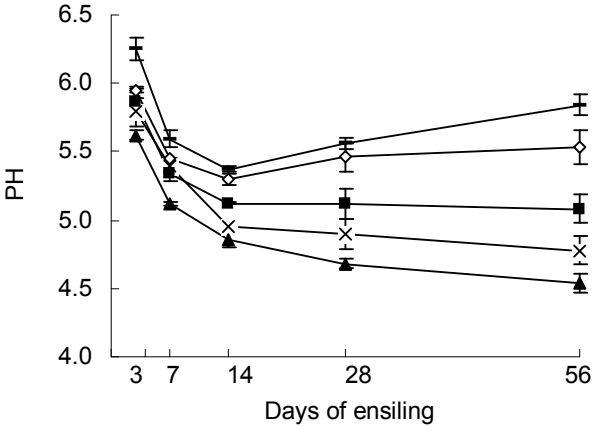

Fig.1 Changes in pH during ensiling of silage mixed by different rates of alfalfa and bromegrass, respectively. 100:0(—); 75:25 (◇); 50:50 (■); 25:75 (×); 0:100 (▲)

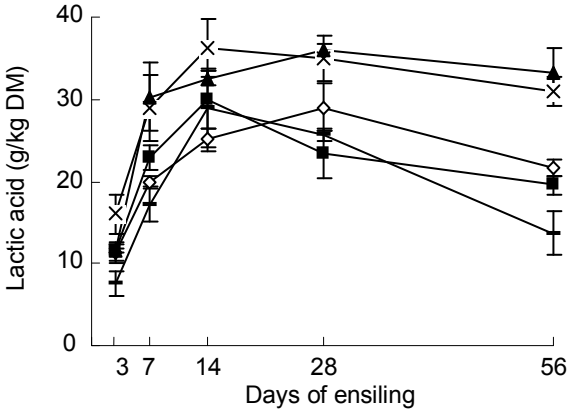

Fig. 2 Changes in lactic acid during ensiling of silage mixed by different rates of alfalfa and bromegrass, respectively. 100:0 (—); 75:25 (◇); 50:50 (■); 25:75 (×); 0:100 (▲)

Effect of rate of application of various commercial exogenous fibrolytic enzymes on fiber hydrolysis and in vitro digestibility of bermudagrass haylage

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Keywords: digestibility, dosing, enzyme, ferulic acid, forage, NDF, sugars.

Introduction Tropical/subtropical grasses are the basis of livestock production in many parts of the world but their quality is usually lower than that of temperate grasses. Exogenous fibrolytic enzymes (EFE) can be used to improve forage quality but the results of recent research with EFE have been equivocal because many factors influence their efficacy. One critical factor is the optimal application rate that maximizes fiber degradation with minimal EFE use. This factor is important because EFE can be ineffective if applied in insufficient or excessive amounts (Beauchemin et al. 2004). The objective was to determine the optimum application rate for five previously selected EFE.

Material and methods Five commercial EFE (E1 - E5) were evaluated for their effect on preingestive hydrolysis and in vitro digestibility of a 4–wk regrowth of Tifton 85 bermudagrass haylage (66.8, 33.2, 3.7 and 18.7 % NDF, ADF, ADL, and CP, respectively). Enzymes were diluted in citrate–phosphate buffer (pH 6) and applied in quadruplicate in each of 2 runs at 0x (control), 0.5x, 1x, 2x and 3x; where 1x was the respective manufacturer–recommended dose (10, 15, 2.25, 2.25, and 15 g EFE/kg substrate). The suspension was incubated for 24 h at 25°C, and for a further 24 h at 39°C after addition of buffered rumen fluid and then filtered. The run was repeated once. Also, pre-ingestive hydrolytic effects were evaluated in a similar manner except that sodium azide antimicrobial agent (0.02% w/v) was used in the EFE solution and after the 24 h enzyme-substrate mixture incubation at 25˚C, 15 mL of water was added and tubes were shaken for 1 h and then filtered. Data for each enzyme were analyzed separately as a completely randomized block design with Proc GLM of SAS v9.1. The model included effects of dose, run, and the interaction. Polynomial contrasts were used to determine dose rate effects. The Fisher's least significant difference test was used to compare EFE means and define the optimal dose, which was the dose with the highest response that was significantly different from the previous lowest dose.

Results and discussion Increasing the dose rate (Table 1) increased ($P < 0.05$) DMD (%) of substrates treated with E2 and E4 (cubic, C) and E1 and E3 (quadratic, Q); increased ($P < 0.05$) NDFD (%) of E1, E2 and E4 (C) and E3 (Q); increased ($P < 0.05$) hemicellulose digestibility (HEMD, %) of E1 and E2 (C), E3 and E5 (Q), and E4 (linear, L); increased ($P < 0.05$) total volatile fatty acids (TVFA, mM) of E1, E2 and E3 (C); increased ($P < 0.05$) acetate (mM) of E1 (C); increased ($P < 0.05$) propionate (mM) of E1, E2 and E3 (C) and decreased ($P < 0.05$) acetate to propionate ratio (A:P) of E1, E2 and E3 (C) and E4 and E5 (L). The optimal dose rate of specific enzymes for improving digestibility was basically the same across most digestibility measures (1x, 0.5x, 2x, 2x and 0.5x, for E1, E2, E3, E4 and E5, respectively). Applying E1 at 1x gave the highest improvement in NDFD (+ 5.7% over control). Optimal doses for improving VFA often differed from those for increasing digestibility perhaps due to partitioning of C skeletons produced by digestion between microbial growth and VFA production. Increasing the recommended dose of E2, E4, and E5 and halving those of E1 and E3 reduced the A:P ratio which could potentially benefit dairy cows by increasing gluconeogenesis. Increasing the enzyme dose rate had the following preingestive effects (Table 2): Increased ($P < 0.05$) DM loss (%) of substrates treated with E3 and E4 (C), E1 (Q), and E2 and E5 (L); decreased ($P < 0.04$) the NDF (%) of all enzyme-treated substrates (C); decreased ($P < 0.01$) ADF (%) of E2, E3, E4 and E5 (C), and E1 (Q); decreased ($P < 0.02$) hemicellulose (%) of E2, E3 and E5 (C), and E1 and E4 (Q); increased ($P < 0.01$) water soluble carbohydrates release (WSC, %) from E2, E3, E4, and E5 (C), and E1 (Q); increased ($P < 0.01$) ferulic acid release (µg/g) of E1, E2, E3 and E5(C), and E4 (L) and increased ($P < 0.01$) *p*-coumaric acid release (µg/g) of E1, E3 and E5 (C) and E2 and E4 (Q). Optimal doses for increasing preingestive fiber hydrolysis were 2x, 0.5x, 3x, 2x, and 3x, for E1, E2, E3, E4 and E5, respectively. Applying E3 at the 3x dose gave the greatest NDF decrease (-8.3%). Applying most enzymes at the 3x dose also optimized WSC and ferulic acid release but the greatest effects were by E1 and E3. The reductions of NDF and increase in WSC could potentially increase intake and ruminal microbial activity, respectively. Also, increased release of ferulic acid by enzyme application partially explains the improvements in digestibility since linking of ferulic acid to hemicellulose impedes fiber digestion.

Conclusions Increasing the dose rate beyond manufacturer recommendations increased preingestive fiber hydrolysis in most cases but did not often increase digestibility or rumen fermentation measures. This suggests that though fiber hydrolysis may increase intake, it is not necessarily an ideal measure of the potential for fibrolytic enzymes to increase fiber digestibility.

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Table1. Effects of EFE dose rate on trends in dry matter (DMD), neutral detergent fiber (NDFD) and hemicellulose digestibility (HEMD), and total volatile fatty acids (TVFA), acetate and propionate concentrations of bermudagrass haylage.

Treatment	DMD (%)	NDFD (%)	HEMD (%)	TVFA (mM)	Acetate (A, mM)	Propionate (P, mM)	A:P
Control[c]	48.6 ± 0.4	35.1 ± 0.6	31.4 ± 0.6	60.7 ± 1.6	37.3 ± 0.9	10.9 ± 0.4	3.43 ± 0.04
E1							
Contrast[a]	Q**	C**	C**	C*	C*	C*	C*
Opt. Dose[b]	1x (+2.8)	1x (+5.7)	1x (+7.1)	0.5x (+5.0)	0.5x (+2.0)	0.5x (+1.6)	0.5x (-0.3)
E2							
Contrast	C*	C**	C**	C*	n.s.	C*	C**
Opt. Dose	0.5x (+2.5)	0.5x (+4.6)	0.5x (+6.2)	0.5x (+4.9)	0.5x (+2.2)	0.5x (+1.3)	3x (-0.3)
E3							
Contrast	Q*	Q**	Q**	C*	n.s.	C*	C**
Opt. Dose	0.5x (+1.2)	2x (+3.8)	2x (+5.1)	1x (+4.2)	none	0.5x (+1.2)	0.5x (-0.4)
E4							
Contrast	C**	C*	L**	n.s.	n.s.	L^{+}	L*
Opt. Dose	2x (+1.1)	2x (+2.5)	2x (+3.6)	none	none	none	2x (-0.1)
E5							
Contrast	n.s.	Q^{+}	Q*	n.s.	n.s.	n.s.	L**
Opt. Dose	none	0.5x (+1.8)	0.5x (+3.1)	0.5x (+4.0)	0.5x (+2.6)	0.5x (+0.8)	2x (-0.1)

a Linear (L), quadratic (Q) and cubic (C) effect (P<0.05). n.s.: non-significant.
[b] Optimal Dose: value in parenthesis is the difference relative to the Control at the optimal dose.
[c] Control: mean ± standard error.

Table 2. Effects of EFE application rate on trends in concentrations of NDF, hemicellulose, ADF, water soluble carbohydrates (WSC) and ferulic acid (µg/g) and DM losses from bermudagrass haylage.

Treatment	DM loss (%)	NDF (%)	HEM (%)	ADF (%)	WSC (%)	Ferulic Acid (ug/g)	*p*-Coumaric Acid (ug/g)
Control[c]	17.2 ± 0.2	71.4 ± 0.3	35.1 ± 0.2	36.3 ± 0.2	2.1 ± 0.1	169 ± 2	152 ± 1
E1							
Contrast[a]	Q**	C*	Q**	Q**	Q**	C**	C**
Opt.Dose[b]	3x (+4.0)	2x (-6.7)	2x (-4.3)	2x (-2.5)	3x (+5.9)	3x (+142)	3x (+37)
E2							
Contrast	L**	C*	L**	C**	C**	C**	Q**
Opt. Dose	3x (+1.5)	0.5x (-1.9)	2x (-0.9)	0.5x (-1.5)	3x (+0.7)	0.5x (+8)	0.5x (+6)
E3							
Contrast	C*	C**	C*	C**	C**	C*	C**
Opt. Dose	3x (+3.7)	3x (-8.3)	3x (-6.7)	0.5x (-1.4)	3x (+5.1)	3x (+218)	3x (+54)
E4							
Contrast	C*	C**	C**	C**	C**	L**	Q**
Opt. Dose	0.5x (+1.4)	2x (-3.8)	2x (-1.8)	0.5x (-1.7)	3x (+2.4)	3x (+47)	2x (+11)
E5							
Contrast	L**	C**	C**	C**	C**	C**	C**
Opt. Dose	3x (+1.7)	3x (-4.0)	3x (-2.6)	0.5x (-1.7)	3x (+1.3)	3x (+157)	2x (+30)

[a] Linear (L), quadratic (Q) and cubic (C) effect (P<0.05). n.s.: non-significant.
[b] Optimal Dose: value in parenthesis is the difference relative to the Control at the optimal dose.
[c] Control: mean ± standard error.

The mixed silage quality characteristics of corn and alfalfa

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Keywords: alfalfa, corn, mixed silage

Introduction It is known that among other things corn has characteristics of high yield, high nutritional value, good palatability and high digestibility (Xu et al. 2009). However, due to the low crude protein (CP) content, it can not meet the needs of modern animal husbandry for high protein feed. Alfalfa (*Medicago sativa* L.) is one of important high protein feed, but because of its low soluble carbohydrate content, high buffering capacity and little lactic acid bacteria, excellent alfalfa silage is difficult to obtain (Yu et al. 1994). The aim of this study was to investigate fermentation quality of mixed alfalfa and corn silage in different proportions in order to find out the reasonable mixing ratio of corn and alfalfa for providing theoretical basis and technology for local forage storage.

Material and methods The raw materials were alfalfa (*Medicago sativa* L.cv.Gannong No.3) and corn (*Zea mays* cv. zhongbei 410), which were obtained from Linxi (43.62 N, 118.02 E; altitude, 900 m) located in Inner Mongolia of China in August, 2009. Corn and alfalfa were cut into about 2-3cm length and appropriate drying, and then mixed and ensiled in different proportions (0:10, 3:7, 5:5, 7:3, 10:0). The nutrition and fermentation quality of all silage treatments were determined after 30 days of the ensilaging. The data were processed and analyzed by Microsoft Office Excel 2003 and ANOVA with SAS.

Results and discussion Compared with alfalfa silage, all three mixed silage treatments improved fermentation quality and attained the goal of excellent silage. CP and ash contents of silage in the ratio of 3:7 treatment were significantly higher than that of the silage treatment of corn but lower than that of the silage treatment of alfalfa ($P<0.05$). Contents of NDF and ADF were lower than the silage treatment of corn and higher than those of the silage treatment of alfalfa ($P<0.05$) (Table 1). The pH values of the mixed silage treatments with the ratio of 5:3 and 7:3 were significantly lower than those of the silage of alfalfa ($P<0.05$). The content of lactic acid of all three mixed silage treatments account for more than 60%, however, the content of acetic acid, propionic acid and butyric acid were reduced comparing with the silage treatment of alfalfa (Table 2).

Conclusions Mixed silage of alfalfa and corn could overcome the shortcomings of alfalfa silage, and modulated silage of high quality more easily, meanwhile, potentially decreased the technical requirements and cost of silage. Nutrition and fermentation quality of mixed silage were between the single silage of corn and alfalfa, and achieved excellent fermentation quality. The mixed silage with the ratio of 7:3 was the best one.

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Table 1. The chemical composition of mixed silage of alfalfa and corn in different proportions.

Corn:Alfalfa	DM[1]	CP[2]	WSC[3]	NDF[4]	ADF[5]	Ash	EE[6]
				DM%			
0 : 10	29.28	19.84 a[7]	1.35 c	39.55 b	25.27 d	9.85 a	1.93 bc
3 : 7	45.16	19.06 b	1.40 bc	42.32 c	32.92 c	9.18 b	2.19 a
5 : 5	42.89	17.72 c	1.46 bc	46.23 c	33.50 c	8.62 c	2.07 ab
7 : 3	41.37	16.28 d	1.63 b	50.91 c	35.23 b	7.84 d	1.98 ab
10 : 0	22.45	10.63 e	2.71 a	58.61 a	37.02 a	5.70 e	1.71 c
MSE		0.012	0.158	4.381	1.270	0.020	0.013

[1]DM = Dry matter [2]CP = Crude Protein [3]WSC = Water-soluble Carbohydrates [4]NDF = Neutral Detergent Fiber [5]ADF = Acid Detergent Fiber [6]EE = Ether Extract; [7]a b c Means in the same column with different superscript letters differ significantly ($P < 0.05$).

Table 2. The fermentation quality of mixed silage of alfalfa and corn in different proportions.

Corn : Alfalfa	pH	Lactic acid	Individual volatility fatty acid (%)			NH_3-N[3]/TN[4] (%)
		(%TA[2])	Acetic acid	Propionic acid	Butyric acid	
0 : 10	4.74 a[1]	58.69 b	24.12 a	17.20 a	1.78 b	12.72 a
3 : 7	4.67 a	65.20 ab	24.54 a	9.97 bc	0.28 a	8.85 b
5 : 5	4.43 b	65.07 ab	20.95 ab	13.70 ab	0.28 a	6.79 c
7 : 3	3.99 c	68.33 a	20.36 b	11.31 bc	0.00 a	5.42 d
10 : 0	3.71 d	72.30 a	18.93 b	8.43 c	0.00 a	3.79 e
MSE	0.003	8.737	2.043	3.716	0.109	0.117

[1]a b c Means in the same column with different superscript letters differ significantly ($P < 0.05$).
[2] TA =Total organic acid [3]NH_3-N = ammonium nitrogen [4]TN = Total Nitrogen

Effect of ensiling of total mixed ration on rumen fermentation profile in vitro

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Keywords: ensiled total mixed rations, in vitro ruminal fermentation, nutritional change

Introduction Ensiling of total mixed rations (TMR) has several advantages compared to normal TMR. TMR silage showed highly aerobic stability after opening silo even under warm climate (Nishino et al. 2004). It is also possible to make TMR in large amount at once and store them (Weinberg et al. 2010), and the TMR silage can be transported to other areas. Nutritional value of TMR is designed in mixing ingredients, thereby it would be expected that some nutrients in TMR silage are different when it is fed to animal. Therefore, we studied that nutritional changes and characteristics of rumen fermentation of TMR silage stored at different temperature and period with compared to non-ensiled TMR.

Material and methods TMR composed with grass silage, maize silage and several kinds of concentrates (forage:concentrate = 40:60, metabolisable energy 11 MJ/kg) were ensiled in 1 L plastic bottles and stored for 30 or 90 days at 15 or 30°C in 5 replicates. The TMR and its silages were oven-dried and ground to analyse nutrient composition. Nitrogen fractions of TMR and TMR silage were determined by the method of Licitra et al. (1995). For in vitro ruminal fermentability test, 1 g of dried and ground samples was incubated anaerobically with 50 mL of buffered rumen fluid (McDougal buffer:rumen fluid = 1:2) in 120 mL glass vial at 39 degree for 24 hours (Uddin et al. 2010). During the incubation, 0.5 mL of medium was withdrawn at 4, 8 and 24 hours after incubation by syringe and determined short-chain fatty acids (SCFA, acetate, propionate and butyrate) and NH_3 concentration. The results were calculated using SAS GLM by one-way ANOVA with Tukey's test to compare before ensiling and other all treatment. Two-way ANOVA was also performed to determine the effect of storage temperature, period and their interaction.

Results and discussion The pH of TMR before ensiling was 5.8 and decreased to around 4.3 after ensiling. TMR silage stored at both temperatures contained high lactate about 100 g/kg DM, whereas lower soluble sugars compared with TMR. Non-fiber carbohydrates (NFC) slightly decreased during ensiling. In nitrogen fractions, the ratio of NH_3-N in total N slightly increased during ensiling but the ratio was relatively small compared to grass silage. The ratio of buffer soluble N compounds was about 300 g/kg N in non-ensiled TMR and it increased 340 to 460 g/kg N in TMR silage. Prolonged ensiling period from 30 days to 90 days clearly enhanced solubilisation of N compounds in TMR silage. The N fraction between buffer insoluble N and neutral detergent insoluble N decreased after ensiling, and the ratio was lower in TMR silage ensiled for 90 days than 30 days.

The SCFA concentration in the in vitro rumen during incubation was not clearly affected by treatment, whereas the molar ration of acetate and propionate was lower in TMR silage compared with non-ensiled TMR. Higher propionate ratio in the medium could be due that high amount of lactate in the silage were metabolised to propionate by rumen microbes. In this experiment, NH_3 concentration was 3.4 mg/dL in the medium at 0 h incubation. The NH_3 concentration from non-ensiled TMR incubation decreased from 0 to 8 h. This result indicated that NH_3 uptake by rumen bacteria was more active than NH_3 release from N compounds in TMR. On the other hand, NH_3 concentration at 4 h incubation of TMR silage was significantly higher than TMR before ensiling, and the trend was enhanced by storage temperature and days. Through the incubation, higher NH_3 concentration was maintained in the in vitro rumen incubated TMR silage than non-ensiled TMR. This result is consistent with in vivo study that also compared TMR and TMR silage (Cao et al. 2010).

Conclusions According to the present study, it concluded that ensiling of TMR decreased soluble sugars, whereas increased soluble N compounds compared with TMR before ensiling. The N solubilisation in TMR silage was enhanced by prolonged storage period. Higher ruminal NH_3 concentration from TMR silage at early phase of in vitro ruminal incubation could be due to shortage of fermentable sugars and enhanced deamination from N compounds.

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Table 1. Fermentation characteristics and nutrient composition of TMR silage.

	Before ensiling	After ensiling				SEM	Stat. Signific.[7]		
		30 Days[5]		90 Days[5]			D	T	DxT
		15°C[6]	30°C[6]	15°C[6]	30°C[6]				
Dry matter (g/kg)	551	547	539	536	548	4.5			
pH	5.79 a	4.44 b	4.28 c	4.26 e	4.20 d	0.01	***	***	***
Lactic acid (g/kg DM)	20.3 d	92.3 c	114.2 a	100.3 b	112.7 a	2.18		***	
Acetic acid (g/kg DM)	12.6 c	35.0 ab	34.1 ab	36.7 a	30.7 b	1.39			
Soluble sugars (g/kg DM)	52.6 a	7.1 b	7.2 b	7.0 b	7.2 b	0.66			
NFC[1] (g/kg DM)	364 a	349 ab	335 bc	321 c	345 abc	7.4			
Total N (g/kg DM)	22.4 d	26.0 ab	26.6 a	24.5 c	25.3 bc	0.35		**	
NH_3-N(g/kg N)	21.0 d	32.5 c	46.0 ab	42.5 b	49.6 a	1.44	**	***	
BSN[2] (g/kg N)	302 d	338 c	354 c	401 b	455 a	9.8	***	**	
BIN[3] - NDIN[4] (g/kg N)	548 a	478 c	510 b	421 d	397 d	7.9	***		**
NDIN[4] (g/kg N)	149 b	185 a	136 b	178 a	148 b	5.2		***	

[1] non-fiber carbohydrates, [2]buffer soluble nitrogen, [3]buffer insoluble nitrogen, [4]neutral detergent in soluble nitrogen, [5]Ensiling period, [6]Storage temperature, [7]D = Day, T = Temperature, D x T = interaction between Day x Temperature

Table 2. In vitro ruminal fermentation characteristics incubated TMR and TMR silage.

	Before ensiling	After ensiling				SEM	Stat. Signific.[5]		
		30 Days[3]		90 Days[3]			D	T	DxT
		15°C[4]	30°C[4]	15°C[4]	30°C[4]				
4 h after incubation									
Total SCFA[1] (mM)	39.8a	37.7b	37.5b	39.3a	38.5ab	0.44			
A:P ratio[2]	3.37a	3.00bc	2.67d	3.09b	2.88c	0.05	*	***	
NH_3-N (mg/dL)	1.42d	3.31c	4.89a	4.23b	4.93a	0.19		***	
8 h after incubation									
Total SCFA[1] (mM)	64.1	65.9	67.0	66.0	67.1	0.69			
A:P ratio[2]	2.65a	2.21b	1.95d	2.19bc	2.07cd	0.04		***	
NH_3-N (mg/dL)	0.15d	1.93c	2.66b	3.61a	3.17ab	0.16	***		*
24 h after incubation									
Total SCFA[1] (mM)	102	98	103	102	103	1.71			
A:P ratio[2]	2.62a	2.28b	2.08c	2.30b	2.07c	0.02		***	
NH_3-N (mg/dL)	4.65b	6.67a	7.75a	7.41a	7.09a	0.52			

[1]short-chain fatty acid, [2]acetate:propionate ratio, [3]Ensiling period, [4]Storage temperature, [5]D = Day, T = Temperature, D x T = interaction between Day x Temperature

Storage duration affects bypass starch of maize silage

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Keywords: bypass starch, maize silage, storage

Introduction Maize silage is often used to complement grass silage in the ration of dairy cows because of its high starch content. Especially its bypass starch fraction is used efficiently as an energy source. However, the bypass starch fraction in maize silage may change during storage (Newbold et al. 2006). In The Netherlands, maize silage is usually sampled in November or December, whereas the silage is fed until the end of the summer. If the content of bypass starch changes during storage, this affects the whole ration, and consequently milk production. The ratio degradable starch fraction (Dstarch) : washable starch fraction (Wstarch), has a major effect on bypass starch. Our goal therefore was to quantify the effect of storage on WStarch of maize silage.

Material and methods Forty maize silages were selected based on their chemical composition (variation in dry matter (DM) and bypass starch), and on their expected stability based on previous research. The silages were representative for Dutch conditions (Table 1). Bypass starch was determined according to CVB method (Van Duinkerken et al. 2011, slightly modified by BLGG AgroXpertus) as follows:

Bypass starch = $(k_{pWs} / (k_{dWs} + k_{pWs})) \times$ Wstarch + $(k_{pDs} / (k_{dDs} + k_{pDs})) \times$ Dstarch, in which

k_{pWs} = passage rate of Wstarch (8%/h),
k_{dWs} = degradation rate of Wstarch (%/h),
Wstarch = washable starch (% of total starch),
k_{pDs} = passage rate of Dstarch (6%/h),
k_{dDs} = degradation rate of Dstarch (%/h),
Dstarch = degradable starch, calculated as 100 – Wstarch (% of total starch)

Table 1. The chemical composition of the selected maize silages (40 samples, December/January 2010).

Parameter	Unit	Mean	Standard deviation	Minimum	Maximum
Dry matter	g / kg	349	35.5	266	443
Crude protein	g / kg DM[2]	72	6.8	61	90
dOM[1]	%	76	2.0	70.3	79
Starch	g / kg DM[2]	351	37.0	244	409
Bypass starch	%	30.1	3.3	21	37
NDF[3]	g / kg DM[2]	380	36.6	325	488

[1] dOM = digestibility of organic matter, [2] DM = dry matter,[3]NDF=neutral detergent fibre

Silage clamps were sampled in December 2010 and January 2011 (P1) and subsequently in February/ March (P2), May (P3), June/July (P4) and September 2011 (P5). Samples were split after receiving. One part of the sample was directly analysed with near infrared spectroscopy (NIRS) on chemical composition, the other part was frozen (-20^{0}C) until further analysis. In June 2011, the Wstarch fractions of P1, P2 and P3 were measured by washing the samples in the washing machine. Subsequently, the residues were dried and analysed for residual moisture and starch. The second batch of samples (P4) was washed and analysed in August/September, and the third batch (P5) in October 2011.

Results and discussion Nine of the selected silage clamps were finished before sampling date P4 and eight before sampling date P5. Consequently, 23 silage clamps were sampled during the entire period of the study. In the statistical tests, all silages that lasted until P4 and longer were included.

There was considerable variation between silages and sampling times. At sampling time P4, digestibility of organic matter (dOM) and starch content were significantly lower than at P1, whereas NDF content and Wstarch were significantly higher ($P < 0.05$ in all cases). This would have a huge effect on degradability. However, only dOM at sampling time P5 appears statistically significant from that at sampling time P1.

Table 2. Average composition ± standard error of mean of the samples at the five sampling times.

	Sampling time[1]				
	P1	P2	P3	P4	P5
Number of samples	40	40	37	30	23
dOM (%)[2]	75.8^{b} ± 0.31	76.6^{c} ± 0.35	75.9^{b} ± 0.37	74.6^{a} ± 0.38	74.6^{a} ± 0.47
Starch (g/kg DM)	351^{b} ± 5.9	350^{b}± 6.4	346^{ab}± 6.2	336^{a}± 6.2	339^{ab}± 9.1
NDF (g/kg DM)	380^{a}± 5.9	387^{ab}± 6.6	376^{a}± 6.6	393^{b}± 6.5	389^{ab}± 8.5
Wstarch (%)[3]	62.9^{a}± 1.41	62.2^{a}± 1.76	64.6^{ab}± 1.83	66.1^{b}± 1.61	63.1^{ab}± 1.98

[1]Different letters in the same row indicate significant difference (P < 0.05), [2] dOM = digestibility of organic matter, [3]Wstarch = washable starch fraction.

Results show a constant Wstarch in the winter months (62.9 and 62.2% Wstarch in P1 and P2, respectively), with an increase in spring and summer (64.6 % and 66.1% Wstarch in P3 and P4, respectively). Although some samples showed no change or a slight decline, on average the observed increase between P1 and P4 appears significant (paired t-test, P = 0.029). No significant differences were observed between P1 and P5: Wstarch in P5 was unexpectedly low, although not statistically different from P4. The relatively low Wstarch at P5 was not in line with dOM or other characteristics.

Of all silages that were available until P4, an linear regression analyses were carried out to estimate the daily change in Wstarch. For ten silages a significant regression (P < 0.10) could be described. Wstarch decreased in one and increased in the other nine silages. Increase was on average 0.06% per day, i.e. 1.8% per month. If this increase is linear with time, this equals an increase of 14.4% over a period of 8 months, which is a normal storage duration of maize silage. Such an increase in Wstarch results in a bypass starch decrease by about 5%. However, this is an average: maximal increase was 0.12% per day = 28.8% in 8 months. This reflects a decrease of bypass starch by almost 11%. This variation necessitates farmers to adapt the composition of the ration of their dairy cows during the year.

The variable dynamics in time of Wstarch may be caused by the type of maize involved. Starch granules in maize are encapsulated by hydrophobic prolamin proteins (zein), which are insoluble in the rumen environment (Larson and Hoffman 2008). Zein in floury maize is markedly lower than in dent maize and this has a clear effect on digestibility. Vitreous endosperm of maize has a lower accessibility, digestibility, and rumen starch disappearance than floury endosperm (Lopes et al. 2009). Whether these cultivar-dependent aspects indeed affect temporal Wstarch dynamics remains to be elucidated.

Conclusions. The Wstarch fraction may increase during storage and bypass starch might thus decrease during the storage period. It can therefore be concluded that bypass starch in summer may be lower than currently assumed. Decrease of bypass starch during storage can be as high as 10%.

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Digestibility of organic matter and neutral detergent fibre of whole maize plants and maize silage at different times of incubation

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Keywords: digestibility, maize, NDF, organic matter, silage

Introduction Genes and phenotypes of maize are extremely diverse allowing an improvement based on increasing grain yield as a way to elevate the energy content of the overall forage. The forage quality increase is apparently due to an increased proportion of grain rather than an increased quality of a stover per se. The study by Lauer et al. (2001) showed that stover neutral detergent fibre (NDF) and *in vitro* digestibility has remained historically unchanged when evaluated for the stover per se at a uniform planting density. Digestibility of both cell and cell wall content decreased with maturity of maize (Khan et al. 2007). There is a significant difference between a 24 and a 48-hour incubation in a rumen liquid of cows (Cole et al. 2001, Justen 2004 and Khan et al. 2007). According to Oba and Allen (1999) an enhanced NDF digestibility of forage significantly increases dry matter intake (DMI) and a milk yield. One-unit increase in the NDF digestibility was associated with a 0.17-kg increase in DMI and a 0.25-kg increase in 4% fat-corrected milk. The goal of this experiment was to compare the digestibility of an organic matter (DOM) and a digestibility of neutral detergent fibre (DNDF) of both the whole maize plants and the maize silage.

Material and methods In this study we used 44 maize hybrids harvested (in the 2/3 milk line of grain) by hand (3x 10 plants). Plants were cut by special cutting machine, dried and sent into laboratory. We used also next 44 maize hybrids planted in the 3 different areas (T1 19, T2 15 and T3 10 hybrids) and harvested by a big cutting machine. From these materials we made silages.

Digestibility of OM and NDF were measured with an *in sacco* (*in situ*) method with 24-h and 48-h incubation using Holstein cows fitted with ruminal cannulas. Nylon bags (size 60 x 150 mm) with 42 microns pore were used. Nylon bags were attached to a carrier (Třináctý et al. 1996). The advantage of the carrier is that the bags are more separated, which provides better conditions for fermentation. The samples which have passed through a sieve 1 mm were used. Degradability of NDF was calculated due Orskov & McDonald (1979). Data were analyzed by statistical software ANOVA using the Tukey test (Statistica 9.1.210, 2010).

Results and discussion The results of the digestibility (with the *in sacco* method at 24-h and 48-h incubation) of organic matter (DOM) and neutral detergent fibre (DNDF) of the fresh whole maize plants (WMP) and maize silage is shown in the Table 1.

Table 1. The *in sacco* digestibility of fresh whole maize plant (WMP) and maize silage.

Kind of	Number of	DOM 24-h incub		DOM 48-h incub		24/48	DNDF 24-h incub		DNDF 48-h incub		24/48
material	hybrids	AVG	SD	AVG	SD	%	AVG	SD	AVG	SD	%
WMP	44	64.4 a	5.5	78.4 b	3.3	82.2	43.1 x	7.4	62.1 y	5.4	69.3
Silage	44	62.4 a	5.9	77.7 b	3.4	81.5	42.8 x	6.2	58.2 y	5.3	71.4

WMP = whole maize plants, DOM = digestibility of organic matter, DNDF = digestibility of neutral detergent fibre, AVG = average, SD = standard deviation. Calculated means in the same column and row bearing different subscripts (a, b for DOM, x, y for DNDF) were significantly (P<0.05) different.

The DOM and DNDF were significantly higher (P<0.05) at 48 hour incubation than at 24 hour incubation in all of our measurements with the whole maize plants, and also the maize silage. Khan et al. (2007) obtained similar results, in his study DOM was in average 64.1 % at 24-h incubation and 76.2 % at 48-h incubation while the DNDF was 43.5 % and 61.6 %, respectively.
The differences between fresh WMP and silage were not significant (P>0.05).
The correlation between 24-h and 48-h was very close, (DOM 24/48 was 81.5 %, the DNDF was 71.4 %, respectively). Khan et al. (2007) obtained similar results where the DOM 24/48 was 84.1 %, and the DNDF was 70.4 %.

The results of DOM and DNDF of maize silage made in the 3 different areas (T1, T2, T3) is shown in the Table 2.

Table 2. The *in sacco* digestibility of maize silage from the different areas.

Trial	Number of	DOM 24-h incub		DOM 48-h incub		24/48	DNDF 24-h incub		DNDF 48-h incub		24/48
	hybrids	AVG	SD	AVG	SD	%	AVG	SD	AVG	SD	%
T1	19	63.6 b	2.0	78.5 a	4.0	81.0	43.9 ab	2.2	56.7 a	5.9	77.5
T2	15	58.7 a	5.9	75.2 a	3.3	78.1	38.1 a	8.1	59.3 a	4.8	64.2
T3	10	65.6 b	2.8	79.8 a	3.0	82.2	47.7 b	5.7	59.4 a	4.7	80.3

T1, T2, T3 = trials with maize silage (44 hybrids) made in the different areas. Calculated means in the same column bearing different subscripts (a, b) were significantly (P<0.05) different.

The significant differences (P<0.05) were found between trials with maize silage in DOM or DNDF measured at 24-h incubation, but not at 48-h incubation.

According to Oba and Allen (1999) the digestibility of NDF *in vitro* or *in situ* might be a better indicator of DMI than NDF digestibility in vivo because forages with high *in vitro* or *in situ* NDF digestibility might have shorter rumen retention times, allowing greater DMI at the expense of NDF digestibility *in vivo*. Given that even a small increase in NDF digestibility resulted in increased performance (Oba and Allen 1999), this kind of research is important. Our data are consistent with those from the literature.

Conclusions The *in sacco* digestibility (DOM, DNDF) were significantly higher at 48-h incubation than at 24-h incubation. The differences between fresh whole maize plant and silage were not significantly different, both DOM and DNDF. The significant differences of DOM and DNDF (measured at 24-h incubation) were found between maize silage ensiled in the 3 different area. DOM and DNDF, measured at 48-h incubation, were not significantly different.

Acknowledgements Supported by project NAZV QI91A240.

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Evaluation of fermentative parameters, aerobic stability and *in vitro* gas production of whole crop maize silage treated with a microbial inoculant containing *Pediococcus pentosaceus* and *Lactobacillus plantarum*

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Keywords: corn silage, fermentative parameters, gas production, lactobacilli

Introduction Homofermentative lactic acid bacteria (^{ho}LAB) are used as silage additives to improve silage fermentation and preservation of energy value. ^{ho}LAB have been used because they are fast and efficient producers of lactic acid, improving natural silage fermentation. Literature review (Muck and Kung 1997) reported that in 60% of the studies reviewed, the inoculated silages, compared with untreated samples, show a lower pH, greater lactic acid content and less ammonia nitrogen. Furthermore, research (Kung and Muck 1997) indicates that microbial inoculation may have a role in increasing animal performances affecting the nutritive value of the silages. The aim of this trial was to determine the effects of a microbial inoculant containing *Pediococcus pentosaceus* and *Lactobacillus plantarum* as maize silage additive (L) in comparison with untreated silage (C) on the fermentative profile at 2, 5, 40 and 110 days of ensiling, and temperature, aerobic stability and multiple points *in vitro* Gas Production (GP) at 110 days of ensiling.

Material and methods Whole crop maize was harvested at milk-dough stage (276 g DM/kg as fed, 55, 240 and 487 g/kg DM, of WSC, Starch and aNDFom, respectively), with a theoretical length of cut of 15 mm. According to the European Food Safety Authority Guidance on technological additives (EFSA 2008), about 1500 g of fresh forage were ensiled in 2 L micro-silos with lid seal and bleed valve, with an average packing dry matter (DM) density of 246 kg/m^3. The inoculation of the fresh forage was made in order to obtain a mixture of lactic acid bacteria concentration of 1.0 x 10^5 CFU g^{-1} of fresh maize, with a ratio of 4 to 1 CFU between *Pediococcus pentosaceus* and *Lactobacillus plantarum.*

Negative control was treated with the same amount of water used to inoculate the treated samples to ensure a similar DM content. Seven replicates for each incubation time (2, 5, 40 and 110 d) and each treatment were prepared. VFA, lactic acid and alcohols were determined by gas chromatography. *In vitro* GP was determined following the method of Menke and Steingass (1988) at 2, 4, 6, 8 and 24 hours of incubation, on the silages after 110 days of ensiling.

Data were analyzed using the General Linear Model procedure of SAS (SAS Institute, Inc. 2001) for the evaluation of treatment effect.

Results and discussion The evaluation of fermentative parameters for C and L silages showed that microbial inoculant utilization significantly reduced pH values at 2, 5 and 40 days of incubation (Table 1). No differences were registered at 110 days.

Lactic acid content resulted higher for C silage after 5 days of ensiling (109 and 92.4 g/kg DM for C and L, respectively; P<0.01); however, the additive determined higher values at 40 (97.2 and 104 g/kg DM for C and L, respectively; P<0.05) and 110 days of incubation (93.9 and 102 g/kg DM for C and L, respectively; P<0.05).

Considering the other fermentative parameters, differences between treatments were detected only for acetic acid and ammonia nitrogen contents at 40 days of incubation. Particularly, lower acetic acid (21.4 and 18.3 g/kg DM for C and L, respectively; P<0.05) and higher N-NH_3 concentration (2.08 and 2.24 g/kg DM for C and L, respectively; P<0.01) were found in the silage treated with microbial inoculant. Propionic, isobutyric and butyric acids were detected only in trace in the samples, with the exception for butyric acid during the first week of incubation; however, at 2 and 5 days, only a very few amount of butyric acid was found (0,20 g/kg DM, on average). Small amounts of ethanol were detected in the silages until forty days of incubation (3.4 g/kg DM, on average); in the last period it increased at 7.49 and 5.75 g/kg DM, for C and L, respectively.

DM losses resulted similar up to 40 days of incubation (1.14 *vs* 2.70% for C and L, respectively), but a significant difference was found at 110 days, with lower DM losses for L treatment (7.12 *vs* 3.53%, P<0.01). Aerobic stability data did not show any difference due to the treatment applied to the forage. In fact, both treatments determined a rise in temperature higher than 3°C above the background temperature (20°C) after 3 days.

L treatment increased significantly GP at 6 and 8 hours (+10%); the same trend, even not significant, was also registered at 4 (+10%; P=0.062) and 24 hours (+5%; P=0.093) (Table 2). These results indicate a possible effect of the inoculum on rumen fermentability of organic matter after 110 days of ensiling.

Conclusions The results of this study confirm that the addition of ^{ho}LAB has positive effects on the silage fermentative pattern and on the reduction of DM loss. Moreover, ^{ho}LAB seems to improve the nutritive value of maize silage increasing *in vitro* rumen fermentability**.**

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Table 1. Fermentative parameters of maize silage treated with the additive (L) compared with untreated control (C) at different days of ensiling.

Days of ensiling	2			5			40			110		
Treatment	C	L	SEM	C	L	SEM	C	L	SEM	C	L	SEM
pH	3.80^{A}	3.76^{B}	0.01	3.64^{A}	3.59^{B}	0.01	3.47^{A}	3.44^{B}	0.01	3.56	3.55	0.02
Temperature, °C *	20.0	20.1	0.14	20.1	20.0	0.16	19.8	20.2	0.15	20.7^{A}	19.2^{B}	0.30
Dry matter1, g/kg	275	273	1.66	275	273	2.41	273	269	1.60	256^{B}	266^{A}	2.15
DM losses, %	0.33	1.00	0.60	0.36	1.04	0.87	1.14	2.70	0.58	7.12^{A}	3.53^{B}	0.78
Lactic acid, g/kg DM	102	95.6	2.14	109^{A}	92.4^{B}	2.08	97.2^{b}	104^{a}	1.75	93.9^{b}	102^{a}	1.99
Acetic acid, g/kg DM	10.4	11.5	0.40	12.2	12.0	0.37	21.4^{a}	18.3^{b}	0.84	22.0	23.2	0.51
Propionic ac., g/kg DM	trace	trace	.	trace	trace	.	trace	trace	.	trace	trace	.
Isobutyric ac., g/kg DM	trace	trace	.	trace	trace	.	trace	trace	.	trace	trace	.
Butyric acid, g/kg DM *	0.20	0.20	0.09	0.26	0.14	0.10	trace	trace	.	trace	trace	.
Ethanol, g/kg DM	3.12	3.56	0.16	3.67	3.54	0.13	3.54	3.04	0.17	7.49	5.75	0.59
N-NH3, g/kg DM	1.52	1.42	0.05	1.59	1.58	0.05	2.08^{B}	2.24^{A}	0.01	2.61	2.57	0.08

[1] Dry matter content corrected for volatiles.
*Variables not normally distributed. In this case the P value reported is that obtained with Kruskal-Wallis Test.
$^{A, B}$: capital letters differ for P<0.01; $^{a, b}$: lower case letters differ for P<0.05.

Table 2. Gas Production (GP) of maize silage treated with inoculants (L) in comparison with untreated control (C) at 110 days of ensiling.

	C	L	SEM	P value
2h GP, mL/200 mg DM	8.8	9.7	0.39	0.139
4h GP, mL/200 mg DM	16.9	18.6	0.57	0.062
6h GP, mL/200 mg DM	23.3	26.0	0.65	0.019
8h GP, mL/200 mg DM	28.4	31.4	0.66	0.005
24h GP, mL/200 mg DM	46.1	48.5	0.92	0.093

Effect of the addition of acetic acid or lactic acid bacteria and enzymes on the chemical composition and *in vitro* gas production of the silage of different hybrid maize varieties

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Keywords: acetic acid, bacteria, enzymes, *in vitro* gas production, maize

Introduction Corn is one of the main cereals grown for food and feed in many countries and it has a great economic importance worldwide. In Mexico, traditionally planted corn native varieties adapted to the region and new varieties of corn hybrids has been improved in yield and nutritional quality. The objectives of this study were to determine the chemical composition and *in vitro* gas production of whole plant corn silage preserved without additive and with adding bacterial or chemical of two varieties of corn hybrids and two local natives, grown in high valleys in Mexico (2.500 m above sea level).

Material and methods Four corn varieties were valuated, two hybrids (H51EA and CLO 80001), and two local native white (LNW) and yellow (LNY) maize. Three forage samples of each variety were chopped (5 cm) and stored in micro-silos (n=9) without additive (NT), with a bacteria additive (SAT, Sill all 4x4 ® 10 g/ton, *Streptococcus faecium, Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus salivarius*, cellulase, pentosanases hemicellulases and amylase, 10 g/ton) or with a chemical additive (AACT, acetic acid 1%) in three replicates of each treatment that were prepared by placing 1.5 kg in a PVC tube covered with a polythene bag. After 60 days silos were opened and pH was determined, dry matter (DM), organic matter (OM), CP (N x 6.25) (Buchi K370), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) were also determined by NIRS (NIR FLEX N400). For *in vitro* gas production technique, two dairy cattle (450 kg BW) fitted with permanent rumen fistula, were used as donors of rumen fluid. Gas production (GP) was determined by the method proposed by Theodorou et al. (1994), registered at 0, 3, 6, 9, 12, 18, 24 and 30 h using a pressure transducer (DELTA OHM, Manometer, 8804). After the incubation was calculated the proportion of dry matter disappeared (DMd%) and relative gas production (RGP, ml gas/ g DMd%). The GP was fitted by the equation proposed by Krishnamoorthy et al. (1991), and metabolizable energy (ME, Mj/kg DM) was estimated by the equation proposed by Menke and Steingass (1988). For statistical analysis a factorial arrangement 4x3 was used, with three replicates per treatment.

Results and discussion There were no differences for pH among varieties and treatments. DM content was higher ($P<0.01$) for CLO80001, and the lower content was for LNY, OM content was higher ($P=0.01$) for CLO80001 compared to LNW and LNY. The CP content of hybrid varieties was higher ($P<0.01$) than LNW and LNY. The contents of NDF and ADF were higher for LNW ($P <0.01$) with respect to LNY and were higher to CLO80001 and H51. The inclusion of SAT or AACT increase ($P<0.01$) the DM content vs NT. OM content was higher ($P=0.01$) for SAT > AACT > NT. The content of crude protein, NDF and ADF decreased ($P=0.01$) with the inclusion of SAT over the control.

The highest gas production (ml gas/g DM) ($P=0.01$) was for hybrid varieties (CLO8001 and H51EA) compared with LNY and LNW. There were not differences for b and lag time ($P>0.05$) between varieties. The DMd was higher ($P=0.01$) for LNW and CLO80001 compared with H51EA. The RGP was higher H51EA ($P<0.01$) compared to the rest. ME (MJ / kg DM) was higher ($P=0.01$) for CLO80001 and H51EA> LNY> LNW. In relation with the effect of the treatments, there were no differences for *in vitro* gas production among treatments, except for ME, witch was higher ($P=0.01$) for SAT and AACT compared with NT, Corral-Luna et al. (2011) found values of ME of 9.62 to 10.46 MJ ME / kg DM in maize silage with out additives similar to those found in the present study. Results indicate that the addition of SAT decreased the amount of CP, NDF and ADF, this may due to the impact of enzymes in inoculums', as lactic acid bacteria that could hydrolyze the forage cell wall constituents.

Conclusions. Native local maize remain a good option for small farmers in the region, the content of crude protein, NDF and ADF decreased with the inclusion of SAT over the control treatment, the use of additives did not improve the nutritional quality of silage.

Table 1. Chemical composition (g / kg DM) and *in vitro* gas production (ml gas g / DM) of four varieties of corn maize (Natives and hybrids), preserved by ensiling with different treatments.

	VARIETY						TREATMENT				
	LNY	CLO8001	H51EA	LNW	SEM	P value	NT	SAT	AACT	SEM	P value
pH	3.96	3.97	4.18	3.96	0.13	0.58	4.04	4.03	4.00	0.12	0.97
Chemical composition											
DM	176.4^{f}	222.9^{d}	185.4^{e}	185.4^{e}	0.60	0.01	171.8^{e}	203.1^{d}	202.6^{d}	0.52	0.01
OM	913.1^{e}	933.2^{d}	922.9^{de}	922.4^{e}	0.27	0.01	91.2^{f}	93.4^{d}	92.2^{e}	0.23	0.01
CP	90.3^{e}	82.2^{f}	104.9^{d}	106.4^{d}	1.81	0.01	97.4^{d}	90.6^{e}	99.9^{d}	1.57	0.01
NDF	554.4^{e}	534.6^{f}	522.2^{f}	574.9^{d}	4.99	0.01	558.7^{e}	532.7^{f}	548.2^{d}	4.33	0.01
ADF	340.5^{e}	317.9^{f}	321.3^{f}	355.5^{d}	3.84	0.01	348.8^{d}	322.1^{f}	332.3^{e}	3.33	0.01
ADL	70.6^{de}	64.6^{e}	75.5^{d}	64.7^{e}	2.02	0.01	71.3	66.8	68.7	1.75	0.22
In vitro gas production											
b	282.9^{e}	319.1^{d}	316.6^{d}	297.3^{e}	9.63	0.01	306.9	300.4	304.8	8.34	0.85
c	0.049	0.050	0.051	0.047	0.004	0.77	0.047	0.053	0.049	0.01	0.50
T	1.36	1.40	1.42	1.34	0.15	1.92	1.44	1.43	1.28	0.13	0.65
DMd%	63.2^{de}	65.0^{d}	59.6^{e}	63.5^{d}	1.03	0.01	61.4	63.7	63.3	0.94	0.16
RGP	340.0^{e}	362.8^{e}	414.5^{d}	341.1^{e}	9.88	0.01	367.3	366.4	360.1	8.56	0.81
ME	9.64^{e}	9.96^{d}	9.88^{d}	9.42^{f}	0.05	0.01	9.52^{e}	9.90^{d}	9.75^{d}	0.05	0.01

Values are expressed as mean. Different letters indicate significance (d < e < f, $P < 0.01$). LNY = Local Native yellow, LNW= Local native with, NT= No treatment silage, SAT = Sill all treatment, ACCT= Acetic acid treatment, SEM= Standard error of mean. b = total gas production (mL gas/g incubated DM); c = fermentation rate (h^{-1}); T = lag time (the time in that the fermentation is initiated); DMd% = Disappeared dry matter proportion; RGP = relative gas production (mL gas/g DMd%), ME= Metabolizable energy (Mj /Kg DM).

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Quality of two types of corn based farm silages in Tianjin area in China

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Keywords: farm silage, fermentation quality, Tianjin

Introduction Silage is nowadays a main resource of livestock fodder for cattle in Tianjin. Therefore, the quality of silage is gradually being taken seriously. The aim of this study was to investigate the silage condition in Tianjin and get deeper understanding on the fermentation and nutritional quality of silage used in the prevailing production practices in farms.

Material and methods The samples of farm silages were collected from Wuqing district of Tianjin (43.62 N, 118.02 E; altitude, 900 m) of China. There were total two silage types of eight samples in Wuqing district including four whole-corn silage and four fresh corn-stover silage, which were ensiled in different farms' silage trenches. Silage fermentation indicators including lactic acid, acetic acid, propionic acid, butyric acid, ammonia and organic acids were determined by liquid chromatography, and fat and ammonia was measured by sodium hypochlorite assay. The nutritional index contained following determinations: dry matter (DM) was determined after drying at 105 ºC for 24h, crude protein (CP) was determined by a Rapid N cube Analyzer (Germany, Eementar company), ether extract was determined by Soxhlet extraction (GB6433-94), water soluble carbohydrates (WSC) was determined using the anthrone method according to Owens (1999), neutral detergent fiber (NDF) was analyzed by the method of Van Soest et al. (1991), acid detergent fiber (ADF) was analyzed sequentially on the same sample by the method of AOAC (2000), ash was indicated by igniting at 550 ºC for 3h in a muffle furnace, and the determination of calcium (Ca) and phosphorus (P) were based on potassium permanganate method according to Zhang (2002). The data were processed by Microsoft Office Excel 2003 and analyzed with Tukey test of ANOVA by SAS.

Results and discussion The pH values of the two type farm silages in Tianjin were less than 4.0 (table 1). The fermentation quality of the silages were evaluated according to the pH value, results showing that quality of both of the two type farm silages was excellent. Lactic acid/total acid content in whole-plant corn silage was significantly greater than that of the silage of green corn stalks in Tianjin (P<0.05). The total score for Fernandez was assessed by the pH value of the results of the same quality (Zimmer 1990), the Fernandez score of volatile organic acids and ammonia nitrogen content indicated that both two type of Tianjin farm silage fermentation quality were excellent. In agreement with this, the whole-plant corn silage and green corn stalks silage fermentation quality had no significant difference (Table 1).

The nutritional content of the two type farm silages in Tianjin in terms of DM, CP, Ca and P showed no significant difference, but the contents of EE, WSC, NDF, ASH and ADF were significantly different (P<0.05). Compared with whole-plant corn silage, contents of NDF and ADF were greater in fresh corn-stove silage indicating that the palatability and feed intake of this silage was significantly better than the green stalks of corn silage (Table 2).

Conclusions There were no major differences in the fermentation quality of the two type of farm silages made of whole-plant corn or of green corn stalks silage in Tianjin. The quality of green stalks corn silage was slightly better than that of whole-plant corn silage.

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Table 1. Fermentation quality of the two type corn based farm silages in Tianjin.

Silage type	pH[1]	La/Ta(%)[2]	Aa/Ta(%)[3]	Pa/Ta(%)[4]	Ba/Ta(%)[5]	VBN/TN(%)[6]	Total score	Grade
Whole-corn silage	3.58±0.16a[7]	74.67±7.68a	21.71±8.99a	1.61±0.74b	2.01±0.96a	6.67±2.19a	90.5	Excellent
Fresh corn-stover silage	3.83±0.09a	68.64±6.8b	25.38±5.90a	4.32±2.75a	1.66±0.92a	6.70±3.30a	89	Excellent

[1]pH = pH-value; [2]La/Ta = lactic acid / total acid; [3]Aa/Ta = acetic acid /total acid; [4]Pa/Ta = propionic acid /total acid; [5]Ba/Ta= butyric acid/ total acid; [6]VBN/TN = volatile basic nitrogen/total nitrogen; [7]a b c different letters in same column indicate significant differences at a level of $P < 0.05$.

Table 2. Chemical composition of the two type corn based farm silages in Tianjin (% in dry matter).

Item	Whole-corn silage	Fresh Corn-stover silage
Dry matter	17.73+2.44 a[1]	15.67+2.41 a
Crude Protein	9.22+0.63 a	10.30+0.77 a
Ether extract	2.28+0.13 a	1.47+0.37 b
Water-soluble Carbohydrates	4.77+0.71 a	3.07+1.17 b
Neutral Detergent Fiber	44.74+4.04 b	51.23+6.53 a
Acid Detergent Fiber	26.84+1.80 b	32.51+3.12 a
Ash	6.13+0.82 b	8.56+1.18 a
Calcium	0.58+0.03 a	0.59+0.04 a
Phosphorus	0.16+0.03 a	0.13+0.04 a

[1]a b c different letters in same row indicate significant differences at a 0.05 level.

Forage maize at northern latitudes (60°N;17°E) harvested and ensiled before and after frost

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Keywords: corn harvest, yield, silage

Introduction The acreage of forage maize is increasing in Sweden although the vegetation period is short and Sweden is situated at the northern border of the maize cultivation zone. In the southern coastal region of Sweden the vegetation period (number of days with average temperature > 5°C) is 240 days and in the north only 150 days. The limit of cultivating maize for silage is regarded to be in central Sweden at 60°N where the vegetation period is about 180 days. The short vegetation period results in risk of frost damages on the maize crop before desirable maturation is reached. In order to investigate the yield, the development of nutrients in the plant, the hygiene and the silage quality, forage maize was harvested at 8 consecutive weeks around the first autumn frost at three sites during two years in mid Sweden (60°N;17°E).

Material and methods Five maize varieties of FAO numbers around 200 (Artist, Mas09A, Density, Agassi and Patrick) were sawn at three different sites at the 15th of May 2009 and at the 6-12th of May 2010. The frost was forecasted to the end of September. Harvests of 5+5 plants took place once a week from mid September to early November both years. The temperature was recorded every hour both at the top level of the plants and at the level of the cobs. The plants were weighed and analyzed with and without the cobs for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), starch and water soluble carbohydrates (WSC) with standard methods and indigestible NDF was analyzed with nylon bags in situ, all according to NorFor (2011). Portions of the complete crops were ensiled in laboratory silos of .1.7 L during 4 months and then analyzed for pH, ammonia-N, VFA with standard methods also described in NorFor (2011). Total lactic acid bacteria were analyzed according to Pahlow (1990). Yeast and mould at serial dilutions were aerobically cultivated and counted on malt extract plates with added penicillium G (30 mg/l) and streptomycin sulphate (30 mg/l) at 25 °C.

Results The temperature development is presented in Table 1. The DM yield increased until the first frost occurred at the third week of the harvest series (6th of October 2009 and 30th of September 2010). After the first frost, defined as colder than 2°C below zero during more than 2 h, the growth was abruptly stopped and the DM yield decreased (Figure 1). At weeks 5-8 the DM yield was 93 % of the maximum yield. The cob yield as a proportion of the total yield increased until week 4 and then remained unchanged (Table 2). The content of WSC of the total crop decreased from 18.1 to 4.7 % of DM over the 8 weeks and the starch content increased from 16 to 26 % of DM over the same period. NDF and CP were unchanged but the indigestible NDF increased from 19.2 to 21.0 % of total NDF (Table 2). The LAB of the fresh crop increased from log 3.4 to log 5.1, the yeasts increased from log 4.4 to log 5.3 and the moulds from log 4.2 to log 5.4. After ensiling the LAB was log 7.8 for silage harvested week 1 and log 8.1 at week 8, Clostridia log 0.0 and log 1.1, yeasts log 1.3 and log 2.2 and moulds log 0.0 and log 0.1 of silages harvested at week 1 and 8, respectively. The pH increased slightly from 3.8 to 3.9 between weeks 1 and 8, ammonia nitrogen was unchanged, lactic acid decreased from 5.9 to 3.4 % of DM, acetic acid decreased from 3.5 to 1.8 %, butyric and ethanol were unchanged and 2,3- buthandiole increased from 0.2 to 0.6 % of DM. After opening the silos the aerobic stability was monitored by temperature registration during 5 days of air exposure. No effects of week of harvest on aerobic stability between silages were detected.

Table 1. Temperature during the 8 experimental weeks around the first frost, the relative total dry matter (DM) yield and an appraisal of the hygienic standard (for figures see text) of the fresh crop at harvest.

Week	Mean temp. [1], °C	Min. temp., °C	Max. temp., °C	DM yield, relative	Hygienic standard
1	11.7	+3.5	+ 19	88 %	OK
2	9.9	0	+22	93 %	OK
3	9.8	- 2	+23.5	100 %	Yeast
4	7.0	-3.5	+24	96 %	OK
5	6.1	- 3	+14.5	96 %	Yeast + Mould
6	2.6	-7	+13.5	90 %	Yeast + Mould
7	-0.5	- 7.5	+ 10	93 %	Yeast + Mould
8	6.2	-5.5	+13	91 %	Mould

[1)]The mean temperature of hourly measured temperature during the week

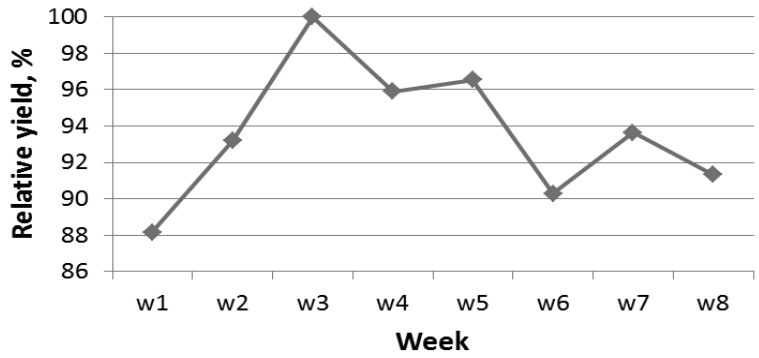

Figure 1. The development of the total relative forage maize yield (kg dry matter) when harvested during 8 weeks around the first frost.

Table 2. Relative yield (from Fig. 1) of forage maize compared to the cob proportion, crude protein (CP), fiber (NDF), insoluble NDF (iNDF), water soluble carbohydrates (WSC) and starch as least square means of three sites over two years. Means within column that do not share the same super-script are statistically different at $p<0.05$. The GLM procedure in SAS was used.

Week	Relative Total yield	Cob prop., % of DM	CP, % of DM	NDF, % of DM	iNDF, % of NDF	WSC, % of DM	Starch, % of DM
1	0.88	51^{a}	8.4^{ab}	$44.1^{a.d}$	$19.2^{a.d}$	18.1^{a}	16.3^{a}
2	0.93	52^{a}	8.4^{ab}	$42.4^{b.c.d}$	18.2^{a}	17.3^{a}	17.5^{a}
3	1.00	$56^{b.c}$	8.1^{ab}	41.6^{b}	18.4^{a}	14.4^{b}	21.4^{b}
4	0.96	55^{b}	8.4^{ab}	$42.2^{b.e}$	$19.0^{a.c}$	11.9^{c}	$23.2^{b.c}$
5	0.96	$57^{b.c}$	8.4^{ab}	$43.9^{a.c.e}$	$19.9^{b.c.d}$	10.1^{d}	$23.1^{b.c}$
6	0.90	58^{c}	8.5^{a}	44.5^{a}	$19.0^{a.d}$	7.5^{e}	$24.9^{c.d}$
7	0.94	58^{c}	8.2^{ab}	44.8^{a}	$19.9^{b.c}$	6.4^{e}	26.1^{d}
8	0.91	58^{c}	8.1^{b}	45.4^{a}	$21.0^{b.c}$	4.7^{f}	25.7^{d}

Discussion and conclusions The results suggest that the starch formation continues after frost on the expense of WSC. The concentration of fiber remains relatively constant but becomes slightly more indigestible. The net growth stops at frost and the DM yield decreases. The low temperature, possibly with assistance of the short daylight inhibits net photosynthesis but cobs continue to grow with available sugars in the plant. However, the cob yield also declines in spite of the higher cob proportion since total DM yield continuously decreases after frost. A lower DM yield accompanied with a lower total content of digestible nutrients results in a recommendation to harvest the maize shortly after frost even though the starch content increases. The deteriorating hygienic condition with higher counts of yeasts and moulds in the green crop after frost also points in the same direction. Maize plants infected with moulds in the field are often found to contain aflatoxin, zearalenone and deoxynivalenol (Driehuis 2011). However, this study also showed that maize harvested long after frost with relatively high numbers of yeast and mould in the green crop proved to produce silage of good standard, with low yeast and mold counts and good aerobic stability. It must be kept in mind though, that toxins produced before the ensiling process will still be present in final silage.

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White-rot fungal digestion of maize stover components harvested at sequential maturities

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Keywords: stover, white-rot fungi, lignin, digestibility

Introduction Maize stover (stem and leaves) silage is not considered a ruminant feed of high value, and generally supports similar rates of animal performance to what could be achieved with average quality grass silage. This herbage generally has a high fibre concentration (575 - 800 g NDF/kg dry matter) containing lignin, which is indigestible to ruminants and forms linkages with cell wall polysaccharides in a matrix that protects a proportion of these carbohydrates from ruminal digestion. The digestion of fibrous agricultural residues with lignin-degrading white-rot fungi (WRF) can result in improvements in feed digestibility. The objective of this study was to describe the changes in chemical composition and digestibility during the digestion of contrasting components of maize stover harvested at sequential stages of maturity and treated with each of two WRF (*Pleurotus ostreatus; Trametes versicolor*).

Material and methods For maize plants from separate plots in each of three replicate blocks of a split-plot design of field plots, three harvest date treatments (7 September, 5 October and 5 November) comprised the three main plots and within which sub-plots were comprised of three stover components. On each harvest date either the leaves, upper stem or lower stem were manually separated from all plants present in a plot and precision-chopped. Samples weighing 700 g were allocated to inoculation with one of two fungal additives (*P. ostreatus,* PO; *T. versicolor,* TV) and one of five digestion durations (0, 1, 2, 3 and 4 months). Prior to inoculation, samples were immersed in 4 litres of room temperature tap water for 20 minutes and autoclaved at 110°C for 1 hour to eliminate epiphytic microflora. Approximately 10 g of fungal additive (i.e. fungus + culture medium), previously cultured on potato dextrose agar at 30°C for 14 days, was thoroughly distributed throughout each of the appropriate samples, with aseptic techniques being applied throughout. Subsequently, for their duration of digestion, samples were stored at *approx*. 20°C in sealed polypropylene bags that had a membrane that facilitated gas exchange and thus provided aerobic conditions. Following the appropriate duration of digestion, samples were weighed to determine recovery rates and stored at -20°C prior to chemical analysis. The *in vitro* DM digestibility (DMD) was determined by the method of Tilley and Terry (1963). Data were analysed using a model that accounted for harvest date (main plot), fungal additive and digestion duration (two x five factorial arrangement of treatments in the sub-plot) for three replicate blocks in a split plot design. All analyses were conducted using the PROC GLM model of the SAS statistical program.

Results and Discussion Later harvesting of leaf, upper stem or lower stem reduced ($P<0.01$) the DMD and water-soluble carbohydrate concentration of these samples prior to inoculation. As interactions between harvest date and other factors were rare and of little biological significance, only the effects of fungal additive and digestion period are discussed.

The lignin concentration of all digested upper stem and lower stem samples, with the exception of upper stem digested for 4 months, was higher ($P<0.05$) than samples prior to digestion. However, all samples digested with PO for greater than two months had lower ($P<0.05$) lignin concentrations than samples digested for shorter periods (Table 1). Therefore, lignin degradation was observed in all sample types digested with PO, with the results indicating that leaf may have undergone more extensive lignin degradation than the stem samples. In addition, decreases in the lignin concentration of leaf, upper stem and lower stem due to increased duration of digestion by PO were only observed after 1 month. This indicates that lignin degradation was initiated later in the storage period as a secondary metabolism.

Despite this, the DMD and neutral detergent fibre digestibility (NDFD) of all samples digested by PO were lower ($P<0.01$) than samples prior to digestion, with the exception of lower stem digested for 4 months which did not significantly differ ($P>0.05$) from pre-digested herbage. This indicates that the selective degradation of lignin over the more rumen-digestible substrates such as hemicellulose and cellulose, in samples digested with PO was low and thus resulted in a reduced DMD and NDFD in PO-digested samples when compared to samples prior to inoculation.

Similar to the effects of digestion of leaf with PO, TV-digested leaf underwent a non-selective degradation of substrate. However, the large increase in the lignin concentration of leaf samples digested with TV was in contrast to the effect observed in PO-digested samples. This indicated that very minor

or no lignin degradation occurred in these samples. The high N concentration (24 to 41 g/kg DM) of the leaf prior to inoculation may have contributed to this effect as Tapia and Vicuna (1995) reported that culturing *Ceriporiopsis subvermispora* in a high N medium negatively affected the activity of ligninolytic enzymes produced by the organism. While this may explain a proportion of the higher lignin concentration observed for TV-digested leaf, this explanation is not apt for describing the low ligninolytic activity observed in the upper stem and lower stem samples, which had a low N concentration (5 to 14 g/kg DM) prior to inoculation. Therefore, non-defined other factors negatively impacted on the ligninolytic ability of TV in the present study.

In addition to their contrasting effects on lignin degradation, PO-digestion of leaf, upper stem and lower stem resulted in a higher ($P<0.05$) NDFD than occurred with TV, when averaged across all digestion periods. This contributed to the higher DMD of PO-digested samples compared to TV-digested samples and was in accord with Rahman et al. (2011) who reported that oil palm fronds digested with *P. ostreatus* had a higher digestibility than samples digested with *T. veriscolor.*

In general, the lack of positive effects of WRF digestion on the digestibility of herbages used in the present study may have been due to the high availability, and subsequent utilisation, of easily digestible substrate substantially reducing the opportunity for lignin degradation to improve the digestibility of these samples. In addition, the higher moisture content in the present study may have resulted in sub-optimal conditions for PO or TV growth.

Conclusions The changes in chemical composition of leaf, upper stem and lower stem of maize stover digested with either *P. ostreatus* or *T. veriscolor* were not beneficial to the feed value of the forage, and incurred high losses in DMD and nutritive value.

Table 1. Digestibility and fibre concentration indices of maize stover components digested with *Pleurotus ostreatus* (PO) and *Trametes veriscolor* (TV).

Digestion	Additive	Leaf			Upper Stem			Lower Stem		
(D)	(A)	DMD[1]	NDF[2]	ADL[2]	DMD[1]	NDF[2]	ADL[2]	DMD[1]	NDF[2]	ADL[2]
At harvest	-	670	691	23	572	739	38	524	766	50
1 month	PO	514	580	55	342	809	67	240	858	87
2 month	PO	557	532	48	436	767	62	281	839	84
3 month	PO	547	481	33	454	747	53	358	813	75
4 month	PO	584	434	20	471	722	46	410	785	72
1 month	TV	512	613	62	392	747	63	295	861	74
2 month	TV	437	572	88	360	750	69	264	844	79
3 month	TV	421	522	109	366	764	69	262	842	79
4 month	TV	360	514	139	378	753	61	261	824	78
S.E.M.	DxA	19.9	15.2	8.8	16.8	18.1	3.5	18.7	10.7	3.6
Significance	D		***		*		**	**	***	
	A	***	***	***	***		**	***	*	
	DxA	***		***	***		*	***		*

[1] g/kg; DMD= dry matter digestibility,
[2] g/kg DM; NDF = neutral detergent fibre, ADL = acid detergent lignin

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Chemical composition and silage fermentation of sweet corn by-products

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Keywords: chemical composition, silage, sweet corn by-product

Introduction Sweet corn (*Zea mays* convar. *saccharata* var. *rugosa* also called Indian corn, sugar corn and pole corn) is a variety of maize with a high sugar content. Sweet corn is traditionally eaten with beans in some countries including Thailand, and their by-products have increased rapidly in recent years. Although a small proportion of the fresh sweet corn by-product is fed to animal, most are generally unused.

From a silage fermentation point of view, to our knowledge, a very few information is available on the silage preparation with sweet corn by-products in Thailand and elsewhere. In the present study, the chemical composition and the effects of LAB inoculants on silage fermentation quality of sweet corn by-products were studied.

Material and methods Sweet corn at milk stage were collected as stover, ear, cob and whole crop in a farm field, Khon Kaen, Thailand (Fig. 1). These materials were chopped into 10 mm and prepared by using a small scale system of silage fermentation (Cai *et al*. 1999). The commercial inoculant Chikuso-1 (CH, *Lactobacillus plantarum*) and Snow Lact L (SN, *Lactobacillus rhamnasus*), were used as silage additives. The inoculum size of LAB was 1.0×10^5 colony forming unit/g of fresh matter (FM). Experimental treatments included control silage without additive or with LAB inoculants Chikuso-1 and Snow Lact. Three silos per treatment were used for chemical analysis. The chemical compositions of silage were determined by conventional methods. The organic acid contents were measured by high-performance gas chromatography. The Tukey test was used to identify differences ($P < 0.05$) between means.

Results and discussion The weight ratios of stover, ear and cob to whole crop were 46.1, 19.6 and 11.0% on fresh matter basis, and 36.5, 16.7 and 10.4% on dry matter (DM) basis. The crude protein and organic matter contents of the whole crop, stover, ear and cob were 5.56, 5.28, 2.33 and 3.73%, and 94.6, 92.7, 96.5 and 96.7% on a DM basis, respectively. The DM of whole crop, stover, ear and cob were 30.5, 24.2, 26.5 and 28.9%, respectively.

Overall, 10^5-10^6 of LAB, 10^4-10^6 of coliform bacteria, 10^5-10^7 of aerobic bacteria and 10^4-10^6 of yeast were found in these materials. Moulds were too few to found to be counted in all samples. All silages were well preserved, with low pH values, ammonia-N and high lactic acid contents. The lactic acid contents of tow LAB-inoculated silage for corn stover were similar to that of control, but for ear, cob and whole crop were higher ($P < 0.05$) than that of control. The Chikuso-1-inoculated silage had a lower ($P < 0.05$) acetic acid than Snow Lact-inoculated and control silages (Table 1).

ConclusionsThe results confirmed that the ratio of sweet corn by-products to whole crop were more than 60% on a DM basis, and these by-products can be preserved as a good quality without LAB inoculant. The sweet corn by-product should be a good potential resource for livestock feed.

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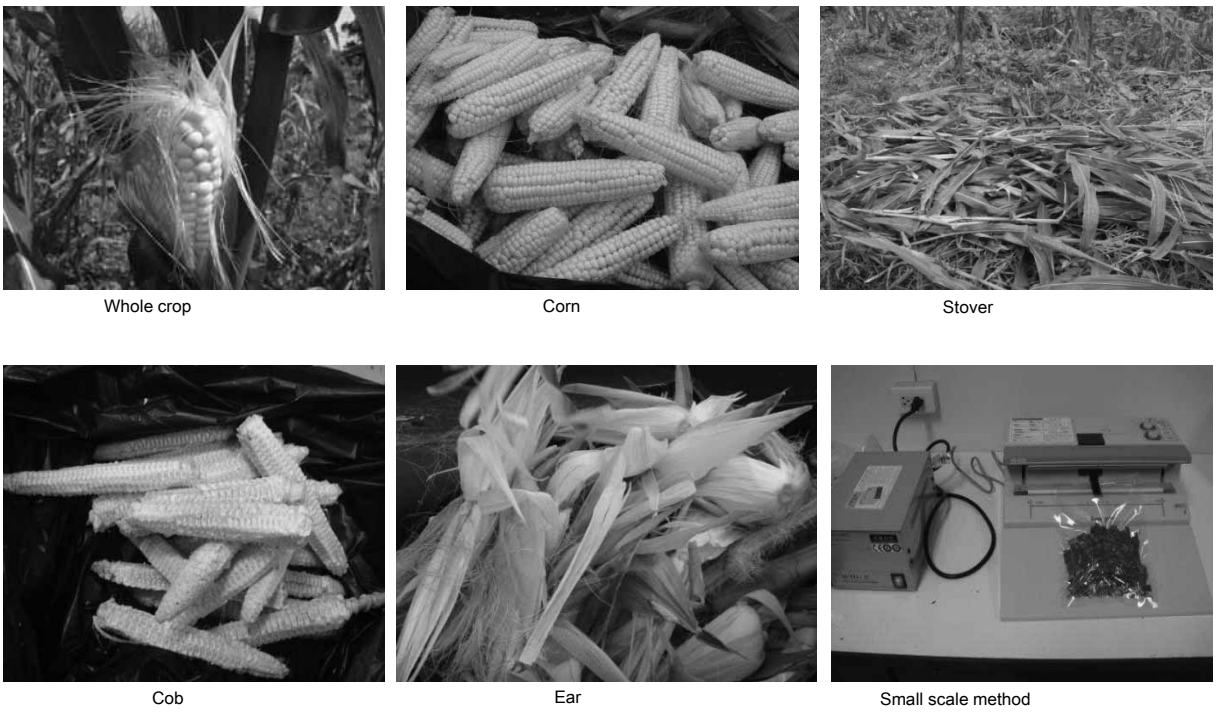

Fig. 1. Sweet corn materials and silage preparation method.

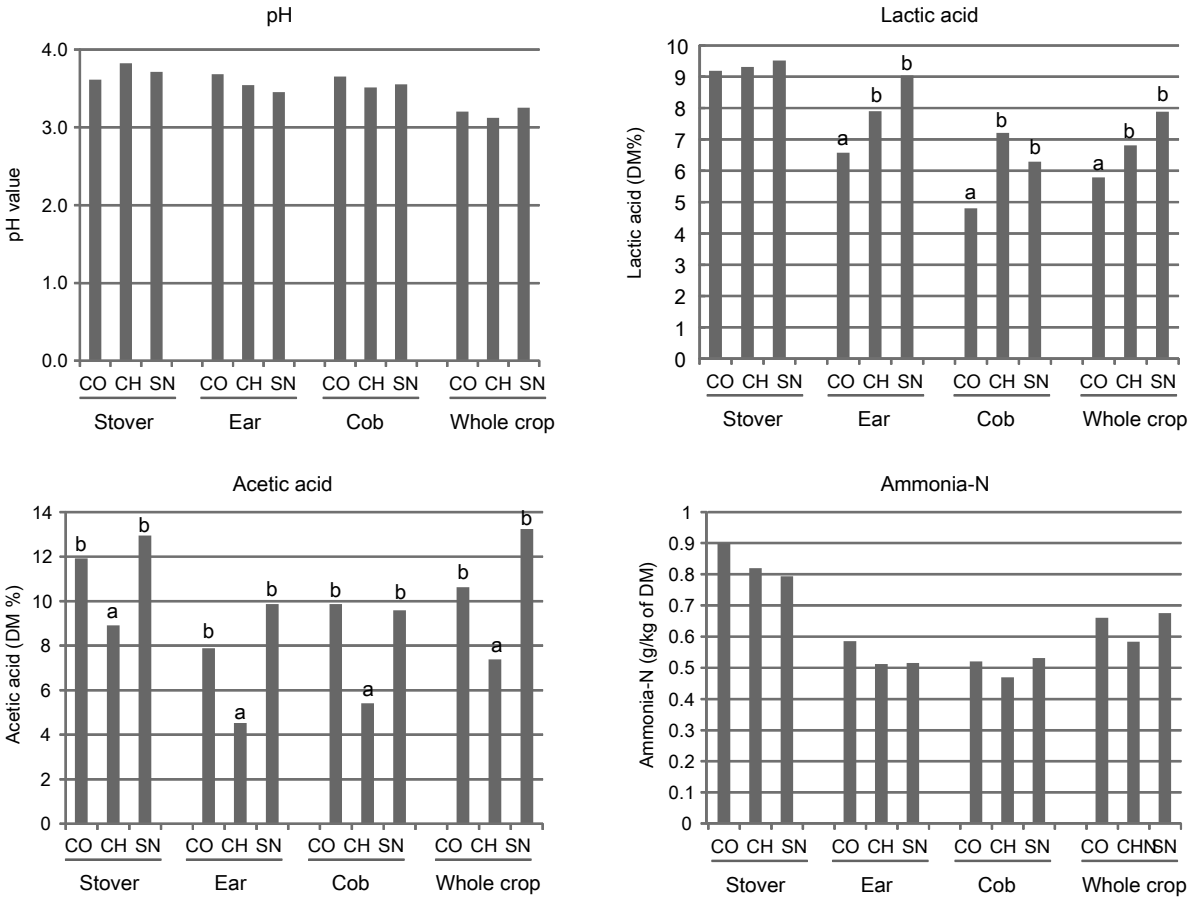

Fig. 2. The fermentation quality of sweet corn silage. Values are means average of three silage samples (P < 0.05). DM, dry matter; CO, control; CH, Chikuso-1; SN, Snow Lact L.

Comparison of chemical and degradability characteristics in three sorghum silage varieties with corn silage using *in vitro* and *in situ* methods

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Keywords: corn silage, forage sorghum varieties, *in situ, in vitro*

Introduction Sorghum (*Sorghum bicolor* [L.] Moench) is one of the important forage sources in arid and semi arid areas of the world. This plant has a great range of phenotypic distribution from 0 (in infertile hybrids) to 10000 kg grain per hectare and from 50 to 400 cm in height. Similar to corn, it is a warm-season plant (C4 photosynthetic pathway) and in general can be classified into two types: forage and grain. Forage type is taller and has better quality than grain varieties and grouped into four types: 1- hybrid forage sorghum, 2- *Sudangrass*, 3- sorghum × Sudan hybrids and 4- *Sweet* sorghum. In the past, sorghum typically is grown in hot regions where was not suitable for cultivating of corn; but nowadays thanks to emergence of forage sorghum hybrids (mostly is known as forage sorghum), in ideal and suitable conditions, their production is similar to corn.

In a previous experiment new sorghum varieties were compared and was shown that all of them have a great concentration of water soluble carbohydrate (WSC) and low content of crude protein (CP) and ash (Miron et al. 2005). Introducing sorghum hybrids with a vast range of performances persuade researcher to conduct studies for comparing them with corn for finding possible alternatives. Therefore, the objective of this study was to compare the chemical composition and degradability characteristics of three forage sorghum varieties with corn silage using *in vitro* and *in situ* methods.

Material and methods Three varieties of forage sorghum (*Sweet*, *Pegah* and *Speedfeed*) and one forage corn variety (*KSC 704*) were planted in the same condition (i. e. similar seeding, fertilizers and irrigation). Forages were harvested (corn in 95 days and sorghums in 110 days of age) in soft dough maturity stage and ensiled in mini-silos in four replicates. The silos were opened on 60th day and concentrations of volatile fatty acids (VFAs) and ethanol concentrations determined by gas chromatography (CP-9002, 259 a.m, Chrompack, Netherlands). Ammonia nitrogen content was estimated by Kjeldahl machine and lactic acid concentration was determined by spectrophotometry.

For the *in situ* study, all silage samples were dried at 60°C for 72 h and ground to pass through 2 mm screen using a wiley mill (Arthur H. Thomas Co., Philadelphia). Duplicate samples were put into Dacron polyester bags (10 mg/cm^2) and incubated for 0, 3, 6, 9, 12, 24, 48 and 72 h in the rumen of two non lactating dairy cows. After removal from the rumen, bags were washed under cold, running tap water and then dried. For each treatment, the equation of $P = a + b\ (1\text{-}e)^{-ct}$ was fitted to the percentage of dry matter (DM) disappearance.

A completely randomized design was used for analyzing the *in vitro* study data. The kinetics of DM disappearance was estimated using repeated nonlinear regression procedure. Tukey's range test was used to differentiate among means and significance was considered at $P < 0.05$.

Results and discussion Corn silage had significantly lower DM than sorghum silages (Table1). The pH in Silages was consistent with loss of water soluble carbohydrate and lactic acid production, however only pH in *Speedfeed* sorghum silage was significantly higher than corn silage. The concentration of neutral detergent fiber had a high linear correlation with acid detergent fiber and acid detergent lignin (ADL) and this correlation with ammonia nitrogen and WSC was very low (0.8, 0.98, 0.16 and 0.03, respectively). ADL content of corn silage was lower than *sweet* and *Speedfeed* sorghum; also the residual WSC was lower in corn silage than *Pegah* and *Sweet* sorghum silage ($P < 0.05$). Low WSC maybe an advantage during secondary fermentation and reduces the growth of yeast and molds. Ammonia N was greater in *Pegah* and *Speedfeed* sorghum varieties than others ($P < 0.05$). Lactic acid percentage in corn and *Pegah* sorghum was significantly greater than in *Speedfeed* and *Sweet* sorghum silage. Considering lactic acid, acetic acid and ethanol in silages showed that quantity of fermentation in corn and *Speedfeed* sorghum silages were highest and lowest, respectively. Greater ratio of lactate to total acid in corn and *Pegah* sorghum may be implies that homo-fermentative bacteria were more active than other

bacteria in these two silages. Clostridium activity in the silages was not detected due to low concentrations of butyric and propionic acids (lower than 0.05 %DM, data are not mentioned in the table).
The *Speedfeed* sorghum had lowest *a* coefficient as compared with other silages ($P < 0.05$). The probable reason for this finding is the low amount of lactate in this silage; acidic environment due to greater concentration of lactic acid causes release of hemicelluloses and consequently increase *a* coefficient in silage. The *b* coefficient for corn silage was significantly greater than sorghum silages which might be related to higher amount of non fiber carbohydrate. Moreover, low ADL level that is the important factors in DM digestibility improved *b* coefficient in corn silage. Greater *b* coefficient might be as determinant reason on higher effective degradability with passage rate of 2% / h in corn silage than sorghum silages. Also greater *a* and *b* coefficients led to greater potential of digestibility in corn silage than sorghum silages.

Table 1. Chemical, fermentative and degradability characteristic of sorghum silage varieties and corn silage.

		Sorghum varities			
	Corn	Sweet	Pegah	Speedfeed	S.E
Chemical composition (% DM)					
Dry matter (%)	30.5b	33.2a	32.5a	33.7a	0.84
pH	3.85b	3.93ab	3.89ab	4.10a	0.08
Crude protein	7.24ab	5.49c	6.41bc	7.63a	0.48
Ash	5.80b	6.48b	8.08a	9.34a	0.5
Neutral detergent fiber	45.2c	51.1b	46.9bc	57.1a	1.99
Acid detergent fiber	23.6b	23.8b	21.7b	28.2a	1.45
Acid detergent lignin	2.10c	3.24b	2.50bc	5.14a	0.57
Water soluble carbohydrate	1.63b	3.36a	3.06a	2.33ab	0.52
Ammonia nitrogen	0.35b	0.40b	0.55a	0.52a	0.06
Non fiber carbohydrate[1]	38.30a	33.46b	35.00ab	21.84c	1.9
Lactate	3.60a	2.19b	3.43a	2.02b	0.24
Acetate	2.14a	2.41a	1.05b	1.32b	0.19
Ethanol	1.53ab	1.73a	1.87a	0.74b	0.35
Coefficients of degradability					
a	35.4a	35.8a	37.0a	32.6b	0.61
b	49.85a	37.65b	35.80b	36.20b	0.88
c	0.041b	0.039bc	0.052a	0.030c	0.003
Effective degradability (2% / h)	68.85a	60.55b	62.90b	54.35c	0.83
Potential of degradability (%)	85.25a	73.45b	72.80b	68.75c	0.92
100 - (a+b)	14.75c	26.55b	27.20b	31.25a	0.85

[a, b, c, d] Means in the same row with different superscripts letters are significantly different ($P < 0.05$). [1]Non fiber carbohydrate = 100 - (Crude protein + Ash + Ether extract + Neutral detergent fiber).

ConclusionsOur results imply that *Speedfeed* sorghum is less suitable than other forage for making silage and *Sweet* and *Pegah* sorghum might be comparable with corn silage, however it should be also studied in practical feeding.

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Nutritive value of silages made with sweet pearl millet and sweet sorghum forage residues obtained after juice extraction

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Keywords: silage conservation, laboratory silos, forage residue after juice extraction

Introduction Sweet pearl millet [*Pennisetum glaucum* (L.) R.Br] and sweet sorghum [*Sorghum bicolor* (L.) Moench] have a high biomass yield in eastern Canada, are highly tolerant to drought and infertile soils, and are rich in readily fermentable sugars. They are considered as dual purpose crops because they can be used to produce both a sweet juice for ethanol production and a forage residue for animal feed. Our objective was to determine the effects of forage species and harvest date on nutritive value and conservation of silages made with forage residues obtained after juice extraction.

Material and methods The crops were seeded on 28 May 2010 at a first site (St-Augustin; 2300-2500 corn heat units, CHU) and on 11 June at another site (Ste-Anne; 2900-3100 CHU) in the province of Québec, Canada, on light soils with a N fertilization of 100 kg N ha^{-1}. The factorial arrangement of treatments was replicated three times in a split-plot design with species as main plots and harvest dates (mid-August and early September at St-Augustin; late August and mid-September at Ste-Anne) as subplots. Forages were cut using a corn harvester and the juice was extracted using a worm drive press. Forage residues were inoculated (1 g Mg^{-1}) or not with *Lactobacillus plantarum* (1×10^{11} CFU g^{-1}). Laboratory mini-silos were filled with pressed forage residues and sealed. Silage samples were taken after 90 days and analysed for nonstructural carbohydrates (NSC), total nitrogen (TN), ADF, and NDF concentrations, *in vitro* true dry matter (DM) digestibility (IVDMD), and *in vitro* digestibility of NDF (dNDF) according to Morin et al. (2011), and also for pH, ammonia nitrogen (NH_3-N) (Tremblay et al. 2001), lactate, and volatile fatty acid (VFA) concentrations (Dionex 2006). The IVDMD and dNDF were measured using a 48-h incubation with rumen fluid. For each site, data were analysed as a split-plot design using a mixed model of SAS with species as main plots and harvest dates as subplots.

Results and discussion Silages made from forage residues of both species were well preserved (pH<4.2; NH_3-N<82 g kg^{-1} TN; lactate>30 g kg^{-1} DM; VFA<20 g kg^{-1} DM; Table 1) with a similar residual NSC concentration of 80 g kg^{-1} DM. Silages made with sweet pearl millet forage residues had greater NH_3-N and lactate concentrations (81.0 g kg^{-1} TN; 39.8 g kg^{-1} DM) than those made with sweet sorghum forage residues (55.5 g kg^{-1} TN; 31.6 g kg^{-1} DM). Delayed harvesting caused an average increase in forage yield in both species of 7.5 Mg DM ha^{-1} at St-Augustin and 6.5 Mg DM ha^{-1} at Ste-Anne. Concentrations of NSC in silages were not affected by harvest dates, but ADF concentration was greater and IVDMD and dNDF were lower for the September harvest at both sites (Table 1). The inoculation with *Lactobacillus plantarum* did not affect silage conservation.

Harvesting the two forage species in September maximized NSC yields, whereas harvesting in August resulted in greater nutritive value of silages made with forage residues. Fibre concentrations of silages made with the pressed forage residues, although lower when the forage was harvested in August than in September, were nonetheless greater than those of silages produced from perennial forage grasses harvested at maturity (ADF>380 g kg^{-1} DM, NDF>650 g kg^{-1} DM; Tremblay et al. 2005) or from corn. Crude protein concentrations in silages made from sweet sorghum and sweet pearl millet forage residues (65 g kg^{-1} DM) were lower than that of silages made from grasses harvested at maturity (140 g kg^{-1} DM) and that of corn silage (90 g kg^{-1} DM; Tremblay et al. 2005).

ConclusionsSilages made with forage residues of sweet pearl millet and sweet sorghum were well preserved and did not differ in nutritive value but harvesting in August resulted in greater silage nutritive value than harvesting in September. The feeding value of these silages should be confirmed with beef and dairy cattle.

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Table 1. Nutritive value of silages made with forage residues obtained after juice extraction of sweet pearl millet and sweet sorghum harvested at two dates and two sites.

Main effects	Treatments	pH	Total N	ADF	NDF	IVDMD	dNDF	NH_3-N	Lactate	VFA	Acetate
			g kg^{-1} DM				g kg^{-1} NDF	g kg^{-1} TN	g kg^{-1} DM		
Site 1 (St-Augustin)											
Species	Pearl millet	4.0	10.5	492	754	691	590	79.6	42.9	13.9	13.5
	Sorghum	3.9	10.4	486	748	689	584	57.5	33.2	14.9	14.5
SEM‡		0.01	0.2	1.6	2.4	3.5	4.1	4.0	2.6	1.3	1.4
Harvest date	mid-August	3.9	10.7	475	755	704	608	75.6	43.3	11.2	10.8
	early September	4.0	10.3	502	747	676	565	61.5	32.8	17.6	17.2
SEM		0.01	0.2	1.9	2.4	3.5	4.1	3.9	2.4	1.3	1.4
Sources of variation	df						*P* value				
Species (S)	1	ns†	ns	0.019	ns	ns	ns	<0.001	<0.001	ns	ns
Harvest date (D)	1	0.003	ns	<0.001	ns	<0.001	<0.001	<0.001	<0.001	0.001	0.001
S × D	1	<0.001	ns	0.018	ns	ns	ns	ns	0.020	ns	ns
Inoculation	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Site 2 (Ste-Anne)											
Species	Pearl millet	4.0	10.4	492	753	688	586	82.3	36.7	12.3	11.9
	Sorghum	3.9	10.6	487	748	691	586	53.6	30.0	12.2	11.8
SEM		0.02	0.3	3.7	3.9	4.4	6.1	1.9	0.9	0.4	0.4
Harvest date	late August	3.9	10.7	476	755	704	608	74.2	38.5	10.5	10.1
	mid-September	4.0	10.3	503	746	675	564	61.6	28.2	14.0	13.6
SEM		0.02	0.3	4.6	4.9	5.0	6.1	1.9	0.9	0.4	0.4
Sources of variation	df						*P* value				
Species (S)	1	<0.001	ns	ns	ns	ns	ns	<0.001	<0.001	ns	ns
Harvest date (D)	1	<0.001	ns	<0.001	ns	<0.001	<0.001	<0.001	<0.001	0.001	0.001
S × D	1	<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns
Inoculation	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ADF = acid detergent fibre, NDF = neutral detergent fibre, IVDMD = *in vitro* true dry matter digestibility, dNDF = *in vitro* digestibility of NDF, VFA = total volatile fatty acids.
‡SEM, standard error of the mean (n = 6, df = 1). †ns, non significant, $P > 0.05$

Nutritional evaluation of winter cereal silages harvested at two stages of maturity and effect of inoculum with lactobacilli and fibrolytic enzymes on wheat silage

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Keywords: winter cereal silages, lactobacilli, fibrolytic enzymes, NDFD, gas production

Introduction In Italy cereals are mainly used for grain production. However, these species are getting popular as forage source used as winter crop before the seeding of corn. They have been reported to generally ensile well and to provide both high forage yield and quality. Several studies showed that delaying cereals harvesting past the flowering or heading stage determines greater forage DM yields but with a reduction of forage fibre digestibility. Due to limited information available on nutritive value of cereal silages in Italy, the aim of this study was to estimate the nutritive value of four winter cereal silages harvested at two different stages of maturity in the Po Plain (Italy) and, on one of these silages, to evaluate the effect of an inoculum containing lactobacilli and fibrolytic enzymes.

Material and methods The experiment was conducted at the experimental station of the Department of Animal Science. Fermentative profile, dry matter losses, chemical composition, 48 hours in vitro dry matter digestibility (DMD) and neutral detergent fibre digestibility (NDFD) (using the Daisy^{II} system, Ankom Technology Corp.), 8 and 24 hours in vitro Gas Production (GP) (Menke and Steingass 1988) were determined on the following whole crop silages: a milling soft wheat (*Triticum aestivum* var. Artico) (WA), a tall growing soft wheat (*Triticum aestivum* var. Sollario) (WS), a triticale (x *Triticosecale* var. Magistral) (TM), and a wheat-barley-triticale mixture (WesternTM) (MW). The forages were harvested at kernels watery ripe (early stage - E -) and at milk-dough stage (late stage - L -), and ensiled in 2 L micro-silos, in triplicate, for a 40 days period. Furthermore, on WA, we evaluated the effect of a commercial inoculum (Advance Whole Crop, Micron Bio-systems Ltd) containing lactobacilli ($1.05x10^5$ cfu *Pediococcus pentosaceus*, $2.0x10^5$ cfu *Lactobacillus brevis* and $0.25x10^5$ cfu *Lactobacillus plantarum,* per g fresh forage) and fibrolytic enzymes (13 IU ß-glucanase, 4 IU Cellulase, 44 IU Mannanase and 30 IU Xylanase, per kg fresh forage) (LE), in comparison with control (C).Data were analysed using the General Linear Model of the Statistical Analysis System (SAS Institute, Inc. 2001) in order to test the effects of harvest date and crop/inoculum, and their interactions.

Results and discussion All forages were well preserved as indicated by low pH (3.88, on average) and moderate-high lactic acid content (50 g/kg DM, on average), even with more proteolytic activity, expressed as N-NH_3 in percentage of total N, at the earlier stage (14.2 vs 9.1, P=0.002). As showed in table 1, dry matter (DM) and crude protein (CP) contents of the silages were affected by maturity stage but not by crop. CP was 25% higher for E than L stage. NDF content was influenced by maturity stage and crop. Acid detergent lignin, as percentage of NDF, was affected by maturity stage (19% higher for L than E stage).

Regarding the NDFD, a significant reduction was observed from E to L stage (50.5 vs 45.0%, P<0.001), whereas DMD was not affected due to the higher starch content at the milk-dough stage, as confirmed by the higher GP 8h value for this stage. However, GP values at 24h were similar due to the higher NDFD of the crops harvested at earlier stage.

Wheat var. Artico, inoculated with lactobacilli and fibrolytic enzyme mixture, showed a significant reduction in dry matter losses at 40 days of ensiling compared to C; this effect was registered at both stages of maturity (2.7 vs 6.4% at E stage and 2.8 vs 4.3% at L stage, for LE and C, respectively). The inoculum addition also showed (table 2) a reduction of fibre fractions and an increase of non-fibre carbohydrates (NFC) of the ensiled crop. LE treatment increased DMD, but not NDFD; it also determined a significant increase in GP 8h at E stage of maturity, probably due to the higher NFC content of LE treatment.

Conclusions Whole crop winter cereal silages harvested at early stage of maturity appear more suitable for high yielding cows due to their higher NDFD that can positively affect DM intake particularly at early stage of lactation. Inoculum addition reduces dry matter losses during the ensiling period, and seems to slightly reduce the fibre fractions.

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Table 1. Chemical analysis, in vitro dry matter digestibility (DMD) and in vitro neutral detergent fibre digestibility (NDFD), in vitro gas production (GP) and estimated nutritive value of whole crop cereal silages harvested at early (E) or late (L) stage of maturity.

Maturity stage (S)	E				L				SEM	P value		
Crop (C)	WA	WS	TM	MW	WA	WS	TM	MW		S	C	S*C
Chemical analysis												
DM (%)	23.7	25.6	30.8	26.2	35.0	31.4	34.4	34.5	1.68	***	NS	NS
CP (% DM)	8.10	8.55	8.50	8.20	6.83	6.95	6.50	6.50	0.13	***	NS	NS
NDF (% DM)	60.6^{ab}	56.4^{bcd}	64.0^{a}	58.4^{abc}	51.8^{d}	53.0^{cd}	59.9^{ab}	51.7^{d}	1.11	***	***	NS
ADF (% DM)	31.1^{bc}	30.3^{c}	33.8^{ab}	29.7^{c}	30.1^{c}	32.1^{abc}	35.4^{a}	31.2^{bc}	0.66	NS	***	NS
Lignin(sa) (% DM)	1.43	1.65	1.50	1.35	1.76	1.55	1.60	1.45	0.10	NS	NS	NS
Lignin (sa) (% NDF)	2.36	2.92	2.34	2.30	3.40	2.92	2.66	2.80	0.18	***	NS	NS
Starch (% DM)	0.06^{e}	0.30^{de}	0.01^{e}	5.25^{cde}	15.0^{a}	9.40^{bc}	5.80^{cd}	12.6^{ab}	1.03	***	***	***
NFC (% DM)	23.3^{cd}	28.1^{bc}	20.6^{d}	26.2^{cd}	34.9^{a}	33.7^{ab}	27.8^{c}	35.3^{a}	1.10	***	***	NS
In vitro 48 h digestibility (%)												
DMD	70.9^{ab}	76.0^{a}	65.3^{cd}	68.3^{bcd}	71.3^{ab}	73.0^{ab}	64.5^{d}	70.3^{bc}	1.08	NS	***	NS
NDFD	51.7^{b}	60.9^{a}	44.5^{bcd}	44.8^{bcd}	45.5^{bcd}	51.0^{bc}	40.5^{d}	43.1^{cd}	1.46	***	***	NS
In vitro Gas Production (mL/200 mg DM)												
GP 8h	17.8	18.4	17.7	19.3	22.5	21.5	20.3	22.5	1.13	***	NS	NS
GP 24h	40.8	44.8	42.8	39.8	42.2	39.1	39.1	40.2	2.12	NS	NS	NS
Estimated Nutritive Value (MJ/kg DM)												
NEL $_{3X}$ [1]	4.43^{bcd}	5.42^{a}	4.03^{cd}	4.17^{cd}	4.63^{bc}	4.87^{ab}	3.79^{d}	4.42^{bcd}	0.12	NS	***	*
NEL $_{GP}$ [2]	4.80	5.20	5.03	4.70	4.87	4.57	4.54	4.66	0.20	NS	NS	NS

[a, b, c] Tukey test was conducted if P<0.05 for crop effect. Different letters in the same row differ significantly (P<0.05).
[1]: NEL $_{3X}$: Net Energy of Lactation calculated according to NRC (2001).
[2]: NEL $_{GP}$: Net Energy of Lactation calculated according to Menke and Steingass (1988), based on GP values

Table 2. Chemical analysis, in vitro dry matter digestibility (DMD) and in vitro neutral detergent fibre digestibility (NDFD), in vitro GP and estimated nutritive value of wheat silage (var. Artico) harvested at early (E) or late (L) stage of maturity and treated with (LE) or without (C) an inoculant.

Maturity stage (S)	E		L		SEM	P value		
Treatment (T)	C	LE	C	LE		S	T	S*T
Chemical analysis								
DM (%)	23.7	25.3	35.0	35.7	1.53	***	NS	NS
CP (% DM)	8.10	8.47	6.83	6.90	0.17	***	NS	NS
NDF (% DM)	60.6	57.4	51.8	50.2	0.67	***	***	NS
ADF (% DM)	31.1	29.1	30.1	29.1	0.49	NS	*	NS
Lignin (% DM)	1.43	1.17	1.76	1.57	0.09	***	*	NS
Lignin (% NDF)	2.36	2.03	3.40	3.11	0.16	***	NS	NS
Starch (% DM)	0.06	0.20	15.0	14.1	0.35	***	NS	NS
NFC (% DM)	23.3	26.1	34.9	36.9	0.65	***	***	NS
In vitro 48 h digestibility (%)								
DMD	70.9	73.4	71.3	71.9	0.44	NS	*	NS
NDFD	51.7	53.4	45.5	44.0	1.56	***	NS	NS
In vitro Gas Production (mL/200 mg DM)								
GP 8h	17.8^{b}	23.2^{a}	22.5^{a}	24.7^{a}	0.64	***	***	*
GP 24h	40.8	46.4	42.2	43.4	2.17	NS	NS	NS
Estimated Nutritive Value (MJ/kg DM)								
NEL $_{3X}$	4.43^{b}	4.90^{a}	4.63^{ab}	4.70^{ab}	0.07	NS	***	*
NEL $_{GP}$	4.80	5.39	4.87	4.99	0.21	NS	NS	NS

[a, b] Tukey test was conducted if P<0.05 for interaction of main effects. Different letters in the same row differ significantly (P<0.05).
[1]: NEL $_{3X}$: Net Energy of Lactation calculated according to NRC (2001).
[2]: NEL $_{GP}$: Net Energy of Lactation calculated according to Menke and Steingass (1988), based on GP values

Nutritive characteristics of sorghum grain silage (whole or cracked) using *in vitro* gas production technique

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Keywords: *in vitro* gas production, sorghum grain, starch, volatile fatty acids

Introduction Sorghum grain (*Sorghum bicolor L. Moench*) is rich in starch and used as a source of energy for industry in ruminant feed. The aim of the present study was to compare whole or cracked sorghum grain treated as silage.

Material and methods Four treatments grain were used: cracked silage sorghum (CSS), dried whole sorghum (DWS), silage whole sorghum (SWS), dry cracked sorghum (DCS), crushed to 3 mm diameter. Sorghum silage was made by adding water to the sorghum until it reached 65 % dry matter (DM). Samples were ensiled for 21 days in CSS and 42 days in SWS. The *in vitro* gas production technique (Theodorou et al. 1994) was used to determine the kinetics of ruminal fermentation, 800 mg DM samples were incubated and gas production was recorded at 3, 6, 9, 12, 18 and 24 h post incubation At the end of the incubation period cumulative *in vitro* gas production (ml gas/ g DM), pH, N-NH_3, VFA, CH_4 (Wolin, 1960), dry matter disappearance (DMd, mg /100 mg) of the residue and relative gas production (RGP, ml gas /g DMd) were determined. The gas production data were fitted to the model proposed by France et al. (1993), $PG = a \{1–e -b^{(tT)} - c (\sqrt{t-T})\}$, where: PG, cumulative gas production (ml gas/g DM), A, asymptote of the curve (total gas production, mL), b (h^{-1}) c ($h^{-1/2}$), gas production constant and time delay before the start of fermentation (T, h). Completely randomized design was used, which includes cereal type (n = 4) and replication (3 rounds of incubation). A comparison between conservation method (dry vs silage) and particle size (whole vs cracked) was performed.

Results and discussion CP content was lower for CSS and DCS compared with DWS and SWS (Table 1). The starch content was highest (P<0.01) in CSS and SWS. Total gas production (ml gas / g DM) was highest (P<0.01) for CSS, while SWS had the lowest value. The DMd was highest (P<0.01) for DWS and DCS compared with the rest of the treatments. The RGP was highest (P<0.01) for CSS and lowest for DCS. (Dry vs Silage) The *in vitro* pH of the silages (Table 2) there were not differences between treatments, whereas, the highest N-NH_3 (P<0.01) was in the SWS compared with the rest of the treatments. For the production of VFA, acetic acid was lowest (P< 0.01) in CSS and SWS, while DCS has the highest value, the proportion of propionic acid was highest for SWS and CSS (P<0.01) while DCS have the lowest value. Methane production was lower (P<0.01) in SWS and CSS. Acetic acid concentration was higher (P<0.01) in dry sorghum vs silage sorghum. When cereals are compared whole vs cracked, propionic acid and the ratio acetic / propionic acid was higher (P<0.01) for silage sorghum, methane concentration was higher (P<0.01) for dry sorghum vs silage sorghum.

ConclusionsReconstitution of sorghum grain silage either whole or broken decrease *in vitro* digestibility, acetic acid and methane production, compared with untreated sorghum. Data demonstrated that sorghum grain silage improve rumen starch availability increasing propionic acid and reducing the methane production as a consequence of a lower DM digestibility.

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Table 1. Chemical composition (g/kg DM) and *in vitro* gas production (ml gas/g DM) parameters of sorghum grains for the different treatments.

Item	DM	OM	CP	Starch	*In vitro Gas production*					
					A	b	*c*	*Lag time*	*DMd*	*RGP*
DWS	977^{d}	985	102^{e}	744^{fg}	106^{g}	0.015^{f}	-0.022^{e}	1.69^{d}	77^{f}	138^{f}
DCS	992^{d}	986	98^{e}	742^{ef}	95^{d}	0.018^{e}	-0.031^{f}	0.74^{e}	80^{f}	119^{g}
SWS	658^{e}	978	111^{d}	752^{e}	89^{e}	0.021^{d}	-0.038^{d}	1.42^{d}	68^{d}	130^{d}
CSS	646^{e}	977	98^{e}	758^{d}	111^{f}	0.015^{f}	-0.023^{e}	0.74^{e}	61^{d}	182^{e}
SEM	0.28	0.02	1.05	0.23	0.23	0.002	0.002	0.07	0.40	0.70
P-value	0.01	0.13	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
D vs S	0.01	0.43	0.07	0.01	0.01	0.38	0.28	0.65	0.01	0.01
W vs C	0.97	0.43	0.01	0.01	0.01	0.34	0.49	0.01	0.04	0.46

WDS: Whole Dried Sorghum, DCS: Dry Cracked Sorghum, WSS: Whole Silage Sorghum, CSS: Cracked Silage Sorghum. D: Dry grain sorghum, S: Silage grain sorghum, W: whole grain sorghum, C. cracked grain sorghum, A: total gas production (ml gas/g DM incubated), b: fermentation rate (h^{-1}), c: fermentation rate ($h^{-1/2}$), lag time (h), DMd_{24h}: DMd disappeared at 24h (mg/100 mg), RGP: (ml gas 24h/g DMd_{24h}). SEM, standard error mean. ^{defg} Different letters between rows differ significantly P<0.01.

Table 2. *In vitro* pH, N-NH_3, volatile fatty acids (VFA) and methane concentration (mol/100mol) in sorghum grains for the different treatments.

	Dry grain		Silage grain			*P-value*		
	WDS	DCS	WSS	SCS	SEM	*Tx*	*D vs S*	*W vs C*
pH	7.0	7.0	7.0	7.0	0.80	0.22	0.07	0.06
N-NH_3 (mg/dl)	11.0^{bc}	9.7^{d}	12.3^{a}	10.0^{cd}	0.21	0.01	0.06	0.44
VFA (mmol/100mol)								
Acetic (A)	58.8^{b}	61.9^{a}	54.9^{bc}	53.3^{c}	0.42	0.01	0.01	0.05
Propionic (P)	33.5^{b}	28.5^{a}	36.8^{c}	36.4^{c}	0.23	0.01	0.06	0.01
Butyric	7.7^{c}	9.6^{abc}	8.2^{bc}	10.3^{a}	0.32	0.01	0.88	0.01
A/P	1.5^{c}	2.2^{a}	1.8^{b}	1.9^{b}	0.04	0.01	0.03	0.01
Methane	0.5^{c}	0.5^{a}	0.4^{b}	0.4^{b}	0.05	0.01	0.02	0.01

SCS: Silage Cracked Sorghum, WDS: Whole Dried Sorghum, WSS: Whole Silage Sorghum, DCS: Dry Cracked Sorghum. D: Dry grain sorghum, S: Silage grain sorghum, W: whole grain sorghum, C. cracked grain sorghum SEM, standard error mean. ^{abcd} Different letters between rows differ significantly P<0.05.

Prediction of sugarcane feed value by stepwise regression

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Keywords: acid detergent fiber, agronomic traits, digestibility, soluble carbohydrates

Introduction Sugarcane is a useful crop of great importance to Brazilian agribusiness. Despite of high biomass productivity, this roughage source presents a high digestibility compared to other tropical forages. Basically, sugarcane is composed of two principal fractions: a) soluble carbohydrates - SC (mainly sucrose) and b) insoluble fiber (neutral detergent fiber - NDF). In early 80´s, Gooding (1982) proposed a functional relationship between NDF and SC as criteria to select varieties to animal feeding purposes. This ratio is widely used in Brazil, but this parameter can be unreliable due to high variation related to both chemical entities.

Stepwise regression has been used as a tool to predict digestibility in other crops (ex. corn, alfalfa). The objective of this study was to establish the main variables (agronomic and chemical traits) involved in the predictions of: a) in vitro organic matter digestibility (IVOMD) and b) digestible dry matter yield (DDMY).

Material and methods Whole plant samples of IAC 93 3046 variety (n = 480) were collected manually from 12 field plots in Piracicaba, SP, Brazil (22°43'S, 47°25'W) during crop harvesting period in 2009 (1st cut): 300, 360, 420 and 540 days after planting. Each experimental plot (150 m^2) consisted of seven rows of 5 m long each spaced 140 cm inter row. Ten plants were randomly harvested, weighed, and fractionated to determine plant agronomic traits. Crop ripening index (RI) was considered the relationship between top vs. base BRIX of stalk (% juice soluble solids). Fresh samples were chopped in a stationary chopper, dried in a forced air oven at 60^{o}C and ground at Wiley mill, with a 1 mm sieve.

The chemical composition and IVOMD of samples were determined by near infrared reflectance spectroscopy (NIRS). Data set of digestibility from NIRS equations were provided from *in vitro* determinations according to Goering and Van Soest (1970). Soluble carbohydrates (SC) were determined by colorimetry after ethanol extraction (Hall, 2000). Associations between agronomic and chemical traits were established through stepwise multiple regressions using the REG procedure of SAS. Models fitting were indicated by R^2 and RMSE (root of mean square error).

Results and discussion In general, the models developed to predict IVOMD fitted better than those used to predict DDMY, as noticed by the higher R^2, despite of higher RMSE values (Table 1). The first independent variable (step 1) considered in the model to predict IVOMD was acid detergent fiber (ADF, R^2 = 0.81, RMSE = 16.28), whereas SC was the best single predictor of DDMY (R^2 = 0.49, RMSE = 3.73). Agronomic traits had more relevance for DDMY than for IVOMD prediction. The number of nodes (NN), stalk weight (SW), and number of plants per 1 m^2 (NPM) were the most important agronomic variables considered by regression models.

The DDMY responded to the dynamics of sugar accumulation and stalk elongation as reported in other studies (Mamet & Galwey, 1999). In this study, fiber quality indicated by ADF seems to be more important than NDF:SC ratio, traditionally adopted as digestibility predictor for selection of sugarcane varieties (Gooding, 1982). However, the summary of stepwise selection showed that NDF was the fourth important prediction trait in both cases (IVOMD and DDMY).

Conclusions Both chemical and agronomical traits are important in the prediction of nutritional value in sugarcane crop. Acid detergent fiber as a single variable was the best predictor of sugarcane nutritive value.

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Table 1. Best regression models developed by stepwise regression from chemical composition and agronomic traits of sugarcane whole plant in predicting in vitro digestibility and digestible dry matter yield.

Factor	Regression model	R^2	RMSE[1]
Organic matter digestibility[2]			
1	IVOMD = 1186.96 – 1.66(ADF)	0.81	16.28
2	IVOMD = 1263.21 – 1.81(ADF) – 1.24(NN)	0.85	14.61
3	IVOMD = 1435.48 – 10.26(CP) – 1.48(ADF) – 1.68(NN)	0.89	12.36
Digestible dry matter yield[3]			
1	DDMY = 14.57+ 0.04(SC)	0.49	3.73
2	DDMY = 10.61 + 0.03(SC) + 16.86(SW)	0.62	3.25
3	DDMY = -9.91 + 0.02(SC) + 1.24(NPM) – 41.78(SW)	0.77	2.50

[1]Root mean square error, [2]IVOMD: in vitro organic matter digestibility (g/kg), ADF: acid detergent fiber (g/kg), NN: number of nodes, CP: crude protein (g/kg), [3]DDMY: digestible dry matter yield (ton/ha), SC: ethanol soluble carbohydrates (g/kg), SW: stalk weight (g), NMP: number of plants per 1 m^2

Influence of waste dates on the *in vitro* ruminal gas production of banana tree by-product silage in cows

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Key words: banana, refusal dates, gas production, ruminant

Introduction Bananas (Musaceae) are produced in large quantities in tropical and subtropical areas. The total planted area of banana in south of Iran (2008) was 5678 hectares (Jehad keshavarzi Organization of Sistan and Blochestan Province, 2009). Banana (*Musa balbisiana colla)* is a traditional plant cultivated widely for its fruits. Banana plants range in height from 0.8m to more than 15m. Each contains a flattened, modified stem, called a pseudostem consisting of concentric layers of leaf sheath and crown of large leaves (Ennos et al. 2000). After harvesting of the fruit, the various other parts of the plant (by-products) are not effectively utilized. It has been estimated that a residual biomass (pseudostem and leaves) of 13-20 tonnes dry matter ha^{-1} $year^{-1}$ is available (Amarnath and Balakrishnan 2007). Ensiling offers many advantages over haymaking. Large quantities of forage can be conserved in a short time, forage conservation is less weather dependent and thirdly, silage is well suited to mechanization. This experiment was carried out to evaluate impact waste dates (WD) on the nutritive value of banana by-products (BP - *Musa balbisiana)* silage, of tree leaves and stems, using the *in vitro* gas production method in cattle.

Material and methods Banana by-product were obtained from banana farms in south of Iran (Chahbahar region). Rumen liquor was taken from two ruminal fistulated male cows before the morning feeding. BP was ensiled for two months without (control) or with 15% of waste dates. After the ensiled period (*i.e*. 2 months), silages were opened for the immediately pH determination as well as for some apparent characteristics of silage quality. Silages chemical compositions, organic matter digestibility and metabolisable energy were also estimated by gas production techniques. Representative samples were dried at 48 ^{0}C in oven for 72 hours. After drying, silage samples were ground through a 1-mm screen for chemical analysis. The dry matter (DM), ash, crude protein (CP), ether extract (EE) by AOAC (1990) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) by Van Soest et al. (1991) were determined. The samples (milled through a 1-mm sieve) were incubated in vitro rumen fluid in calibrated glass syringes following the procedure of Menke and Steingass (1988). Approximately 200 mg dry matter weight of sample was weighted in triplicate into calibrated glass syringes of 100 ml. Total gas values corrected for blank incubation. Cumulative gas production data were fitted to the modified model of Ørskov and McDonald (1979) ($y = b(1-e^{-ct})$).One-way analysis of variance (ANOVA) was carried out to compare chemical composition, gas production, gas production parameters using SAS software (SAS, 2002).

Results and discussion Chemical compositions of banana by-products (i.e., BP) silage with and without WD are presented in Table 1. The addition of WD improved silage quality by decreasing neutral detergent fiber ($p<0.05$) and increasing water soluble carbohydrates ($p<0.05$). Also, adding of WD decreased pH in BP silage and improved nutritional quality of silage. The low pH may be caused by the fermentation of high sugar content of WD (McDonald et al. 1991). It is known that the quality of silage can change depending upon the type of additive (Filya 2001). Addition of WD increased DM content which could be related to the high DM content of the WD used. Gas production volume, organic matter digestibility and metabolisable energy increased 55.10, 33.29 and 43.82%, respectively in WD compared with control. This may be caused by high sugar content of WD leading to a rapid production of alcohol as well as of VFA's and lactic acid (Leroy and Zelter 1954).

Conclusions There are large quantities of banana crop residues available in Iran with substantial potential for contributing to its livestock industry. Even though this by-product alone is a poor quality feed, it becomes extremely important for maintenance purposes during the dry season when little or no grass is available.

Overall, results of chemical compositions and *in vitro* gas production revealed that 15% WD was useful for ensiling of banana by-products and could be consider for ruminants feeding. From this experiment it was concluded that banana crop residues are likely to ferment successfully when a fermentable carbohydrate source such as waste date or molasses is added.

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Table1. The effect of adding of waste date (WD) on chemical composition of banana by-products silage.

Silage	DM	OM	ash	CP	EE	ADF	NDF	WSC	pH
Without WD	11.1[b]	81.5[b]	18.5[a]	6.1[a]	2.4[a]	39.0[a]	55.6[a]	4.4[b]	7.5a
With WD	20.4[a]	82.4[a]	17.6[b]	5.0[b]	2.4[a]	29.8[b]	44.5[b]	27.3[a]	4.6b
SEM	0.223	0.257	0.257	0.230	0.125	0.276	0.294	0.217	0.058

Mean of three observation, Means with different superscript within same column significantly differ (P<0.05), DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSC, water soluble carbohydrates, SEM, standard error mean.

Table 2. The effect of adding of waste date (WD) on *in vitro* gas production and estimated parameters of banana by-products silage.

	Incubation time					Estimated gas production			
Silage	12	24	48	72	96	b	c	OMD	ME
Without WD	16.1[b]	28.7[b]	36.4[b]	39.2[b]	40.9[b]	42.7[b]	0.046[b]	55.2[b]	6.5[b]
With WD	39.6[a]	50.6[a]	58.1[a]	61.2[a]	63.5[a]	57.4[a]	0.088[a]	73.6[a]	9.4[a]
SEM	0.46	0.67	0.88	0. 08	0.87	0.79	0.001	0.68	0.10
% increase	145.76	76.11	59.73	56.30	55.09	34.37	91.30	33.29	43.82

Mean of three observation, Means with different superscript within same column significantly differ (P<0.05).

Effect of molasses and polyethylene glycol on dry matter degradability of pistachio by products silage in cows

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Key words: pistachio by-product, silage, tannins, PEG, molasses, ruminant

Introduction Pistachio (*Pistachio vera*) is from Anacardiaceae family and according to the FAO (2003) report, Iran is the largest pistachio producer in the world (more than 310,000 tons). About 150,000 tons in DM of pistachio by-product (PB) is produced from dehulling process in Iran, annually. This by-product is mainly consisted of pistachio hulls, and then peduncles, leaves and a little amount of mesocarp and kernels. The results of some studies found that this by-product could be used as a feedstuff for ruminants (Shakeri and Fazaeli 2005; Vahmanet al. 2005). However, PB contains a high level of tannins (Seied Moemen 2003) and several methods have been used previously to deactivate tannins and improved its nutritional value (Makkar 2003), such as anaerobic storage (silage), drying and/or addition of polyethylene glycol (PEG).The aim of this study was to determine the effect of molasses or PEG addition during ensiling process on the chemical composition, phenolic compound, *in situ* dry matter degradability (DMD) of pistachio by-product (PB) silage and to improve nutritional quality of PB silage.

Material and methods Fresh PB, which contains hulls, twigs, leaves, shells and green kernels, was collected from the 20 samples of PB were collected from pistachio farms in khorasan razavi (Bardaskan) in Iran. Treatments were PB without additive (control), PB with 5% molasses and PB with 9% PEG. After the ensiled period (i.e., 2 months), silages were opened and samples were taken for evaluations. Ensiled samples were freeze-dried, ground to pass a 1 mm sieve and stored at −20 ◦C. The freeze-dried, ensiled PB samples were analyzed for dry matter (DM), ash, crude protein (CP) (AOAC 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined according to Van Soest et al. (1991). Silage pH was determined on expressed juice obtained by thorough mixing of 50 g of fresh silage with 125 ml of distilled water and allowing standing at 25 ◦C for 1 h. After decanting the silage extract into a small beaker, the pH was measured using a portable digital pH meter. Five grams of each silage treatments were incubated in nylon bags (10×21cm – in duplicated) and incubated in the rumen of fistulated native cows (two ruminal fistulated cows) for 3, 6, 12, 24, 48, 72 and 96 h (Ørskov and McDonald 1979). Neway program was used to determine the DMD fractions (a, b, c and ED). The data was analyzed using completely randomized design by using the procedure of SAS (2002).

Results and discussion Ensiling with molasses and PEG decreased ($p<0.05$) total tannin, hydrolysable tannins and condened tannin, as well as crude protein and neutral detergent fiber contents. The highest *in situ* DMD value (at 96h) and fraction (*a*) was in PB treated with molasses. Potential degradability (*a* + *b*) and effective degradability were higher ($p<0.05$) in PB with PEG. Addition of PEG led also to a larger decrease in phenolic compounds than addition of molasses. Results of this study were similar to finding of Yousef Elahi and Rouzbehan (2010) that the addition of PEG improved OMD and ME of oak leaves.

Conclusions This study illustrates that detanification of PB can improve its nutritive value. Overall, PB with PEG silage was the best treatment in improving the nutritive value of PB. Also, molasses can decrease phenolic compounds in tannin- containing forages. The improvement in DM degradability with PEG emphasizes the negative effect of tannins on digestibility.

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Table 1. The effect of molasses or polyethylene glycol (PEG) addition on chemical composition of pistachio by-products (PB) silage.

Silage	DM	OM	ash	CP	ADF	NDF	pH	TT	HT	CT
Control	26.1^{b}	88.7	11.3	11.0^{a}	21.4^{b}	32.8^{a}	4.7^{a}	4.53^{a}	3.90^{a}	0.43^{a}
Molasses	28.6^{a}	88.6	11.3	10.8^{a}	18.6^{c}	32.7^{a}	4.0^{b}	4.29^{b}	3.63^{b}	0.34^{b}
PEG	26.1^{b}	89.5	10.4	9.06^{b}	23.3^{a}	30.7^{b}	4.5^{a}	3.96^{c}	3.22^{c}	0.32^{b}
SEM	0.57	0.34	0.34	0.17	0.45	0.46	0.02	0.31	0.096	0.015

Mean of three observation, Means with different superscript within same column significantly differ (P<0.05), DM, dry matter; OM, organic matter; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; TT, total tannin; HT, hydrolysable tannin; CT, condensed tannin; standard error mean..

Table 2. The effect of molasses or polyethylene glycol (PEG) addition on DM degradability and estimated parameters of pistachio by-products (PB) silage in cows.

	Incubation time					Estimated parameters				
Silage	12	24	48	72	96	a	b	a + b	c	ED
Control	63.7^{b}	76.6^{c}	83.1^{b}	83.2^{b}	86.0^{b}	44.1^{b}	40.6^{a}	84.7^{b}	0.034^{b}	60.5^{b}
Molasses	69.3^{a}	79.7^{b}	83.2^{b}	85.2^{a}	87.8^{a}	59.9^{a}	27.4^{c}	87.3^{a}	0.038^{b}	71.8^{a}
PEG	68.8^{a}	81.0^{a}	84.0^{a}	85.5^{a}	87.7^{a}	57.1^{a}	30.4^{b}	87.5^{a}	0.051^{a}	72.5^{a}
SEM	0.93	0.92	0.46	0.59	0.68	2.02	1.78	1.29	0.001	1.04

Mean of three observation, Means with different superscript within same column significantly differ (P<0.05); a water soluble fraction (%); b, insoluble but fermentable fraction (%); c, the degradation rate of b (/h); a +b, the potential degradability (%); ED the effective degradability of DM calculated for an outflow rate of 0.05/h (%).

Chemical composition and digestibility of ensiled pistachio by-products

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Keywords: ensilage, nutritive value, phenolics, pistachio by-product.

Introduction De-hulling of pistachio soon after harvest produces up to 400,000 tonnes/year of pistachio by-products (PB) in Iran. The crude protein (CP) content of PB varies from 92 to 120 g/kg on a dry matter (DM) basis, NDF from 300 to 360 g/kg DM and the DM degradability of PB was 463 g/kg (Forough Amery and Fazaeli 2005). Storage of this by-product is difficult due to its high moisture content of 700 g/kg. Ensiling is one method of preserving PB. PB contains a high level of tannins (Seied Moemen et al. 2003) which can have an adverse effect on nutrient utilization and can be toxic at high intake levels (Reed 1995). Several methods have been used to deactivate tannins to improve their nutritional value such as ensilaging. Ben Salem et al. (2005) noted that anaerobic storage of shrubs decreased the concentration of phenolic compounds. The present study was conducted to determine chemical composition, phenolic compounds and digestibility of PB ensiled for 30 and 60 days.

Material and methods Fresh PB containing hulls, twigs, leaves, shells and green kernels was collected from 20 pistachio gardens in Rafsanjan (Iran), and directly two samples of these byproducts were ensiled for 30 and 60 days in five polyethylene bags into 3 l capacity plastic containers with tight lids. The samples received no mechanical or additive treatment prior to ensilage. Ensiled samples were freeze-dried, ground to pass a 1mm sieve and stored at −20 °C. Samples were analyzed for DM, ash, ash-free acid detergent fiber (ADFom) and N (AOAC, 1990). Ash-free neutral detergent fiber (NDFom) was determined according to Van Soest et al. (1991). Lignin(sa) was determined by solubilization of ADF with sulphuric acid (Robertson and Van Soest 1981). Silage pH was measured using the digital pH meter. Water soluble carbohydrates (WSC) were measured using the anthrone method (MAFF, 1982). Total phenolics (TP) were measured using the Folin Ciocalteau method. Total tannin (TT) was determined after adding insoluble PVPP and reacting with Folin Ciocalteau reagent. The CT standard was separated from non-tannin phenolics using Sephadex LH20 and soluble condensed tannins (SCT) were extracted and measured. Hydrolysable tannins (HT) were analysed using the Rhodanine assay, and results are expressed as gallotannin. Protein-precipitable phenolics (PPP) were determined according to Makkar (2000). All phenolics were determined using spectrophotometric method. For ensiled samples, *in vitro* digestibility and ME were determined (Tilley and Terry, 1963). Obtained data were subjected to analysis using the GLM procedure of SAS, based on the statistical model $Y_{ij} = \mu + T_i + e_{ij}$ where Y_{ij} is the observation, μ the general mean, T_i the ith effect of treatment and e_{ij} the error term. Means were compared using t-test.

Results and discussion The CP content of ensiled PB was more than 80 g/kg DM (Table 1) which, according to Norton (1998), should provide ruminal ammonia levels above the minimum required by rumen microorganism to support optimum growth. The increasing time of ensiling decreased the content of the NDFom and ADFom and it may be due to the hydrolysis of a small amount of hemicellulose and/or cellulose during ensiling. The concentration of WSC at 60-d ensiling was lower than that at 30-d ensiling. This was probably due to the consumption of WSC during the fermentation process (McDonald et al., 1991). The TP content of PB was relatively high. The reduction in the levels of CT and HT with increasing time of ensilage may be due to oxidation of tannins in PB (Ben Salem et al., 2005). Ensiling for 60 day compared to 30 day had no effect on in vitro digestibility of PB.

Conclusions The CP concentration and OM digestibility of ensiled PB suggest that this by-product has a potential as livestock feedstuff. Increasing time of ensilage decreased concentrations of condensed and hydrolysable tannins, without negative effect on digestibility.

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Table 1. Chemical composition, phenolics compounds (g/kg DM) and digestibility of freeze-dried and ensiled pistachio by-products.

	Freeze-dried (n=3)	Silage		SED	Probability
		30-d ensiling	60-d ensiling		
Chemical composition					
DM	338	323	315	2.82	*
OM	889	883	881	1.65	NS
CP	91.6	92.1	92.9	1.72	NS
NDFom	321.3	311	272	1.21	*
ADFom	210	207	184	0.98	*
Lignin(sa)	56.6	57.5	58.3	0.34	NS
WSC	30.4	28.3	18.3	0.84	*
pH	5.2	4.9	4.6	0.09	NS
Phenolics					
TP	142	141	133	2.32	NS
TT	97.1	94.1	93.0	1.21	NS
CT	9.14	8.91	6.16	0.61	*
HT	83.4	65.1	55.0	0.92	*
PPP	54.2	34.9	22.2	0.76	*
Digestibility					
DMD	536	518	512	2.86	NS
OMD	512	489	484	2.46	NS
ME	7.15	6.91	6.81	0.35	NS

DM, dry matter (g/kg fresh weight); OM, organic matter; CP, crude protein; NDFom, neutral detergent fiber; ADFom, acid detergent fiber; lignin(sa), lignin determined by solubilization of cellulose with sulphuric acid; WSC, water soluble carbohydrates; SED, standard error of difference; TP, total phenols; TT, total tannins as tannic acid equivalents; CT, condensed tannins; HT, Hydrolysable tannins; PPP, protein-precipitable phenolics; DMD, DM digestibility (g/kg DM); OMD, OM digestibility (g/kg); ME, metabolisable energy (MJ/kg DM).

The mixed silage nutrient composition of maize and *Astragalus adsurgens* Pall.

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Keywords: *Astragalus adsurgens* Pall., maize (Keduo 8), mixed silage

Introduction *Astragalus adsurgens* Pall. is one of the important forage resources of legume which is distributed in China, Mongolia, Korea, Russia and Japan. However, it is nutritionally deficient in methionine that is an essential amino acid for animal nutrition and must be acquired from the food supply, see Bai et al*.* (2009). The purpose of this study was to understand the nutritive value of the mixed silage of maize and *Astragalus adsurgens* Pall.

Material and methods The research was performed at Linxi (43.62 N, 118.02 E; altitude, 900 m) located in Inner Mongolia of China. Keduo 8 was harvested at kernel milky maturity stage and *Astragalus adsurgens* Pall. was harvested after three growing years at the bud stage. Maize and *Astragalus adsurgens* Pall. were cut into 2~3 cm particles by a straw chopper, then all materials were mixed in the ratio as followed: single maize (Keduo 8) silage; 2/3 maize and 1/3 *Astragalus adsurgens* Pall. mixed silage; 1/2 maize and 1/2 *Astragalus adsurgens* Pall. mixed silage; 1/3 maize and 2/3 *Astragalus adsurgens* Pall. mixed silage; and single *Astragalus adsurgens* Pall. silage. The mixed material was ensiled in plastic bags (20×28 cm) without additives. Silage samples were analyzed using standard procedures of AOAC (2000): DM (920.36) after drying at 105 ºC for 24 h, ash (923.03) by igniting at 550 ºC for 3 h in a muffle furnace. Neutral detergent fiber (NDF) was analyzed by a method of Van Soest et al. (1991). Acid detergent fiber (ADF) was analyzed sequentially on the same samples according to the method of AOAC (2000). Crude protein (CP) was determined by a Rapid N cube Analyzer (Germany, Eementar company).Water soluble carbohydrates (WSC) was determined using the anthrone method, see Owens (1999). Amino acid was determined by a High Speed Amino Acid Analyzer (Hitachi 835-50). The data were processed by Microsoft Office Excel 2003 and analyzed with Tukey test of ANOVA by SAS.

Results and discussion Mixed silage with increasing proportions of *Astragalus adsurgens* Pall. showed an increasing trend for CP. The CP content's silage treatments of maize single silage and 2/3 maize and 1/3 *Astragalus adsurgens* Pall. mixed silage were significantly lower than others (P<0.05). With increasing proportions of *Astragalus adsurgens* Pall., NDF showed a decreasing trend, which suggested that the mixed silage could decrease the fiber content of feeds and increase digestibility (Table 1). Amino acids showed an increasing trend, except for leucine content of maize silage, and mixed silage of 2/3 maize and 1/3 *Astragalus adsurgens* Pall. was lower than other amino acid (P<0.05). *Astragalus adsurgens* Pall. silage's amino acid was the highest, which indicated the mixed silage would increase the amino acid content of maize silage. The 8 kinds of amino acid content and the total amino acid content of the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. were higher than that of other two (2/3 maize and 1/3 *Astragalus adsurgens* Pall. mixed silage and1/3 maize and 2/3 *Astragalus adsurgens* Pall. mixed silage) mixed silage treatments. The methionine content of single maize silage was up to 0.0443% (Table 2), higher than other treatments while that of single *Astragalus adsurgens* Pall. silage was 0.0197% and significantly different from other treatments (P<0.05), suggesting that methionine in *Astragalus adsurgens* Pall. was scarce, mixed silage with maize could increase its methionine content.

Conclusions Mixing of *Astragalus adsurgens* Pall. and maize can improve silage nutritive value. 1/2 maize and 1/2 *Astragalus adsurgens* Pall. was the ideal mixed silage treatment.

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Table 1. pH and nutrient composition of maize and *Astragalus adsurgens* Pall. in mix ed silage.

Treatment	DM[1] %	Ash	CP[2]	WSC[3]	ADF[4]	NDF[5]
		DM %				
Maize	25.07[a6]	7.16[a]	7.28[a]	3.82[c]	30.26[a]	54.23[c]
2/3 Maize +1/3 *Astragalus adsurgens* Pall.	26.08[a]	6.12[a]	10.72[b]	2.32[b]	28.67[a]	50.33[c]
1/2 Maize +1/2 *Astragalus adsurgens* Pall.	27.37[ab]	7.05[a]	14.69[c]	2.31[b]	25.93[a]	41.92[bc]
1/3 Maize +2/3 *Astragalus adsurgens* Pall.	27.65[b]	7.64[a]	14.86[c]	2.28[b]	25.88[a]	41.93[bc]
Astragalus adsurgens Pall.	30.48[c]	8.10[b]	15.03[c]	1.87[a]	23.98[a]	32.51[a]

[1]DM = Dry matter [2]CP = Crude Protein [3]WSC = Water-soluble Carbohydrates [4]ADF = Acid Detergent Fiber [5]NDF = Neutral Detergent Fiber [6]a b c Means in the same column with different superscript letters differ significantly ($P < 0.05$).

Table 2. Amino acid content of maize and *Astragalus adsurgens* Pall. in mixed silage.

Treatment	LYS[1]	TRY[2]	PHE[3]	MET[4]	THR[5]	ILE[6]	LEU[7]	VAL[8]
	DM%							
Maize	0.0887[a9]	0.0233[a]	0.0898[a]	0.0443[b]	0.0739[a]	0.0976[a]	0.1498[a]	0.1351[a]
2/3 Maize +1/3 *Astragalus adsurgens* Pall.	0.0827[a]	0.0199[b]	0.0866[b]	0.0294[b]	0.0536[b]	0.0865[b]	0.1280[a]	0.0997[b]
1/2 Maize +1/2 *Astragalus adsurgens* Pall.	0.1395[b]	0.0695[c]	0.1863[c]	0.0351[b]	0.1471[c]	0.1888[cd]	0.2977[a]	0.2193[c]
1/3 Maize +2/3 *Astragalus adsurgens* Pall.	0.1287[c]	0.0619[c]	0.1517[c]	0.0254[b]	0.1182[c]	0.1470[c]	0.1300[a]	0.1791[c]
Astragalus adsurgens Pall.	0.1753[d]	0.0810[d]	0.1648[c]	0.0197[a]	0.1345[d]	0.1619[d]	0.2536[a]	0.1928[c]
s.e.m.	0.01	0.004	0.01	0.004	0.01	0.01	0.01	0.02

[1]LYS = Lysine [2]TRY = Tryptophan [3]PHE =Phenylalanine [4]MET = Methionine [5]THR = Threonine [6]ILE = Isoleucine [7]LEU = Leucine [8]VAL = Valine [9]a b c Means in the same column with different superscript letters differ significantly ($P < 0.05$).

Effects of wilting and additives on fermentation quality of *Amaranthus Retroflexus* silage

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Keywords: additives, *Amaranthus Retroflexus*, moisture content, silage.

Introduction Although being as one of the worst weed, the high contents of Fe, Ca, and P elements, and the crude protein (CP) content about 16 to 18 percent of *Amaranthus Retroflexus* means that the *Amaranthus Retroflexus* may play a role as one possible forage resource.

Material and methods The *Amaranthus Retroflexus* were harvested by hand during flowering stage. The *Amaranthus Retroflexus* material were separated two piles, one for fresh material, and the other for wilted material, which were wilted about three hours. The material were cut about 1 cm length, then mixed with formic acid or sucrose. The amount of formic acid added was 6 ml per kg of fresh or wilted material, while that of sucrose was 2 percent on fresh weight basis. No additives were used for control treatment. The fresh or wilted materials were sampled for analysis. After mixing the material was enclosed into plastic bags, then vacuumed and sealed. The silages were sampled after storage period of 360 d.

The contents of dry matter (DM), CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, ether extracts (EE), water soluble carbohydrates (WSC), nitrate, nitrite, and buffering capacity of *Amaranthus Retroflexus* material were analysed. Except for the content of DM, CP, NDF, ADF, ash, EE, nitrate, and nitrite, the pH, and lactic acid, acetic acid, propionic acid, butyric acid, and ammonia nitrogen content of *Amaranthus Retroflexus* silage were determined. The results were calculated using SAS GLM.

Results and discussion The DM content of *Amaranthus Retroflexus* material increased after wilting, while the contents of CP, NDF, ADF, ash, EE, WSC, nitrate, nitrite, and buffering capacity of *Amaranthus Retroflexus* material were not affected significantly (table 1).

The pH and the content ammonia nitrogen of *Amaranthus Retroflexus* silage decreased significantly (P<0.01) with formic acid or sucrose, while the content of lactic acid increased significantly (P<0.01) (table 2). The content of CP, NDF, ADF, ash, EE, WSC, and nitrite were not affected significantly owing to the use of additives or wilting (table 3). Compared with the fresh material, the nitrate content of *Amaranthus Retroflexus* silage decreased about 50 percent.

Conclusions The less than 5 percent WSC content of *Amaranthus Retroflexus* material during flowering stage and the buffering capacity of 500 mE •kg/DM indicated that the fermentative quality of *Amaranthus Retroflexus* silage without additives was low. Using formic acid and sucrose additives improved the fermentative quality of *Amaranthus Retroflexus* silage. The nitrate content of *Amaranthus Retroflexus* was decreased post-ensilage.

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Table 1. Characteristics of *Amaranthus Retroflexus* material.

Item	Treatment	
	Fresh material	Wilted material
DM (%)	21.52±1.09	32.48±1.27
CP (% DM)	11.34±0.49	11.44±0.38
NDF (% DM)	43.10±2.10	43.37±1.01
ADF (% DM)	31.91±0.28	41.12±1.37
Ash (% DM)	14.37±1.03	14.57±0.38
EE (% DM)	3.21±0.23	3.11±0.58
WSC/%DM	4.45±0.91	4.25±0.36
Buffering capacity (mE·kg^{-1}·DM)	512.44±12.34	567.04±25.11
Nitrate (mg·kg^{-1}·DM)	889.89±8.12	893.19±24.17
Nitrite (mg·kg^{-1}·DM)	1.34±0.19	1.51±0.37

Table 2. Fermentation characteristics of *Amaranthus Retroflexus* silage using formic acid or sucrose as additives.

Item	Fresh material			Wilted material			Significance of effects	
	Control	Sucrose	Formic acid	Control	Sucrose	Formic acid	wilting	Additives
pH	5.33±0.09	4.13±0.12	3.84±0.07	5.21±0.15	4.23±0.09	4.01±0.08	NS	**
Lactic acid (% DM)	0.40±0.03	1.54±0.19	1.84±0.43	0.35±0.05	1.65±0.19	1.58±0.28	NS	**
Acetic acid (% DM)	0.26±0.06	0.19±0.11	0.11±0.01	0.74±0.16	0.92±0.06	0.05±0.02	**	**
Propionic acid (% DM)	0.04±0.01	-	-	-	-	-	-	-
Butyric acid (% DM)	0.10±0.04	-	-	0.04±0.03	-	-	-	-
Ammonia nitrogen (% of total nitrogen)	2.44±0.21	1.04±0.04	0.89±0.08	1.87±0.22	1.03±0.33	0.93±0.01	*	**

Table 3 Nutrient contents (% DM) of *Amaranthus Retroflexus* silage using formic acid or sucrose as additives

Item	Fresh material			Wilted material			Significance of effects	
	Control	Sucrose	Formic acid	Control	Sucrose	Formic acid	Wilting	Additi-ves
DM (%)	21.51±1.28	22.28±1.65	21.85±0.98	32.32±1.22	32.09±0.79	33.30±1.63	**	NS
CP	11.28±0.23	11.38±1.07	11.22±0.83	10.28±0.06	10.81±0.23	10.53±0.14	NS	NS
NDF	44.63±1.21	43.04±1.18	42.30±0.63	43.23±1.51	44.69±1.26	42.08±0.70	NS	NS
ADF	31.53±0.88	30.29±1.47	32.38±0.52	30.60±1.12	31.91±1.06	32.92±1.17	NS	NS
Ash	14.09±0.54	13.44±1.01	13.84±1.33	13.94±129	13.34±0.14	14.32±0.62	NS	NS
EE	3.14±0.22	3.14±0.77	3.05±0.25	3.06±0.40	3.19±0.76	3.04±1.20	NS	NS
Dry matter recovery (%)	92.94±0.82	93.06±1.72	93.53±1.01	91.20±0.77	92.42±0.93	92.09±1.16	NS	NS
nitrate (mg/kg DM)	489.06±28.74	428.30±15.06	364.85±78.47	403.57±41.70	376.14±18.42	365.29±14.55	*	NS
Nitrite (mg/kg DM)	2.21±0.42	2.38±0.13	2.32±0.37	2.24±0.33	2.14±0.52	2.38±0.32	NS	NS

Chemical composition and *in vitro* gas production of tree leaves ensiled with urea and molasses in growing lambs

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Keywords: gas production, silage, urea, molasses, sheep

Introduction Mexico as an edaphic and topographic country and it's the characteristic of climatic presents an important abundance of natural resources, mainly by the diversity of vegetal species. Nonetheless, in tropical and sub tropical zones many of the native forages exhibit low quality. Mexican browse species differ in their nutritional content depending on the time of the year and the zone where they grow. Tropical grasses and legumes are not natural material for silage, largely because at cutting, they have a low content of water soluble carbohydrates, which are essential to successful ensilage (McDonald et al. 1991). There is very little information on their nutritional value and degradation at ruminal level. The technique of *in vitro* gas production (Menke and Steingass 1988) allows us to predict fermentation and degradation with small samples. The aim of this study was to evaluate the ensiling of *Prunus persica* (Pp), *Leucaena esculenta* (Le)*, Acacia farnesiana* (Af)*, Prunus domestica* (Pd) leaves with urea (UR), molasses (MO) and their mixture (URMO) on *in vitro* ruminal fermentation in lambs.

Material and methods Leaves of *Prunus persica* (Pp), *Leucaena esculenta* (Le)*, Acacia farnesiana* (Af) and *Prunus domestica* (Pd) trees were collected from the Mexico state region, Mexico. For each treatment, 100 g of fresh tree leaves of each species were collected from different trees and chopped to pass a 1-3 cm sieve. Treatments were: dried leaves (30ºC for 72 h) or ensiled with 40ml of urea solution (UR- 5% of urea), molasses solution (MO- 10%) and URMO (40ml of 5% UR and 10%MR) in nylon bags (20x50cm) for 30 days. After the ensiling period, bags were opened and silage quality was immediately determined. Bags contents of each treatment were dried a 30ºC for 72 h and ground to pass a 1mm sieve and stored at ambient temperature (25–27ºC) in sailed plastic bags. Chemical composition, secondary metabolites (total phenols (TP), saponins (SP) and aqueous fraction (AF)), *in vitro* gas production (IVGP) and DM degradability (DMD) were determined, while the OM digestibility (OMD), metabolizable energy (ME) and short chain fatty acids (SCFAs) were estimated. Rumen liquor was collected from 4 lambs fed on a total mixed ration (21 and 141g/kg DM of CP and NDF, respectively).

Results and discussion Silage pH was highest ($P<0.001$) with URMO and lowest with drying treatment. CP was decreased ($P<0.001$) with UR and decreased ($P<0.001$) with drying treatment, while ADF and NDF were not affected ($P=0.633$) among treatments. Synman et al. (1987) reported that ensiling of maize residues had no effect on silage. This could be attributed to the fact that ADF does not provide sugars for lactic acid production during fermentation. Also, a decrease in CP content during ensiling is contrary to the results reported by Keady and Murphy (1998). This reduction in CP could be attributed to the degradation of protein during ensiling which resulted in higher non protein nitrogen in the silage than in the herbage before ensiling (Whittenbury et al. 1967). TP and SP were higher in Pp compared with the other species. Heights decreased ($P<0.001$) in TP and SP was with the URMO, while the lowest decreased was with drying treatment. IVGP (b), ME, DMD, OMD and SCFA were highest with MO and lowest ($P<0.001$) with drying treatment. Within the tree species, Pp had the heights nutritive value (IVGP, ME, DMD, OMD and SCFA), while the lowest value was in Af or Pd species (Table 1).

Conclusions Data suggested that ensiling leaves of tree species with molasses was the most effective and can decrease partly anti-nutritional impacts of secondary metabolites containing tree species. *P. persica* was the better species affected by all treatments during ensiling than other species in lambs feeding and could be useful as a good source for ruminant feeding.

Table 1. Secondary metabolites, chemical composition, *in vitro* gas production volume in incubation different times and estimated parameters of tree species leaves ensiled with urea, molasses or the mixture of urea and molasses.

	Tree species					Treatments				
	Pp	*Le*	*Af*	*Pd*	SEM	Control	Urea (U)	Molasses (M)	UM	SEM
Secondary metabolites and nutrients levels										
TP	13.2[a]	10.4[b]	9.7[b]	11.1[b]	0.85	14.6[a]	11.3[b]	10.1[cb]	8.3[c]	0.60
SP	12.9[a]	7.8[b]	11.9[a]	5.4[c]	0.96	11.6[a]	10.0[ab]	7.5[c]	8.9[bc]	0.68
AF	61.1[a]	35.8[b]	45.1[b]	39.3[b]	6.54	51.1	43.6	40.0	46.6	4.62
Ash	8.4[b]	6.6[c]	5.7[d]	16.9[a]	0.14	9.7b	8.6[c]	10.4[a]	8.8[c]	0.10
CP	22.9[a]	16.9[c]	21.3[b]	13.6[d]	0.62	15.8[c]	22.0[a]	18.2[b]	18.7[b]	0.44
NDF	29.8	30.96	31.2	31.1	4.29	25.6	30.2	32.8	34.4	3.04
ADF	17.5	21.2	21.1	19.7	3.11	16.7	17.9	20.8	24.1	2.20
pH	6.9[c]	7.0[b]	7.0[ab]	7.1[a]	0.02	6.7[d]	7.2[b]	6.9[c]	7.2[a]	0.02
In vitro gas production (ml/g DM)										
6	35.4[a]	11.7[b]	11.2[b]	13.9[b]	3.85	28.5[a]	9.4[b]	24.8[a]	9.6[b]	2.72
12	57.5[a]	20.90[b]	20.4[b]	25.1[b]	4.92	46.1[a]	17.2[b]	42.9[a]	17.6[b]	3.48
24	82.5[a]	33.9[b]	34.3[b]	41.0[b]	4.9	66.5[a]	29.2[b]	66.1[a]	29.9[b]	3.43
48	103.4[a]	47.5[c]	50.9[bc]	57.8[b]	4.16	84.0[a]	43.8[b]	86.7[a]	45.1[b]	2.94
72	111.3[a]	53.2[c]	59.6[bc]	64.9[b]	3.96	90.4[a]	51.3[b]	94.0[a]	53.3[b]	2.80
In vitro gas production parameters										
B	118.4[a]	57.9[c]	73.6[b]	70.3[b]	3.94	94.7[a]	61.0[b]	99.1[a]	65.5[b]	2.79
c	0.055[a]	0.036[b]	0.027[c]	0.038[b]	0.004	0.052[a]	0.028[b]	0.047[a]	0.029[b]	0.003
L	2.080[a]	0.70[b]	1.67[a]	1.92[a]	0. 87	2.07[a]	1.48[ab]	1.74[a]	1.07[b]	0.203
ME	5.7[a]	4.1[b]	4.3[b]	4.1b	0.13	4.9[a]	4.2[b]	5.0[a]	4.1[b]	0.09
OMD	40.4[a]	28.9[c]	30.9[b]	28.8[c]	0.86	34.3[a]	30.4b	35.3[a]	29.0[b]	0.61
SCFA	1.81[a]	0.73[b]	0.74[b]	0.89[b]	0.108	1.46[a]	0.63b	1.44[a]	0.64[b]	0.076

[1]TP is the total phenols (TP); SP is the saponins; AF is the aqueous fraction; [1]CP is the crude protein; NDF is the natural detergent fibre; ADF is the acid detergent fibre; pH is the ruminal pH; [3] Different superscripts following means within a row indicate differences at P<0.01; *b* is the asymptotic gas production (ml/g DM); *c* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h); ME is the metabolizable energy (MJ/kg DM); IVOMD is the *in vitro* organic matter digestibility (g/kg MS); SCFA is the short chain fatty acids (mmol/g DM); *Prunus persica* (Pp), *Leucaena esculenta* (Le)*, Acacia farnesiana* (Af)*, Prunus domestica* (Pd) .

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Potassium, sulphur, chlorine and sodium levels in maize silage from five regions in Brazil

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Keywords: corn silage, DCAD, minerals, nutrition

Introduction Maize silage use in the Brazilian dairy production is constantly growing by increasing both the cropping area and its productivity. Several factors are important to achieve the maximum amount and quality of this forage, mainly the plant nutrition. Variability in the nutrient content of the plants depends on its availability in the soil, absorption capability, weather changes during the crop development, besides cultivars being used. Minerals in silages such as potassium, sodium, chlorine, and sulfhur are responsible for the cation-anion difference (CAD) balance in the transition period of dairy cows, and may be associated with some metabolic disorders, particularly hypocalcemia. This trial aimed to measure potassium, sodium, chlorine, and sulfhur levels in maize silage from 109 farms in different regions of Brazil, and to estimate the CAD for these silages.

Material and methods The research was performed by the Centro de Pesquisa em Forragicultura (CPFOR), of Federal University of Paraná, in Curitiba, PR, Brazil. Three samples per silo (1 kg, vacuum sealed) were collected in 109 dairy farms located in five regions (four states) of Brazil (*Castro*, *Minas Gerais*, *Goiás, Toledo* and *Santa Catarina*), totaling 327 samples. After oven dying (55^{o}C) samples were 1-mm ground and sent to Laboratory of Plant Tissue Analysis of University of São Paulo. Potassium (K), sodium (Na), chlorine (Cl), and sulphur (S) levels were determined by atomic absorption spectrophotometry. The cation-anion difference (CAD - mEq 100 g^{-1} of Dry Matter (DM)) was estimated using the equation: CAD = (K + Na) - (Cl + S) (Horst et al. 1997). The results were submitted to analysis of variance and the mean values of each region were compared by Tukey test using the GLM procedure of SAS. Data of different regions were compared using the mean value of the three samples for each farm.

Results and discussion Large variation was observed among silage samples for mineral concentrations (Table 1). The mean values of 327 samples were different from those described by NRC (2001) for maize silage (12.0; 2.9; 0.1 and 1.4 g kg^{-1} of DM for K, Cl; Na, and S, respectively). Potassium and S levels were lower while the Cl and Na were higher than the NRC values. All the minerals showed a large variability between the minimum and maximum values. Probably this variability is related to different fertility and soil fertilization strategies among farms.

Differences among regions were found for Cl, Na and S levels (Table 2). However, the potassium levels were similar, and this mineral is a major factor influencing CAD balance. No explanation of these differences can be stated only according to mineral evaluation of the silages.

The variability in mineral composition of silages leads to a great amplitude on CAD (Table 3), and most of them were cationic. Diets for prepartum dairy cows should be anionic, varying between -10 and -15 mEq 100 g^{-1} DM (Moore et al. 2000), which is difficult to achieve using silages with high levels of strong cations such as K and Na. Only six of 109 farms showed negative CAD, probably related to poor fertilization of corn field.

The region of *Castro* has the highest productivity for maize silage in Brazil. The high level of potassium and the low level of chloride in the silages of this region lead to the highest mean value of CAD, which increase the risk of hypocalcemia in peripartum cows. Transition diet with high cationic forage is associated with metabolic alkalosis and consequently contributes to the inhibition of bones calcium reabsorption (Goff 2008), which is important to the homeostasis of this element in tissues and fluids. Thus, highly cationic forages must be avoided in this period, besides dairy producers should evaluate the supplementation of an anionic salt.

Conclusions High variability of mineral composition was detected for maize silages of different Brazilian farms and regions. The monitoring of forages CAD is necessary to avoid the risks of hypocalcemia in peripartum dairy cows.

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Table 1. Means (g kg^{-1} DM) and standard deviations, minimum and maximum values of minerals in 327 samples of maize silage in Brazil.

Variable	Mean ± SD[1]	Minimum	Maximum	Variation
Potassium	8.04 ± 2.1	1.02	16.83	1550%
Chloride	3.53 ± 1.3	0.80	6.90	763%
Sodium	0.64 ± 0.2	0.23	3.56	1451%
Sulfhur	0.23 ± 0.1	0.07	1.24	1671%
Dry matter	324 ± 50	198	491	148%
Ash	33 ± 10	11	109	891%
CAD[2]	7.67 ± 5.4	-5.04	30.95	714%

[1] SD – standard deviation
[2] CAD – Cation-anion difference (mEq 100 g^{-1} DM)

Table 2. Means (g kg^{-1} DM) and standard deviations of CAD minerals in maize silages from five Brazilian regions (109 farms).

Mineral	Region[1]					CV[2]
	Castro	*Minas Gerais*	*Goiás*	*Toledo*	*Santa Catarina*	(%)
Potassium	8.39 ± 2.1	8.59± 1.7	7.70± 1.5	7.64± 1.4	7.41± 2.0	25.8
Chloride	2.51^{c}± 0.9	4.20^{a}± 0.8	4.03^{a}± 0.7	4.14^{a}± 1.0	3.41^{b}± 0.8	35.9
Sodium	0.32^{a}± 0.1	0.29^{a}± 0.1	0.20^{b}± 0.1	0.12^{c}±0.1	0.17^{b}± 0.1	33.9
Sulfhur	0.59^{b} ± 0.1	0.62^{b}± 0.2	0.60^{b}± 0.1	0.65^{ab}± 0.1	0.76^{a}± 0.1	54.4

[1] Means followed by different superscripts are different according Tukey test (P<0.05)
[2] CV – Coefficient of variation

Table 3. Means and standard deviations of cation-anion difference (CAD – mEq 100 g^{-1} DM), minimum and maximum CAD values of maize silages from five Brazilian regions (109 farms)

Mineral	Region					CV[1]
	Castro	*Minas Gerais*	*Goiás*	*Toledo*	*Santa Catarina*	(%)
CAD	12.10 ± 5.5	7.59 ± 4.7	5.48 ± 3.9	4.32 ± 4.1	5.40 ± 6.1	70.8
Minimum	2.45	-0.96	-2.16	0.74	-5.04	-
Maximum	30.95	16.93	13.29	11.03	15.38	-

[1] CV – Coefficient of variation

Effects of plant species, stage of maturity and level of formic acid addition on plant mediated lipolysis during ensiling

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Keywords: grass, lipolysis, polyphenol oxidase, red clover

Introduction Polyphenol oxidase (PPO) has been shown to lower proteolysis and plant mediated lipolysis of grasses (Lee et al. 2006) and red clover (*Trifolium pratense;* Lee et al., 2007) in silo. Decreases in the extent of lipolysis during ensiling may also result in higher ruminal escape of forage lipids and increase the amount of 18:2n-6 and 18:3n-3 available for incorporation in ruminant meat and milk (Dewhurst et al. 2006). However, little is known about the influence of stage of maturity and ensiling additive on the extent of lipolysis during ensiling of red clover and grasses grown in Northern climates. In this investigation, red clover or a mixture of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) were harvested at three stages of maturity and treated with different amounts of formic acid (FA) to study the abundance of esterified lipid and non-esterified fatty acids (NEFA) before and after ensiling to characterize factors affecting lipolysis in silo.

Material and methods The experiment was conducted as a randomized block design with a 2×3×4 factorial arrangement of treatments; two forages (red clover and timothy/fescue), harvested at three growth stages and treated with four levels of formic acid (FA) addition (0, 2, 4 and 6 l/t). Three replicates were made for each ensiling treatment. Fresh forages were harvested at the early leaf stage (5 June 2008) and at 7–12 day intervals thereafter. Silos were maintained under anaerobic conditions for 7 months before opening. The activity of PPO in fresh herbage collected at the time of ensiling was determined according to Lee et al. (2007). Bound phenol contents in silages collected at the time of ensiling were determined according to Potty (1969). Due to the number of samples generated, only herbage treated with 0 or 6 l FA/t were submitted for lipid analysis. Forage lipids were extracted with chloroform and methanol and separated into: di- and triacylglycerols (DAG and TAG), polar membrane (galacto and phospho) lipid and NEFA by thin layer chromatography. The polar membrane lipid (PL) fraction also included monoacylglycerols (MAG). Bands were recovered, converted to fatty acid methyl esters (FAME) using methanolic sulphuric acid as a catalyst and analysed by GC. Owing to the inability to separate PL and MAG the possible recycling of NEFA liberated during the lipolysis of PL and incorporation into TAG via the activity of diacylglycerol acyltransferase could not be accounted for. Therefore, the net balance between lipolysis of esterified lipid and resynthesis of TAG during ensiling was calculated as the difference in the total esterified lipid (TEL) content of fresh chopped herbage with that in the resultant silage (distribution of NEFA in silage g/100 TEL – distribution of NEFA in fresh herbage g/100 TEL). Experimental data were evaluated by ANOVA using orthogonal contrasts for post-ANOVA comparisons (SAS Institute Inc.).

Results and discussion Concentrations of 18:3n-3 and TEL were higher ($p<0.001$) in red clover compared with grass. For both forage species, 18:3n-3 and TEL content decreased with advancing growth stage (Table 1). Concentrations of 18:3n-3 and TEL were lower in red clover silages than grass silages ($p<0.001$). In all cases, 18:3n-3 and TEL content were higher in ensiled than fresh herbage consistent with previous reports (Boufaïed et al. 2003). Addition of FA decreased the TEL content of red clover silage at all growth stages, whereas the influence of FA on the TEL content of grass silage was dependent on growth stage. Ensiling with FA resulted in marginal changes in the 18:3n-3 content of grass silage, but decreased the amount of 18:3n-3 in red clover silage. Ensiling red clover with FA was associated with an increase in the proportion of lipid as DAG and PL and a decrease in the net increase in proportion of NEFA in silage.

Mean activity of PPO was higher for red clover than grass (2.96 vs. 0.11 μkatal g fresh weight). The activity of PPO increased in red clover with advancing growth stage ($P<0.001$). The content of bound phenol is known to reflect the activity of PPO of these forage species (Lee et al. 2004). The content of bound phenol was higher in red clover silages than in grass silages (2.34 vs. 1.22 mg/g DM), except the grass silage harvested at the third growth stage and treated with formic acid addition 0 or 2 l/t ($p<0.001$). Furthermore, the content of bound phenols was dependent on three factors ($p<0.05$ for interaction of plant species, growth stage and FA). Net lipolysis of esterified lipid decreased during en-

silage of red clover, but increased for grass when FA was used ($p<0.001$ for interaction of plant species and FA). Increases in forage maturity were associated with an increase in net lipolysis of lipid in grass, whereas the reverse was true for red clover ($P<0.01$).

Conclusions Concentrations of 18:3n-3 and TEL were higher in red clover than in grass. For both forage species, 18:3n-3 and TEL contents decreased with advancing growth stage. Ensiling decreased the concentration of 18:3n-3 in red clover silage, but the changes in grass were marginal. Use of formic acid during ensiling of red clover was associated with a decrease in net lipolysis in silo, whereas for grass net lipolysis increased.

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Table 1. Concentration of 18:3n-3 and total esterified lipids (g/kg DM) and amount of lipid (g/100 lipids) in fractions in fresh and ensiled red clover and timothy-meadow fescue grass and influence of forage species, maturity and formic acid ensiling additive on net lipolysis of esterified lipid in silo (%).

	Fresh herbage							Silage						NLP
	TEL	18:3n-3	TAG	DAG	PL	NEFA		TEL	18:3n-3	TAG	DAG	PL	NEFA	
TF GS 1	16.0	11.6	2.1	4.2	89.1	4.6	TF GS1 F0	23.9	14.5	10.6	7.9	10.9	70.6	66.0
TF GS 2	13.7	9.9	1.6	3.1	91.6	3.7	TF GS1 F6	23.0	14.3	2.6	5.4	18.3	73.7	69.1
TF GS 3	10.3	7.6	1.7	1.9	92.0	4.4	TF GS2 F0	21.4	13.0	7.9	7.1	13.4	71.7	67.9
RC GS 1	17.2	12.4	1.4	2.1	93.4	3.1	TF GS2 F6	21.6	13.2	2.4	4.9	17.8	74.9	71.2
RC GS 2	16.5	11.2	2.0	2.0	93.3	2.7	TF GS3 F0	17.0	10.1	10.5	4.8	9.2	75.6	71.9
RC GS 3	12.7	8.3	2.3	2.4	93.1	2.1	TF GS3 F6	18.4	11.1	4.0	4.1	11.2	80.6	75.9
							RC GS1 F0	21.8	12.8	9.7	5.0	11.0	74.3	71.2
							RC GS1 F6	18.0	10.0	7.0	13.2	18.3	61.5	58.4
							RC GS2 F0	21.3	12.5	8.9	6.1	13.4	71.6	68.9
							RC GS2 F6	19.8	11.0	4.2	16.0	16.0	63.7	61.0
							RC GS3 F0	19.1	10.7	6.7	4.8	12.5	76.0	73.7
							RC GS3 F6	16.0	8.6	3.9	15.3	22.4	58.4	56.3
SEM	0.51	0.63	0.26	0.31	0.71	0.49		0.66	0.77	0.90	0.48	1.97	0.84	1.86
Statistical significance														
Plant species (1)	***	*		**	**	**		***	***		***	o	***	***
Growth stage L(2)	***	***		**	o			***	***	*	o		o	*
Growth stage Q(3)	*							**	**	*	**			
Formic acid (4)				**	*			**	**	***	***	***	***	***
Interaction 1 × 2			*					**	**	**	***	*	*	**
Interaction 1 × 3												o		
Interaction 1 × 4								***	***	**	***		***	***

DM = dry matter, TF = timothy/meadow fescue, RC = red clover, GS = growth stage, F0/F6 = formic acid addition 0/6 litre/tonne, TEL = total esterified lipids, TAG = triacylglycerols, DAG = diacylglycerols, , NEFA = non-esterified fatty acids, NLP = Net lipolysis, L = linear effect, Q = quadratic effect, PL = polar membrane lipids and monoacylglycerols. Interactions other than 1×2 and 1×4 were not significant ($P>0.05$).

Fatty acids composition of a variety of forages before and after ensiling

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Keywords: fermentation, linoleic acid, n-3 fatty acids, wilting

Introduction There has been indications that dairy and beef products from grazing animals displayed improved long-chain fatty acids (LCFA) profile with regards to human's health than products that originate from animals fed with a diet with conserved forage or high-concentrate (Elgersma et al., 2004a; Fredriksson and Pickova 2007). However, the grazing season is limited, and the majority of the forage used in beef and milk production is consumed as preserved forage, mainly as silage. Since LCFA composition differs among forage crops, the aim of the study was to screen LCFA profile in forages commonly used in feeding of ruminants and monitor changes of LCFA during their ensiling process.

Material and methods Forages represented by rye-grass, meadow fescue, tall fescue, timothy, red and white clover, and bird's-food trefoil were manually harvested during the period 4-12 of June (first harvest). Forage crops were wilted in a drying cupboard at 25-27 °C to target dry matter (DM) level of approx. 350 g/kg. All crops were mechanically untreated. Samples were collected at the harvest time, after the wilting and at the end of ensiling period, and kept frozen at -18 °C. Wilting time for each forage crop was recorded. Forages were ensiled in black PVC silos without additive treatments for 120 days. Crude fat was extracted according to Lourenco et al. (2007). FA analyses were performed using gas chromatography as described by Apelqvist (1968). Fermentation profile of silage samples was determined in silage juice using HPLC.

Results and discussion Fermentation parameters indicated that clostridia activity in silages was very low. Concentrations of butyric acid, a typical marker of clostridia activity (Pahlow et al. 2003), were detected in trace amounts with the highest concentration of 2.3 g/kg dry matter (DM) in timothy silages. The average pH in all silages was found to be 5.2±0.4, with the lowest value in tall fescue silages 4.5±0.05. The LCFA profile and total fatty acids (FA) concentration of all forage crops during the ensiling process are demonstrated in Table 1. Grass forages, except timothy were found to have higher proportion of C18:3n-3 ($P<0.001$) but lower C18:2n-6 ($P<0.001$) than legumes. In contrast to the finding of Van Ranst et al. (2009), wilting of forages did not significantly reduce either the total FA content or proportions of C18:3n-3 and C18:2n-6, except in white clover (C18:3n-3) and bird's-food trefoil (C18:2n-6). Wilted meadow fescue, tall fescue, and timothy displayed even higher proportion of C18:3-n3 than in fresh crops. A possible explanation of the results can be fast wilting process in drying cupboard in the absence of light. Wilting time of forages did not exceed 24 hours, except for white clover and bird's-food trefoil (48 hours). The decrease in C18:3n-3 proportion ($P<0.001$) was, however observed in all silages, except for white clover. The proportion of C18:2n-6 in all grass silages was higher ($P<0.001$) in comparison with fresh and wilted forages. The effect of interaction between ensiling stage (wilting + ensiling) and forage type was demonstrated on C16:0, where proportion of C16:0 in grasses were lower ($P<0.001$) when wilted and ensiled, whereas the opposite trend was observed in legumes. Proportion of C18:0 in legumes followed the same trend ($P<0.001$) as C16:0. Furthermore, a significant effect of ensiling stage on reduction of C18:1cis-9 proportion in grasses was obtained.

Conclusions There were differences in LCFA profile prior and during ensiling process between grasses and legumes. Proportions of C18:3n-3 from total LCFA content in silages were lower than in fresh crops, except for timothy and tall fescue. Unexpectedly, the impact of wilting on reduction of total FA content and favourable C18:3n-3 was not observed which could be the result of fast wilting process in drying cupboard.

Table 1. Total fatty acid (FA) content and fatty acid composition in tested forages during ensiling process.

Forage	Ensiling stage	DM	C16:0	C18:0	C18:1 cis-9	C18:2 n-6	C18:3 n-3	Total FA
		g/kg	g/100g total FA					mg/g DM
Rye-grass	fresh	191	17.3[a]	1.4[b]	3.4[a]	12.7[b]	62.2[a]	14.7
Rye-grass	wilted	365	17.6[a]	1.6[a]	2.4[b]	11.9[b]	62.6[a]	13.0
Rye-grass	silage	349	16.4[b]	1.4[b]	2.0[b]	14.1[a]	60.7[b]	18.0
SEM			0.20	0.03	0.15	0.28	0.38	1.74
Meadow f.	fresh	191	17.6[a]	1.2[b]	3.5[a]	12.5[b]	62.9[b]	16.3
Meadow f.	wilted	385	16.5[b]	1.4[a]	2.4[b]	12.3[b]	64.3[a]	16.7
Meadow f.	silage	362	17.5[a]	1.1[b]	2.5[b]	14.3[a]	60.4[c]	17.3
SEM			0.15	0.03	0.07	0.14	0.28	2.57
Tall fescue	fresh	229	17.5[a]	1.5[ab]	5.6[a]	10.9[b]	61.8[c]	13.9[b]
Tall fescue	wilted	374	16.4[b]	1.6[a]	3.2[b]	10.1[b]	65.7[a]	13.4[b]
Tall fescue	silage	343	15.6[b]	1.2[b]	3.0[b]	12.4[a]	63.4[b]	17.4[a]
SEM			0.23	0.08	0.27	0.23	0.32	0.89
Timothy	fresh	261	17.4[a]	1.8	3.8[a]	19.6[b]	53.7[b]	11.6
Timothy	wilted	400	16.0[b]	1.9	2.9[b]	18.8[b]	56.6[a]	12.7
Timothy	silage	374	16.3[b]	1.7	3.1[b]	21.1[a]	52.0[b]	13.7
SEM			0.13	0.06	0.17	0.30	0.69	0.98
red clover	fresh	169	16.1[b]	2.2[b]	2.0	19.5	57.2[a]	19.8
red clover	wilted	346	17.7[a]	2.7[a]	1.7	18.0	56.2[a]	16.1
red clover	silage	311	17.7[a]	2.7[a]	2.1	19.3	52.8[b]	17.2
SEM			0.16	0.08	0.34	0.45	0.86	0.93
white clover	fresh	165	16.7[b]	2.2[b]	3.1[a]	16.7	57.3[a]	14.7[a]
white clover	wilted	462	19.7[a]	3.0[a]	2.8[ab]	16.8	52.2[b]	10.0[b]
white clover	silage	447	19.3[a]	2.8[a]	2.8[b]	16.6	51.9[b]	15.0[a]
SEM			0.17	0.05	0.09	0.34	0.46	0.21
Bird's-food	fresh	179	16.7[b]	1.7[b]	1.5	17.7[a]	58.0[a]	16.2
Bird's-food	wilted	535	16.5[b]	1.8[b]	1.3	16.3[b]	59.0[a]	15.0
Bird's-food	silage	521	18.9[a]	1.9[a]	1.4	17.3[a]	53.8[b]	16.6
SEM			0.16	0.06	0.11	0.11	0.30	1.79
Probability	Forage		***	***	***	***	***	***
	Stage		**	***	***	***	***	***
	Int.		***	***	***	***	***	NS

[a,b,c] Significant (P<0.05) differences within forages and between ensiling stage; *, ** and *** at P<0.05, P<0.01 and P<0.001, respectively.; NS – not significant; FA – fatty acids; DM – dry matter.

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Characterisation of long-chain fatty acids in mixture silage of erect milkvetch and perennial ryegrass

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Keywords: Perennial ryegrass, erect milkvetch, mixture silage, long-chain fatty acids

Introduction Ensiling erect milkvetch (*Astragalus adsurgens*) as a mixture with perennial (ryegrass *Lolium perenne* L.) can result in the production of a high quality silage with the supply of nutrients from each forage species complimenting one another. Research has indicated that altering diet composition feed could enhance unsaturated fatty acids in animal products. These fatty acids have been proved to be beneficial to human health, especially the conjugate linoleic acid (CLA). This experiment was carried out to investigate the composition of long-chain fatty acids in erect milkvetch and perennial ryegrass when ensiled separately or as a mixture of the two.

Material and methods The mixing ratios on a fresh weight basis of erect milkvetch and perennial ryegrass at ensiling were set at the rates of 100:0, 70:30, 50:50, 30:70 and 0:100, respectively. Triplicate silos for each treatment were stored for 60 d at room temperature, then sampled for the analysis of fermentation quality and content of long-chain fatty acids (Sukhija and Palmquist 1988, Zhu Yu *et al*. 1999). Data were analysed using SAS statistical Software.

Results and discussion Table 1 showed that these two grasses are rich in long-chain fatty acids. The pH value, concentration of acetic acid and ammonia N in the ensiled perennial ryegrass were significantly lower (P<0.05) than ensiled erect milkvetch, whereas, lactic acid in perennial ryegrass was significantly higher (P<0.05) than ensiled erect milkvetch. In addition, lactic acids in the three mixture silage groups were markedly higher (P<0.05) than the two forage ensiled solely (Table 2). In the table 3, C16:0 and total long-chain fatty acids concentrations in ensiled erect milkvetch were increased compared with that in the fresh forage, but in the perennial ryegrass these fatty acids reduced. The C16:1 and the C18:1 content in the direct silage of erect milkvetch was higher than other treatment group ensilage obviously (P<0.05), and there was no difference between other treatment group (P>0.05). Higher C16:0 and C18:0 in the ensiled erect milkvetch than in the ensiled perennial ryegrass was observed (P<0.05), but for C18:2 and C18:3, it was on the other way.

Table 1. Long-chain fatty acids content of fresh grasses (mg/g DM).

Fresh grass	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TFA
Erect milkvetch	2.85	0.11	0.71	1.04	1.92	3.66	10.29
Perennial ryegrass	5.67	0.04	0.44	0.74	2.31	5.88	15.08

TFA: total long-chain fatty acids.

Table 2. Fermentation characteristics of experimental silages.

Erect milkvetch : Perennial ryegrass	pH	%DM				AN/TN(%)
		LA	AA	PA	BA	
100:0	4.77^{a}	7.63^{b}	2.83^{a}	0	0.05^{a}	8.47^{a}
0:100	4.02^{d}	11.16^{a}	0.97^{c}	0	0.00^{c}	2.77^{d}
70:30	4.09^{c}	13.46^{a}	1.30^{bc}	0.36	0.00^{c}	2.90^{cd}
50:50	4.14^{c}	13.43^{a}	1.65^{bc}	0.22	0.00^{c}	3.47^{c}
30:70	4.36^{b}	13.28^{a}	1.99^{ab}	0.29	0.03^{b}	4.47^{b}
Significances	*	*	*	NS	*	*

NS: non-significant, *: significant at P<0.05, LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid, AN: ammonia nitrogen, TN: total nitrogen.

Table 3. Long-chain fatty acids content of experimental silages (mg/g DM).

Erect milkvetch : Perennial ryegrass	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	TFA
100:0	4.58^{a}	0.30^{a}	1.25^{a}	1.21^{a}	1.94^{c}	3.25^{d}	12.53
0:100	3.28^{c}	0.22^{b}	0.72^{b}	0.71^{b}	2.48^{a}	5.05^{a}	12.46
70:30	3.55^{bc}	0.20^{b}	0.63^{b}	0.83^{b}	2.17^{b}	4.85^{ab}	12.23
50:50	3.45^{c}	0.18^{b}	0.73^{b}	0.81^{b}	2.23^{b}	4.40^{bc}	11.8
30:70	3.77^{b}	0.23^{b}	1.04^{a}	0.90^{b}	2.10^{b}	3.95^{c}	11.99
Significances	*	*	*	*	*	*	

*: significant at P<0.05, TFA: total long-chain fatty acids.

Conclusions The ensilage process may enhance the concentration of C16:0 and total long-chain fatty acids, but reduce them as compared to perennial ryegrass ensiled solely. The C18:2 and the C18:3 content of ensiled perennial ryegrass was high. Regarding these fatty acids, if the pasture (erect milkvetch or perennial ryegrass) had more long-chain fatty acids and its mixing ration was high, the higher was the concentration of long-chain fatty acids in the mixture silage.

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Degrading mimosine and tannins of *Leucaena leucocephala* by ensiling

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Keywords: additives, *Leucaena leucocephala,* mimosine, silage, tannin

Introduction *Leucaena leucocephala* (leucaena), belonging to the family Leguminosae, is vigorous, rapidly growing, drought-tolerant, highly palatable, protein rich and high yielding and can be grown on a wide range of soils (Gupta and Atreja 1999). It is a widely used species as a valuable fodder shrub for increased animal production in the tropics (Khamseekhiew et al. 2001). However, as feed, leucaena may have harmful effects on animals because of the presence of mimosine and tannin. The objective of this study was to investigate the silage fermentation characteristics and ensiling effects on mimosine and tannin degradation of leucaena with or without the addition of sucrose and lactic acid bacteria (LAB).

Material and methods Tender branches and leaves of leucaena were obtained from green trees on July 14 in 2008, grown on the central campus of South China Agricultural University (Guangzhou, China). The material was chopped into 2 to 3 cm lengths, and sucrose and/or LAB were added at 2% and 1×10^5 cfu/g fresh material, respectively. The control was sprayed with the same amount of distilled water alone. The treated material was ensiled with plastic film bags. Each treatment was made 21 bags and they were kept in the room at ambient temperature. Three bags were opened for analyses after ensiling for 1, 3, 7, 15, 30, 60 and 90 days, respectively.

The contents of dry matter, crude protein, crude fat, crude fiber, crude ash, neutral detergent fiber, acid detergent fiber, water-soluble carbohydrates (WSC), mimosine and tannin of leucaena were determined. The pH values, organic acids contents and contents of mimosine and tannin in leucaena silages ensiled for different days were measured.

Results and discussion Leucaena contained high contents of crude protein (25.7% DM) and low WSC content (3.7% DM). Mimosine content was near to 5% DM and tannins content was 1.3% DM.The pH values dropped sharply during the initial period of ensiling (0 to 3 days) and tended to be stable after ensiling for 15 days in all the treatments. The pH values of silages added with sucrose and sucrose + LAB were significantly lower than those of control and LAB alone during the whole ensiling period ($p < 0.01$).

Lactic acid content rapidly increased during the first 7 days, and then rose slowly for all the treatments. During the whole ensiling process, all the silages added with sucrose had the highest lactic acid content. LAB inoculation alone did not increase lactic acid content. As ensiling time prolonged, lactic acid, acetic acid, propionic acid and butyric acid were increased. Adding sucrose and LAB + sucrose reduced the contents of acetic acid, propionic acid and butyric acid compared to the control (Figure 1). Both mimosine content and tannin content were gradually decreased as the fermentation time increased for all the silages. After ensiling for 30 days, the drop rates slowed down, and at the end of ensiling mimosine degradation rates in sucrose and sucrose + LAB added silages were 49.0% and 48.0%, respectively, while the control and LAB inoculation alone were 25.5% and 30.7%, respectively. The degradation rates of tannin of sucrose and sucrose + lactic acid treatments were 52.4% and 54.7%, respectively, while the control and LAB alone were 40.0% and 42.3%, respectively. The degradation rates of mimosine and tannin was negatively related to pH values ($p < 0.01$) (r = -0.824 and -0.844), positively related to lactic acid content ($p < 0.01$) (r = 0.961 and 0.957).

Conclusions The mimosine and tannin contents of leucaena could be markedly declined by ensiling, and sucrose addition promoted their degradation. The degradation rates of mimosine and tannin were significantly related to pH values (negatively) and significantly related to the content of lactic acid (positively).

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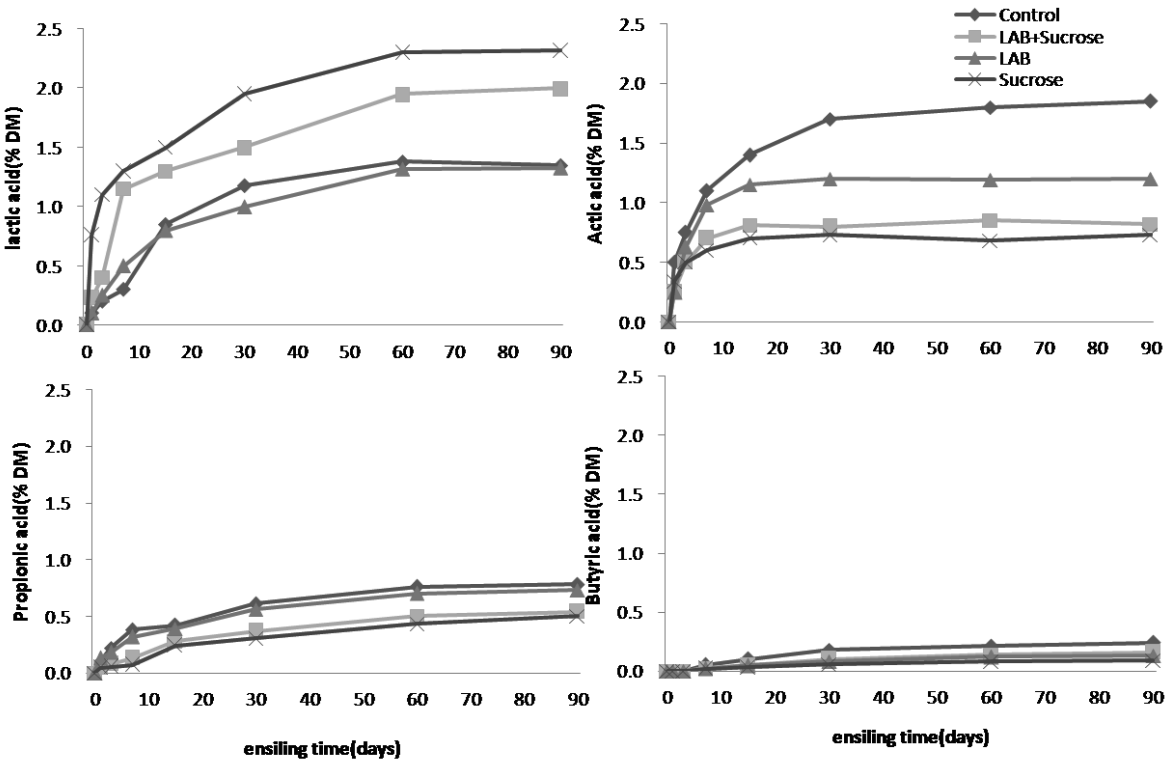

Fig. 1. Changes of organic acid contents during ensiling of *L. leucocephala*. LAB: lactic acid bacteria.

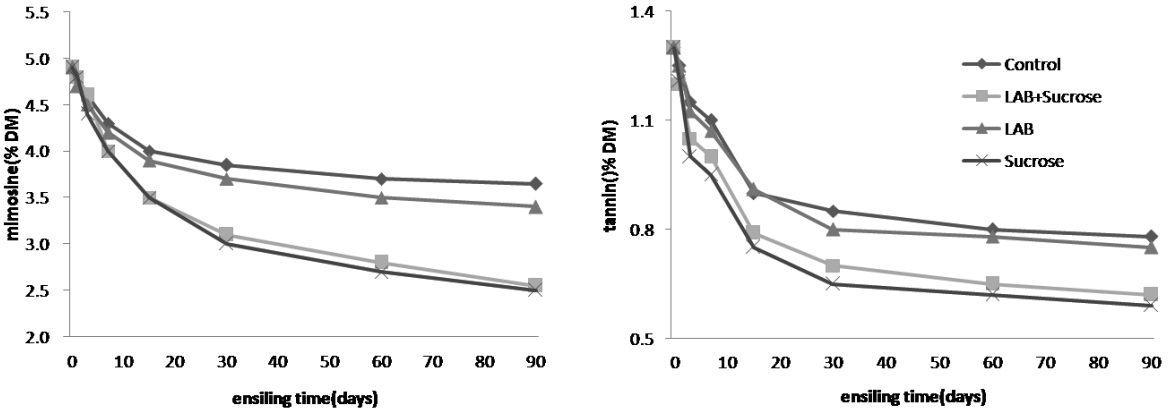

Fig. 2. Changes of mimosine content and tannin content during ensiling of *L. leucocephala*. LAB: lactic acid bacteria.

The influence of ensiling method on the composition of nitrogen fractions in red clover, alfalfa and red fescue silage

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Keywords: alfalfa, bales density, nitrogen fractions, red clover, red fescue

Introduction During ensiling plant proteases and peptidases hydrolyze most of the plant protein to free amino acids, ammonia and other forms of non-protein nitrogen (NPN). As a result, high levels of NPN in the total nitrogen (TN) of silage are among the main reasons for low efficiency of nitrogen utilization in rumen (Givens & Rulquin, 2004; Slottner & Bertilsson, 2006). Particular nitrogen fractions have different impact on the efficiency of microbial protein synthesis by their differential degradability. The degree of bales density can affect the thermal conditions and the dynamics of fermentation process, which has an impact on protein solubility and the activity of plant proteases. Most of the grass and legumes in Poland is ensiled in large round bales. Used balers are typical of silage production, however, a large proportion of farms use balers originally used for harvesting straw. Therefore, silage in bales produced in Poland is often characterized by a diverse and low density. The extent of proteolysis during ensiling may depend mainly on the combination of three factors: plant species, bales density of raw material and/or the presence of an inhibitor of proteolysis. The aim of this study was to determine the effect of ensiled plant species, density of bales and the addition of formic acid on the composition of nitrogen fractions in red clover, alfalfa and red fescue silage.

Material and methods The experimental silage (2 x 3 x 2=36 bales) was produced using two types of balers: SIPMA Z 230 (SIPMA S. A. Poland) and Claas Variant 160 (Claas KgaA mbH Germany) in bales with two degrees of density (low and high squeeze, respectively) with red clover, cv. Nike (L - 123.1, H - 190.2 kg/m^3), alfalfa cv. Alba (L - 122.5, H - 189.2 kg/m^3), red fescue cv. Godolin (L - 127.3, H - 189.7 kg/m^3), with or without the additive (5 l/t FM, 80% formic acid). Nitrogen fractions (Hedqvist &Udén, 2006) were determined in silage after 120 days of storage. The analysis of the nitrogen fraction included the following indications: protein nitrogen, buffer soluble nitrogen (BSN), buffer soluble protein nitrogen (BSPN), non-protein buffer soluble nitrogen (NPBSN), free amino acids nitrogen (free AA-N), acid detergent insoluble nitrogen (ADIN), biogenic amines (histamine, tyramine, cadaverine, putrescine).

The data was analysed according to the model: y = Sp + D + A + Sp x D + Sp x D + D x A which in addition to the impact of the species, density and additive includes interaction: species x density, species x additive, additive x density. Statistical analysis was performed using the GLM procedure (SAS 9.3.)

Results and discussion Analysis of nitrogen fraction of silage in big bales after 120 days of storage showed the greatest impact of ensiling plant species on the contribution of individual fractions of nitrogen (Table 1). Alfalfa silage was characterized by a lower ($P < 0.01$) contribution of insoluble fractions (protein nitrogen and ADIN) compared with red clover and red fescue silage, while the highest contribution of soluble fraction (BSN, BSPN, NPBSN, free AA-N) in the total nitrogen ($P < 0.01$). It is admitted that red fescue silage had a higher degree of deamination than in legume silage (N-NH_3, $P < 0.01$) and a lower degree of decarboxylation (contribution of biogenic amines $P < 0.05$). Between grass silage and red clover silage differences were found only in relation to the contribution of BSN. Red fescue silage was characterized by a higher content of BSN ($P < 0.05$) compared to red clover silage, but there was no evidence of differences in content of protein and non-protein BSN. Density of bales from various plant materials influenced significantly only the contribution of soluble fractions in total nitrogen. Silage bales with a greater density contained less BSN, BSPN and free AA-N ($P < 0.05$) in total N. In regard to the contribution of free AA-N there was an interaction ($P < 0.05$) between the species of ensiled plant and the density of the bale. A higher contribution of protein nitrogen and a lower content of ADIN in higher density bales were not confirmed statistically.

No effect of formic acid addition on the composition fraction of nitrogen was observed. Noteworthy is a high protein nitrogen fraction in each type of silage, which probably was associated with a high fraction of ADIN. This may be confirmed by the relationship between protein nitrogen and ADIN in silage with high and low density, which indicates that the reduction of proteolysis (high levels of protein nitrogen) in the low density was due to a greater amount of oxygen contained in bales and the temperature increase during ensiling. Species differences in ADIN fraction content may indicate a greater sensitivity of clovers and grasses protein on the growth temperature.

Table 1. Chemical composition of experimental silage.

	Species			Density		Additive		SEM	Interaction
	Red clover	Alfalfa	Red fescue	Low	High	Without FA	With FA		
DM (g kg^{-1}FM)	436	438	452	444	440	444	440	7.4	NS
CP (g kg^{-1}DM)	161^{B}	176^{A}	108^{C}	145^{b}	151^{a}	148	148	5.1	NS
TP (g kg^{-1}DM)	96.6^{A}	79.3^{B}	59.4^{C}	72.8^{b}	84.1^{a}	80.2	76.7	4.4	NS
WSC (g kg^{-1}DM)	25.6^{b}	25.6^{b}	45.5^{a}	34.3	30.1	30.2	34.3	4.6	NS
pH	4.6	4.6	4.7	4.7	4.6	4.6	4.6	0.05	DA*
Lactic acid (g kg^{-1}DM)	49.0^{A}	42.4	36.9^{B}	45.9	39.7	46.8^{a}	38.8^{b}	2.9	DA*
Acetic acid (g kg^{-1}DM)	18.8	19.7	23.3	18.2^{b}	23.0^{a}	25.0^{A}	16.2^{B}	2.1	SpD*DA*
Butyric acid(g kg^{-1}DM)	2.3	2.0	1.8	2.2	1.9	1.7	2.4	4.2	NS
Total VFA (g kg^{1}DM)	32.6	33.5	33.4	31.7	34.6	36.4^{a}	29.9^{b}	2.6	SpD**
Total acids (g kg^{-1}DM)	81.6	75.9	70.3	77.6	74.3	83.2^{A}	68.7^{B}	4.6	SpD**
Total N (g kg^{-1}DM)	25.8^{B}	28.1^{A}	17.0^{C}	23.2^{b}	24.1^{a}	23.7	23.5	0.9	NS
Nitrogen fractions (g kg^{-1}TN)									
Protein N	597^{A}	449^{B}	561^{A}	512	560	543	529	21.1	NS
BSN	192^{Bb}	338^{A}	234^{Ba}	273^{a}	236^{b}	259	251	15.2	NS
NPBSN	137^{B}	241^{A}	166^{B}	193	170	182	181	11.6	NS
BSPN	55.3^{B}	96.6^{A}	68.0^{B}	80.1^{a}	66.5^{b}	77.0	69.7	5.4	NS
Free AA-N	60.2^{B}	173^{A}	75.9^{B}	126^{a}	79.8^{b}	117	89.6	18.9	SpD*
ADIN	172^{A}	108^{B}	157^{A}	154	137	140	151	10	NS
N-NH_3/TN (g kg^{-1}TN)	0.02^{B}	0.02^{B}	0.03^{A}	0.03	0.03	0.03	0.03	0.001	NS
∑ amines (mg kg^{-1}DM)	192^{b}	297^{a}	170^{b}	255	184	329^{A}	110^{B}	39.9	DA*

DM=dry matter; CP=crude protein; TP=true protein; WSC=water soluble carbohydrates; VFA=volatile fatty acids; TN= total nitrogen; NS=non-significant; FA=formic acid
Means in the same line with different superscripts differ significantly at ^{ABC} ($P < 0.01$), ^{ab} ($P < 0.05$)

Conclusions The results showed a dominant influence of the species on composition of the nitrogen fractions in silage. They also confirmed a greater resistance of red clover and grass protein to proteolysis as compared to alfalfa. The species of ensiled plants and the density of bales had a stronger impact on the composition of nitrogen fractions than the addition of formic acid.

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Acknowledgements Supported by the State Committee for Scientific Research, Grant No. N N311 234238

Comparison of free amino acid composition in fresh herbage and red clover, alfalfa and red fescue silage

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Keywords: alfalfa, composition, free amino acids, red clover, red fescue

Introduction In ruminant nutrition, preserved forages play an increasingly important role. Thus, knowledge concerning the effects of forage preservation on the content of particular amino acids is vital. The process of fermentation and the extent of proteolysis may affect qualitative changes in the composition of nitrogen fractions (Arrigo 2006, Huhtanen and Shingfield 2005). An important part of the non-protein nitrogen (NPN) in the silage includes free amino acids. The majority of research concerns changes of contribution in nitrogen compounds in the fraction of free amino acids and changes in the composition of total amino acids (Guo et al. 2008). There is no data on the composition of this fraction and its changes in relation to the total content of amino acids. The knowledge of the amino acid composition of this fraction is the basis for estimating the composition of protein and peptide forms contained in the silage. The aim of the present study was to compare the composition of free amino acids fraction in fresh, wilted herbage and silage made of red clover, alfalfa and red fescue and to determine the contribution of these amino acids in the free or bounded form (proteins, peptides).

Material and methods The plant material were fresh red clover cv. Nike, alfalfa cv. Alba and red fescue cv. Godolin collected in full flower, and silage from these raw materials produced in bales. Dry matter content was 460, 454, 468 g/kg respectively and density 190, 189, 190 kg DM/m^3 respectively. In samples of fresh and wilted herbage and silage (after 120 days of storage) the content of total amino acids in the hydrolysates (acid hydrolysis) was determined by amino acid analyzer (AAA 400 INGOS, Czech Republic), in accordance with the procedures recommended by the manufacturer, using a column of sodium. The free amino acid composition was determined in deproteinated samples using an amino acid analyzer (AAA 400 INGOS, Czech Republic), equipped with a column of lithium.

Results and discussion The highest proportion of free amino acids was found in fresh herbage of red clover, and the lowest in fresh herbage of red fescue (Table 1, Table 2). The comparison of free amino acids composition of fresh, wilted herbage and silage showed the smallest contribution and the extent of changes in the content of free amino acids in red clover. It also confirmed an increase (P<0.05) of contribution of free lysine in silage compared to fresh herbage. The increased contribution in the form of free amino acids during ensiling was confirmed (P<0.05) for alfalfa (108 vs. 24.9 g/kg N) and red fescue (100 vs. 19.7 g/kg N), while the changes in the contribution of free amino acids in relation to the total content were for alfalfa (0.17 vs. 0.03) (P<0.01) and for red fescue (0.12 vs. 0.03) (P<0.05). Winters et al. (2001) reported about sixfold increase in this fraction in ensiled fresh Italian ryegrass. The highest contribution of free amino acids in silage concerned proline, isoleucine, lysine (red clover and alfalfa) or proline, isoleucine, valine (red fescue).

Conclusions The increased contribution of amino acid free forms during wilting concerned alfalfa and clover but in red fescue it occurred primarily during ensiling. Significant changes in contribution of amino acids in alfalfa and red fescue were in the same single amino acids: proline, valine, isoleucine, leucine and lysine. Those amino acids where the contribution increased in the smallest degree during ensiling in bales in all plant raw materials were cystine, methionine, histidine and arginine.

Table 1. Composition of free amino acids in fresh and wilted herbage and in silage (g kg^{-1} N).

	Red clover			Alfalfa			Red fescue			SEM
	Fresh	Wilted	Silage	Fresh	Wilted	Silage	Fresh	Wilted	Silage	
Thr	0.14	0.13	0.21	0.12	0.19	0.41	0.10	0.13	0.54	0.06
Pro	1.36	2.28	1.73	0.35^{b}	3.39^{a}	3.16^{a}	0.22^{b}	1.17^{a}	3.59^{a}	0.38
Val	0.24	0.26	0.32	0.13^{b}	0.22	0.57^{a}	0.14^{b}	0.12^{b}	0.69^{a}	0.07
Cys	0.05	0.05	0.02	0.07	0.04	0.04	0.04	0.02	0.02	0.01
Met	0.02	0.01	0.01	0.01	0.04	0.04	0.02	0.02	0.02	0.01
Ile	0.13	0.13	0.25	0.10^{b}	0.21	0.41^{a}	0.09^{b}	0.03^{b}	0.41^{a}	0.04
Leu	0.12	0.14	0.39	0.21^{b}	0.24^{b}	0.7^{a}	0.16^{b}	0.12^{b}	0.6^{a}	0.07
Phe	0.12	0.14	0.21	0.13^{b}	0.13^{b}	0.37^{a}	0.08^{b}	0.08^{b}	0.29^{a}	0.04
Lys	0.06^{b}	0.10	0.27^{a}	0.20^{b}	0.11^{B}	0.56^{Aa}	0.14^{b}	0.11^{b}	0.26^{a}	0.04
His	0.05	0.05	0.04	0.04	0.09	0.09	0.02	0.03	0.04	0.02
Arg	0.15	0.18	0.12	0.10	0.08	0.25	0.09	0.05	0.10	0.03
∑ free AA[1]	35.6	46.6	60.7	24.9^{b}	60.1	108^{a}	19.7^{b}	27.0^{b}	100^{a}	10.9

[1] ∑ free AA contain 36 amino acids determined in accordance with the method

Means in the same line with the different superscripts differ significantly at ^{AB} (P<0.01), ^{ab} (P<0.05)

Table 2. The ratio of free amino acids to total amino acids.

	Red clover			Alfalfa			Red fescue			SEM
	Fresh	Wilted	Silage	Fresh	Wilted	Silage	Fresh	Wilted	Silage	
Thr	0.04	0.04	0.05	0.07	0.05	0.28	0.04	0.09	0.14	0.03
Pro	0.24^{b}	0.35^{Aa}	0.23^{B}	0.06^{B}	0.44^{A}	0.62^{A}	0.06^{B}	0.16^{B}	0.44^{A}	0.03
Val	0.04	0.05	0.06	0.03^{b}	0.04^{b}	0.15^{a}	0.03^{b}	0.03^{b}	0.12^{a}	0.01
Cys	0.06^{a}	0.04	0.02^{b}	0.04	0.02	0.06	0.02	0.01^{b}	0.04^{a}	0.01
Met	0.02^{a}	0.01^{b}	0.00	0.01	0.02	0.08	0.01	0.01	0.02	0.01
Ile	0.04	0.03	0.09	0.03^{B}	0.06^{b}	0.22^{Aa}	0.03^{B}	0.01^{B}	0.14^{A}	0.01
Leu	0.02	0.02	0.05	0.03^{b}	0.04^{b}	0.12^{a}	0.03^{b}	0.02^{b}	0.09^{a}	0.01
Phe	0.04	0.03	0.05	0.03^{b}	0.04^{b}	0.11^{a}	0.02	0.02	0.07	0.01
Lys	0.02^{b}	0.03	0.06^{a}	0.04^{B}	0.02^{B}	0.16^{A}	0.04	0.02^{b}	0.07^{a}	0.01
His	0.01	0.01	0.01	0.01	0.03	0.05	0.01	0.01	0.02	0.01
Arg	0.06	0.05	0.03	0.03	0.02	0.09	0.03	0.01	0.04	0.01
free/total AA	0.05	0.05	0.10	0.03^{B}	0.07^{b}	0.17^{Aa}	0.03^{b}	0.04^{b}	0.12^{a}	0.01

Means in the same line with the different superscripts differ significantly at ^{AB} (P<0.01), ^{ab} (P< 0.05)

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Acknowledgements Supported by the State Committee for Scientific Research, Grant No. N N311 234238

Effect of a mixture of lactic acid bacteria on the amount of protein degradation in grass silages of different raw material

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Keywords: proteolysis, lactic acid bacteria, protein fractions

Introduction The extent of protein degradation during ensiling is dependent on many factors such as field retention period before harvesting, time of acidification at the beginning of ensiling process and the quantity of undesired microorganisms in the silage (Hoedke et al., 2010).

In the present study, different first cut grass material of Infeld research farm of the Chamber of Agriculture of Niedersachsen was ensiled either as untreated or treated with a biological silage additive in order to assess, whether the mixture of lactic acid bacteria has the potential to improve the fermentation pattern of the silage and whether it has an effect on the extent of proteolysis occurring during the ensiling process.

Material and methods In 2009, six first cut grass materials (mainly perennial ryegrass) were ensiled in triplicate laboratory silage vessels either as untreated control or treated with a biological silage additive (*Bonsilage Plus*: Five homo- and heterofermentative lactic acid bacteria with the dosage of $1*10^5$ cfu/ t fresh matter)). The six grass material variants differed in level of dry matter content (I) 20-30%; II) 30-40%; III) 40-50%) and level of fertilization (I) 25 m^3 slurry/ha + 27 kg N/ha mineral fertilization; II) 25 m^3 slurry/ha + 68 kg N/ha mineral fertilization). Laboratory vessels were analysed after 90 days of storage at 20 °C. Determination of plant nutrients, pH-value, fermentation pattern and Ammonia-N were carried out and fractionation of crude protein was performed according to Licitra et al.1996.

Results and discussion In terms of each dry matter class and fertilization level the mixture of lactic acid bacteria improved the fermentation pattern. The DLG score for fermentation quality was 99.5 of 100 points on average of the six variants in contrast to 71.3 on average of 100 points for the untreated variants.

Table 1. DLG score for fermentation quality of the different trial variants.

		DLG-score		
Level of fertilization	DM range (% FM)	Control	Treatment	Stat. Signific.
	20-30	37	100	**
I	30-40	50	100	**
	40-50	90	100	*
	20-30	67	97	*
II	30-40	94	100	°
	40-50	90	100	*
Mean		71.3	99.5	

DM = dry matter; FM = fresh matter

Furthermore, all treated variants showed significantly ($p<0.05$) lower contents of ammonia-N. In addition, with exception of dry matter class 20-30% within fertilization level II, the treatment of grass material with lactic acid bacteria significantly ($p<0.05$) reduced crude protein fraction A (NPN-compounds, immediate degradation in rumen) and increased ($p<0.05$) crude protein fraction B2 (true protein, potentially complete, but slower degradation in rumen). As an example figure 1 shows the crude protein fractions and NH_3-N for dry matter content II and level of fertilization I.

For a grass silage, a shift of crude protein fraction A to fraction B2 may lead to a lower exposure of cow's liver due to a reduced extent of ruminal ammonia, which has to be detoxificated to urea via liver. Feeding cows a high ratio of grass silage, this effect becomes more relevant. Furthermore, grass silage with high extent of crude protein fraction A is associated with increasing amounts of biogenic amines and this is often assumed to have negative impact on cow's health status (Hoedtke et al. 2011, Theermann et al. 2011).

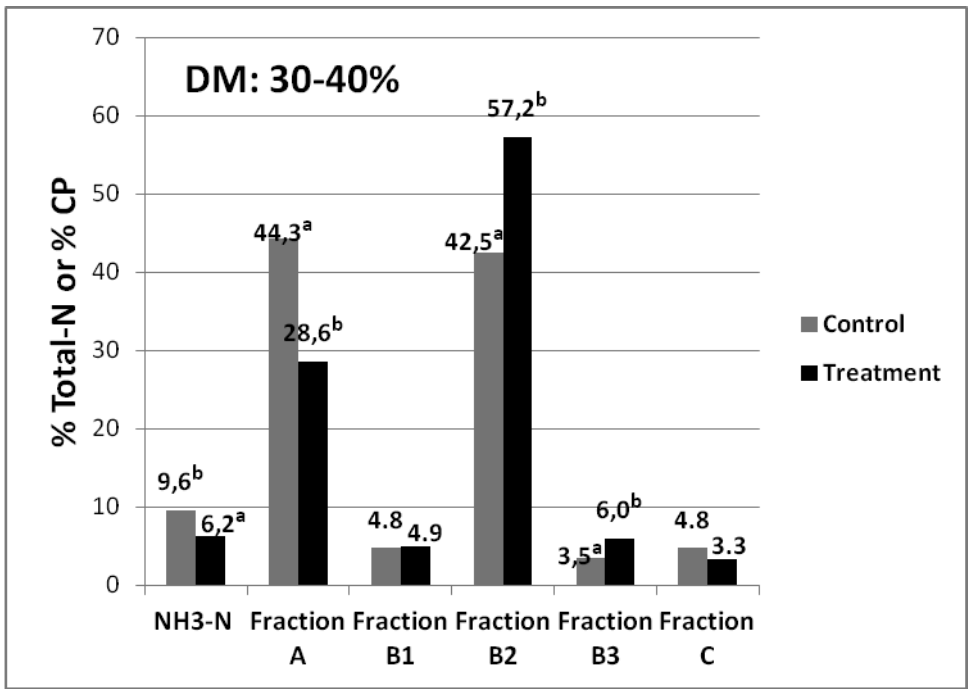

Figure 1. Crude protein fractions and NH_3-N for control and treatment within level of dry matter content II and for level of fertilization I.

Conclusions The results of the present study may indicate that the extent of proteolysis can be reduced by an application of selective mixtures of lactic acid bacteria. In order to verify the results of the present study more research in this field is as necessary as the establishment of a standard method for a better description of protein quality of silages.

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Influence of homolactic acid bacteria (*Lactobacillus plantarum* DSMZ 8862 and 8866) in combination with molasses or partly neutralized formic acid while ensiling of nearly unfermentable feedstuffs on the content of biogenic amines and clostridia spores

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Keywords: biogenic amines, clostridia, ensiling, formic acid, homolactic acid bacteria, molasses

Introduction The increasing importance for protein supply of high yielding-cows goes along with an increasing need for proper silage quality in nearly unfermentable feedstuffs. This includes the limitation of protein degradation, maintenance of palatability as well as minimizing the concentration of enterotoxins, biogenic amines and the number of clostridia in the silage. In previous studies (Pieper and Korn, 2009), we have demonstrated the synergistic effect of the combined application of homolactic acid bacteria (*Lactobacillus plantarum* DSMZ 8862 and 8866) and molasses or partly neutralized formic acid (FA-NA; Amasil® NA of BASF AG) while ensiling nearly unfermentable feedstuffs. The pH-value, dry matter loss, volatile fatty acids and the fodder value indicated that untreated silages had very poor quality, whereas silage treated with LAB alone showed slightly improved values. Treatment with FA-NA alone and LAB in combination with molasses revealed improved silage quality as indicated by the above-mentioned measures. Only the combination of FA-NA and LAB enabled the production of high quality silages from the nearly unfermentable feedstuffs. The combination of LAB and molasses improved the results of FA-NA application alone regarding the energy content, protein quality and a reduced butyric acid formation. Hoedtke et al. (2011) and Söffing et al. (2012) demonstrated similar effects by combination of the same bacterial strains and the acid mixture Promyr NT 570 (>75 % formic acid and formate and <25 % propionic acid; Perstorp, Sweden). Continuing the experiments of Pieper and Korn (2009), the results of analyses of biogenic amines and clostridial spores ensiling the nearly unfermentable feedstuffs were determined.

Material and methods Alfalfa, rough blue-grass, creeping soft grass, perennial ryegrass and red clover of different dry matter content and maturity were ensiled in 1.5 L laboratory silos. Plant materials were mixed with soil containing clostridial spores to increase the potential of butyric acid production during ensiling (Table 1). Treatments were: Control, LAB (3 x 10^{11} cfu per t fresh matter (FM), *L. plantarum* DSMZ 8862 and 8866), FA-NA (3.25 – 4.5 l/t FM Na-formiate), LAB+FA-NA, LAB +molasses (35-40 kg/t FM), and a chemical preservative (Chem. Add.; Kofasil liquid, 3 l/t FM, 30% $NaNO_2$ and 20% Hexamethylenetetramine), respectively. All compounds were added separately to the harvested material. The fermentation characteristics were determined according to standard procedures; biogenic amines were extracted with perchloric acid and quantified with HPLC; clostridial spores were incubated under anaerobic conditions in DRCM-bouillon at 37°C for 3 days.

Table 1. Characteristics of feedstuffs and factors affecting ensiling process.

Feedstuff / Parameter	Alfalfa 1	Alfalfa 2	Rough blue-grass	Red clover	Cocks foot	Creeping soft grass	Peren. rye grass 1	Peren. rye grass 2
Treatment with clostridia cfu/g FM[1]	-	2.000	2.000	2.000	2.000	2.000	2.000	2.000
DM (g/kg)	255	188	205	186	191	208	201	181
Crude protein (g/kg DM)	16.0	23.5	18.4	18.5	19.1	16.8	15.8	16.9
Sugar (g/kg DM)	3.4	4.3	4.3	12.6	0.5	5.4	11.8	10.7
Buffering capacity[2]	5.5	7.9	5.3	4.4	5.3	5.0	6.0	7.2
Sugar/buffer. capacity	0.6	0.5	0.8	2.9	0.1	1.1	2.0	1.5
NO_3 (g/kg)	0.4	15.0	18.4	11.2	4.3	10.4	0.4	0.2
Epiphytic LAB (log cfu/g)	n.d.[4]	0.4	4.7	5.9	4.8	6.4	4.9	5.0
Experimental site [4]	AUL	SH	SH	SH	SH	SH	RIS	RIS

[1] clostridial spores containing sand (4.6 x 10^4 cfu/g), [2] g lactic acid/100 g DM, [3] not determined, [4] AUL- Landwirtschaftliches Zentrum Baden-Württemberg, D-88326 Aulendorf (Dr. H.-J. Nußbaum), SH- Landwirtschaftskammer Schleswig-Holstein, D-24768 Rendsburg (Dr. J. Thaysen), RIS- Landwirtschaftskammer Nordrhein-Westfalen, 47533 Kleve (Dr. M. Pries, Dr. K. Hünting). We thank the institutions for excellent collaboration.

Results Protein degradation was characterized using the parameters biogenic amines (decarboxylation) (Fig. 1a) and NH_3-N of total N (deamination) (Fig 1b). The lowest concentration of these metabolites and consequently the lowest protein degradation was observed with LAB+FA-NA. It was also established that decarboxylation of amino acids without simultaneous deamination is unlikely in silages with low dry matter content as revealed by correlation analysis of NH_3-N of total N and biogenic amines (Fig. 1c). This was also true when additional data from other experiments were included in the analysis. An effective suppression of clostridial growth was only achieved by the combination of FA-NA and LAB (Fig. 1d).

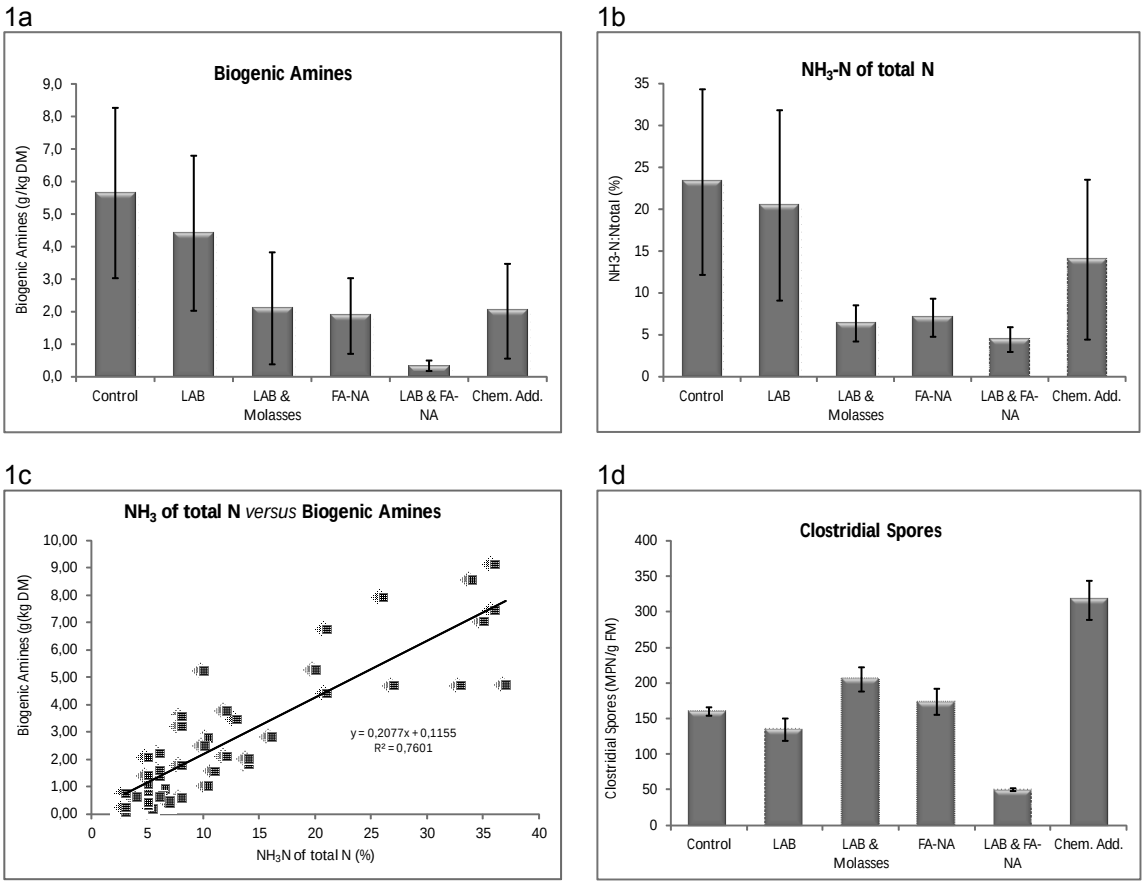

Figure 1. Biogenic amines (a), NH_3-N of total N (b), correlation of amines and NH_3-N of total N (c) and number of clostridial spores (d) in nearly unfermentable feedstuffs depending ensiled with different additives and their combination. Results are presented as means ± SD.

Conclusions The correlation analysis indicates that silages with low values for NH_3-N of total N (<7%) likely also contains low levels of biogenic amines (<5 g/kg DM), thus being an easy indicator for protein degradation in silage through both decarboxylation and deamination. Based on fermentation parameters, fermentation losses, the concentration of biogenic amines and clostridia, ensiling of nearly unfermentable feedstuffs appears easily achievable using the combination of *Lactobacillus plantarum* DSMZ 8862 and 8866 and buffered formic acid.

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Ammonia-N and α-amino-N in silage determined on either water extracts or solubilized freeze-dried samples

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Keywords: alpha-amino-N, ammonia, drying losses, freeze-drying, silage

Introduction Drying of silage samples causes losses of volatiles, of which ammonia is reported to disappear completely by oven drying at 60° C (Porter and Murray 2001) as well as partially by drying at 80° C (Sorensen 2004). Freeze-drying is commonly not assumed to cause losses of ammonia, although freeze-drying studies on silage generally report total weight losses rather than ammonia losses. Fecal samples are another sample category where ammonia losses would be likely to occur. Spanghero and Kowalski (1997) reported that 12% of total N content disappeared by freeze-drying dairy cow fecal samples. In research work it is usual that ammonia N is analyzed together with α-amino-N, which, although not being a volatile fraction, still could undergo changes during freeze-drying. The objective of this study was to assess the changes in concentration of ammonia N and α-amino-N when analyzed in water extracts obtained either from fresh material or from freeze-dried samples of whole silage.

Material and methods One batch from the primary growth from each of timothy grass (22.0 g N/kg DM) and red clover (33.2 g N/kg DM), respectively, was subjected to ten different combinations of wilting length (0-52 h) and target dry matter (130-750 g/kg) before ensiling in triplicates for 115 d in 4.5 L PVC silos. This created a variation in fermentation profiles, with the sum of acids and alcohols ranging from 11 – 221 g/kg DM, pH 4.38 – 5.50 and with NH_3-N constituting 14 – 207 g/kg N.

After silo opening, the samples were prepared for determination of solubles by the routine procedure of our lab. This comprises weighing 100 g fresh material into ziplock bags, addition of equal weights of deionized water, freezing, thawing and hydraulic pressing of juice from the bag after puncturing it. Parallel samples of whole silage were freeze-dried in a CD 8 freeze-drier (HETO, Birkerød, Denmark) and milled on a hammer mill to pass a 1 mm screen whereafter 1.2 g sample was weighed into a 50 mL conical plastic centrifuge tube with 40 mL of deionized water. The tubes were agitated for 60 min on a tube shaker/rotator with 360° vertical rotation at 21 rpm, centrifuged for 5 min at 1800 × g on a swing-out centrifuge and the supernatant was decanted to 7 mL tubes, subsequently transferred to Eppendorf tubes and centrifuged for 5 min at 13000 × g. The solubilized samples, as well as centrifuged juice samples from hydraulic pressing, were then diluted with deionized water at ratios 1:4 and 1:19 (v/v), respectively. Both sample types were analyzed for NH_3-N and α-amino-N on a Technicon AutoAnalyzer with phenol-hypoclorite and ninhydrin as main reagents (Broderick and Kang 1980). The results were recalculated to a per kg DM basis after DM determination at 60°C on the milled samples without employing any DM correction for losses of volatiles.

Results Average sample contents of NH_3-N were 2.96 g/kg DM (range: 0.32 – 7.88 g/kg DM) determined on pressed juice and 2.99 g/kg DM (range: 0.50 – 7.82 g/kg DM) from analysis on solubilized freeze-dried samples. Corresponding results for α-amino-N were 7.26 g/kg DM (range: 1.21 – 11.99 g/kg DM) determined on pressed juice and 8.32 g/kg DM (range: 2.16 – 12.66 g/kg DM) on solubilized freeze-dried samples. Regressions of pressed juice results (y) against solubilized freeze-dried sample results (x) on all 60 samples are presented in Figure 1. Slopes were different from 1 ($p < 0.001$ and $p < 0.05$ for NH_3-N and α-amino-N, respectively). The largest difference between methods for a treatment mean (n = 3) was 0.55 g kg/DM lower NH_3-N for freeze-dried samples than for pressed juice, corresponding to 7% of total NH_3-N content in the treatment. For α-amino-N, the largest difference was 1.69 g/kg DM less in pressed juice than in freeze-dried samples, equal to 25% of total α-amino-N in the treatment.

Discussion The moderately positive slope implies that ammonia N disappeared to a proportionally larger extent when concentration was high. However, this was not very pronounced. The absence of a treshold effect, with more or less complete disappearance above a certain ammonia concentration, may be due to that extensive acid production usually accompanies ammonia formation. This means that more anions for salt formation also would be available when ammonia levels increase.

The larger N losses (and hence ammonia losses) found from freeze-drying fecal samples (Spanghero and Kowalski 1997) are probably explained by the compared to silage much higher pH of fecal samples, typically >7 for high yielding cows (Mgbeahurike 2007). Since each pH unit equals a factor 10, equilibrium in a silage sample would favour the ionized and non-volatile ammonium form by about 10^3 compared to a fecal sample.

There was no obvious explanation for the apparent increase in α-amino-N values after freeze-drying. A possibility is that oligopeptides during freeze-drying to some extent are cleaved to free amino acids and shorter peptides that will give response in the ninhydrin assay. There is also a larger degree of uncertainty involved in α-amino-N determination than in NH_3-N determination with the current method, because the result is obtained after deduction of the ninhydrin response to ammonia.

Conclusions The results suggest that only minor losses of ammonia occur during freeze-drying of timothy/red clover silage with the characteristics described here. The α-amino-N values increased by freeze-drying.

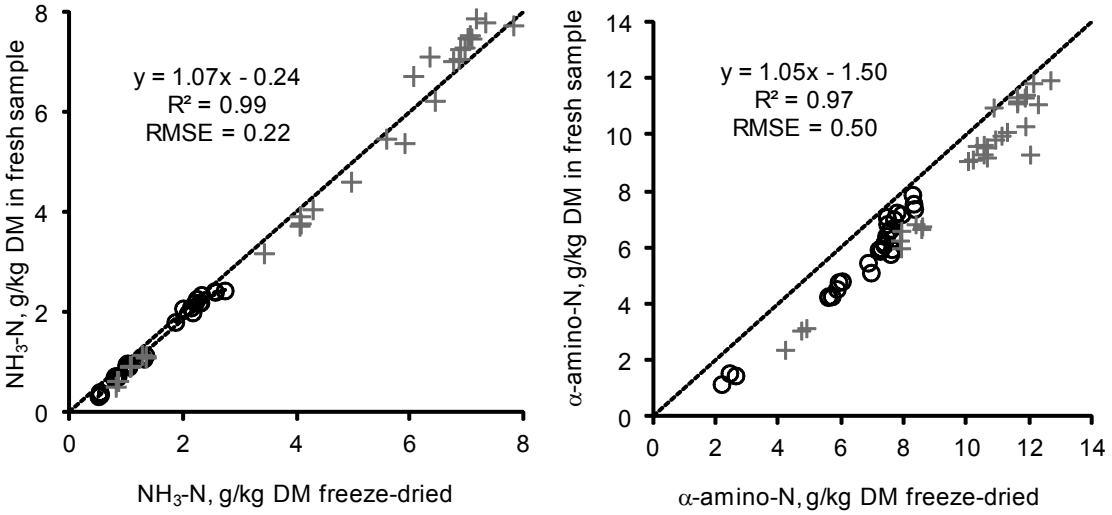

Figure 1. Concentrations of silage N fractions determined in water extracts obtained either from fresh samples or from freeze-dried and milled samples of timothy (**O**) or red clover (**+**). Dashed line represents y = x. RMSE = Root Mean Square Error. N = 60.

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Evaluation of some aspects of in situ and in vitro techniques in ruminant feed evaluation

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Keywords: digestibility, digestion rate, feed evaluation, gas production, in situ, in vitro

Introduction In situ and in vitro methods have been developed with the primary aim to estimate digestibility of feeds for diet formulation in ruminant livestock production systems. The objective with this study was to compare digestion rate (k_d) of NDF for different feeds estimated with the in situ method or derived from an automated gas in vitro system. Further, in vitro true digestibility of the feed samples incubated in filter bags or freely floated was compared, and k_d for insoluble and soluble components of those feeds were estimated. In addition, proportion of methane to total gas of the samples was measured in the automated gas in vitro system.

Material and methods Four different concentrates and four forages were used in this study. Samples of barley, oat and rapeseed meal were collected from AB Västerbottens Fodercentral in Umeå, Sweden. A sample of dried sugar beet pulp shreds was from the commercial feed Betfor® (Nordic Sugar A/S, Copenhagen, Denmark). Samples of 2 grass silages harvested on June 21 and July 21 in 2010 from a mixed sward of timothy and meadow fescue at Röbäcksdalen research farm in Umeå, and hay from a timothy dominated sward harvested in July 2010 were included in this study. Further, lucerne harvested on July 3 in 2009 in early flower stage at the farm of Institute of Animal Science in Prague-Uhříněves was also used. Two lactating Swedish Red cows fed a diet of 60% grass silage and 40% concentrate on DM basis were used for in situ incubations and for collection of rumen fluid. In situ nylon bags (pore size 38 µm) were introduced in reverse sequence into the rumen of each cow and incubated for 3, 6, 12, 24, 36, 48, 72 and 96 h for determination of k_d of NDF. The 96 h incubation was used for forages. Triplicates of zero hour samples were determined for all feeds. Additional kinetic data of the feeds were produced from isolated forage NDF and intact samples subjected to in vitro incubations in which gas production was automatically recorded for 72 h. Samples were weighed directly in the bottles or in F57 filter bags (for fiber and in vitro studies; Ankom Technology Corp., Macedon, NY) that were placed in the bottles. Feed samples were analyzed for chemical composition and in vitro residues for NDF and ash. Further, gas samples were drawn from each bottle at 24 and 48 h of the incubation, and methane was determined by gas chromatography. Total gas was recorded by the automated in vitro gas system.

Results and discussion Digestion rate of NDF estimated with the in situ method was higher across all feeds than when derived from the automated gas in vitro system ($P<0.01$; Table 1). The interaction between feed and method was significant ($P<0.01$; Table 1); k_d of NDF for grass hay tended ($P=0.06$) to be lower, while k_d of NDF for lucerne, barley grain, rapeseed meal and dried sugar beet pulp was higher ($P<0.01$) estimated with the in situ method than when derived from gas production recordings. Digestion rates of the intact feeds and of feed neutral detergent solubles were lower for all feeds, except for the hay, when samples were incubated in the bags compared to freely floated ($P<0.01$; Table 1). Significant ($P<0.01$; Table 1) interactions between incubation method and feed indicated that k_d was equal or lower for feed NDF incubated in bags compared to freely floated except for the first cut grass silage, the barley and the oat. This was explained from the lower asymptotic gas volumes when isolated NDF was incubated in bags compared to as free samples, with a concomitant faster digestion of this smaller fraction (data not presented). The proportion of methane to total gas was not different between feeds ($P\geq0.34$; Table 1) and more methane of total gas was produced for the feeds incubated in bags compared to free samples ($P<0.01$; Table 1). This despite less OM and NDF were digested for all feeds when incubated in bags than freely floated ($P<0.01$; Table 1). Measured proportion of methane to total gas at the 2 different time points gave similar results.

Conclusions There were highly significant interactions between feeds and methods, irrespective if the bags were incubated in situ in the rumen or in the in vitro bottles. Interactions in k_d could be related to secondary particle loss from the bags, especially from some concentrate feeds, during the ruminal incubation or from altered microbial activity inside the bags in vitro. It is concluded that incubating feeds in bags in situ and in vitro can result in biased estimates of digestion kinetic parameters.

Table 1. Digestion rate (kd) of NDF determined in situ or derived from in vitro gas production profiles, and kd, CH4:Total gas, and in vitro digestibility of OM (OMD) and NDF (NDFD) of samples incubated freely floated or in filter bags.

	Lucerne	Grass silage[2] 1	Grass silage[2] 2	Grass hay	Barley grain	Oat grain	Rapeseed meal	Sugar beet pulp	SE	*P*-value[1]		
										Feed	Method	F × M
In situ k_d of NDF (%)	11.7	5.8	5.5	3.8	16.3	3.6	16.6	11.8	0.73	<0.01	<0.01	<0.01
Gas in vitro k_d of NDF (%)	7.7	5.9	6.2	6.0	5.3	2.8	9.9	6.6	0.73	<0.01	<0.01	<0.01
Freely floated												
k_d of DM (%)	11.8	7.9	7.3	7.1	7.6	9.3	9.5	12.2	0.31	<0.01	<0.01	<0.01
k_d of NDF (%)	7.7	5.9	6.2	6.0	5.3	2.8	9.9	6.6	0.64	<0.01	0.15	<0.01
k_d of NDS (%)	25.8	16.4	9.5	9.8	14.9	21.0	12.9	21.3	0.84	<0.01	<0.01	<0.01
CH_4: Total gas at 24 h (%)	14.6	11.6	12.0	13.3	14.3	15.0	14.7	14.3	1.98	1.00	<0.01	0.86
CH_4: Total gas at 48 h (%)	15.0	12.9	13.6	13.8	14.6	15.6	16.5	14.4	1.43	0.34	<0.01	0.82
In vitro OMD (%)	76.6	87.7	84.8	76.5	91.6	81.0	88.8	97.3	1.12	<0.01	<0.01	<0.01
In vitro NDFD (%)	52.0	81.3	76.9	65.2	64.9	46.4	52.0	92.4	2.24	<0.01	<0.01	<0.01
In filter bag												
k_d of DM (%)	8.1	6.3	6.0	6.9	3.5	6.6	6.3	4.6	0.31	<0.01	<0.01	<0.01
k_d of NDF (%)	6.8	9.8	7.5	6.0	7.5	6.9	5.3	4.3	0.64	<0.01	0.15	<0.01
k_d of NDS (%)	8.3	8.0	5.7	10.0	8.0	8.3	10.4	8.5	0.84	<0.01	<0.01	<0.01
CH_4: Total gas at 24 h (%)	20.3	20.3	20.8	20.3	19.1	18.9	19.5	19.9	1.98	1.00	<0.01	0.86
CH_4: Total gas at 48 h (%)	21.5	18.7	18.7	20.0	17.1	18.8	21.3	18.9	1.43	0.34	<0.01	0.82
In vitro OMD (%)	72.6	68.5	57.1	58.7	84.4	71.5	83.9	91.1	1.12	<0.01	<0.01	<0.01
In vitro NDFD (%)	43.8	51.8	34.7	38.9	34.8	19.5	30.9	74.9	2.24	<0.01	<0.01	<0.01

[1]Probability of a significant effect of feed, method, and interaction between feed and method (F × M) analysed using the GLM procedure in SAS (2002-2003).
[2]Grass silage 1 and 2 were harvested as a first and second cut, respectively.

Effects of corn silage sample handling on fermentation parameters

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Keywords corn silage, fermentation, sample handling

Introduction Field nutritionists submit samples to a laboratory for analysis prior to ration balancing. Many times, samples must be shipped to the laboratory as laboratory services may not be readily available nearby. Samples may arrive at the laboratory anywhere from a few hours post-collection to a few days post-collection. There is no standardized protocol for silage sample handling between sample collections until they arrive to the laboratory. University Extension Service bulletins (Barnhart, 2010; Garthe and Zummo, 1990) are available regarding the taking of representative silage samples, but provide no supporting data for their recommendations. Cotanch et al. (2009) reported on the type of bag to place the sample for mold and yeast count. Alomar et al. (1999) evaluated the effect of freezing and grinding method on near infrared spectra variation and chemical composition of fresh silage. Kung and Shaver (2001) suggest freezing samples for chemical fermentation analysis immediately after being taken, but provide no supporting data for their recommendation. We are not aware of any study available to quantify how post-collection sample handling affects wet chemical analytical results in terms of fermentation parameters. Therefore, a study was undertaken to determine the effect of silage sample handling on fermentation parameters measured by wet chemistry at a commercial laboratory.

Material and methods Corn silage samples were collected at a single farm in mid-August 2011. Eleven silage storage bags (with an approximate storage capacity of 750-800 metric tons each) were sampled with a silage probe approximately 15 m from the south end of the bag. Each individual silage bag sample was thoroughly mixed and subdivided into 6 sub-samples that were treated in each of the following manners: 1) Silage sample placed in air tight bag and ice immediately, taken to laboratory within 3.5 hrs (ICE); 2) Silage sample placed in air tight bag, taken to laboratory within 3.5 hrs (NO-ICE); 3) Silage sample placed in air tight bag, taken to post office for overnight delivery (24H); 4) Silage sample placed in air tight bag, iced, taken to freezer overnight and taken to post office for overnight delivery (48HFZN); 5) Silage sample placed in air tight bag, kept at room temperature in garage and taken to post office for overnight delivery 48 h post-sampling (48H); and 6)Silage sample extracted 10:1 with distilled water in the field and iced immediately, taken to laboratory within 3.5 hrs (EXT).

The field extraction procedure was as follows: after weighing the sample, it was brought to the final weight with distilled water to achieve a 10:1 dilution. Sample was stirred for 15 sec manually with a chopstick, allowed to rest for 1 min prior to filtering through 2 layers of cheese cloth. This differed from the laboratory extraction were the sample was stirred with a laboratory blender.

All 66 silage samples were analyzed at a properly accredited commercial laboratory (Rock River Laboratory, Inc., Watertown, WI) for: dry matter, pH, lactic acid, acetic acid, butyric acid and propionic acid. From these results, the following calculations were made: total VFA, lactic acid as a proportion of total VFA (Lac:VFA) and the ratio of lactic acid to acetic acid (Lac:Acetic). Statistical analysis was performed using SAS (1990) with a model containing terms for silage bag number and treatment. Separation of treatment means was conducted using Tukey-Kramer's test.

Results and discussion Dry matter content was similar regardless of sample handling. There were significant differences in pH ($p<0.002$). Field extraction resulted in the highest pH value, while 48HFZN was the lowest. There was a significant difference between 48HFZN and 24H, which does not support the recommendation provided by Kung and Shaver (2001) of freezing samples immediately after collection. There were no pH differences among the other three treatments. There were significant differences ($p<0.03$) in propionic acid content. Field extraction yielded the highest value while there were no differences among the other five treatments. There were statistical differences for butyric acid ($p<0.002$). Field extraction was not able to extract any butyric acid as it was not detectable during testing. There was a tendency ($P<0.10$) for NO-ICE to increase butyric acid compared to 24H. Field extraction was lower ($p<0.05$) than NO-ICE and 48HFZN for LAC:VFA. Field Extraction was lower ($p<0.10$) than ICE, 24H and 48H for LA:VFA. There were no statistical differences among treatments for lactic acid ($p>0.89$), acetic acid ($p>0.99$) and total VFA ($p>0.99$).

Data from this experiment do not support field extraction as a viable alternative for measuring fermentation parameters. Furthermore, data indicates that frozen samples arriving at the laboratory after 24 h post-collection are subject to decreases in pH. Finally, data indicates that upon aerobic exposure, when the sample is not kept cold, there is a change in the content of butyric acid from 24 to 48 h

post-collection. Corn silage samples should arrive to the laboratory within 24 h post-collection in order to avoid changes in the results of fermentation parameters and to allow for proper interpretation of the analytical results.

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Table 1. Effect of silage sample handling on fermentation parameters measured by wet chemistry.

	Experimental Treatment							
Item	ICE	NO-ICE	24H	48HFZN	48H	EXT	P	SEM[1]
N	11	11	11	11	11	11		
Dry matter, %	37.81	38.66	38.92	37.42	37.54	*	0.468	.721
pH	3.86^{b,c}	3.83^{b,c}	3.87^{b}	3.80^{c}	3.84^{b,c}	3.93^{a}	0.002	.021
Lactic acid, %	2.53	2.62	2.42	2.60	2.46	2.43	0.894	.156
Acetic acid, %	.652	.679	.654	.659	.641	.671	0.991	.042
Propionic acid, %	0.117^{b}	0.004^{b}	0.096^{b}	0.085^{b}	0.113^{b}	0.226^{a}	0.024	.05
Butyric acid, %	0.067^{a,y}	0.079^{a,y}	0.052^{a,z}	0.065^{a,y}	0.056^{a,y}	0.000^{b}	0.001	.009
Total VFA[2], %	3.37	3.39	3.22	3.41	3.27	3.37	0.997	.272
Lac:VFA[3], %	75.41^{z}	77.67^{b}	75.17^{z}	76.73^{b}	75.20^{z}	72.32^{a,y}	0.048	1.17
Lac:Acetic[4]	4.49	4.50	4.20	4.57	4.42	3.93	0.189	.193

[1] Standard error of the mean [2]Total VFA = Total volatile fatty acids [3]Lac:VFA = Percentage of the total volatile fatty acids represented by lactic acid [4]Lac:Acetic = Ratio of lactic acid to acetic acid

[a,b,c] Means with unlike superscripts in the same row differ P<0.05

[y,z] Means with unlike superscripts in the same row differ P<0.10

*DM for the ICE treatment was used to calculate values on a DM basis

Silage Analysis- Comparison of 58 Welsh farm silages analysed either by traditional wet chemistry or Wet NIRs

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Keywords: analysis, clover, grass, NIRs, silage, wet chemistry

Introduction Silage analysis in the UK is based on wet near infra red spectroscopy (NIR) which relies on analyses of silages of known quality as the reference point. Consultants working across Wales have become increasingly concerned that the NIR analysis is not giving reliable estimates of the crude protein concentrations in silages containing significant proportions of white or red clover. These silages would be expected to contain in the range of 14-18% protein, but many NIR analyses are indicating levels of 10-14%. This is leading to over supplementation with costly protein concentrates. The reasons for this perceived poor analysis is likely to be due to the data base of standard silages not being sufficiently comprehensive or representative to include high clover silages which have become incorporated into many farm silage making practices today with in the original calibration work. The aim of this study was to assess the accuracy of fresh (wet) NIRs silage analysis compared to conventional wet chemical methodologies. In order to obtain a range of silages 58 samples were collected from commercial farms in Wales. The samples were collected both from bale and clamp silages and from grasses that contained different levels of clover.

Material and methods Silages were selected to provide both baled and clamp silages and a range of cuts through the season. In total 25 farms were visited within a 24 h period between 3 pm on the 3rd Jan 2011 and 3 pm the following day. In total 58 silages were collected. Upon collection the samples were well mixed and divided into two portions, one for wet chemical analysis by standard analytical methods the other for wet NIR analysis. The samples were delivered to the laboratory by 1 pm on the 5th Jan. Wet chemical analysis included pH, dry Matter, crude protein, ammonia-N, lactic acid and neutral detergent fibre. The NIRs analysis gave the full chemical analyses. Data were analysed using linear mixed models (the REML procedure of Genstat 13th Edition). Analysis type (wet chemistry or NIR) was the fixed effect, and sample number was the random effect. Initially, all data were included in the data analysis; subsequent analyses separated first and second cut silages (of both silage types), and baled versus clamped silages (of both cuts). The wet chemistry results were regarded as the 'absolute' values, and the effects of NIR analysis on the data were tested for significance. Statistical significance was declared at $P < 0.05$.

Results A range of different silages were table 1 shows the mean silage analysis. The silages as assessed by NIR analysis had the following composition had a mean DM of 41% (range 20.7 – 86%), mean pH 4.61 (range 3.50 – 5.63), Crude protein 13.4% (range 8.1% – 19.4%), NDF 52% (Range 37% – 64). Table 2 shows the comparison of means for the two analytical methodologies.

Significant differences in mean composition were observed for pH, crude protein and lactic acid. NIR analysis under-predicted pH by an average of 0.48 units, and under-predicted CP concentrations by an average of 22 g/kg DM. Lactic acid concentrations, on the other hand, were over-predicted by NIR by an average of 15 g/kg DM. No significant differences in mean DM contents, ammonia-N or NDF concentrations were found.

Table 1. Indicating mean (and range) silage fresh NIR analyses for all 58 silages sampled.

	Mean	Maximum	Minimum
DM (g/kg FM)	412.20	860.60	207.90
pH	4.61	5.63	3.50
D-value	68.23	78.40	54.57
Crude protein (g/kg DM)	134.82	194.80	81.66
NDF (g/kg DM)	521.93	641.14	371.59
ADF (g/kg DM)	348.58	593.73	184.15
ME (mJ/Kg)	10.92	12.54	8.73
Ash (g/kg DM)	77.98	106.71	46.45
WSC (g/kg DM)	58.37	202.84	2.01
Lactic acid (g/kg DM)	66.26	187.78	7.55
Acetic acid (g/kg DM)	18.02	56.41	5.00
Butyric acid (g/kg DM)	6.18	23.66	3.00
Ammonia-N (% TN)	9.69	28.40	1.08

Table 2. Predicted means and effects of analysis method for all silages in the study (n=58).

	Analysis method				
	Wet Chemistry	NIR	SED	Effect	P
Dry matter, g/kg	422.4	412.2	9.11	-10.3	0.265
pH	5.09	4.61	0.091	-0.48	<0.001
Crude protein, g/kg DM	157.1	134.8	3.75	-22.3	<0.001
Ammonia-N, g/kg DM	8.47	9.69	0.764	1.22	0.116
Lactic acid, g/kg DM	51.2	66.3	3.90	15.1	<0.001
Neutral detergent fibre, g/kg DM	508.0	521.9	8.96	14.0	0.125

Conclusions The results shown indicate that there is a significant problem with the use of wet NIR prediction of silage composition, particularly in the case of crude protein which across all silages was underestimated by 22 g/kg DM. There were also significant differences, depending on silage selection pool (ie, clamp or bale, first or second cut), with all the analyses except NDF which at times is very close to being significantly different. For these reasons, it is somewhat of an arbitrary exercise to calculate the costs associated with these poor analyses as so much must to be done to rectify the situation. Interestingly most of continental Europe uses dry NIR not wet NIR for their analysis due to the unreliability of the wet NIR methodology, where there is likely to be greater heterogeneity in the sample, which is removed by the drying and grinding process used in the dry NIRs methodology.

Acknowledgements This project has received funding through the Rural Development Plan for Wales 2007 - 2013 which is funded by the Welsh Government and the European Union.

Dry matter determination in silage samples with freeze-drying or oven drying with or without correction for volatile losses

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Keywords: Drying losses, freeze-drying, Norfor, silage

Introduction Dry matter (DM) concentration of a feed sample represents the non-water proportion, which in routine analysis is usually determined with oven drying to constant weight. In silage samples DM determination is compromised by volatile compounds, which are nutritive constituents of DM but are partially or totally lost in oven drying. Various correction equations are in use to compensate for the volatile losses, either based on the volatilization coefficients and the concentration of the respective volatile compound, or empirically based on oven DM because the concentration of volatiles and hence also drying losses generally decrease with increasing DM. Direct use of the freeze-dried weight is an alternative means of obtaining a DM value. This paper reports DM concentration of grass and clover silage samples with freeze-drying compared to five different oven drying methods.

Material and methods The primary growths of one pure stand of timothy grass and one pure stand of red clover were subjected to ten different combinations of wilting lengths (0-52 h) and target DM (130-750 g/kg) and ensiled without additives in triplicates (N = 60) for 115 d in 4.5 L PVC silos. At silo opening, pH range was 4.38 – 5.50 and HPLC analysis on water extracts gave the following range for the concentration of volatiles (g/kg DM as determined by freeze-drying (FDM)): lactic acid 0 – 109; succinic acid 0 – 32; acetic acid 0.5 – 10.7; propionic acid 0.0 – 4.2; butyric acid 0.0 – 19.0; ethanol 2.5 – 21.5 and 2,3 butanediol 0 – 41. Silages were dried in a CD 8 freeze-drier (HETO, Birkerød, Denmark), promptly weighed after removal from the freeze-drier to get FDM, equilibrated with ambient air, weighed again, milled and oven dried. Oven drying was done according to the current standard procedure for forage samples at our laboratory, where the milled sample is dried at 60°C overnight, weighed to achieve a NorFor DM (Åkerlind et al., 2011) and dried at 103°C overnight to get a DM value that is compatible with our previously used routine.Total DM was calculated as: equilibrated weight proportion × oven dried weight proportion and corrections were applied as displayed in the Result section. The weight loss between 60 and 103°C was compared with values calculated from the volatility coefficients of Porter & Murray (2001).

Results Linear regressions (Table 1) with freeze-drying DM as y values against the oven-drying methods (x) yielded significant intercepts and also slopes different from 1, except for the Norfor advanced equation. The mean prediction error (MPE) reflects the relative difference between methods in DM estimate (g DM/kg DM), although it cannot be stated that DM determined by freeze-drying is more correct than by any of the other methods, because no measurements of water content in dried residues were performed. However, oven drying at 60°C without correction had the smallest MPE and oven drying at 103°C without correction had the highest. Oven drying at 60°C gave DM values very similar to freeze-drying, while continued drying to 103°C resulted in a further loss of 72 g ± 8 g DM/kg DM (Figure 1). Even if these losses diminished with increased silage DM, applying the volatility coefficients of Porter and Murray (2001) for lactic acid and total volatile fatty acids (VFA) still left a disappearance of 50 – 80 g DM/kg DM unexplained.

Discussion The comparable results between freeze-drying and oven drying at 60°C suggests loss of volatiles also from freeze-drying. The weight loss of 72 g/kg DM between 60 and 103°C is considerably larger than the 28 – 30 g that Porter & Murray (2001) reported. It is also more than the disappearance of 58 g DM/kg DM that Dewhurst et al. (2003) found, when comparing oven drying at 130°C and freeze-drying for a range of grass and legume silage samples. Increased disappearance of sugars with increased drying temperature is previously reported (Jones, 1962), but it is unclear if this really would lead to DM loss or if the material is recovered in the bulk DM.

Conclusions Weight losses were similar with freeze-drying and oven drying at 60°C. Further drying at 103°C gave weight losses of 50-100 g/kg DM. Assessment of the proportion between water and volatiles in the weight loss requires analysis of volatiles and water determination in residues from freeze-drying and oven drying. Applying the Norfor advanced equation resulted in DM estimates slightly higher than direct weighing from the freeze-drier.

Table 1. Linear regressions for DM value obtained by freeze-drying determinationson 60 silage samples against DM from different oven drying methods. RMSPE = Root Mean Square Prediction Error; MPE = Mean Prediction Error.

Method	RMSPE g /kg FW	MPE, g /kg DM	Intercept, g/kg FW	Slope	R^2
DM = Oven drying 60°C residue	3.1	8.1	3.6	0.996	1.000
DM = Oven drying 103°C residue	30.4	86.9	9.3	1.055	0.999
DM = Oven drying 103°C residue + 14 g/kg (Lingvall and Ericson 1981)	17.9	49.1	-5.5	1.055	0.999
DM = Oven drying 60°C residue + analyzed volatiles × coefficients (Norfor advanced equation; Åkerlind et al. 2011)	6.7	17.5	-4.5	0.997	1.000
DM = (Oven drying 60°C residue) × 0.99 + 10 g/kg (NorFor simple equation; Åkerlind et al. 2011)	4.7	12.4	-6.9	1.007	1.000

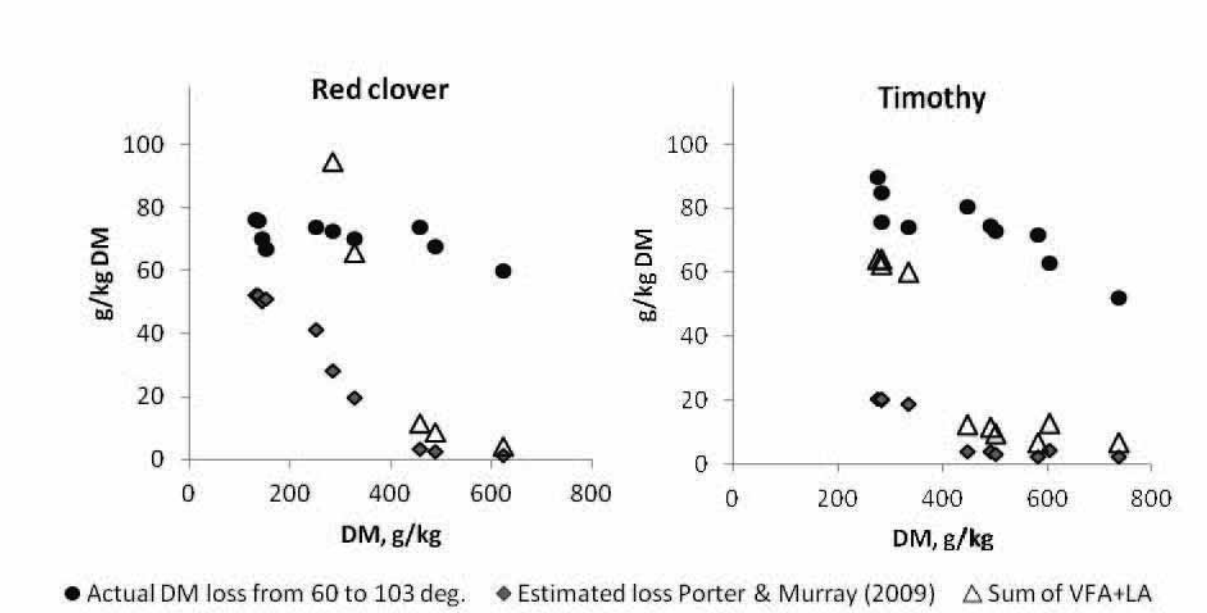

Figure 1. Actual weight loss from silage samples during drying from 60 to 103°C. Sample contents of lactic acid (LA) and volatile fatty acids (VFA) are displayed as well as estimated loss when applying the coefficients of Porter and Murray (2001). All values are expressed on a DM basis determined with freeze-drying. Each point is the mean of 3 observations.

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Lignin analyses in timothy (*Phleum pratense*) clones of different digestibility

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Keywords: digestibility, lignin amount, lignin quality, timothy

Introduction Grass silage is the most important source of metabolised energy in milk and beef production. The goal in forage production is to obtain high herbage mass with high nutritive value. Lignin content in grasses increases with maturity, as the need for structural strength increases during stem elongation. This reduces digestibility of cell wall polysaccharides as lignin makes them inaccessible to rumenal enzymes that would normally digest them. Also cross-linkages between ferulic acid bound to hemicellulose arabinoxylan exist further impeding the digestion of cell wall polysaccharides. The aim of this work is to evaluate whether it is lignin amount and/or quality that leads to different digestibility in different timothy (*Phleum pratense*) clones. One aim is also to develop analytical methods that could be later utilised for quick analysis of plant material for digestibility.

Material and methods Timothy clones that have either very low or high digestibility in relation to their stem proportion were used as a material (material rights: Boreal Plant Breeding; called from now on as different digestibility groups). The clones were cultivated in field conditions in MTT Maaninka in East Finland. Clones were collected at three different developmental stages (45, 56 and 58 according to Simon and Park 1981) during mid-June 2011. This time corresponded to time of spring harvesting. Their lignification stage was observed by microscopy using Alcian blue–Safranin O staining. As different quantitative methods are known to give different results (Hatfield and Fukushima 2005) we compared several methods for lignin determination (acetyl bromide (AcBr), acid detergent (ADL), acid dioxane, Klason, permanganate) using extractive-free stem powder (alcohol insoluble residue, AIR) of Tammisto II cultivar as a material (leaf sheaths removed). Clonal material (stems and leaf sheaths separately) was then analysed with the AcBr method (Klason lignin and acidic dioxane lignin as standards; Hatfield et al. 1999). FTIR spectra were run from lignins prepared with different methods and from the original plant powder.

In vitro organic matter digestibility (OMD, cellulase; Nousiainen et al. 2003) and neutral detergent fibre (NDF) were assayed from the same clonal material for the corresponding developmental stages at MTT. The data was subjected to analysis of variance where phenological stage and digestibility group and their interaction were fixed effects and clones were considered as a random factor.

Results and discussion In accordance to literature information (e.g. Hatfield and Fukusima 2005) different lignin quantitative methods gave different results. Klason lignin and AcBr lignin (with acid dioxane standard) methods gave 3.5 to 6 times higher lignin concentrations than ADL, permanganate lignin and acid dioxane lignin methods (Fig. 1). The effect of phenological stage was evident for tiller weight, tiller height, NDF, OMD and digestibility value (D-value) in the clone material (Table 1). The samples of high digestibility group had lower NDF and higher OMD and D-values. The differences between groups in OMD and D-value were more evident at development stage 58 than at stage 45. AcBr method (both Klason lignin standard and acid dioxane lignin standard) was chosen for lignin analyses of clonal samples as it requires only a small amount (5 mg) of plant material. Neither the phenological stage nor the digestibility group had any effect on lignin concentration in NDF of whole tiller or in AIR of stem. Lignin concentration in AIR of leaf sheaths was higher in tillers at stage 58 compared to tillers at stage 45. In general, lignin content of AIR of leaf sheaths was higher than that of stems. However, there was no difference between the digestibility groups. The OMD correlated negatively with NDF (-0.93, $p < 0.001$), stem height (0.91, $p < 0.001$) and lignin in sheath AIR (0.68, $p < 0.015$). Leaf sheath is an important structural support for the growing stem. The systematic difference between phenological stages 45 and 58 as well as between the high and low digestibility groups in OMD and D-value but not in AcBr lignin content of stem AIR may be partly explained by the procedure: in lignin analyses stems and leaf sheaths were analysed separately and in OMD, NDF and D-value analyses they were analysed together. The results suggest that stem NDF was not as lignified as NDF in leaf sheaths. It must be underlined that the data set is limited and thus further evidence is needed. At next step we will assay lignin quality by copper oxidation method (Goni and Montgomery 2000) as the lignin subunit composition may vary.

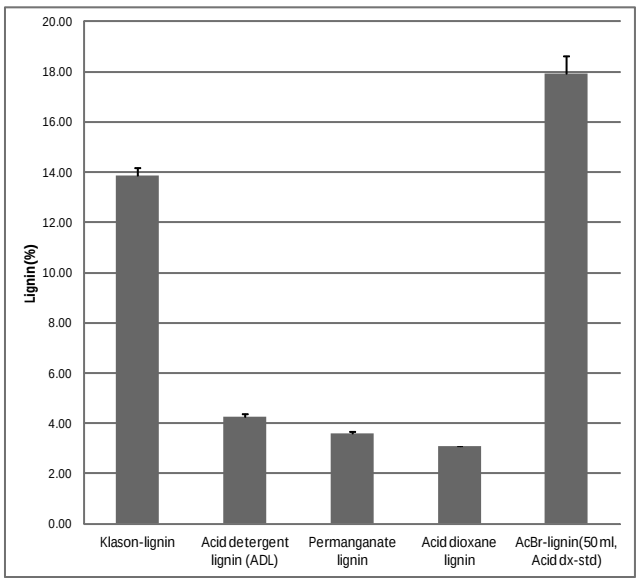

Fig. 1. Lignin concentration in stems of Tammisto II cultivar by using several quantitative methods.

Table 1. NDF and AcBr lignin (Klason standard) content of high or low digestibility timothy clones at two phenological stages.

Digestibility group	Stage[1]	Tiller DM weight,	Stem height,	NDF[2]	OMD[2]	D-value[2]	Lignin in NDF,	Lignin in stems,	Lignin in leaf sheaths,
		mg tiller^{-1}	cm	--------	g kg^{-1} DM	--------	g kg^{-1} NDF	g kg^{-1} AIR	g kg^{-1} AIR
High	B	382	35	641	705	653	168	101	120
Low	B	296	36	670	685	632	176	106	135
High	FH	606	47	660	664	615	163	97	134
Low	FH	652	52	695	614	568	166	102	141
SEM		38.1	1.8	3.6	7.9	7.4	7.2	6.4	4.6
P values									
Phenological stage		<0.001	<0.001	<0.001	<0.001	<0.001	0.37	0.53	0.004
Digestibility group		0.67	0.22	0.004	0.026	0.024	0.49	0.48	0.15
Stage x group		0.107	0.17	0.43	0.031	0.043	0.76	1.00	0.084

1) Phenological stage B = booting, FH =full heading; 2) stem + sheath, 3) AIR = alcohol insoluble residue

ConclusionsLignin quantitation methods vary in the principle how lignin is assayed. As results obtained with various methods clearly differ from each other the method used should always be mentioned when reporting the results. AcBr method is a suitable quantitative method to determine lignin in small amounts (<50 mg) of grass material. Our results underline the importance of the structure and digestibility of leaf sheaths on digestibility of timothy tillers.

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Optimizing silage harvesting with an intelligent machinery control system

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Keywords: automation, forage harvesting, fuzzy logic, loader wagon, precision agriculture

Introduction The agricultural industry has striven to improve functions and features of their products by researching the human-machine interface. Control and automation can increase the efficiency of agricultural machines, which is a strong selling feature. For example, forage harvesting with a tractor and loader wagon often requires many observations and simultaneous corrections or adjustments by the driver. The ISO11783 (a.k.a. ISOBUS) standard for communication between tractors and agricultural implements provides a common platform for implementing new control systems and accelerates their adoption.

Maintaining the optimal load of loader wagon feeding unit can prevent problems such as overloading, blockages, or unevenly chopped grass from underloading. The chop length is an important factor, which can affect silage quality and the performance of feeding equipment (Suokannas and Nysand 2008). The use of the precise amount of additive applied at the harvesting phase requires continuous monitoring by the driver. This paper presents a solution for optimizing the forage harvesting process with an intelligent control system developed for controlling the driving speed of the machine combination and the dosing of silage additive. In this study the test tractor had an ISO11783 class 3 TECU (Tractor electronic control unit) and an ISO11783 bus was fitted to the loader wagon.

Material and methods The research platform was based on an evaluation version of a Valtra T132 tractor equipped with a continuous power transmission and a Krone ZX 45-GL loader wagon equipped with hydraulic suspension. The swath cross-sectional area was measured with a laser scanner in front of the tractor and the moisture of the forage with a NIR (near infra-red) sensor fitted to the front wall of the wagon. The weight of the load was measured with three pressure sensors, two in the rear axles hydraulic circuits (160 bar) and a third at the front of the wagon (250 bar). The position of the pick-up unit of the wagon was monitored with a sensor, and the speed of the scraper floor was measured with a pulse sensor. The additive pump was equipped with its own ECU (electronic control unit) and a PID (Proportional-Integral-Derivative), which is the most widely used feedback controller. The additive was applied through four nozzles situated in the fixed boom above the pick-up. The loader wagon ECU estimated mass flow of forage using a Kalman filter based on three inputs: volume flow of swath, total mass of collected forage in the wagon and the density of forage in the swath. All measurements were delivered via an ISOBUS installed in loader wagon.

The weighing systems were calibrated with concrete weights varying from 1000 to 8000 kg and a NIR -sensor with oven-dried grass samples varying from 15 to 50 % DM. The harvesting trials were done mainly in two fields: a uniform 5 ha field in second cut of mixed timothy *(Phleum pratense)* and meadow fescue (*Festuca pratensis)* sward and a 2 ha field in second cut of red clover *(Trifolium pratense)*. The forage was mown with a 3.2 m mower conditioner. After wilting for 3 to 24 hours, it was windrowed in widths of 9 to12 m.

Results and discussion When the area of the swath decreased, the speed of the tractor was increased (Fig.1) and the target of 30 kg s^{-1} constant mass flow was achieved with relatively low variation in the measured mass flow; average measured mass flow was 29.1 kg s^{-1}.

The control system kept the mass flow of grass in the feeding unit constant and optimal regardless of the swath area and mass, thus preventing blockages, improving work efficiency and quality of grass chop. The control of additive application prevents too low or too high consumption (Fig. 2). The optimized process is easier to operate.

The NIR moisture readings deviated between -6.17 and 5.49% from the oven-dried samples. Research by Thurner et al. (2011) found the absolute deviation for the DM content in online measurement in self-propelled forage harvester for system A (NIR sensor) of between -0.97 and -6.81 % and for system B (dielectric conductivity and temperature of the crop) between +0.46 and -6.57 % when compared with the reference values.

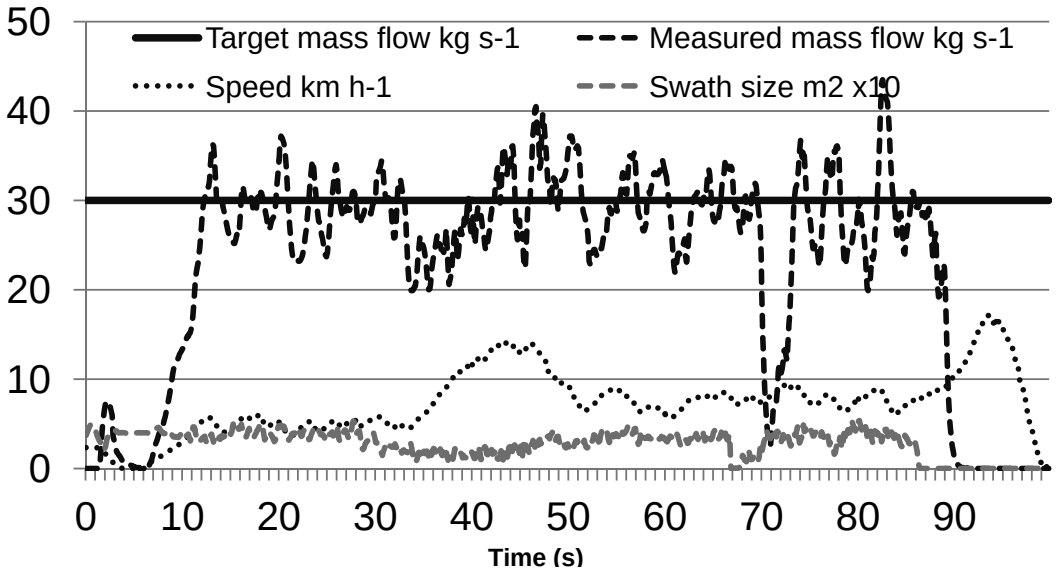

Figure 1. Optimized speed control based on mass flow of grass. Notice the scale of the swath area is multiplied by 10 to distinguish it from the X-axis.

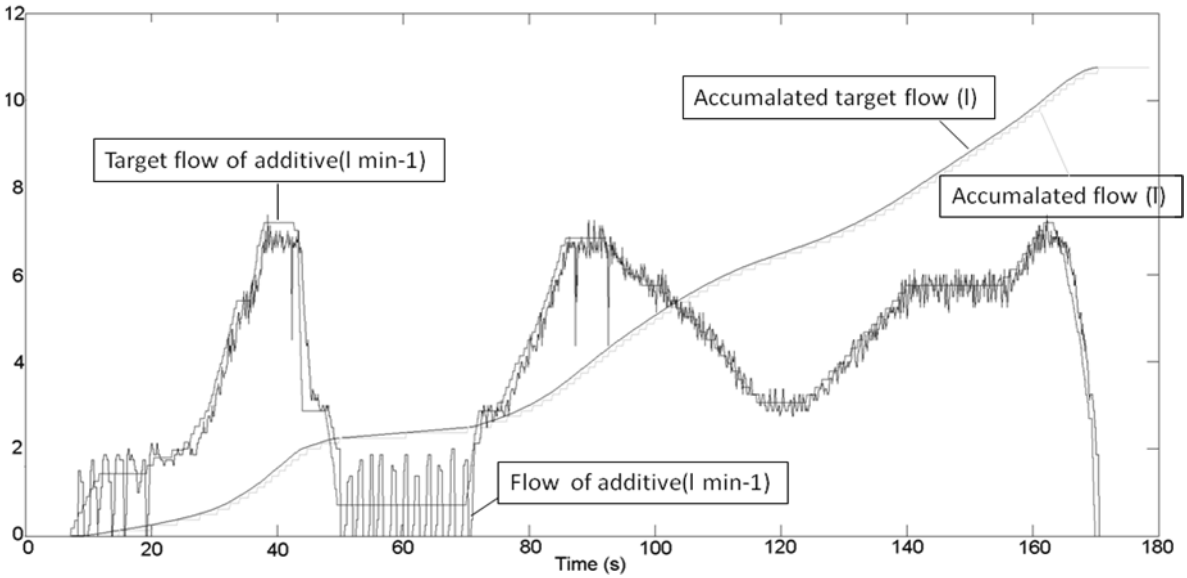

Figure 2. Measured flow of additive compared with the target flow applied to grass.

Conclusions There are several different kinds of yield measurement and control systems in forage harvesting machines, but no others use a forage mass estimator and apply a fuzzy logic speed controller and precision additive application. The speed controller was able to maintain the mass flow at a desired level and apply additive with an accurate ratio.

Acknowledgements A part of this research is done in the project Agromassi that is part of FIMECC-program EFFIMA.

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Effect of processing on fermentative quality of rice grain silage

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Keywords: fermentative quality, fully ripened rice, processing method, rice grain silage

Introduction In Japan, forage rice grain has drawn attention in recent years as a concentrated feed. Forage rice grain can be harvested using a head-feeding combine, as is used for food rice; thus a dedicated harvester is not needed. However, it is difficult to store forage rice grain in the same way as food rice because the drying process is not suitable for postharvest storage due to problems such as the high cost and contamination of forage rice grain with food rice in rice processing facilities or country elevators. It has been proposed that rice grain silage is a useful storage method that does not require drying by heating. Uegaki et al. (2010) demonstrated a processing method for rice grain silage, although the fermentation quality after a long storage period and the appropriate processing method are not clear.

The aim of this study was to develop a storage method of forage rice grain which allows us to use the rice as feed throughout the year. We examined the fermentative quality of rice grain silage stored for 40 or 120 days while using various pretreatments. The effects of processing, moisture control, the crushing of grains, lactic acid bacteria (LAB) additive, and glucose additive on the fermentative quality of fully ripened rice grain silage were analyzed.

Material and methods The rice cultivar used was *Oryza sativa* 'Momiroman', which was developed as a feed crop. The plants were cultivated in Ohtawara, Tochigi, Japan, and rough rice was harvested at the fully ripe stage. Some of the rough rice was then crushed by a feed rice crusher (DHC-2000, DELICA Corp., Nagano, Japan) with a 0.5-mm distance between the crushing rollers.

The silage was prepared in small-scale silos (200 g). The grain material (rough rice and crushed rough rice) was ensiled in plastic pouches with or without moisture control, added glucose, and added lactic acid bacteria (LAB; 10^5 colony-forming units g^{-1} fresh matter [FM], *Lactobacillus plantarum,* Chikuso–1). After 40 or 120 days of storage, the fermentation quality (pH, content of organic acids, and volatile basic nitrogen [VBN]) and microbial flora were analysed.

Results and discussion The results of the fermentative quality and microorganism compositions of rough rice silage are shown in Tables 1 and 2. Troller (1980) reported that a low water activity (a_w) value inhibits the growth of microorganisms. In this study, regardless of crushing or storage time, no moisture additive resulted in high pH (6.82–7.26) or low lactic acid contents (not detected). Moisture additives significantly (P<0.05) lowered the pH value compared to the use of no additive. Brown rice includes 71.8% carbohydrates, mostly starch. The addition of starch is not effective for enhancing lactic acid fermentation (Hattori et al. 1993). Furthermore, brown rice contains little free sugar. However, Miyamori (2003) reported that glucose may be produced via enzymatic activities in rice by soaking it in water, and LAB can grow through the use of this released glucose. Moisture additives should activate microorganisms and enzyme, therefore, silage fermentation was enhanced.

Long storage time and crushing resulted in not only high lactic acid content but also high butyric acid and VBN contents. This result showed that long storage time, crushing, and moisture additive enhanced fermentation. In 120 days of storage with crushing and moisture additive, the butyric acid and VBN content were 0.79% and 0.44 g^{-1} kg FM, respectively. With crushing and added glucose, the butyric acid and VBN content were 0.41% and 0.36 g^{-1} kg FM, respectively. With crushing and added LAB, the butyric acid and VBN content were 0.05% and 0.30 g^{-1} kg FM, respectively. As a result, when butyric acid and VBN content increased in the long storage period, LAB additive inhibited the amount of this increase.

With both 40 and 120 days of storage of rough rice with moisture additive, crushing significantly (P<0.05) increased the lactic acid contents. Furthermore, the interaction effect (P<0.01, P<0.05) between crushing and additives was a factor in increasing the lactic acid contents. Crushing removed the pericarp, endosperm cell wall, and hull in rough rice, allowing LAB access to the substrate. Therefore, the combined use of crushing and LAB addition enhanced lactic acid fermentation.

Conclusions In this study, it was found that moisture additive, crushing, and LAB additive enhanced lactic acid fermentation and provided rich fermentative quality in rice grain silage. During the long storage period, not only lactic acid but also butyric acid and VBN increased, but LAB additive can inhibit increases in butyric acid and VBN.

Table 1. Fermentation quality of rough rice silage.

Storage period	Pretreatment	Additive	pH	Organic acids (% FM)				VBN	DM
				Lactic	Acetic	Propionic	n-Butyric	(g/kgFM)	(%)
40 days	No crushing	None	7.26^{a}	nd^{c}	nd	nd^{b}	nd^{b}	nd^{c}	82.2
		Water	4.69^{b}	0.37^{b}	0.02	nd^{b}	0.13^{a}	0.01^{a}	64.2
		Water+Glucose	4.41^{b}	0.47^{b}	0.01	0.01^{a}	0.03^{b}	nd^{b}	65.8
		Water+LAB	4.37^{b}	0.69^{a}	0.04	nd^{b}	0.06^{ab}	0.01^{a}	63.8
40 days	Crushing	None	7.08^{a}	nd^{b}	nd^{b}	nd	nd^{b}	nd^{c}	82.1
		Water	4.92^{b}	0.55^{b}	0.13^{a}	nd	0.32^{a}	0.03^{a}	63.7
		Water+Glucose	4.80^{b}	0.61^{b}	0.01^{b}	0.03	0.25^{ab}	0.02^{a}	65.4
		Water+LAB	3.92^{c}	3.06^{a}	0.03^{b}	0.02	nd^{b}	0.02^{b}	63.6
120 days	No crushing	None	6.82^{a}	nd^{b}	nd^{b}	nd	nd^{b}	nd^{b}	82.6
		Water	4.56^{b}	0.60^{ab}	0.07^{ab}	nd	0.20^{a}	0.08^{a}	64.0
		Water+Glucose	4.53^{b}	0.68^{ab}	0.04^{ab}	0.04	0.12^{a}	0.06^{a}	65.7
		Water+LAB	4.20^{b}	0.93^{a}	0.10^{a}	nd	0.11^{ab}	0.10^{a}	64.6
120 days	Crushing	None	7.05^{a}	nd^{b}	0.02^{b}	nd	nd^{b}	0.02^{c}	82.7
		Water	4.89^{b}	0.90^{b}	0.18^{a}	nd	0.79^{ab}	0.44^{a}	63.2
		Water+Glucose	4.36^{c}	2.40^{a}	0.06^{ab}	nd	0.41^{a}	0.36^{ab}	65.1
		Water+LAB	3.95^{d}	2.54^{a}	0.08^{ab}	nd	0.05^{b}	0.30^{b}	65.1

VBN, volatile basic nitrogen; DM, dry matter; nd, not detected; LAB, lactic acid bacteria
A significant mean difference (P<0.05) exists between values with different signs in the same pretreatment and the same storage period, and nd (< 0.01 % fresh matter) was regarded as 0.

Table 2. Microorganism compositions of rough rice silage (log CFUs g^{-1} FM).

Storage period	Pretreatment	Additive	Lactic acid bacteria	Aerobic bacteria	Coliform bacteria	Bacilli	Clostridia	Yeast	Fungi
40 days	No crushing	None	5.4	>8.6	>8.6	2.3	nd	3.8	2.8
		Water	8.2	8.1	5.9	3.8	3.1	nd	nd
		Water+Glucose	8.0	8.0	2.9	nd	nd	5.1	nd
		Water+LAB	7.7	3.8	nd	3.2	3.4	nd	nd
40 days	Crushing	None	7.3	7.8	5.5	4.6	nd	2.6	2.6
		Water	8.5	4.5	5.3	nd	2.8	nd	nd
		Water+Glucose	>8.6	7.3	>8.6	3.9	nd	nd	nd
		Water+LAB	>8.6	>8.6	nd	2.3	4.1	nd	nd
120 days	No crushing	None	5.9	8.1	6.3	nd	nd	3.3	2.7
		Water	7.5	6.5	nd	nd	nd	nd	nd
		Water+Glucose	7.0	6.3	5.5	4.3	nd	nd	nd
		Water+LAB	7.5	7.0	nd	3.0	3.8	nd	nd
120 days	Crushing	None	5.7	7.7	6.2	3.9	nd	nd	nd
		Water	6.7	6.7	nd	5.0	2.7	3.3	4.7
		Water+Glucose	8.0	7.6	7.5	nd	nd	7.7	nd
		Water+LAB	>8.6	7.0	nd	nd	nd	nd	nd

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Effects of various commercial inoculants on the fermentation, aerobic stability and nutritional quality of rolled and ground high moisture corn

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Key words: high moisture corn, inoculants, aerobic stability, particle size, starch availability

Introduction High moisture corn (*Zea mays* L.; HMC) is an important source of dietary energy for ruminants due to the high starch content, but it is often prone to aerobic spoilage with negative effects on its feeding value and animal performance. Bacterial inoculants have the potential to improve both the aerobic stability and nutritional quality of silage, increasing animal performance (Kung et al. 2003), but little research has been published on their effects on HMC. The aim of the study was to evaluate the effects of (i) various commercial inoculants and (ii) two grinding sizes on dry matter (DM) losses, fermentation characteristics, and nutritional quality of HMC.

Material and methods Corn grain harvested at a DM content of 745 g/kg was rolled to crack kernels or ground through a hammer mill at final size of 6.70 ± 0.41 mm and 3.84 ± 0.65 mm, respectively, and then ensiled in laboratory silos without or with one of five different commercial inoculants following the producer instructions (Lallemand Animal Nutrition, Milwaukee) to obtain the final microbial concentrations suggested. Treatments were completely randomly assigned as follows: Control, corn grain without microbial additive; LB, *Lactobacillus buchneri* at $6.0X10^5$ cfu/g fresh forage; LPLB, *L. plantarum* at $1.0X10^5$ cfu/g fresh forage and *L. buchneri* at $5.0X10^5$ cfu/g fresh forage; LBPP, *L. buchneri* at $7.5X10^5$ cfu/g fresh forage and *Pediococcus pentosaceus* at $1.5X10^5$ cfu/g fresh forage; LPPF, *L. plantarum* and *Propionibacterium freudenreichii* both at $1.1X10^5$ cfu/g fresh forage; PPPF, *P. pentosaceus* and *P. freudenreichii* both at $1.2X10^5$ cfu/g fresh forage. Four replications were prepared for each treatment, totaling 48 silos. Silos were stored at room temperature and opened after 130 days of conservation. Silages were analyzed for DM content, DM losses, pH, fermentation products, ammonia, microbiological counts, crude protein, prolamin, ash and starch contents, and starch availability. The starch availability was determined as proposed by Blasel et al. (2006) and expressed as degree of starch access (DSA).

Results and discussion Inoculants and grinding size significantly affected pH, fermentation products, mould counts, and aerobic stability of HMC (Table 1). Grinding to a smaller particle size increased silage fermentation as indicated by lower pH and higher lactic acid concentrations. The *L. buchneri* alone or in mixture with homofermentative lactic acid bacteria (LAB) increased silage concentrations of acetic acid and 1,2-propanediol. Furthermore, higher values of 1,2-propanediol were observed in rolled than ground HMC. These differences could be due to an increased activity of anaerobic conversion of lactic acid to acetic acid in the rolled HMC, with higher production of 1,2-propanediol, another major end-product from the anaerobic conversion of lactic acid to acetic acid by *L. buchneri* (Oude Elferink et al., 2001). Although two inoculants tested were based on propionic acid bacteria, propionic acid was not detected, except for small amounts in ground HMC treated with LB and LBPP. Unexpectedly at opening, yeasts were not detected except for rolled HMC treated with PPPF and LPPF (data not shown). These data are in contrast with literature where the presence of yeasts in HMC was also related with a short aerobic stability. Conversely, we observed an aerobic stability higher than 300 hours except for rolled HMC treated with PPPF and LPPF.

The DM contents were significantly affected by grinding size, but the differences were numerically small (Table 2), whereas the concentrations of crude protein, prolamin and ash didn't show differences due to inoculants or grinding size. Inoculants and grinding size affected the starch content and DSA. The DSA was higher when corn was ground at smaller size as reported by Blasel et al. (2006), who observed a strictly positive relationship between DSA and decrease in particle size of HMC. Furthermore, in both rolled and ground HMC the DSA increased with ensiling. The DSA in average increased from 469 to 677 g/kg of total starch, and from 701 to 919 g/kg of total starch for rolled and ground HMC, respectively.

Conclusions Results showed that grinding corn grain to a smaller particle size before ensiling provides an opportunity to increase fermentation, conservation and starch availability of high moisture corn. Furthermore, positive or negative effects on aerobic stability and starch availability were observed when commercial inoculants were added to high moisture corn.

Table 1. Fermentation profiles, mould counts, aerobic stability, and dry matter (DM) losses of rolled and ground high moisture corn untreated or treated with different commercial inoculants.

Treatment	pH	Lactic acid	Acetic acid	1,2-Propanediol	Ethanol	Ammonia	Moulds	Aerobic stability[1]	DM losses
		g/kg DM				g/kg NT	log (cfu/g)	hours	%
Rolled									
Control	4.42	12.8	3.8	0.68	4.66	25.9	2.41	>300	3.50
LB[2]	4.73	5.5	9.6	7.08	3.28	48.3	1.10	>300	3.84
LPLB	4.65	5.8	9.3	7.96	1.93	31.8	2.10	>300	3.31
LBPP	4.79	4.2	11.6	9.33	3.39	40.0	1.78	>300	4.22
PPPF	4.26	15.0	2.7	0.80	2.86	30.8	1.50	238	3.18
LPPF	4.17	16.4	1.9	0.51	2.26	21.7	2.90	155	2.98
Ground									
Control	3.96	23.5	6.7	0.53	2.48	23.4	1.12	>300	3.21
LB	4.08	20.8	9.2	1.33	2.00	47.9	1.54	>300	3.70
LPLB	3.98	23.3	6.9	1.04	2.47	33.4	1.51	>300	3.08
LBPP	4.12	17.4	10.5	1.63	2.34	34.3	1.09	>300	3.30
PPPF	3.97	23.3	5.5	0.44	1.78	30.9	1.49	>300	3.42
LPPF	3.94	24.8	5.0	0.53	1.62	43.7	2.24	>300	3.01
SEM	0.044	0.106	0.045	0.047	0.014	0.193	0.127	-[3]	0.097
Inoculum (I)	***	***	***	***	***	**	*		
Grinding size (GS)	***	***	***	***	***		*		
Interaction (IxGS)	***	***	***	***	**				

1 Aerobic stability given in hours until temperature rose 2°C above ambient temperature

2 LB, *Lactobacillus buchneri*; LPLB, *L. plantarum* + *L. buchneri*; LBPP, *L. buchneri* + *Pediococcus pentosaceus*; LPPF, *L. plantarum* + *Propionibacterium freudenreichii*; PPPF, *P. pentosaceus* + *P. freudenreichii*

3 Statistical analysis not performed

Table 2. Nutritional characteristics of rolled and ground high moisture corn untreated or treated with different commercial inoculants.

Inoculant	Dry matter (DM)	Ash	Crude Protein	Prolamin	Starch	Degree of starch access
	g/kg	g/kg DM				g/kg starch
Rolled						
Control	726	12.7	72.3	24.8	723	645
LB[1]	723	13.3	73.2	25.3	717	714
LPLB	725	14.8	73.1	23.2	715	696
LBPP	719	13.8	75.8	24.4	712	703
PPPF	724	14.0	74.1	22.4	706	653
LPPF	724	13.4	73.5	25.1	705	653
Ground						
Control	727	14.1	74.1	25.7	716	942
LB	727	13.9	72.8	23.4	737	929
LPLB	728	14.9	74.1	24.1	712	923
LBPP	728	14.8	73.9	22.3	732	931
PPPF	727	14.4	74.7	21.6	717	903
LPPF	727	14.3	74.7	23.2	707	887
SEM	0.062	0.019	0.242	0.038	0.184	1.852
Inoculum (I)					**	*
Grinding size (GS)	**				*	***
Interaction (IxGS)					*	

1 LB, *Lactobacillus buchneri*; LPLB, *L. plantarum* + *L. buchneri*; LBPP, *L. buchneri* + *Pediococcus pentosaceus*; LPPF, *L. plantarum* + *Propionibacterium freudenreichii*; PPPF, *P. pentosaceus* + *P. freudenreichii*.

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Test of snow groomer ”Pistenbully 300 Greentech“ for use in bunker silos at harvesting different crops

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Keywords: bunker silo, compaction, distribution, silage harvest, silage quality, snow groomer

Introduction Modern self-propelled precision-cut forage harvesters, when combined with large transport units, yield a high harvest performance with more than 100 to 150 tons of fresh material per hour (Shinners 2003). As a consequence, the distribution and compacting of the feed in bunker silos is becoming increasingly problematic. Low density accelerates the risk of aerobic deterioration at the feeding out period (McDonald et al. 1991). If several pack tractors are working simultaneously on the bunker silo, the risk of accidents increases (Holmes and Bolsen 2009). Therefore a snow groomer was tested in practice at silo filling. The following questions were asked: 1. How well is the material spread and 2. In what extent are compaction, silage temperature and fermentation quality affected?

Material and methods The snow groomer ”PistenBully 300 GreenTech“ was tested for performance, silage density, fermentation quality and aerobic stability on five different crops (sorghum, green rye, grass, triticale, maize) and under field conditions in bunker silos (Nussbaum and Urfell 2010). The machine used in the experiment had the following technical specifications: power output 240 kW, tare weight 9.3 tons, X-track chains (specific ground pressure 0.16 kg/cm²), variable speed (0-19 km/h), clearing blade 4.6 m, width (over X-track chains) 3.0 m, air-conditioned cab, multi-function joystick.

At harvest, we measured the time during which harvested volumes were delivered, as well as observing the behaviour of the vehicle in the bunker (Urfell and Nussbaum 2010). Each transport vehicle was weighted and a sample was taken from every tipper’s content to determine dry matter (DM) content, feeding value and fermentability. During unloading, the quality of the work was evaluated and the fill level in the silo was documented every hour. At feeding out period, the mean density in the silo was determined and silage sampling was conducted three times (first third, middle, last third in the bunker silo) at three positions (50 cm and 100 cm from side wall, middle of the pile) and three layers (30 cm and 100 cm from top, 50 cm above floor), using a sample borer (Pioneer™). The samples were analyzed to determine DM content, feeding value and fermentation quality. Temperature was measured at three depths (15 cm, 50 cm, 150 cm from the front edge of the bore hole).

Results and discussion The main results are presented in Table 1 and the overall valuation in Table 2. The total harvest quantities for grass were 500 tons of fresh material (FM) and up to 2151 t FM of green rye. The machine’s hourly performance varied from 50 t FM (grass) up to 109 t FM (maize), or 15 t DM/h (sorghum) up to 25.3 t DM/h (triticale). A total of 719 (grass) up to 2700 cubic meters (green rye) were filled in the bunker silos. The fermentation coefficient was very low for green rye (25.9), but very good (> 40) for the other crops. The DM content ranged from 16.3 % (sorghum) to 37.7 % (triticale).

During unloading no technical problems were caused. Neither the tracks or engine nor the cooler became dirty or blocked. The PistenBully was very agile, handled easily and was able to spread the feed well up to the side walls and into the corners of the bunker silo. This machine gained a high stability on the silage slopes. The total fuel consumption was 10 to 13 litres per hour, or rather 0.11 to 0.24 l/t FM. Wheel loaders, which are normally used, consume 15 to 20 litres per hour per machine.

The anaerobic storage period (end of filling to the beginning of feeding out) was very short for maize (immediate feeding out) and triticale (28 days), but more than 70 days for the other crops. This had an impact on the aerobic stability (Table 2). In addition, a correlation between storage period, density and the measured temperature (Table 1) was noticeable. The fermentation quality was very good, excluding the green rye silage. The poor fermentation quality of this silage with predominantly too much butyric acid and ammonia is not due to use of the snow groomer, but rather to the poor fermentation characteristics of the harvested crop. The target of well compacted silage (Honig 1991) was nearly achieved in grass silage (92 %). The other silages were at 49 % (sorghum) up to 87 % (maize). The lowest compaction was measured at the silo wall, whereas the best mean compaction was found in the middle of the silo. The snow groomer used in the test yielded a very good distribution performance, the compacting performance was good, but can be improved through technical changes (different blade, more additional weight). Volumes of up to ~50 to 70 tons of fresh material per hour are spread very well and evenly; above 70 and up to 100 tons less so. Spreading is still guaranteed with higher outputs

(100 – 150 t FM/h), but use of an additional rolling vehicle is recommended. Meanwhile, more than 60 PistenBully vehicles are in use to distribute and compress silage.

Table 1. Effect of the snow groomer on harvest and silage performance.

Attribute	Sorghum[1]	Green Rye	Grass	Triticale	Maize
Crop and harvest performance					
DM[2] %	16.3	17.8	32.6	37.7	31.1
Chop length mm	not detected	25 - 50	5	6	6 – 8
t FM total	1931	2151	501	1130	871
t FM/h	93	101	50	66.9	109
t DM/h	15	17.7	16.3	25.3	22.9
Volume total m³	not detected	2700	716	1835	1270
Fuel l/t FM	not detected	0.13	0.24	0.15	0.11
Crop fermentability					
WSC % i.DM	not detected	4.9	6.9	8.5	5.7
Buffer capacity	not detected	6.3	8.1	2.5	7.4
WSC/BC	not detected	0.8	0.9	3.5	0.8
Ferment. coeff.	not detected	25.9	40.3	66.3	38.1
Silage at feeding out period					
Storage period days	70	75	105	28	0
DM %	16.3	20.4	32.9	33.9	30.8
Density kg DM/m³	69	119.2	183.2	186.2	194.5
Temperature (mean) °C	9,7	26,2	20,1	24,8	28,0

[1]Sorgum: research started at the feeding out period. 2DM: dry matter; FM: fresh matter; WSC: water soluble carbohydrates; BC: buffer capacity; Ferment.coeff.: Fermentation coefficient (8 x WSC/BC + DM)

Table 2. Overall judgement for the suitability of using snow groomers at silage harvest.

Attribute	Sorghum	Green Rye	Grass	Triticale	Maize
Practical use at harvest					
Harvesting silage	not detected	+ (+ +)	+ +	+ +	+ +
Distribution performance	not detected	+ +	+ +	+ +	+ +
Compacting performance	not detected	+	+ +	+ +	+
Silage controlling					
Fermentation quality	+ +	-	+ +	+ +	+ +
Feeding value	not detected	o	+ +	+ +	+
Density	o (+)	o	+	o (+)	o (+)
Aerobic stability	+ +	o	+ (+ +)	o	(-) o

Legend: + + = very well / + = good / o = standard / - = insufficient / - - = poor

Conclusions The snow groomer "*PistenBully 300 GreenTech*" is suited for silage harvesting. The technique worked accurately and without disturbance. The silage fermentation and density was good overall. It can be derived from these results that use of this snow groomer is suitable to spread and compact high harvest volumes. There were no technical problems during unloading. The fuel consumption was a third lower than using a typical wheel loader. In the test, a distance of at least 40 to 50 cm between wall and vehicle remained when the blade was retracted. At present, a new blade is used, which does not exceed the track width when retracted. Additionally the vehicle's weight was increased (up to total 11 tons).

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Three safety issues for large-scale bunker silos and drive-over piles in North America

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Keywords: accidents, silage, avalanche, bunker silo, drive-over pile

Introduction Few farming operations invite as many different opportunities for injury or fatality as a silage program. From harvesting forage in the field, transporting it to the farm, placing it into storage, and then removing the silage at feedout, employees are exposed to numerous serious risks. Silage-related tragedy knows no age boundary as workers and bystanders of all ages have been injured or killed during crop harvest and silage feedout (Murphy and Harshman 2006). Increasingly, stories involve bunker silos and drive-over piles (Bolsen and Bolsen 2010). Consistently protecting employees, equipment, and property throughout harvesting, filling, and feeding does not occur without thought, preparation, and training. Presented here are several major hazards involved with managing silage in bunkers and piles, and ways these hazards can be eliminated, reduced, or controlled.

Material and methods Three bunker silo and drive-over pile hazards are presented and discussed: 1) fall from height, 2) avalanche or collapsing silage, and 3) complacency.

Results and discussion *Fall from height***.** It is easy to slip on plastic when covering or uncovering a bunker or pile, especially in wet weather. Standard guardrails should be installed on all above ground level walls. Use caution when removing plastic, tires, or pea gravel bags near the edge of the feedout face, and never stand on top of a silage overhang, as a person's weight can cause it to collapse. Where necessary, use equipment operating from the ground to remove spoiled silage from the surface of bunker silos and drive-over piles. Never allow a person to ride in the bucket of a front end loader!

Crushed by an avalanche/collapsing silage. A major factor contributing to injury or fatality from silage avalanche/collapsing silage is over-filled bunkers and piles (Holin 2010a). A nutritionist had the following near miss, "I was taking a core sample at one of our large dairy customers and had just moved away from the face when a large section just fell off. This was a very well packed silo and had immaculate face management" (Bolsen and Bolsen 2010).

Sugar Valley Volunteer Fire Company responded to a farm accident in Greene Township after Kenneth R Hettinger, 63, of Rebersburg, PA became entrapped under three tons of silage (The Express 2007). Fire personnel said Hettinger was removed from the silage by farm personnel. Fire company personnel attempted to resuscitate Hettinger but were unsuccessful, and he was pronounced dead at the scene.

It started out as a typical day for dairy nutritionist Doug DeGroff of Tulare, California (Holin 2010b). He pulled up to a client's corn silage pile for a forage sample, bucket and pitchfork in hand. After filling the bucket, he turned to walk back to his pickup to mix the samples. "The sun basically went out – I could not see any light and the feed hit me on my head and covered me completely," says DeGroff. "I knew what was happening before I hit the ground. The entire face fell on me ... about 20 tonnes broke away." DeGroff was caught in a silage avalanche, and he offered these additional comments: "This particular pile did not look unsafe at all. It was only 3.3 to 3.6 m tall at the time that I sampled it and was mechanically shaven. I personally have taken feed samples from piles where I should not have been. I knew they were not safe, but I took the risk. This pile looked safe from any angle you looked at it from. I feel very blessed to be here and that everything still works. Yes, it was a broken back, but it could have been so much more. I am not on pain medication, and I don't think there are going to be long-term issues."

An 11-year old boy died from injuries suffered after a feed pile collapsed on top of him at a Claremont farm (WMUR TV 2010). Andy Wheeler had previously been listed in critical condition at Dartmouth-Hitchcock Medical Center. Police said it took as long as 20 minutes to find and free Wheeler from the feed pile after the accident Tuesday. Police said the boy was on vacation from Maple Avenue School which is why he was hanging out at the MacGlaflin Farm, where his father works. He was riding his bike near a silage crib, where livestock feed is stored, police said. "The boy was in a silage crib where there was a large pile of silage, and that overhang collapsed," said Police Chief Alexander Scott. Scott said it took some time for anyone to realize there was a problem. "No one was working on it and no one saw what happened," he said. When he was found, an adult started CPR, and the boy was taken to a hospital. "It's probably a pile close to 7.6 meters high, so when they are taking silage out, they are digging it

out and that can result in an overhang," he said.

Avalanche/collapsing silage does not have to happen. Bunkers and piles should not be filled higher than the unloading equipment can reach safely, and typically, an unloader can reach a height of 3.5 to 4.5 meters. Use proper unloading technique that includes shaving silage down the feedout face and never "dig" the bucket into the bottom of the silage. Undercutting, a situation that is quite common when the unloader bucket cannot reach the top of an over-filled bunker or pile, creates an overhang of silage that can loosen and tumble to the floor. Never allow people to stand near the feedout face, and a rule-of-thumb is never stand closer to the face than three times its height. When sampling silage, take samples from a front-end loader bucket after it is moved to a safe distance from the feedout face.

Complacency. Always pay attention to your surroundings and be alert! A dairy nutritionist almost lost his life the day he took silage samples from a bunker silo with a 9-m high feedout face (Schoonmaker, 2000). "Even though I was standing 6 m from the feedout face, 12 tonnes of silage collapsed on me. I did not see or hear anything. I had been in silage pits hundreds of times, and you just become kind of complacent because nothing ever happens. It just took that one time".

"The accident happened on June 14, 1974 while making silage at Kansas State University's Beef Cattle Research Farm. The blower pipe plugged for about the eighth time that afternoon, and I started to dig the forage out from the throat of the blower. The PTO shaft made one more revolution. Zap! The blower blade cut the ends off three fingers on my right hand". The injured person, Keith Bolsen, said later, "I was complacent and did something pretty stupid" (cited by Bolsen and Bolsen 2010).

Conclusions Even the best employee can become frustrated with malfunctioning equipment and poor weather conditions and take a hazardous shortcut, or misjudge a situation and take a risky action. It is best to take steps to eliminate or control hazards in advance than to rely upon yourself or others to make the correct decision or execute the perfect response when a hazard is encountered. Only experienced people should be permitted to operate equipment associated with harvesting, filling, packing, sealing, and feeding in a silage program. The correct sizing of bunkers and piles can reduce the risk of an accident, and spreadsheet software is available to assist producers to better design and manage bunker silos and drive-over piles (Holmes and Bolsen 2009). Think safety first. The silage industry has nothing to lose by practicing safety - it has everything to lose by not practicing it.

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Economics of sealing maize silage in bunker silos and drive-over piles: an Excel spreadsheet

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Keywords: silage, surface spoilage, dry matter loss, bunker silo, drive-over pile

Introduction Between 2007 and 2011, the USA produced an average of 98.4 million tonnes of whole-crop maize silage annually (United States Department of Agriculture 2011). Approximately 82 to 84 % of this silage was made in bunker silos and drive-over piles. However, the failure to implement proper silage management practices, especially proper sealing technique, resulted in the unnecessary loss of approximately 10 to 12 million tonnes annually. Standard polyethylene (plastic), weighted with discarded full-casing tires or tire sidewalls, has been the most common method used to seal bunkers and piles, but dry matter losses in the original top 0.91 metres can exceed 30.0 % (Holmes and Bolsen 2009). The use of an oxygen barrier (OB) film (www.silostop.com) as an alternative to standard polyethylene for sealing bunker silos and piles was introduced at the XII International Silage Conference held in Uppsala, Sweden (Degano 1999). This paper presents an Excel spreadsheet, which estimates the economic benefit of sealing ensiled forage or high moisture grain in bunker silos and drive-over piles, and compares two sealing methods - standard plastic and OB film.

Material and methods The spreadsheet was developed from research conducted at Kansas State University from 1989 to 1995, and equations published by Huck et al. (1997). In the first section of the spreadsheet, the user enters values for the following: depth from the original surface to be evaluated, silage price, as-fed silage densities, bunker or pile dimensions, percent of the silage in the original depth lost during the storage and feedout phases, and cost of the sealing materials. The results are calculated and reported in the second section. Examples from the spreadsheet compare sealing bunker silos and drive-over piles with either standard plastic or OB film. The examples use whole-plant maize silage, which has a value of 65 US dollars per tonne on a fresh weight basis.

Results and Discussion In a large (18.3 m wide x 76.2 m long) bunker silo, which has an average depth of 3.66 m, the OB film would save an additional 4,055 US dollars of maize silage in the original top 0.75 m compared to standard plastic. In a 27.7 m wide x 62.0 m long drive-over pile, which has an average depth of 1.98 m, the OB film would save an additional 4,732 US dollars of silage in the original top 0.75 m compared to standard plastic. The OB film reduced the total shrink loss by 2.36 and 4.43 percentage points in the bunker silo and drive-over pile, respectively, compared to standard plastic.

Conclusions The economics of properly sealing bunker silos and drive-over piles makes it clear that farmers should pay close attention to the details of this troublesome task. Sealing with OB film has a greater economic benefit than sealing with standard plastic.

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Table 1. Profitability of sealing maize silage in bunker silos and drive-over piles with either standard plastic or oxygen barrier (OB) film (maize silage: 65 US dollars per tonne on a fresh weight basis).[1]

	Bunker 1 plastic	Bunker 2 OB film	Pile 1 plastic	Pile 2 OB film
Density in the top **0.75** m, kg fresh wt per m^3	**700**	**700**	**675**	**675**
Density below top 0.75 m, kg fresh wt per m^3	**775**	**775**	**750**	**750**
Bunker/pile depth, m	**3.66**	**3.66**	**1.98**	**1.98**
Bunker/pile width, m	**18.3**	**18.3**	**27.7**	**27.7**
Bunker/pile length, m	**76.2**	**76.2**	**62.0**	**62.0**
Silage lost in original top 0.75 m, % of crop ensiled[2]	**25.0**	**12.5**	**25.0**	**12.5**
Silage lost below original top 0.75 m, % of crop ensiled[2]	**8.0**	**8.0**	**8.0**	**8.0**
Cost of covering sheets, US $ per sq m	**0.48**	**1.35**	**0.48**	**1.35**
Total value of silage in the bunker/pile, US $	250,002	250,002	159,495	159,495
Silage in the original top 0.75 m, tonnes	732	732	869	869
Silage below the original top 0.75 m, tonnes	3,145	3,145	1,584	1,584
Silage saved by sealing, US $	9,517	15,465	11,303	18,367
Sealing cost, US $	2,344	4,238	2,887	5,219
Net value of silage saved by sealing, US $	7,173	11,228	8,416	13,148
Net benefit from oxygen barrier film, US $	---	4,055	---	4,732
Total silage lost in the bunker/pile, % of the crop ensiled	11.21	8.85	14.03	9.60

[1]Values in **bold** are user inputs. [2]Values derived from Bolsen et al. (1993); Kuber et al., (2008); Holmes & Bolsen (2009); and Rich et al. (2009).

Effect of pressing instruments on feed structure of maize silage during the compaction of bagging technology

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Keywords: bagging technology, compaction, feed structure, maize silage, silage bags

Introduction Two main advantages of bagging technology are flexible applicableness and good preservation results of silage. In the process of ensilaging maize in plastic bags the substrates are getting embedded in the press tunnel by conveyor and moulding technique. The compression of the ensiled material by a press rotor leads to a stronger mechanical load of harvest crops than with other compression procedures. It is well-known from practice that this process may reduce the particle size of maize and consequently leads to a poorer feed structure. Particularly for reasons of ruminal physiology it is essential to preserve an adequate feed structure up to the feeding table (Steingaß and Zebeli 2008). The objective of this examination was to evaluate the effect of the pressing tools on harvested material during ensilaging process. Further on it was evaluated if changes in structure of silage material influences on ingredients, silage nutritive value parameters and acidifying speed.

Material and methods The study was conducted with maize silage at two different farms with two types of bagging machines. At the first farm a G 6700 bagging machine from BAG Budissa Agro Service was used. The theoretical cutting length l_{th} of the forage harvester was set at 6 mm. At the second farm a rotor machine type G 7000 from the same company was used and the l_{th} was set at 8 mm.

Table 1. Overview of breadboard construction.

	Farm 1	Farm 2
Machine type	G 6700	G 7000
Harvesting stage	34 % DM	33% DM
Maize sort	Patrick	Tiago
Cutting length l_{th}	6 mm	8 mm

Fresh maize samples were taken from the fall shelter (n=12) of the bagging machine. After ensiling process the samples of pressed material were taken from the filled bags by means of drilling a stick in the feed stock ((n=12) Fig. 1). The samples were dried and sieved in the laboratory for the determination of the particle structure.

Figure 1. Fall shelter (left) and drilling stick (right) to take samples in front of and behind pressing rotor while harvesting.

Sieving analyses were executed with the help of a sieving tower by standard methods (Kromer 1993). The tower was equipped with seven floors with round holes which have a diameter of 40, 25, 15, 10, 6, 3 and 2 mm. It takes three minutes per passage. After sieving the mass fractions were weight separately.

The test series for acidifying speed were made according to DLG (2000). Therefore micro-silos were filled up with 1 kg fresh weight in 1.5 l glasses. To determine the effect of the press rotor glasses were filled with fresh (n=4) and pressed maize (n=4) respectively. After three days of airproof storing under constant 25°C pH- measurements were made according VDLUFA- instructions (VDLUFA 1997). To analyse fermentation quality further long term experiments micro-silos were performed (n=4). After 90 days of storing the samples were analysed by Association of German Agricultural Analytic and Research Institutes (VDLUFA) by means of near infrared spectroscopy.

Results and discussion There was a visual difference of particle structure between maize which was taken in front of and behind the press rotor. The proportion of large particles decreased and proportion of medium-size particles increased as a consequence of pressing. The proportion of fine particles (< 2mm) was unchanged (Tab.2).

At the second farm the differences between pressed and not pressed maize were even more obvious. Especially the proportion of large particles (6-10mm, 10-15mm and 15-25 mm) decreased. Mass fractions decreased in size group 10-15 mm from 26 to 4 % and increased in size group 6-10 mm from 32 to 53 %.

It was obvious that a part of large particles shifted into smaller fractions. Thus, the pressing tools affected mainly the longer sheet components by pressing procedure.

Table 2. Effect of pressing on particle size distribution (% of DM) at two theoretical cutting length (l_{th} = 6 mm and l_{th} = 8 mm; n = 12; T-test). SD = standard deviation.

Particle length in mm	l_{th} = 6 mm					l_{th} = 8 mm				
	Before pressing		After pressing			Before pressing		After pressing		
	Mean	SD	Mean	SD	P	Mean	SD	Mean	SD	P
<2	8.9	1.7	8.7	1.7	n.s.	3.0	0.4	5.4	1.2	***
2-3	4.8	1.7	3.7	0.4	*	2.3	0.2	3.2	0.6	***
3-6	22.5	4.5	26.3	3.1	*	12.6	1.4	15.6	3.5	*
6-10	49.3	6.4	52.1	3.8	n.s.	31.6	6.1	52.8	3.2	***
10-15	10.6	3.1	7.5	2.0	**	21.7	9.4	18.8	4.6	n.s.
15-25	3.4	1.4	2.0	0.7	**	26.0	7.7	4.0	2.4	***
25-40	1.2	1.0	0.3	0.3	*	3.0	1.3	0.6	0.5	***
>40	0.4	0.2	0.1	0.1	n.s.	0.7	0.6	0.3	0.4	n.s.

n.s.= not significant; *= P<0,05; **P<0,01; ***P<0,001

Micro-silo analyses were used to observe the acidifying speed of the samples before and after pressing. PH-measurements after three days of storing in micro-silos showed a significant faster process of acidifying for pressed substrates (pH 4.2) than for not pressed silage (pH 4.3, P<0.05). Due to the more intensive mechanical load of substrate in the bag it is assumed that the fermentation goes off faster.

After 90 days storing these samples were analysed by VDLUFA. Significant differences between samples before and after pressing could be determined regarding crude ash, crude fibre and ruminal nitrogen balance. Values after pressing were 4.4 %, 8.6 %, and -7.8 g N kg^{-1}. Thus the values were only minimally higher than values before pressing (4.1 %; P<0.05, 8.3 %; P<0.05, -8.5 g N kg^{-1}; P<0.01). Energy content was after pressing 6.7 MJ NEL kg^{-1} i.e. 0.1 MJ NEL kg^{-1} higher than before pressing. Regarding structure, ADF and NDF, there were no significant differences between the before and after pressing samples.

These results show that pressing tools did not affect analysed structure of maize silage.

Conclusions Used pressing technique decreased the proportion of large particles and increased the proportion of medium and small size particles of maize silage. Squishing of pressed substrate was not detected. The structural value and fermentation quality were not affected by the technique.

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Influence of covering strategies on feed losses and fermentation quality of maize silage stored in bunker silos

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Keywords: aerobic deterioration, maize silage, oxygen transmission rate, plastic sheet

Introduction It has long been known that the practice of sealing bunker silos with a covering improves silage quality. The most common method of covering has been polyethylene sheeting to create a boundary between the anaerobic environment of the silo and the aerobic conditions of the atmosphere surrounding (Bolton and Holmes 2004). Usually, the plastic sheet used to cover silos has oxygen permeability and small amounts of air can penetrate into the forage mass. Thus, the objectives of this trial were to evaluate different plastic sheets used to seal bunker silos and to investigate their influence on silage fermentation and microbiology profile.

Material and methods The trial was carried out in Piracicaba, Brazil, during 14 weeks of silage feed-out. Four Bunker silos (one per treatment) were filled with approximately 75 metric tons of maize silage harvested at 33.5 % of dry matter (DM). Treatments were defined according to silo covering method: OB+BW - oxygen barrier film (thickness 45 µm) + black-on-white polyethylene film (200 µm) over the OB film; BW - black-on-white polyethylene film (200 µm); B - black polyethylene film (200 µm); RB+SB - recycled black polyethylene film (200 µm) + sugarcane bagasse over the RB film. The deteriorated silage was weighted every day and the results were expressed in relation to fresh matter (FM) and DM basis. The oxygen permeability of the covering strategies was determined at 25 ºC and 35 ºC by ASTM (2010). To evaluate losses of organic matter (OM) during feed out, ash content was determined on silage samples in a muffle furnace, at 550 °C for 4h. During the 14 weeks, silage samples were collected from the core and top zones of the silos to determine the pH, fermentation end products (acetic and lactic acids) and the profile of microbial population (lactic acid bacteria, yeasts and filamentous fungi). One sample per location (core and top zone) was collected weekly and analyzed using the MIXED procedure of SAS (2003). For silage pH and microbial counts the model included random effect of time and fixed effects of treatment, silo zone and its interaction, whereas for oxygen permeability and proportions of inedible silage, fixed effect of treatment and random effect of time were stated in the model. Means were compared by Tukey test and effects were declared significant at $P<0.05$.

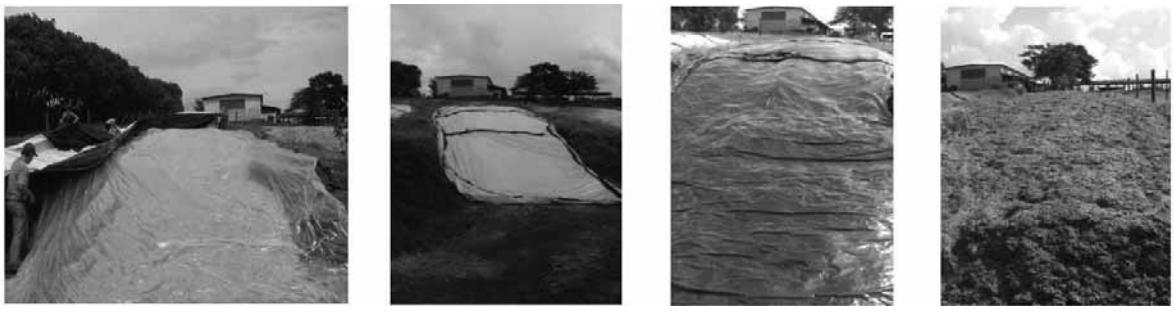

Figure 1. Sealing strategies of bunker silos used in the trial. From left to right: oxygen barrier film (thickness 45 µm) + black-on-white polyethylene film (200 µm) over the oxygen barrier film; black-on-white polyethylene film (200 µm); black polyethylene film (200 µm); recycled black polyethylene film (200 µm) + sugarcane bagasse over the recycled black polyethylene film.

Results and discussion There was a significant reduction in the percentage of inedible silage with the utilization of sugarcane bagasse over the RB and with the OB+BW when compared with BW and B (Table 1). The rate of oxygen permeability was higher for B and BW covering methods at both 25 and 35 °C. Pahlow et al. (2003) reported that as a result of air infiltration, acid tolerant aerobic (facultative) microorganisms present in silage start to proliferate, oxidizing sugars, lactic acid, acetic acid and ethanol as substrates. When the microbial mass formed is large enough, the heat released from oxidation gives rise to a measurable increase of temperature. Thus, sealing the silage mass with OB+BW and RB+SB

films maintained a less aerobic atmosphere at the top with a more appropriate temperature profile. For losses of OM (Table 1) and for pH, lactic acid and acetic acid (Table 2), no difference among covering strategies was observed. Silage samples collected on the top zone of the bunker silo had higher concentration of acetic acid and yeast counts when compared with samples collected on the core zone, indicating a poor fermentation and microbiology profile in the silage located on the top of the bunker (Table 2). The pH, lactic acid content, lactic acid bacteria and filamentous fungi was not affected by the location of sampling collection.

Table 1. Plastic film permeability and parameters of silage deterioration during feed out.

Parameter[1]	Treatments[2]				SE	P
	OB+BW	BW	B	RB+SB		
Inedible silage, % as fed	5.61[b]	7.78[a]	8.96[a]	3.38[c]	0.46	**
Inedible silage, % DM	3.86[b]	5.96[a]	7.42[a]	2.87[b]	0.40	**
Oxygen permeability at 25 °C, $cm^3.m^{-2}.d^{-1}$	92.6[c]	1006.2[a]	1038.7[a]	622.9[b]	46.48	**
Oxygen permeability at 35 °C, $cm^3.m^{-2}.d^{-1}$	208.0[c]	1700.8[a]	1707.9[a]	1052.6[b]	65.73	**
Losses of OM, %	27.44	29.19	34.86	28.41	3.47	NS

[a,b,c] Means within a row with unlike superscripts differ $P < 0.05$.
[1] OB + BW = oxygen barrier film 45 µm thick + black-on-white polyethylene film 200 µm thick over the OB film; BW = black-on-white polyethylene film 200 µm thick; B = black polyethylene film 200 µm thick; RB + SB = recycled black polyethylene film 200 µm thick sugarcane bagasse over the RB film

Table 2. The chemical (DM basis) and microbial composition (log_{10} cfu/g) of maize silages sealed with different covering methods.

Parameter	Treatments[3]					*P*	Sampling locations[4]			P
	OB+BW	BW	B	RB+SB	SE		C	T	SE	
pH	4.17	4.23	4.35	4.27	0.05	NS	4.23	4.21	0.04	NS
Acetic acid	1.31	1.44	1.08	1.14	0.10	NS	0.97[b]	1.43[a]	0.09	**
Lactic acid	2.91	3.08	3.35	3.78	0.44	NS	3.92	3.12	0.38	NS
LAB[1]	5.56	6.01	6.13	5.41	0.32	NS	5.29	5.90	0.28	NS
Yeasts	3.25	3.47	4.23	4.51	0.67	NS	2.41[b]	3.56[a]	0.55	**
FF[2]	3.62	2.93	4.36	3.83	0.68	NS	2.99	3.88	0.61	NS

[a,b] Means within a row with unlike superscripts differ $P < 0.05$.
[1] LAB = Lactic acid bacteria; [2]FF = Filamentous fungi; [3] OB + BW = oxygen barrier film 45 µm thick + black-on-white polyethylene film 200 µm thick over the OB film; BW = black-on-white polyethylene film 200 µm thick; B = black polyethylene film 200 µm thick; RB + SB = recycled black polyethylene film 200 µm thick sugarcane bagasse over the RB film; [4] C = core zone; T = top zone.

Conclusions In summary, the oxygen barrier film and sugarcane bagasse protection over the plastic film represented efficient strategy to reduce the proportion of deteriorated silages. Maize silage from the top zone of silo showed poor quality, which might be due to the proximity with the surface and occurrence of gas exchange.

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Oxygen barrier film improves fermentation, microbial status and aerobic stability of maize silage in the upper 30 cm of the silo

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Keywords: maize silage, oxygen barrier film, fermentation, moulds, aerobic stability

Introduction The extent of diffusion of oxygen through the top surface seal of bunker silos depends on the permeability of the plastic film and the efficiency of the sealing process, providing conditions for aerobic microorganisms to proliferate and resulting in aerobic deterioration of the top region of the silage. Berger and Bolsen (2006), in a survey of 127 bunker silos and clamp silos of maize silage in Kansas, USA, reported losses of dry matter (DM) in the top 46 cm layer ranging from 36 to 52% in unsealed silage and from 14 to 28% in silage covered with a single sheet of 100 to 150 μm polyethylene. Borreani et al. (2007) found that loss of DM from the upper 40 cm layer was 10% for maize ensiled with no additive treatment and covered with a coextruded oxygen barrier (OB) film (125 μm thickness, 100 cm3/m2 per 24 h oxygen permeability at 1 bar, 23°C, 85% relative humidity) under farm-scale conditions in Italy. Comparable loss of DM was higher ($P<0.05$), averaging 38%, for the same crop ensiled under conventional polyethylene film (180 μm thickness, 990 cm3/m2 per 24 h oxygen permeability at 1 bar, 23°C, 85% relative humidity).
An experiment was conducted to test the hypothesis that a bunker silo sealing system comprising a thin oxygen barrier film with protective woven polypropylene restricts the development of undesirable micro-organisms in the upper 30 cm layer of the silo to a greater extent than conventional thicker black polyethylene film (with protective tyres under large-scale farm conditions).

Material and methods The trial was carried out on a commercial large-scale farm in Hungary with a herd size of 600 dairy cows and an average 305-day lactation yield in 2010 of 12,146 litres per cow. Forage maize was ensiled in a bunker silo 25 m x 70 m without additive on 16th September 2009 (372 g DM/kg fresh matter (FM), 443 g NDF/kg DM) with a precision chop harvester fitted with a corn-cracker (Claas Jaguar 840). Estimated chop length was 8 to 19 mm. The target packing density was 200 to 250 kg DM/m^3. Consolidation was carried out using two tractors with a weight of 10 tonnes per tractor (Raba Steiger). Sealing was completed within 4 hours. The total storage period was 365 days.

The oxygen barrier (OB) silo sealing system consisted of a thin co-extruded plastic film of 45 μm thickness (“Silostop”, 2Gamma, Mondovi, Italy), a close weaved polypropylene net to protect the film, and gravel bags. The gravel bags not only sealed the edges but they also prevented the film and net blowing off the silage. The standard (C) sealing system comprised a single black coloured standard plastic sheet (thickness of 125 μm) covered with an average of 1.6 used car tyres per m^2.

Ten initial samples, each of 2.5 kg fresh matter (FM) were taken by corer in both the OB and C areas of the silo before sealing, after the completion of consolidation but before sealing, to a depth of 30 cm from the top surface, for laboratory analyses and density estimation. A further ten samples were taken on the 16th of September 2010 (12 months after filling and first sampling) from both the OB and C areas, within 50 cm of where the initial samples were taken. These samples, (2.5 kg FM,) were also taken to a depth of 30 cm from the top surface for laboratory analyses, density estimation and assessment of aerobic stability.

Results and discussion There were no significant differences between the two sealing treatments in either the density of the top 30 cm or the temperature at 30 cm depth. FM and DM densities of silage in the top 30 cm averaged 543 and 196 kg/m^3 respectively, and were very similar between the two treatments which indicated consistency across the two treatments with respect to compaction during filling. Analysis of silage samples taken to 30 cm depth showed no significant differences in pH or lactic acid between the two sealing systems (Table 1). However, concentrations of acetic acid, propionic acid and ethanol were lower for OB than for C ($P<0.05$), whilst the ratios of lactic to acetic acid and of lactic to total fermentation products were higher for OB than for C ($P<0.05$). There were no significant differences in the aerobic mesophyl bacterial count between silages. Whilst an average 2.56 log10 CFU/g FM moulds was found in samples of silage sealed with C, no moulds were found in any of the samples from silage sealed with OB ($P=0.008$). Lower populations of *Clostridium perfringens* were found in the silage stored under OB than under C ($P=0.008$). Aerobic stability, defined as the number of hours which elapsed before the temperature of samples of silage rose by 2°C above ambient when exposed to air at a constant

temperature of 20°C, averaged 249 hours and 184 hours for OB and C, respectively (P=0.002). Possibly most importantly, the OB system improved the aerobic stability of the maize silage by 65 hours. These results are particularly interesting as it would generally be expected that the silage under C, which had higher acetic and propionic acid concentrations than the silage under OB, would provide a greater degree of aerobic stability. Whilst oxygen concentrations in the silo were not measured, and no differences were found in aerobic mesophylic bacterial numbers, differences in the number of samples containing moulds and yeasts were found.

Conclusions It is concluded that the OB silo sealing system had a beneficial effect on the fermentation and hygienic status of the top 30 cm of silage. It is likely that the shift in fermentation characteristics and the increased aerobic stability in the upper layer of silage stored under the OB system are due to reduced oxygen permeation through the silo seal during the storage period. The OB system probably inhibited the development of the micro-organisms responsible for the initiation of aerobic deterioration to a greater extent than the conventional silo sealing system.

Table 1. Fermentation characteristics, microbiological composition and aerobic stability of samples of the top 30 cm of maize silage stored under conventional (C) or oxygen barrier (OB) film (n=10).

Sealing system		C	OB	s.e.d.	Sig.
pH		3.73	3.80	0.038	NS
Lactic acid	g/kg DM	45.0	47.1	3.280	NS
Acetic acid	g/kg DM	32.3	24.7	2.108	P=0.002
Propionic acid	g/kg DM	0.9	0.4	0.192	P= 0.017
Butyric acid	g/kg DM	0.0	0.0		
Ethanol	g/kg DM	11.3	6.5	1.011	P=0.005
Aerobic mesophylic bacteria	$\log_{10}$ CFU/g FM	4.71	4.12	0.539	NS
Moulds	$\log_{10}$ CFU/g FM	2.56	0.0	0.426	P=0.008
Yeasts	Number of positive samples	1	3		
Clostridium perfringens	$\log_{10}$ CFU/g FM	1.93	0.56	0.458	P=0.008
Aerobic stability	No. hours to +2 °C above ambient	184	249	16.630	P=0.002

s.e.d. = standard error of difference. NS = Not significant P>0.05, FM - fresh matter.

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Testing inoculant and chemical additives in round bales in comparison to laboratory silos

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Keywords: silage additives, round bales, fermentation quality, aerobic stability

Introduction The DLG approval scheme for silage additives takes into account different aims of actions, especially the two main actions, which are improving the fermentation process on the one hand and improving aerobic stability on the other hand (Staudacher et al. 1999). The tests were mainly carried out in small scale laboratory silos. In round bales, the distribution of silage additives is more difficult than in other systems as round bales are often made of unchopped forage with high dry matter content. The DLG plans to elaborate a test scheme for the approval of silage additives for round bales (Pauly and Rubenschuh 2009). The aim of different trials, carried out in Germany (D), Sweden (S) and Switzerland (CH) was to study the efficacy of silage additives in round bales in comparison to laboratory silos. In 2010, in each country one trial was carried out and in 2011, only in Germany and Switzerland one trial was carried out.

Material and methods In 2010, forage of leys was ensiled in laboratory silos of 1.5 l content and in round bales. In Germany and Sweden the leys mainly consisted of grasses. In Switzerland the ley contained grasses and clover. The fermentation coefficients of the forage was between 53 and 71, which means, that the forage was easy to ensile. Besides a control without additive, variants were either treated with an inoculant or with a chemical silage additive. The DM content of the forage at ensiling was 35.1, 50.2 and 38.6 % in Germany, Sweden and Switzerland.

In 2011, only variants with and without an inoculant were tested. But additionally, round bale silages were exposed to a special air stress. One week before the samples were taken, holes were punched into the stretch-film. In the variant with stress 1, four holes (diameter 2 cm) were made and after 24 hours the holes were closed. In the variant with stress 2, 20 holes were made with a nail (diameter 0.2 cm). Here the holes were not sealed until the samples were taken. The DM contents of the forages were 45.0 and 40.5 % in Germany and Switzerland, respectively. The fermentation coefficients amounted 51 and 57.

The inoculant contained the strains *L. plantarum, L. rhamnosus, P. pentosaceus, L. buchneri* and *L. brevis.* The application rate was 1 g per tonne, respectively 100'000 cfu/g. The product was diluted with water. The chemical product contained hexamine, sodium nitrite, sodium benzoate and sodium propionate. It was used undiluted in doses of 4 litres per tonne of fresh forage.

In order to determine DM losses, the small scale silos respectively the bales were weighed before and after the storage period. Samples were taken to analyze the DM contents, nutrient contents and fermentation parameters as well as pH, silage acids and ammonia. Based on these results, DLG points were calculated (DLG 2006). These points are based on butyric and acetic acids as well as pH. In addition, the aerobic stability was recorded. Silages were instable, when the temperature was 3 degrees above ambient. For the laboratory silos the storage time was always 90 days. The samples in the round bales were taken between 56 and 180 days after ensiling.

Results and discussion The application of the silage additives in round bales was not always simple. Particularly achieving the recommended dose rate turned out to be difficult. In 2010, the dosage for the inoculant amounted 118, 148 and 108% and for the chemical product 131, 138 and 103% of the recommended doses in the three countries. In 2011, the dose rate for the inoculant amounted 67 and 113%. A special point is also the DM content, because the forage dries up during the baling process and therefore DM contents can vary considerably between treatments and within bales.

In general, the silages from the laboratory silos and round bales had a good fermentation quality. The silages had between 82 and 100 DLG points, the maximum scaling up to 100 points. The fermentation was more intensive and the pH was lower in the silages of the small scale laboratory silos compared with the round bale silages (Table 1 and 2). This can be partly explained by the different chopping length of the forage. In some trials, the addition of the inoculant (with heterofermentative lactic acid bacteria) produced higher acetic acid contents. As it can be seen from the DLG points the air stress did not negatively influence the silage quality. With one exception (trial 2011 in Germany) DM losses were very

similar between the laboratory silos and the round bales.

The results of the aerobic stability tests differed a little between trials. In 2010 and 2011, the inoculant did improve the aerobic stability of the silages from the laboratory silos and the round bales in 83% (Table 1) and in 75% (Table 2) of the trials. In 66% of the trials, the chemical product improved the aerobic stability in the laboratory silos and the round bales (Table 1). With the different air stresses the silages without additives heated up earlier in comparison to the bales without air stress in both trials 2011 (Table 2). In the trial in Switzerland the aerobic stability was improved in the silages treated with the inoculant with and without air stress. But in the trial in Germany, the relation between inoculant and air stress was not evident. The low application rate of the inoculant can be the reason for this effect.

Table 1. Silage quality and aerobic stability of the silages 2010.

Country	Parameter	Laboratory silos			Round bales		
		no add.	Inoculant	Chemical product	no add.	Inoculant	Chemical product
D	DM content, g	351	364	366	379	372	382
	pH	4.3	4.1	4.3	4.7	4.5	5.3
	DLG points	82	100	87	94	98	91
	DM losses, %	7.6	9.7	6.5	6.1	7.8	4.5
	Aerobic stability, days	6.5	16.8	14.4	6.0	13.6	5.7
S	DM content, g	453	450	451	439	470	463
	pH	5.0	4.1	5.1	5.7	4.4	5.9
	DLG points	90	100	90	90	100	90
	DM losses, %	4.4	5.0	3.8	5.7	5.7	4.1
	Aerobic stability, days	1.4	7.0	7.0	1.5	7.0	4.0
CH	DM content, g	378	379	379	356	366	396
	pH	4.6	4.4	4.6	5.0	4.6	5.3
	DLG points	96	98	95	91	97	90
	DM losses, %	5.4	5.5	5.2	4.8	5.2	4.2
	Aerobic stability, days	14.0	14.0	14.0	12.1	14.0	14.0

Table 2. Silage quality and aerobic stability of the silages 2011.

Country	Parameter	Laboratory silos		Round bales					
		no stress		no stress		stress 1		stress 2	
		no add.	Ino-culant	no add.	Ino-culant	no add.	Ino-culant	no. add.	Ino-culant
D	DM content, g	368	399	393	431	418	437	431	482
	pH	4.3	4.2	5.2	4.7	5.1	4.9	5.1	4.8
	DLG points	100	100	90	93	90	91	90	91
	DM losses, %	4.5	4.9	9.1	9.2	9.8	9.3	9.5	9.6
	Aerobic stability, days	7.1	13.5	10.5	7.0	9.5	9.3	7.9	11.0
CH	DM content	399	400	379	398	400	394	383	405
	pH	4.4	4.1	4.6	4.4	4.7	4.4	4.6	4.4
	DLG points	100	100	91	100	84	100	94	100
	DM losses	5.3	5.2	5.4	5.3	5.4	5.3	5.4	5.3
	Aerobic stability, days	8.6	14.0	8.5	14.0	8.1	14.0	6.4	12.8

Conclusions The results indicate, that silage additives can also be tested in round bales provided that treated and untreated forages have the same DM content and that silage additives have been applied homogeneously and in the recommended dose. Furthermore, it is also possible to make an air stress to the round bales and thereby to make the conditions more difficult for the silage additives.

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Fermentation pattern and fungal growth in haylage bales according to number of film layers and use of preservative

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Keywords: additive, fermentation, fungi, haylage, horse, polyethylene

Introduction To ensure a successful preservation and prevent aerobic detoriation and fungal growth during storage, haylage bales are commonly wrapped in many layers (10-16) of polyethylene film. From an environmental perspective, efforts should be made to reduce the use of polyethylene in agriculture. If shown to be effective, preservatives with inhibitory agents against aerobic detoriation and fungal growth, e.g. propionic acid, sodium benzoate and/or sodium propionate, might become a replacement for more or less of the polyethylene film layers. One such preservative containing all the above mentioned agents (KOFA®Grain –pH 5) has been examined for this purpose in a Norwegian trial in which haylage bales were preserved with or without additive and sealed with 12 vs 4 polyethylene film layers. Main results from the trial which was part of a joint Norwegian-Swedish project will be presented here.

Material and methods The trial was conducted in the 1st and 2nd cuts in 2010 in Central Norway following a 2 x 2 factorial design with number of polyethylene layers (12 and 4) and use of additive (- no additive, + additive) as treatment factors. The 1st cut was taken at full heading of timothy (*Phleum pratense*) and the 2nd cut 46 days later, by use of a disc mower with conditioner. The mower left the grass in rows which were tedded twice (1st cut) or once (2nd cut). When the dry matter (DM) content was approximately 60%, the grass was baled and wrapped (Orkel HiQ Smart baler) with and without addition of 3-4 L of Kofa® Grain–pH5 (Addcon Nordic AS) per tonne to every second bale. The 12-layer treatment was finished before the 4-layer treatment started. The baling and sealing was finished within two hours. The driving pattern was such that within each treatment, bales originated from different parts of the field by chance. The ley which was dominated by timothy was fertilized with 100 kg N ha^{-1} in the spring and 90 kg N ha^{-1} after the 1st cut. The bales were stored outdoors for a minimum of 110 days in upright position in one layer. At opening, 39 bales (1st cut: 20, 2nd cut: 19) were visually inspected and the relative coverage of moulds on the surface was noticed before aseptic collection of forage samples (36 bales) using a sharpened stainless steel cylinder (diameter 4.5 cm). Eight sample cores from each bale were pooled to produce one sample for analysis of chemical and microbial composition.

Samples for microbial analyses (moulds, yeast, and lactic acid bacteria) were brought fresh within few hours to the laboratory (LabNett AS, Stjørdal) where cultivation started immediately. Samples for chemical analyses were frozen until measurement of pH and analysis of DM, ash, crude protein, sugars, Neutral Detergent Fibre (NDF), volatile fatty acids (VFA), ethanol (LabNett AS), ammonia-N and *in vitro* organic matter (OM) digestibility (Kungsängen Laboratory, SLU, Sweden). At LabNett AS the analyses were made according to NMKL standards (www.nmkl.org). For detailed descriptions of the analyses conducted at Kungsängen and the computation of metabolizable energy (ME) for horses, see Müller (2009). When contents or number of colony forming units (CFUs) were below detection limits, values were set to half the limit before further statistical analyses according to two-way ANOVA. The model included the main effects, cuts, interactions and error term (haylage bales within treatment and cut). The interactions film layers * cut and additive * cut were insignificant for all variables and removed from the model. Here, only the overall results over both cuts are presented.

Results and discussion The average contents of ash, crude protein, NDF, sugar and ME were 55 g, 101 g, 564 g, 91 g and 10.7 MJ kg DM^{-1} respectively, with no differences between treatments (results not shown). Due to the relatively high DM content (59 %), the fermentation seemed to have been restricted with low contents of lactic acid, VFA and ammonia-N in all treatments (Table 1). No interactions between main effects were obtained for fermentation characteristics. Higher contents of propionic acid in bales with added Kofa® Grain –pH 5 (+) compared to bales without additive (-) seems reasonable, as propionic acid is one of the main constituents of the additive (370 g kg^{-1}). Moreover, use of additive restricted ethanol fermentation and tended to give higher contents of residual sugars (95 g vs 88 g kg DM^{-1}, results not shown). Higher DM contents in bales with 4 polyethylene layers vs 12 layers may partly be due to the slight difference in time of baling (< 2 hours), according to the experimental design.

Until opening, all bales were intact without damages or holes in the polyethylene film. Surface mould was recorded on 14 bales out of 39 bales with an average coverage of 1.4 %. Thirteen of these bales were from the 4 layer treatment, with equal distribution between '+' and '-'(results not shown).

Analytically, mould was detected in eight samples only, out of which five from the 4+ treatment, two from 4- and one from 12-. In statistical analyses, mean contents of mould were found to be higher in bales with 4 layers compared to bales with 12 layers. Addition of preservative did not affect the counts of neither moulds nor yeasts (Table 2) at the time the haylage bales were opened.

These results, indicating that sealing with many layers with polyethylene film is more efficient to prevent fungal growth than application of preservative, are in well agreement with results from a laboratory scale experiment where the addition of Kofa ® Grain –pH5 was found to have limited effect on fermentation characteristics, mould- and fungal counts at silo opening of heavily wilted forages (Müller and Johansen, unpublished). However, in the laboratory experiment, KOFA®Grain-pH5 was found efficient to prevent temperature increment and fungal growth during aerobic storage of haylages with low DM contents (30 and 50 %). In the present experiment, the dosage of KOFA®Grain followed the recommendations given by the producer, i.e. 3-5 Litre tonne^{-1} for wilted grass with 40-70 % DM. It remains to investigate whether higher doses might improve the results, and to answer the question; how many layers of polyethylene film are required?

Table 1. Dry matter (DM) contents, pH and contents of lactic acid, volatile fatty acids, ethanol and ammonia-N in haylage with (+) or without (-) additive, sealed with 4 or 12 layers of polyethylene film. Means of 1st and 2nd cut. N=36 (DM) and N=24 (others).

	DM		Volatile fatty acids, g kg DM^{-1}				Ethanol	Ammonia-N
Treatment	g kg^{-1}	pH	Lactic	Acetic	Propionic	Butyric	g kg DM^{-1}	g kg^{-1} total N
4 –	598	5.27	0.48	0.53	0.21	0.17	3.56	28.4
4 +	601	5.29	0.46	0.42	0.35	0.17	3.12	23.2
12-	577	5.11	0.73	0.61	0.17	0.17	3.49	28.8
12+	598	5.08	0.65	0.56	0.39	0.18	2.94	29.3
Statistical significance:								
Effect of additive	NS	NS	NS	NS	***	NS	*	NS
Effect of film layers	**	**	***	*	NS	***	NS	NS
Interactions	NS	NS	NS	NS	NS	NS	NS	NS

Table 2. Surface coverage (%) with mould and colony forming units (CFU/g) of moulds, yeasts and lactic acid bacteria (LAB) in haylage with (+) or without (-) additive, sealed with 4 or 12 layers of polyethylene film. Means of 1st and 2nd cut. N=39 (surface coverage) or 36 (others)

Treatment	% surface coverage with mould	Mould	Yeast	LAB
4 –	0.71	1.99 a	5.31 a	5.91
4 +	1.08	2.78 a	5.46 a	5.96
12-	0.01	1.84 b	4.11 b	6.70
12+	0	1.73 c	2.97 c	6.34
Statistical significance:				
Effect of additive	NS	NS	NS	NS
Effect of film layers	***	***	*	**
Interactions	NS	O	*	NS

Conclusions Sealing with 12 layers of polyethylene film was effective to prevent fungal growth during long term (anaerobic) storage of haylage bales with 60% DM. Application of 3-4 Litre tonne^{-1} of a preservative containing fungi inhibitory agents could not replace eight out of 12 layers with polyethylene film.

Acknowledgments The experiment was financed by the joint Norwegian-Swedish research programme 'Hästforskning' (see: www.hastforskning.se), with additional support from Addcon Nordic AS.

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Microbiological and fermentative quality of maize silage conserved under new bio-based biodegradable films

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Keywords: maize silage, biodegradable film, plastic film, silage quality

Introduction The agricultural plastic demand in the world greatly increased in the last decade, reaching 3.6 million tons in 2008. A large proportion of the plastics used in agriculture are agricultural films. Plastic films are used in greenhouses, tunnels, silage covers, bale-wrap films, and mulching films to cover the soil (Briassoulis 2007). The plastic films utilized to cover silages influence the preservation efficiency of the system in terms of degree of anaerobiosis reached in the silo. The plastic can be basically used only once, plastic disposal has become more and more severe and could represent a potential environmental concern (Kyrikou and Briassoulis 2007). An alternative way of disposing agricultural plastic wastes is through biodegradation. Most experts define a fully biodegradable polymer as a polymer that is completely converted, by microorganisms, into carbon dioxide, water, minerals and microbial biomass, without leaving any potentially harmful substances (Kyrikou and Briassoulis 2007). Mater-Bi® is the first completely biodegradable and compostable bio-polymer ever invented and, recently, it has been shown that it can be utilized to produce film suitable for covering silage (Borreani et al. 2010).

The aim of this research was to study whether polyethylene (PE) film used to cover maize silage can be replaced with bio-based biodegradable films and to determine the effects on fermentative and microbiological quality of resulting silages.

Material and methods The trial was carried out at the experimental farm of the University of Turin in the western Po plain, northern Italy (44°50'N, 7°40'E, altitude 232 m a.s.l.) on maize harvested as a whole-crop, at about 50% milk-line stage and at 353 g DM kg^{-1} of fresh forage. Three different plastic films were compared: a standard PE film of 120 μm thick (PE) and two different Mater-Bi® biodegradable plastic films of 120 μm thick (MB1, mono-layer and MB2, multilayer co-extruded). The maize crop was chopped with a precision forage harvester to a 10 mm cut length, ensiled in plastic bags, with four replications for each treatment. Each bag was inserted into a portion of a PVC tube (internal dimensions: 300 mm diameter and 300 mm height, 21 l volume) so that just the top and the bottom of the bag had access to air. All bags were then filled with about 12 kg of fresh forage, which was compacted manually, and secured with plastic ties. The silos were stored indoors at ambient temperature (18 to 22°C) and opened after 170 days. At silo opening, approximately 5 cm of silage from the top of each silo were separately sampled and then the silage in the top half of the bags (5 to 20 cm) was mixed thoroughly and subsampled. All samples were analyzed for DM concentration, microbiological counts and fermentation end-products. The silages from the top half of the bags were subjected to an aerobic stability test and aerobic stability was determined by monitoring the temperature increases due to the microbial activity. The difference between silage temperature and ambient temperature was defined as dT. Other indices of aerobic stability were expressed as the maximum temperature rise (°C), the interval until the maximum temperature was reached (h), and the hourly accumulated dT (°C) in the first 120 h of aerobiosis. Silages were sampled after 2, 5, and 7 d of aerobic exposure to quantify microbial and chemical changes in the silage during exposure to air. Significant differences between means were identified from the *P*-values of the analysis of variance and effects were considered significant at $P < 0.05$. When the calculated values of *F* were significant, the Duncan range test ($P < 0.05$) was used to interpret any significant differences among the mean values.

Results and discussion The fermentation and microbial quality of the silages stored close to the films are reported in Table 1. The MB films are characterised by a lower oxygen permeability (about 500 vs. 1200 cm^3 m^{-2} 24 h at 23°C, 1 bar and 90% RH), whereas they were more permeable to water vapour. This led to a drying silage layer in the first 5 cm close to the film. The pH was lower in MB2 and PE than in MB1 and lactic acid content was similar in all the treatments, both for the top 5-cm layer and the 5-20 cm layer. The MB1 showed a higher yeast and mould count both in the top 5-cm layer and in the 5-20-cm layer. The worst quality of MB1 film was due to the partial microbial degradation of the film that occurred in the last month prior to sampling. For these reasons the aerobic stability test was performed only on the silage from one bag out of four bags, because the other three bags were already deteriorated (Table 2). The aerobic stability of the MB2 showed higher aerobic stability than those of PE as also showed by the evolution of dT, mould count and pH during the aerobic stability test (Figure 1).

Conclusions The MB2 film allowed to obtain a good silage quality even in the mass close to the film over 170 days of conservation, with comparable or even better results than those obtained with the PE film. Furthermore aerobic stability increased for silages conserved under the MB2 film. These promising results indicate that the development of new degradable materials to cover silage could be possible. Further researches should be undertaken to find new bland and film make coextrusion that enhance microbiological film stability.

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This work was funded by the Regione Piemonte POR. FESR 07-13-ASSE I.1.1 - Agroalimentare, Years 2011–2013 project "Feed & Food packaging: film biodegradabile per la sostenibilità ambientale della filiera agro-alimentare."

Table 1. Fermentation and microbiological characteristics of the silages stored under different plastic films in the top 5 cm and in the lower part of the silo (from 5 to 20 cm), after 170 days of conservation.

Zone	Treatment	DM	pH	Lactic acid	Acetic acid	Lactic/acetic ratio	Yeast	Mould
		(g kg^{-1})		(g kg^{-1} DM)	(g kg^{-1} DM)		(log cfu g^{-1})	(log cfu g^{-1})
top 5-cm	MB1	500a	3.96a	40.9	8.8b	4.8a	3.95a	4.90°
	MB2	522a	3.68b	56.8	14.3b	4.3a	1.13b	1.26b
	PE	341b	3.76b	53.0	22.8a	2.4b	2.35ab	0.88b
	sem	2.06	0.042	4.51	1.85	0.33	0.47	0.61
	P	*<0.001*	*0.008*	*NS*	*0.002*	*<0.001*	*0.034*	*0.004*
5-20 cm	MB1	329	3.82a	35.6	18.2	2.0b	4.59a	4.92a
	MB2	345	3.61b	45.7	13.9	3.3a	2.33b	0.72b
	PE	321	3.66b	36.7	17.7	2.2b	2.32b	0.70b
	sem	2.92	0.028	2.07	1.17	0.16	0.31	0.56
	P	*NS*	*0.002*	*NS*	*NS*	*<0.001*	*<0.001*	*<0.001*

MB1, mono-layer Mater-Bi®; MB2, multilayer co-extruded Mater-Bi®; PE, polyethylene.

Table 2. Indices of aerobic stability of the silages stored under different plastic.

Treatment	Aerobic stability	Peak temperature	Interval to peak temperature	Accumulated dT for 120 h
	(h)	(°C)	(h)	(°C)
PE	59	42.5	117	788
MB2	94	36.8	122	216
sem	5.8	1.74	3.7	94
P	*<0.001*	*NS*	*NS*	*<0.001*

The aerobic stability test was not performed on MB1 because 3 out of four bags were already deteriorated at silo opening.

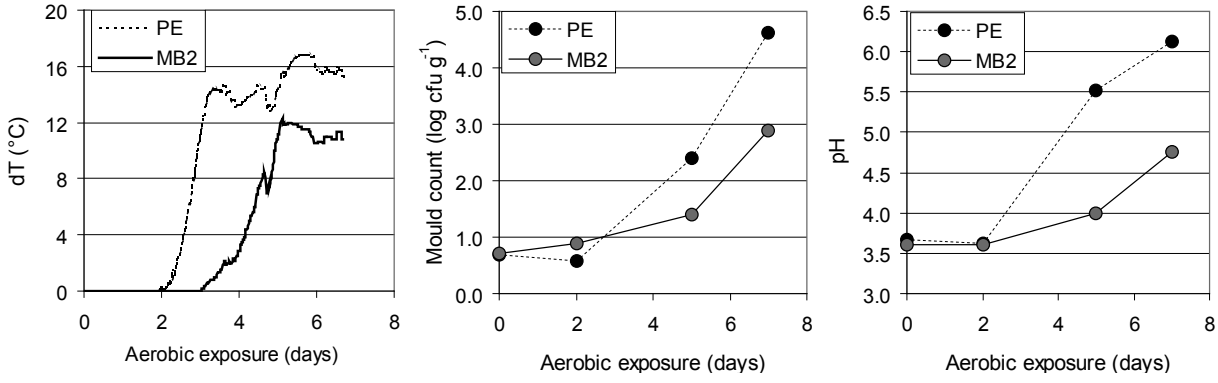

Figure 1. Evolution of dT, mould count and pH in the PE and MB2 silages during the aerobic stability test.

Using a special EVOH grade in stretch film manufacturing reduces dry matter losses and spoilage and increases hygienic quality of baled silages

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Keywords: Italian ryegrass, red clover, haylage, wrapped bales, high barrier stretch film

Introduction Bale silage making is based on a well-established procedure that usually consists of wilting forage up to 500-600 g DM kg^{-1}, baling and then wrapping it with 4 to 6 layers of a stretch polyethylene film (PE). The bale silage technique is particularly prone to spoilage and the challenge to the oxygen barrier is much greater than with conventional silage, because of the proximity of most of the silage to the plastic film and the thinness of the film (Forristal and O'Kiely, 2005). For these reasons, in baled haylage for horse feeding the number of layers applied can range from a minimum of 8 up to 20 (Muller et al. 2011). In recent years, the stretch polyethylene wrapping system has shown some limits with regards to sealing efficiency, the high permeability to oxygen of the stretch film utilized for wrapping (Borreani and Tabacco, 2008), and the non-uniform distribution of the plastic film between the ends and the side of the bale (Borreani et al. 2007). These problems lead to an undesirable air exchange over the conservation period and suggest the necessity of applying an increasing number of plastic film layers, thus increasing costs and labour time (Borreani and Tabacco, 2010).

The aim of this work was to compare the microbial and fermentation quality of Italian ryegrass and red clover baled silages, wrapped with commercial PE film and with a new oxygen high barrier stretch film (HOB), made with a special EVOH grade, that was specifically developed for stretch film manufacturing, with permeability 250-fold lower than PE.

Material and methods Two trials were performed in a commercial farm in northern Italy on two producing Italian ryegrass and red clover fields. Two day field wilted forage at a DM content around 550 g DM kg^{-1} was baled (small round bale 600-mm-long and 600-mm-diam.). Bales were individually wrapped using four, six, or ten layers of commercial PE stretch film (white linear low density PE, 250 mm wide × 25 µm thick), with an oxygen permeability at 1 bar overpressure, 20°C, and 65% RH of 7989 $cm^3\ m^{-2}\ 24h^{-1}$) or four, six, or ten layers of HOB stretch film PE-EVOH-PE (SG611 Soarnol) coextruded high oxygen barrier film (white UV protected, 250 mm wide × 25 µm thick; Nippon Gohsei Europe GmbH, Germany) with an oxygen permeability at 1 bar overpressure, 20°C, and 65% RH of 32 $cm^3\ m^{-2}\ 24h^{-1}$) (Table 1). Four individual randomly selected bales were wrapped for each treatment. Bales were stored on their ends for 140 d of conservation. At opening the bales were weighed and sampled to analyze the DM content, pH, lactic and monocarboxylic acids, ammonia, yeast and mould counts, and DM losses according to Borreani and Tabacco (2010). On removal of the plastic film, all visible moulds on the bale surface were located and measured, according to O'Brien et al. (2008). The percentage of the total surface area affected by fungal growth was then calculated for each bale. The chemical compositional data and microbial counts were analyzed for their statistical significance via ANOVA, with significance reported at the 0.05 probability level.

Results and discussion The EVOH layer reduced the oxygen permeability of the HOB film by 96 folds compared with the commercial PE film tested (Table 1). No holes were observed in any of the bales for both crops (data not shown). The fermentation quality of the haylages is reported in Table 2. The DM content was around 600 and 500 g kg^{-1} for ryegrass and red clover, respectively. The DM content restricted the fermentation profiles for both the crops and addressed the fermentation towards ethanol production. The HOB film reduced ethanol production for both the crops. Italian ryegrass showed very low percentage of bale surface covered by mold for both the films, whereas red clover reached 18% of the surface in bales wrapped with 4 layers of PE film (Table 3). On red clover, increasing the number of layers decreased the surface covered by mould for both the films. The film type and the number of layers clearly affected the DM losses, which were always lower in the bales wrapped with the HOB film and decreased with increasing the amount of plastic used to wrap the silage. Baled silage of both crops showed no fungal growth on the surface when ten layers of plastic wrap were applied. When the DM losses were related to the ethanol concentration of the silages a high correlation was found (0.90). Ethanol is produced mainly through fermentation of sugars by yeasts, whose survival and growth during storage could be enhanced by the presence of little amount of oxygen, as could happen with the PE stretch film. The activity of yeast is undesirable since ethanol is produced in a metabolic process which leads to proximately 49% loss of substratum.

Conclusions The HOB film reduced DM losses and mould spoilage in comparison to the PE commercial film in both crops. The results clearly indicated that the film permeability to oxygen is a key factor to successfully ensile forage in wrapped bale allowing to reduce the plastic usage.

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Table 1. Characteristics of the 25 µm stretch films utilized in the trials.

Characteristics	PE	HOB
Oxygen permeability at 20°C cm^3/m^2/24 h at 1 bar and 65% RH	7989	32
Force at break (N)	6.4	4.4
Energy to break (J)	0.08	0.04
Elongation at break, MD (%)	534	733
Elongation at break, TD (%)	1015	858

HOB, high barrier film; MD, machine direction; PE, standard polyethylene film; TD, transverse direction.

Table 2. Fermentation profile (g kg^{-1} DM) in relation to the type of stretch film and number of layers of bale silage after 140 d of conservation.

		Italian ryegrass					Red Clover				
Film	L	DM (g kg^{-1})	pH	Lactic acid	Acetic acid	Ethanol	DM (g kg^{-1})	pH	Lactic acid	Acetic acid	Ethanol
PE	4	606	5.62	5.9	0.4	48.9	515	5.43	8.1	2.8	29.9
	6	587	5.67	4.9	0.4	47.1	526	5.41	6.6	3.4	17.1
	10	574	5.73	5.9	0.4	50.3	515	5.47	5.7	2.5	28.6
HOB	4	590	5.86	2.3	0.5	37.8	515	5.25	5.1	2.8	18.6
	6	615	5.79	2.4	0.6	41.7	523	5.50	6.4	1.8	4.3
	10	596	5.82	4.5	0.6	35.0	537	5.18	11.6	3.9	8.7
sem		16.8	0.019	2.21	0.064	3.55	26.1	0.053	2.91	0.62	4.48
F		*NS*	*NS*	*NS*	*	*	*NS*	*NS*	*NS*	*NS*	**
L		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
F x L		*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

PE, polyethylene film; HOB, high oxygen barrier film; F, type of film effect; L, number of layers effect.

Table 3. Percentage of bale surface covered by visible mold and DM losses in relation to the type of stretch film and number of layers.

		Italian ryegrass						Red lover					
		Bale surface covered by mold (%)			DM losses	Yeast	Mould	Bale surface covered by mold (%)			DM losses	Yeast	Mould
Film	L	side	ends	Tot	(g kg^{-1})	(Log cfu g^{-1})		side	ends	Tot	(g kg^{-1})	(Log cfu g^{-1})	
PE	4	0.3	0.0	0.2	80	3.49	1.82	34.7	9.8	18.1	62	4.74	3.21
	6	0.9	0.0	0.6	78	3.17	1.95	8.3	4.9	6.0	48	5.08	2.68
	10	0.5	0.0	0.3	73	3.12	1.72	0.0	0.0	0.0	43	4.57	2.86
HOB	4	0.0	0.0	0.0	55	2.59	1.97	15.2	0.0	5.1	41	3.91	2.20
	6	0.0	0.0	0.0	42	2.53	1.89	2.2	0.0	0.7	29	3.48	2.69
	10	0.0	0.0	0.0	39	2.30	1.97	0.0	0.0	0.0	23	2.00	2.37
sem		0.17	-	0.11	1.33	0.30	0.09	0.39	1.86	0.78	4.22	0.25	0.20
F		**	-	**	***	**	*NS*	***	***	**	***	***	*
L		*NS*	-	*NS*	***	*NS*	*NS*	***	***	***	**	***	*NS*
F x L		*NS*	-	*NS*	*NS*	*NS*	*NS*	***	***	***	*NS*	**	*

PE, polyethylene film; HOB, high oxygen barrier film; F, type of film effect; L, effect of the number of layers.

Special EVOH-based films with lowered oxygen permeability reduce dry matter losses and increase aerobic stability of farm maize silages

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Keywords: maize silage, oxygen barrier film, plastic film, silage quality, aerobic stability

Introduction The most important factor that influences the preservation of forage ensiling is the degree of anaerobiosis that is achieved during conservation. Polyethylene (PE) films have been used for many years to seal bunker silos and drive-over piles because of their suitable mechanical characteristics and low costs. The high O_2 permeability of PE films can contribute to the low quality of silage in the top layer of horizontal silos (Borreani et al. 2007). Polymers different from PE, such as ethylene-vinyl alcohol (EVOH), help create an excellent barrier against oxygen, combined with good mechanical characteristics (puncture resistance), and are suitable for blown coextrusion with PE to produce 45- to 200-µm-thick plastic films. Recently, it has been shown that the use of oxygen barrier plastic films for ensiling can ensure a longer shelf life of silage, protecting it from spoilage and delaying the growth of pathogenic molds, which are able to produce mycotoxins that are harmful to animals and humans (Dolci et al. 2011). The aim was to assess the effects of new oxygen barrier film (HOB), based on PE coextruded with EVOH, on the fermentation quality, DM losses and yeast and mold counts at opening of whole-crop maize bunker silos compared to conventional polyethylene (PE).

Material and methods Two trials were carried out in two commercial farms (Farm 1 and 2), in the western Po plain of Italy (44°27´ N, 7°43´ E, altitude 408 m above sea level, Farm 1, and 44°40´ N, 7°32´ E, altitude 310 m a.s.l., Farm 2) on maize harvested as a whole-crop at 368 and 356 g DM kg^{-1} of fresh forage for Farm 1 and 2, respectively. The two farms manage maize silage very well. The bunkers were divided into two parts along the main direction and were half covered with PE film and half with HOB film in order to allow silage sampling at the same time during feed-out phase. The 2 sealing treatments were 1) a single sheet of 200-µm-thick black-on-white PE; and 2) a single sheet of 125-µm-thick black-on-white coextruded polyethylene-EVOH film, with an enhanced oxygen barrier property (Table 1). Plastic net bags with well mixed fresh material were weighed and buried in the upper layer of the bunker, at different distances from the wall (0.5, 1, 2, and 3 m). Bags were also buried in the central part of each bunker. The silos were opened for summer consumption, the bags were unloaded, weighed and sub-sampled to analyze the DM content, pH, lactic and monocarboxylic acids, ammonia, yeast and mold counts, and aerobic stability (as the number of hours the silage remained stable before rising more than 2°C above the ambient temperature). The DM losses were calculated as the difference between the amount of DM placed in each bag at ensiling and the DM removed at the end of conservation. The chemical compositional data and microbial counts were analyzed for their statistical significance via independent samples T-test, with significance reported at the 0.05 probability level.

Results and discussion The EVOH layer reduced the oxygen permeability of the of HOB film by 96 folds compared with commercial PE film (Table 1). Increased oxygen impermeability greatly improved the microbial and fermentation quality of the maize silage stored in the top layer of the silo (Table 2). The pH, mould and yeast counts, and DM losses were reduced, whereas lactic and acetic acids and aerobic stability were increased in silage stored under the HOB film compared with PE film in both studied silos. As an example, in Figure 1 the lactic acid concentration, pH, mould count, and DM losses of maize silage in the top peripheral area of the Farm 2 silo are reported in relation to the distance from the bunker wall. It is clear that the quality of the silage was improved by the use of HOB film, especially in the corner areas close to the bunker walls, where the DM density was lower and there are more difficulties in sealing the silos. Improving the silage quality in the bunker top layer is crucial for nutritional and hygienic quality of feed, since microbial populations increase during aerobic deterioration in an exponential manner, and, in farm conditions, the improper mixing of different parts of the silage, when being incorporated into the feed-mixer, could enhance the final feed contamination with undesirable microrganisms (i.e. fungi and aerobic and anaerobic spores) and harmful mycotoxins. The HOB film, contributed to create a more anaerobic environment, thus increasing the aerobic stability of silage and confirmed the possibility of ensuring a longer shelf life of silage during consumption, by delaying the growth of pathogenic aerobic microrganisms.

Conclusions The results indicate that the new oxygen barrier film HOB reduced spoilage and DM losses in the peripheral area of the silos on farm, even when a proper harvest-to-feedout management was implemented.

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Table 1. Characteristics of the films utilized in the trials.

Characteristics	PE	HOB
Thickness (µm)	200	130
Oxygen permeability at 20°C (cm^{-3} m^{-2} 24 h at 1 bar and 65% RH)	846	8.8
Force at break (N)	16	21
Energy to break (J)	0.14	0.14
Elongation at break, MD (%)	601	1113
Elongation at break, TD (%)	1381	1176

HOB, high barrier film; MD, machine direction; PE, standard polyethylene film; TD, transverse direction.

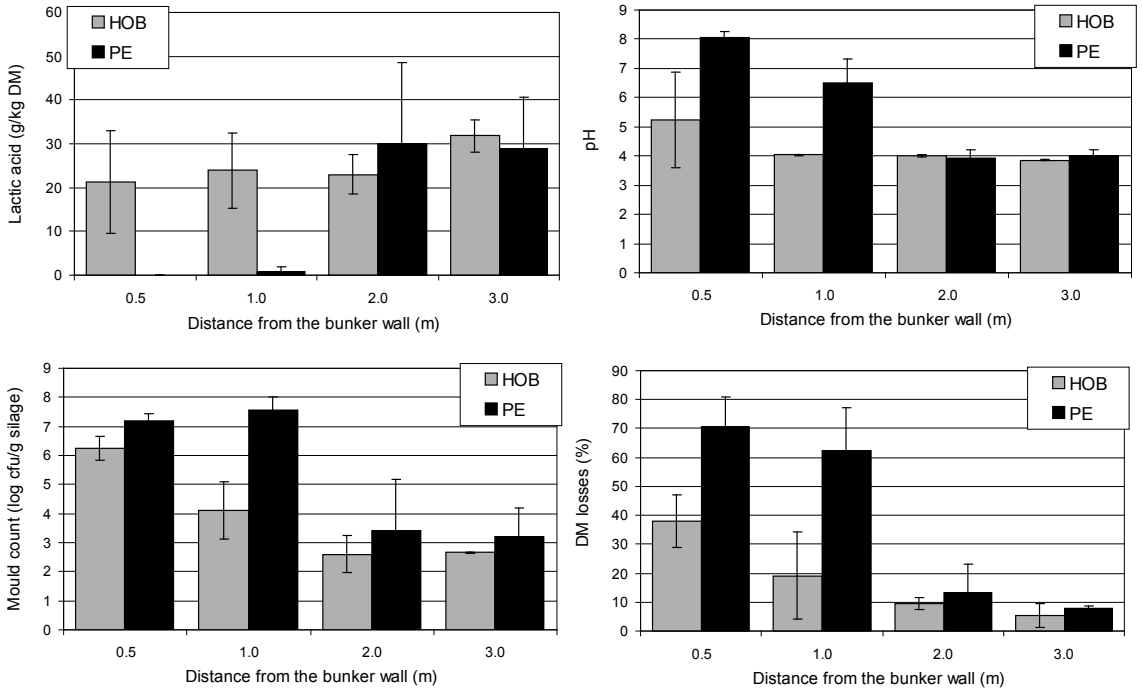

Figure 1. Lactic acid concentration, pH, mould count, and DM losses of maize silage in the peripheral area of the silo covered with high barrier film (HOB) and polyethylene film (PE) in relation to the distance from the bunker wall (Farm 2).

Table 2. Fermentation characteristics, concentration of moulds and yeasts in the top layer of the bunker silo covered with oxygen barrier (HOB) and standard polyethylene (PE) films 1 meter from the wall for Farms 1 and 2.

	Farm 1				Farm 2			
Item	HOB	PE	sem	*P* value	HOB	PE	sem	*P* value
DM (g kg^{-1})	360	235	32.9	**	324	202	20.9	**
pH	3.79	5.85	0.39	***	4.02	5.70	0.38	**
Lactic acid (g kg^{-1} DM)	33.1	7.1	3.68	***	25.1	6.0	3.22	**
Acetic acid (g kg^{-1} DM)	19.1	6.0	2.09	***	40.0	24.1	*4.85*	*NS*
DM losses (%)	4.2	33.6	5.66	***	14.3	48.2	6.38	**
Moulds (log cfu g^{-1})	2.15	6.16	0.58	***	3.36	6.59	0.57	**
Yeasts (log cfu g^{-1})	3.40	5.26	0.53	*NS*	1.18	4.30	0.49	**
Aerobic stability (h)	72	0 [1]	-	-	49 [2]	22 [2]	-	-

NS = P > 0.05; * = P < 0.05, ** = P < 0.01, *** = P < 0.001
[1] the measurement was not performed since the four bags below PE film were already deteriorated at unloading.
[2] the measurement was only performed on two out of four bags for PE, and three out of four bags for HOB, as two and one bags proved to be completely deteriorated at unloading, for PE and HOB, respectively. To the already deteriorated bags a value of 0 h of aerobic stability was assigned.

The use of plastic film instead of net to secure baled silage before wrapping

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Keywords: baled silage, silage quality, tying film, tying system

Introduction The bale silage technique is characterized by its unique individual-package storage system, but is particularly prone to spoilage, since each ton of baled silage usually has 6 to 8 times the surface area in contact with plastic film compared to clamp silage and about half of the silage stored is within a 120 mm distance of the film cover (McEniry et al. 2011). Small holes, that can occur on farm due to both mechanical and wildlife factors, and the reduced total thickness of the combined layers of stretch film on bale side, typically 70 µm (four layers) to 105 µm (six layers), might be expected to make individually wrapped bales more susceptible to permitting O_2 ingress (Forristal and O'Kiely 2005). The commercial availability of round balers equipped with film-tying attachments, suggests the possibility of replacing the standard net-tying system with a film tying system, in order to improve the airtightness of the plastic cover on surface of the bale (Bisaglia et al. 2011).

The aims of this research were to study the effect of the material used to secure the bale in the baler chamber (net vs. film) and of the number of layers (4 vs. 6) on fermentative profiles, microbiological quality, plastic consumption and DM losses of baled silage made from permanent meadows in Italy.

Material and methods The research was carried out on a permanent meadow grown in a commercial dairy farm near Bergamo (Northern Italy, 45°32' N, 09°38' E, altitude 114 m above sea level). Two effects were studied: the method used to secure the bale inside the baler chamber (net vs. film), and the number of layers of polyethylene film (four vs. six), with four bales for each treatment. Furthermore, to better understand if the plastic film that was used to secure the bale contribute to create a more anaerobic environment, each bale was sampled in three different zones to determine the microbiological quality of the forage (outer layer from 0 to 120 mm, inner layer from 121 to 540 mm and spoiled spot from 0 to 120 mm). The forages were mown with a rubber roll conditioner machine, spread with a conventional rotating tedder in the same day of cutting. After 24 hours of wilting, the forages were raked and then baled in 1.3 m-diameter and 1.2 m-wide hard-core round bales. Four bales were alternatively tied with either 3 wraps of commercial net or 3 wraps of Manustif (Manuli Stretch SpA, Isernia, Italy, made with standard polyethylene, white colour, 1.25 m wide and 16 µm thick). The round bales were then transported to the storage area, cored to a depth of 540 mm, weighed, and individually wrapped with four or six layers of polyethylene plastic film (Agriflex, Manuli Stretch SpA, Isernia, Italy, 0.5 m wide x 25 µm thick and light green pigmented). At the end of storage (270 days) the bales were weighed and the DM losses were calculated based on the DM at ensiling minus the DM after ensiling. The percentage of the total surface area affected by fungal growth was calculated for each bale. For each sample DM content, fermentative profile and microbial count were determined. The total plastic consumption were determined by separately weighing the plastic film that was used to wrap the bales, and the plastic film or plastic net that was used to secure the bale in the baler chamber. All the film were dried and weighed. The thickness of the films (both stretch film and tying film) which cover the curved side of the bale were measured on each bale with 12 replicates using a micrometer. The data were analysed by ANOVA utilizing the type of tying (T), the number of film layers (L), and the bale sampling zone (Z) as the fixed factors, with four replicates.

Results and discussion The bale weight at baling ranged from 527 to 548 kg bale^{-1}, with no difference between treatments (Table 1). The DM densities of the bales ranged from 230 kg m^{-3} to 247 kg m^{-3} and the values were higher than bale silage densities usually reported in the range of 150 to 200 kg DM m^{-3}. This was probably due to the low operating speed of the baler during baling and to the cutting of the crop made by knives in the baler chamber, that is known to increase the density of the bale to some extent. The amounts of stretch film used were about 0.90 kg bale^{-1} and 1.27 kg bale^{-1} for the four- and six- layer settings, respectively. The plastic used to tie the bale was about 0.22 kg bale^{-1} and 0.27 kg bale^{-1} for the net and film tying systems, respectively. The film used to tie the bale increased the actual number of layers on the curved side of the bale (from 4 to almost 7 and from 6 to almost 9 in the four- and six- layer settings) and it likely contributed to creating an enhanced barrier which helped to prevent air entering at the curved side of the bale. This was reflected in a reduction of the bale surface covered by mould, that was particularly evident in bales secured with film and wrapped in four layer of plastic stretch. The high DM content of the silages restricted fermentation and resulted in high pH and low concentrations of acids (Table 2). The spoiled spots were highly contaminated by yeast and mould, especially those from bales wrapped in four layers. Irrespective of the treatments, yeast counts were always higher than 5 log

cfu g^{-1} of silage and mould counts were always higher than 6 log cfu g^{-1} of silage. It was concluded that to increase hygienic quality of the silage the moulded spot should be reduced to the minimum since, in farm conditions, the improper mixing of different parts of the baled silage when being incorporated into the feed-mixer, could enhance the final feed contamination with fungi and mycotoxin.

Conclusions The results have shown that the use of a film to secure the bales prior to wrapping could contribute towards increasing the anaerobic conditions of the bale throughout the conservation period. This reduces undesirable growth of mould on the bale surface, especially when four layers of polyethylene stretch film is applied. This study shows that film-tying is an interesting and feasible alternative to net-tying when round bales are chosen as the storage option for silage, without greatly increase plastic consumption.

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Table 1. Bale weight, bale density, plastic consumption, plastic thickness on the curved side of the bale, surface covered by mould and dry matter (DM) losses in relation to the tying method and the number of plastic layers applied.

Items	Tying method				Level of significance		
	Net-tying		Film-tying		Tying method	Number of layers	Interaction
	4 layers	6 layers	4 layers	6 layers	(T)	(L)	(T) x (L)
Bale weight (kg)	548	543	547	527	NS	NS	NS
Bale density (kg DM m^{-3})	230	231	247	234	NS	NS	NS
Tying plastic per bale (kg)	0.22	0.21	0.26	0.27	*	NS	NS
Stretch plastic per bale (kg)	0.90	1.28	0.90	1.26	***	***	NS
Total plastic per bale (kg)	1.12	1.48	1.16	1.53	NS	***	NS
Thickness of stretch film (µm) [1]	79	121	81	119	NS	***	NS
Thickness of tying film (µm) [1]	-	-	43	46	-	-	-
Micro-holes in the plastic cover	14	5	7	3	*	**	NS
Surface covered by mould (%)	25.3	2.6	3.3	0.8	***	***	***
DM losses (g kg^{-1} DM)	5.6	3.0	2.9	2.8	*	*	*

[1] Average values were measured on the curved side of the bale.

Table 2. Fermentation and microbiological characteristics in relation to the tying method, the number of plastic layers applied and the sampling position on the bale.

Tying method	Plastic Layers	Bale zone	DM (g kg^{-1})	pH	Lactic acid (g kg^{-1} DM)	Acetic acid (g kg^{-1} DM)	Water activity	Yeast (log cfu g^{-1})	Mould (log cfu g^{-1})
Film	4	outer	636	5.70	2.47	10.29	0.92	3.60	1.59
Film	4	inner	660	5.68	1.38	4.53	0.91	1.95	2.04
Film	4	spoiled	615	6.12	0.58	0.51	0.93	6.97	6.86
Film	6	outer	650	5.73	1.43	9.21	0.91	2.09	1.28
Film	6	inner	642	5.59	3.36	9.57	0.93	1.30	0.87
Film	6	spoiled	640	5.93	0.96	0.66	0.92	7.62	5.80
Net	4	outer	679	6.28	1.04	9.25	0.94	4.76	6.24
Net	4	inner	698	5.70	1.50	8.26	0.87	1.83	3.09
Net	4	spoiled	608	6.53	0.07	0.26	0.92	6.53	6.94
Net	6	outer	667	5.74	0.98	9.64	0.93	3.28	3.19
Net	6	inner	675	5.67	1.64	5.04	0.91	1.80	1.53
Net	6	spoiled	643	6.88	0.24	0.59	0.93	7.92	5.55
	Level of significance								
	Tying method (T) [1]		NS	**	NS	NS	NS	NS	**
	Number of layers (L)		NS	NS	NS	NS	NS	NS	***
	Sampling zone (Z)		NS	***	NS	***	NS	***	***
	T x Z		NS	*	NS	NS	NS	NS	**

[1] NS = P > 0.05; * = P < 0.05, ** = P < 0.01. The interactions TxL, LxZ, and TxLxS were all NS.

Recovery and PCR-based characterization of *Listeria* strains and investigation on managerial factors influencing its occurrence on farm baled silages

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Keywords: baled silage, *Listeria* spp., silage quality, aerobic deterioration

Introduction Anaerobiosis is critical for successful ensilage, and it can be more difficult to achieve adequate anaerobic conditions with baled silages compared with conventional bunker or clamp silages (O'Brien et al. 2007, Borreani and Tabacco 2008). The penetration of air into the silage stimulates aerobic bacteria, yeasts and moulds and causes aerobic deterioration. This aerobic activity results in dry matter (DM) and nutrient losses, the accumulation of pathogens and mycotoxins, and reduced DM intake. Among pathogens, *Listeria monocytogenes* is a potentially dangerous foodborne pathogen that represents a primary concern in the production of Gorgonzola, a Protected Designation of Origin (PDO) Italian blue veined cheese produced in the Piedmont and Lombardy regions (Italy).
The aim of this research was to investigate the occurrence of *Listeria* spp. strains in baled silage fed to cows that produce milk destined to Gorgonzola production and to characterize by PCR-based method all isolated strains.

Material and methods A survey was carried out over 2 years in the western Po plain (Italy) on 20 dairy farms (mainly Italian Friesian cows) that give milk (about 25,000 kg d^{-1}) to a Gorgonzola producing plant. Each farm was visited four times and, on each visit, one sealed bale was examined in detail (for a total of 80 bales). A questionnaire was completed on each studied farm with information on the baled silage-making process, bale storage and management. Bales were carefully examined for visible holes or damages to the cover and sampled for fermentation and microbiological analyses. The percentage of the total surface area affected by fungal growth was calculated for each bale. Four samples were taken from the bale surface (0-540 mm), in four positions where no mould or spoilage were visible and from 2 to 6 samples were also taken from the surface that was covered by mould (0-120 mm). ISO 11290-1:1996/Amd 1:2004 (2004) method was applied to all collected samples for the isolation of *Listeria* spp. and PCR for the identification of *L. monocytogenes* (D'Agostino et al. 2004). The other *Listeria* species were identified after 16S rRNA gene sequencing. Statistical analyses were performed in order to explore differences between spoiled and non spoiled areas. In order to investigate effects of managerial factors on spoilage (expressed as percentage of the total surface area affected by fungal growth), a Kruskall-Wallis test was used. Signed Rank test and χ^2 McNemar test were used for quantitative and frequency data, respectively. Results were considered significant when $P < 0.05$. Interquartile range (IQR) was used as a measure of statistical dispertion. *Listeria* characterization was performed using ERIC and REP primers, following the protocol of Jersek et al. (1999) with minor modifications. The results were then combined and a dendrogram was generated by the Unweighted Pair Group Method with Arithmetic mean (UPGMA). Shared profiles were defined those shared between one or more strains, whereas unique profiles those which were characteristic of a single strain.

Results and discussion Fungal growth was observed on the surface of 62 of the 80 bales examined. Bale management factors and storage characteristics and their relationships with the extent of the spoiled area of the baled silages are reported in Table 1. Lower time of conservation (lower than 200 days) and higher amount of plastic used for wrapping bales (i.e. at least 6 layers of plastic) are the most influencing factors that can contribute to reduce the incidence of fungal growth on the bale surface. Furthermore, in agreement with O'Brien et al. (2007), bales with damaged plastic cover had a higher proportion of their surface area visibly contaminated with fungi than bales where the film appeared intact. *Listeria* spp. and *L. monocytogenes* were detected in 21 and in 6 out of 80 bales, respectively, and 67% of the positive samples were collected in moulded zones. Visible fungal-contaminated silages had lower DM content and higher water activity, pH, yeast and mould counts than silages that were free of visible fungal contamination sampled from the same bale (Table 2). A total of 56 and 24 strains of *Listeria* spp. and *L. monocytogenes* were PCR-typed, respectively. The technique was preferred over the PFGE because this method implies a higher level of expertise, higher costs and technology, therefore it was not a suitable alternative for the screening-typing. Results showed that the method could be successfully applied to all tested strains, with high repeatability level. The analysis of *Listeria innocua* and *Listeria seeligeri* strain clustering evidenced that the overall similarity between strains was 75,9%, indicating high genetic homogeneity between isolates recovered from the 20 studied farms as evidenced by the

observation that 7 PCR profiles grouped strains belonging to more than one farm. Results were similar when considering the grouping of *L. monocytogenes* strains with a similarity level of 79%. Two major clusters grouped isolated retrieved in different farms, with similarity values as high as 95%.

Conclusions Bale area affected by fungal growth was confirmed to be at risk of *Listeria* contamination. The application of the PCR typing in *Listeria* spp. allowed the timely typing of strain and the setting up of a database needed for characterize the primary production of a well defined Gorgonzola PDO production chain. This database will be used in future study in order to systematically type *Listeria* spp. (and mostly *L. monocytogenes*) in the food chain, in order to understand the putative transmission/dissemination pathways.

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Table 1. Bale management factors and relationship with extent of spoiled area on the bale surface.

Management factor		Bale surface covered by mould	Proportion of bales
Plastic layers applied (n)	4 - 5	14.4 [a]	0.18
	6 - 7	3.5 [b]	0.51
	> 8	2.3 [b]	0.31
P value		*0.0017*	
Days of conservation	< 100	1.2 [c]	0.22
	100-200	2.6 [c]	0.51
	201-300	9.0 [b]	0.16
	> 300	18.2 [a]	0.11
P value		*< 0.001*	
Bale orientation	Flat end	5.6	0.55
	Curved side	4.6	0.45
P value		*NS*	
Storage location	On farm	4.9	0.44
	In the field	5.3	0.56
P value		*NS*	
Visible holes in the plastic cover (n)	No holes	1.0 [b]	0.48
	1-2	4.4 [b]	0.22
	> 2	13.3 [a]	0.30
P value		*< 0.001*	

Table 2. Characteristics of non spoiled and spoiled samples collected on the same bale in the 2-years survey at Novara, Italy (IQR = interquartile range).

	Non visible spoiled areas		Visible spoiled areas		
Variables	Median	IQR	Median	IQR	P value
DM content (g kg^{-1})	559	210	487	186	*
pH	5.78	0.77	6.25	1.60	*
Water activity (aw)	0.94	0.053	0.96	0.038	*
Nitrate (mg kg^{-1} DM)	0	457	0	696	NS
Yeast (log cfu g^{-1})	3.75	3.04	6.31	3.00	*
Moulds (log cfu g^{-1})	2.13	1.60	4.93	3.48	*
Lactic acid (g kg^{-1} DM)	2.90	12.24	3.48	13.13	NS
Acetic acid (g kg^{-1} DM)	3.69	5.32	4.01	6.33	NS
Propionic acid (g kg^{-1} DM)	0	2.91	0	1.66	NS
Butyric acid (g kg^{-1} DM)	0	0	0	0	NS
Ethanol (g kg^{-1} DM)	9.25	17.23	1.39	7.85	*
Ash (g kg^{-1} DM)	10.7	3.75	10.7	2.81	NS
Positive to *Listeria spp.*	12.9%		27.4%		*
Positive to *L. monocytogenes*	6.5%		8.1%		NS

The effects of varying vacuum levels during packing on the chemical composition and feed quality class of previously ensiled silage

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Keywords: silage, vacuum, packed silage, storage in vacuum bag

Introduction Silage has been made with traditional silage making techniques that usually uses above-ground horizontal silage silos and silage is made in the farms to meet their own needs in Turkey. Trade of silage, is not common, yet. The main barrier for trade in silage is the current traditional silage making technique in which silage is not sold and transported to long distances because the silage is not packaged. Silage that is bought from close distances has to be consumed within a few days, otherwise silage will be spoiled. Using silage packing method would help to overcome this problem. There are two silage packing application methods available. First method: fresh material is packed directly from the field during harvest period. Second method: fermented silage on bunker silo is packed and offered for sale. In the first method, packed silage is not ready for feeding only after the fermentation is completed inside package. But in the second method, packed silage is ready for feeding. In this study, the aim was to determine the effects of varying vacuum levels during packing on the chemical composition and quality class of previously ensiled silage as discussed in the second methods.

Material and methods This study was done at Ataturk University, Faculty of Agriculture in Erzurum, Turkey. In this study maize (*Zea mays*), sunflower (*Helianthus annuus*), sorghum (*Sorghum vulgare*) and sorghum-sudangrass hybrid (*Sorghum sudanense* Staph.) were used as silage materials. Plants of silage were harvested at about dough stage by tractor pull-type silage harvester equipped with chopper. Plant material of each species was filled in a separate horizontal bunker silo and compressed by tractor. The silos were covered by plastic cover and were left to fermentation for 60 days. This method is called traditional silage making technique. After the fermentation period, bunker silos were opened and 20 kg silage material was taken from each silo (fresh silage – FS). Chemical compositions of fresh silages were determined by using 5 kg silage material. The remained 15 kg silage was filled 15 vacuum bags that size of 200 × 300 mm and thickness of 90 microns, each bag was filled with 1 kg FS. Vacuum bags were closed in three different vacuum levels by vacuum machine (Multivac C 200). Vacuum levels were a) non-vacuum (V_0), b) 41.5 kPa vacuum (V_1) and c) 83.0 kPa vacuum (V_2) (Figure 1). The experimental plan was designed as 4 different species × 3 different vacuum level × 5 repetitions. Silage samples were preserved inside vacuum bags for one year under room temperature. After this period, the bags were opened and pH, dry matter content (DM), crude protein (CP), ADF and NDF, levels of organic acids (LA, AA, PA, BA) were determined. Silage quality class was determined by DLG score (DLG = [220 + (2 × silage dry matter (%) – 15)] – 40 × silage pH value (Kilic, 1986).

Chemical composition of initial fresh silage and vacuum silages were compared by analysis of variance (SAS 9.0 software package; SAS 1982). Each species was analyzed separately.

Vacuum machine	V_0 : non-vacuum silage	V_1:41.5 kPa vacuum silage	V_2:83.0 kPa vacuum silage

Figure 1. Vacuum machine and vacuum silage samples used in this study.

Results and discussion The hypothesis was that when concrete silos were first time opened and the samples would be contact with air during the packing period it should be causing increase pH and DM of vacuum silages. Furthermore, the vacuum levels was supposed to lessen the negative effect of air.

Generally, dry matter content and pH value of both fresh and vacuum silages were compatible with good-quality silage (recommendation 3,5 - 4.5 pH and 25 - 35% DM; Mc Donald, 1981; Roth, 2001). When fresh silage samples from concrete silo were transferred to bags for second time packing the numerical values of pH and DM content increased in all silages. However, these changes were significant only for sunflower and sorghum silages ($P < 0.05$). The level of vacuum application had no significant

effect on pH and DM content of silages ($P > 0.05$). According to DLG scores all silages were very good quality to over 80 points. Vacuum application did not affect the quality class of silages ($P > 0.05$). Values of CP, ADF and NDF of vacuum silages numerically reduced in the fresh silage of the four species, but these changes were not statistically significant ($P > 0.05$). Values of LA, AA, PA and BA of both fresh and vacuum silages, generally, they were compatible with silos acids of good and quality silages [(LA > 20 g/kg DM, AA < 20 g/kg DM, PA and BA < 10 g/kg DM) - (Mc Donald 1981; Roth 2001)]. The vacuum levels had no significant effect on the silo acids ($P > 0.05$).

Conclusions Pre-fermented silage on the concrete silo, packing of different vacuum level had not significant effect on the chemical composition. Silages obtained from all vacuum levels were in very good quality class.

Table 1. Effect of vacuum treatment on chemical composition (g kg^{-1} DM) and quality class of silages made of four plant species. Trea =treatment; FS=fresh silage; V_0, V_1 and V_2 = levels vacuum treatments for 365 d study period (see text for details).

Type of silage	Trea.	pH	DM	CP	ADF	NDF	LA	AA	PA	BA	DLG	SQC
Corn	FS	3.83 ^{b}	308	80	310	526	50	9	5	3	113	Very good
	V_0	3.94 ^{a}	312	80	307	520	51	13	7	4	110	Very good
	V_1	3.92 ^{ab}	329	80	305	517	52	12	6	4	114	Very good
	V_2	3.90 ^{ab}	339	80	305	515	52	12	6	4	117	Very good
Sunflower	FS	4.29 ^{b}	290 ^{b}	95	430	435	20	22	14	12	92	Very good
	V_0	4.38 ^{a}	307 ^{a}	90	420	430	19	25	17	15	91	Very good
	V_1	4.36 ^{a}	315 ^{a}	91	415	428	20	23	16	15	93	Very good
	V_2	4.36 ^{a}	318 ^{a}	91	415	428	20	23	16	14	94	Very good
Sorghum	FS	4.06 ^{b}	380 ^{c}	73	338	540	38	14	9	7	119	Very good
	V_0	4.16 ^{a}	394 ^{a}	71	336	537	35	19	12	11	117	Very good
	V_1	4.13 ^{a}	401 ^{ab}	72	334	535	36	18	9	11	120	Very good
	V_2	4.09 ^{b}	407 ^{bc}	72	334	535	36	17	9	11	123	Very good
Sorghum-sudangrass hybrid	FS	3.90 ^{b}	340	68	378	565	36	15	5	6	117	Very good
	V_0	4.02 ^{a}	354	66	370	560	33	18	6	8	115	Very good
	V_1	3.99 ^{a}	360	66	368	558	34	17	6	8	117	Very good
	V_2	3.95 ^{ab}	364	66	368	557	34	16	6	8	120	Very good

*: Each species was evaluated separately. Different letters in the same column indicated significant difference between the average ($P<0.05$). LA = Lactic acid; AA= acetic acid; PA= propinatic acid; BA= buturic acid; DLG= DLG score; SQC= silage quality class.

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Factors affecting estimation of spoilage indices in silage: Effects of amount of silage evaluated and type of container

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Keywords: aerobic stability, methodology, corn silage

Introduction Aerobic stability is a measure of the shelf life of silage and an indirect measure of the likelihood of undesirable microbial activity, which predisposes to heating, nutrient depletion and growth of pathogenic organisms. Different methods are used to perform this assay, which likely affects the outcome. This project aimed to examine effects of container type and amount of silage evaluated on the aerobic stability of corn silage.

Material and methods Corn silage was collected from the face of a bunker silo after removal of the daily feeding amount. Three different amounts of corn silage, 1, 2, or 3 kg, were packed at the same density (1000 kg/m^3) into 20 L plastic buckets (PB) or 20 L styrofoam containers (SC) in quadruplicate. Wireless temperature sensors were placed in the center of the silage mass in each container and set to record temperatures every 30 min for 14 d. Containers were covered with two layers of cheesecloth to avoid dehydration and placed in an enclosed room. Three additional sensors were placed at different locations around the containers for determination of ambient temperature. Aerobic stability was denoted as the length of time that elapsed before silage and ambient temperatures differed by more than 2ºC (Pedroso et al. 2010).Temperature data were plotted against 'hours of aerobic exposure' to calculate the area under the aerobic stability curve. This stability index accounts for temperature fluctuation during the entire length of time of this assay. Maximum and minimum temperatures were recorded and temperature range calculated. The experiment had a randomized complete block design and a 2 (container type) x 3 (amount of silage) factorial treatment arrangement. The statistical model contained silage amount, container type and their interaction. The GLM procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC) was used to analyze the data. Mean separation was performed using Tukey's test.

Results and discussion Minimum temperature was generally lower for PB versus SC silages and the effect of the silage amount differed with container type (P=0.019). The PB silages also tended to have a greater temperature range than SC silages (P=0.07). These results indicate that PB allowed higher heat exchange between the silage and the surrounding environment than SC. Maximum temperature was unaffected by container type but was highest when silage amount was greatest (P=0.008). Temperature range was also widest when the silage amount was greatest (P = 0.01). The area under the aerobic stability curve, which reflects the heating duration, was greatest when the silage amount was greatest, particularly in SC silages (P= 0.073). Aerobic stability was greater for silages in SC than PB (P=0.003) but was unaffected by silage amount.

ConclusionsThis study shows that container type and silage amount influenced heat production during aerobic exposure. Furthermore, container type affected the aerobic stability result. These procedural differences may confound the validity of comparing aerobic stability and heat production results from different laboratories.

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Table 1. Effects of container type and silage amount aerobically exposed on spoilage measures of corn silage.

	Plastic bucket			Styrofoam container			SEM	Container type (Cont)	Quantity (Qt)	Cont. × Qt[1]
	1 kg	2 kg	3 kg	1 kg	2 kg	3 kg				
Min. temp.[2], ºC	16.4	15.2	16.8	19.1	17.8	17.5	0.37	0.0001	0.016	0.019
Max. temp.[3], ºC	26.6	31.3	40.5	31.4	24.5	34.4	2.92	0.270	0.008	0.111
Temp. range[4],ºC	10.2	16.1	23.6	12.2	6.6	16.9	3.01	0.071	0.010	0.165
Area[5] x10[4], ºC x h	2.2	7.2	16.6	6.0	1.2	9.6	2.39	0.149	0.002	0.073
Aerobic Stability[6],h	78.4	97.4	64.0	203.7	168.8	134.2	31.34	0.003	0.390	0.618

[1]= Interaction between container type and quantity of silage, [2] minimum temperature observed, [3] = Maximum temperature observed, [4] = difference between maximum and minimum temperatures, [5] = area under the aerobic stability curve, [6]= length of time that elapsed before silage and ambient temperatures differed by more than 2ºC.

Precision farming – online determination of yield and dry matter and yield-depending silage additive application in grass and maize

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Keywords: application, precision farming**,** silage additives

Introduction Several companies offer agricultural equipment using different technological principles which enables the continuous online determination of DM and yield during the chopping process of grass and maize and other silage crops (Anon. 2012). The yield measurement system used by John Deere identifies the volume of crop entering the chopper based on the displacement of the feedrolls as well as their speed. Until not very long ago, the moisture content of the crop at harvest could not be measured, but was rather estimated by the driver of the chopper, which ultimately resulted in insufficiently accurate information. The use of the technology of Near-Infrared-Reflection-Spectroscopy (NIRS) can significantly improve the precision of moisture and dry matter (DM) determination, respectively.

The continuous determination of DM offers the opportunity to permanently monitor the actual crop moisture level and its unavoidable fluctuation during harvest. The recorded data are saved by the computer system of the chopper and can be allocated during the chopping process, or afterwards, to specific locations by GPS (Global Positioning System). Based on this technology, the mapping is possible of fresh matter (FM) and DM yield, moisture/DM levels, fuel consumption as well as silage additive use across fields and field segments. The basic NIRS system includes a technology which can contribute to optimizing the efficiency of the machinery and of the total harvest procedure. Exemplarily, the DM based, automatic adjustment of chop length during harvest is possible within a range of 4-38 mm. The system functions absolutely remotely, and based on different input variables on chop length, makes adjustments in short intervals (per second) without the driver´s interference. Thus, fuel consumption can be decreased and capacity and performance of the machinery be increased.
The online recording of yield and DM during harvest of grass and maize is a crucial pre-requirement for precise silage additive application. Only recently have agricultural machinery and equipment become available to measure those parameters online. The aim of the trials was to compare the accuracy of data of DM determination and silage additive application obtained by automatic recording on the chopper and by analyzing in a laboratory.

Material and methods The trials on grass (*Lolium multiflorum*) and maize (*Zea mays*) were carried out in October 2008 on a dairy farm in Pasenow, Mecklenburg-Pomerania, Germany. A John Deere chopper of the 7050*i* series was used in the harvest of both crops. This chopper was equipped with an automatic system (Harvest lab) for determination of DM based on NIRS technology. Additionally, a silage applicator (Comfort Data, SILA GmbH) was mounted on the chopper and connected to its software enabling the automatic application of silage additives, which were all provided by ADDCON EUROPE GmbH (grass: chemical additive KOFASIL ULTRA-KU and the biological additive KOFASIL LIFE-KL; maize: chemical additive MAIZE KOFASIL LIQUID-MKL and biological additive KOFASIL LIFE M- KLM).

Forage yield was measured by recording the time to fill forage trailers (grass: 10 trailers; maize 15 trailers) and the weight of the content by using a calibrated scale. DM was continuously recorded by harvest lab (3600 measurements per hour) and on 5 samples per trailer in the oven at 60 °C (grass: 50 measurements; maize: 75 measurements). The intended application rates of KL and KLM were set at 0.5 and 2.0 l/t, giving $4x10^5$ cfu lactic acid bacteria per g fresh forage for KL and $1x10^5$ cfu lactic acid bacteria per g fresh forage for KLM. For both chemical additives KU and MKL, a dosage of 4.0 l/t was used. Both products contain, among other substances, a specific concentration of the non-volatile active ingredient sodium benzoate which was used as an internal marker for the determination of application rate by chemical analysis in the laboratory.

The precision of the application was tested in two steps. Firstly, the comparison was made between the set application rate and that measured online by the harvest lab system of the John Deere chopper in connection with the Silaspray applicator. Secondly, the actual application rate recorded by the board computer was related to the values determined by analytical methods. Lactic acid bacteria counts were determined according to ISO 15214, whereas sodium benzoate was analyzed by HPLC.

Results and discussion There was no difference in recorded yield between online measurement on chopper and scale (table 1). Determination of DM by Harvest lab and oven resulted in identical values in maize, whereas a difference was found of 2% DM units in grass.

Table 1. Yield and DM as affected by analytical method.

Parameter/Analytical method	Grass		Maize	
	Mean	SD	Mean	SD
Yield (ton forage per trailer)				
Harvest lab	14.8	1.7	14.2	3.7
Scale	14.8	1.4	14.2	3.7
DM (%)				
Harvest lab	20.1	0.2	30.1	0.2
Laboratory	18.1	0.7	30.1	0.9

Yield-dependent silage additive application worked well in grass for both additive types with relative deviation of <10% from the intended dosage (table 2). The actual application rate for the inoculant in maize was identical to the intended dosage. MKL was underdosed by about 30%, which can be attributed to too a low capacity of the applicator regarding the required throughput based on the harvesting capacity.

Table 2. Yield-dependent silage additive application – comparison between set and online measured application rate.

Parameter	Grass			Maize		
	KL (l/t)		KU (l/t)	KLM (l/t)	MKL (l/t)	
Set application rate	0.5	2.0	4.0	0.5	2.0	4.0
Application rate Harvest lab	0.46	1.84	3.65	0.50	2.00	2.63

KL KOFASIL LIFE, KU KOFASIL ULTRA, KLM KOFASIL LIFE M, MKL MAIZE KOFASIL LIQUID

The calculated application rates based on sodium benzoate concentration in the sample were in good agreement with the actual application rate given by harvest lab (Table 3). The relative deviation between the two values was 3.8% in grass and 8.4% in maize, respectively.

Unfortunately, this kind of comparison was not possible to be carried out for biological additives on account of too a high epiphytic lactic acid bacteria (LAB) count on the fresh crop. Thus, the difference between LAB count of untreated and treated material lied within the typical error of the analytical method.

Table 3. Yield-dependent silage additive application – comparison between harvest lab and chemical analysis.

Parameter	Grass	Maize
	KOFASIL ULTRA (l/t)	MAIZE KOFASIL LIQUID (l/t)
Harvest lab	3.65	2.63
Chemical analysis	3.51	2.41

Conclusions It can be concluded from this trial that the system of online yield and DM determination is sufficiently precise under practical conditions, and that the software of the 7050*i* chopper series in connection with the applicator Comfort Data enables the accurate automatic application of silage additives based on yield. This technology makes possible the optimization of costs associated with the use of silage additives.

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Influence of seasonal temperature differences on maximum storage time of maize silage when using automatic feeding systems (AFS) for dairy cattle- first results

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Keywords: automatic feeding, dairy cattle, feed quality, microbiological status, silage temperature

Introduction Feeding is responsible for the majority of the costs of dairy farming, accounting for between 40 and 45 % of the whole-life costs of milk production alone. Moreover, besides milking, feeding is the most time-consuming activity in milk production, accounting for approx. 20 % of the total working-time requirement (Schick 2006). According to the claims of the manufacturers, automatic feeding enables a significant easing of the workload, better feed hygiene, and lower feed loss (Grothmann et al. 2011). With automatic feeding, the feed components are stored for a minimum of 24 hours in various filling devices. Particularly in the summer, contact with air during transport from the feed store to the filling device as well as lengthy storage in filling devices – especially in the case of maize silage – can bring about a significant reduction in the feed quality. This may result in reduced feed consumption as well as repercussions for animal health.

This study helps to establish vital information for the operation of automatic feeding systems. In terms of animal health and the saving of labour, it is important to obtain information on the maximum storage time of the various feed components in these filling units. The influence of high ambient temperatures in summer, especially on the quality of maize silage, must be clarified. The aim of this study was to determine the influence of the high temperatures in summer and the low temperatures in winter on the potential storage time in an automatic feeding system (AFS) filling units.

Material and methods The investigations took place over eight weeks in July and August 2011 on the ART experimental farm, using AFS produced by Pellon, the Finnish manufacturers. For a period of two days, the Pellon filling devices were filled with maize silage from a tower silo as well as square-bale silage. Three filling passes per week were carried out. During this time, the temperature of the maize silage, the ambient temperature and the atmospheric humidity in the barn were measured every 15 minutes. In addition, temperatures were recorded in the maize silage and in the barn. The temperature in the maize silage was measured by means of temperature sensors and saved via an Eltek Squirrel Meter. Three temperature sensors per feed type and batch were positioned in the feed, enabling a measurement to be recorded over two days with no need to move the sensors. Two sensors were positioned in the top third and one sensor was positioned in the centre of the feed.

In order to assess initial feed quality, samples were taken directly after filling and after two days' storage from all feed batches. Samples were deep-frozen before being collected and transported to the laboratory (Chemistry Lab, University of Hohenheim), where they were examined for dry matter as well as crude protein, crude fibre, crude fat and crude-ash content. Other feed samples were analysed for the occurrence of yeast and mould, energy content, pH values and fermentation acids to evaluate altered feed quality. Directly after these samples were taken they were packed, vacuum-sealed and cooled. The samples for analysis for yeasts and mould were driven directly to the UFA Laboratory in Sursee after packaging and cooling.

For further characterisation of the starting material, aerobic stability under controlled conditions was determined with the aid of a standard laboratory method (Honig 1990). The samples for the aerobic-stability testing were vacuum-packed, cooled and dispatched by courier to the Agroscope Liebefeld-Posieux Research Station. Aerobic stability was defined as ending at a feed temperature of 3°C above the ambient temperature (DLG 2000; McDonald et al. 1991; Woolford 1984).

Results and discussion Initial results from the measurements taken in summer 2011 with its high temperatures show that the method of filling the maize silage (loose maize silage from the tower silo, square bales) has a considerable influence on the maximum possible storage time. Under laboratory conditions, the aerobic stability of the starting material was in most cases lower for square-bale silage than for tower silage. By contrast, temperature measurements in the filling device showed a greater temperature increase for the tower silo variant, as very high temperatures occurred after only a short time for the loose maize silage from the tower silo and only low temperature differences existed for the most part with the square bales(Figure 1). Feed provision and feed intake of the animals was reduced by up to 20% with the tower-silo variant, with the result that a significant influence on the animal was already recognisable.

The presence of air has an adverse effect on silage. This was described *inter alia* back in 1964 by Beck & Gross, as well as in 1990 by Woolford. Oxygen enables various aerobic spoilage microorganisms which survived in the anaerobic phase of silage-making to become active and multiply (Woolford, 1990; Borreani & Tabacco, 2010).

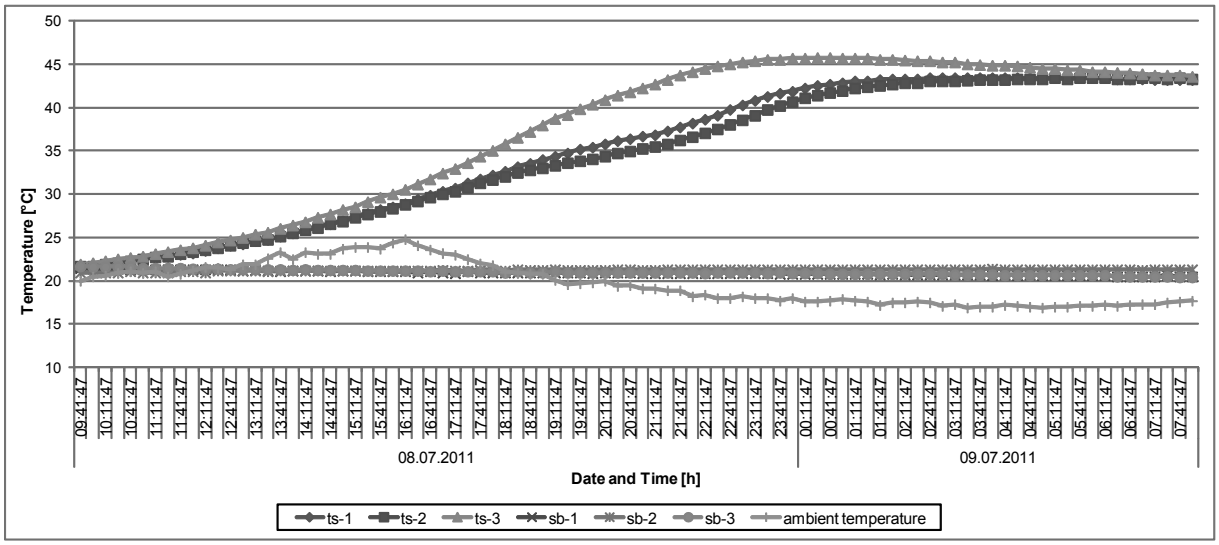

Figure 1. Measurement of temperature in maize silage: tower silo (ts) and square bale (sb)

Conclusions The initial results show that for feed which has not been loosened and mixed with air, storage in the filling devices over two days may also be possible. The potential for easing the workload (by filling with several daily portions) even at higher summer temperatures can therefore be achieved via the appropriate course of action when filling the filling devices. On the basis of our experiences in this trial, animal-specific parameters such as feed consumption and milk yield are also recorded. In addition, the trial was expanded by the 'silo blocks from bagged silage' variant. On this basis, after the 2012 winter measurements the experiment will continue with the same methods in summer 2012.

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Sensor controlled total-mixed-ration for nutrient optimized feeding of dairy cattle

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Keywords: feed mixer wagon, near infrared spectroscopy, feed ration improvement, total-mixed-ration

Introduction The exact regulation of dry matter, energy and ingredients in green fodder has a large advantage in the ration optimization of economical animal nutrition. The production of in-house fodder is connected with varying contents of dry matter, energy and ingredients in the individual green fodder of each farmer.

The near infrared spectroscopy (NIRS) is used in many agricultural ranges. NIRS is a fast and not destructive method to determine substrate-specific characteristics (Stockl et al., 2011). With the employment of NIRS today the dry matter content can be measured with accuracy by 2 % on the forage harvester (Keller, 2009). The documentation of contents in case grass, silage and hay in round bundles was likewise accomplished on basis of NIRS successfully (Walther et al., 2011).
Main object of the project "SenToMiRa" is a nutrient optimized total mixed ration for feeding dairy cattle. By installation of a near infrared sensor (MUT-Group) on the material flow at a self-propelled feed mixer wagon (STRAUTMANN) it will be tested to fill and analyze the individual ration components according to the specific needs of the respective performance group. Particularly the in-house basal feed can be analyzed real time regarding the content of dry weight, nutrients and energy content. The adaptation of the ration has to be done by balancing the components depending on the actually measured values of the different parameters. The parameters of the desired ration should be nearly consistent at every feeding time. So with the developed technology it should be ensured that at each time and each feeding place, a qualitatively homogeneous TMR is mixed. This TMR is adapted to the performance level and specific ration requirement of the respective group of dairy cattle.

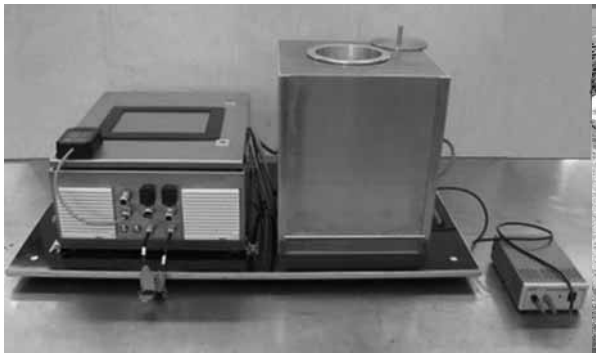

Figure 1. Turn table with NIRS

Figure 2. Self-propelled feed mixer wagon

Material and methods For a meaningful measurement of fodder it is important to have a good calibration. To get a good calibration model 400 - 500 samples are needed. The samples are measured with the near infrared sensor and calibrated by the wet chemistry analyzes. For the calibration of the NIRS we measured the samples with an offline method by using a developed turntable. Figure 1 presents the turntable for simulation. Figure 2 shows the self-propelled feed mixer wagon where the near infrared sensor will be installed. With a modification the NIR sensor hang under the fodder samples. Through this process we simulated the silage-taking from the silo surface into the fodder mixer. Reference measurements of dry weight, nutrients and energy content with wet chemistry analysis in the laboratory completed the components for the calibration. The wet chemistry analysis will be performed by a certificated laboratory (LKS Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH). By the measurements we took of each silo surface different samples.

Results and discussion By analyzing the off line measurements we noted that in the surface of the silo it was a high variability. The contents of dry matter changed significantly between maize silage and grass silage (Figure 3 and 4). The results show the dry matter contents of 35 samples taken at 10 maize silos. The dry matter changed from 270 to 400 g DM kg^{-1} FM. The dry matter of grass silage changed from 270 to 570 g DM kg^{-1} FM, by 15 silos with 45 samples. In the Figures one point presenting one sample.

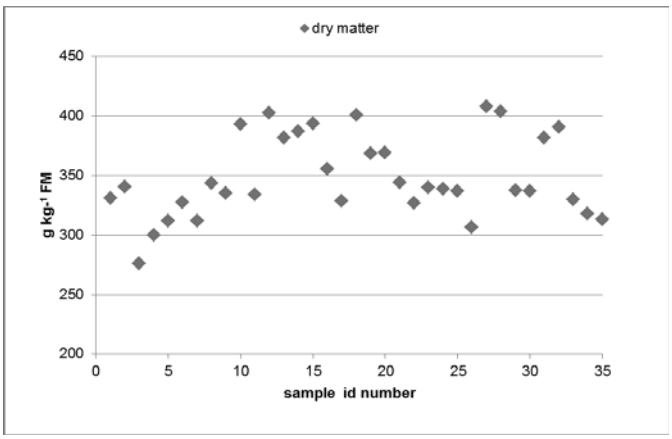

Figure 3. DM content in different grass silage silos.

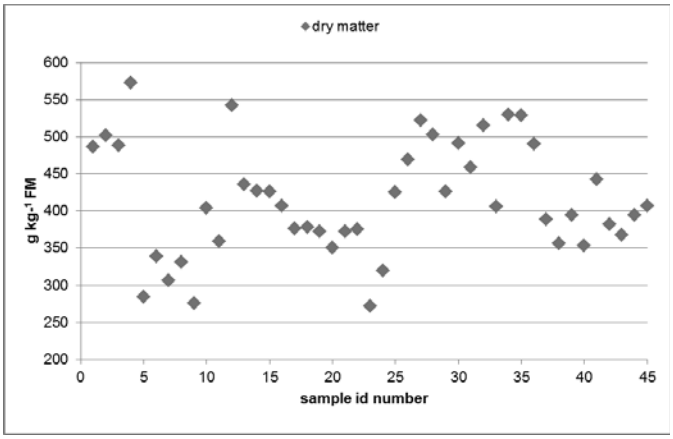

Figure 4. DM content in different maize silage silos.

The permanent and detailed qualitative analyses allow during the feed out, conclusions on preservation, and storage problems and thus support the optimization of the basal feed preparing. The next step will be to install the near infrared spectroscopy system on the self-propelled feed mixer wagon. The Sensor of the near infrared spectroscopy system will be installed in the area where the milling machine tool is working. So the sensor can measure online the material flow from the silo into the feed mixer.

Conclusions By the enumerating of the different contents of dry matter in the same silo and in different silos it is important to know the true contents of dry matter, energy and ingredients. Only by this the quality parameters of the desired ration should be nearly consistent at every feeding time.

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Dry matter losses of grass and maize silages in bunker silos

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Keywords: bunker silo, dry matter losses, grass silage, maize silage, silage

Introduction Agricultural and food production faces worldwide challenges due to climate change, population growth and loss of agricultural area. Therefore the optimization of the efficiency in feed management is highly relevant. One major possibility to increase the feed production efficiency is the reduction of feed losses, on field but eminently at storage. Several authors reported mean dry matter (DM) losses during ensiling of about 16 to 32 % (Watson and Nash 1960, Bastiman and Altman 1985), whereas only 7 % of them are supposed to be unavoidable (Zimmer 1980). Because those results are obtained from measurements in bench-scale silos, transferability of test results to practice is questionable. Mayne and Gordon (1986) reported 6 % DM losses of wilted grass silages in silos of 100 t capacity. However, aerobic deterioration as discussed by Spiekers et al. (2009) as a major source of DM losses has not been taken into account. Therefore, concerning DM losses of large scale silos, reliable values still are not available. For this reason, in the present investigation 48 silos where studied by the total in/total out procedure to get clear information about acceptable dimensions of DM losses for farm scale bunker silos and to find out promising starting points for the improvement of the efficiency of feed management.

Material and methods The mass flow from field to bunk was examined on one organic and two conventional farms of the Bavarian State Research Centre for Agriculture. Data was collected over a period of four years (2008 - 2011) from 26 grass, four lucerne and 18 maize silos, and DM losses were determined. The feed management enfolded permanent grassland, maize (all farms) and other forage crops (e.g. grass-clover-mixtures, perennial grass or lucerne on two farms). The permanent grassland was dominated by grass species, in particular by *Alopecurus pratensis*. The proportion of legumes in the ensiling materials was of significance on the organic farm, exclusively. Five cuttings a year from permanent grassland and one-day-silage are common practice on all involved farms. Mean yield of silo maize on these farms accounts for 15 t DM/ha. The good practice of ensiling on the farms is geared to the guidelines of the German research and advertiser group feed preservation (DLG 2011). All crops were harvested with a self-propelled forage harvester, cut to a theoretical chopping length of 20 - 50 mm (grass and lucerne) or 4 - 9 mm (maize) and analyzed for their characteristics (Table 1). Grass and maize silages were conserved in side walled bunker silos using silo pit foils, underlay films, silage films and protection covers and weighted down with gravel bags. The bunker silos capacities ranged from 170 to 690 m³. The mass harvested or rather the transportation per hour normally didn't exceed the fourth of weight used for compaction.

Table 1. DM and ADFom[1] contents and fermentation coefficients[2] of three different types of ensiling material.

	Grass (n = 26)			Maize (n = 18)			Lucerne (n = 4)		
	DM	ADFom[1]	FC[2]	DM	ADFom	FC	DM	ADFom	FC
	%	% DM		%	% DM		%	% DM	
Mean	31	30	42	36	25	54	32	37	36
Range	22-44	21-34	30-55	30-49	19-29	44-69	22-40	36-38	26-45

[1] ADFom: acidic detergent fiber, without residual crude ash in % of the DM content; [2] FC: fermentation coefficient = DM % + 8 x (sugar/buffering capacity) (according to Weißbach et al. 1974 cited in DLG 2011)

DM losses were determined by using the total in/total out procedure. During harvesting, each wagonload was weighed on a 40 t cart scale (measurement accuracy ± 10 kg). One sample was taken from each load and pooled for each field. These pooled samples were analyzed for crude nutrient concentrations. At least four samples per ha were analysed for DM content. During the period of removal (on average three months), all feedstuff taken out of the silos was weighed by means of a fodder mixer wagon equipped with digital weighing systems. The DM content of the silages was determined weekly. The values were corrected for volatiles. Samples were taken from the face of the silos, using a special silo auger for sampling (drilling depth 40 cm). The amount of DM losses was calculated by subtracting the removed DM masses from the ensiled ones, summing up all single values, respectively. Evaluation of all data was carried out by statistical analysis, based on a SAS macro program.

Results and discussion DM losses determined for 48 farm scale silos are presented in figure 1. The collected data followed a normal distribution. For maize silages an average of 10 % DM losses was observed, for grass- and lucerne silages 9 % and 12 %, respectively. Compared to the values reported by Zimmer (1980), the average DM losses of these silages appear to be low. This finding can likely be attributed to the good ensiling practice and the high feed out rate on the involved farms. Nevertheless, silos with 26 % losses of DM were observed. According to the present results, a benchmark of maximum 8 % unavoidable DM losses can be set for bunker silos, independently from the crop. This was unexpected as DM losses of grass silages in laboratory scale exceeded those of maize silages (Thaysen et al. 2007).

Grass silages, which were shown to be most heterogeneous, exhibited the widest range of DM losses (-2 - 26 %), followed by maize (-4 - 23 %) and lucerne silages (6 - 15 %). As negative values for DM losses are impossible, they have to be ascribed to the precision of the method, which is strongly dependant on the accurateness of data collection. Potential sources of error are an inadequate distribution of control points for DM determination, as well as inaccurate weighing by fodder mixer wagon.

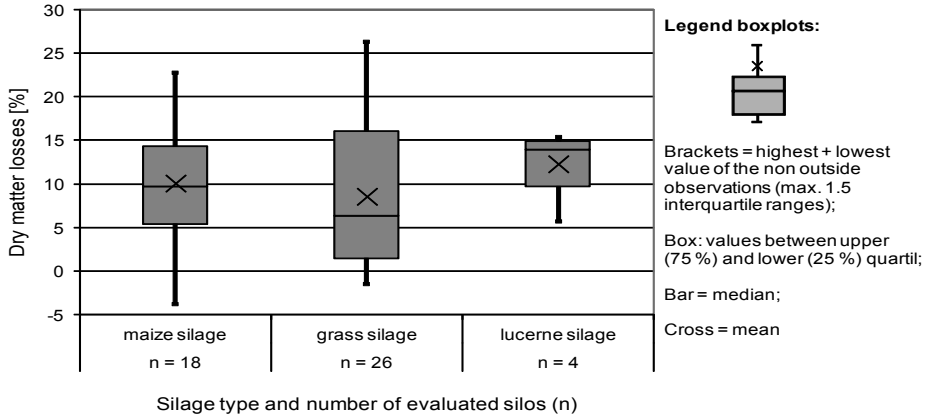

Figure 1. Dry matter losses (%) of silages determined by the total in/total out procedure.

ConclusionsA method for the determination of DM losses in bunker silos has been described. The DM losses in grass silages from field to bunk seem to be comparable with those of maize, assuming good ensilage practice. As a benchmark for maximum DM losses in bunker silos, **8 %** emerged to be an adequate value. In spite of problems concerning data collection, the method seems to be adaptive to commercial farms as a tool of control.

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Modelling working time requirement and work performance using a mowing system as an example

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Keywords: working time requirement, work performance, modelling, silage-making, mowing

Introduction Quality management and time management are closely linked in the silage-making process chain (Wagner 2005). If timely harvest for optimal forage quality is constrained by available machinery or labour, increases in machinery investment can have a positive effect on farm profit (Rotz et. al. 2003). The exact modelling of agricultural work processes constitutes an essential basis for the use of agro-economic data for work budgets or in farm planning. In addition to *working time requirement* per reference value, the importance of the *work performance* to be expected increases, in particular for contractors. The approach in data collection, preparation and data modelling for calculating time requirement and performance of work procedures is described in the following, using the example “mowing with rotary mower”.

Method Key work-economics data are systematically recorded, prepared and analysed according to a standardised procedure (Table 1). This default method for work-economics analyses forms the basis of numerous budgeted labour times and model calculations (Schick 2011, Heitkämper 2010). Analysis of the individual work procedures in terms of workflow and influencing factors affecting the latter is followed by the actual measurement of the time input and the relevant influencing factors. Data are statistically analysed and stored in a database table. For easy comprehensibility, the database table also contains a description of the beginning, end, and content of the *work element* in question. Using the PROOF model calculation system, developed at the ART, the *standard times* are then assembled into workflow models and work procedures and logically linked with the relevant influencing factors. Results are illustrated in a tabular and graphic format. The use of the computer-assisted *work budget* system allows a comparison of work routines and production processes to be made right up to whole farm level, all other things being equal. Sectoral statements about labour potential in agriculture can also be made.

Table 1. Method for analysing work processes and compiling key work-economics figures (Schick 2011).

Level	Step	Description
ACTUAL	Selection of farm	Use of available contacts and databases
↓	Description of farm	Recording of farm-specific parameters (questionnaires); description of workflow
↓	Determination of influencing factors	Influencing factors affecting the work elements (number of production units, distances, measures, etc.)
↓	Data collection	Work observation at part-time level; measurement of working-time input and relevant influencing factors using an electronic horologe and REFA standardised time-recording software (Ortim b3) with original-report generation (traceability)
↓	Analysis	Data preparation with standard software according to REFA (OrtimZeit) and table-calculation software (Excel 2007) with inbuilt VBA support; statistical evaluations with statistics software (Regressa 5.0)
↓	Compilation of budgeted times	Processing of the secured data from the working-time measurements into budgeted-time values and functions; budgeted-time values are assigned a unique code and made available in a database table.
↓	Model calculation	Creation of the calculation models in a format that the table-calculation software can read and edit.
TARGET	Work Budget System	Database application

Results and discussion “Mowing with a rotary mower” is a widely used process in the forage preservation. The work performance of mowing is mainly influenced by the field structure and increases with the field size (Figure 1). The intensity of this effect is depending on the working width and working speed of the machines. Due to a minor working width (2.5 m) the proportion of the turn-around time increases with the field size, thus reducing the increase of work performance. Additionally the types of mowers

have an effect on the work performance. Even with a small field-size the use of modern technique (self propelled mower, 12 m working width) becomes more efficient.

Conclusions This example shows how the potential for savings of working time requirement can be analysed by a model. The model allows to evaluate the work performance of different measures (mechanization; organisation, e.g. work done by a contractor etc.). In order to control factors affecting silage quality (harvest, transport, compression, covering) the model is also a planning tool to coordinate mass flow rates throughout the process chain, in this way, a part of the quality assurance in the forage preservation.

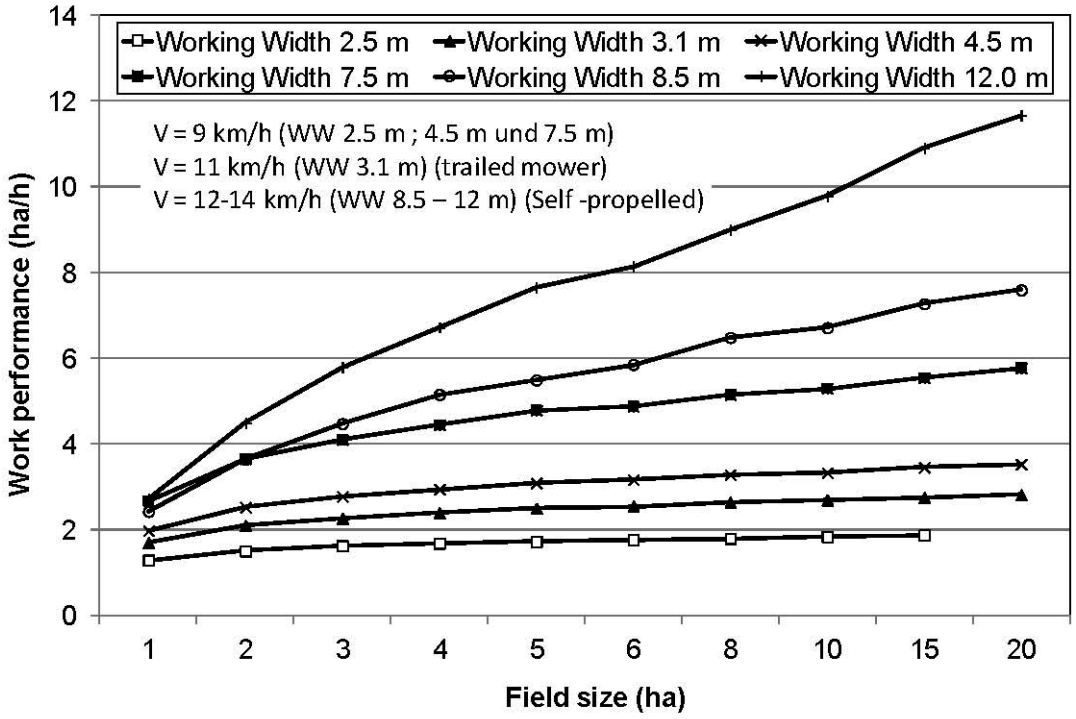

Figure 1. Work performance of the process ”mowing with a rotary mower” with different working widths and field sizes (without consideration of transit and preparation time)

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A snapshot of maize silage quality on dairy farms in South Brazil

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Keywords: chopping, dairy nutrition, forage harvester, maize silage, management practices

Introduction Whole-plant maize silage is the major forage and energy source in the Brazilian dairy industry. Economic pressures have resulted in a renewed interest in maize silage quality. This scenario is particularly important in our country because the cultivated maize is predominantly of hard texture. Furthermore, pull-type forage harvesters without crop processor are still widely used by farmers. Most of the research on maize silages in Brazil has been performed in laboratory-scale silos, but limited research has been conducted on farm-produced silages. Given the need for information on the dairy industry in South Brazil, our objective was to assess the quality of commercial maize silages and the management practices that involve their production. The collected data will facilitate the design of industry-oriented research and aid in development of strategies for making silage.

Material and methods A survey was carried out on 120 dairy farms in Paraná State, the main milk-producing basin in South Brazil. The majority of farms had Holstein cows, which produced about 10.000 kg milk/cow/yr. A total of 142 commercial maize silages were sampled during a 2 yr-period, 53 in 2009 and 89 in 2010. Samples were taken at 5 locations across the feed-out face to determine dry matter (DM), pH, crude protein (CP), starch, neutral detergent fiber (NDF), and particle size distribution. The silage from top, bottom and sides of each silo (0 to 40 cm) was avoided. At sampling, other data were collected from participating producers; these data included maize hybrid (MH), forage harvester (pull-type or self-propelled), and the use of silage inoculants. In 2010, the silo type, effluent production, number and type of plastic sheets used to cover the silos, and the material used to weight down the silo surfaces were recorded. All data were tabulated in an Excel Spreadsheet. The number of responses, mean, minimum value, maximum value, and standard deviation were calculated. Correlation analysis was performed with PROC CORR (SAS Institute, 2001) to determine the relationship between NDF and DM, starch and DM, and coarse fiber fraction (≥ 19.1 mm) and DM of the silages. Significance was declared when the *P*-value was less than 0.05.

Results and discussion Twenty-four MH were purchased by producers, which were classified by companies as flint type. The three hybrids (MH1, MH2, and MH3) most commonly used (n = 39, 30, and 20, respectively), which accounted for two thirds of the samples, were compared. MH2 silages showed lower concentrations ($P < 0.05$) of NDF and starch than MH1 and MH3 silages. NDF and starch concentrations of the three hybrids were correlated with DM concentrations. There was a decrease in NDF ($r^2 = 0.613$; $P = 0.021$) and an increase in starch concentration ($r^2 = 0.808$; $P = 0.006$) in MH2 silages while increasing stages of maturity but this was not shown by the other hybrids. Sixty-four silage samples (45%) had less than 30% of DM (Table 1). In 2010, twenty-six silos (29%) had effluent production. This shows that many farmers harvested the maize at early stages of development when the grains were still immature. The aim of the farmers is to enhance starch digestibility because of hard texture of the grains. Particles remaining on top screen (≥ 19.1 mm) were positively correlated with DM concentrations. This correlation was higher in silages from pull-type ($r^2 = 0.359$; $P = 0.005$) than self-propelled harvesters ($r^2 = 0.275$; $P = 0.012$). The current recommendations indicate that the amount of maize silage retained on the top screen should be 3-8% (Heinrichs and Kononoff 2002). In this survey, only 13% of the sampled silages from pull-type harvesters reached these values. On the other hand, 62% of the silages from self-propelled harvesters were within this range (Figure 1). Twenty-six (18%) of the sampled silages were treated with homolactic inoculants. The pH values and DM concentrations were similar between treated and untreated silages (on average 3.86 vs. 3.87; 30.9 vs. 30.6%, respectively). The use of homolactic inoculants did not seem to be an efficient management tool to improve fermentation profile in maize silages. All farms (n = 89) used horizontal silos. A bunker silo was most commonly found (n = 35), followed by a trench silo (n = 33), and a stack silo (n = 20). All silages were covered with a single sheet (60% with black and 40% with black-on-white polyethylene film). Soil was the preferred material by producers to weight down the silo surface (n = 78; 88%), followed by tires (n = 2), sand bag (n = 1), and sawdust (n = 1). Seven silos were sealed with plastic film only.

Conclusions The hybrids affected the nutritive value of the commercial maize silages. When dairy producers are considering hybrid selections, it is important that they consider both yield and composition. Pull-type forage harvesters caused a long final particle size with wide distribution. Homolactic inoculants

had no effects on pH values of the silages. Most farmers rated sealing as an important practice and placed soil on plastic sheets.

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Table 1. Characteristics of the commercial corn silages (n = 142) from dairy farms in South Brazil.

Item	Average	Range	Standard Deviation
Dry matter (%)	30.6	21.3-45.3	4.10
pH	3.87	3.61-4.50	0.13
Crude protein (% DM)	7.45	5.55-8.90	0.75
Starch (% DM)	33.3	15.3-47.4	5.35
Neutral detergent fiber (% DM)	44.7	35.3-61.5	4.95
Particle size distribution (%)			
>19.1 mm	5.70	0.91-20.7	3.70
7.9 to 19.1 mm	52.0	16.6-75.5	14.5
<7.9 mm	42.3	16.9-78.3	14.4

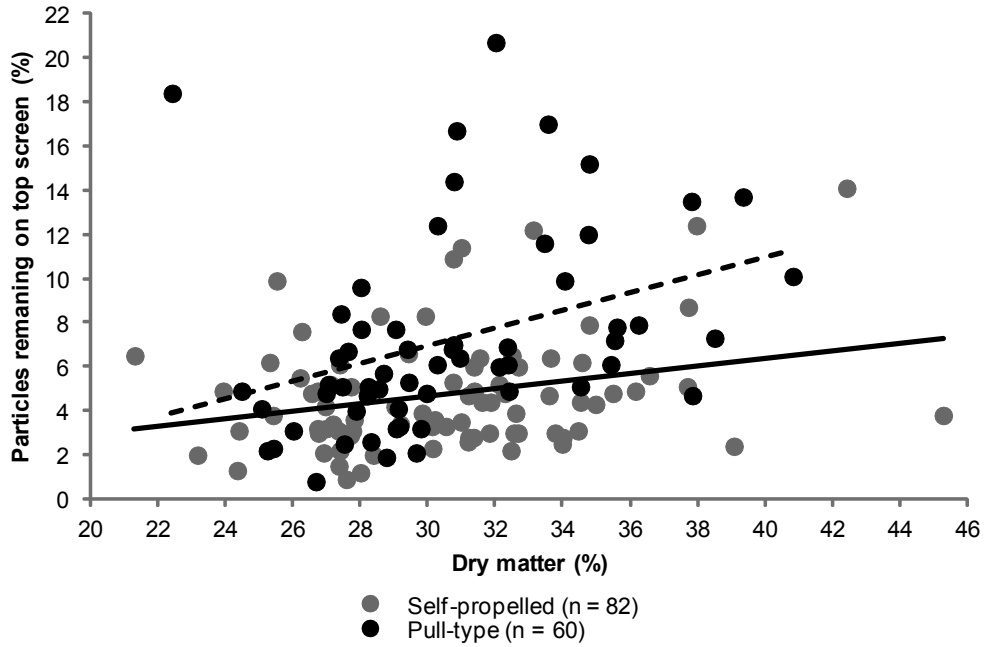

Figure 1. Relationship between particles remaining on top screen (≥ 19.1 mm) of a particle separator and dry matter of the maize silages as affected by forage harvester type. Regression equation for pull-type (- - -): y = 0.4019x – 5.0818; r^2 = 0.359. Regression equation for self-propelled (—): y = 0.1713x – 0.4802; r^2 = 0.275.

Mycotoxin survey in Europe 2010

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Keywords: ELISA, Europe, HPLC, mycotoxins

Introduction Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by animals and humans. More than 300 different mycotoxins have been identified until now. However for a practical consideration in the feed-manufacturing process only a small number of toxins are of relevance, with aflatoxins (Afla), trichothecenes, zearalenone (ZON), ochratoxins and fumonisins (FUM) being of particular interest, although it has to be mentioned that the extent of harm each toxin (group) can cause is highly species-dependant (Erber and Binder 2004).

The aim of the current study is to report the results of a survey of the occurrence of mycotoxins relevant to the feed industry in Europe.

Material and methods A total of 1166 samples were analyzed from north Europe, central Europe and south Europe for the most important mycotoxins in terms of agriculture and animal production: aflatoxins (Afla), zearalenone (ZON), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). Analyses were performed either by HPLC or ELISA according to standard procedures. Raw materials and grains were preferably analyzed by ELISA, whereas for compound feed and feed premixes HPLC was used. Samples tested were diverse, ranging from cereals such as corn, wheat and rice to processing by-products, namely soybean meal, corn gluten meal, dried distillers grains with solubles (DDGS) and other fodder such as straw, silage and finished feed. HPLC analyses were performed as published in Binder et al. (2007) using an HPLC series 1100 from Agilent® technologies (Germany), comprising a micro vacuum degasser, a binary capillary pump, a micro autosampler, column oven and an API-ES interface in case of fumonisin analysis, a variable wavelength detector for DON, and a fluorescence detector for zearalenone, derivatized fumonisins, aflatoxins and ochratoxin A determination. All mycotoxin analyses in the European region were performed according to the internal referenced methods of ROMER Labs Diagnostic GmbH (Austria).

ELISA (Enzyme linked immunosorbent assay) analyses were performed with a commercially available test kit (AgraQuant®Assay, Romer Labs® Diagnostic GmbH, Tulln, Austria). For the purpose of data analysis, non-detection levels are based on the detection limits of the test method for each mycotoxin: HPLC: aflatoxins < 0.5 µg/kg; zearalenone < 20 µg/kg; deoxynivalenol < 50 µg/kg; fumonisins < 25 µg/kg; ochratoxin A < 1 µg/kg; ELISA: aflatoxins < 1µg/kg; zearalenone < 40 µg/kg; deoxynivalenol < 250 µg/kg; fumonisins < 250 µg/kg; ochratoxin A < 2 µg/kg.

Results and discussion From all tested samples in the European region the most prevalent mycotoxins were DON (59%) and FUM (50%) (Table 1). Samples from Northern Europe were contaminated mainly with DON (62%) and FUM (40%) and the highest contamination by DON was 10440 ug/kg (Table 2). Out of 1166 samples tested during the 12 month period, 49% of tested samples were positive for, at least one mycotoxin and 15% were contaminated with two and more mycotoxins. On the other hand 83% of all tested samples from southern Europe were contaminated with FUM. Results from central Europe show that two most prevalent mycotoxins in this region were DON (60%) and FUM (32%). Maximum levels of FUM in all European regions were diverse. While maximum level of FUM in south Europe was 7260 ug/kg, in north Europe it was 236 ug/kg.

The situation in Europe is distinct depending on latitude. If in northern Europe DON is the most prevalent mycotoxin (62%), at lower latitudes (nearer equator), mycotoxins such as Afla have greater importance. It is important to point out the great incidence and high averages of DON in north and central Europe and of DON and FUM in south Europe. From all samples tested positive for DON in all Europe, an average contamination of 907 ug/kg was analyzed. The most prevalent mycotoxins in silage samples were DON, where 48% of all silage samples were contaminated with this particular mycotoxin, and ZON with 36% positive samples (Table 3).

Conclusions The complex diet of ruminants consisting from forages, silages and concentrates may give us a diverse mixture of different mycotoxins in daily rations of dairy and beef cattle. This survey gives us a picture of different representation of various mycotoxins in several European regions and points to the need of continuous mycotoxin risk management in ruminants.

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Table 1. Mycotoxin contamination levels detected in samples from all Europe.

	Afla	ZON	DON	FUM	OTA
Total samples analysed	74	759	1103	70	90
Positive samples [%]	28	23	59	50	28
Mean [ug/kg]	2	26	907	1131	5
Median of positive [ug/kg]	1.7	78.4	560.5	1807	2.7
Maximum level [ug/kg]	103	1045	49000	7260	331

Afla - aflatoxins, ZON - zearalenone, DON - deoxynivalenol, FUM - fumonisins, OTA - ochratoxin A

Table 2. Mycotoxin contamination levels detected in samples from northern Europe.

	Afla	ZON	DON	FUM	OTA
Total samples analysed	11	69	68	5	20
Positive samples [%]	0	3	62	40	25
Mean [ug/kg]	0	1	665	92	1
Median of positive [ug/kg]	0	49.5	526.5	230	5
Maximum level [ug/kg]	0	65	10440	236	9.6

Afla - aflatoxins, ZON - zearalenone, DON - deoxynivalenol, FUM - fumonisins, OTA - ochratoxin A

Table 3. Mycotoxin contamination levels detected in silage samples.

	Afla	ZON	DON	FUM	OTA
Total samples analysed	253	320	359	239	233
Positive samples [%]	2	36	48	19	14
Mean [ug/kg]	0	80	637	166	1
Median of positive [ug/kg]	6	139	462	533	3
Maximum level [ug/kg]	9	2146	14326	3134	35

Afla - aflatoxins, ZON - zearalenone, DON - deoxynivalenol, FUM - fumonisins, OTA - ochratoxin A

Infrared thermography to indicate the presence of mycotoxins in maize silage

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Keywords: aflatoxin, corn silage, ruminants, zearalenone

Introduction The use of different ingredients in ruminant feed (forage and concentrate) increases the risk of mycotoxins intake. The mycotoxins cause important economic losses and can be harmful to animal and human health. Mycotoxins in silages can be related to both the growth of toxigenic fungi in preharvest silage crops and to the postharvest contamination of the silo face (Storm et al. 2008). Deterioration after opening the silo is a result of microorganisms' growth under aerobic conditions usually manifested by a rise in temperature and by the appearance of moulds (McDonald et al. 1991).

This trial aimed to use the Infrared Thermography (IRT) to identify heating sites in the silo faces, and to correlate the temperatures with mycotoxin content in maize silages from five regions in Brazil.

Material and methods The research was conducted by the Centro de Pesquisa em Forragicultura (CPFOR), of Federal University of Paraná, in Curitiba, PR, Brazil. A total of 109 dairy farms were surveyed in five Brazilian regions (four states). In each farm one silo was chosen and scanned by infrared thermography (Fluke Ti25FT) to identify three different sites on silo faces: the highest (H), the mean (M) and the lowest (L) temperature sites. At each site, the external temperature was measured using an IR thermometer (Fluke 66), as well as the internal temperature with a temperature probe (15 cm). Ambient temperature was collected by a digital thermometer kept under shadow. A sample of silage (1 kg, vacuum sealed) was collected at each site. After oven drying (55°C), forage samples were ground through a Wiley Mill (1-mm screen) and sent to the Laboratory of Mycotoxicology Analysis of Federal University of Santa Maria, RS, Brazil. Aflatoxins B1, B2, G1 and G2 (AFB1, AFB2, AFG1 and AFG2), zearalenone (ZEA), deoxynivalenol (DON), ochratoxin A (OTA) and fumonisin B1 and B2 (FB1 and FB2) were determined using a *High Performance Liquid Chromatography* (HPLC) and *Liquid Cromatography – Mass Spectrometry* (LC-MS/MS). All mycotoxins were determined in parts per billion (ppb).

Pearson correlation coefficients (r) were calculated to determine the relationship between temperature (internal or external), pH and mycotoxins. Data were analyzed using STATGRAPHICS plus 4.1.

Results and discussion Aflatoxins B2, G1 and G2 were not detected in any sample. Only 3 of 327 samples were positive for AFB1, at low concentration (6, 2 and 1 ppb). These values and incidence are in according to those reported by Acosta-Aragón and Rodrigues (2009) for Asian maize silage (1% of incidence, mean value 27 ppb). Although farms with either good or poor silo management were surveyed, aflatoxins seem not to be a problem in Brazilian maize silages.

Zearalenone showed the highest incidence (72.8%), followed by FB1 (48.6%) and DON (33.6%) (Table 1). Regardless the incidence, the mean values were lower than the limits established by most of international regulations.

Infrared thermography was effective to show differences of temperature in the silo face. However, poor negative correlation coefficients were found between external temperature and ZEA (r = -0.26) or FB1+B2 (r = -0.08) (Table 2).

The unknown origin of mycotoxins (pre- or postharvest) can lead to the poor correlation with silo heating. Zearalenone showed high incidence in both H and L sites and probably has strong influence of preharvest period (Storm et al., 2008).

A moderate correlation coefficient (r = 0.54) between external temperature of the silo and ambient temperature indicates that ambient conditions such as luminosity, cloudiness, wind speed, interfere over IRT measurements, which leads to a poor correlation between external and internal temperature (r = 0.36) for open air measurements. In a temperature-controlled room (25°C), poor correlation coefficient (r = 0.04) was found between external temperature of maize silage by IRT and the room temperature (Junges et al. 2011).

Conclusions Mycotoxins are present in the most of the maize silages in Brazil, but at low concentration. The IRT is ineffective for detecting the presence of mycotoxins, suggesting that temperature of silo face is not a good indicator of these compounds in maize silages.

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Table 1. Means (ppb) and standard deviations, minimum and maximum values of mycotoxins in 327 samples of maize silage in Brazil.

Mycotoxin	Mean ± SD[1]	Incidence (%)	Minimum[2]	Maximum
Aflatoxin B1	3 ± 3	0.92	1	6
Zearalenone	181 ± 278	72.8	10	1830
Ocratoxin	11 ± 13	6.1	2	62
Deoxynivalenol	259 ± 124	33.6	140	648
Fumonisin B1	369 ± 401	48.6	124	2310
Fumonisin B2	261 ± 215	25.1	113	1380

[1]Mean and standard deviation calculated only for positive samples [2] Minimum and maximum values only for positive samples

Table 2. Pearson correlation coefficients (*r*) between mycotoxin, temperature and pH.

Variables[1]	External	Internal	pH	Ambient	ZEA	FB1+B2	DON
External		0.36	0.10	0.54	-0.26	-0.08	0.05
Internal	0.36		0.40	0.10	-0.11	-0.03	-0.09
pH	0.10	0.40		0.00	-0.01	0.15	-0.05
Ambient	0.54	0.10	0.00		-0.30	-0.05	0.12
ZEA	-0.26	-0.11	-0.01	-0.30		0.07	-0.06
FB1+B2	-0.08	-0.03	0.15	-0.05	-0.07		0.09
DON	0.05	-0.09	-0.05	0.12	-0.06	0.09	

[1]External – external temperature of the silage; Internal – internal temperature of the silage; Ambient –ambient temperature; ZEA – Zearalenone; FB1+B2 – sum of fumonisin B1 and B2; DON – deoxynivalenol.

Changes of fumonisin production in rice grain silage during ensilage

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Keywords: ensiling, fermentative quality, fumonisin, *Fusarium* fungus, rice grain silage

Introduction In recent years, fluctuations in the prices of cereals have driven increases in the use of forage rice (*Oryza sativa* L.) grain in Japan as a feed concentrate. Several methods are used for the preparation and storage of forage rice grain, including ensilage. Silage in particular offers benefits in terms of storage, product quality, and convenience of use.

During the cultivation of rice in Japan and Asia, *Fusarium* head blight and bakanae disease are caused by *Fusarium fujikuroi*. *F. fujikuroi* and related fungi are also known for producing fumonisin (FUM), an important mycotoxin in cereal production. FUM comprises the related compounds fumonisin B_1 ($FUMB_1$), B_2 ($FUMB_2$) and B_3. *Fusarium* fungi that produce mycotoxins such as FUM grow in cereal grain. Therefore, FUM production might increase in forage rice during ensiling, but this phenomenon has not been elucidated. If the conditions for FUM accumulation could be clarified, it may be possible to develop methods to suppress FUM production during the ensilage of rice. In this study, we ensiled rice grain under 6 conditions and investigated whether FUM increased during ensiling.

Material and methods The rice cultivar 'Momiroman' was grown in a paddy field in Ohtawara, Tochigi, Japan, in 2010. The grain was harvested at the full ripe stage on 7 October. The rough rice and crushed rough rice were ensiled under the treatments described below. The experiment was performed in a small-scale plastic bag fermenter using 6 treatments: (1) no fungus added, anaerobic condition; (2) no fungus added, aerobic condition; (3) water added, anaerobic condition; (4) water and FUM-producing fungus added, anaerobic condition; (5) water and FUM-producing fungus added, aerobic condition; and (6) FUM-producing fungus added to autoclaved material, aerobic condition. In treatments (1) and (2), 200 g of rice was packed in plastic bags (18 cm × 26 cm; Hiryu BN-12, Asahi Kasei, Tokyo, Japan), which were degassed and sealed. For treatment (2), a hole was made in center of the bag with an injection needle (22 G, 0.7 mm). For treatment (3), 155 g of rice and 45 mL of purified, sterilized water were packed in plastic bags, which were degassed and sealed. Treatments (4) and (5) were prepared as in treatment (3), with the addition of 200 µL of a suspension of the fungus body of FUM-producing fungus (*F. fujikuroi* MO409, details described below). For treatment (5), a hole was then made in the center of the bag as described above. For treatment (6), 1 g of rice was packed in a test tube with a silicone stopper and autoclaved at 121 °C for 15 min. Then 10 µL of the suspension of fungus and 1 mL of sterile purified water were added to the cooled test tube. All containers, prepared in triplicate, were stored in the laboratory (20–25 °C) in darkness for 40 days.

FUM-producing fungus was used isolate of MO409 in this study. The fungus was isolated from rice leaves sampled from a paddy field in Nasushiobara, Tochigi, Japan in August 2009.

Fermentative quality of silage: Fermentative quality of silage was evaluated as following parameters; pH, organic acid content and Volatile basic nitrogen (VBN).

Quantification of FUM in silage: Fresh and silage samples were dried in a forced-air oven at 65 °C for 48 h, ground and passed through a 1-mm screen. Then 30 mL of methanol–water (3:1) was added to 2 g of each sample. The mixture was shaken well and filtered through filter paper (Advantec No. 5A). The filtrate was purified with an ion-exchange column (Bond Elut SAX®; Varian Technologies, Palo Alto, CA, USA), and was subjected to LC/MS/MS (Acquity UPLC®; Waters, Milford, MA, USA). The integrated value of the two compounds of FUM (B_1 +B_2) is shown as the total FUM in this paper.

Results and discussion In treatments (1) and (2) with no moisture control, the fermentation of silage did not progress in both of the rice materials because the pH was around 7, and the lactic acid content was not detected or was around the detection limit level (<0.01 %) (Table 1). In treatments (3) to (5), some fermentation occurred because the pH was around 4 to 5, and some lactic acid was produced. The concentration of FUM did not increase in both materials in treatments (1) to (4) (Fig. 1). In treatments (3) and (4), water was added, with the difference between the treatments being whether the FUM-producing fungus was added or not (Table 1). The concentration of FUM did not increase in either treatment (3) or (4) in both of the materials, even in treatment (4) containing the FUM-producing fungus. In treatment (5), water and the fungus were added under aerobic conditions, and the concentration of FUM tended to increase, but not significantly, in the crushed rough rice. It is interesting that FUM accumulation did not progress greatly, even though some colonies of fungi were observed around the holes on the bags. Treatment (6) was actually set up under impossible conditions, in order to produce of FUM. Since the study was performed in a small-scale fermentation system, no analysis of fermentative quality was conducted. The concentration of FUM increased substantially only in treatment (6), in which the

material was sterilized. Even in the presence of FUM-producing fungi, the concentration of FUM in rice grain silage did not increase during anaerobic storage. In aerobic conditions, such as when the seal of the silo is broken, the concentration of FUM can increase marginally. In particular, aerobic conditions may allow the population of FUM-producing fungi to increase is dominant in the silage process. Therefore, anaerobic conditions are necessary during ensiling in order to reduce the FUM content.

ConclusionsEven in the presence of FUM-producing fungi, the concentration of FUM in rice grain silage did not increase during anaerobic storage. In aerobic conditions, such as when the seal of the silo is broken, the concentration of FUM can increase marginally. In particular, aerobic conditions may allow the population of FUM-producing fungi to increase is dominant in the silage process. Therefore, anaerobic conditions are necessary during ensiling in order to reduce the FUM content.

Table 1. Fermentative quality and microbial flora of rice grain silage.

Material	Treatment No.		Moisture control (Water added)	FUM-producing fungi †1	Condition	pH			Organic acid (fresh matter %) Lactic			Butyric			Volatile basic nitrogen (fresh matter %)		
						AV	SD†2		AV	SD		AV	SD		AV	SD	
Rough rice		Fresh				7.55	-		ND†4	-		ND	-		ND	-	
	(1)	Ensiled†4	Not added	Not added	Anaerobic	7.26	0.13	a†5	ND	-	b	ND	-	b	ND	-	a
	(2)	Ensiled	Not added	Not added	Aerobic	7.21	0.28	a	ND	-	b	ND	-	b	ND	-	a
	(3)	Ensiled	Added	Not added	Anaerobic	4.69	0.18	b	0.37	0.07	a	0.13	0.06	a	< 0.01	0.00	a
	(4)	Ensiled	Added	Added	Anaerobic	4.80	0.34	b	0.25	0.18	ab	0.13	0.04	a	< 0.01	0.00	a
	(5)	Ensiled	Added	Added	Aerobic	5.07	0.34	b	0.20	0.12	ab	0.01	0.01	b	< 0.01	0.00	a
	(6)	Ensiled	Added	Added	Aerobic	-	-		-	-		-	-		-	-	
Rough rice crushed		Fresh				7.35	-		ND	-		ND	-		ND	-	
	(1)	Ensiled	Not added	Not added	Anaerobic	7.08	0.02	a	0.01	0.00	b	ND	-	b	ND	-	a
	(2)	Ensiled	Not added	Not added	Aerobic	7.41	0.03	a	0.01	0.00	b	ND	-	b	ND	-	a
	(3)	Ensiled	Added	Not added	Anaerobic	4.92	0.30	bc	0.55	0.46	ab	0.32	0.15	a	0.03	0.00	b
	(4)	Ensiled	Added	Added	Anaerobic	4.15	0.24	c	2.13	0.54	a	0.14	0.13	ab	0.03	0.00	b
	(5)	Ensiled	Added	Added	Aerobic	5.58	0.61	b	0.30	0.33	ab	0.02	0.03	b	0.04	0.02	b
	(6)	Ensiled	Added	Added	Aerobic	-	-		-	-		-	-		-	-	

†1: Fumonisin-producing fungus: *Fusarium fujikuroi* MO409, isolated from rice grown in paddy field in Nasushiobara, Tochigi, Japan, in 2009.
†2: AV, average; SD, standard deviation.
†3: ND, not detected.
†4: Ensiled at room temperture (20-25 °C) for 40 days.
†5: Values followed by the same letter are not significantly different within a material ($P < 0.05$). The value for statistical analysis was assumed to be 0.

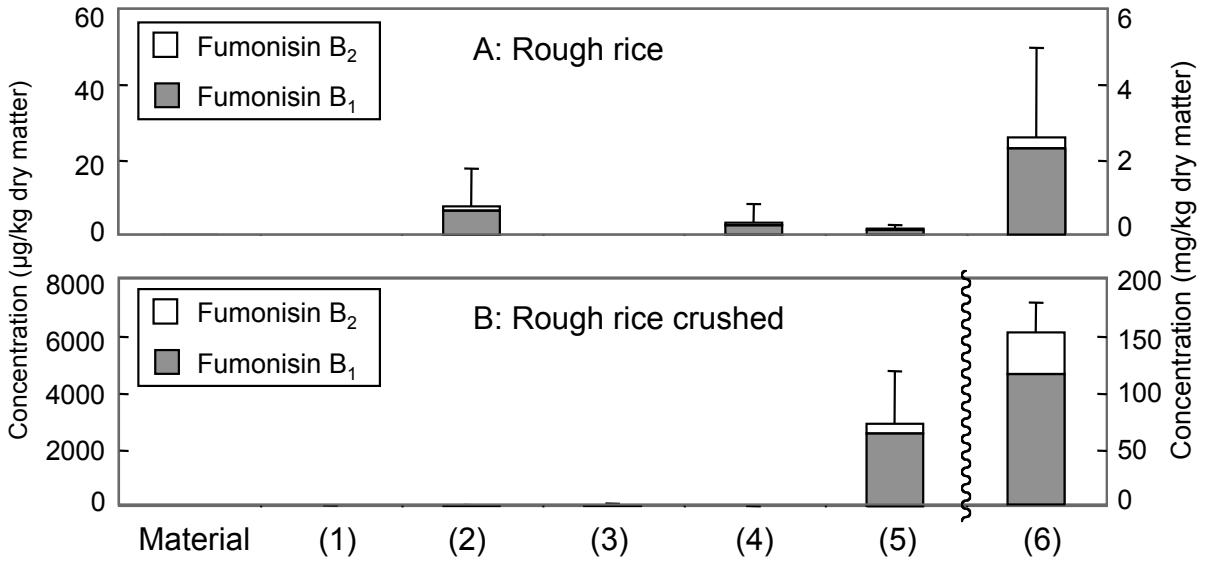

Figure 1. Concentrations of fumonisin in rice grain silages. (1) No fungus added, anaerobic; (2) no fungus added, aerobic; (3) water added, anaerobic; (4) water and FUM-producing fungus added, anaerobic; (5) water and FUM-producing fungus added, aerobic (left-side scale); (6) FUM-producing fungus added to autoclaved material, aerobic (right-side scale). Letters indicate significant differences between treatments ($P < 0.05$). Error bars show standard deviation of fumonisin B1 + B2.

Pathogenic *E. coli* survival in corn silage with various bacterial inoculants at two stages of contamination.

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Keywords : STEC, silage, bacterial additives, inhibition

Introduction Shiga-toxin producing *E. coli* (STEC) is a recent emerging group of food-borne pathogens. These bacteria are responsible for severe human illness, such as hemorrhagic and uremic syndrome (HUS). The ruminant gastro-intestinal tract is considered as the main reservoir of STEC and so uncooked beef, raw dairy products or vegetables contaminated by faeces of infected cattle are frequent sources of human STEC infection (Karmali *et al.* 2010). STEC could infect cattle by their ability to survive and replicate in variety of cattle feedstuffs, particularly in poorly preserved silages. Therefore, silages have been recognized as possible vehicles for STEC spread.

Microbial additives such as Lactic Acid Bacteria (LAB) are commonly used for silage preservation to achieve a rapid pH decrease through organic acids production, and some strains have demonstrated their efficacy to improve silage aerobic stability and increase microbiological quality of silages by inhibiting spoilage moulds and yeasts. Some LAB are also known to produce antimicrobial compounds active against other microorganisms.

Our objective was to identify bacterial strains able to show inhibitory properties against STEC development, and then to evaluate the efficacy of a few selected candidates to interact with inoculated STEC in corn silage in mini silos experiments; we also compared the effects of these strains when STEC contamination occurred at silo opening or after a long term aerobic exposure.

Material and methods A total of 268 strains of bacteria belonging to several taxons were evaluated following different steps: *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Propionibacterium, Vagococcus* and 84 not yet identified strains. They were tested against 36 STEC strains belonging to different origins (environment, feces, meat, cheese, milk or unknown) and serogroups (O157, O26, O145, O103, O111).

For detection of antagonistic activity, an agar spot test was performed according to Tagg and McGiven protocol (1971). The test was performed in triplicate and the bacterial strain was considered as effective when an inhibition zone of >2 mm could be measured for the 3 repeats. To confirm the inhibitory potential of the most interesting bacterial candidates, growth kinetics of STEC were studied in absence or presence of these strains on MRS/BHI medium.

For silage experiments, whole corn material was inoculated in triplicate with 3 different bacterial additives (10^6 CFU/g); 2 of them were selected within the most inhibitory against STEC strains. The third one was a commercial microbial silage additive (*Lactobacillus buchneri* NCIMB 40788, Lalsil Fresh Lallemand Animal Nutrition, Blagnac, France), which has demonstrated its efficacy on aerobic stability (Tabacco *et al.* 2011). Forage was loaded in 2 kg-mini-silos and stored for 4 months. Non inoculated mini silos were also prepared as controls. At opening, a portion of the different silages was contaminated independently by 3 STEC O26 strains (10^5 CFU/g).

Numeration and detection of pathogens were performed during 48h after contamination. The same protocol was applied for contamination after a long term (144h) aerobic exposure, as described in Pedroso *et al.* (2010).

Results Among the 268 strains tested, 26 showed STEC growth inhibition against the 5 serogroups included in our study (inhibition zone was higher than 2 mm). No significant influence of neither origin nor STEC serogroup was highlighted on the inhibitory effect. However, 3 groups of STEC strains could be characterized by their sensitivity to LAB strains. Most of the O157:H7 strains belonged to group B (intermediate sensitivity), O26 strains were predominant in group C (weak sensitivity), and strains of other serogroups were divided into group A (strong sensitivity) and group C.

From this first screening step, *Leuconostoc mesenteroides* 6A1MLB4 and *Propionibacterium* sp.

R0410 were identified as potential candidates. *L. mesenteroides* 6A1MLB4 effect was further tested in a co-culture experiment with STEC O26 growth and confirmed its inhibitory potential (figure 1). The latter strain, *Propionibacterium* sp. R0410 and *L.buchneri* 40788 were further evaluated in mini silo experiments. Our results showed that 48 hours after their inoculation at silo opening, the three strains of *E. coli* O26 were totally eradicated from corn silage previously treated with *L. mesenteroides* 6A1MLB4 (figure 2). This was not observed after a long term aerobic exposure in the silages which, however, were greatly deteriorated as demonstrated by a sharp increase in pH (up to 7.16 +/- 1.6).

In our experiment, the other bacterial additives did not have the same effect on STEC O26 elimination. Nevertheless, Dunière *et al.* (2011) did show that *L.buchneri* 40788 had a positive effect on STEC survival when the two bacteria were co-inoculated at ensiling, and Pedroso et al. (2010) did show a positive effect of the same strain on O157:H7 contamination at silo opening.

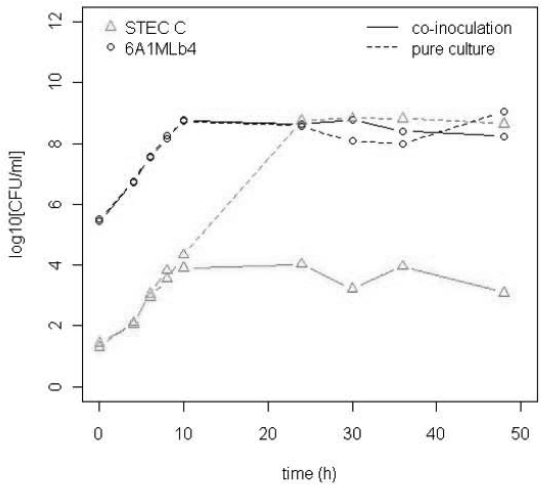

Figure 1 : Effect of L.mesenteroides 6A1MLB4 on growth kinetics of O26 STEC strain (group C). results are shown as the mean growth curves of triplicate independent cultures.

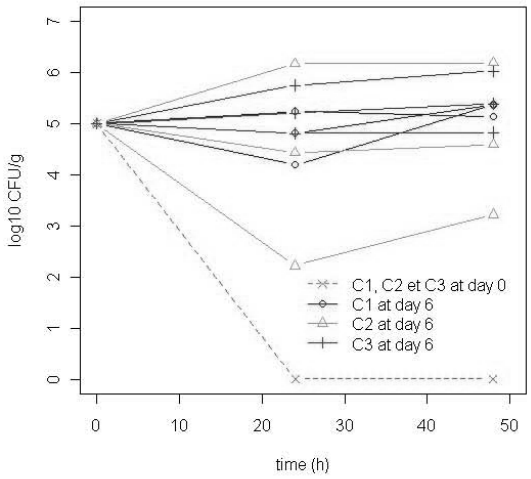

Figure 2 : Fate of O26 (strains C1, C2 and C3) in corn silage inoculated with L. mesenteroides 6A1MLB4 at opening (day 0) or after 144h of aerobic exposure (day 6). Results are shown as individual mini silo values.

Conclusions Further research is needed to better understand the effect of *L. mesenteroides* 6A1MLB4 on STEC survival in silage after aerobic exposure, and a deep analysis of its metabolic properties and its capacity to survive in silage needs to be performed in future studies. However our results suggest that the utilization of selected bacterial inoculants can represent a good strategy to guarantee hygienic quality of cattle feed, by limiting the entry of pathogenic *E. coli* into the epidemiological cycle to improve microbial safety of the food chain.

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Animal feed types and sources in Nandi and Makueni Counties, Kenya: aflatoxins and fumonisins contamination

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Keywords: aflatoxin, fumonisin, feeds, contamination, Kenya

Introduction Nandi and Makueni counties in Kenya occupy different agro-ecological zones. Nandi is in the high potential area –the highlands, while Makueni is in the eastern lowlands. The livestock production systems vary. In 2004 to date Makueni has had infrequent outbreaks of human aflatoxicosis while no such outbreaks have been reported in Nandi despite being a major maize producing area.
The quality of feed is important in the nutrition of livestock and greatly affects the productivity in terms of meat and milk. Kang'ethe et al. (2007,2009) showed that feeds given to livestock in Kenya are highly contaminated with aflatoxins but no study yet has investigated the contamination level of fumonisins in animal feeds. Wakhisi et al. (2005) showed that esophageal cancers incidences were higher in Nandi than other parts of Kenya. Sydenham et al. (1991) associated these cancers with people consuming maize based diets contaminated with fumonisins.

Feed contaminated with aflatoxins has been shown to have varied affects on cattle (Bonomi et al. 1994 Brown et al. 1982; Guthrie 1979; Masri et al.1969). Fumonisins have varied effects in animals: encephalomalacia in horses, pulmonary edema in pigs and liver and kidney damage in most other species. The objective of this investigation was to identify the feed types and sources and contamination level with aflatoxins and fumonisins in the two counties of Kenya.

Material and methods *Household selection* The two counties were selected because of past incidences of aflatoxin (Makueni) and maize and dairy production activity (Nandi). Households selected for the study were those with cattle, children under five years and growing maize. This study was part of a larger project examining the risk factors for human exposure to aflatoxin and fumonisins. Households fulfilling the criteria were randomly sampled from a sampling frame that was provided by village elders in the study sites.

Data collection A structured questionnaire was administered to household heads or their spouses via personal interviews in all the selected households. Information sought included, among others, feed types and sources, fodder preservation, numbers and types of livestock kept and husbandry practices
Sampling Feed samples from the households were collected for aflatoxin and fumonisin analysis.
Laboratory analysis Aflatoxin and fumonisin levels in the samples were analyzed using competitive ELISA kits from r-biopharm, Germany.

Results and discussion A total of 541 households were sampled, 280 and 261 in Makueni and Nandi, respectively. Cattle, sheep and goats were the main livestock targeted in this study. On average, there were 1.76 and 3.53 heads of cattle per household in Makueni and Nandi, respectively (Table 1). Goats were more common in Makueni with 3.02 goats per household, while sheep more in Nandi with an average of 1 sheep per household.
Four husbandry practices (zero grazing, semi-zero grazing, pasture and tethering) were evaluated. Farmers practiced pasture and tethering more than others depending on season and labour availability.

Feed was mainly home grown or purchased, while a small group of farmers in Makueni bartered milk, manure and draught power for feed. About 73% of the farmers in Nandi grew their own feed compared to 27% in Makueni. However, majority also purchased feeds.

The major feed types were maize crop residue, free range and spoilt maize grains. Commercial feeds were mainly used by 50% of farmers in Nandi and by only1.4% of farmers in Makueni. This is a reflection of the husbandry practices.

A total of 2 and 207 feed samples were collected from Makueni and Nandi, respectively. In Makueni both two samples were positive for fumonisin and exceeded the FAO limit of 2 ppm. One sample exceeded the 10 ppb limit for animal feeds by Kenya Bureau of Standard for aflatoxin. In Nandi, 96.6% of the samples were positive for fumonisins, 25.6 % exceeded the 2 ppm limit while 51.2% were positive for aflatoxin with only 2.9% exceeding the 10 ppb limit. Maize and sorghum samples taken from the same households showed similar levels of contamination of both toxins as feed samples.

Fodder preservation as means of evening out feed supplies throughout the seasons was not widely practiced in Makueni or Nandi. The majority of farms only stored feed for a short while as stovers in the field. During severe periods of drought, especially in Makueni, whole herds of livestock are wiped due to lack of feed.

Conclusions Contamination of animal feeds with fungal toxins appears highly common in the areas surveyed in this study. Transfer of toxins through the feed-food chain is, therefore, probable and causes a health risk to both livestock and humans. There is a need to educate and provide appropriate technologies for fodder preservation to increase the quality and availability of feeds and reduce the health risks associated with fungal toxins.

Acknowledgements This study was supported by the Ministry for Foreign Affairs of Finland.

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Table 1. Household characteristics in regard to husbandry practices, feed types and analysis in Nandi and Makueni sites.

Characteristics	Nandi	Makueni
Livestock per household		
Households	261	280
Cattle	3.6	1.6
Goats	0.2	3.0
Sheep	1.0	0.2
Husbandry practices (% households practicing)		
Zero grazing (n=43)	11.6	88.4
Pasture (n= 500)	82	18
Others (n= 293)	40.6	59.4
Feed types (% households using)		
Commercial feeds (n=160)*	77.1	22.9
Maize stovers (n=454)	62.6	37.4
Pasture (grazing) (n=340)	62.6	37.4
Spoilt maize (n=349)	96.8	3.2
Others (n=494)	67.8	32.2
Feed Analysis (% exceeding statutory limits)	n=207	n=2
- Aflatoxin	2.9	50
- Fumonisin	25.6	100

* Households using combination of husbandry and feed types

Composition of fungi in wrapped forages of high dry matter content in Sweden and Norway

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Keywords: haylage, hygienic quality, microbial enumeration, mould,

Introduction The use of forage with dry matter (DM) contents above 500 g/kg, also referred to as haylage, has increased during the latest years, particularly for feeding of equines. Generally, haylage contain lower concentrations of fermentation products and has a higher pH compared to silage, and is heavily dependent on an air-tight seal for a successful conservation and to avoid mould growth (Clarke 1988, Behrendt et al*.* 1997). Some moulds can act as allergenic pathogens and produce toxic fungal metabolites, mycotoxins. Both can cause health problems in equines and other farm animals (Clarke 1988, Wilkinson 1999). The fungal flora in baled haylage is not well-studied at present. The aim of this study was therefore to increase the knowledge of the composition of the fungal flora in baled grass haylage in Sweden and Norway by screening bales at farm level.

Material and methods Sample collection of a total of 375 bales of haylage from 125 farms took place in Sweden in 2010 and in Sweden and Norway in 2011. Three bales per farm were examined for incidence of fungal growth, and visible colonies were sampled. The bales were examined for bale properties such as tightness of wrapping (measured by gas entry rate), number of stretch film layers, overlap of stretch film layers, stretch film width and visible damages of the wrappings. Forage samples were collected from all three bales at each farm using a core sampler. The farmers were asked to fill in a questionnaire to retrieve background information about the forage production and bale management practices.

Enumeration of yeast and fungi was done using tenfold dilution series prepared from the core samples, and inoculated on malt extract agar (MEA) and Dichloran 18% glycerol (DG18) agar at two temperatures; 25°C and 37°C for seven days. Yeasts were counted after two days and fungi after seven days. Direct plating of samples from visible colonies on bales was cultivated on MEA at 25°C for seven days (Seale et al. 1986). Fungal colonies from the same farm sharing the same macroscopic characteristics (colour, conidia, mycelium and medium buckling) were pooled and three of those isolates were re-cultivated on MEA for seven days and considered to represent one fungal species present of that farm. Characterizations of *Aspergillus* spp. were performed on macro- and microstructures as described by Klich (2002) and characerization of *Penicillium* spp. on macro- and microstructures as described by Pitt (2000).

Molecular identification of the fungal isolates by polymerase chain reaction (PCR) was performed to confirm the identification of fungal species previously done in the microscope, and to identify fungal isolates that were not possible to identify by microscopic examination. The fungal DNA was extracted according to Stewart and Via (1993). *Fusarium* spp. isolates were sequenced in the translation elongation factor (EF) 1α coding region using the primers EF-1 and EF-2 (O´Donnell et al*.* 1998). *Aspergillus* spp. and *Penicillium* spp. isolates were sequenced in the β-tubulin gene using the primers Bt2a and Bt2b (Glass and Donaldson 1995). Unknown fungal isolates were sequenced in the internal transcribed spacer (ITS) region using the primers ITS1F (Gardens and Bruns 1993) and ITS4 (White *et al.* 1990). All fungal amplicons were sequenced by Macrogen (Seoul, South Korea) in 5´and 3´ directions. Sequences 5´and 3´ were assembled and ends were manually trimmed based on peak quality in SeqMan (DNA-Star) Sequences were compared with GenBank data base sequences from NCBI´s homepage (http://www.ncbi.nlm.nih.gov/BLAST) using the BLASTN algorithm (Altschul et al*.* 1997).

Results and discussion Visible fungal contaminations on haylage bales showed to be common in this survey. Haylage samples collected in Sweden in 2010 showed that 28 of 50 farms had visible fungal growth in their bales. The most common visible fungus was *Penicillium roqueforti*, which was found on 17 farms. Haylage samples collected in 2011 showed that 20 of 50 Swedish farms and 9 of 25 Norwegian farms had visible fungal growth in their bales. The most common visible fungus was *P. roqueforti*, which was found on 7 farms in Sweden and 6 farms in Norway. The findings of *P. roqueforti* in wrapped forages have also previously been reported by for example Skaar (1996) in Norway and Obrien (2008) in Ireland. Other fungi contaminants found in the haylage bales in Sweden and Norway were *Aspergillus fumigatus, Cladosporium cucumerinum, Fusarium* spp. and *Mucor* spp.

Feed of high hygienic quality is important to maintain good animal husbandry. For example *P. roqueforti* produce toxic metabolites that are known to cause problems in farm animals (Scudamore

and Livesey 1996. Results from the questionnaire showed that some farmers stored the haylage bales out on the field where there is a greater risk of holes caused by rodents and other animals. To store and manage haylage bales properly play a major factor, since damage to the plastic layers is one of the biggest factors that promote mould growth in haylage bales. Other factors that are important to prevent mould growth are for example grassland crop composition, cutting time, DM-content and the number of plastic layers.

Conclusions The results of this study showed that visible mould growth on haylage bales is a big problem for the farmers. The most common visible mould found was *P. roqueforti* both in Sweden and Norway. Storing and managing the bales carefully is of big importance to prevent mould growth. Hopefully, results from this study will provide information to the farmers on which bale management practices that restricts the mould growth in wrapped haylage.

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Silage extracts used to study the mode of action of silage inoculants in ruminants

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Keywords: inoculant, *in vitro* digestibility, lucerne, maize

Introduction Microbial inoculants can enhance animal performance (Weinberg and Muck 1996), but the mechanisms involved in these effects are not clear. Previous studies indicated that lactic acid bacteria (LAB) survived in rumen fluid and resulted in a small but consistent increase in pH (Weinberg et al. 2003). In addition, LAB and extracts of silages treated with LAB inoculants exhibited antimicrobial activity (Gollop et al. 2005). In other studies, LAB inoculants did not have a consistent effect on *in vitro* dry matter digestibility (IVDMD) or volatile fatty acids (VFA) in the rumen fluid (Muck et al. 2007), but various inoculated silages resulted in reduced gas production (GP) and higher microbial biomass yields (MBY) as compared with untreated control silages (Muck et al. 2007). These studies suggest that the effects of inoculated silages on animal performance are due to changes in rumen microbial fermentation by an unknown mechanism. The objective of the current experiments was to study the effect of extracts from silages treated with *Lactobacillus plantarum* MTD1 (Ecosyl, UK) on GP and MBY as compared with control silages. This inoculant has had consistent effects on cow performance (Weinberg and Muck 1996) and *in vitro* studies above. Our hypothesis was that the factor in inoculated silage that enhances rumen microbial activity should be extractable from silage.

Material and methods One lucerne haylage (58% dry matter (DM)) and two maize silages (30% and 50% DM) were made in mini-silos with two treatments: untreated control and *Lactobacillus plantarum* MTD/1 (LP, Ecosyl). Three silos of each treatment were frozen (-20°C) after 4 and 60 days of fermentation until analysed. The silages were analysed for pH, fermentation products, *in vitro* true dry matter digestibility, *in vitro* MBY, and standard nutritive characteristics. In addition, 1:1 aqueous and 80% ethanol extracts of control and inoculated silages and suspensions of *L. plantarum* MTD/1 were prepared to study their effects on *in vitro* ruminal MBY and GP. The MBY was determined in tubes and GP in 160 ml bottles, as described previously (Muck et al. 2007). Each tube or bottle contained 12 ml rumen fluid, 17 ml buffer, 1 ml extract and 2 mg/ml glucose. In an additional *in vitro* treatment, *L. plantarum* suspension was added in place of extract to the buffered rumen fluid at 10^6 cfu/ml. Gas production and MBY were measured after 24 h incubation at 39˚C.

Results and discussion Dry matter concentration was different among crops ($P < 0.05$), but the interaction of crop by treatment was not ($P > 0.810$, Table 1). Fermentation characteristics were affected differently among crops as results of this DM concentration. However, crop by treatment interaction was significant for pH, lactic acid, and ethanol, but not for acetic acid (Table 1). In all three crops, pH was greater in control than in LP. Lactic acid concentration was greater in LP than control in lucerne, whereas the opposite was true in both maize trials. Fermentation differences among crops and between the two treatments were expected. Lucerne usually has greater pH than maize (Contreras-Govea et al. 2011), and microbial inoculated crops normally have lower pH than non-inoculated crops (Muck and Kung 1997). Silage *in vitro* digestibility and the VFA produced during *in vitro* digestion were different among crops ($P < 0.05$), but the crop by treatment interaction was not significant ($P > 0.05$, Table 2). Both maizes had greater IVDMD and VFA concentrations than lucerne; however the MBY estimated was greater in lucerne than both maizes. Lucerne treated with LP had the highest MBY, greater than the lucerne control ($P < 0.05$). These results agree with those of Contreras-Govea et al. (2011) who found that some microbial inoculants increased MBY and such increases were different among crops. The *in vitro* MBY and GP determined from the water or ethanol silage extracts were different among crops ($P < 0.05$) and the crop by treatment interaction was also significant ($P < 0.10$, Table 2). For lucerne, the LP extract had reduced MBY in the water extract and higher MBY in the ethanol extract than control. In maize, there was no effect of treatment on MBY from the extracts. LP tended to increase GP in the water extracts whereas in the ethanol extracts LP had higher GP in lucerne and one of the maizes. The direct application of LP to the rumen inoculum had the lowest values of MBY and GP. These results indicate that substrate availability limited rumen bacterial growth.

Conclusions In the lucerne silage where inoculation with *Lactobacillus plantarum* (MTD/1) affected *in vitro* MBY, ethanol extracts of inoculated silage increased both MBY and GP, suggesting the ethanol extract may contain the factor in inoculated silage that improves rumen microbial growth.

Table 1. Fermentation profiles (g/kg DM) of lucerne, maize-HighDM, and maize-LowDM inoculated with *Lactobacillus plantarum* MTD/1.

	Lucerne		Maize-HighDM[1]		Maize-LowDM		SEM	P-value
Constituent	Control	LP	Control	LP	Control	LP		
Dry matter	575.0	570.0	494.0	490.0	306.0	296.0	4.89	0.810
aNDF	345.0	350.0	401.0	393.0	376.0	360.0	11.72	0.664
pH	5.301	4.992	4.193	4.175	3.787	3.774	0.008	<0.001
Lactic acid	24.41	35.33	33.56	31.71	46.39	42.34	1.75	0.0005
Acetic acid	4.63	4.38	7.28	5.95	10.80	9.14	0.429	0.249
Ethanol	0.92	0.58	3.17	3.49	7.29	10.1	0.368	0.007

[1]Maize-HighDM=50% DM maize silage; Maize-LowDM=30% DM maize silage; LP=treated with *L. plantarum* MTD/1; aNDF=neutral detergent fibre assayed with heat stable amylase.

Table 2. In vitro rumen fermentation profile, microbial biomass estimate (MBY, mg/g DM) and gas production (GP, mL/g DM) at 24 h of Lucerne, Maize-HighDM, and Maize-LowDM inoculated with *Lactobacillus plantarum* MTD/1.

	Lucerne		Maize-HighDM[1]		Maize-LowDM		SEM	P-value
Constituent	Control	LP	Control	LP	Control	LP		
Whole plant silage								
IVDMD 24h	645.3	660.9	846.1	835.9	767.9	760.3	11.45	0.473
Acetate (mM)	53.8	52.7	73.0	71.8	60.9	62.2	1.18	0.502
Propionate (mM)	14.6	15.0	26.2	26.5	20.7	21.2	0.71	0.998
Butyrate (mM)	8.0	8.6	20.6	21.0	13.7	13.9	0.55	0.929
MBY[2] (mg/g DM)	314.1	347.2	241.3	241.3	195.9	192.8	8.32	0.074
Silage extract								
MBY water extract	20.7	15.7	15.9	16.5	16.6	15.9	1.32	0.023
MBY ethanol extract	16.4	18.8	19.9	18.1	17.1	15.7	1.32	0.023
MBY LP-DA[3]	13.5		13.3		12.0		1.31	0.699
GP water extract	35.2	36.0	19.8	21.3	25.4	27.2	1.06	0.082
GP ethanol extract	33.7	37.3	21.9	19.7	24.5	28.2	1.06	0.082
GP LP-DA	24.7		16.8		21.6		1.49	0.007

[1] Maize-HighDM=50% DM maize silage; Maize-LowDM=30% DM maize silage; SEM=standard error of the mean; P-value=significance of crop by treatment interaction; LP=treated with *L. plantarum* MTD/1.

[2] Microbial biomass yield=*in vitro* true digestibility minus *in vitro* apparent digestibility.

[3] LP-DA= direct addition of LP to rumen inoculum, no silage extract.

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Improved silage fermentation often results in silage with a low pH – So what does pH in silage actually relate to?

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Keywords: Fermentation, Grass Inoculants, Lucerne, Maize Red Clover, Silage

Introduction. Traditionally the main aim in producing a good silage was to encourage a rapid fermentation (Merry et al.1995). This approach, supported by many research studies, (Cussen et al. 1995; Davies et al.1998) inhibits the activity of undesirable processes mediated by both plants and microorganisms. The result being silages with significantly lower concentrations of ammonia-N, acetic and butyric acids and higher levels of true protein with better animal performance measured in terms of intake and animal production (Winters et al. 2001). As a result inoculants were developed containing homo-fermentative lactic acid bacteria. Today lactic acidosis is a major problem in the dairy industry world-wide which is a consequence of too much lactic acid being produced in the rumen. Low pH silages, associated with a rapid silage fermentation, are highlighted as one of the causes on farm. However there is little scientific evidence to indicate that a low pH silage actually contains more acid than average pH silages or what the relationship is between pH and lactic acid concentration in silage. Many additive manufacturers have now introduced hetero-fermentative species of lactic acid bacteria for a number of reasons one being to produce silages with higher pHs, unfortunately this results in a slower pH decline and greater nutrient loss. The aim of this study was to assess the relationship between pH, final lactic acid concentration and silage quality.

Material and methods Silages were prepared from 1 of 4 crops namely Lucerne, Red Clover, Perennial Ryegrass and Maize and each crop was treated with 1 of 6 treatments, either untreated or different inoculant lactic acid bacteria. The inoculants were chosen so they would produce different rates of pH decline and as such inhibit undesirable processes within the silo at different rates. Thus giving rise to silages with varying levels of protein breakdown and ammonia-N. This approach, alongside the 4 different crops which also result in different challenges to speed of pH decline and thus silage quality allow a wide range of pH, lactic acid concentrations and protein breakdown as measured by Ammonia-N concentrations to be assessed. All silages were made in 1.5 litre Weck silos and were opened after 90 days and the pH, lactic acid, acetic acid and ammonia-N concentrations were measured by standard wet chemical methods.

Results The mean results for pH, Ammonia-N, Lactic and acetic acid and the combined lactic and acetic acid concentration from the 24 silages are shown in Table 1.

Table 1. Showing the mean and range of silage analyses.

	pH	Ammonia-N g/kg TN	Lactic acid g/kg DM	Acetic acid g/kg DM	Lactic plus acetic acid g/kg DM
Mean	3.88375	11.55	92.85	17.15	110.01
Max	6.18	25.2	133.5	33.62	150.78
Min	3.35	6.72	1.7	6.21	22.23

The mean results were used to perform regression analyses to elucidate which factors have the biggest effect on silage pH. The results are shown in Table 2.

Table 2. Showing the regression analyses for pH versus the major fermentation end-products.

	pH versus Lactic	pH versus ammonia-N	Lactic versus ammonia-N	pH versus acetic	pH versus acetic + lactic
r^2 all silages	0.637	0.883	0.392	0.159	0.507
r^2 Maize and grass silages	0.010	0.806	0.003	0.001	0.012

The results show that the r^2 value for pH against lactic acid for all silages was 0.64 whereas that for pH versus ammonia-N was far better at 0.88. Interestingly when the legume silages were removed from the regression analysis the r^2 value for pH against lactic acid was very poor at 0.01 whereas that for pH against ammonia was 0.80. The results indicate that the easier to ensile crops that are often identified as having too low a pH, have the worst correlation between pH and lactic acid concentration.

Discussion It is well known that a slower fermentation produces a poorer quality silage with higher ammonia-N (Merry et al. 1995). However it is less well understood that the result of higher ammonia-N in silage is associated with higher buffering capacity in these silages. Higher buffering capacity therefore requires more acid production to attain a pH sufficiently low enough to maintain an anaerobically stable silage (McDonald et al. 1991). Thus a silage with a higher ammonia-N concentration with the same level of lactic acid will produce a silage with a higher pH or more lactic acid will be required to produce a silage with the same pH. The results of this study clearly indicate a stronger relationship between silage pH and ammonia-N concentration than to lactic acid concentrations. Thus supporting the hypothesis that silage lactic acid concentration is more closely associated to protein breakdown products such as ammonia than it is to concentration of lactic acid.

Conclusions Future research is required to elucidate exactly how silages with different pH and different concentrations of lactic acid and ammonia actually affect rumen acidosis and whether a low pH silage with better nutrient status is a positive or negative for rumen health and thus animal welfare.

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A survey on fermentation quality and bacterial community of bunker-made maize silage in China

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Keywords: bacteria, fermentation, maize, silage

Introduction In China, it is increasingly important to produce highly digestible forage to support the expanding dairy industry. Many farmers make silage from whole-crop maize (Wc) and maize stover (St), but there is much room for improvement with regard to silage management. To evaluate current practice, samples of bunker-made maize silage were collected from 14 farms within a 300 km radius of Beijing for analysis of fermentation quality and bacterial communities.

Material and methods Of the 14 silage samples, 3 were prepared from Wc and 11 from St. Five grab samples were taken from each bunker silo and thoroughly mixed to prepare a representative sample. The numbers of lactic acid bacteria (LAB) and yeast were determined by using MRS agar and YM agar, respectively, and fermentation products were determined by ion-exclusion polymeric HPLC of water extracts. Bacterial communities were determined by denaturing gradient gel electrophoresis (DGGE) (Wang and Nishino 2010); PCR was used to amplify a variable (V3) region of the bacterial 16S rRNA gene with the forward primer GC357f and reverse primer 517r. The GC-clamp PCR products were separated according to their sequences by using a DCode Universal Mutation Detection System (Bio-Rad Ltd, Tokyo, Japan). BLAST searches were performed against the GenBank database to determine the closest relatives of the partial 16S rRNA gene sequences.

Table 1. Microbial counts and fermentation products of bunker-made whole crop maize (Wc) and maize stover (St) silage produced in China.

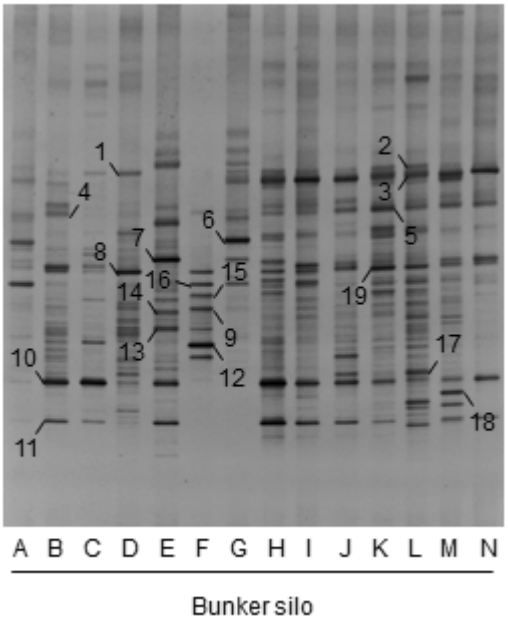
Farm	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Maize silage	St	St	St	St	St	St	St	Wc	Wc	St	St	St	St	Wc
Dry matter[1]	253	276	340	207	172	362	281	259	226	191	216	183	174	273
pH	4.65	4.24	4.02	4.41	5.23	4.20	4.83	3.80	3.67	4.35	4.24	4.85	4.22	3.72
LAB[2]	7.25	8.02	5.53	7.79	7.40	4.30	7.86	4.48	5.48	7.16	5.49	6.69	7.00	5.18
Yeasts[2]	6.32	6.47	5.75	5.91	7.85	4.67	7.14	5.90	6.74	6.91	6.00	6.05	5.93	4.52
Lactic acid[3]	28.7	26.6	44.2	4.10	1.80	32.9	15.9	52.2	85.9	22.8	25.3	5.76	34.6	56.8
Acetic acid[3]	36.2	58.9	21.0	73.3	22.5	16.6	29.8	66.2	81.6	77.5	53.3	80.7	115	62.7
Propionic acid[3]	8.64	10.9	0.00	13.4	6.78	0.00	5.97	0.00	29.7	19.5	6.34	18.8	9.83	0.00
Butyric acid[3]	1.94	1.90	0.42	11.9	5.30	0.73	2.74	0.19	0.82	2.94	7.95	28.0	14.7	0.00
EtOH[3]	0.86	4.08	3.02	3.48	0.00	0.68	0.94	4.14	3.46	7.66	5.10	4.45	25.3	12.9
1,2-PD[3]	5.63	6.33	0.84	1.53	0.00	1.69	0.00	26.7	0.00	0.00	6.57	0.00	0.00	27.5
1-PrOH[3]	0.44	3.15	0.13	4.26	0.00	0.00	1.03	0.51	7.96	7.71	6.90	10.4	18.5	3.84
L/A ratio	0.79	0.45	2.10	0.06	0.08	1.98	0.53	0.79	1.05	0.29	0.47	0.07	0.30	0.91

[1] g/kg, [2] log cfu/g, [3] g/kg DM. EtOH; ethanol, 1,2-PD; 1,2-propanediol, 1-PrOH; 1-propanol, L/A ratio; lactic to acetic acid ratio.

Results and discussion Mean values for DM content were as low as 250 g/kg in both Wc and St silages, and pH values averaged 3.73 and 4.48, respectively (Table 1). The lactic and acetic acid contents averaged 65.0 and 70.2 g/kg DM in Wc silage and 22.1 and 53.2 g/kg DM in St silage. Only 3 of the 14 silages exhibited a lactic-to-acetic acid ratio of more than 1.0, indicating that acetic acid predominated in fermentation. Yeast populations were high both in Wc and St silages (>10^5 cfu/g except in silages F and N). Although 1,2-propanediol content was marginal in most cases (<5.0 g/kg DM), 2 Wc silages had diol levels of more than 25 g/kg DM. In contrast, 3 St silages had large amounts (>10 g/kg DM) of butyric acid, and 2 of the 3 butyrate silages also had high concentrations of 1-propanol.

The DGGE profiles appeared similar in 10 out of the 14 silage samples (except for silages A, D, F and G; Figure 1). Distinctive bands indicative of *Lactobacillus acetotolerans* and *Acetobacter pasteurianus* were found in silages H, I, J, K, L, M and N; thus, differences between Wc and St silages were small with regard to major species in the bacterial community. Although *Bacillus smithii* was the predominant species in silage F, the silage was regarded as well fermented on the basis of the pH and composition of the fermentation products. The presence of *L. acetotolerans* and *A. pasteurianus* may account for the

high acetic acid content found across the silage samples. LAB and acetic acid bacteria were also identified in bunker-made Wc silages produced in Japan (Li and Nishino 2011); thus, further study is needed to understand their function during the ensiling of maize in bunker silos.



1 Uncultured bacterium
2 *Lactobacillus acetotolerans*
3 *Lactobacillus acetotolerans*
4 *Lactobacillus buchneri*
5 *Acinetobacter* sp.
6 *Kurthia* sp.
7 *Pseudomonas* sp.
8 Uncultured bacterium
9 *Bacillus smithii*
10 *Acetobacter pasterianus*
11 *Acetobacter pasterianus*
12 *Bascillus smithii*
13 *Enterobacter cloacae*
14 *Paenibacillus barengoltzii*
15 *Bacillus smithii*
16 *Geobacillus pallidus*
17 *Klebsiella pneumoniae*
18 *Acetobacter pasteurianus*
19 *Clostridium acidisoli*

Figure 1. Bacteria community of whole crop maize silage (H, I and N) and maize stover silage (A–G and J–M) produced using a bunker silo near and around Beijing, China.

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Microbial communities and aerobic stability of whole crop corn and wilted Italian ryegrass silage inoculated with and without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*

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Keywords: Bacteria, fungi, denaturing gradient gel electrophoresis, silage

Introduction It is not clear why lactic acid bacteria (LAB) inoculants sometimes fail to improve fermentation of crop silage. A general explanation may be that the indigenous LAB population is high is pre-ensiled crops and can compete with inoculant LAB for the water soluble carbohydrates (WSC); hence, the success rate might be enhanced if the rate of LAB inoculation is increased to a level greater than that of the epiphytic LAB. Because many microorganisms are thought to be either non-cultivable or difficult to cultivate, conventional culture-dependent analysis may not provide a complete view of the microbial community. The technique of denaturing gradient gel electrophoresis (DGGE) is useful in detecting shifts in microbial populations over time and in understanding how LAB inoculants may function under various ensiling conditions (Li and Nishino 2011). In this study, two types of microbial inoculants were added to wilted Italian ryegrass and whole crop corn during ensiling to investigate shifts in microbial communities that can account for changes or lack of changes in fermentation and aerobic stability.

Material and methods Wilted Italian ryegrass (386 g kg^{-1} DM) and whole crop corn (253 g kg^{-1} DM) were stored in laboratory silos with and without inoculation of *Lactobacillus rhamnosus* (10^6 cfu g^{-1}) and *Lactobacillus buchneri* (10^6 cfu g^{-1}). The silos were opened after 14, 56 and 120 days and then subjected to aerobic deterioration for 7 days. Microbial counts and fermentation products were determined before and after exposure to air, and bacterial and fungal communities were determined by denaturing gradient gel electrophoresis. Species identification was performed based on 16S rRNA (bacteria) and 18S rRNA (fungi) gene sequences.

Results and discussion Intensive alcoholic fermentation was found in untreated Italian ryegrass silage; the sum of ethanol and 2,3-butanediol content at day 14 was about 7 times higher than that of lactic and volatile fatty acids. Alcoholic fermentation was suppressed by *L. rhamnosus* and *L. buchneri* inoculation, and lactic acid and acetic acid became the dominant fermentation products, respectively. Italian ryegrass silages were deteriorated in untreated and *L. rhamnosus*-inoculated silages, whereas no spoilage was found in *L. buchneri*-inoculated Italian ryegrass silage. In whole crop corn silage, inoculation of *L. rhamnosus* did not affect the fermentation, while that of *L. buchneri* decreased the lactic acid content and increased the acetic acid content. Aerobic stability was enhanced in whole crop corn silage stored for a long period (120 days), with increases in the acetic acid content even without *L. buchneri* inoculation. A high aerobic stability was achieved with a shorter ensiling period (56 days) in *L. buchneri*-inoculated whole crop corn silage. Both bacterial and fungal communities were stable in *L. buchneri*-inoculated Italian ryegrass silage silage during the ensiling process and after exposure to air. Few changes occurred in the bacterial and fungal communities in corn silage due to LAB inoculation. Bacterial communities were mostly retained even when the corn silage was spoiled, whereas substantial changes occurred in the fungal community upon spoilage.

Conclusions Inoculation of *L. buchneri* can inhibit aerobic deterioration of grass silage, with no specific effects observed on lactic acid-assimilating species. Bacterial and fungal community analyses help us to understand how inoculated LAB can function to improve the fermentation and aerobic stability of silage. The results also indicated that LAB inoculation produces few changes in bacterial and fungal communities in corn silage and that substantial changes occur in the fungal community when the silage is spoiled.

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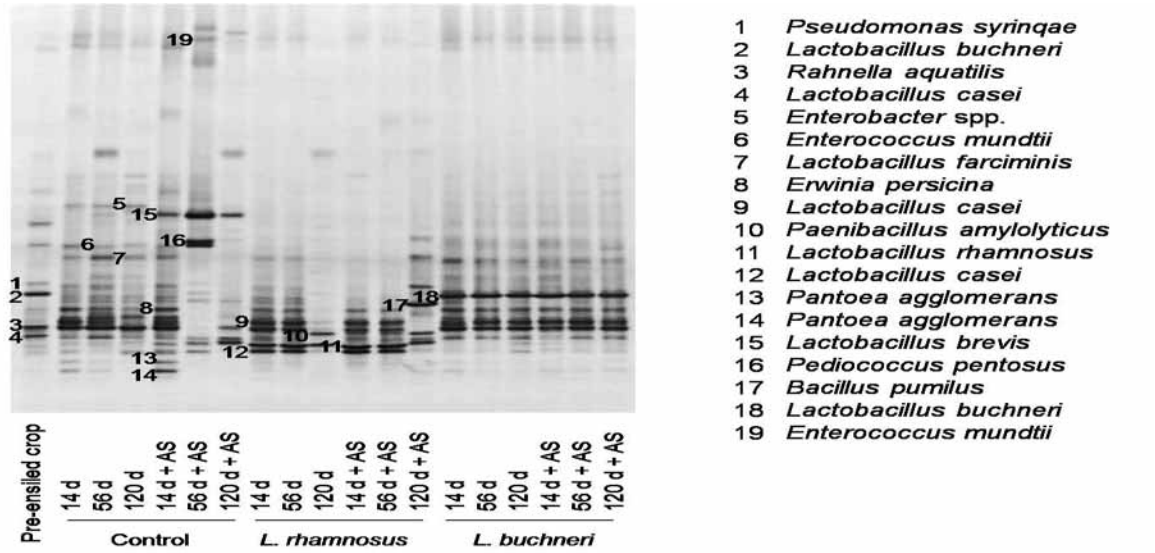

Figure 1. Bacterial communities in wilted Italian ryegrass silage inoculated without and with *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. Silages were sampled at silo opening and after conducting a 7-day aerobic stability (AS) test.

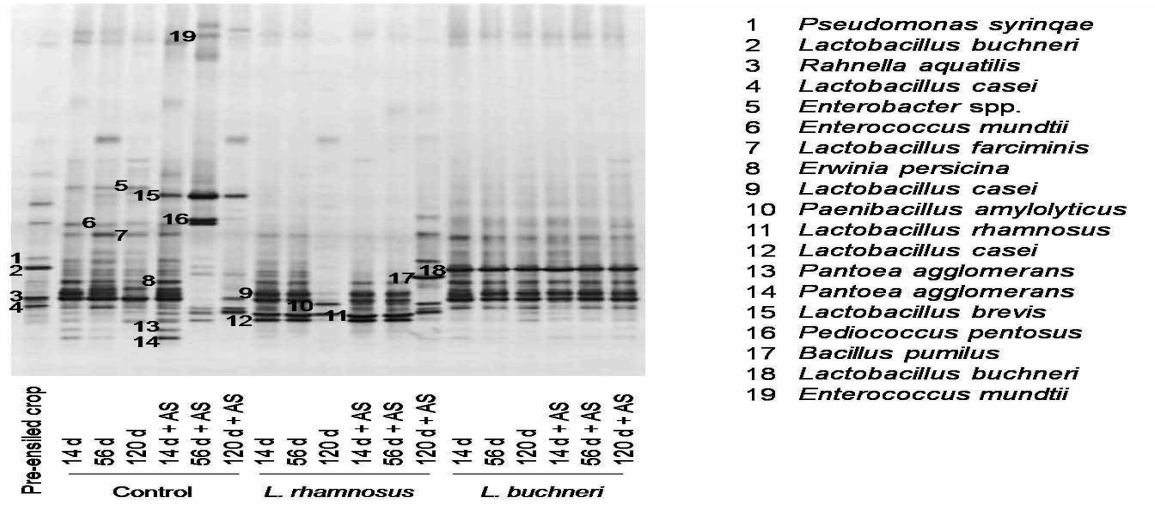

Figure 2. Bacterial communities in whole crop corn silage inoculated with and without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. Silages were sampled at silo opening and after conducting a 7-day aerobic stability (AS) test.

Characteristics of lactic acid bacteria from alfalfa silage

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Keywords: alfalfa, characteristics, Lactic acid bacteria, silage

Introduction Alfalfa (*Medicago sativa*) is a widely used as silage in China. Lactic acid bacteria (LAB) present on forge crops and grass plays an important role during the silage fermentation. The silage contains many kinds of LAB, including *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Weissella*, see Cai et al., (1998). The aim of this study was to understand the characteristics of LAB isolated from alfalfa silage at the beginning of ensiling.

Material and methods Six alfalfa cultivars were all harvested from Linxi (43.62 N, 118.02 E; altitude, 900 m) located in Inner Mongolia of China in July 2009. Materials were cut into about 2-3cm, and then about 150 g of material was compacted into polyethylene bags that were closed airtight, repeated three times. LAB were separated and identified after ensiling of 1, 3 and 45 days. Gram stain of LAB and morphological characteristics were determined after 24 h. Catalase activity and gas production from glucose were determined. Growth at different temperatures was detected in MRS broth after incubation grown at low temperature of 10°C and 15°C for 14 d, and grown at high temperature of 40°C and 45°C for 7d. Growth at pH 3.0, 3.5, 4.0, 4.5, 5.0, 7.5, 8.0 was observed in MRS broth after incubation at 37°C for 7d. Salt tolerance of LAB was tested in MRS broth containing 3.0% and 6.5% NaCl. Carbohydrate assimilation and fermentation of 49 different compounds with one control were identified on API strips, see Ennahar et al. (2003). The data were analyzed with Tukey test of ANOVA by SAS.

Results and discussion 39 strains of LAB were isolated from alfalfa after silage 60 days and they were all gram-positive, catalase negative and homofermentative LAB. These strains grew well at 10°C, pH 5.0 and 7.5, and in MRS broth containing 3.0% and 6.5% NaCl. All LAB grew weakly or could not grow at 5°C, 10°C, 40°C, 45°C and pH 3.0~8.0, also, strain GI44 grew weakly at 40°C and pH 3.5, 4.5, and could not grow at 45°C, pH 3.0, 4.0, and 8.0. These LAB were divided into seven groups (A-G) according to their morphological and biochemical characters. Groups A and B belong to the cluster of genus *Pediococcus*, group C, D and E were identified as *Enterococcus*, group F and G were placed in *Lactobacillus*.

Conclusions The results confirmed that alfalfa silage contains abundant kinds of LAB. 39 strains of LAB from 6 alfalfa silage were clustered into 7 groups. These strains grew well at temperature of 10°C, pH 5.0 and 7.5, endured in MRS broth containing 6.5% NaCl.

References

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Table 1. Alfalfa cultivars and LAB strains isolated from alfalfa silage.

Samples	Cultivars	Kinds	Strain Number
1	Rangelander	*Medicago varia* Martin	GI14, 15, 16, 17
2	Algonquin	*Medicago sativa* L.	GI18, 19, 20, 21, 23, 24, 25
3	Rambler	*Medicago sativa* L.	GI46, 47, 48, 49, 62
4	Zhungeer	*Medicago sativa* L.	GI26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36
5	Gannong No.3	*Medicago sativa* L.	GI37, 38, 39, 40, 42, 44, 45, 69
6	Aohan	*Medicago sativa* L.	GI6, 7, 9, 10, 11, 12

Table 2. Characteristics of representative strain of each group from alfalfa silage.

Characteristics	Group A	Group B	Group C	Group D	Group E	Group F	Group G
	GI36	GI69	GI6	GI30	GI14	GI62	GI44
Shape	Coccus	Coccus	Coccus	Coccus	Coccus	Coccus	Rod
Gram stain	+[1]	+	+	+	+	+	+
Catalase activity	-[2]	-	-	-	-	-	-
Fermentation type	Homo	Homo	Homo	Homo	Homo	Homo	Homo
Grown at low temperature of 5 ° C	-	+	-	+	+	+	+
Grown at low temperature of 10 ° C	+	+	+	+	+	+	+
Grown at high temperature of 40 ° C	+	+	+	+	+	+	W[3]
Grown at high temperature of 45 ° C	+	+	+	+	+	+	-
3.0% NaCl	+	+	+	+	+	+	+
6.5% NaCl	+	+	+	+	+	+	+
PH3.0	w	-	w	w	w	w	-
PH3.5	w	w	w	+	+	+	w
PH4.0	-	+	w	+	+	+	-
PH4.5	+	+	w	+	+	+	w
PH5.0	+	+	+	+	+	+	+
PH7.5	+	+	+	+	+	+	+
PH8.0	+	+	+	+	+	+	—

[1]+: positive reaction, [2]-: negative reaction, [3]w: weakly positive reaction.

16S rDNA analysis and characterization of lactic acid bacteria associated with corn

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Keywords: corn, corn silage, lactic acid bacteria, 16S rDNA sequence

Introduction The objective of this study was to screen t hoLAB and heLAB from corn and corn silage, to isolate the strains involved in the ensiling process and increase microbial resources.

Material and methods Corn and corn silage were collected from some dairy farms around Beijing. Samples of corn or corn silage (20g) were removed and mixed with 180ml of sterile physiological saline and shaken for 30 min. Sequential 10-fold dilutions were poured into a culture dish on MRS agar containing 0.3% (w/v) $CaCO_3$ at 37° for 48h. Selected bacterial colonies that exhibited a clear zone on the plates, catalase negative, Gram-positive were checked for their purity by streaking on MRS agar.

Each selected strain was cultured for 48 h at 37°C in MRS broth to study LAB growth and pH reduction ability. Approximately 107cfu/ml of selected LAB were inoculated into 250ml MRS both. Each culture was incubated for 48 h at 37°C. At 2 h intervals aliquots were taken for measurements of the absorbency (620nm) and pH of the media, and the description of growth and acid yield curve (Figures 1 and 2).

The bacterial colonies of catalase negative and Gram-positive were isolated for 16S rDNA analysis. The genomic DNA was extracted from LAB by the phenol-chloroform method. Primers LPW5 (5'-AGTTTGATCCTGGCTCAG-3') and LPW205 (5'-CTTGTTACGACTTCA CCC-3') were used for PCR analysis (Patrick et al. 2002). The sequence was compared with 16S rDNA sequences available in GenBank (http:// www.ncbi.nlm.gov/BLAST/) using the BLAST programme (Camacho et al. 2009).

Results and discussion LAB are the most important group of microorganisms in silages and LAB play an important role during ensiling. In this study, eight Lactobacillus strains were identified as MRS-03, MRS06, MRS-10, MRS-17, MRS-20, MRS-28, MRS-35 and MRS-39. Comparison of lactic acid bacteria growth and acid yield curves show that MRS-3 and MRS-35 growth rate and the acid yield rate was significantly lower than the other six; MRS-20 and MRS-39 show the most powerful growth and acid yield in all of this 8 bacteria. By DNA analysis, MRS-3 and MRS-35 were identified as ^{ho}LAB, while the others were ^{ho}LAB.The ^{ho}LAB fermentation resulted in lactic acid as the primary fermention product, but ^{ho}LAB also result in the production of acetic acid, etc, with a lower growth and acid yield than ^{ho}LAB. Comparison with GenBank database strains, revealed all Lactobacillus strains had a similarity of 99% with the 16S rDNA of the reference cultures. MRS-03 is *Lactobacillus buchneri*, and showed a similarity of 99% to AB425940.1; MRS06 and MRS-10 are *Lactobacillus lactis*, 100% to AB572041.1 and 99% to CP002365.1, respectively; MRS-17 is *Enterococcus sp.*,100% to DQ469877. 1,MRS-20 and MRS-39 are *Lactobacillus plantarum*,100% to AL935263.2 and 99% JN560899.1, respectively; MRS-28 is *Lactobacillus casei*,100% to JN560922.1 ,MRS-35 is *Weissella sp.*, 99% to AB291633.1.

Conclusions Eight LABS were identified in fresh corn or corn silage. Six were hoLAB, while MRS06 and MRS-10 are Lactobacillus lactis, MRS-17is Enterococcus sp., MRS-20 and MRS-39 are Lactobacillus plantarum, MRS-28 is Lactobacillus casei. The others, heLAB, and MRS-03 are Lactobacillus buchneri and MRS-35 is Weissella sp.. The effect of LABS on silage fermentation quality is under further research.

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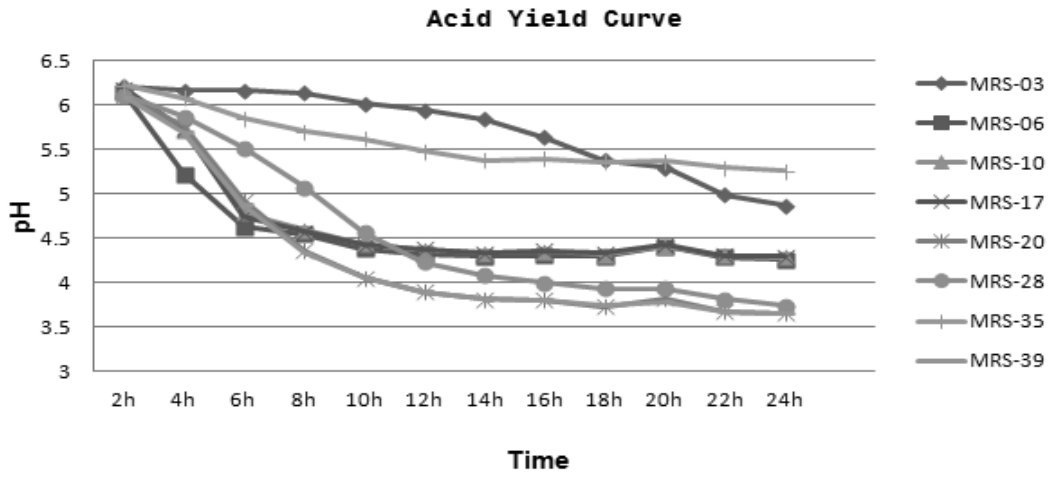

Figure 1. LAB acid yield curve.

Note: Approximately 10^7cfu/ml of selected LAB were inoculated into 250ml MRS and incubated for 48 h at 37°C. At 2h intervals aliquots were taken for measurements of the pH of the media, use the train time as the X-coordinate and pH value as the Y- coordinate description the acid yield curve.

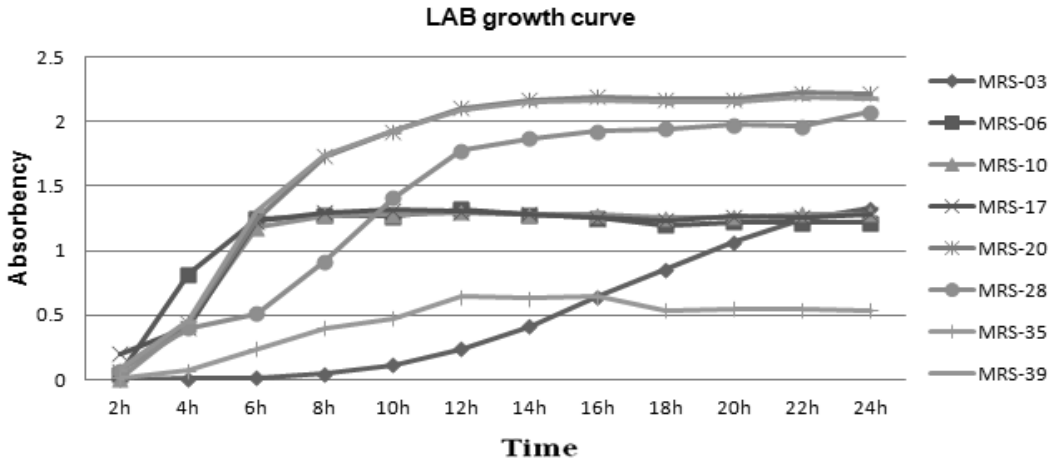

Figure 2. LAB growth curve.

Note: Approximately 10^7cfu/ml of selected LAB were inoculated into 250ml MRS and incubated for 48 h at 37°C. At 2h intervals aliquots were taken for measurements of the absorbency (620nm) of the media, use the train time as the X-coordinate and OD_{620} as the Y- coordinate description the growth curve.

Identification and characterization of lactic acid bacteria isolated from mixed pasture of timothy and orchardgrass silage

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Keywords: characterization, genotype, lactic acid bacteria, phenotype, silage

Introduction Timothy and orchardgrass are perennial, cool-season grasses that are widely distributed in several regions, including Europe, North America and northern Japan. Utilizing grass combinations should provide more uniform long-term and nutritive forage production, making up for the cultivation and nutritional shortcomings of individual grasses. Although mixed pasture of timothy and orchardgrass is a valuable forage crop, less information is available on the microbial ecology of the mixed pasture silage. In the present study, we isolated and characterized the lactic acid bacteria (LAB) strains inhabiting mixed pasture of timothy and orchardgrass and its silage, which were not preserved well with relatively higher pH values and lower lactic acid contents, taking particular interest in the development of a microbial silage inoculant and the relationship between LAB species and silage fermentation. To determine their taxonomic status, the isolates were also studied using 16S ribosomal DNA (rDNA) sequence and PCR-based analyses.

Material and methods Mixed pastures of timothy at the late vegetative stage and orchardgrass at the heading stage were obtained from a local field at the Kozu dairy Farm (Shimonita, Gunma, Japan) on June 3, 2008 (first cutting grass); August 18, 2008 (second cutting grass); and October 29, 2008 (third cutting grass) as previously described (Tohno et al. 2012a). Silage was also prepared and stored in a small-scale fermentation system as previously described (Tohno et al. 2012b). All samples were transferred into sterile homogenization bags, suspended 1: 10 w/v in sterilized distilled water and chopped for 1 min in a Promedia SH-II M homogenizer (ELMEX, Tokyo, Japan). Serial dilutions were used for isolation of LAB using Lactobacilli de Man Rogosa Sharpe (MRS) agar (Difco, Detroit, MI, USA) at 30°C for 48 h under anaerobic conditions. The isolation process was carried out as follows: 5 to 20 colonies (10-20% of the total) on MRS agar medium were picked randomly from each sample (n=3), and a total of two hundred and sixty-two strains were collected and purified twice by streaking on MRS agar. Of these, sixty-five isolates were considered to be LAB, as determined by Gram-stain appearance, catalase test and 16S rDNA sequencing. Morphological, physiological and biochemical tests, 16S ribosomal RNA gene sequencing analysis as well as PCR analysis were conducted as previously described (Tohno et al. 2012a).

Results and discussion According to phenotypic and genetic analysis, the isolates were divided into thirteen groups (a-m), including *Enterococcus gallinarum*, *Lactobacillus acidipiscis*, *L. coryniformis* subsp. *coryniformis*, *L. coryniformis* subsp. *torquens*, *L. curvatus*, *L. paraplantarum*, *L. plantarum* subsp. *argentoratensis*, *L. plantarum* subsp. *plantarum*, *L. sakei* subsp. *carnosus*, *Lactococcus garvieae*, *Lactococcus lactis* subsp. *cremoris*, *Leuconostoc pseudomesenteroides*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Weissella hellenica*, *Weissella paramesenteroides* and *Carnobacterium divergens* (Figure 1). Thus, LAB isolates from mixed pasture of timothy and orchardgrass as well as its silage displayed considerable diversity, being distributed among seven genera and more than 10 species. This is the first report to document that *C. divergens, L. acidipiscis*, *L. sakei* subsp. *carnosus*, *L. garvieae*, phenotypically novel *L. lactis* subsp. *cremoris*, *E. gallinarum* and *W. hellenica* are present in vegetative forage crops. *L. plantarum* group strains were most frequently isolated from the badly preserved silages. Some isolates showed a wide range of growth preferences for carbohydrates utilization, optimal growth pH and temperature *in vitro*, indicating that they have a high growth potential. These results are useful in understanding the diversity of LAB associated with decayed silage of timothy and orchardgrass. A slight difference in carbohydrate fermentation patterns was observed among the different LAB strains despite the higher similarity of their genetic backgrounds, supporting the idea that suitable candidates for LAB in the fermentation process should be considered not in a species- but a strain-specific manner.

Conclusions We found that LAB in decayed silages of timothy and orchardgrass are diverse, both at the species and subspecies levels. For the first time, we also isolated certain species previously unknown in vegetative samples. Understanding the cellular and molecular mechanisms of insufficient fermentation process by LAB isolates which have a high growth potential, and the regulation of undesirable LAB isolates should help in the development of higher quality silages in the future.

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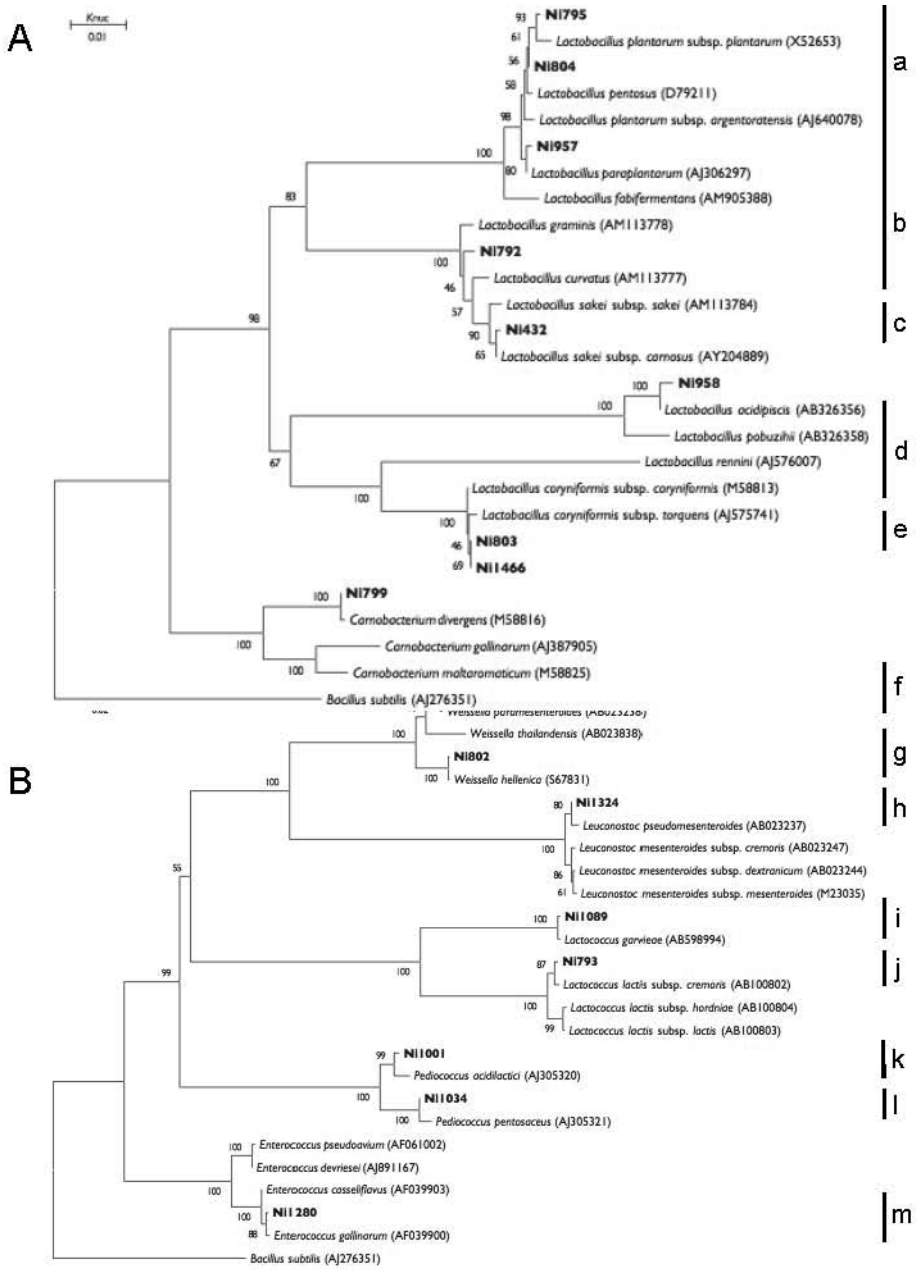

Figure 1. Phylogenetic tree showing the relationship between the complete 16S rDNA sequences of rod- (A) and cocci- (B) shaped strains obtained in this study (boldface type). Numbers at nodes are the percentage of replicates in which the associated taxa clustered together based on a neighbor-joining bootstrap analysis with 1,000 replications. Reference sequences of type strains from GenBank are used for comparison. Bacillus subtilis is used as the outgroup. The width is proportional to terminal branch lengths (Knuc, the number of nucleotide substitution per site shown as a scale bar at upper left).

Metagenomic analysis of a microbial community isolated from silage

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Keywords: Metagenome, silage, lactic acid bacteria

Introduction Lactic acid bacteria (LAB) based fermentation of plant materials is one of the most prevalent methods for the preservation of animal feed as well as for the preservation of substrates for biogas production. Silages comprise a population of different microorganisms, usually containing a mixed population of lactic acid bacteria, depending on the substrate and the site (Pang et al. 2011), however the full microbiome is yet unknown.

In order to investigate the role, growth dynamics and distribution of microbes within a silage process we analyzed the metagenome of two different silage fermentations (spontaneous and inoculated grass-silage sample). Analysis of the data allows for taxonomic as well as functional insights into the complex community involved in the silage process.

Material and methods Isolation Method: The cell disruption of the grass-silage probes was done with the liquid nitrogen method. The isolation of genomic DNA was done following the manufacturer´s protocol Easy DNA Kit (Invitrogen) with prolonged incubation steps and additional protein degradation steps. 100 µl of isolated genomic DNA were mixed with 6.75 ml of extraction buffer (McHugh et al. 2003), 50 µl Lysozyme [100 mg/ml] and 50 µl Proteinase K [20 mg/ml]. The samples were incubated for 2 hrs at 37°C with gentle end-over-end inversion every 15 min. After centrifugation the supernatant was collected and added to equal volumes of 24:1 chloroform:isoamyl alcohol. After centrifugation the aqueous phase was removed and DNA was precipitated using 0.6 volumes of isopropanol for 1 h at room temperature. The extracted nucleic acids were pelleted by centrifugation, two times ethanol-washed and then resuspended in sterile deionised water. The genomic DNA was additionally purified through a column cleaning step using NucleoBond AX-G20 (Macherey and Nagel) and then resuspended in TE buffer. The isolated genomic DNA was analyzed by agarose gel electrophoresis and spectophotometrically (Fig. 1).

To get an overview of the taxonomic composition of the silage a shot gun sequencing of the genomic DNA was performed. Therefore isolated genomic DNA (sample 1, spontaneous silage) was partial digested to 1000 bp - 3000 bp fragments and cloned into a pJET 1.2 vector. After transformation into *E.coli* plasmid was isolated and sequenced (n=20; see Fig. 2). The sequencing results show that about 70% of the analysed clones harbour a part of Lactobacillus DNA. The remaining sequence results belong in equal shares to Enterobacteriaceae, Actinobacter, Insecta and Streptophytina.

Table 1. Results of 454 Sequencing: Contig statistics; genomic DNA isolated from a spontaneous (sample 1) or inoculated grass-silage (sample 2).

Sample	Bases in all contigs	Number of contigs	Average number basis in all contigs	Largest contig
Sample 1	7 596 102 bp	6168	1231.5 bp	17 511 bp
Sample 2	6 726 670 bp	5731	1173.7 bp	12 056 bp

bp…base pair

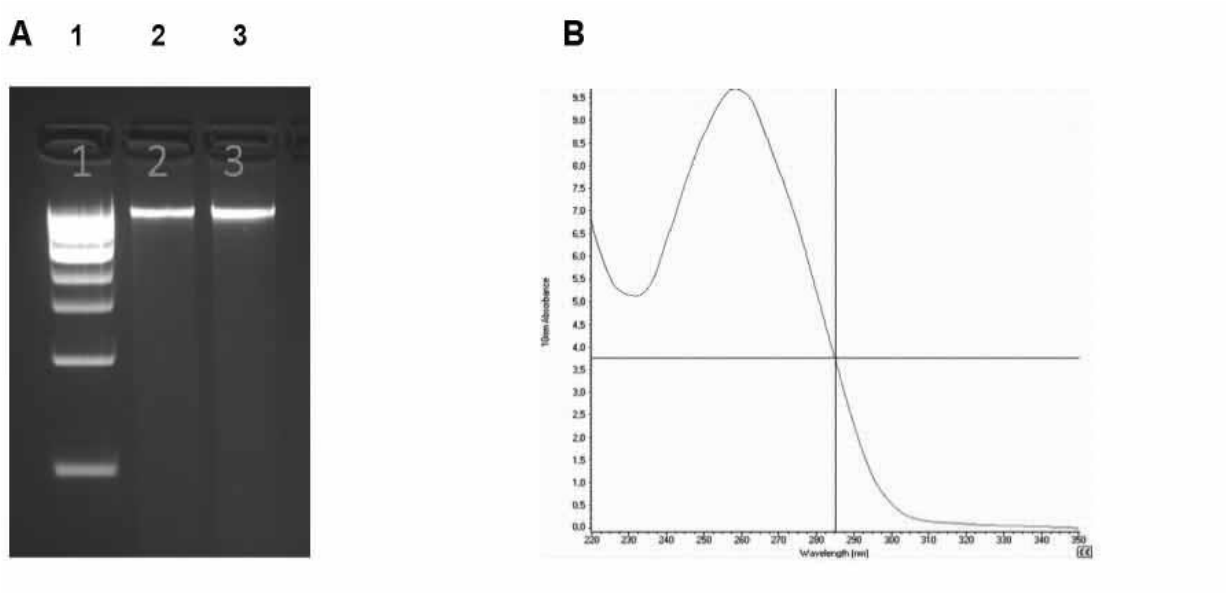

Figure 1. Quality analysis of isolated genomic DNA;
A: Agarosegel: genomic DNA isolated from spontaneous (2) or inoculated silage (3)
B: Spectometrical analysis by NanoDrop (480 ng/µl; A260/280: 1.85; A260/230: 1.89)

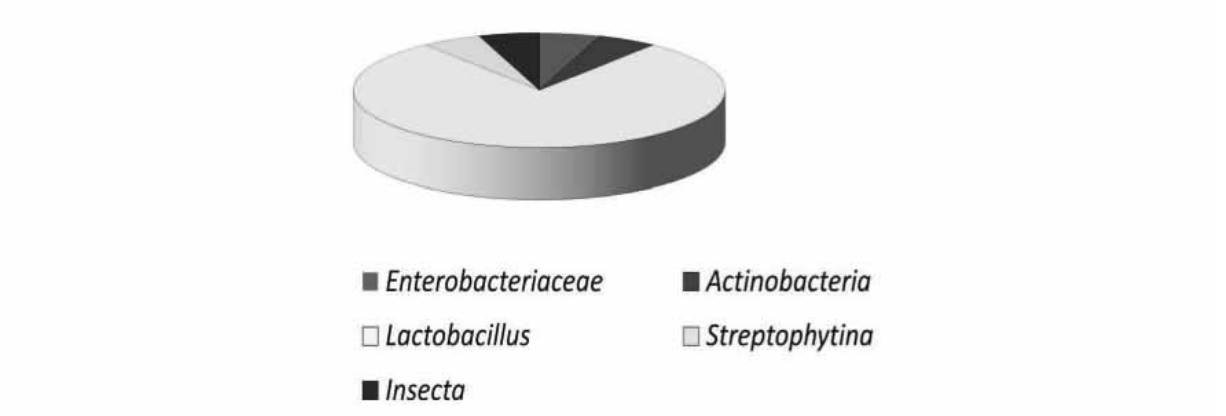

Figure 2. Sequencing results of shot gun cloning: Isolated genomic DNA (sample 1, spontaneous silage) was partial digested to 1000 - 3000 bp fragments and cloned into pJET 1.2 vector. After transformation into *E.coli* plasmid was isolated and sequenced (n=20).

Results and discussion Isolation Problems: The isolation of genomic DNA from silage is difficult and challenging because of the vast diversity of microorganisms that live in the microbial ecosystem. This diversity makes it hard to establish a method which allows the isolation of genomic DNA in good quality from every microorganism. Another challenge in this field is the contamination of the DNA with humic acids. They are produced by biodegradation of dead organic matter and are a complex mixture of many different acids containing carboxyl and phenolate groups. As humic acids disturb the 454 pyrosequencing, it is necessary to get rid of them. The results of the 454 sequencing are listed in table 1.

Conclusions It could be clearly demonstrated that it is possible to isolate high quality genomic DNA from different silage fermentations (spontaneous and inoculated grass-silage sample). Metagenomic DNA samples of silage were sequenced on the Roche GS FLX platform using the Titanium chemistry. About 200 000 reads with an average sequence length of 390 bp were obtained for both samples.
By making a shot gun sequencing of the genomic DNA it could be demonstrated that about 70% of the analysed clones harbour a part of Lactobacillus DNA.

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The effect of adding ferulate esterase producing *Lactobacillus* strains during ensiling on the quality of grass silage

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Keywords: cell wall digestibility, ferulate esterase, *Lactobacillus,* perennial ryegrass, silage

Introduction Grass silage is an important component in the ration of cattle especially during the winter time. Silage quality determines the need for supplementary feed, the performances of the animals and hence the income of the farmer. The quality of grass silage depends to a large extent on the growth stage of the grass at harvest, the weather conditions during field wilting and the ensiling practices. Good silage preservation requires rapid exclusion of air and a fast acidification by lactic acid bacteria. In case of unfavourable ensiling conditions, several silage additives may be used. A specific type of additives are cell wall degrading enzymes, which not only may release more fermentable carbohydrates during the ensiling process providing extra substrate for lactic acid fermentation, but may also result in a pre-digestion of the plant cell walls, so increasing the extent and rate of degradation in the rumen and hence improve digestibility and nutritive value (Rodrigues et al. 2001). The latter mode of action may offer farmers more flexibility to postpone harvest in case of bad weather conditions. Pioneer has developed a silage additive containing living strains of *Lactobacillus buchneri* and *L. plantarum* which produce ferulate esterase, an enzyme able to break down the linkages between (hemi)cellulose and lignin.

The objective was to examine the effects of Pioneer® 11GFT on the fermentation characteristics of grass silage as well as on its chemical composition, in situ rumen degradability and in vitro digestibility. The interaction with growth stage and ensiling period was studied.

Material and methods First cut perennial ryegrass (*Lolium perenne*) was harvested at four growth stages between the end of April and early June 2010. All stages were pre-wilted at a similar dry matter (DM) content of respectively 364, 336, 365 and 360 g/kg; wilting lasted for respectively 1.5, 2.0, 3.5 and 1.5 d. The sugar content of the grass amounted to 225, 184, 150 and 201 g/kg DM respectively. The grass was ensiled in micro-silos (cylindrical tubes with a volume of 2.75 L provided with a CO_2-lock) at a density of about 180 kg DM/m³. Therefore, the grass was chopped at a length of 24 mm and a solution of 10 ml/kg grass containing 1 mg of Pioneer® 11GFT (recommended dose: 1 g/ton grass) was sprayed on half of the grass, whereas the same quantity of water was added to the other half of the grass. The micro-silos were opened, sampled and analyzed after either 60 or 150 days.

Four micro-silos per treatment were weighed weekly to determine fermentation losses and were analyzed for moisture content, pH, ammonia-N, lactic acid, acetic acid, propionic acid, butyric acid and alcohols as well as aerobic stability (time in hours until temperature of silage rises 3°C above ambient temperature). The degradation characteristics of organic matter (OM) and neutral detergent fibre (NDF) were determined on three micro-silos per treatment by incubating frozen and finely cut silage samples in nylon bags in the rumen of two cannulated cows for 8, 24, 48, 72 and 336 hours. The degradation rate (kd) was derived using an exponential model without lag phase. To calculate the fermentable NDF (FNDF) content, a variable passage rate (kp = 0.1775xkd+1.39) was used, whereas FOM content was calculated using a constant kp of 4.5% per hour (Tamminga et al. 2007). Mean data within growth stage and ensiling period were compared by a t-test. Finally, on pooled material of 3 micro-silos per treatment crude protein (CP) and sugars were determined and total OM digestibility (OMd) was estimated by an in vitro cellulase technique.

Results and discussion The addition of 11GFT resulted in silage with better fermentation characteristics (Table 1). In general, the effects were more pronounced for the first two than for the latter two growth stages and also more after 150 than after 60 days of ensiling. With 11GFT the fermentation losses were 18 to 64% lower and DM-content of the silage (corrected for volatiles) was 2 to 12% higher than untreated. Addition of 11GFT decreased pH (from 0.4 to 2.0 units), because of higher contents of lactic and acetic acid. On the other hand, the alcohol content of treated silage was lower. Propionic acid was absent in all silages and also butyric acid was not detected except small amounts in the untreated silages of the last 2 growth stages after 150 d of ensiling. Compared to untreated silage the ammonia fraction after treatment was clearly lower, meaning less protein degradation in the silo. Though, crude

protein content of the resulting silage tended to be somewhat lower. Finally, also aerobic stability of the treated silage was better.

The more intensive fermentation in the treated silage can partly be explained by a more extended sugar fermentation. Moreover, treatment with 11GFT significantly decreased NDF-content in the silage of the first two growth stages, the effect being more pronounced after 150 d of ensiling; NDF-content of the last two growth stages was not affected. On the other hand, treatment tended to slow down NDF degradation in the rumen, resulting in less fermentable NDF. On the contrary, the FOM content as well as total OM digestibility of treated silages was in most cases higher.

Table 1. Fermentation characteristics, chemical composition and degradability of grass silage at four growth stages either untreated (U) or treated (T).

	Stage 1		Stage 2		Stage 3		Stage 4	
	U	T	U	T	U	T	U	T
After 60 d ensiling								
Ferment. loss (%)	0.9	0.8^{ns}	1.5	1.2^{ns}	1.9	1.4***	2.1	1.5***
DM (g/kg)	387	397***	342	361***	370	380**	359	365**
pH	4.93	3.93**	4.60	3.84***	4.41	3.93***	4.42	4.04***
Lactic ac. (g/kg DM)	32	87***	40	83***	46	71***	45	52^{ns}
Acetic ac. (g/kg DM)	26	24^{ns}	11	32***	11	27***	11	34***
Alcohols (g/kg DM)	38	21**	27	21*	34	22**	42	26**
NH_3-N/N %	4.5	2.7***	6.3	3.8***	7.6	6.3**	8.3	5.5***
Aerobic stability (h)	30	127*	24	153**	31	150**	32	>170***
NDF (g/kg DM)	367	336**	414	402*	517	514^{ns}	544	540^{ns}
kd_{NDF} (%/h)	6.87	6.63^{ns}	6.51	5.74^{ns}	3.81	4.06^{ns}	4.34	4.34^{ns}
FNDF (g/kg DM)	247	219*	261	245^{ns}	274	277ns	284	274^{ns}
OM (g/kg DM)	882	886*	902	908**	915	920**	914	917^{ns}
kd_{OM} (%/h)	7.45	7.80^{ns}	7.35	7.17^{ns}	4.22	4.58^{ns}	4.72	5.17^{ns}*
FOM (g/kg DM)	665	672^{ns}	640	637^{ns}	517	533^{ns}	511	510^{ns}
CP (g/kg DM)	231	226	169	167	141	138	135	129
Sugars (g/kg DM)	127	74	159	41	61	18	55	15
OMd (%)	91.8	92.4	88.8	88.1	76.9	79.1	73.1	74.6
After 150 d ensiling								
Ferment. loss (%)	3.3	1.2***	3.2	1.4***	2.4	1.7***	2.6	1.8***
DM (g/kg)	353	397***	313	350***	355	368**	338	351***
pH	5.92	3.95***	5.07	3.85***	4.62	4.01***	4.82	4.07***
Lactic ac. (g/kg DM)	31	91**	28	89***	35	66***	34	56***
Acetic ac. (g/kg DM)	17	29***	9	33***	16	30**	11	41***
Alcohols (g/kg DM)	78	24**	79	24***	43	27***	50	26***
NH_3-N/N %	4.76	3.58***	6.74	4.52***	7.14	6.37**	8.56	5.97***
Aerobic stability (h)	94	>170***	43	>170***	76	>170**	39	>170***
NDF (g/kg DM)	377	334**	448	406***	522	515^{ns}	538	552^{ns}
kd_{NDF} (%/h)	7.76	7.31^{ns}	6.36	5.50**	4.47	4.18^{ns}	4.70	4.36^{ns}
FNDF (g/kg DM)	249	222***	279	255**	287	283^{ns}	283	301*
OM (g/kg DM)	872	883**	894	907***	913	916**	915	916^{ns}
kd_{OM} (%/h)	8.96	8.49^{ns}	7.10	6.45^{ns}	4.78	4.56^{ns}	5.11	4.69*
FOM (g/kg DM)	646	684**	613	639**	537	535^{ns}	522	519^{ns}
CP (g/kg DM)	245	225	186	167	144	142	129	129
Sugars (g/kg DM)	53	50	71	28	38	11	35	10
OMd (%)	90.7	93.0	86.8	88.6	75.2	79.0	73.6	74.9

ns: not significant, *P<0.05, **P<0.01, ***P<0.001

Conclusions By treating prewilted grass with Pioneer® 11GFT more sugars and apparently also more easy degradable cell walls were fermented to lactic and acetic acid, resulting in a lower pH, less DM-losses and protein degradation and a better aerobic stability. The treated silage contained less rumen degradable NDF, but more degradable OM and had also a higher total OM digestibility. These effects were more pronounced for grass cut in a young growth stage and after a long ensiling period.

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Effect of additives on fermentation process of maize silage with different dry matter content

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Keywords: additive, dry matter, maize, silage

Introduction Maize was grown on 280 thousand hectares in Slovakia in 2011. Of this area, 86 thousand ha were maize for silage, which represents 0.164 ha per one head of cattle. Maize silage is irreplaceable in nutrition of dairy cows; therefore it is necessary to pay great attention to its quality. The level of dry matter at which forage crop is ensiled has profound effects on silage fermentation because a lack of moisture in dry forages restricts the overall fermentation process.

Therefore, the objective of the study was to evaluate the effect of dry matter content on the fermentation process of whole crop maize silage treated with various commercial antifungal additives.

Material and methods Whole crops of maize for silage were harvested in two stages of maturity. The first harvest was performed at the early dough stage, when the dry matter content in maize represented by 329-g/kg fresh matter (FM). The second harvest was done at late dough stage of maize with dry matter content 404 g/kg FM. The silage was prepared in laboratory conditions with three various treatments: Control – without an additive, T1 – with 22.9 % sodium benzoate, 8.3 % sodium propionate (4.0 l/t FM), T2 – with *Lactobacillus buchneri* DSM 13573 (2.0 l/t FM).

During the fermentation, changes in silage weights that were expressed as dry matter losses in % were observed. The laboratory silos were opened after 90 days of ensilaging. The samples were chemically analysed and statistically evaluated. Results were statistically processed using the method of variance analysis and differences between the experimental groups compared by Student t-test.

Results and discussion Maize harvested at the early dough stage had higher content of crude protein, fibre, sugars and ash, and lower content of starch than maize harvested at late dough stage. It was reflected also in the energy content, where higher NEL and PDI were observed for maize harvested at the early dough stage, having lower dry matter content. It appeared from this that the feeding value of maize with 329 g/kg of dry matter was higher than that of maize with dry matter content 404 g/kg of fresh matter. The composition of the fresh forage in this experiment was normal for whole plant maize (Table 1). Our results correspond with results in studies of other authors (Bíro et al. 2007, Ranjit and Kung 2000).

Table 1. Chemical composition of fresh whole crop maize in g/kg DM.

Parameters	Early dough stage	Late dough stage	Parameters	Early dough stage	Late dough stage
Dry matter	328.85	404.32	Sugar total	117.57	85.22
Organic matter	960.38	933.83	Sugar	97.90	66.17
Crude protein	89.04	80.46	Fat	30.07	29.97
ADF	201.02	186.22	Ash	39.62	31.25
NDF	520.69	477.26	ME, MJ/kg DM	10.73	10.43
Nitrogen-free extract	670.45	704.44	NEL, MJ/kg DM	6.44	6.26
Starch	305.66	368.48	PDI, g/kg DM	53.98	48.78

DM – Dry matter, ADF – Acid detergent fibre, NDF – Neutral detergent fibre, ME – Metabolizable energy, NEL – Nett energy of lactation, PDI – Protein digested in the small intestine

When ensiling the maize with 329 g DM/kg we found that the application of chemical additive on the basis of salts (T1) decreased highly significantly the DM losses, pH and alcohol (Table 2.). Lower content of acids was also found in chemically treated silage. The increase of DM losses to 9 % occurred with application of *Lactobacillus buchneri* (T2). Content of lactic acid decreased and content of acetic acid increased compared to the control silage. Increase in alcohol concentration was detected as well.
In silages produced of maize with higher content of dry matter (404 g DM/kg) was found lower content of fermentation products, which gives evidence of lower intensity of the fermentation that passed through. pH varied from 3.57 (T1) to 3.72 (Control). Application of T1 and T2 additives decreased level of losses of DM. Content of acids was the highest in silage treated with *Lactobacillus buchneri,* especially of acetic acid.

Schmidt and Kung (2010) suggested that the application of *Lactobacillus buchneri* increased levels of acetic acid in maize silages compared with untreated silages. Acetic acid has been reported as a potent inhibitor of fungi (Moon, 1983), which play an active role in aerobic deterioration. (McDonald et

al. 1991). Highly significant differences were also observed in the alcohol content between the silages, with highest content in control silage. Concentration of alcohol was markedly lower in silages treated with additive, which contained of sodium benzoate. Similar results have been in study of Kleinschmit et al. (2005).

Table 2. Fermentation parameters in whole crop maize silage

Index, n = 6	Control		T 1		T 2		Statistical significance		
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	C/T1	C/T2	T1/T2
DM of fresh maize 328.85 g/kg									
DM of silage	307.12	4.20	313.19	1.79	302.54	4.33	*		**
Losses of DM in %	7.36	1.39	5.09	0.67	9.00	1.39	**		**
pH	3.67	0.05	3.50	0.03	3.65	0.01	**		**
Acids in g/kg DM									
- lactic	78.84	2.23	76.61	1.30	54.46	3.10		**	**
- acetic	22.48	1.98	15.59	1.48	29.60	0.31	**	**	**
- propionic	0.83	0.41	0.49	0.06	0.38	0.09	**	**	
- butyric + isobutyric	0.05	0.04	0.20	0.10	2.67	0.49		**	**
VFA total in g/kg DM	23.63	2.30	16.53	1.34	32.84	0.20	**	**	**
Alcohol in g/kg DM	4.57	0.45	1.90	0.10	8.11	0.08	**	**	**
DM of fresh maize 404.32 g/kg									
DM of silage	373.59	7.28	385.47	7.51	377.79	0.92	*		
Losses of DM in %	10.03	2.69	4.88	1.85	7.27	0.21	**	**	**
pH	3.72	0.09	3.55	0.03	3.57	0.01	**	**	
Acids in g/kg DM									
- lactic	39.90	3.82	34.86	2.24	42.85	1.13	**		**
- acetic	9.67	0.80	8.89	1.85	17.06	1.67		**	**
- propionic	0.26	0.04	1.33	0.53	0.52	0.13	**	**	**
- butyric + isobutyric	0.79	0.47	0.35	0.15	3.21	0.69		**	**
VFA total in g/kg DM	11.00	0.62	11.84	1.83	21.26	2.15		**	**
Alcohol in g/kg DM	4.19	0.15	0.96	0.17	2.81	0.01	**	**	**

DM – Dry matter, VFA – Volatile fatty acids, Control, T1, T2 – variants of silages, SD – Standard deviation, C/T1 – Control vs. T1, C/T2 – Control vs. T2, T1/T2 – T1 vs. T2, * – P<0.05, ** – P<0.01

Conclusions The obtained results indicate that the feeding value of maize with dry matter content 329 g/kg was higher than in maize with dry matter content 404 g/kg fresh matter. The application of additive, which contains sodium benzoate and sodium propionate, highly significantly improved the course of fermentation process in whole crop maize silage. Application of the additive with *Lactobacillus buchneri* influenced positively only the fermentation in silage with higher dry matter content. With both levels of dry matter content there was found highly significantly higher concentration of acetic acid compared with the control silage and silage treated with the chemical additive. Therefore the positive influence of *Lactobacillus buchneri* on aerobic stability in whole crop maize silage shall not be forgotten.

Acknowledgements This publication/article was written during realization of the project „BELNUZ č. 26220120052" supported by the Operational Programme Research and Development funded from the European Regional Development Fund and project MOBILITY 7AMB12SK178.

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Fermentation potential of corn silage

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Keywords: buffering capacity, carbonated lime, corn silage; fermentation potential, organic acids, proteolysis

Introduction Within good agricultural practice, corn (Zea mays) shows good fermentation properties with a rapid drop of pH due to a low buffering capacity and a sufficient concentration of sugar (Wilkinson et al. 2003). Fermentation results in moderate concentrations of lactic and acetic acid (Kung and Shaver; 2001). Fermentation conditions increase with advancing maturity (Buxton and O'Kiely 2003). To demonstrate the change in the fermentation potential of corn during advancing maturity, corn was harvested at the different stages of growth and treated with increasing amounts of carbonated lime. The addition of lime increases buffering capacity and this decreases fermentation conditions (Neureiter et al. 2005).

Material and methods The trials were conducted at the research centre "Haus Riswick" of the agricultural Chamber of North-Rhine-Westphalia, Kleve, Germany. Aim was to harvest corn at three different stages of maturity: 25, 30 and 35 % of dry matter (DM) concentration. At all three stages of maturity untreated control silages were compared with silages treated with either 15, 25 or 35 g carbonated Lime ($CaCO_3$) per kg fresh material (FM). Each treatment was done in three repetitions. Corn plants were harvested by hand and chopped with a laboratory scale chopper. Immediately after chopping the different treatments were applied and the corn ensiled in 1.5 l glass jars and stored for a 90 days period at a temperature of 25°C in a temperature-controlled room according DLG-regulations for testing silage additives (DLG 2000). After ensiling the following fermentation parameters were determined: lactic-, acetic- butyric- and propionic acids, ethanol, 1-2 propanediol and ammonia-N.

Results and discussion The composition of the fresh materials is shown in table 1. With advancing maturity fresh materials showed an expected increase of starch and a decrease of water soluble carbohydrates (WSC) concentration due to the transformation of WSC to starch. Buffering capacity (BC) decreased because of decreasing CP concentrations. Higher DM content and low BC resulted in an increasing fermentation coefficient (FC = DM+8x(WSC/BC). Fresh materials with a FC higher than 45 are rated as "most likely ferment without the occurrence of butyric acid" (DLG 2012).

Table 2 shows the fermentation parameters of the silages after a storage period of 90 days. At maturity stage 1 addition of 25 or respectively 35 g $CaCO_3$ /kg FM led to poor fermentation with high pH values and increasing amounts of butyric and acetic acid. Also ethanol concentrations increased from 2 to 31 g/kg DM. The trials conducted at a later maturity showed different results. The corn silage harvested at maturity stage 2 showed increasing concentrations of lactic and acetic acid by increased amounts of added lime. While in untreated Control about 80 g Lactic acid /kg DM were determined the concentration increased up to nearly 180 g/kg DM by applying 35 g lime /kg FM. At this stage of maturity only the untreated control silage showed traces of butyric acid. Untypical high for corn silage was the concentration of acetic acid of 30 g/kg DM. Similar like the lactic acid content the concentrations of acetic acid increased at this stage of maturity too. Up to 70 g/kg DM were determined for variants treated with 25 resp. 35 g lime/kg FM. Higher concentrations of lime led to pH-Values not higher than still sufficient values of pH 4.6. For control variant moderate 4% of NH_3-N (of total N) was analysed. The concentrations of detected ammonia decreased by addition of more lime. The corn harvested at 35 % DM showed comparable results based on a even lower level of available water soluble carbohydrates in the fresh material. Even with added 35 g of lime/kg FM pH-Values dropped to pH4.7. Ethanol production was on low level for the last two stages of maturity and was only slightly affected by the adding of lime (from 3 resp. 4 up to app. 11 g/kg DM). It is to assume that the decreasing values for ammonia determined in these trials are more likely related to the formation of (not analyzed) products like ammonium lactate or ammonium acetate than to decreased proteolysis (Pahlow, G. 2011, personal message).

Conclusions From on a certain level of maturity (app. 30 % of DM) corn shows a high fermentation potential and thus it is most likely more important to secure aerobic stability of corn silage than to enhance fermentation quality.

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Table 1. Composition of the fresh material.

	Maturity stage 1	Maturity stage 2	Maturity stage 3
Dry matter (g/kg DM)	247	289	351
Crude ash (CA; g/kg DM)	42	39	35
Crude protein (CP; g/kg DM)	77	73	68
Crude fiber (CF; g/kg DM)	221	212	198
Crude starch (CS; g/kg DM)	191	237	305
Water soluble carbohydrates (WSC; g/kg DM)	141	120	90
Buffering capacity; (BC; g lactic acid /100g DM)	2.6	2.1	1.3
Fermentation coefficient (FC)	68	75	90

Table 2. Organic acids, alcohols, ammonia-N concentrations and fermentation losses of the fermented material.

	Maturity stage 1				Maturity stage 2				Maturity stage 3			
Treatment $CaCO_3$ (g/kg FM)	0	15	25	35	0	15	25	35	0	15	25	35
Lactic acid (g/kg DM)	85	181	30	9	80	119	149	175	62	126	157	160
Acetic acid (g/kg DM)	26	24	57	38	30	45	60	61	34	31	35	54
Propionic acid (g/kg DM)	1	3	8	12	2	6	8	10	2	4	5	6
Butyric acid (g/kg DM)	8	0	44	98	1	0	0	0	0	0	0	0
Ethanol g/kg DM)	2	9	32	31	4	10	11	11	3	6	8	11
1-2 Propanediol (g/kg DM)	17	0	1	4	26	5	1	0	14	4	1	0
pH	3.58	4.06	5.54	5.99	3.67	4.28	4.59	4.64	3.70	4.07	4.18	4.71
NH_3-N of total N	2.0	4.2	0.2	0.2	4.9	3.7	1.4	0.2	5.1	5.0	3.5	1.5
DM -Fermentation losses* (%)	4.76	8.38	17.57	21.03	5.30	8.23	10.34	11.50	4.81	6.59	8.06	10.31

*Weissbach 1998

New mixtures of additives containing lactic acid-producing bacterial strains enhance the fermentation characteristics and aerobic stability of tropical maize silage

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Keywords: additives, aerobic stability, fermentation, lactic acid bacteria, tropical maize

Introduction In tropical environments ensiling of forages results in less accumulation of lactic acid, higher pH, lower lactic acid-producing bacteria populations (LAPB), and short aerobic stability relative to temperate regions (Prieto, 2007; Arias 1998). The objective of this study was to determine the ensiling characteristics and aerobic stability of tropical maize (TM; *Zea mays*) fermented with new mixtures of additives containing the LAPB strains; *Lactobacillus plantarum* DSM 16568 (LP); *Enterococcus faecium* DSM 22502 *(*EF), *Lactococcus lactis* DSM 11037 (LL1) and NCIMB 30177 (LL2) and *Lactobacillus buchneri* DSM 22502 (LB) added alone or in combination. The new additives were compared to a negative (NC, no additive) and a positive control (PC; commercial inoculant containing *Lactobacillus plantarum*).

Material and methods Tropical Maize (26.1% DM) was chopped at 2.5 cm and ensiled with one of seven additives; NC (T1), LB (T2), mixtures of EF, LB, and LP (T3), blends of LP, EF, and LL1 with sodium benzoate added at 400 g/Ton (T4), blends of LP, EF, and LL1 (T5), mixtures of LP, EF, and LL2 (T6) and PC (T7). Additives were added to weighed portions of TM and packed into PVC micro-silos (1.8 kg) to ferment for 90d at 25-27°C. Five samples of fresh forage and silage from each treatment were analyzed for pH, fermentation products (organic acids and NH_3), and yeast and mold populations. Statistical analysis for pH, microbial populations, and fermentation products data was performed as a completely randomized design (CRD) with a 7 (additives) by 2 (0 and 90 days of fermentation) factorial arrangement. Dry matter loss data was analyzed as a CRD with 5 replicates per treatment. For aerobic stability, temperature was monitored every 6 hours in 5 samples from each treatment (1000 g) during 168 h. A rise in temperature of 3°C or more above background was taken as indicative of aerobic instability. Responses were measured using data loggers that recorded temperature readings once per six hours from thermocouple wires placed in samples aerated in open polystyrene boxes kept at room temperature (25-27°C), with monitoring of actual ambient temperature. Statistical analysis was performed as a split plot design with a 7 (additives) by 29 (0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 128, 132, 138, 144, 150, 156, 162 and 168 hours of aerobic exposure) factorial arrangement using the silo as repetitive measurement. Main effect differences among means were separated using Tukey t-test. To separate significant differences within day of fermentation as a result of the interaction fermentation day * silage additive, or hour of aerobic exposure * silage additive, Least Square Means were compared using the pdiff option of SAS (1990).

Results and discussion Initial chemical composition of the vegetative material utilized in this experiment is in agreement with values previously reported for maize harvested in tropical climates (Prieto, 2007; Sandoval, 2007; Arias, 1998). Compared to typical maize harvested in temperate climates, vegetative material utilized in this experiment had higher contents of structural carbohydrates and lower of cell soluble components (61.36 % Neutral Detergent Fiber, 36.67% Acid Detergent Fiber, 7.26% Crude Protein, and 2.01% Water Soluble Carbohydrates). Only minor differences between treatments were seen concerning fermentation characteristics after 90 days of fermentation (Table 1). The pH values in T5-T7 treated silages were lower ($P<0.05$) than in the untreated silage (T1). Over the entire fermentation period tropical maize inoculated with T2 had yeast and mold populations (4.29 cfu/g) that were significantly lower ($P<0.05$) than those of forage treated with T7 (4.93 cfu/g) and numerically lower than those of the other remaining treatments. This effect was also observed in the interaction silage additive by day of fermentation. Experimental treatment did not affect the population of clostridia. Lactic acid content was numerically higher in silage treated with T5-T7 than in that of T1-T4. The concentration of acetic acid indicated that maize treated with T2 tended ($P<0.08$) to have greater acetic acid content than maize treated with T5 and T7, and greater numerical values than that these of the remaining treatments. Small concentrations of propionic and butyric acid ranging from 0.01 to 0.06 and 0.10 to 0.50%, respectively, were detected in the silages. However, concentrations of these two acids were not sufficiently high to play a major role in the fermentation process. Experimental treatment did not affect NH_3-N/Total-N ratio, however, dry matter losses were higher ($P<0.05$) in T1 than in T3 to T7. Compared

to control silage, additives containing LB (T2-T3) had lower (P<0.05) temperature than T1 after 66 and 48 h of aerobic exposure, respectively (Table 2). However, with additives T3-T4 temperature was lower than control within the first 36 h after silages were exposed to air. Temperature after aerobic exposure was always higher in silage treated with T6-T7 than that of control silage. The untreated silage also reached an increase of 3°C over ambient temperature at 30 h after opening of the silos, whereas the T2 silage did so after 60 h and the T3 after 54 h.

Conclusions The new additives containing LP, EF, LL1, and LL2 improved the fermentation characteristics of tropical maize silage as evidenced by lower pH, higher lactic acid concentration, and lower dry matter losses. Likewise, treatments containing LB delayed the aerobic instability of the exposed silage.

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Table 1. Characteristics of tropical maize silage when adding different blends of bacterial strains after 90 days of ensiling.

	Experimental Treatment							Probability		
Item	1	2	3	4	5	6	7	T[1]	D[2]	T*D
pH	4.02[a]	4.00[a,b]	4.02[a,b]	3.96[a,b]	3.88[b,c]	3.74[d]	3.79[d]	.001	<.01	.001
Microbial populations[3]										
Yeast and molds	4.40	3.74	4.43	4.50	4.44	4.41	4.49	.053	<.01	.765
Clostridia	1.06	1.37	0.78	1.01	0.69	0.53	0.40	.747	<.01	.669
Fermentation products[4]										
Lactic acid[5]	4.46[b,c]	4.55[b,c]	4.46[b,c]	4.26[c]	5.13[a,b,c]	5.21[a,b]	5.29[a,b]	.053	<.01	.013
Acetic acid[5]	1.07[y,z]	1.37[y]	1.12[y,z]	0.84[z]	0.97[z]	1.11[y,z]	0.93[z]	.001	<.01	.088
Propionic acid[5]	0.01[b]	0.03[b]	0.06[a]	0.01[b]	0.01[b]	0.01[b]	0.04[b]	.021	<.01	.001
Butyric acid[5]	0.20	0.23	0.50	0.33	0.10	0.12	0.33	.403	<.01	.428
NH_3-N/total-N	7.40	6.80	6.80	6.00	6.40	6.20	6.60	.523	-----	------
Dry matter losses,%	3.72[a]	3.07[a,b]	2.73[b,c]	2.05[c]	2.04[c]	2.46[b,c]	1.98[c]	.001	-----	------

[1] Effect of treatment [2] Effect of day of fermentation, [3]log cfu/g, [4] %, [5]Dry matter basis

[a,b,c,d] Means with unlike superscripts in the same row differ (P<0.05)

[x, y ,z] Means with unlike superscripts in the same row differ (P<0.10)

Table 2. Time with lower temperature than control and time to reach 3°C increase in temperature of tropical maize silage when adding different blends of bacterial strains after 90 days of ensiling and exposure to air during 7 days.

	Experimental Treatment						
Item	1	2	3	4	5	6	7
Time with lower temperature than control silage (h)	-----	66	48	24	36	0	0
Time to reach 3°C increase in temperature (h)	30	60	54	30	36	18	24

The effect of different types of chemical silage additives on dry matter losses, fermentation pattern, volatile organic compounds (VOC) and aerobic stability of maize silage

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Keywords: chemical additives, ethanol, ethyl esters, maize silage, volatile organic compounds (VOC)

Introduction Volatile organic compounds (VOC), e.g. alcohols, organic acids and esters thereof, are frequently found in silages and may detrimentally affect feed intake by dairy cattle (Weiss et al. 2009; Weiss and Auerbach 2012). As the knowledge of the formation of VOC in silages is still very limited, it was the aim of this study to test the effects of chemical silage additives on fermentation pattern, production of VOC and aerobic stability of maize silage.

Material and methods Forage maize (332 g DM/kg, 11 g water-soluble carbohydrates/kg DM) was chopped to a theoretical particle size of 20 mm and subsequently filled into 1.5 l glass jars. Silages were anaerobically stored at 25 °C for 97 days, and three replicates per treatment were prepared. The following treatments were tested: CON - Control, KST – liquid mixture of 21.9% sodium benzoate and 13.2% potassium sorbate, 2 l/t (KOFASIL STABIL, ADDCON EUROPE GmbH, Germany), GSP – liquid mixture of 35 % formic and 12% propionic acids, 25.5 % sodium formate, 1.5 % sodium benzoate, 4 l/t (GrasAAT SP, ADDCON NORDIC AS, Norway); PROM – liquid mixture of 48.8 % formic acid/ formate, 18.4 % propionic acid/propionate, 6.1 % sodium, 4 l/t (PROMYR XR 680, Perstorp AB, Sweden).
Chemical analysis of the fresh crop was performed according to official German standards for feed evaluation. DM of silages was measured and corrected for the loss of volatiles during drying according to Weissbach and Strubelt (2008). Determination of pH was done potentiometrically using a calibrated pH electrode. Lactic acid was analyzed by HPLC (Weiss and Kaiser 1995); volatile fatty acids, alcohols and VOC were determined by GC as described by Weiss (2001). Ammonia concentration was determined photometrically by Scalar (CFSA) based on the Berthelot reaction. Losses of DM during fermentation were calculated according to Weissbach (2005). Aerobic stability was measured for 14 days by the temperature method according to Honig (1990).

Data were subjected to statistical analysis by employing the procedure MIXED of SAS. Differences among means were tested by Tukey test, and significance declared at P≤0.05.

Results and discussion Treatment had significant effects on all parameters tested, except pH, which was very low in all silages (table 1). DM losses were highest in acid treatments, whereas a significant reduction in DM loss was found by KST. These observations can be explained by differences in ethanol concentrations, whose formation always results in CO_2 release, which escapes from the silo. The most significant reduction in ethanol was caused by the chemical additive KST, whereas acid additives stimulated ethanol production.

Table 1. Effects of silage additives on DM losses, fermentation pattern, volatile organic compounds and aerobic stability of whole-crop maize silage.

Parameter	Treatment				SED	Significance
	CON	KST	GSP	PROM		
DM loss (%)	6.5^{b}	4.3^{a}	7.5^{c}	7.5^{c}	0.31	***
WSC[1,2]	13.9^{a}	17.3^{ab}	20.5^{b}	19.2^{b}	1.28	**
pH	3.65	3.53	3.63	3.63	0.05	*
NH_3-N (g/kg total N)	108^{a}	106^{a}	90^{b}	87^{b}	3.28	***
Lactic acid[2]	86.5^{a}	118.8^{b}	84.6^{a}	78.2^{a}	7.31	**
Acetic acid[2]	22.2^{b}	13.1^{a}	5.7^{a}	5.7^{a}	2.53	***
Propionic acid[2]	0.5^{a}	0.3^{a}	1.2^{b}	1.7^{c}	0.08	***
Ethanol[2]	23.2^{b}	6.5^{a}	49.1^{c}	46.5^{c}	1.57	***
1,2-propanediol[2]	0.3^{b}	0.4^{b}	0^{a}	0^{a}	0.08	***
Ethyl lactate[3]	398^{b}	166^{a}	617^{c}	612^{c}	39.3	***
Ethyl acetate[3]	223^{b}	123^{a}	189^{b}	184^{b}	16.1	**
Total ethyl esters[3]	621^{b}	289^{a}	806^{c}	795^{c}	31.3	***
ASTA[4] (days)	5.9^{a}	12.7^{b}	14.0^{b}	14.0^{b}	1.17	***

[1]water-soluble carbohydrates; [2]g/kg DM; [3]mg/kg DM; [4]aerobic stability; means in columns with unlike superscripts differ significantly at P<0.05 (Tukey test)

All treatments significantly improved aerobic stability when compared to untreated silages. The inhibiting effect on fungi was caused by sodium benzoate and potassium sorbate in treatment KST, whereas in treatments GSP und PROM mainly the added propionic acid, and to a lesser extent formic acid, resulted in fungal suppression.

In agreement with data by Weiss et al. (2009) and Weiss and Auerbach (2012) concentration of ethyl esters in this study were also clearly affected by the concentration of ethanol and the respective organic acids.

In general, lactate content was high, and KST increased the concentration of this fermentation acid over that of silages of all other treatments. Acetic acid concentration was reduced by all used additives. As contents of lactic acid were higher than those of acetic acid, the formation of ethyl lactate was also more pronounced than that of ethyl acetate. KST decreased contents of ethanol, ethyl lactate (EL) and ethyl acetate (EA). GSP and PROM stimulated the production of ethanol and EL, whereas no effect was found on EA. By using all experimental data from all individual silages of all treatments (n=12), a very high correlation was found between ethanol and total ester concentrations (r^2=0.985, figure 1). These findings are in line with earlier observations (Weiss et al. 2009), thereby confirming the primary role of ethanol in the formation of ethyl esters in silages. Ester formation is a pure chemical reaction, which depends on the presence of the reaction partners and certain environmental conditions. High ester levels are associated with increased ethanol contents. Elevated ethanol production in anaerobic conditions can be attributed to the activity of yeasts, which may have been present at high numbers during the initial stages of fermentation but died off during later storage. Also, heterofermentatiive lactic acid bacteria as well as enterobacteria may form ethanol. However, it still remains to be elucidated which microbial population contributed most to overall ethanol production.

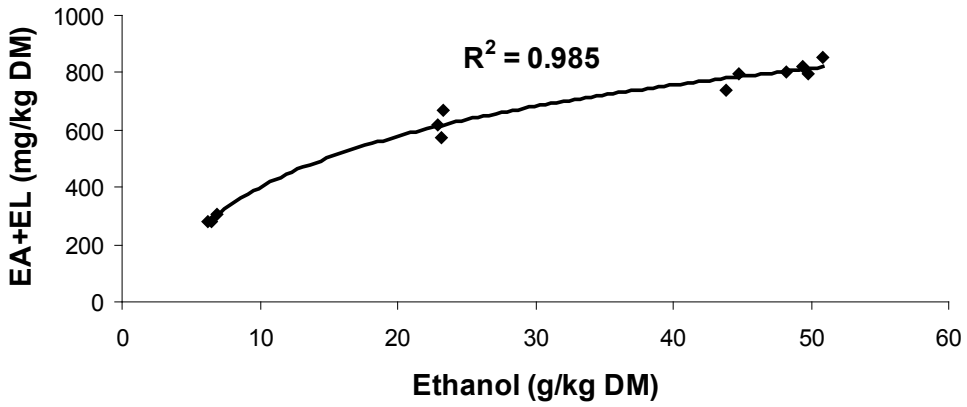

Figure 1. Correlation between ethanol and total esters (sum of ethyl acetate and ethyl lactate) (n= 12)

Conclusions It can be concluded that the silage additive KST, containing sodium benzoate and potassium sorbate, was superior to all the other treatments regarding DM losses, ethanol production as well as ester formation. It can, therefore, be highly recommended to alleviate the detrimental effects of VOC on feed intake.

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The effect of *Lactobacillus buchneri* 40788 on aerobic stability of corn silage

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Keywords: Corn silage, *Lactobacillus buchneri* NCIMB 40788, chop length, opening time, dry matter

Introduction Aerobic stability is of great importance because of the consequent losses of nutrients and DM and the potential development of molds. Silages of corn are especially sensitive to aerobic deterioration because of the high concentration of substrates such as organic acids and starch, which are utilized by undesirable microorganisms (Schmidt and Kung, 2010). A meta-analysis showed that inoculation with *L. buchneri* enhances aerobic stability in a variety of forages (Kleinschmit and Kung 2006). The aim of this trial was to evaluate the effect of inoculation with *L. buchneri* NCIMB 40788 on the aerobic stability of corn silage in relation to different parameters at harvest and conservation.

Material and methods The trial was a multi-factorial design. The following factors were observed: (i) dry matter (DM) of the silage at ensiling (33 vs 40% DM), (ii) chop length (short vs longer theoretical length of cut – respectively 10.3% and 16.3% of total particles collected upon a sieve of 10 mm, (iii) no silage additive (C) vs *L. buchneri* NCIMB 40788 (LB) (300 000 000 CFU/kg treated material), (iv) distance to the top (top (T) vs bottom (B)), and (v) length of conservation (winter (W) vs spring (S) silo opening). Two farm silos with DM content of 33 and 40% were used in this trial. The same material as the farm silo (whole plant corn silage) was harvested at two chop length, received additive or no, and then packed into polypropylene bags (n=16 for each silo). The bags were buried in the correspondent farm silo (25 x 8m) at 4 places (top or bottom, next to or far from the front end). At feed-out, the propylene bags were removed from each silo at two dates and the chemical compositions (DM, pH; N-NH_3, lactic acid and VFA C2, C3 and C4) were determined on samples taken from each bag. From each bag the remaining material was placed loosely into isolating boxes and allowed to deteriorate at room temperature (T°); others samples were taken at days 4 and 14, and analyzed. The T° was recorded continually every 10 minutes in each box. These data were synthesized into 2 parameters: time for the silage to reach a T° >2°C than ambient T° and the cumulative excess of T° of the silage over the 13 days. Results on the aerobic stability were analyzed according to non parametric Mann Whitney test. Data on chemical composition of the samples were submitted to an analysis of variance.

Results and discussion In terms of aerobic stability (Table 1), it was observed that silage heating was higher in the spring when compared to the winter. Aerobic stability of a sample is expressed as the total number of samples with a T° exceeding at least 2°C of the ambient T° (5/16 for winter versus 15/16 for spring). Silages also started heating earlier in the spring (first silos start warming up 4 days after opening in spring versus 10 days after opening in winter). These observations are in line with the results of Ashbell *et al.* (2002), who found that ambient T° has a significant effect on silage aerobic stability. It was also observed that the top layers were more affected by heating then the bottom layers. In spring for the low DM silage aerobic stability was significantly lower for the top layer compared to the bottom layer. Not only was the time to exceed a 2°C difference with the ambient T° much lower, the cumulative of the T° over the 13 day period was also significantly higher. It was also observed that heating of silo occurred mostly in the C samples (without LB). At winter feed-out, 4 C-silages heated vs. 1 LB-treated silage. In the spring and at silo opening, most silages were aerobically unstable, however the last silages to start heating were the LB-treated ones. The meta-analysis performed by Kleinschmidt and Kung (2006) showed that the aerobic stability of silages was consistently improved with inoculation of LB.

Regarding the chemical composition of the samples, for the silages with a pH above 4, a correlation could be observed between the rise in pH and the decrease in lactic acid content of the silage (R^2=0.84). Ohyama et al. (1975) observed a similar relation in grass silage and Weinberg et al. (2010) in corn silage, *i.e*. for deteriorated silages a rise in pH value marked a decrease in lactic acid.

For samples from stable silages (*i.e.* pH equal or below 4), lactic acid content could be explained by the chemical composition. Lactic acid contents (expressed in g/l) increased with higher DM content, decreased with higher non starch organic matter content and decreased in each case when treated with LB (R^2=0.91). Kleinschmidt and Kung (2006) observed similar results and explained this decrease of lactic acid in presence of LB is a consequence of the fact that this micro organism is an obligate heterolactic acid bacteria that anaerobically degrades lactic acid into acetic acid.

The chemical composition at opening of the silos is summarized in Table 2. The difference in chop length did not show any significant differences for the measured parameters. For the other factors (DM, presence of additive, distance to the top and opening time) there were no significant differences observed for pH. The addition of LB had a significant effect on all the other parameters (DM, lactic and

acetic acid and NH_3). The content of lactic acid and NH_3 was significantly different for top vs bottom samples, whereas opening time has a significant effect on the content of acetic acid and NH_3.

Table 1. Results aerobic stability – average for all factors (hours of aerobic exposure at which Δ(T°silage-T°room) > 2°C (cumulative T° x hours in 13 days)).

		40% DM - winter	33% DM - winter	40% DM - spring	33% DM - spring
Average C	Δ(T°silage-T°room) >2°C	289 ± 22 h	237 ± 43 h [C]	165 ± 33 h	132 ± 45 h
	Cumulative T°	161 ± 80 °C [A]	455 ± 231 °C	686 ± 105 °C	586 ± 314 °C
Average LB	Δ(T°silage-T°room) >2°C	> 300 h	288 ± 24 h [D]	190 ± 35 h	164 ± 122 h
	Cumulative T°	68 ± 55 °C [B]	181 ± 162 °C	645 ± 139 °C	783 ± 679 °C
Average Long	Δ(T°silage-T°room) >2°C	288 ± 22	266 ± 42	184 ±29	130 ± 89
	Cumulative T°	162 ± 83	291 ± 210	649 ± 147	699 ± 431
Average Short	Δ(T°silage-T°room) >2°C	> 300 h	259 ± 48	170 ± 42	166 ± 93
	Cumulative T°	67± 49	345 ± 289	682 ± 96	670 ± 631
Average T	Δ(T°silage-T°room) >2°C	289 ± 22	282 ± 35	165 ± 30	80[a] ± 36
	Cumulative T°	98 ± 109	220 ± 270	731 ± 120	1092[c] ± 332
Average B	Δ(T°silage-T°room) >2°C	> 300	243 ± 43	190 ± 38	216[b] ± 62
	Cumulative T°	132 ± 50	416 ± 176	600 ± 73	277[d] ± 182

Means within the same factor with no common superscripts differ significantly: [a,b]=P≤0.05; tend to differ:[A,B]=P≤0.1

Table 2. Chemical composition of samples at day of opening - average values for different factors

		33% DM		33% DM		40% DM		40% DM		SEM	P			
		C*B	C*T	LB*B	LB*T	C*B	C*T	LB*B	LB*T		DM	C vs LB	T vs B	Time
DM	W	35.1	34.5	33.5	32.6	42.1	41.1	40.2	39.6	0.7	<0.001	<0.01	NS	NS
%	S	34.3	33.4	32.4	31.8	40.4	39.4	39.4	38.5					
pH	W	3.88	3.89	3.86	3.93	3.91	3.98	3.94	3.96	0.04	NS	NS	NS	NS
	S	3.79	3.87	3.83	3.84	3.88	3.89	3.86	3.93					
Lactic acid	W	17.9	16.8	16.2	13.8	17.6	15.2	14.6	13.5	0.9	NS	<0.01	<0.05	NS
g/kg DM	S	18.7	16.3	15.7	14.5	17.1	16.0	15.4	13.0					
Acetic acid	W	5.7	6.1	7.0	7.7	5.2	5.8	6.7	7.1	0.5	NS	<0.01	NS	<0.05
g/kg DM	S	4.8	5.5	6.4	6.7	4.5	4.9	5.8	6.5					
NH_3	W	5.3	4.4	5.0	4.1	6.1	5.2	5.8	4.9	0.1	<0.001	<0.001	<0.001	<0.01
% N total	S	5.1	4.2	4.8	3.9	5.8	5.0	5.6	4.7					

In this trial a clear link was also observed between the aerobic stability of the silages and the evolution of their chemical composition. For most silages a reduction in the lactic acid content was observed during the aerobic exposure. This reduction was on average higher for the C-silages compared to the LB-treated (-11.15 g lactic acid /kg DM vs. -6.72 g lactic acid / kg DM for C and LB respectively). At desiling the acetic acid content was lower for the C samples than the LB treated samples (5.30 g acetic acid / kg DM compared to 6.72 g acetic acid /kg DM). Little variation was observed in the acetic acid content during aerobic exposure. Weinberg *et al.* (2010) also reported a relation between the evolution of some chemical components of corn silage and its aerobic stability.

Conclusions It can be concluded that a good management of all the study factors is needed to ensure silage that is aerobically stable (appropriate DM content at ensiling, *etc*.). The addition of a silage additive like *Lactobacillus buchneri* can help optimizing these good management practices.

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Effects of a ferulate-esterase producing inoculant on aerobic stability, fermentation products, and nutritive value of maize silages harvested at different dry matter contents

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Keywords: aerobic stability, ferulate-esterase, *Lactobacillus buchneri*, maize silage, nutritional quality

Introduction Whole-crop maize silages are often the major forage fiber sources in dairy cow diets, as well as, they are prone to aerobic deterioration with decrease of their nutritive value. Bacterial inoculants containing *Lactobacillus buchneri*, a heterofermentative lactic acid bacterium (LAB), have been developed and its effect, as an additive that could improve the aerobic stability of silages, has been extensively studied (Kleinschmit and Kung 2006). Recently, dual-purpose inoculants containing homofermentative and heterofermentative LAB have been developed to overcome the limitations of inoculants containing either type of bacteria alone and positive effects on the aerobic stability of maize silage have been reported (Kang et al. 2009; Tabacco et al. 2011).

The aims of this research were to study the effects of a commercial inoculant containing a strain of *L. buchneri* that produces ferulate-esterase in combination with a homofermentative LAB on aerobic stability, microbial status, dry matter (DM) losses, fermentation products and nutritional characteristics of maize silages ensiled at different DM contents.

Material and methods The experiment was performed at the experimental farm of the University of Turin in the western Po plain, northern Italy (44°50´ N, 7°40´ E, altitude 232 m above sea level, annual mean temperature 11.7°C, and annual average rainfall 739 mm). Four whole-crop maize crops (trial I, II, III, and IV), differing in stage of maturity and in DM content at harvest, were chopped with a precision forage harvester, untreated or treated with inoculant (11CFT inoculant, Pioneer Hi-Bred International, Des Moines, IA), and ensiled in 20 l laboratory silos, with three replicates for each treatment. The inoculant was applied at the recommended rate of 1 g Mg^{-1} of fresh forage to achieve a final application rate of 1×10^4 cfu g^{-1} of *L. casei* strain LC32909 and 1.0×10^5 cfu g^{-1} of *L. buchneri* strain LN40177. All silos were weighed and ensiled for about 10 months. After conservation, silos were weighted, opened and aerobic stability, pH, mould and yeast counts, fermentation products and nutritional characteristics of silages were determined. Aerobic stability was determined by monitoring temperature increases due to microbial activity and it was defined as the number of hours the silage remained stable before rising more than 2°C above room temperature. All silages were analyzed for NDF (aNDF), and in vitro NDF digestibility after 48 hours (NDFD).The significance of the inoculation effect was tested separately for the four trials and between-treatment comparisons were made using the unpaired Student's t-test. Aerobic stability and yeast count from the four trials were regressed on the content of acetic acid as independent variable. A regression analysis was performed to select the best regression model at $P < 0.05$.

Results and discussion Whole-crop silage DM contents encompassed all the full range in silage DM content (from 250 to 450 g kg^{-1} DM) that can occur on farm (Table 1). The application of the bacterial inoculant containing *L. buchneri* resulted in silages with lower concentration of lactic acid, higher concentrations of acetic acid, lower lactic-to-acetic acid ratio, higher aerobic stability and higher weight losses in three out of four trials. In treated silages of trials I, II, III yeast counts were always under the detection level. No effect of inoculation was observed in trial IV characterized by the highest DM content at ensiling. When all the data were analyzed together it was observed that the effect of *L. buchneri* decreased with increasing DM content of the crop at ensiling and it was negligible at the highest DM content of 440 g kg^{-1}. When the yeast counts at silo opening were related to the acetic acid concentrations, it was noted that yeast fell under the detection limit when the acetic acid content exceeded 25 g kg^{-1} DM (Figure 1) and, consequently, aerobic stability of silage increased to over 200 hours. The strong relationships between the content of acetic acid and the yeast count and between the content of acetic acid the aerobic stability of silages is in agreement with the results of Schmidt and Kung (2010). No effects were observed on NDF-D of silages, measured after 48 hours of incubation, in any of the four trials. These results partially agree with findings of Kang et al. (2009), who reported an increase in NDF-D at 48 h only in one out of two trials on maize silage.

Conclusions Inoculation with *L. buchneri* that produces ferulate-esterase increased aerobic stability in three out of four trials, by shifting the fermentation of silage towards an heterolactic pathway (i.e. increased acetic acid content that decreased yeast count), but did not affect NDF digestibility of silage at

opening. Furthermore, there were some evidences that the effect of inoculation decreased with increasing DM content at ensiling and that it was ineffective at DM contents higher than 400 g kg^{-1}.

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Table 1. DM, pH, microbial counts, fermentative profiles, weight losses, aerobic stability, and NDF-D of treated and untreated whole-crop maize silages at silo opening in four trial at Turin.

Trial	Treatment	DM	pH	Yeast	Mould	Lactic acid	Acetic acid	1,2 Propanediol	Weight losses	Aerobic stability	NDFD 48-h
		g kg^{-1}		-- log cfu g^{-1} --		---------- g kg^{-1} DM ----------				h	% NDF
I	Untreated	277	3.56	<1.00	1.41	104	21	0.5	31.9	153	50.1
I	Treated	274	3.86	<1.00	1.91	54	46	0.8	48.0	246	54.4
	P value	NS	<0.001	-	NS	<0.001	<0.001	NS	<0.001	0.006	NS
II	Untreated	323	3.57	1.18	1.13	86	18	1.0	28.1	94	56.1
II	Treated	326	3.77	<1.00	1.51	59	30	7.5	36.5	226	53.9
	P value	NS	<0.001	-	NS	0.012	<0.001	<0.001	0.001	0.008	NS
III	Untreated	385	3.79	4.06	1.74	54	12	0.0	27.8	39	48.1
III	Treated	386	3.88	<1.00	1.71	44	29	10.9	32.3	242	51.3
	P value	NS	0.039	-	NS	0.008	<0.001	0.026	0.038	<0.001	NS
IV	Untreated	440	3.78	2.55	2.34	66	12	0.1	23.3	95	49.6
IV	Treated	439	3.81	2.41	1.55	64	13	0.1	21.7	81	48.0
	P value	NS	NS	NS	0.041	NS	NS	NS	NS	NS	NS

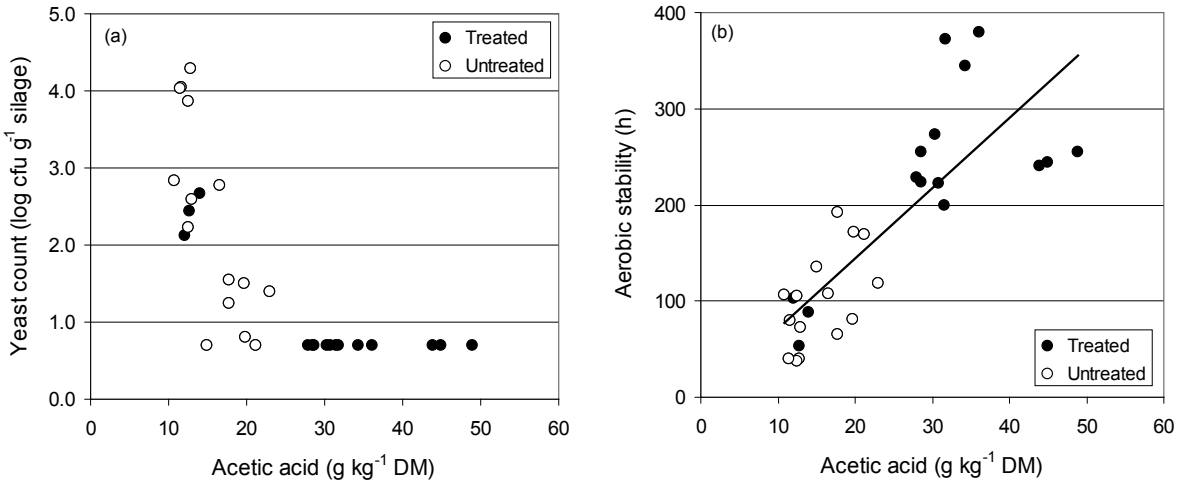

Figure 1. The yeast count (a) and the aerobic stability (b) of all the maize silage samples from the four trials correlated to the acetic acid content at silo opening.

Fermentation losses and dry matter recovery of corn silage inoculated with *Lactobacillus buchneri* and exogenous fibrolytic enzymes

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Keywords: cellulase, effluents, fermentation, microbial inoculant, xylanase

Introduction The ensilage is an alternative to minimize the seasonality effects of forage production of pastureland. The corn culture is most widely used worldwide and meets the requirements for a proper ensilability, however has shown a greater tendency to development aerobic deterioration due to the action of undesirable microorganisms and high levels of preserved nutrients presented. Different types of additives are commonly used to minimize nutrient losses and improve the silage quality among them, the enzymatic additives stand out by increase fiber degradation and available simple sugars, facilitate the action of desirable bacteria. Bacterial additives like as *Lactobacillus buchneri* are used to improve aerobic stability of silage. The aim of this research was to evaluate the losses by gases (LG), effluent (LE) and dry matter recovery (DMR) of corn silage inoculated with *Lactobacillus buchneri* or not, associated with different levels of enzymatic extract, during the fermentation.

Material and methods The experiment was conducted according to a completely randomized design with eight treatments and 3 replicates. The treatments were: S - silage without enzymes or inoculants; SLB - Corn Silage with $1x10^5$ CFU *Lactobacillus buchneri*/g of forage; SLBE1, SLBE2 and SLBE3 – (SLB + 5, 10 and 15 mL of enzymatic extract/kg of forage, respectively); SE1, SE2 and SE3 - 5, 10 and 15 mL of enzymatic extract/kg of forage, respectively. To determine the best level of enzymatic extract and enzymatic extract with inoculants we divided the treatments into two groups: Group 1 – S, SE1, SE2 and SE3; Group 2 – SLB, SLBE1, SLBE2 and SLBE3. After the best level choice, we procedure a ANOVA with Tukey's test (Pr<0.05) with S, best level of enzymatic extract in the silage, best level of enzymatic extract with inoculant in the silage and only with SLB. The inoculants used were Katec Lallemand™ and enzymatic extract was obtained from *Aspergillus niger* by submerged fermentation in a liquid medium containing 1% of wheat bran as substrate. Xylanolytic and cellulolytic activities were determined by 3´,5´-dinitrosalicylic acid (DNS) (Miller, 1959), with 1% (v/w) birchwood xylan and CMcellulose as substrates, at 39°C, respectively. One unit (U) was defined as the amount of enzyme that releases 1 Lmol of reducing sugar per minute. The material was ensiled with 29.41%±0.15 of DM and additives were sprayed on the forage, which was homogenized and stored. We used plastic experimental silos with 5 liter of capacity . It was added 0.5 kg of sand in the silos to determine the effluent losses. The silos were closed with plastic cover sealed with adhesive tape and stored at ambient temperature. After 2, 7, 14, 21 and 60 days of fermentation specific silo for each day were opened and the effluent losses were determined by weight difference. Gas losses were calculated according to equation described by Mari (2003) and the dry matter recovery was determined according to equation described by Jobim et al. (2007). For statistical analysis, we used the procedures PROC REG and PROC GLM from SAS™ software (Statistical Analysis System 2002). The regression analysis was used to determine the optimum level for each group based on the lowest gas and effluent losses and greater recovery of dry matter.

Results and discussion Enzyme extract used as additive in this research presented the 25.78 UmL and 1.54 UmL of the xylanase and celulase activities in pH 6.0 and temperature of 39°C, respectively. The pH values and bromatological composition of corn silage studied within the limits set of good quality silage (pH=3.59±0.01; DM=28.6%±0.13; OM=92.80%±0.08; EE=1.50%MS±0.04; CP=9.82%MS±0.11 and NDF=58.83%MS±0.40).

The regression equations adjusted for choosing the best level were:
Group 1: $LG=1.028+0.00234*Level$, ($R^2=0.61$) and $LG=0.996+0.00864*Level-0.00014*Level^2$, ($R^2=0.99$); $DMR=94.354+0.311*Level-0.0283*Level^2 + 0.00047*Level^3$, ($R^2=0.77$).
Group 2: $LE=2.405+0.353*Level-0.02*Level^2+0.000295*Level^3$, ($R^2=0.99$); $LG=1.047+0.00254*Level$, ($R^2=0.92$) and $LG=1.036+0.00469*Level-0.000048*Level^2$, ($R^2=0.98$) and $DMR=94.625-0.551*Level+0.0325*Level^2-0.000468*Level^3$, ($R^2=0.97$).

The treatments that showed the best levels were SE1 and SLBE2. Table 1 shows the losses by gases, effluents and dry matter recovery by each treatment and fermentation times. The largest gas losses were observed for treatments with the use of fibrolytic enzymes, which can be justified by the highest level of soluble carbohydrate such silages, providing losses increase. The effluent losses in the treatment SLB had smaller values. It was observed the largest effluents losses over the days of

fermentation due to increased of the liquid portion drained from the forage. For the dry matter recovery, the increased of levels during the fermentation days could be justified by higher effluent losses. There was no significant difference when the fibrolytic enzymes were associated or not to microbial inoculant.

ConclusionsThe addition of fibrolytic enzymes associated or not with *Lactobacillus buchneri* resulted in highest fermentative losses. The dry matter recovery was not affected in response by the additive utilized.

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Table 1. Losses by gas, effluents and dry matter recovery of corn silage containing fibrolytic enzymes associated or not with *Lactobacillus buchneri*.

Time (days)	treatments[1]				
	S	SLB	SE1	SLBE2	Mean
			Gas losses (%DM)		
2	0.93	1.01	1.08	1.14	1.04
7	0.99	1.01	1.08	1.10	1.04
14	0.95	1.03	1.08	1.10	1.04
21	1.01	1.06	1.17	1.21	1.11
60	1.10	1.06	1.07	1.08	1.08
Mean	1.00^{C}	1.03^{BC}	1.09^{AB}	1.13^{A}	
CV(%)					8.30
			Effluent losses (Kg/ton GM)		
2	0.00	0.77	0.00	2.19	0.99C
7	1.36	0.64	1.52	0.65	1.04C
14	2.46	2.04	3.59	2.06	2.54B
21	1.94	2.07	2.50	2.32	2.21B
60	8.21	6.50	8.79	7.37	7.72A
Mean	2.99^{AB}	2.40^{B}	3.78^{A}	2.92^{AB}	
CV(%)					31.5
			Dry Matter Recovery (%)		
2	94.19	92.93	93.04	96.37	94.24
7	91.69	92.42	97.12	98.36	95.64
14	94.22	96.09	91.87	92.12	93.68
21	96.26	96.37	95.14	94.58	94.55
60	95.12	96.28	92.85	95.03	94.98
Mean	94.35	94.63	94.26	94.80	
CV(%)					3.34

Medium followed by letters the same, do not differ by Tukey Test (Pr<0,05). [1]S - silage without enzymes or inoculants; SLB-corn silage with *Lactobacillus buchneri* 1 x 10^5 CFU/g of forage; SE1-corn silage with 5 mL of enzymatic extract/kg of forage; SLBE2-corn silage with *L. buchneri* 1 x 10^5 CFU/g of forage containing 10 mL of enzymatic extract/kg of forage. [2]CV: coefficient of variation.

Short and long time effects of multi-species lactic acid bacteria inoculant on fermentation characteristics and aerobic stability of whole corn silages harvested at different maturities

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Keywords: aerobic stability, corn silage, frost killed corn, Lactisil Maize, *Lactobacillus buchneri*

Introduction Homofermentative lactic acid bacteria (LAB) can enhance the rate of acidification and reduce the final pH and protein breakdown in silages. However, these LAB strains induce aerobic deterioration of whole-crop cereal silages, because not enough volatile fatty acids are produced to inhibit fungi. Heterofermentative LABs (like *Lactobacillus buchneri*) produce more acetic acid than homofermentative LAB and improve aerobic stability (Kleinschmit and Kung Jr. 2006). The aim of this study was to investigate the short and long time effects of a new commercial multi-species LAB inoculant (Lactisil Maize) (consists both homofermentative (*Enterococcus faecium* M74, *Lactobacillus plantarum* LS1, *Lactobacillus casei* and *Pediococcus pentosaceus*) and heterofermentative (*Lactobacillus buchneri*) LAB) on fermentation characteristics and aerobic stability of corn silages at different maturities and frost killed corn.

Material and methods The corn crops was harvested at hard dough (253 g dry matter (DM)/kg) (HD), one-third milkline (294 g DM/kg) (ML) and one-third milkline with a killing frost (298 g DM/kg) (FKML) maturity stages and were chopped at 2.5 cm of theoretical cut length. Lactisil Maize (Medipharm, Kågeröd, Sweden) was suspended in sufficient double distilled water according to manufacturer´s recommendations and sprayed over whole corn crops using a pressure sprayer and mixed thoroughly to get final inoculation rate of 1×10^5 colony forming units (cfu)/g and were filled in triplicate in laboratory PVC silos (70 cm in height and 10 cm in diameter). The same amount of distilled water was applied to the control silages. The laboratory silos were unsealed after 42 and 160 days of preservation and chemical analysis and aerobic stability measurement was done. Analysis of variance and General Linear Model of SAS (2003) was used for analyses of chemical composition and aerobic stability of silages.

Results and discussion Fermentation characteristics and aerobic stability of FKML forages was similar as ML (Table 1) maybe due to neglible difference of harvesting time between ML and FKML crops in this study. Inoculation increased lactate content during whole fermentation period of HD, but a reduction occurred in ML and FKML silages between 42 and 160 d of ensiling. These results agree with Filya (2003) who reported higher concentrations of lactate and acetate in *L. buchneri* + *L. plantarum*-inoculated silages as compared to control. After whole fermentation period, greater content of lactic acid were found in HD in comparison with ML and FKML. Studies have demonstrated that chemical changes occurs in the corn plant as it matures and leads to less fermentable substrates being available for lactic acid producing bacteria (McDonald et al. 1991). Acetate concentration was increased in response to the inoculant in current study. Nishino et al. (2003) suggested that high acetic acid in inoculated silages attributed mainly to lactic acid degradation and not to heterolactic fermentation. Increase in acetate concentration in response to inoculant occurred in all silages but it was more evident in ML and FKML silages. In general, these findings imply that this inoculant dominated heterofermentative lactic acid bacteria to homofermentative LAB only in ML and FKML silages at late phase of ensiling. It is maybe because of earlier suspension in activity of homofermentative LAB in high dry matter crops due to relatively less available water soluble carbohydrates (WSC) and lower water activity.

The pH of control silages decreased from 42 to 160 d after ensiling probably due to continuing in lactate production. In inoculated silages pH increased as a result of lactate conversion to acetate. NH3-N accumulated slowly in silages with inoculant from 42 to 160 d of ensiling but elevation in concentration of NH3-N appeared more extensive in control silages. As soon as silage pH falls rapidly after ensiling, the aerobic microorganisms and plant enzymes are inhibited more rapidly, which results in reduced proteolysis (Kleinschmit and Kung Jr. 2006). Inoculation improved aerobic stability only after 160 d of fermentation just in ML and FKML. Within each maturity, silages with greater aerobic stability had greater acetate concentration and lower ratio of lactate to acetate. Lower wet pack density in ML and FKML silages declined air infiltration into the silo, and subsequently resulted in silages with lower aerobic stability (Weinberg et al. 2010).

Table 1. Fermentation characteristics of corn silages at different maturity stages with and without inoculant.

Maturity	Treatment	Day	DM (g/kg)	Lactate (g/kg DM)	Acetate (g/kg DM)	Lactate/ Acetate	pH	NH_3-N (% N)	Aerobic Stability (h)
HD	control	42	234.9	78.1	11.5	6.81	3.78	5.31	161
HD	inoculated	42	238.2	101.2	11.6	8.75	3.67	5.27	137
ML	control	42	240.8	63.5	11.0	5.79	3.78	5.63	56
ML	inoculated	42	240.5	103.4	14.2	7.31	3.78	4.93	35
FKML	control	42	262.2	49.5	12.9	3.85	3.80	3.96	82
FKML	inoculated	42	266.2	76.8	14.8	5.20	3.67	4.89	70
HD	control	160	226.4	129.9	13.2	9.88	3.71	6.69	178
HD	inoculated	160	222.9	158.7	15.8	10.06	3.85	5.51	172
ML	control	160	231.0	68.9	14.3	4.83	3.76	6.21	76
ML	inoculated	160	233.6	80.3	18.4	4.37	3.86	5.73	108
FKML	control	160	239.7	54.6	13.9	3.92	3.69	4.76	89
FKML	inoculated	160	239.0	57.1	19.3	2.96	3.83	5.40	102
Standard error			3.1	2.9	0.8	3.4	0.4	1.7	5.55
Day			**	**	**	*	**	**	**
Maturity			**	**	**	**	*	**	**
Treatment			NS	**	**	**	NS	*	NS
Maturity by day			**	**	NS	**	NS	NS	**
Treatment by day			NS	**	**	**	**	**	**
Maturity by treatment			NS	**	**	*	NS	**	**
Maturity by treatment by day			NS	**	NS	NS	*	**	**

HD=hard dough stage; ML=one-third milkline stage; FKML=one-third milkline with a killing frost; DM=dry matter; NH_3-N=ammonia nitrogen

Conclusions Stage of maturity affected fermentation characteristics of silages. Silages at earlier maturity stage had extended rate of fermentation. Inoculation improved fermentation characteristics of silages and aerobic stability was improved after long time preservation with inoculant. Better aerobic stability in HD silage was associated with lowest concentration of acetate and highest lactate to acetate ratio.

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Effects of pre-treating whole crop maize with fungicides on the fermentation quality of ensiled maize

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Keywords: abacus, disease, duett, fungi, residuals, silage

Introduction Maize silage is one of the major forage sources in diets of high producing ruminants in South Africa (Cilliers et al., 1998, Meeske and Basson, 1998). One of the major problems faced with the production of maize for silage production in South Africa is the prevalence of grey leaf spot, that reduces maize yields (Ward et al., 1996, Ward and Nowell, 1998) and silage quality. Certain fungicides can control diseases but their effectiveness lie with correct application. In South Africa, fungicides such as Abacus® and Duett™ are used to control fungi in whole crop maize. However, research that evaluates the effect of pre-treating whole crop maize with these fungicides on the ensilibility and nutritive value of whole crop maize in South Africa is limited. Consequently, this study was done to evaluate the effects of pre-spraying of Abacus® and Duett™ on the fermentation quality of ensiled whole crop maize.

Material and methods Batches (± 15kg each) of whole crop maize (241 g dry matter (DM)/kg, 109 g water soluble carbohydrates (WSC)/kg DM and 7.05 pH) that were treated with or without fungicides were harvested from a farm in the Free State Province. The whole crop maize was treated with: i) untreated (control), ii) Abacus® (early application at 1.6 L/ha, denoted as Ab (E)), iii) Abacus® (early application + late application at 1.6 L/ha, denoted as Ab (E+L)), and iv) Abacus® (early application at 1.6 L/ha) + Duett™ (late application at 1.2 L/ha), denoted as Ab+Duett. The harvested maize was chopped to achieve a 10 mm theoretical chop length, and ensiled in 1.5 L anaerobic jars. Each jar was filled with approximately 850 g (wet weight) of chopped maize without headspace, and a packing density of 567 kg DM/m^3 was obtained. A sample of the freshly chopped material from each treatment was collected on day 0 (pre-ensiled materials) while the ensiled material (3 replicates per treatment) was collected on day 42 for the determination of fermentation characteristics. Data was analysed in a completely randomised design for ANOVA using SAS (1999) and significance was declared at 5 % probability level.

Results and discussions The Ab+duett® had lower ($P<0.05$) residual WSC and higher LA content compared to the other treatments (Table 1), indicating that more sugar was utilized by lactic acid bacteria (LAB) to produce LA during fermentation in this treatment (McDonald et al., 2002). In addition, CF was reduced by this treatment. The Ab (E) had higher ($P<0.05$) CF content compared to other treatments, indications of lack of fibryolytic activity with this treatment. The Ab (E+L) had higher ($P<0.05$) contents of ammonia-N compared to the other treatments, indicating higher proteolytic activity in this treatment compared to other treatments. Nevertheless, the silage produced was of good quality as indicated by the lack of butyric acid, reduced pH and lactic acid contents of acceptable levels (Kung and Shaver, 2001).

Conclusions Good quality silage was produced irrespective of the treatments. However, the aerobic stability of silage and the fungicide residuals in the silage still need to be determined to ensure safe feed for ruminants. Further work to elucidate the results of the present study on a larger scale and on animal production is needed.

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Table 1. Effects of treatments on the nutritive value and fermentation dynamics (g/kg DM unless stated otherwise) of ensiled whole crop maize (n=3).

	Treatments				SEM	P
	Control	Ab (E)	Ab (E+L)	Ab+Duett		
DM, g/kg	193.4^{c}	217.4^{a}	213.2^{b}	209.5^{b}	1.358	0.001
CP, g/kg DM	71.0^{c}	79.9^{a}	73.2^{b}	63.4^{d}	0.49	0.001
CF, g/kg DM	265.1^{b}	283.4^{a}	244.2^{c}	228.4^{c}	5.41	0.001
NSC, g/kg DM	128.6^{c}	316.2^{a}	163.2^{b}	165.8^{b}	4.89	0.001
WSC, g/kg DM	84.9^{a}	79.0^{b}	75.1^{b}	66.6^{c}	9.16	0.032
pH	4.16^{b}	4.23^{a}	4.15^{b}	4.13^{b}	0.0148	0.006
LA, g/kg DM	98.2^{c}	87.2^{d}	109.0^{b}	125.2^{a}	0.602	0.001
AA, g/kg DM	18.3	26.2	11.6	13.1	5.11	0.228
PA, g/kg DM	1.10	1.62	1.57	1.30	0.404	0.779
NH_3-N %TN	24.97^{b}	22.96^{b}	29.74^{a}	24.79^{b}	1.265	0.015

$^{a-c}$ Means with different letters in a row differ significantly (P<0.05)

Treatments: Control (no fungicide); Ab(E), abacus early application; Ab (E+L), abacus (early and late applications); Ab+Duett, abacus and Duett applications.

DM, dry matter; CP, crude protein; CF, crude fibre; NSC, non-structural carbohydrates; WSC, water-soluble carbohydrate; LA, lactic acid; AA, acetic acid; PA, propionic acid; NH_3-N %TN, ammonia-nitrogen as % of total nitrogen.

Conservation characteristics of maize stover ensiled with the addition of *Lactobacillus plantarum* MTD-1, *L. plantarum* 30114 or *L. buchneri* 11A44

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Keywords: stover, additive, lactic acid bacteria, aerobic stability

Introduction The use of bacterial additives, selected to dominate the epiphytic bacteria on herbage and alter the silage fermentation process, can reduce conservation losses and improve nutritive value. Those which promote highly efficient lactic acid dominated fermentations, such as *Lactobacillus plantarum,* have the potential to reduce fermentation losses, whereas lactic acid bacteria (LAB) that promote the production of anti-fungal compounds such as acetic acid (e.g. *L. buchneri*) during ensilage can increase the aerobic stability of maize silage and reduce aerobic losses. Forage maize (*Zea mays* L.) plants consist of two physical components with contrasting chemical compositions, the cob (grain and rachis) and the stover (stem, leaves, husks and tassel). Maize stover is considered to have a nutritive value for ruminants comparable to average quality grass silage. Despite this, information on the effects of added bacterial on the ensilage of maize stover is not readily available. The aim of this study was to investigate the effects of inoculating maize stover with three different LAB on the fermentation profile, chemical composition and aerobic stability of the subsequent silages.

Material and methods Stover was separated from whole-crop maize plants obtained from three replicate field blocks. Sub-samples were precision-chopped and allocated to one of the following treatments: no additive (control), *Lactobacillus plantarum* MTD-1 (LP1), *L. plantarum* 30114 (LP2) and *L. buchneri* 11A44 (LB). Each bacterial additive was applied of 1 x 10^6 colony forming units/g fresh herbage. Triplicate samples of each treatment were ensiled in laboratory silos at 15ºC for each of 3, 10, 35 or 130 days. The fermentation products after each ensiling duration and the chemical composition, dry matter (DM) recovery and aerobic stability after 130 days ensilage were measured. In addition, LAB plate counts and real-time quantitative PCR (qPCR) were carried out at each time-point to enumerate the *L. plantarum* and *L. buchneri* populations. Data were subjected to analysis of variance for a 4 (additive treatment) × 4 (ensilage duration) factorial arrangement of treatments using the PROC GLM procedure of SAS.

Results and Discussion The DM, acetic acid, butyric acid and total fermentation products (TFP) concentrations of stover silages were unaffected (P>0.05) by LB, LP1 or LP2, when compared to uninoculated silages (Table 1). Silages made with the LB or LP2 and ensiled for 35 days had lower (P<0.05) lactic acid concentrations. In addition, stover silages made with LB had a higher (P<0.05) pH than silages made with either LP1 or LP2. Populations of *L. buchneri* in stover were more abundant (P<0.01) after 130 days ensilage than after shorter durations.

The lactic acid concentration of uninoculated silages was greatly reduced when ensilage continued for 130 days. Muck and Shinners (2006) stated that the secondary fermentation of lactic acid by epiphytic *L. buchneri* likely contributed to the heterolactic nature of relatively immature (300 g DM/kg) stover silage fermentations. *L. buchneri* populations were high in uninoculated stover silages in the present study and following the 130 day ensilage it appears that lactic acid was almost exhausted as a substrate. This finding is supported by the corresponding large increase in acetic acid concentrations between 35 and 130 days ensilage.

The lack of difference between the fermentation dynamics, DM recovery or aerobic stability of stover silages made with LP1 was related to it not having an effect on *L. plantarum* numbers, compared to the uninoculated silages, indicating that added LAB failed to successfully dominate the fermentation. Stover silages made using LP2 had a higher proportion of acetic acid in TFP than silages made without an additive or with LP1, but did not differ from the LB treatment after 35 days ensilage.

Conclusions The aerobic stability and DM recovery of stover silages in this study were not improved when made with LB, LP1 or LP2, due to the indigenous highly heterolactic fermentation that prevailed in the uninoculated stover during 130 days ensilage.

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Table 1. Fermentation products and microbial enumerations of stover silages made with different bacterial additives at four stages of ensilage

Stage of ensiling	3 days				10 days				35 days				130 days					Sig[13]		
Additive[1]	C	LB	LP1	LP2	C	LB	LP1	LP2	C	LB	LP1	LP2	C	LB	LP1	LP2	s.e.m.[12]	A	E	AxE
DM[2]	190	186	186	192	198	188	183	194	193	183	200	193	175	176	189	185	6.0			
pH	4.1	4.5	3.9	3.8	3.9	3.9	3.9	3.9	4.0	4.4	3.7	4.0	4.6	4.6	4.4	4.5	0.17	*	***	
Lactic acid[3]	25	17	18	16	31	24	25	21	27	12	43	14	1	0	3	1	9.2	*	***	
Acetic acid[3]	10	7	9	8	12	12	13	14	19	27	17	26	46	43	44	43	2.3		***	
Ethanol[3]	6	5	5	5	7	8	6	6	11	15	10	11	28	29	33	25	1.0	*	***	**
Propionic acid[3]	0.5	0.4	0.5	0.2	0.3	0.9	0.9	1.0	1.8	3.3	0.8	2.7	3.5	4.5	6.3	3.5	0.41		***	**
Butyric acid[3]	0.1	0.0	0.0	0.0	0.1	0.6	0.3	0.4	0.1	0.2	0.0	0.6	0.5	1.3	0.5	0.3	0.27		*	
TFP[3]	42	30	33	29	51	45	45	42	59	57	71	54	80	78	87	72	9.7		***	
NH_3-N[4]													97	102	71	76	12.2			
LA/TFP[5]	0.59	0.57	0.53	0.53	0.60	0.49	0.51	0.51	0.47	0.21	0.60	0.21	0.02	0.01	0.04	0.01	0.042	**	***	**
AA/TFP[6]	0.25	0.22	0.28	0.28	0.25	0.29	0.32	0.32	0.32	0.47	0.24	0.52	0.58	0.54	0.51	0.59	0.029	**	***	**
LAB (plate count)[7]	8.46	8.78	8.92	9.54	8.43	8.56	8.46	9.02	8.02	8.66	7.74	7.56	8.15	8.09	8.15	8.31	0.203		***	*
L. buchneri (qPCR)[8]	4.64	5.69	3.79	4.37	4.85	5.27	4.12	3.78	5.31	7.34	5.57	4.89	6.03	6.96	6.57	7.07	0.497	**	***	
L. plantarum(qPCR)[8]	6.27	5.30	6.90	6.15	6.48	5.19	6.96	5.97	6.50	7.00	7.09	6.98	6.16	6.03	6.37	6.79	0.254	***	**	*
DM recovery[9]													840	812	907	884	26.5			
Hours > 2°C[10]													>192	>192	>192	>192	0			
ACT 120h[11]													1	1	2	2	0.3			

[1] C= uninoculated control, LB= *Lactobacillus buchneri* 11A44, LP1= *L. plantarum* MTD-1, LP2= *L .plantarum* 30114

[2] g/kg; DM= dry matter

[3] g/kg DM; TFP= total fermentation products (Lactic acid + acetic acid + propionic acid +butyric acid + ethanol)

[4] g/kg N; only analysed after 130 days ensilage

[5] g lactic acid/ g TFP

[6] g acetic acid/ g TFP

[7] Log_{10}(colony forming units using agar/g herbage); LAB=lactic acid bacteria

[8] Log_{10}(estimated colony forming units using real time quantitative PCR/g herbage); *L.*= *Lactobacillus*

[9] g silage/kg herbage ensiled (dry matter basis); Only determined after 130 days ensilage

[10] Interval (h) until temperature rises more than 2°C above reference temperature (index of aerobic stability); only determined after 130 days ensilage

[11] Accumulated temperature rise during 120 hours exposure to air (index of aerobic deterioration); only determined after 130 days ensilage

[12] Standard error of the mean for stage of ensilage and additive

[13] A=additive, E= stage of ensilage;

The effect of lactic acid bacteria and enzymes on ensiling of corn stover and wet corn distillers grains

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Keywords: corn stover and wet distillers grains, ensiling, enzymes complex, lactic acid bacteria

Introduction The effects of additives depend upon the properties of the crops (Nadeau et al., 1998), *lactobacillus* and enzymes are fermentation stimulators for silage (Whiter, 2001; Muck, 2004). Cellulase, xylanase and pectinase are cell wall-degrading enzymes which can degrade the fibrous material and increase apparent digestibility of cellulose (Ridla,1993). Corn stalks (CS) are a low quality feedstuff remaining after corn ears have been harvested. Wet corn distillers grains (WCDG) have a higher content of readily degradable cell contents, such as protein. The objective of this study was to evaluate the effects of lactic acid bacteria (LAB) and enzyme complex (EC) in mixture of CS and WCDG (as dry matter base to 4:1) by ensiling.

Material and methods A 3×2 factorial design was conducted to study three inoculants levels of LAB (0, 1×10^5, 1×10^6 CFU/g) and two levels of EC (0, 2200 IU/g), and 6 treatments were LAB0EC0 (I, as the control), LAB0EC1(II), LAB1EC0(III), LAB1EC1(IV), LAB2EC0(V) and LAB2EC1(VI). The LAB inoculants contained *Lactobacillus plantarum, L. buchneri* and *Pedicoccus acidilactici* isolated from corn silage; the proption of EC contained cellulose, xylanase and pectinase were screened by a trial in our laboratory, and enzymes were bought from TAKARA BIOTECHNOLOGY (DALIAN) CO., LTD. The corn stover was chopped into lengths of 3 to 5 cm with a simple straw chopper, the portion of silage material was calculated according to 10 kg chopped CS assorted with 2.5 kg WDGS on DM-basis, additives as solution were blended for modulating CS and WCDG moisture to 67%, manually packed into high density polyethylene bags (100×120cm), four replicates in a batch of treatments were compacted and sealed with elastic string respectively. The fermenting silos were kept indoors (22°C). Samples (5 kg) were collected from each silo and frozen (-20°C) for later chemical analysis after 2, 4, 8, 16, 32 and 64 days of ensiling.

The composition of CS and WCDG mixture was analysed, concentrations of neutral-detergent fibre (NDF) and acid detergent fibre (ADF) were 605.1 g/kg DM and 338.5 g/kg DM determined by Van Soest (1991), and water-soluble carbohydrates (WSC) were 220.5 g/kg DM analysed using method described as Chen (1994). The frozen silage samples were prepared using homogenizer for content of DM, pH, NH3-N, and concentrations of lactic acid, acetic acid and butyric acid were determined using gas chromatogram, samples were dried at 55°C oven to dry for NDF, ADF and WSC analysing (Yang 1993). Data were analyzed using ANOVA using GLM procedure of SAS 9.0 for a repeated measurement.

Results and discussion The composition of the silage were list in Table1. During ensiling for 64 d, inoculants LAB and EC significantly decreased the silage pH, butyric acid, ammonia-N, NDF and ADF concentrations ($p<0.01$), increased WCS, lactic acid and acetic acid concentrations respectively ($p<0.01$). The DM content of silage was from 31.63% to 33.02%, there were less loss among the groups ($p>0.05$), it was indicated that CO_2 was formed less during the conversion of lactic acid to acetic acid and 1,2-propanediol. The pH of the treatments III, IV, V and VI declined more rapidly compared to the control during 64d ensiling, the pH of silage IV with LAB and EC was the lowest one to 3.95 same as VI 3.96, and moreover, the silage IV pH had reached toward 4.20 when ensiling was at the 16 d, and the pH of VI was only 4.26 at the 32 d, it indicated that EC was effectively hydrolyzing cellulose to enhance LAB secreting acid like matter, especially LAB1 level. Compared to the control, NH_3-N content of the silages treated with LAB or LAB and EC decreased in IV and VI ($p<0.01$),and the two additives were related to a more rapid and higher acidification that probably inhibited the activity of aerobe and proteolysis in silage. Concentration of lactic acid and acetic acid in silages IV,V and VI increased rapidly from the 2 d to 64 d, the butyric acid in silage added inoculants and EC were lower for the silage IV, V and VI, the silage VI was the lowest one. From 4 d to 64 d fermentation, WSC content in silage with two additives were significantly higher than of the untreated one($p<0.01$), far more WSC were tested in treatments contained EC rather than LAB ($p<0.01$), these demonstrated that EC exerted enzymolysis to cellulose under this condition, furthermore, less NDF content were observed in silage II, IV and VI treated with EC, the NDF of silage VI reduction is the lowest level ($p<0.01$), it demonstrated indirectly EC function to some extent, the ADF concentration had a minor change to initial material, though there was a statistic significance in treatments.

Conclusions All the additives improved the fermentation quality compared with the control silage. The combination of LAB1EC1 level (1×10^5 CFU/g, 2200 IU/g) was, in the mean, the most rapid fermenting in this experiment. Enzymes shorten the time of ensiling with different LAB level, improved the fermenting quality and ratio. Silages with LAB and EC additives were steadily when ensiling was at 60 d, it was that interaction between LAB and EC played an important role to lead well ensiling results, and this also demonstrated LAB separated from our laboratory and the portion of EC screened were all efficiently.

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Table 1. The content of dry matter (DM) and concentrations of water-soluble carbohydrates (WSC), NDF, ammonium-N (NH3-N), pH, fermentation products (lactate, acetate and butyrate) either untreated or treated with enzymes complex or lactic acid bacteria.

	Control	Treatments					Significance[a]		
	I	II	III	IV	V	VI	LAB	EC	LAB×EC
Dry matter, g/kg	324.9	329.3	320.1	316.3	324.9	330.2	NS	NS	NS
WSC, g/kg DM	33.1^{c}	216.2^{a}	78.7^{b}	234.2^{a}	81.6^{b}	218.6^{a}	**	**	**
NDF, g/kg DM	602.5^{a}	563.5^{b}	571.7^{b}	530.2^{c}	572.9^{b}	534.3^{c}	**	**	**
ADF, g/kg DM	347.6^{a}	308.3^{c}	328.0^{b}	319.7^{bc}	334.5 ^{ac}	322.8^{bc}	**	**	**
Lactic acid, g/kg DM	19.5^{d}	21.6^{b}	20.3^{c}	22.9^{a}	21.2^{b}	23.1^{a}	**	**	**
Acetic acid, g/kg DM	13.9^{cd}	13.3^{d}	14.4^{c}	19.0^{a}	15.5^{b}	18.6^{a}	**	**	**
Butyric acid, g/kg DM	0.84^{a}	0.75^{ab}	0.70^{b}	0.61^{c}	0.67^{bc}	0.50^{d}	**	**	**
Ammonia N, g/kg total N	40.0^{a}	32.7^{bc}	30.3^{c}	29.7^{d}	34.5^{b}	30.2^{c}	**	**	**
pH	4.50^{a}	4.18^{b}	4.20^{b}	3.95^{c}	4.29^{b}	3.96^{c}	**	**	**

a.*p<0.05, **p<0.01; NS, Nonsignificant.

The effect of two bacterial strains on the fermentation characteristics and aerobic stability of grass silages

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Keywords: aerobic stability, grass silage, *Lactobacillus buchneri*, *Propionibacterium acidipropionici*

Introduction Additives are expected to ensure a more efficient fementation phase as well as reduce the risk of aerobic deterioration when silages are exposed to air. Recently the heterofermentative *L. buchneri* is regarded to be the most promising lactic acid bacteria for increasing aerobic stability. Applied by itself it may show a negative effect by reducing the speed of fermentation. According to Ruser and Kleiman (2005) it takes effect on stability in the 2nd phase: during the 1st phase lactic acid originates from sugar and in the 2nd phase acetic acid and 1,2- propandiol are generated from lactic acid. Oude Elferink et al. (2001) emphatize the role of propionic acid originating from 1,2 -propandiol and 1 -propanol in stability. (1,2-propandiol and 1-propanol are not found in untreated silage). *L.buchneri* may produce other yet unindentified metabolites with antifungal activity. Some studies showed that the primery reasons for the ineffectiveness sometimes for the fermentation of propionic acid bacterias such as *Propionibacterium acidipropionici* include the facts that they are strict anaerobes, slow growing, relatively acid intolerant and they have proteolytic activity. They are able to convert lactic acid and glucose to acetic and propionic acids that are more antifungal than lactic acid, therefore can improve the aerobic stability of silage (Filya and Sucu 2007).

Material and methods Second cut grass containing some leguminous species was mechanically harvested at a chop lenght of 2.4 cm and ensiled at a dry matter (DM) content of 24 % and a sugar content of 2,9% water soluble carbohydrates (FM basis).

The applied treatments were: *L. buchneri* NCIMB 40788 at 2 dosages: 1.0 x 10^5 cfu/g FM (T2) or 3.0 x 10^5 cfu /g FM (T3), and *P. acidipropionici* MA 26/4U at 1.0 x 105 cfu/g FM.(T4). Each bacteria was diluted in distillated water and then sprayed onto the fresh forage. The negative control (T1) received the same amount of water than the other 4 treatments. For each treatment, 6 small sized containers of 4.2 l cubic capacity each were used, closed by screwed cap (altogether 24 pieces). The mini-silos were stored at 20-22 C° ambient temperature and opened after 135 and 185 days ensiling (3 replicates each). The laboratory examinations focused primarily on the fermentation products and the microbiological analysis. Lactic acid and volatile fatty acids were determined using gas chromatography (Young Lin6100 Acme 6100 gas chromatograph with FID detection). Ethanol content was determined on a watery extract with K2Cr2O7 solution and through titration with Mohr-salt-solution. NH3 content was determined by the use of an ammonium electrode (OP-264/2, Radelkis Ltd. Budapest). Microbiological analysis (for lactic acid bacteria (LAB), yeasts and moulds) were performed using traditional plate count methods.
The aerobic stability of silages was determined by the system of Völkenrode (Honig, 1990) and set for the time to reach a 3°C increase above ambient temperature.

Data were compared using the student-t- test of Microsoft Excel program. Significance was declared for $P<0.05$.

Results and discussion The density of silos was 470-485 kg/m^3 which corresponds to a density of 113-116 kg DM /m^3. Lactic acid content was lower respectively in the treated T2, T3 and T4 silages than that of T1 control silage ($P<0.001$). Acetic acid content was essentially higher in all treated silages compared to the control. The difference was very strong ($P<0.001$) in case of T2 and T3 treatments and still pronounced ($P<0.01$) in case of T4 silages. Propionic acid concentration was significantly increased for all treated silage compared to T1 control silage. This is in line with the observations of Fylia and Sucu (2007) on whole crop cereal silages. They observed that *L. buchneri* inoculated silages had the highest levels of acetic acid, whereas the *P. acidipropionici* inoculated silages showed increased propionic acid levels compared with the control.Ethanol production was almost twice as high ($P<0.05$) in treated silages than in control. These findings are in contrast to earlier research where inoculation with either *L. buchneri* or *P. acidipropionic* did not affect the ethanol content of the silage (Fylia *et al,* 2004; Fylia and Sucu, 2006).

Control silage was stable for 146 hours, while it lasted 234 hours for T4 treated silages. The stability of T2 and T3 silages surpassed the control and T4 silages, they remained unspoiled longer than the experimental period (>240 hours) ($P<0.001$). *L. buchneri*,and *P. acidipropionici* improved the aerobic stability of the silages by causing more extensive heterolactic fermentation that resulted in the silages with high levels of acetic and propionic acid (Fylia and Sucu, 2007). Less DM losses were detected in

aerobic condition for all treated silages. (P<0.001) This is in line with the higher aerobic stability as aerobic deterioration is associated with dry matter losses (Woolford, 1990).

The fresh material and the treated silages were healthy, containing very small number of yeasts and moulds. The microbial profile of silages showed that the LAB treated forages did not increase the number of mesophyl LAB at the end of fermentation and storage (Ns).

Table 1. Fermentation products and aerobic stability of *Lactobacillus buchneri* and *Propionibacterium acidipropionici* treated grass silages on DM basis

Parameters		Treatments T1 Untreated control		T2 LB 1x10^5 cfu/g FM		T3 LB 3x10^5 cfu/g FM		T4 PA 1x10^5 cfu/g FM	
		Mean	sd	Mean	sd	Mean	sd	Mean	sd
Dry matter	%	24.91	1.97	22.43	0.18	23.95	0.51	24.19	0.29
pH		4.53^a	0.30	4.96^b	0.06	5.02^b	0.04	4.93^b	0.07
Lactic acid	%	7.21^a	0.89	3.03^b	0.23	2.94^b	0.72	2.61^b	0.36
Acetic acid	%	1.92^a	0.70	3.31^b	0.31	3.28^b	0.22	2.77^c	0.13
Butyric acid	%	0.000	0.00	0.000	0.00	0.000	0.00	0.007	0.02
Propionic acid	%	0.02^a	0.04	0.13^b	0.05	0.09^b	0.04	0.14^b	0.05
Ethanol	%	1.05^a	0.14	1.83^b	0.49	2.03^b	0.30	1.67^b	0.31
Ammonia	% of total N	17.36	3.43	18.56	1.09	19.61	0.85	17.56	1.38
DM losses	%	6.09^a	1.95	0.00 ^{b}	0.00	0.00^b	0.00	0.75^b	1.83
Aerobic stability	hours	146^a	27.7	>240^b	-	>240^b	-	234^b	14.3

a,b,c: mean values within a row with no common superscripts differ significantly (p<0.05)

Conclusions Treatments with biological inoculants improved fermentation and aerobic stability of grass silages. *Lactobacillus buchneri* (NCIMB 40788) on grass compared to *Propionibacterium. acidipropionici* (MA 24/4U) resulted in longer aerobic stability of silage.

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The effect of lactic acid bacteria-based additives and wilting on grass silage fermentation characteristics

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Keywords: lactic acid bacteria, silage, silage additive, fermentation quality, aerobic stability, wilting

Introduction Silage additives containing lactic acid bacteria (LAB) contribute to improve ensiling process (Reich and Kung Jr 2010). The use of homofermentative LAB results in lactic acid production and rapid drop of feed pH. Heterofermentative LAB produce volatile fatty acids or other components preventing the feed from heating up and improving aerobic stability of the forage during feed-out (Oude Elferink et al. 2001, Ranjit et al. 2002). Using LAB additives, the water soluble carbohydrate concentration and the dry matter (DM) content of the raw material are crucial for the fermentation success. Especially the effects of heterofermentative *Lactobacillus buchneri* on low dry matter (DM) silage was studied in this experiment, because weather conditions in Finland do not always allow sufficient wilting during harvesting period.

Material and methods The plant material of the two trials consisted predominantly of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). The first trial comprised two levels of grass DM concentrations (239 g/kg DM and 371g/kg DM) and five additive treatments: 1) untreated silage (CON), 2) formic acid (FA) (4L/tn 100% acid), 3) homofermentative *Lactobacillus plantarum* and *Pediococcus acidilactici* ($1x10^6$ cfu (colony forming units)/g forage) and the enzymes pectinase, xylanase and cellulase (LAB), 4) heterofermentative *Lactobacillus buchneri* ($3x10^5$ cfu/g) (BUC), 5) mixture of LAB ($7x10^5$ cfu/g) and BUC ($3x10^5$ cfu/g) (LBU). The second trial included one DM level (208 g/kg) and six additive treatments: 1) CON, 2) FA, 3) LAB, 4) *L. plantarum* ($1x10^6$ cfu/g) (LP6), 5) *L. plantarum* ($1x10^5$ cfu/g) (LP5), 6) a mixture of *L. plantarum* ($1x10^5$ cfu/g) and BUC ($1x10^5$ cfu/g) (LB).

Results and discussion In both trials the water soluble carbohydrate (WSC) content of the plant material was high (trial 1: 33.7 g/kg fresh matter (FM), 34.5 g/kg FM; trial 2: 40.8 g/kg FM). The first trial exposed different silage fermentation patterns between the two DM levels. The use of BUC alone resulted in lower WSC content, higher pH values and higher concentrations of acetic acid and ethanol as compared to other treatments. The interaction between DM and BUC revealed lower amounts of fermentation products on higher DM levels. When BUC was used in a mixture with LAB, the fermentation characteristics were improved. No differences in aerobic stability were observed. In the second trial the LAB treatment improved fermentation quality of silages as compared to untreated control. All the silages revealed a low pH value. The untreated control silage showed the highest ammonia concentration and FA silage exposed elevated acetic acid values. Aerobic stability was better in FA silage as compared with other treatments. The significantly better aerobic stability of FA treated silage compared to other silages cannot be explained by elevated acetic acid amounts alone. Acetic acid amounts of BUC silage were of equal levels with FA silage, but BUC aerobic stability was poorer than that of FA silage.

Conclusions *L. buchneri* treated forage did not demonstrate any effect on aerobic stability, because in the first experiment all feeds were stable and in the second experiment *L. buchneri* treated forage did not differ significantly from LP silages. Silages based on *L. plantarum* additives were of good quality. Higher application levels did substantially affect fermentation quality.

The addition of BUC to forage alone or combined with lactic acid bacteria resulted in silages with high ammonia-N and acetic acid amounts. The quality of *L. buchneri* silage was not as good as those treated with homolactic additives or formic acid. Even the untreated control was of better quality. The results suggest that the use of a pure *L. buchneri* additive on forages with low dry matter content is not appropriate due to the excessive fermentation and furthermore, high acetic acid concentration is not necessary for aerobic stability improvement. The interaction of DM and BUC favours the use of BUC for silages with higher DM content.

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Table 1.Chemical composition of silages in trial 1 (g/kg dry matter (DM), unless otherwise stated) and statistical significances.

	Dry matter 1					Dry matter 2					
	CON	FA	LAB	BUC	LBU	CON	FA	LAB	BUC	LBU	SEM
Dry matter, g/kg	237	241	241	263	241	359	354	361	363	372	1.3
Ash	75.1	75.2	76.7	85.2	78.8	77.8	76.0	77.5	80.8	78.1	0.43
pH	4.16	3.88	4.06	4.73	4.16	4.40	4.36	4.03	4.85	4.06	0.006
Ammonia-N, g/kg N	78.0	28.2	65.1	111.0	66.9	59.3	32.4	51.8	78.3	58.3	3.56
WSC	9.6	9.4	24.3	2.0	6.8	38.4	42.3	29.2	2.5	11.5	1.01
Lactic acid	93.2	65.4	118.0	14.1	101.0	69.6	32.6	105.0	36.2	97.8	1.52
Acetic acid	18.3	14.6	15.5	55.9	29.5	11.6	7.4	10.4	38.7	16.1	0.29

	DM 1 vs. DM 2 (1)	FA vs. others (2)	CON vs. BIOL (3)	LAB vs. BUC (4)	LAB & BUC vs. LBU (5)	DM x 2	DM x 3	DM x 4	DM x 5
Ash		***	***	***	**		***	***	
pH	***	***	***	***	***	***	***	***	***
Ammonia-N, g/kg N	***	***		***	**	**			
WSC	***	***	***	***	***	***	***	*	
Lactic acid	***	***	*	***	***	***	***	***	**
Acetic acid	***	***	***	***	***	***	***	***	**

CON=untreated control; LAB=*L.plantarum* and *Pediococcus acidilactici* and enzymes; FA=formic acid; BUC=*L.buchneri*; LBU= LAB and BUC; BIOL=LAB, BUC, LBU; others=CON, LAB, BUC, LBU; DM = dry matter; WSC=water soluble carbohydrates

Table 2. Chemical composition of silages in trial 2 (g/kg dry matter, unless otherwise stated) and statistical significances.

	CON	FA	LAB	LP6	LP5	LB	SEM	FA *vs.* others	CON *vs.* BIOL	LAB *vs.* BIOL	LP6 *vs.* LP5, LB	LP5 *vs.* LB
Dry matter, g/kg	206	211	206	208	206	207	0.8	***				
Ash	67.7	65.9	69.8	70.0	69.8	70.7	0.68	***	**			
pH	3.77	3.69	3.70	3.69	3.71	3.78	0.006	***	***	***	***	***
Ammonia-N, g/kg N	89.3	25.4	44.7	30.1	46.1	65.9	1.50	***	***		***	***
WSC	19.0	3.9	60.0	34.2	26.6	11.8	0.82	***	***	***	***	***
Lactic acid	141	77	141	140	137	132	1.9	***		*		
Acetic acid	13.9	19.3	9.8	11.0	11.5	18.5	0.13	***	***	***	***	***
Temperature diff. 2 ^{o}C, 24h	3.45	-	3.39	2.33	4.36	7.04	1.890	***				

CON=untreated control; FA=formic acid; LAB= *L.plantarum* and *Pediococcus acidilactici* and enzymes; LP6= *L.plantarum* 1x10^{6} cfu/g forage; LP5=*L.plantarum* 1x10^{5} cfu/g forage; LB=*L.plantarum* and *L.buchneri* 1:1, 2x10^{5} cfu/g forage; cfu= colony forming units; others=CON, LAB, LP6, LP5, LB; BIOL=LAB, LP6, LP5, LB; WSC=water soluble carbohydrates

Effects of additive and particle size on fermentation characteristics and aerobic stability of grass silage

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Keywords: additive, aerobic stability, fermentation, grass silage, particle size

Introduction Silage fermentation can be influenced by the particle size of the forage being ensiled (McEniry et al. 2008, Rinne and Seppälä 2011) and variations in silage fermentation affect intake of cattle (Huhtanen et al. 2007, Krizsan and Randby 2007). Furthermore, silage particle size affects chewing activity, intake and performance of cattle (Nørgaard et al. 2011, Rustas and Nadeau 2011). However, only limited information is available on the effectiveness of biological and chemical additives in silages differing in particle size. The aim of this experiment was to study the effects of silage additive and particle size and their interaction on fermentation characteristics and aerobic stability of grass silage.

Material and methods A grass sward was mowed on 6 June, 2010 and wilted to a dry matter (DM) content of 330 g/kg. Wilted forage was run through a baler and half of the long forage of 250 mm length was chopped to 20 mm. Both long and chopped forages were treated with additives. Bacterial inoculants used were KOFASIL COMBI (*Lactobacillus plantarum* DSM 3676, 3677 at 100,000 cfu/g forage, 240 g/t sodium benzoate and 30 g/t potassium sorbate) and KOFASIL LIFE (*Lactobacillus plantarum* DSM 3676, 3677) at 400 000 cfu/g forage (ADDCON EUROPE GmbH). Acid-based additives were GrasAAT SP (35% formic acid and 12% propionic acids; 25.5% sodium formate, 1.5% sodium benzoate) at 3 L/ton forage (ADDCON NORDIC AS, Norway), PROMYR NT 570 (50.0% formic acid, 17.1% propionate, 5.6% sodium) at 3 L/ton forage (Perstorp Inc., Sweden). Salt-based additives were KOFASIL LP (20.2% sodium nitrite, 13.5% hexamethylene tetramine, 5.0% sodium benzoate) at 2 L/ton forage, KOFASIL ULTRA K (16.5% sodium nitrite, 11.0% hexamethylene tetramine, 8.1% potassium sorbate, 2.2% sodium benzoate, 0.8% sodium propionate) at 2 L/ton forage (ADDCON EUROPE GmbH) and SAFESIL (18.0% sodium benzoate, 7.4% potassium sorbate, 5% sodium nitrite) at 3 L/ton forage (Hanson & Möhring, Sweden). Treated forages were compared to untreated forages after ensiling in 1.7-L silos for 90 days (d). Fermentation products were analysed and DM losses were calculated according to Weissbach (2005). Contents of ammonia-N in silages treated with KOFASIL LP, KOFASIL ULTRA K and SAFESIL were corrected for the ammonia-N produced from the additives. Aerobic stability of the silages was measured as the number of d reaching a temperature of 2°C above ambient temperature during a 10-d period (Honig, 1990). Acidification rate was measured as silage pH after 3 d of fermentation in 0.5-L silos. Data were analysed as a completely randomized design in PROC GLM of SAS 9.2, with additive treatment and particle length as fixed factors, using three replicates per treatment. When the overall *P* – value was significant at 5% level, pair wise comparisons between LSMEANS of treatments were done using Tukey´s test.

Results and discussion Concentrations of neutral detergent fibre, water soluble carbohydrates (WSC) and crude protein of wilted forage were 487, 180 and 142 g/kg DM, respectively. *In vitro* organic matter digestibility was 917 g/kg (Lindgren, 1979). The acidification rate was slower in long than in chopped silage (pH 4.78 vs. 4.55, $P < 0.0001$) but final pH after 90 d of ensiling was low for all treatments (Table 1). Lactic acid concentration was high in relation to acetic acid in all silages with KOFASIL LIFE resulting in the highest lactic acid concentration (106 g/kg DM) of all treatments, when averaged over particle size ($P < 0.0001$). GrasAAT SP and PROMYR NT 570 had lower acetic acid concentrations than the control in both chopped and long silage (Table 1). KOFASIL LP, KOFASIL ULTRA K and SAFESIL had lower ethanol concentrations than the control in both chopped and long silage, whereas GrasAAT SP and PROMYR NT 570 only decreased the ethanol concentration of chopped silage (Table 1). Also, KOFASIL COMBI decreased the ethanol concentration of long silage. Silages treated with GrasAAT SP and PROMYR NT 570 had more residual WSC in chopped than in long silage (Table 1). Averaged over particle size, KOFASIL LP and KOFASIL ULTRA K were most effective in decreasing the proteolysis of untreated silage (48 vs. 73 g NH_3-N/kg total N, $P < 0.0001$). Analysed counts of lactate assimilating yeasts (LAY), total yeasts and clostridia were low, showing a maximum of log 3.7 cfu/g for LAY, log 4.8 cfu/g for total yeasts and log 1.2 cfu/g for clostridia. Butyric acid was not detected in the silages. Silages treated with KOFASIL LP, KOFASIL ULTRA K and SAFESIL had lower DM losses than the control silage, when averaged over particle size (48 vs. 75 g/kg, $P < 0.0001$). Aerobic stability was improved in the silages treated with KOFASIL ULTRA K and SAFESIL compared to the control, when averaged over particle size (9.2 d vs. 5.5 d, $P < 0.0001$).

Conclusions Additives can further improve fermentation characteristics, thereby decreasing DM losses, and improve aerobic stability of well fermented untreated grass silage with the greatest effects by the chemical additives, especially the salt-based additives. Acidification rate was increased by chopping and utilisation of sugars was more efficient in acid-treated chopped silage than in acid-treated long silage.

Acknowledgements This experiment was funded by Agroväst and SLU. Dr. Kirsten Weiss, Humboldt University, Berlin, Germany, is acknowledged for conducting the analyses of fermentation products in the silages.

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Table 1. Water soluble carbohydrates (WSC), pH, lactic acid, acetic acid and ethanol, in g/kg DM, and ammonia-N (NH_3-N, in g/kg total N), in chopped and long grass silages treated with or without additive.

	WSC	pH	Lactic acid	Acetic acid	Ethanol	NH_3-N
Chopped silage						
CONTROL	50^{e}	4.10^{d,e}	91	15^{c,d,e}	30^{a}	70
KOFASIL COMBI	67^{d,e}	4.05^{e,f}	92	15^{a,b,c,d,e}	22^{a,b,c}	68
KOFASIL LIFE	63^{e}	4.02^{f}	106	18^{a,b,c}	27^{a,b}	58
GrasAAT SP	171^{a}	4.12^{c,d}	89	7^{i}	12^{c,d,e}	66
PROMYR NT 570	152^{a,b}	4.11^{c,d,e}	83	8^{h,i}	11^{c,d,e}	66
KOFASIL LP	133^{a,b,c}	4.24^{a}	77	19^{a}	11^{c,d,e}	44
KOFASIL ULTRA K	123^{a,b,c,d}	4.19^{a,b,c}	77	12^{e,f,g}	4^{e}	49
SAFESIL	122^{a,b,c,d}	4.12^{c,d}	86	17^{a,b,c}	4^{e}	63
Long silage						
CONTROL	46^{e}	4.10^{d}	96	14^{c,d,e}	29^{a}	76
KOFASIL COMBI	54^{e}	4.10^{d}	96	13^{d,e,f}	15^{b,c,d,e}	68
KOFASIL LIFE	47^{e}	4.03^{f}	105	12^{e,f,g,h}	20^{a,b,c,d}	55
GrasAAT SP	98^{b,c,d,e}	4.12^{c,d}	84	9^{f,g,h,i}	19^{a,b,c,d}	60
PROMYR NT 570	76^{c,d,e}	4.13^{c,d}	79	9^{g,h,i}	29^{a}	62
KOFASIL LP	96^{b,c,d,e}	4.20^{a,b}	80	13^{d,e,f}	8^{d,e}	49
KOFASIL ULTRA K	96^{b,c,d,e}	4.16^{b,c}	78	13^{d,e}	4^{e}	51
SAFESIL	99^{b,c,d,e}	4.12^{c,d}	82	16^{a,b,c,d}	7^{d,e}	64
SEM	11.1	0.1	2.1	0.7	2.5	2.3
P - value	0.017	0.008	0.104	< 0.0001	0.0002	0.105

^{a,b,c,d,e,f,g,h,i}LSMEANS with different superscripts within a column differ significantly at $P < 0.05$.

The effects of wilting and additives on the number of lactic acid bacteria in alfalfa forage and silage

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Keywords: alfalfa, lactic acid bacteria, fermentation quality, inoculant, chemical additive

Introduction Alfalfa (*Medicago sativa*) presents a substantial part of livestock diets because of its high protein content. However ensiling of alfalfa is difficult due to its high buffering capacity and low content of water-soluble carbohydrates (WSC). The addition of silage additives thus would exerts a positive effect on the fermentation process.

Lactic acid bacteria (LAB) are distributed at different quantities throughout the environment. They ferment soluble sugars and produce lactic acid from water-soluble carbohydrates as the main fermentation end-product. Lactic acid is the primary acid responsible for a decrease in the pH of silage. Although it is well recognized that epiphytic LAB have an important role in silage fermentation, their numbers in the standing crop are limited and variable (Muck 1990; Lin et al. 1992). The objectives of the study were to evaluate the effects of wilting and additives on the number LAB in alfalfa forage and silage.

Material and methods Alfalfa was harvested at the small-bud growth stage and wilted in the swath to a dry matter (DM) content of approximately 38 % before ensiled. Forage was chopped with a conventional forage chopper to a length of 30 mm and ensiled 1) without any additive (C), 2) with a commercial biological inoculant (I) and 3) with a chemical additive (Ch). The commercial bacterial inoculant, added at 1 g/t of forage, contained the homofermentative LAB *Lactobacillus paracasei*, *Lactobacillus plantarum* and *Pediococcus pentosaceus* at concentrations of 3 x 10^5 cfu/g of forage. The chemical additive, containing formic acid (55 %), propionic acid (5 %), ammonium formate (24 %) and benzoic acid (2.2 %) was applied at 4 l/t of forage. Treated forage (700 g) was vacuum sealed in polyethylene bags and stored at 18 to 20 °C.

The number of LAB was counted on Petri dishes after addition of Rogosa agar (Oxoid) and incubated in a thermostat at 30 °C for 72 h. Numbers of LAB were counted in fresh and wilted alfalfa, and silages at 2, 10 and 90 days of the fermentative process. Differences between fresh and wilted alfalfa were evaluated by unpaired t-tests. For silages ANOVA was applied followed by Tukey tests to identify differences between means.

Results and discussion The number of LAB was higher in wilted than fresh alfalfa. Whereas fresh alfalfa presented LAB numbers less than the detectable (< 10), 78 cfu/g of LAB was found for wilted alfalfa. However large variability in LAB numbers for wilted alfalfa caused that differences were not statistically significant.

Table 1. Silage fermentation characteristics.

	Control (C)	SD	Biological additive (I)	SD	Chemical additive (Ch)	SD
			2-day silage			
pH	5.11[a]	0.04	4.75[a]	0.02	4.51[b]	0.14
Lactic acid (%)	2.17	0.22	3.15	0.10	3.13	0.46
Acetic acid (%)	0.40	0.05	0.43	0.03	0.37	0.01
Propionic acid (%)	0	0	0	0	0.23	0.01
			10-day silage			
pH	4.90[a]	0.09	4.73[b]	0.03	4.28[c]	0.03
Lactic acid (%)	3.22	0.15	3.62	0.06	3.40	0.34
Acetic acid (%)	1.10	0.04	0.74	0.02	0.41	0.06
Propionic acid (%)	0	0	0	0	0.25	0.01
			90-day silage			
pH	4.85[a]	0.03	4.68[b]	0.05	4.33[c]	0.03
Lactic acid (%)	3.23	0.04	3.33	0.41	3.20	0.11
Acetic acid (%)	0.58	0.17	0.51	0.09	0.26	0.03
Propionic acid (%)	0	0	0	0	0.16	0.01

[a,b,c]Values in the same row with the different letters are significantly different ($P < 0.01$)

Chemical additives decreased the pH of silage (Table 1). Two days after the start of ensiling the pH in treatment Ch was the lowest ($P < 0.01$). At 10 days of ensiling biological additives have started to decrease ($P < 0.01$) silage pH compared to the control. However treatment with chemical additives resulted in lower ($P < 0.01$) silage pH than biological treatment at both 10 and 90 days of ensiling.

Table 2. Number of LAB in alfalfa silage.

	LAB at silage (cfu/g)					
	Control (C)	SD	Biological additive (I)	SD	Chemical additive (Ch)	SD
2-days silage	$7.92 \times 10^{5\,b}$	5.97×10^{4}	$6.2 \times 10^{9\,a}$	4.91×10^{8}	$1.80 \times 10^{5\,b}$	8.87×10^{3}
10-days silage	$1.65 \times 10^{8\,c}$	7.08×10^{7}	$1.15 \times 10^{12\,a}$	1.52×10^{11}	$7.30 \times 10^{11\,b}$	1.51×10^{11}
90-days silage	$7.98 \times 10^{7\,a}$	1.28×10^{7}	$1.39 \times 10^{6\,b}$	1.46×10^{5}	$7.60 \times 10^{7\,a}$	5.46×10^{6}

[a,b]Values in the same row with the different letters are significantly different ($P < 0.01$)

After 2 days of ensiling the number of LAB was higher ($P < 0.01$) in the I treatment than treatments C or Ch (Table 2). Addition of chemical additives to silage (Ch) resulted in the lowest numerical number of LAB at 2 days. At 10 days of ensiling biological additives still caused the highest ($P < 0.01$) number of LAB, although chemical additives at this stage also increased ($P < 0.01$) the number of LAB when compared to the control. However, after 90 days of ensiling biological treatment resulted in the lowest ($P < 0.01$) number of LAB when compared to both the chemical and control treatments.

Conclusions Wilting improved the number of LAB in alfalfa. The addition of biological additives to alfalfa silage can be recommended for the beginning phase of ensiling due to an increase in LAB numbers compared to a control treatment without any additives. However, at 90 days of ensiling it caused a decreased in LAB numbers. Chemical additives would resulted in a decrease in silage pH compared to a control treatment.

Acknowledgments Supported by the Ministry of Agriculture of the Czech Republic (Project No. MZE 0002701404).

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The effects of wilting and additive treatments on the quality of *Bothriochloa ischaemum* silage

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Keywords: additives, *Bothriochloa ischaemum*, formic acid, moisture content, silage, wilting

Introduction Bothriochloa ischaemum is a species of perennial grass in the Poaceae family, found throughout much of the world and grows in conditions of warm and drought area. It can be used as a forage for ruminant animals, and aids the conservation of water, and plays important roles in maintaining an ecological balance of grassland. The utilization by sheep or cattle of *Bothriochloa ischaemum* as grazed grass has been studied earlier (Xu et al. 2004) while the effects of moisture content and additives of formic acid or sucrose on *Bothriochloa ischaemum* silage have been reported rarely.

Material and methods The *Bothriochloa ischaemum* was harvested by hand during heading stage. The grass was separated into two piles, one for fresh material, and the other was wilted about three hours. The material was cut into about 1 cm long particles and then mixed with formic acid or sucrose. The amount of formic acid was 6 ml per kg of fresh material, while 20 g sucrose per kg of fresh material was used. A control group without any additives was also prepared. The materials were sampled for analysis. After mixing, the material was placed in plastic bags (three replicates per treatment were prepared), vacuumed and sealed. The silages were sampled post 360 d.

The content of DM (dry matter), CP (crude protein), NDF (neutral detergent fiber), ADF (acid detergent fiber), ash, EE (ether extract), WSC (water soluble carbohydrate), nitrate, nitrite, and buffering capacity of *Bothriochloa ischaemum* material were analysed according to the methods of Yang and Owens (1993). For the silages, the contents of DM, CP, NDF, ADF, ash, EE, nitrate, and nitrite were determined, the pH, and lactic acid, acetic acid, propionic acid, butyric acid, and ammonia nitrogen concentrations were analysed according to Xu's (2007) methods. The results were calculated using SAS GLM.

Results and discussion The DM content of *Bothriochloa ischaemum* material increased in response to wilting while the contents of CP, NDF, ADF, ash, EE, WSC, nitrate, nitrite, and buffering capacity were not affected significantly (Table 1). Increased silage DM concentration restricted silage fermentation (lower lactic and acetic acid concentrations) and improved silage fermentation quality (lower ammonia nitrogen concentration) when wilted silages were compared with directly ensiled silages (P<0.05; Table 2).

Compared with the control group, the pH and the contents of acetic acid and ammonia nitrogen decreased significantly (P<0.01) in the silage with formic acid, while the content of lactic acid increased significantly (P<0.01). Moreover, the pH and the content of ammonia nitrogen decreased significantly (P<0.01) of that with sucrose, while the content of lactic acid as that of formic acid (Table 2). The content of CP, NDF, ADF, ash, EE, WSC, nitrate, and nitrite were not affected significantly with the additives or wilted (table 3). The nitrate content of *Bothriochloa ischaemum* silage decreased about 50 percent after ensilaged.

Conclusions The low WSC content (about 5 percent) of *Bothriochloa ischaemum* material during heading stage indicated that the fermentation quality of *Bothriochloa ischaemum* silage without additives would be poor. The formic acid and sucrose additives improved the fermentation quality of *Bothriochloa ischaemum* silage. The nitrate content of *Bothriochloa ischaemum* decreased during ensilage. Wilting restricted silage fermentation and improved silage fermentation quality.

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Table 1. Characteristics of *Bothriochloa ischaemum* material.

Item	Treatment	
	Fresh material	Wilted material
Dry matter (%)	38.7±1.28	50.1±0.39
Crude protein (% DM)	9.37±1.21	9.95±0.87
Neutral detergent fiber (% DM)	59.4±1.33	60.2±0.47
Acid detergent fiber (% DM)	40.5±0.78	39.2±1.42
Ash (% DM)	7.26±0.27	7.50±0.18
Ether extract (% DM)	3.21±0.25	3.22±0.03
Water soluble carbohydrate (% DM)	4.27±0.81	4.79±0.28
Buffering capacity (mE·kg^{-1}·DM)	214±11.3	233±21.0
Nitrate (mg·kg^{-1}·DM)	882±29.2	944±41.3
Nitrite (mg·kg^{-1}·DM)	2.18±0.16	2.16±0.10

Table 2. Fermentation characteristics of *Bothriochloa ischaemum* silage with additives of formic acid or sucrose.

Item	Fresh material			Wilted material			Significance of effects	
	Control	Sucrose	Formic acid	Control	Sucrose	Formic acid	DM	Additives
pH	5.15±0.03	4.46±0.07	4.09±0.23	5.14±0.01	4.70±0.30	4.30±0.03	NS	**
Lactic acid (% DM)	0.52±0.20	1.38±0.08	1.58±0.07	0.33±0.05	0.91±0.02	0.66±0.05	**	**
Acetic acid (% DM)	0.70±0.01	0.74±0.14	0.21±0.12	0.40±0.02	0.39±0.06	0.10±0.01	*	**
Propionic acid (% DM)	0.11±0.03	0.21±0.09						
Butyric acid (% DM)								
Ammonia nitrogen (% Total nitrogen)	1.30±0.06	0.77±0.20	0.47±0.16	1.01±0.45	0.45±0.01	0.43±0.12	**	**

Note: NS, no significance; ** means $P<0.01$; * means $P<0.05$, the same as below.

Table 3. Chemical composition of *Bothriochloa ischaemum* silage with additives of formic acid or sucrose.

Item	Fresh material			Wilted material			Significance of effects	
	Control	Sucrose	Formic acid	Control	Sucrose	Formic acid	DM	Additives
Dry matter (%)	29.6±0.76	30.8±0.88	28.6±0.87	50.1±1.52	50.0±1.45	52.3±1.98	**	NS
Crude protein (% DM)	9.49±0.58	9.57±0.26	9.34±0.12	9.94±0.24	9.91±0.45	10.0±0.12	NS	NS
Neutral detergent fiber (% DM)	60.8±0.80	60.4±1.63	59.2±2.00	60.4±1.47	59.3±0.21	60.5±0.93	NS	NS
Acid detergent fiber (% DM)	40.3±1.54	38.6±1.73	40.5±0.90	40.5±0.83	41.7±1.54	40.8±1.01	NS	NS
Ash (% DM)	7.82±0.74	7.79±0.95	7.50±0.69	7.51±0.67	8.40±0.86	7.90±1.30	NS	NS
Ether extract (% DM)	3.18±0.25	3.18±0.08	3.27±0.09	3.31±0.21	3.30±0.13	3.50±0.69	NS	NS
Nitrate (mg·kg^{-1}•DM)	468±54.5	436±15.9	442±2.88	480±55.1	458±17.9	464±2.94	NS	NS
Nitrite (mg·kg^{-1}•DM)	3.32±0.22	3.12±0.72	3.18±0.31	3.19±0.41	3.38±0.45	3.25±.0.48	NS	NS

Efficacy of three different silage inoculants on the fermentation quality and aerobic stability of ryegrass ensiled with three different prewilting degrees

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Keywords: inoculants, silage quality, aerobic stability, pre-wilting degree

Introduction Inoculants containing lactic acid bacteria (LAB) are the most common additives used in silage making. The homofermentative strains promote an intensive lactic acid production and a rapid decrease in pH. The inoculants with heterofermentative LAB especially improve the aerobic stability of silages. In heavily wilted forage, the water availability becomes a limiting factor for the development of LAB (Pahlow and Weissbach1996). Under these conditions osmotolerant LAB are more active.

The objective of the study was to investigate the efficacy of three different silage inoculants on fermentation quality and on aerobic stability of ryegrass silage with three different prewilting degrees.

Material and methods Italien ryegrass of the first cut was wilted to three different dry matter levels, chopped and ensiled in 1.5 l laboratory silos. The DM contents amounted 34, 46 and 61%. Fermentation coefficients (FC) were calculated with DM, water soluble carbohydrates (WSC) and buffering capacity in the fresh forage. Besides a control treatment without additive, three treatments were inoculated with LAB (Table 1). One inoculant contained homo and heterofermentative LAB. The two others contained only homofermentative LAB. After a storage period of 3, 49 and 91 days three silos per treatment were opened. The silos that were opened at day 49 were exposed two times to an air stress and aerobic stability was measured. DM losses, nutrient contents, fermentation parameters and aerobic stability were analyzed after a storage period of 91 days. DLG points were calculated on the basis of the results for butyric and acetic acids as well as for pH values (DLG 2006). Concerning the aerobic stability test, silages were instable, when the temperature was 3 degrees above ambient. The trial was carried out according to the methods of DLG (Staudacher et al. 1999). The data were analysed with SYSTAT 12 using two-way ANOVA.

Table 1. Treatments.

Treatment	LAB-strains	CFU/g fresh forage
Control	-	-
Inoculant 1	*L. plantarum, L. rhamnosus, P. pentosaceus, L. buchneri* and *L. brevis*	100‘000
Inoculant 2	*L. plantarum, E. faecium, P. acidilactici* and *L. lactis*	495’000
Inoculant 3	*E. faecium, L. plantarum, P. Acidilactici* and *L. salivarius*	1’000’000

CFU: colony forming unit

Results and discussion The DM and nutrient contents at ensiling time are presented in Table 2. The forage was characterized by high contents of water soluble carbohydrates and high values of fermentation coefficients. These parameters indicate, that the forage was easy to ensile. The DM losses decreased in the treatments without additives with increasing DM content (Table 3). The three inoculants reduced the DM losses in the treatments with 34 and 46% DM. In the treatment with 61% DM only the inoculant 1 with homo and heterofermentative LAB reduced strongly the DM losses.

Table 2. Dry matter (DM), nutrient contents (g/kg DM) and fermentation coefficient (FC) of ryegrass ensiled at three different DM levels.

DM level	DM g/kg	Ash	Crude protein	Crude fiber	ADF	NDF	WSC	FC
1	344	71	60	260	289	478	345	97
2	460	71	62	259	282	471	344	110
3	611	70	59	258	280	469	370	134

Concerning the fermentation quality, most silages showed high DLG points and therefore a very good quality except for the treatment without additive and 34% DM. Here, butyric acid was produced. When the inoculants were applied, the pH decreased already after 3 days in the forages with 34 and 46% DM, but not in the forage with 61% DM. The addition of the inoculants increased the lactic acid production in all three wilting degrees. Acetic acid was mainly produced in the treatment with the inoculant 1, which contained homo and heterofermentative LAB. This additive limited also the ethanol production in all si-

lages. For the other treatments, the highest ethanol production was found in the silages with the highest DM content.

After 91 days and without air stress the silages were more stable in comparison to the one after 49 days and with air stress (Figure 1 and 2). The bad wet silage without additive showed a very good aerobic stability. But here the butyric acid was responsible for the good stability. The silages treated with the inoculant 1 showed a very good aerobic stability. This can be explained by the higher acetic acid contents. The aerobic stability of the silages treated with the two homofermentative inoculants increased with increasing prewilting degree in the silages after 49 days with air stress and also 91 days without air stress.

Table 3. Fermentation quality of the different silages and a storage period of 91 days.

Treatment	DM level	DM	pH Day 3	pH Day 91	Lactic acid	Acetic acid	Butyric acid	Ethanol	NH_3-N N total	DLG points	DM losses
		g/kg			g/kg DM				%		%
Control	1	307	6.0	4.9	35	2	17	24	14	38	11.7
Inoculant 1	1	333	4.3	3.9	118	15	0	3	7	100	4.5
Inoculant 2	1	330	4.3	4.0	114	5	0	9	5	100	4.4
Inoculant 3	1	331	4.4	4.0	111	6	0	6	6	100	4.4
Control	2	431	6.1	5.8	21	3	1	55	9	90	10.5
Inoculant 1	2	452	4.9	4.1	100	16	0	2	6	100	4.6
Inoculant 2	2	442	5.0	4.2	92	4	0	3	5	100	3.8
Inoculant 3	2	452	5.3	4.2	92	4	0	2	5	100	3.9
Control	3	593	6.1	6.0	4	1	0	46	3	90	8.1
Inoculant 1	3	594	6.1	4.4	47	24	0	3	4	100	5.0
Inoculant 2	3	596	6.1	5.3	36	2	0	38	6	90	7.6
Inoculant 3	3	586	6.1	4.9	46	2	0	30	5	90	6.7
SD		3	0.4	0.6	2	1	1	3	1	1	1
Treatment (T)		***	***	***	***	***	***	***	***	***	***
DM level (D)		***	***	***	***	*	***	***	***	***	***
Interaction TxD		***	***	***	***	***	***	***	***	***	***

SD: standard deviation; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

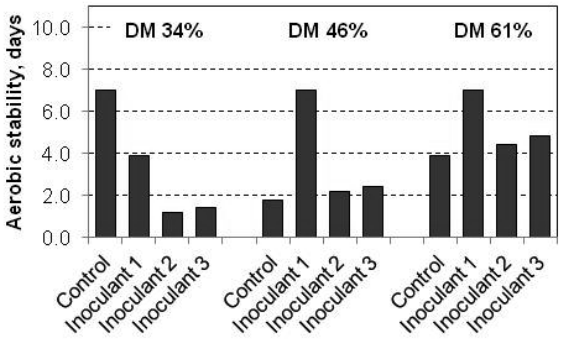

Figure 1. Aerobic stability of the silages with a storage period of 49 day and air stress.

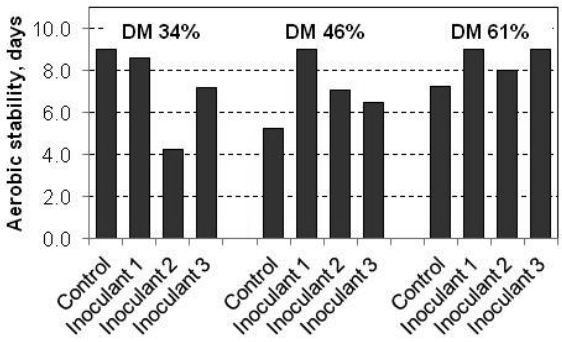

Figure 2. Aerobic stability of the silages with a storage period of 91 days without air stress.

Conclusions The addition of the three different inoculants reduced the pH of the silages and improved the silage quality mainly in the forage with 34 and 46% DM. In addition, the inoculant with homo and heterofermentative LAB improved the aerobic stability of all silages. On the other hand, the variants treated only with homofermentative LAB and with 34% DM heated up earlier in comparison to the control.

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Effect of different chemical additives on silage quality and aerobic stability

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Keywords: additive, aerobic stability, fermentation, formic acid, hydrogen peroxide-sodium benzoate

Introduction Silage additives are used to improve the fermentation quality and aerobic stability of silage to ensure good milk quality and economic production. The aim of this experiment was to study the effect of an additive containing hydrogen peroxide-sodium benzoate on the fermentation quality and aerobic stability of slightly and heavily wilted grass. Hydrogen peroxide is a disinfectant having a high oxidation potential decomposing to water and oxygen. Sodium benzoate is used commonly in food preservation and also in silage additives. Sodium benzoate possesses antimicrobial properties and is more effective at lower pH values (Woolford 1975, Krebs et al. 1983, Lambert and Stratford 1999). Both additives were initially notified as existing silage additives under EU Regulation on feed additives (EC/1831/2003) but only sodium benzoate has been applied for the reauthorisation (EU Register of Feed Additives).

Material and methods First cut timothy-meadow fescue grass (*Phleum pratense* L.-*Festuca pratensis* Huds.) was cut with a mower conditioner in windrows, wilted for 19 and 45 h and harvested with a precision-chop forage harvester. Grass was ensiled in triplicate cylindrical (12 l) pilot scale silos with three additive treatments: no additive, stabilised aqueous solution of hydrogen peroxide-sodium benzoate, 5 l/t (Solvay H_2O_2 Forage: 195 g/kg hydrogen peroxide, 150 g/l sodium benzoate, 5 g/l stabiliser) and formic acid (850 g/kg) 5 l/t. At ensiling slightly and heavily wilted grass, respectively, contained dry matter (DM) 296 and 448 g/kg, water soluble carbohydrates (WSC) 121 and 105 g/kg DM, crude protein 148 and 165 g/kg DM, neutral detergent fibre 557 and 561 g/kg DM, soluble nitrogen 445 and 453 g/kg N. *In vitro* organic matter digestibilities were 0.68 and 0.69. After 110-138 days the silos were opened one replicate at a time and sampled for fermentation quality and aerobic stability measurements in duplicates for 10 days as described by Saarisalo et al. 2006.

Results and discussion Fermentation and organoleptic quality of all the silages were good. Higher DM concentration and formic acid restricted fermentation of the silages. No differences (P>0.05) were found between silages treated with no additive and hydrogen peroxide-sodium benzoate (H_2O_2-NaB) in silage pH, lactic, acetic, propionic and total fermentation acids and proportion of ammonium and soluble N of total N. WSC content was even lower in H_2O_2-NaB than in no additive silages. Formic acid silages had (P<0.001) higher WSC and lower ammonium N of total N than others (Table 1).

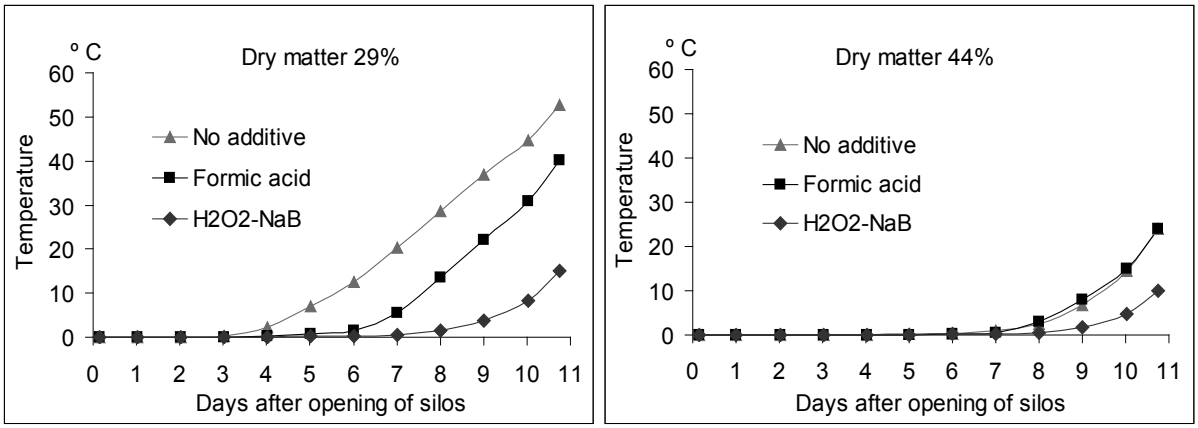
Table 1. Fermentation quality of silages ensiled with no additive, hydrogen peroxide-sodium benzoate (H_2O_2-NaB) and formic acid (FA) additives.

Additive	pH	WSC	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Etha-nol	NH_4-N	Soluble N
		g / kg DM						g / kg N	
				DM 29%					
No additive	4.27	46.9	70.2	17.3	0.14	0.16	9.4	61.3	701
H_2O_2-NaB	4.22	35.5	69.1	19.1	0.22	0.12	11.6	60.7	672
Formic acid	4.14	86.5	33.2	9.7	0.13	0.12	8.9	26.1	622
				DM 44%					
No additive	4.72	60.5	46.0	11.7	0.10	0.19	6.5	48.8	673
H_2O_2-NaB	4.73	58.1	43.8	10.9	0.09	0.14	7.2	48.5	679
Formic acid	4.63	119.8	4.8	5.4	0.13	0.15	3.1	22.0	573
SEM	0.02	2.10	1.11	0.28	0.03	0.02	0.40	0.78	6.8
Statistical significance									
Dry matter (DM)	***	***	***	***	*		***	***	**
Additive	***	***	***	***		*	***	***	***
DM *Additive interact		**		***	o		*	***	**
H_2O_2-NaB / No additive		**		o		*	**		
H_2O_2-NaB / FA	***	***	***	***			***	***	***
FA / No additive	***	***	***	***		*	***	***	***

WSC = water soluble carbohydrates, SEM = standard error of the mean

However, hydrogen peroxide-sodium benzoate additive significantly improved the aerobic stability of the silages compared with no additive (P<0.001) or formic acid (P<0.01) especially in lower DM silage (DM*additive interaction, P<0.05). The lower dry matter silages had lower pH than the higher dry matter silages. The lower DM silages warmed up faster and more than the higher DM silages (Figures 1-2). Within the lower DM silages, the no additive silage started to warm up fast from the third day and the hydrogen peroxide-sodium benzoate silage from the sixth day slower, the formic acid silage being in between them. No differences were found in warming of the no and formic acid additive treated higher DM silages which started warming from the sixth day and the hydrogen peroxide-sodium benzoate silage a day later.

The antimicrobial potential of sodium benzoate as weak-acid preservative depends on the proportion of undissociated acids which increases as the pH declines (Krebs et al. 1983, Lambert and Stratford 1999). Sodium benzoate has been found to improve aerobic stability of silage e.g. when combined with other chemical additives (Lingvall and Lättemäe, 1999) or with inoculants (Saarisalo et al. 2006).



Figures 1-2. Aerobic stability of the silages presented as cumulative temperature difference (sample temperature minus ambient temperature, + 20 ± 1 °C).

ConclusionsThe silages were of good fermentation quality with all the studied additive treatments. The hydrogen peroxide-sodium benzoate additive did not improve the silage fermentation quality compared with the no additive treated silages. The formic acid treatment resulted in typical restrictively fermented and better quality silage with higher WSC content and less protein degradation. However, the hydrogen peroxide-sodium benzoate additive significantly improved the aerobic stability of the silages compared with no additive and formic acid which was probably due to the sodium benzoate.

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Fermentation characteristics and aerobic stability of guinea-grass fermented with a microbial additive containing lactic acid-producing bacterial strains

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Keywords: aerobic stability, fermentation, guinea-grass, lactic acid-producing bacteria, microbial additive

Introduction Ensiling of tropical grasses generally results in silage with high acetic acid content, poor palatability and low animal consumption. Tropical grass silage is also often associated with an elevated rate of protein degradation and ammonia formation and high dry matter losses during fermentation (Rodriguez 1996; Panditharatne et al. 1986). The objective of this study was to determine the ensiling characteristics and aerobic stability of guinea-grass (*Panicum maximum cv.* Tanzania) fermented with a microbial additive containing the lactic acid-producing bacterial strains *Lactobacillus plantarum* MiLAB 393; *Pediococcus pentosaceuos*, *Lactococus lactis*, and *Enterococcus faecium* (LAPBI; Feedtech® CustomChop F-20).

Material and methods Guinea-grass (GG; 259 g/kg dry matter; DM) was chopped at 2.5 cm and assigned to one of two treatments; Control (No additive) and LAPBI. Additives were added to weighed portions of GG and packed into PVC micro-silos (1.8 kg) to ferment for 45d at 25-27°C. The LAPBI was applied at a rate of $2x10^5$ cfu/g of fresh forage. Five silos from each treatment were analyzed for pH, chemical composition, fermentation products (organic acids and NH_3), and DM losses (%). Statistical analysis was performed as a completely randomized design. For aerobic stability determination, temperature was monitored every 6 hours in five samples from each treatment (1000 g) during 168 h. A rise in temperature of 3°C or more above background was taken as indicative of aerobic instability. Responses were measured using data loggers that recorded temperature readings once per six hours from thermocouple wires placed in samples aerated in open polystyrene boxes kept at room temperature (25-27°C). Statistical analysis was performed as a split plot design with a two treatments (additives) by 29 (0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144, 150, 156, 162, and 168 hours of aerobic exposure) factorial arrangement, using the silo as repetitive measurement.

Results and discussion Vegetative material utilized in this experiment had a nutrient content typical of tropical grasses. Prior to ensiling the GG showed initial values of 872.5 g/kg organic matter, 127.4 g/kg inorganic matter, 547 g/kg crude protein, 760 g/kg neutral detergent fiber, 496 g/kg acid detergent fiber, and 11.7 g/kg water soluble carbohydrates. After 45 d of fermentation, the microbial additive evaluated in this experiment did not influence the chemical composition of the resulting GG silage (Table 1). Acidity measured by pH was also similar between experimental treatments. The effect of the LAPBI on the microbial groups studied included had lower ($P<0.05$) yeast and mold populations in inoculated GG than untreated silage, but lactic acid-producing bacteria and clostridia populations were similar. Acetic acid was the major fermentation product associated with ensiled GG, however, the content of this organic acid was lower ($P<0.05$) in vegetative material treated with the LAPBI than in the control silage. The GG fermented with LAPBI was numerically higher in lactic acid content and tended ($P = 0.11$) to increase the lactic acid:acetic acid ratio; it also had a lower ($P<0.05$) propionic acid content than untreated silage, but similar butyric acid concentration. Addition of the microbial additive also decreased ($P<0.05$) the ratio NH_3-N/Total- N content of the silage and the dry matter losses associated with the fermentation process.

Related studies have shown that silages with higher acetic acid content are more stable to aerobic conditions than those with lower acetate concentration (Hu et al. 2009). In this experiment, acetic acid content in GG ensiled with the microbial additive was lower than in untreated vegetative material, however, both silages were stable to aerobic conditions as evidenced by similar temperatures over the entire seven days of aerobic exposure (28.02 and 28.15 °C for untreated and treated silages, respectively.)

Conclusions Results from this experiment show that the addition of the LAPBI improved the fermentation characteristics of GG silage as evidenced by lower acetic acid content, lower NH_3-N/Total–N ratio, lower yeast and molds populations, and higher lactic:acetic ratio and dry matter recovery. Likewise, GG fermented with the microbial additive resulted in silage more stable to aerobic conditions.

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Table 1. Characteristics of a guinea-grass (*Panicum maximum*) treated with a microbial inoculant containing lactic acid-producing bacterial strains (LAPBI) after 45 days of ensiling.

Item	No Additive	LAPBI[1]	SD
Chemical composition (g/kg)			
Dry matter	262.9	264.9	7.2
Organic matter[2]	862.7	860.5	14.3
Inorganic matter[2]	137.2	139.4	14.3
Crude protein[2]	71.0	71.0	4.0
Crude fat[2]	17.0	15.4	2.4
Crude fiber[2]	338.2	333.0	18.6
NFE[2,3]	436.4	441.0	2.54
NDF[2]	695.6	716.5	20.8
ADF[2]	506.3	506.3	12.2
Hemicelulose[2,4]	184.3	225.6	22.0
WSC[5]	6.2	6.9	3.9
Microbial Populations (log cfu/g)			
Lactic acid-producing bacteria	5.70	5.66	0.25
Yeast and molds	4.85[a]	3.67[b]	0.79
Clostridia	3.02	2.84	0.81
pH	4.73	4.76	0.07
Fermentation Products (g/kg)			
Lactic acid[1]	1.5	2.0	1.1
Acetic acid[1]	19.9[a]	9.0[b]	6.1
Lactic acid: Acetic acid Ratio	0.7[y]	2.2[z]	1.8
Propionic acid[1]	10.2[a]	1.1[b]	4.8
Butyric acid[1]	16.9	17.2	2.4
NH_3 - N/Total - N	119.3[a]	61.7[b]	31.9
Dry matter losses (%)	17.1[a]	8.8[b]	5.3

[1] DeLaval Manufacturing, [2] Dry Matter Basis, [3] NFE calculated as organic matter – crude protein – crude fiber – crude fat, [4] Hemicelulose = NDF – ADF, [5] Water Soluble Carbohydrates,
[a,b,] Means with unlike superscripts in the same row differ (P<0.05)
[y,z] Means with unlike superscripts in the same row differ (P<0.10)

Fermentation characteristics of purple guinea grass silage treaded with or without lactic acid bacteria inoculant

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Keywords: guinea grass, lactic acid, silage

Introduction Purple guinea grass (PGG, *Pannicum maximum* TD 58) is high dry matter yield, drought tolerant, growing in wide range of soils, and widely used as ruminant feed in the tropical area including Thailand. Usually, the PGG contains high moisture and a low water-soluble carbohydrates content and its silage may be difficulty to prepare as a good quality (WTSR, 2010). However, there is limited research information on the PGG silage preparation and fermentation quality in the tropical condition. In the present study, PGG were prepared with or without commercial lactic acid bacteria (LAB) inoculants and their fermentation qualities were studied.

Material and methods PGG at cutting age with 60-day growth was harvested in the rainy season (September, 2011) in an experiment field of Khon Kaen University, Khon Kaen, Thailand. PGG was cut into about 10 mm and the silages were prepared by using a small scale fermentation system (Cai et al., 1998). The silage treatments were designed as Untreated (control), Chikuso-1 (*Lactobacillus plantarum,* Snow Brand Seed Co., Ltd, Sapporo, Japan) and Snow Lact L (*L. rhamnosus,* Snow Brand Seed Co., Ltd), the LAB was inoculated at a rate of $1.0x10^5$ colony forming unit (cfu)/g of fresh matter (FM). Seven silos were prepared per treatment and ensilage using a laboratory scale fermentation silo system (Cai et al., 1998). Approximately 100-g FM portions of materials were packed into plastic bags (Hiryu, KN type, 180×260 mm; Asahikasei, Tokyo, Japan) with a vacuum sealer (BH 950, Matshushita, Tokyo, Japan). The silos were stored at room temperature (27 to 33 °C) and one bag on day 3, 7, 10, 30, and three bags on day 55 of each treatment were opened for fermentation characteristic evaluation. PGG was analyzed for DM, OM, CP, EE, NDF, ADF and lactic acid buffer capacity (LBC). The silage effluent samples were analyzed for pH, lactic acid, volatile fatty acids and ammonia-N. Data from the bag opened on day 55 was subjected to analysis of variance and treatment means were compared using a Tukey's test at $P<0.05$.

Results and discussion Dry matter of PGG was 24%, their organic matter, crude protein, ether extract, natural detergent fiber and acid detergent fiber compositions were approximately 92, 4, 0.8, 70, and 41% on a DM basis, respectively (Table 1). LAB and aerobic bacteria were 10^6, coliform bacteria were 10^5, and mold and yeast were10^3 cfu/g of FM on the fresh PGG, respectively. After 55 days of fermentation, 10^5 LAB, 10^3 to 10^4 aerobic bacteria and 10^3 to 10^4 coliform bacteria were found in the 55 days silages, but mold and yeast could not detected (Figure 1). As show in Table 2, after fermentation of 3 to 30 days, the Chikuso-1 inoculated silage showed trend lower pH than the Snow Lact L inoculated and control silage. However, two LAB-inoculated silages and control were not well preserved after 55 days of fermentation, had a very high pH values, ammonia-N and butyric acid contents, but low or too few to found the lactic acid content ($P>.05$). Based on the silage fermentation analysis, we have found that the high moisture of guinea grass cannot be well preserved by preparing silage in this experiment. Therefore, it is necessary to develop preparation techniques of promoting lactic acid fermentation as moisture adjustment and sugar addition for PGG silage in tropical area.

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Table 1. Chemical compositions of purple guinea grass at 60-day cutting age[1].

Item	DM (g/kg FM)	OM	CP	EE	NDF	ADF	LBC (meq/kg DM)
		(g/kg DM)					
Guinea grass	235	918	40	8	700	411	1701

[1]DM = dry matter, OM= organic matter, CP = crude protein, EE = ether extract, NDF = natural detergent fiber, ADF = acid detergent fiber, LBC = lactic acid buffer capacity.

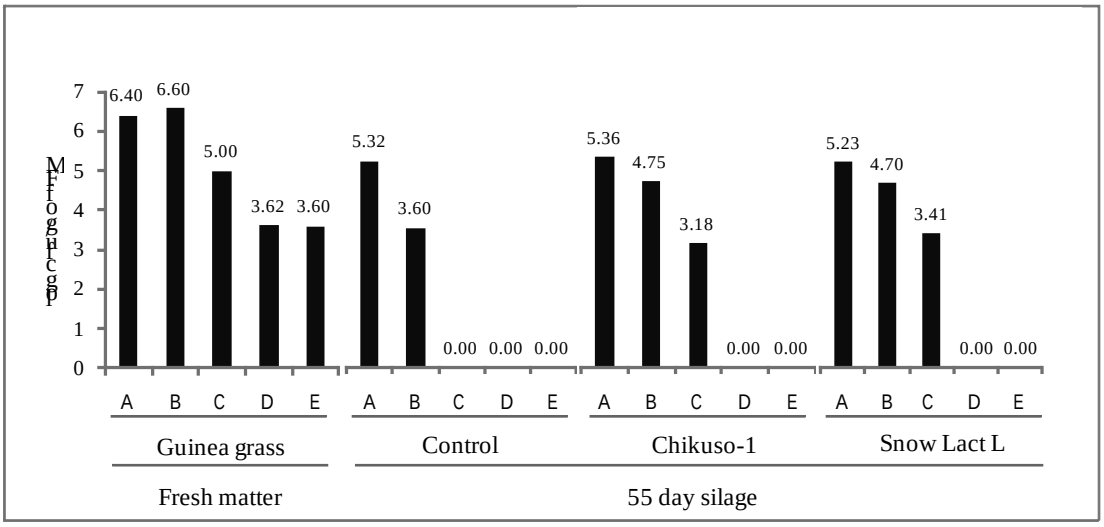

Figure 1. Microbial quantity in purple guinea grass silage after 55 days. (A = Lactic acid bacteria, B = Aerobic bacteria, C = Coliform bacteria, D = Mold and E = Yeast)

Table 2. Fermentation characteristics of purple guinea grass silage at 60-day cutting age[1].

Treatment	DM (g/kg FM)	pH	Organic acid (g/kg DM)					Ammonia-N (g/kg DM)
			Lactic acid	Acetic acid	Propionic acid	Butyric acid	Valeric acid	
3-day silage								
Control	205	5.9	ND	ND	ND	ND	ND	1.1
Chikuso-1	199	4.6	ND	ND	ND	ND	ND	1.6
Snow Lact L	202	5.3	ND	ND	ND	ND	ND	1.3
7-day silage								
Control	189	6.7	ND	ND	ND	ND	ND	2.4
Chikuso-1	189	5.3	ND	ND	ND	ND	ND	1.5
Snow Lact L	198	5.4	ND	ND	ND	ND	ND	1.9
10-day silage								
Control	186	6.7	ND	44.5	11.8	9.3	1.1	4.0
Chikuso-1	198	4.8	ND	32.6	0.0	6.3	0.0	2.1
Snow Lact L	183	5.9	ND	36.0	10.2	8.4	0.0	2.8
30-day silage								
Control	172	5.3	ND	55.7	11.2	19.6	4.5	6.7
Chikuso-1	199	4.8	13.0	37.6	0.0	10.4	0.0	3.1
Snow Lact L	176	5.2	2.8	54.4	11.4	24.6	4.4	7.7
55-day silage								
Control	179	5.2	0.1	71.1[a]	12.4	23.7	4.8	7.8
Chikuso-1	184	5.3	2.5	55.8[b]	11.9	22.2	4.5	6.9
Snow Lact L	172	5.1	6.6	60.2[ab]	12.9	22.8	3.4	5.1
SEM	34.3	0.1	3.8	3.1	0.3	2.5	0.5	0.8
P-value	0.11	0.22	0.52	0.03	0.18	0.88	0.21	0.27

[1]FM = fresh matter, DM = dry matter, ND = not detected.

Effects of crude glycerol addition on silage fermentation

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Keywords: crude glycerol, silage, fermentation quality, microbiology

Introduction The intake and utilization of nutrients is related to the quality of silage. Several industrial by-products are used as alternative additives, and have been added to forage at ensiling to improve fermentation for some time (Salsbury et al. 1949, Cajarville et al. 2012). The use of crude glycerol, a by-product of the biodiesel industry, as a feed component for ruminants is not novel. However, interest in extending the use of this by-product in silage production is novel. The chemical composition of crude glycerol suggests its potential use as a silage additive. There are limited data reporting the effect of glycerol treatment on sugarcane silage (Dias Jr et al. 2010) and corn silage (Krempser et al. 2011, Oliveira et al. 2011). The objective of this study was to determine the fermentation quality of ensiled materials treated with crude glycerol.

Material and methods The fresh material was collected from the first (grass) and third (grass to legume ratio 30:70) cuts, and was wilted for 24 h after harvesting. The treatments were: a control, and treatment with crude glycerol added before ensiling, at a rate of 1.4% to the first cut material and 1.0% to the third cut material on a fresh material basis. The fresh material and silages were analysed for chemical composition and fermentation parameters according to generally accepted methods (AOAC, 2005). The crude glycerol contained 76% glycerol, 17% water, 6.3% salts, 0.2% crude fat and 0.5% methanol.

Lactobacilli spp, *Lactococci spp* and *Enterobacteria* and *Clostridium spp* were enumerated in the fresh materials and silage samples by a conventional cultivation method. Serial dilutions of the silage samples were prepared with sterile saline, and 0.1 ml aliquots of these were inoculated onto MRS and M17 agar (Oxoid, UK) for lactic acid bacteria, and Violet red bile agar with glucose for *Enterobacteria*. The presence of vegetative cells and spores of *Clostridium spp* were evaluated using the MPN method in Bryant Burkey Broth, with Rezurine and Lactate according to the producer's instructions (Merck Germany). The inoculate plates and test tubes were incubated at 37°C for 48 h, (incubator IG 150, Jouan, France). Provisional identification of lactobacilli and lactococcal isolates was based on gram-positive-shaped non-sporing cell morphology and a negative catalase reaction.

Statistical differences within cuts between control and crude glycerol treatments were analysed with the t-test.

Results and discussion Fresh material from both cuts were moderately ensilable: the water soluble carbohydrate content of the first cut was 2.2%, and 1.9% for the third cut. Crude glycerol treatment decreased ethanol ($p=0.006$) and lactic acid ($p=0.006$) content in the first cut silage (Table 1). There was also a tendency for a decrease in butyric acid ($p=0.08$) in the first cut silage. A tendency for an increase in lactic acid content was noted ($p=0.06$) in the silage from the third cut.

To characterize the effect of glycerol on microorganism populations in raw materials and silages, microbiological analyses were carried out. The raw material of the first cut was found to be contaminated with *Enterobacteria* which, however, were suppressed during fermentation. In the first cut, adding glycerol significantly increased the lactobacilli count ($p=0.007$), in comparison with the control samples (Table 1). No differences in the microbial counts were found in the third cut silages. However, in a previous study, glycerol addition decreased the CFU of several microorganisms (Krempser et al., 2011) and increased corn silage aerobic stability (Oliveira et al. 2011) after opening of the silos.

Glycerol has been used to compensate for energy losses during the silage fermentation process (Dias Jr et al. 2010). In the present study, results showed no clear evidence of a common effect of crude glycerol treatment on silage fermentation, and microbiological composition, of silage harvested at different times. There was no clear evidence of how lactic acid bacteria use glycerol for their maintenance. However, the lactobacilli count increased, but the effect on the concentration of lactic acid was different for the different cuts; higher in the first but lower in the third cut silage. Therefore, the effect on the mode of action of the glycerol on silage microflora and fermentation needs additional investigation.

Conclusions The composition of lactic acid bacteria in silage was not negatively affected by the addition of glycerol, but this seemed to depend more on the time of the silage cut and, or, its botanical composition. Crude glycerol addition had no clear effect on silage fermentation across cuts, and with silages

of different composition. Adding glycerol to late cut silage, of mixed grass and legumes, improved some silage quality parameters. Further research is needed to evaluate the potential of crude glycerol addition to silage, and its effect in different ensiling conditions and amounts of glycerol added.

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Table 1. The chemical composition, fermentation characteristics and microbiological composition (log10 CFU/g) of fresh materials and silages[1].

	First cut (S.E.)			Third cut (S.E.)		
	Fresh material	Silage		Fresh material	Silage	
		Control	Glycerol		Control	Glycerol
Chemical composition						
Dry matter, g/kg	372	367±2.2	382±1.7	366	346±1.1	348±1.4
In dry matter, g/kg						
Crude protein	90	99±0.3	95±1.7	142	144±0.6	142±1.1
Crude ash	70	73±0.5	73±0.6	88	91±0.5	91±0.5
Crude fibre	310	329±3.9	309±0.5	246	261±0.1.2	260±3.8
N-free extractives	503	467±4.5	490±2.8	494	472±0.9	476±3.4
Fermentation parameters						
Ethanol		10.5±6.0*	7.1±2.3*		7.7±4.9	7.1±5.2
Acetic acid		9.9±2.5	9.9±2.3		16.8±8.5	18.6±3.4
Butyric acid		0.7±1.7	0.1±0.0		0.1±0.0	0.1±0.0
Lactic acid		50.1±6.1*	44.8±8.3*		59.7±23.4	66.5±10.9
pH		4.2±0.0	4.2±0.0		4.5±0.3	4.4±0.1
NH_3-N/ total N,%		3.0±0.9	3.0±1.0		3.7±0.9	3.9±0.3
Microbiological composition (log10 CFU/g)						
Lactobacilli	5.3±0	6.7±6.7*	7.0±7.0*	4.0±0	6.5±8.7	6.6±6.6
Lactococci	5.0±3.3	6.0±6.3	5.5±5.1	0.0	7.0±7.1	6.8±6.8
Clostridia	4.0±0	3.0±3.1	3.0±3.1	4.0±3.3	3.0±2.7	2.0±2.6
Clost. Spores	3.0±0	0.0*	3.0±2.8*	0.0	0.0	0.0
Enterobacteria	4.6±0.4	0.0	0.0	0.0	0.0	0.0

[1]Statistical differences within cuts between control and treatment silages

* $p<0.05$

Improvement of haylage quality using a *L. plantarum* strain optimized for osmotolerance

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Keywords: haylage, *L. plantarum*, osmotolerance, fermentation quality

Introduction Silages with high dry matter contents (≥50% DM), commonly referred to as haylage (García, A. 2003), traditionally do not only suffer from poor compaction properties and subsequent yeast and mould contamination, but also suffer from inefficient acidification and poor fermentation quality. The latter is a consequence of low viability of the desired microbiology due to high osmotic pressure caused by restricted moisture availability. For the same reason biological silage inoculants are usually less effective in haylages. Therefore a fermentation process to enhance osmotolerance of silage inoculants was developed by cultering *L. plantarum* in a medium with higher salt content.The aim was to test the effectiveness of *L. plantarum* strain raised in high osmolality solutions compared to a strain processed under standard conditions in a laboratory scale silage trial.

Material and methods A laboratory scale silage trial, with sample silos of 0.5 and 2.0 kg was carried out in strongly wilted meadow grass from permanent grassland predominated by species Kentucky Bluegrass (*Poa pratensis*), Orchard grass (*Dactylis glomerata*), Medow fescue (*Festuca pratensis*), White clover (Trifolium repens) and herb species Yarrow (*Achillea millefolium*), Dandelion (Taraxacum officinale), Ribwort plantain (*Plantago lanceolata*) with a dry matter content of 55% DM. Two treatment groups were compared to an untreated control silage (CT). Inoculants used included *L. plantarum* "LpN" (normal standard fermentation procedure) and *L. plantarum* "LpO" (enhanced osmotolerant fermentation procedure) at a rate of 3 x 10^5 cfu/g of raw material. The difference between LpN and LpO is that LpO is cultured in a medium with higher salt content.

The trial ran over 3 months and sampling was done 3, 7, 42 and 93 days after ensiling. Sample silos were prepared in triplicates per treatment and sampling day. An aqueous extract was prepared to analyse pH, organic acids and carbohydrates. Organic acids and carbohydrates were detected using HPLC-RID method. Further silage samples were analysed for a number of parameters including, among others, DM loss, counts of beneficial microorganisms and energy parameters (ME, NEL). Digestibility and energy parameters were external analysed and calculated according to "Weender-analysis". Statistics on trial data were performed with SPSS 10,using Analysis of Variance (ANOVA followed up by Tukey-HSD test).

Results and discussion Both treatments with *L.plantarum* (LpN and LpO) showed sufficient acidification to ensure anaerobic stability compared to CT, which had a much delayed pH drop (Figure 1).
Particularly treatment with LpO had a pH significantly lower than the CT already 3 and 7 days after ensiling. At mid-trial both treatments with lactic acid bacteria had a significantly lower pH than CT. On day 7 lactic acid amount was significantly higher for LpO compared with that in CT. That tendency was kept after 42 and 93 trial days (Figure 2).
LpO was more efficient in lactic acid production and consequently faster in lowering the pH-value (Figure 1 and Figure 2).

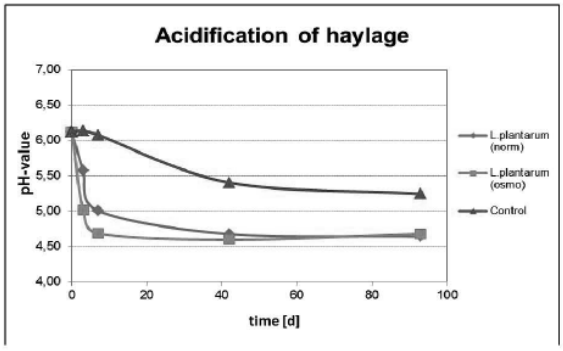

Figure 1. pH values during 93 days of ensiling.

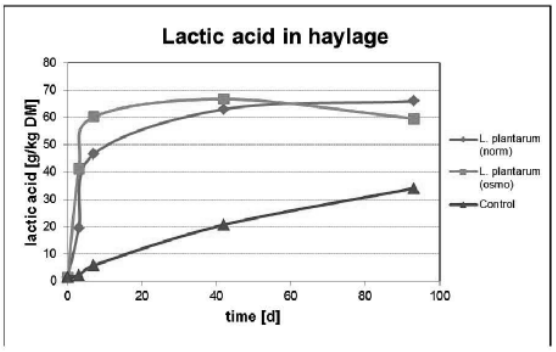

Figure 2. Lactic acid production during 93 days of ensiling.

Results of microbial counts (lactic acid bacteria) at end of trial showed a higher survival of the LpO than LpN (8.71 x 10^6 *vs*. 3.06 x 10^6 cfu/g of silage respectively). Concerning nutrients and energy at trial end both net energy and digestible energy were significantly improved in inoculated treatments *vs*. CT. Even though the results of ammonia-N content were not significant, there was a clear trend that both groups treated with *L. plantarum* had lower ammonia-N contents and dry matter losses than the CT at the end of the trial after 93 days (Table 1).

Table 1. Digestibility, energy, DM losses and ammonia-N of trial treatments on day 93.

Parameters	Unit	*L. plantarum* (norm)	*L. plantarum* (osmo)	Control
Digestibility	%	68.9	68.7	67.7
Metabolizable energy	MJ/ kg DM	9.22	9.14	8.94
Net energy lactation	MJ NEL/ kg DM	5.44	5.38	5.25
Dry matter loss	%	2.74	2.83	5.15
Ammonia-N	% of total N	1.84	2.40	3.35

Although LpO was comparable to LpN in energy content and digestibility at the end of the trial, the advantage of LpO over LpN is in terms of acidification. The efficient lactic acid production and consequent faster lowering of the pH within the first 7 days leads to inhibition of anaerobic spoilage organisms and consequently minimises butyric acid production. It is therefore probable that the preconditioning of LpO during cultivation with higher salt content leads to more efficient adaptation to environments with higher osmotic pressure.

Conclusions A new process could be established to enhance osmotolerance in silage inoculants. The resulting product had a higher survival rate and was faster and more efficient at lactic acid fermentation and consequently faster at lowering the pH-value.

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The benefits of adding a multi-strain homo-fermentative biological additive on the silage quality of a range of forage crops

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Keywords: biological silage additive, fermentation, grass legumes, , silage,

Introduction Many silage inoculant manufacturer's have taken the approach that different crop types require different silage additives in order to enhance silage quality. However, it is often questionable whether there is strong scientific evidence for this approach and whether such an approach enables the end-user to make a more informed decision on their additive choice or not.

The aim of the study was to assess the efficacy of a biological silage additive containing four species of homofermentative inoculant and four enzymes on the fermentation and silage quality of either grass, red clover or lucerne compared to an untreated control silage. The crops were chosen to provide a range of ensilabilities from easy (grass), moderate (red clover) and difficult (lucerne).

Material and methods Pure swards of Perennial ryegrass, lucerne and red clover were mown on the 19th May 2009 and wilted over night prior to being chopped through a precision chop forage harvester. The crops were ensiled in (5 replicate silos per treatment) in 1.5 litre Weck jars either without an additive treatment (control) or after the addition of Sil-All 4x4 (Alltech Inc, Kentucky, USA). Sil-All 4X4 contains a mix of *Enterococcus faecium, Pediococcus acidlactici, Lactobacillus plantarum, Lactobacillus salivarius,* and amylase, hemicellulase, cellulase, pentosanase, applied as directed by manufacturer but to supply 1 x 10^6 CFU/g FM. After 90 days of ensilage the silos were opened and silage was analysed for DM, pH, water soluble carbohydrate, ammonia-N, lactic and acetic acids and DM losses by standard wet chemical methodologies. Treatment effects were examined within each forage type by one-way analysis of variance. Forage means were compared using the Student Newman Keuls test to take into account multiple comparisons.

Results The results of the silage analyses are shown in Table 1. The results show that the inoculant treatment improved many aspect of silage quality across the range of silage analytes examined and across the three crop types tested. The pH was significantly lower with all crops in the inoculant treated silage compared to the untreated silage. The concentration of water soluble carbohydrate was also significantly increased by the use of inoculant compared to no treatment in all forage types.

In both the lucerne and ryegrass forage types there was significantly lower ammonia-N and significantly higher lactic acid with inoculated silages compared to untreated silages. However in the red clover silages there were no significant differences in these two analytes.

Dry matter losses were also significantly reduced with the inoculant treatment compared to no treatment for both of the legume silages.

Table 1. Silage quality and dry matter losses after ensiling lucerne, red clover or perennial ryegrass with or without a biological silage additive.

	Lucerne		Red Clover		Perennial Ryegrass		S.E.M.
	Untreated	Sil-All 4x4	Untreated	Sil-All 4x4	Untreated	Sil-All 4x4	
DM (g/kg FM)	157.5[a]	172.3[b]	122.2	131.9	262.1	266.2	3.7
pH	6.18[a]	3.99[b]	3.83[a]	3.60[b]	3.62[a]	3.45[b]	0.209
WSC[1]	11.26[a]	12.58[b]	10.93[a]	17.44[b]	27.9[a]	127.4[b]	9.67
Ammonia-N[2]	231.4[a]	158.0[b]	135.2	94.6	99.4[a]	82.0[b]	0.72
Lactic acid[1]	1.7[a]	107.4[b]	122.6	133.5	86.4[a]	113.6[b]	17.52
Acetic Acid[1]	20.53	21.10	24.32	17.28	19.63[a]	6.50[b]	1.845
%DM Losses	17.08[a]	8.00[b]	17.51[a]	12.28[b]	4.19	2.42	1.738

DM = Dry matter, WSC = Water soluble carbohydrates, S.E.M. = Standard Error of the Means. Units [1] =g/kg DM, [2]=g/kg total N Values within rows and within crops with different superscripts were significantly different (P<0.001).

Conclusions The results indicate that many of the parameters that are used as indicators of fermentation are improved by the use of the Sil-All 4x4 silage additive and so show that one additive type can be effective across a range of crops. The results indicate the benefits of homofermentative silage inoculants of improving silage quality particular with difficult to ensile crops such as lucerne. The data also indicates that one additive can have a beneficial effect across a range of different forage types without the need to make crop specific formulations.

Acknowledgements This work was sponsored by Alltech Inc.

Fermentation profile of grass-legume forage ensiled with different additives

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Keywords: additive, aerobic stability, fermentation profile, silage

Introduction Efficient fermentation of sugar to lactic acid and minimal proteolysis are crucial for silage preservation (Nadeau et al., 2000). Information about the timing of these events and about the effects of additives on the fermentation profile is important to have a better understanding of silage quality. The objective of this experiment was to evaluate the effects of different additives and storage lengths on the fermentation profile of grass-legume forage.

Material and methods A sward (77% grass, 18% clover, 5% lucerne) was mowed on 3 June, 2010 and wilted to a dry matter (DM) content of 340 g/kg. Wilted forage was precision chopped and ensiled in 1.7-L silos at Lantmännen Dairy Research Farm Nötcenter Viken, Falköping, Sweden. The forage was treated with KOFASIL® LIFE, containing *Lactobacillus plantarum* DSM 3676, 3677 at an application rate of 400 000 cfu/g of forage or with KOFASIL® ULTRA K, containing sodium nitrite, hexamethylene tetramine, potassium sorbate, sodium benzoate and sodium propionate, at 2 L/ton forage (ADDCON EUROPE GmbH). The treated silages were compared to untreated silage. After ensiling periods of 5, 10, 30 and 125 days (d) the forages were analysed for fermentation characteristics at Humboldt University, Berlin, Germany. The content of ammonia-N in silage treated with KOFASIL ULTRA K was corrected for the ammonia-N produced from the additive. Aerobic stability of the silages after 125 d of storage was measured as the number of days reaching a temperature of 2°C above ambient temperature during a 10-d period (Honig, 1990). Acidification rate was measured as silage pH after 3 d of fermentation in 0.5-L silos. Data were analysed as a completely randomized design in PROC GLM of SAS 9.2, with treatment and storage length as fixed factors, using three replicates per treatment. Only pH and ethanol had significant interactions between treatment and storage length. When the overall *P* – value was significant at 5% level, pair wise comparisons between LSMEANS of treatments were done using Tukey´s test.

Results and discussion Concentrations of neutral detergent fibre, water soluble carbohydrates (WSC) and crude protein of wilted forage were 375, 212 and 143 g/kg DM, respectively. Silage treated with KOFASIL LIFE had a higher acidification rate with a pH of 4.23 after 3 d of fermentation compared to 4.56 of the untreated silage ($P < 0.0001$). The higher acidification rate was related to a faster lactic acid production with a more homolactic fermentation of the silage treated with KOFASIL LIFE compared to the control silage (Figure 1a; 7.4 vs. 10.1 g acetic acid/kg DM, $P < 0.05$ at 5 d of fermentation). The control silage had as much lactic acid as the inoculated silage, averaging 96 g/kg DM at 125 d of storage. The inoculated silage also had less ethanol than the control silage (Figure 1b, $P < 0.001$) at similar WSC concentrations of the treatments at all storage times, averaging 105 g/kg DM at 125 d of storage. The higher acidification rate resulted in less proteolysis of the inoculated silage than of the control silage at all storage times (Figure 1c, $P < 0.05$). KOFASIL ULTRA K resulted in a lower ethanol concentration compared to the control at all storage times (Figure 1b, $P < 0.001$). Silage treated with KOFASIL ULTRA K had less ammonia-N being produced at 5 and 10 d of fermentation compared to the control (Figure 1c, $P < 0.001$). The pH was 4.02, 4.02 and 4.14 ($P < 0.01$) and the acetic acid content was 13, 9 and 12 g/kg DM ($P = 0.12$) of the control, KOFASIL LIFE and KOFASIL ULTRA K treatments, respectively, at 125 d of storage. No butyric acid was detected in the silages. Counts of total yeast (log 3.1-3.9) and lactate assimilating yeast (log 1.8-2.5) in the silage at 125 d of storage were low with no differences between treatments. Aerobic stability of the silages tended to be better for the treated silages than for the control (KOFASIL LIFE: 8.4 d, KOFASIL ULTRA K: 9.8 d. vs. control: 7.4 d at 125 d of storage, $P = 0.07$).

Averaged across treatments, lactic acid concentration increased up to 30 d of storage, resulting in a simultaneous pH decline, whereas concentration of ammonia-N increased until 125 d of storage ($P < 0.0001$).

Conclusions Fermentation characteristics and aerobic stability of well fermented grass-legume silage can be further improved by use of KOFASIL LIFE and KOFASIL ULTRA K as compared to silage ensiled without additives.

Acknowledgements This project was funded by Agroväst, ADDCON EUROPE GmbH, VL-foundation, Lantmännen R & D, AIC and SLU.

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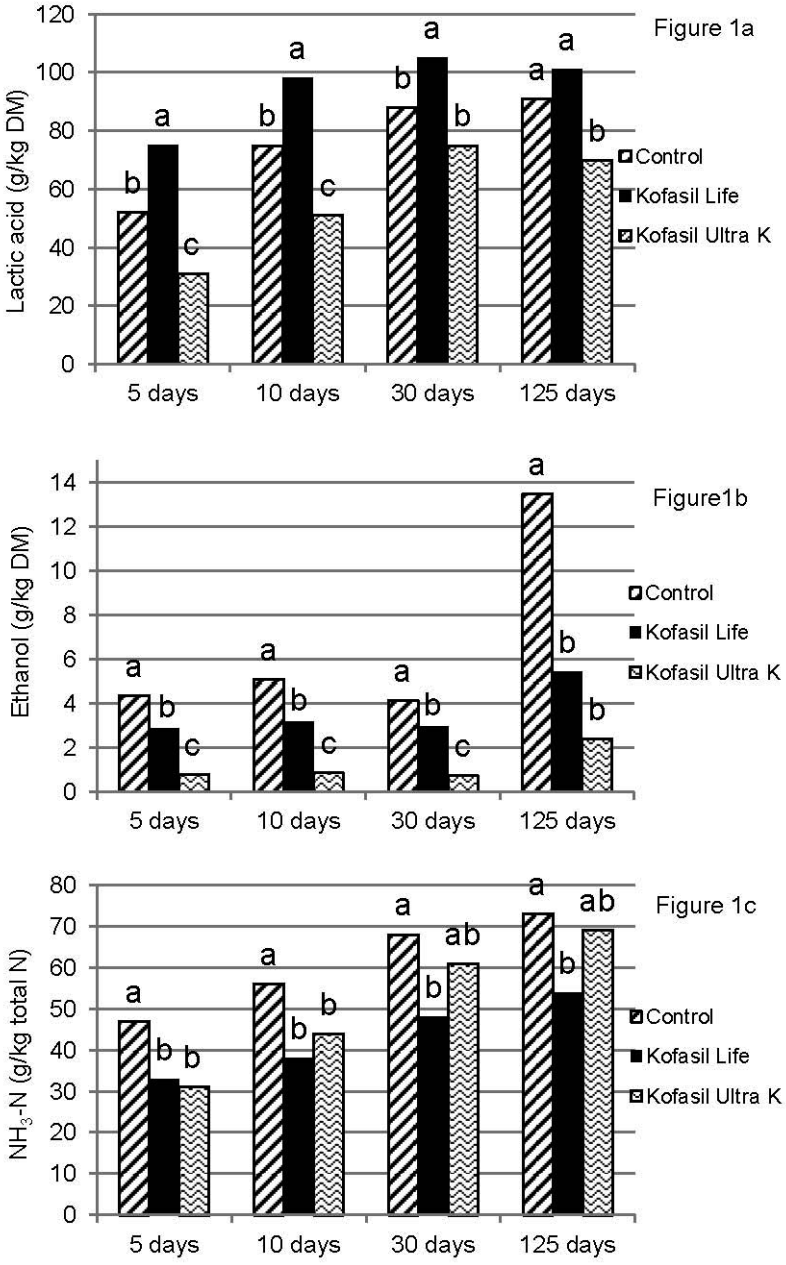

Figures 1a, 1b and 1c. Concentrations of lactic acid, ethanol and ammonia-N (NH_3-N) of grass-legume silage at different storage lengths. Bars with different letters (a, b, c) within storage length differ significantly at $P < 0.05$.

Effects of mixtures of lactic acid bacterial strains in grass, clover-grass and maize on silage fermentation parameters

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Keywords: fermentation, grass**,** inoculant, maize, silage additive

Introduction Lactic acid is the most commonly identified organic acid that reduces the pH in the silage. Therefore, lactic acid bacteria (LAB) are the primary type of bacteria utilized in most bacterial inoculants and can result in a faster decrease in pH, lower final pH values, higher lactate:acetate ratios, lower ethanol and ammonia nitrogen, and improvement in DM recovery. Moreover, inoculants can provide substantial benefit by reducing the risk of the growth of other harmful spoilage organisms such as butyric acid bacteria including clostridia by reducing the pH (Pauly 1999). A combined culture of LAB species as a silage inoculant may be more beneficial than using a single species alone due to differences in growth pattern and positive interaction among bacteria. Differences have been shown to be also not limited only on specie level, but also on strain level. Some silage producers feel the inclusion of an enzyme in inoculants can help the fermentation and digestibility of silages. Enzymes can improve silage fermentation when the substrate of WSC is limiting. Confirming efficacy and commercial value of such mixtures were the targets in this investigation.

Material and methods The experiment was conducted at the Institute of Animal Science of Lithuanian University of Health Sciences. The following LAB combinations were tested: *Enterococcus faecium* NCIMB 11181/ DSM 22502 at 40 %, *Lactococcus lactis* NCIMB 30117 at 30 %, and *Lactobacillus plantarum* DSM16568 at 30 % (FLP); *L. plantarum* DSM16568 at 40%, *E. faecium* NCIMB 11181/ DSM 22502 at 30 % and *L. lactis* NCIMB 30117 at 30 % (PFL) and in one experiment with red clover-grass *E. faecium* NCIMB 11181 at 30 %, *L. lactis* NCIMB 30117 at 30 % and *L. plantarum* DSM 16568 at 40 % supplemented with EC 3.2.1.8. Xylanase at 0.5 HEC/g forage (FLP+X).

All inoculants were diluted with distilled water and applied as suspension at the same rate (4ml solution/kg of crop) or/and in total 150 000 cfu/g fresh forage. The untreated control received 4 ml of distilled water/kg of crop. The 3-liter mini silos were filled with chopped ryegrass – timothy from the third cut (R:T, 70:30), whole crop maize at the dough stage of maturity (M) and red clover-ryegrass from the second cut (RC:R, 50:50). Each treatment and crop was replicated 5 times and were stored at 20°C for 90 days before determining DM, pH, DM losses corrected for volatiles, lactic acid (LA), acetic acid (AA), butyric acid (BA), ethanol, ammonia-N, number of clostridia spores, yeasts and moulds. One way analysis of variance with multiple comparisons was used separately for each treatment and forage type combination. Data were statistically analyzed as a randomized complete block by using the GLM procedure of SAS.

Results and discussion Overall, microbial inoculants generally had a positive effect on grass, whole crop maize and clover-grass silages. Results on the fermentation parameters, dry matter loss and microbiological quality of the grass, maize and clover-grass silages are shown in Table 1.The DM content of the pre-ensiled R:T, M and RC:R was 26.5 %, 27.6 % and 26.5 %, respectively. The WSC content of pre-ensiled R:T, M and RC:R was 102, 100 and 89 g/kg DM, and buffer capacity was 353, 217, and 450 mEq/kg DM, respectively. Yeasts were detected at 10^5 CFU/g in all crops, while mold levels of less than 10^2 CFU/g were measured in pre-ensiled fresh forage.

Addition of inoculants FLP, PFL and FLP+X resulted in a significant decrease in pH and significant increase in lactic acid concentration compared with untreated silages. However, bacterial inoculation did not influence the pH of the maize silage (M), which is in agreement with previous studies (Muck 2010). Inoculants FLP and PFL did not affect acetic acid concentration in ryegrass-timothy silages, but decreased the concentration of acetic acid in maize silages when compared with control silages. Inoculation had a significant effect on protein degradation as measured by ammonia-N concentration with significantly lower values (17- 21 g/kg N for FLP and PFL treatments and 55 g/kg N for FLP+X treatment) compared with control silages after 90 days ensilage ($P<0.05$). Additives FLP, PFL and FLP+X designed to improve fermentation, resulted in significantly smaller DM loss. The DM loss in FLP and PFL treated grass and maize silages was 1.8-2.6 percent units smaller ($P<0.05$), and the DM loss in FLP+X treated silages was 7.3 percent units smaller ($P<0.05$) than in the untreated silages. In general, silages were characterized by low numbers of yeasts, moulds and clostridial spores. However, all inoculants significantly decreased numbers of yeasts and molds present in the silages. The butyric acid and alcohols concentrations of grass, whole-crop maize and clover-grass silage were reduced ($P<0.05$) with FLP,

PFL and FLP+X inoculation. This is in agreement with other studies (Kaldmäe et al. 2007) that reported reduced butyric acid concentration of silage with a homofermentative LAB inoculation. It would appear that homofermentative LAB blends can reduce the risk of clostridial fermentation and enhance the nutritive value of the silages. Dry matter digestibility was numerically improved in all of the inoculated silages. The enzyme xylanase added to homofermentative LAB mixture (FLP+X) had tendency to increase organic matter digestibility in red clover-ryegrass silages.

Table 1. Silages parameters of trials with grass (Ryegrass:Timothy 70:30), maize (M) and Redclover: Ryegrass (RC:R 50:50) ensiled with or without LAB combinations FLP, PFL or FLP+Xylanase.

Item	R:T (70:30)			M			RC:R (50:50)	
	Control	FLP	PFL	Control	FLP	PFL	Control	FLP+X
DM (%)	25.0	28.7*	25.4*	26.4	27.1*	27.0*	23.9	25.4*
DM loss (%)	6.3	4.0*	4.5*	5.5	2.9*	3.1*	12.3	5.0*
pH	4.6	4.0*	4.0*	3.7	3.6	3.6	5.6	4.2*
NH3-N (% total N)	6.0	4.1*	4.3*	5.8	3.7*	4.1*	9.2	3.7*
LA (% DM)	3.1	5.8*	5.8*	5.6	6.9*	7.1*	1.4	6.4*
AA (% DM)	2.8	2.8	2.5	2.6	1.9*	1.9*	1.8	3.1*
BA (% DM)	0.25	0.04*	0.06*	0.05	0.00*	0.01*	3.7	0.04*
Ethanol (% DM)	0.7	0.5*	0.5	1.0	0.6*	0.5*	1.5	0.6*
Clostridia (logCFU/g)	1.0	1.0	1.0	1.0	1.0	1.0	-	-
Yeast (log CFU/g)	3.2	1.5*	1.6*	1.9	1.3*	1.4*	-	-
Moulds (log CFU/g)	3.0	1.4*	1.6*	2.0	1.1*	1.4*	-	-
OMD (%)	63	64	64	66	67	69	66	69

Conclusions The results of the study showed that inoculation with homofermentative LAB mixture or with homofermentative LAB mixture in combination with enzyme xylanase significantly improved the fermentation quality of grass, whole crop maize and clover-grass silages and reduced the number of yeasts and molds. FLP, PFL and FLP+X silage inoculants contain an good blend of lactic acid-producing organisms that have been shown to significantly decrease silage pH for proper fermentation and higher levels of dry matter and lower nutrient losses, ensuring a better silage quality. According to individual strain knowledge, the mode of action of different strains could be different. However, ability of these bacterial strains mixtures to affect fermentation has been observed and was evident. It can, therefore, be concluded that inoculants, which contained *Enterococcus faecium* NCIMB 11181/DSM 22502, *Lactococcus lactis* NCIMB 30117, and *Lactobacillus plantarum* SM16568 are a promising preservative for grass, legume-grass and whole crop maize, because this accelerates the initial lactic acid fermentation process, giving less protein degradation and DM loss. In Red Clover Rye:Grass the LAB mixture added xylanase gave similar good quality silage, with markly reduced DM loss %.

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The effects of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silage

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Keywords: aerobic stability, fermentation, lactic acid bacteria, silage additive

Introduction To achieve high quality silage it is important at the preparation of the silage to have a fast pH reduction, to avoid growth of undesirable microorganisms and fermentation end products, which else can result in dry matter (DM) loss and aerobic stability. Aerobic spoilage by yeasts and moulds is a major cause of reduced nutritional value of silage and increase the risk of potential pathogenic microorganisms. Next to good management the inoculation of lactic acid bacteria (LAB) will secure high quality silage. Making a combined culture of lactic acid bacterial species as a silage inoculant may be more beneficial than using a single species alone due to differences in growth pattern and positive interaction among bacteria. Recent studies have shown that *Lactobacillus. buchneri* inhibit yeast and mould growth and increase aerobic stability of silages and these effects are retained when *L. buchneri* is added in combination with homofermentative lactic acid bacteria. Further more, *in vitro* studies exist indicating superior oxygen scavenging properties of certain *Lactococcus lactis* species. Inoculants containing a mixture of homofermentative or heterofermentative LAB with specific characteristics can reduce DM losses by increasing the acidification rate and increase the aerobic stability. Another alternative is to use additives containing other antimicrobial compounds like sodium benzoate. Our objective was to evaluate the variety of LAB strains on fermentation characteristics and especially aerobic stability in the lucerne, ryegrass, ryegrass-timothy, red clover-ryegrass and maize. The additives were compared to an untreated reference in laboratory-scale experiment.

Material and methods The experiment was conducted at the Institute of Animal Science of Lithuanian University of Health Sciences. The following LAB combinations were tested: *Lactobacillus buchneri* CCM 1819 (TA); *Lactobacillus buchneri* CCM 1819, *Enterococcus faecium* NCIMB 11181 and *Lactobacillus plantarum* DSM 16568 (TB); and *Enterococcus faecium* NCIMB 11181*, Lactobacillus plantarum* DSM 16568 and *Lactococcus lactis* DSM 11037 supplemented with sodium benzoate at 400 g/ton forage (TC). All inoculants were diluted with distilled water and applied at the same rate (4ml solution/kg of crop) or/and 150 000 cfu/g fresh forage. The untreated control (UT) received 4 ml of distilled water/kg of crop. The 3-liter mini silos were filled with chopped lucerne (32.8 % DM; 1.2 % water soluble carbohydrate (WSC) of fresh matter (FM) (L), ryegrass (30.8 % DM; 2.6 % WSC FM) (R), red clover:ryegrass (31.7 % DM, 2.9 % WSC FM) (RC:R), ryegrass-timothy (26.5 % DM; 2.7 % WSC FM) (R:T), red clover-ryegrass (26.5 % DM; 2.4 % WSC FM) (RC:R) and whole crop maize 32.8, 29.5 and 27.6 % DM; 2.7, 2.8 and 3.6 % WSC FM) (M). Each treatment and crop was replicated 5 times and were stored at 20°C for 90 days before determining DM, pH, DM losses, lactic acid (LA), acetic acid (AA), butyric acid (BA), ethanol, ammonia-N, and aerobic stability (AS), defined as a temperature increase of 2 °C above the ambient temperature. Data were statistically analyzed as a randomized complete block by using the GLM procedure of SAS.

Results and discussion From a silage fermentation standpoint, ensiling lucerne, grass, clover-grass, and whole crop maize with three silage inoculants (TA, TB and TC) offered the advantage of better-preserved silage with a significantly lower pH, concentration of ammonia-N, butyric acid and ethanol compared with untreated silages (see Table 1 and Table 2). The main indicators of clostridial infection are high levels of butyrate and ammonia-N. The mode of action of the additives applied to herbage during silage making can reduce respiration and/or proteolysis by plant enzymes, manipulating fermentation, inhibiting the activity of clostridia and aerobic micro-organisms such as yeast and mould (Kung et al. 2003). On an average, the use of additives reduced fermentation losses, by 2.7 % units ($P<0.05$) (variation from 0.5 to 6.6 % units) compared to the untreated control silages.

At the same time more ($P<0.05$) lactic acid (except additive TA and TB for maize with 27.6 % DM) and more ($P<0.05$) acetic acid (except additive TC for ryegrass-timothy and maize with 27.6 % DM) was produced in the inoculated silages compared to untreated silages.

The present study demonstrated that inoculation of grass, clover-grass and whole crop maize with different LAB or/and LAB in combination with sodium benzoate results in different metabolites and different aerobic stabilities. RC:R silage treated with TC resulted in significantly ($P<0.05$) lower DM loss than the untreated silage or the other treatments. The most obvious difference between the inoculants TA, TB and TC used was the variation in the amount of lactic acid and acetic acid during silages fermen-

tation. Inoculation with homofermentative LAB in combination with sodium benzoate (TC) led to silages with higher lactic acid contents, inoculation with heterofermentative LAB (TA) or homo-and heterofermentative LAB (TB) resulted in higher levels of acetic acid. The higher amount of acetic acid in the TA and TB inoculated silages was expected because heterofermentative lactic acid bacteria *Lactobacillus buchneri* can result in high levels of acetic acid (Oude Elferink et al. 2001). Acetic acid is fungicidal agent and can inhibit yeasts and molds growth, in response to increasing aerobic stability of silages. Silage inoculated with additives TA, TB and TC designed for improved aerobic stability, had lower temperatures ($P<0.05$) when exposed to air, and aerobic stability was increased from 2 to 9 days compared with untreated silage. The aerobic stability of silages treated with *Lactobacillus buchneri* (TA) was greatest among all treatments.

Table 1. Silages parameters of trials with grass and clover-grass ensiled with or without inoculants.

Item	L		R		RC:R (70:30)		R:T (70:30)			RC:R (50:50)		
	UT	TA	UT	TA	UT	TA	UT	TA	TC	UT	TB	TC
DM (%)	31.3	31.9*	29.2	30.1*	29.4	29.9*	25.0	25.2	25.3	23.9	25.0*	25.2*
DM loss (%)	6.8	4.6*	7.0	4.1*	10.2	6.7*	6.3	5.8	4.9*	12.3	6.7*	5.7*
pH	5.4	5.0*	5.1	4.3*	4.7	4.3*	4.6	4.2*	3.9*	5.6	4.7*	4.4*
NH_3-N (% total N)	10.2	7.9*	5.9	4.5*	5.7	4.0*	6.0	5.1*	4.4*	9.2	5.2*	5.2*
LA (% DM)	1.7	3.9*	2.3	4.0*	2.7	3.2*	3.1	3.4	7.2*	1.4	3.8*	5.7*
AA (% DM)	3.4	4.9*	2.2	3.5*	2.9	3.8*	2.8	4.6*	1.9*	1.8	3.2*	3.3*
BA (% DM)	1.4	0.1*	0.47	0.04*	0.65	0.03*	0.25	0.19	0.07*	3.75	0.19*	0.03*
Ethanol (% DM)	1.2	0.7*	0.9	0.7*	1.1	0.7*	0.7	0.6*	0.5*	1.4	0.6*	0.4*
AS (days)	-	-	2.6	10*	6.6	>19*	4	>13*	7	7.9*	>18*	15*

Abbreviations: L= Lucerne, R = Ryegrass, RC:R = Red clover : Ryegrass, R:T = Ryegrass : Timothy, UT = untreated, TA, TB & TC = different mixtures of LAB strains, LA = Lactic acid, AA = Acetic acid, BA = Butyric acid, AS = Aerobic stability

Table 2. Silages parameters of trials with maize ensiled with or without inoculants

Item	M (DM 32.8%)		M (DM 29.5%)		M (DM 27.6%)			
	UT	TA	UT	TA	UT	TA	TB	TC
DM (%)	31.2	31.4	28.2	28.7*	26.4	26.8	26.9	26.9*
DM loss (%)	7.4	5.8*	5.9	3.8*	5.5	3.8*	3.7*	3.6*
pH	4.0	3.7*	3.9	3.7*	3.7	3.6*	3.6*	3.6*
NH_3-N (% total N)	6.1	5.2*	8.0	5.7*	5.8	4.8*	4.6*	4.7*
LA (% DM)	2.8	3.4*	4.0	5.0*	5.6	5.0*	5.6	7.4*
AA (% DM)	2.6	3.6*	1.9	1.8	2.6	3.8*	3.3*	2.2*
BA (% DM)	0.19	0.02*	0.05	0.02*	0.05	0.01*	0.01*	0.00*
Ethanol (% DM)	2.7	1.8*	1.1	0.8*	1.0	0.7*	0.6*	0.7*
AS (days)	2.5	5.8*	1.8	2.9*	2	6.3*	5.5*	3.8

Abbreviations: M = maize, UT = untreated TA, TB & TC = different mixtures of LAB strains, LA = Lactic acid, AA = Acetic acid, BA = Butyric acid, AS = Aerobic stability

Conclusions In this study, selected lactic acid bacteria inoculants were efficient in improving fermentation, reducing protein breakdown and nutrient losses of grass, clover-grass, lucerne and maize silages. Homofermentative LAB in combination with sodium benzoate led to silages with higher lactic acid content, when inoculation with single heterofermentative LAB or with mixture of homo-and heterofermentative LAB resulted in higher levels of acetic acid. An enhancement of aerobic stability by additive application was obtained in all inoculated silages. Single strain *L. buchneri* was more effective in improving silages aerobic stability.

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Chemical composition and fermentative profile of elephant grass and Campo Grande *Stylosanthes* mixed silages

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Keywords: lactic acid bacteria, mold, pH, propionic acid

Introduction Grass silages possess advantages such as high annual production per area, perenniality, low risk of losses and greater harvesting flexibility. However, they present some unfavorable aspects, such as the low content of water soluble carbohydrates (WSC) and dry matter at the adequate moment of ensilage, high buffering capacity and low autochthonous population of bacteria that produce lactic acid. Among the tropical forage grasses, elephant grass (*Pennisetum purpureum* Schum.) stands out as the most studied species for ensilage, due to its easiness of cultivation, good acceptability, high dry matter yield and adequate content of WSC. Although leguminous plants also present limitations to the ensilage process, recently, the interest in their ensilage, in Brazil, has grown. *Stylosanthes* Campo Grande is the main legume currently used under grazing in the savannas of Brazil. Since its release in 2000, its use has increased steadily due to its superior performance and development of the technology in close association with seed producers and farmers. By increasing productivity and sustainability while delivering significant economic benefits to the farmer, *Stylosanthes* Campo Grande offers high hopes of restoring international competitiveness to the Brazilian Beef Industry (Fernandes et al. 2005). Thus, because of the shortage of information on the mixed silages of leguminous plants and tropical forage grasses, the present study was conducted, in order to evaluate the chemical composition, the quantification of microorganisms and the fermentation characteristics in silages containing different proportions of *Stylosanthes* Campo Grande and elephant grass, with and without bacterial inoculant.

Material and methods A 5 × 2 factorial arrangement (five levels of elephant grass × with and without microbial inoculant), in a completely randomized block design with three replicates was utilized. Before the ensilage, both forages were chopped in stationary forage machine. Treatments consisted of the following *Stylosanthes*:elephant grass ratios: 100:0; 75:25; 50:50; 25:75 and 0:100%, with and without bacterial inoculant Sil All (Alltech do Brasil). The forage was ensiled in 20-L buckets provided with Bünsen valve on the lids, adopting a density of 650 kg/m^3 of the silo. The opening of the silos was carried out 84 days after ensilage, removing two samples; the first was dried in forced ventilation oven at 55 ºC, then ground in Wiley mill with 1-mm sieve. In these samples, the dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent insoluble nitrogen (ADIN) were determined according to methodologies described by AOAC (1990). Next, 25 g of silage were homogenized for one minute in blender containing 225 mL of buffer (Ringer's) solution. In the aqueous extract, pH was measured and the organic acid, the ammonia and the population of microorganisms were evaluated. The isolation of microorganisms of the silages were performed through plating in Rose Agar selective medium for quantification of lactic acid bacteria (LAB), Violet Red Bile overlay for quantification of enterobacteria and Potato Dextrose Agar for quantification of fungi and yeasts. The counting of enterobacteria was performed after 18 hours of incubation, while the counting of LAB, yeasts and fungi occurred after 36 hours of incubation at 32 ºC. Plates containing between 30 and 300 cfu were susceptible to counting. The data were submitted to variance and regression analyses and to comparison of means utilizing the F test.

Results and discussion Effects of inoculant and elephant grass were observed on the DM and ADIN contents. Dry matter, CP, and ADIN contents decreased linearly, while NDF and ADF increased linearly with increase in the elephant grass levels in the ensiled mass (Table 1). Populations estimated of LAB in the *Stylosanthes* and elephant grass before ensilage were 5.25 and 5.48 log cfu/g, whereas in the mixed silage, maximum LAB population of 8.19 log cfu/g was estimated using the generated regression equations for the 44.8% elephant grass level. Fungi populations in the *Stylosanthes* and elephant grass before ensilage were 5.00 and 5.54 log cfu/g, respectively. The fungi population in the silages decreased linearly with increase in the elephant grass levels. Yeasts populations in the *Stylosanthes* and elephant grass before ensilage were 6.13 and 5.39 log cfu/g, respectively; these populations were affected by the interaction of elephant grass levels × inoculant in the silage. Thus, in the absence of inoculants and in the inoculated silage, the yeast population of the silages decreased linearly with addition of increasing elephant grass levels. Maximum pH value of 4.82 was estimated for the 38.7% elephant grass level in

the mass ensiled. Interaction effect was found between elephant grass level × inoculant on the ammonia contents, with maximum content of 11.8% with 14.4% elephant grass, in the inoculated silage. Quadratic effect of elephant grass was observed on the lactic and propionic acids contents. For the lactic acid, minimum content of 2.53% was estimated with the 31.2% elephant grass level and for the propionic acid, maximum content of 1.53% was estimated with the 51% elephant grass level. There was effect of interaction on the contents of butyric acid; maximum content of 0.28% was obtained with 49.9% elephant grass, and minimum content of 0.12%, with 48.4% elephant grass in the mixture, without and with inoculant, respectively. It could be verified that non-inoculated silages presented the highest butyric acid contents.

Table 1. Mean contents of dry mater (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent insoluble nitrogen as proportion of total nitrogen (ADIN/Total-N), latic acid bacteria (LAB), fungi, yeasts, lactic acid (LA), propionic acid (PA), butyric acid (BA), pH and ammonia (NH_3/N) of Campo Grande *Stylosanthes* silages with increasing elephant grass cv. Cameroon proportions (0; 25; 50; 75 and 100%), without and with inoculants.

	Elephant grass proportions (%)						Inoculant		Level of significance[1]				
	0	25	50	75	100	SEM	without	with	P	I	PxI	L	Q
Chemical Composition (%)													
DM	23.4	22.7	21.3	20.3	21.1	0.11	21.9	21.6	***	***	NS	*	NS
CP	13.5	12.5	11.0	9.1	8.6	0.24	10.9	11.0	***	NS	NS	***	NS
NDF	587	62.9	67.1	71.0	74.0	0.63	66.7	66.8	***	NS	NS	***	NS
ADF	42.7	43.2	46.8	46.6	46.3	0.58	45.6	44.7	***	*	NS	*	NS
ADIN	21.5	17.2	16.6	16.3	10.1	0.71	15.6	17.1	***	*	NS	*	NS
Populations of microorganisms (log cfu/g)													
LAB	7.36	7.82	8.03	8.14	6.60	0.19	7.5	7.7	***	NS	NS	NS	***
Fungi	4.41	4.26	3.77	3.46	2.10	0.21	3.2	3.7	***	NS	NS	***	***
Fermentative characteristics													
LA (%)	3.58	3.02	3.92	3.25	8.78	0.52	5.1	3.9	***	*	NS	NS	**
PA (%)	0.90	1.40	1.58	1.16	1.05	0.13	1.2	1.2	*	NS	NS	NS	*
pH	4.74	4.58	4.67	4.85	3.76	0.032	4.4	4.5	***	***	NS	NS	**
Without Inoculant													
Yeasts	0.98	1.05	0.42	0.20	-	0.076	-	-	***	NS	***	***	NS
BA (%)	0.18	0.23	0.38	0.23	0.18	0.028	-	-	NS	***	*	NS	*
NH_3 (%)	12.5	5.3	12.8	9.0	11.3	1.48	-	-	***	NS	***	NS	NS
With Inoculant													
Yeasts	0.65	0.78	0.59	0.46	-	0.076	-	-	***	NS	***	*	**
BA	0.18	0.11	0,.14	0.14	0.16	0.028	-	-	NS	***	*	NS	*
NH_3/N	11.0	12.1	12.8	5.0	6.2	1.48	-	-	***	NS	***	***	**

[1]P = elephant grass proportions; I = Inoculant; P x I = Interaction between elephant grass proportions and inoculant. L = linear effect; Q = quadratic effect. *, P < 0·05; **, P < 0·01; ***, P < 0·001.

Conclusions The Campo Grande *Stylosanthes* improved the nutritive value of elephant grass silages and all of them presented satisfactory fermentative profile.

Acknowledgements Minas Gerais Research Foundation/FAPEMIG.

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Study of the effect of *Lactobacillus buchneri* inoculation on the aerobic stability and fermentation characteristics of alfalfa-ryegrass, red clover and maize silage

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Keywords: aerobic stability, alfalfa-ryegrass, *Lactobacillus buchneri*, maize, red clover.

Introduction Acetic acid inhibits the growth of spoilage organisms and increases aerobic stability of silage (Danner et al. 2003). Therefore, the effect of a heterofermentative lactic acid bacteria (LAB) inoculant (Lalsil Fresh®, Lallemand) containing *Lactobacillus buchneri* was assessed in alfalfa-ryegrass silage, red clover silage and maize silage.

Material and methods In 2008, trials were performed with red clover and with a mixture of alfalfa and Italian ryegrass, while the trial with maize silage was performed in 2009. All trials were conducted using microsilos (content 2.75 litre) equipped with a CO_2-valve. Besides two dosages of *L. buchneri* (low dosage (LD) : 5.00 log cfu/g fresh matter (FM), high dosage (HD) : 5.48 log cfu/g FM), a control treatment of sterile distilled water was included. All solutions were applied on chopped material by hand held sprayers in a ratio of 100 ml of solution per 10 kg of FM. The characteristics of the starting material of the three trials are given in Table 1.

Table 1. Starting material of ensiling trials with alfalfa-ryegrass, red clover and maize.

	alfalfa-ryegrass	red clover	maize
Dry matter (DM) (g/kg FM)	382.8	371.2	351.1
Sugar (g/kg FM)	14	26	25
Yeasts (log cfu/g FM)	<2	<2	5.7
Moulds (log cfu/g FM)	4.0	4.2	4.8
LAB (log cfu/g FM)	6.7	7.0	5.5
Enterobacteria (log cfu/g FM)	<1	<1	4.8

Per treatment, 6 microsilos were ensiled at a mean silo density of respectively 164.7, 154.15 and 196.25 kg DM/m³ for alfalfa-ryegrass, red clover and maize silage. Microsilos were weighed on a weekly basis to monitor the fermentation losses. All microsilos were subjected to aerobic stress during 24 hours at 71-72 days after ensiling and desiled after 90 days. Samples were taken from 5 microsilos per treatment for chemical analysis (DM), pH, lactic acid, acetic acid and butyric acid), microbial analysis (counts of yeasts, moulds and lactic acid bacteria) and determination of the aerobic stability according to Honig (1990). DM content at desiling was corrected for volatile compounds (Dulphy and Demarquilly 1981).

Obtained data were statistically analyzed by SAS 4.1. Normally distributed, homoscedastic data were subjected to one way ANOVA with Tukey as *post hoc* test. Otherwise, data were subjected to a non-parametric test according to Kruskal-Wallis with Bonferroni correction.

Results and discussion In the trials with alfalfa-ryegrass and red clover, there were no significant differences between treatments for the microbial counts. In the trial with maize, yeast and mould counts did not differ significantly between treatments, but the number of lactic acid bacteria was significantly higher in case of high dosage of *L. buchneri* (7.5 log cfu/g FM) compared to the control treatment (6.7 log cfu/g FM).

Results of the aerobic stability determination and chemical analyses are presented in Table 2. In the trials with alfalfa-ryegrass and red clover, the aerobic stability of the untreated control silage was significantly lower compared to both treatments inoculated with *L. buchneri*. This was not the case for the maize silage : only inoculation with low dosage of *L. buchneri* resulted in a significantly higher aerobic stability than the control treatment, although the acetic acid content in case of high dosage inoculation with *L. buchneri* was significantly higher than in the control treatment. The standard deviations for aerobic stability were very high, which might explain these observations.

In the trial with alfalfa-ryegrass, the untreated control had a significantly lower pH than the inoculated treatments, which can be explained by the significantly higher lactic acid content and the significantly lower acetic acid content of the control treatment. A similar pattern was observed for the red clover trial. In the maize trial, the lowest pH was observed in silage inoculated with the high dosage of *L. buchneri*, but differences in absolute value were very small. The control treatment contained significantly less lactic acid than the inoculated treatments and significantly less acetic acid than the treatment with high dosage of *L. buchneri*.

Butyric acid could not be detected in any sample of the trials with red clover and maize. In the alfalfa-ryegrass trial, low amounts of butyric acid were found only in control samples, but there were no significant differences in butyric acid content between treatments.

Table 2. Results of aerobic stability determination and chemical analysis.

Treatment			aer.stab. (h)		pH		lactic acid [a]		acetic acid [a]	
Alfalfa -ryegrass	control	mean	144.71	a	4.54	a	4.83	a	1.85	a
		st.dev.	97.17		0.01		0.13		0.30	
	L. buchn. LD	mean	360.00	b	4.58	b	4.12	b	3.02	b
		st.dev.	- [b]		0.01		0.25		0.37	
	L. buchn. HD	mean	360.00	b	4.59	b	4.08	b	3.11	b
		st.dev.	- [b]		0.02		0.43		0.41	
				***		***		**		**
Red clover	control	mean	295.89	a	4.36	a	6.49	a	2.21	a
		st.dev.	22.24		0.02		0.14		0.11	
	L. buchn. LD	mean	360.00	b	4.46	b	5.60	ab	3.07	ab
		st.dev.	- [b]		0.02		0.35		0.37	
	L. buchn. HD	mean	360.00	b	4.47	b	5.09	b	3.41	b
		st.dev.	- [b]		0.06		1.12		0.95	
				***		**		*		*
Maize	control	mean	85.89	a	3.77	ab	4.13	a	1.59	a
		st.dev.	15.58		0.01		0.31		0.25	
	L. buchn. LD	mean	126.59	b	3.77	a	4.50	b	1.95	ab
		st.dev.	28.68		0.02		0.24		0.26	
	L. buchn. HD	mean	115.33	ab	3.74	b	4.54	b	2.13	b
		st.dev.	15.99		0.01		0.17		0.16	
				***		*		*		**

[a] % of corrected DM

[b] no heating within 15 days → 360 hours as fixed value

Conclusions It can be stated that inoculation of an alfalfa-ryegrass mixture or red clover with *L. buchneri* resulted in an altered fermentation pattern and a higher aerobic stability compared to the untreated control. Maize silage was clearly more sensitive to heating than alfalfa-ryegrass silage and red clover silage. Inoculation with *L. buchneri* increased the aerobic stability of maize silage by increasing the acetic acid content, but it did not have a systematic effect on lactic acid content and pH in this experiment.

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Effects of *Lactobacillus rhamnosus* inoculation and molasses addition on fermentation, aerobic stability and bacterial community in direct-cut and wilted lucerne silage

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Keywords: aerobic stability, bacteria community, lucerne, silage

Introduction Silage prepared from legume crops can resist deterioration after exposure to air. Stability is unaffected by DM, pH, yeast counts and sugar addition at ensiling (O'Kiely and Muck, 1992a), as well as by concentrations of lactic acid, volatile fatty acids and ethanol in the silage (O'Kiely and Muck 1992b). Because microorganisms associated with stability have not been identified, we prepared laboratory-scale silage from direct-cut and wilted lucerne, then performed microbial counts and analysed the fermentation products and bacterial communities. *Lactobacillus rhamnosus* and molasses were added at the time of ensiling to facilitate lactic acid fermentation and subsequent spoilage after silo opening.

Material and methods First-growth lucerne harvested at the early blooming stage was ensiled directly (DM 256 g/kg) or after wilting for 6 h (DM 522 g/kg), with and without *L. rhamnosus* (10^6 cfu/g) and/or molasses (10 g/kg). The pre-ensiled crop was chopped at a theoretical cut length of 17 mm using a forage cutter. A 300 g sample was placed in a laminated plastic pouch and tightly packed with a commercial vacuum sealer. Silages were made in triplicate, and silos were stored at ambient temperature for 60 days. Microbial counts, fermentation products and bacterial communities were determined by plate-culture, ion-exclusion polymeric HPLC with refractive index detection and denaturing gradient gel electrophoresis, respectively (Nishino et al. 2012). BLAST searches were performed against the GenBank database to determine the closest relatives of the partial 16S rRNA gene sequences.

Table 1. Microbial counts and fermentation products of direct-cut and wilted lucerne silage added with and without *Lactobacillus rhamnosus* and molasses.

	Direct-cut silage					Wilted silage				
	C	M	L	L+M	SE	C	M	L	L+M	SE
Dry matter (g/kg)	207	211	215	217	1.80	537	526	535	544	3.78
pH	5.65^{a}	4.97^{b}	4.94^{b}	4.90^{b}	0.08	5.81^{x}	4.67^{y}	4.40^{y}	4.26^{y}	0.16
Lactic acid (g/kg DM)	25.6^{b}	51.7^{ab}	82.8^{a}	57.9^{ab}	7.14	13.4^{y}	17.0^{y}	48.0^{x}	53.5^{x}	2.12
Acetic acid (g/kg DM)	38.5^{b}	57.5^{a}	42.0^{ab}	49.2^{ab}	3.54	3.79^{y}	8.22^{x}	2.38^{yz}	1.74^{z}	0.40
Ethanol (g/kg DM)	4.13^{ab}	6.00^{a}	1.77^{b}	3.96^{ab}	0.67	0.74^{y}	2.98^{x}	0.88^{y}	0.00^{z}	0.02
LAB (log cfu/g)	7.58	8.80	8.31	8.76	0.17	6.71^{y}	7.83^{x}	7.84^{x}	5.77^{z}	0.10
Yeasts (log cfu/g)	<2.00	<2.00	<2.00	<2.00	---	<2.00	<2.00	<2.00	<2.00	---
ENB (log cfu/g)	<2.00	<2.00	<2.00	<2.00	---	<2.00	<2.00	<2.00	<2.00	---

C; untreated control, M; molasses addition (10 g/kg), L; *Lactobacillus rhamnosus* inoculation (10^5 cfu/g). LAB; lactic acid bacteria, ENB; enterobacteria.

Mean of 3 silages. Values in the same row with different superscript letters (a-c, x-z) are significantly different ($P<0.05$).

Results and discussion In the absence of *L. rhamnosus*, acetic acid predominated in the fermentation of direct-cut lucerne silage (Table 1). Lactic and acetic acid production was enhanced in silage to which molasses was added, whereas only lactic acid production was increased in silage to which *L. rhamnosus* was added. High pH (about 4.9) was seen even in *L. rhamnosus*- and molasses-added silages. Yeasts and enterobacteria were not detected upon the opening of silos and no distinctive deterioration was observed for any of the direct-cut silages.

Wilting suppressed acetic acid rather than lactic acid production; lactic acid was the predominant product in all wilted lucerne silages. Similar to direct-cut silage, lactic and acetic acid production was enhanced in molasses-added silage. Intensive lactic acid fermentation occurred in silage to which *L. rhamnosus* or both *L. rhamnosus* and molasses were added. These wilted silages were supposed to deteriorate after silo opening, but no heating was seen over 7 d in the presence of air. Yeast numbers were below detectable levels at silo opening and after the 7-day aerobic spoilage test.

Lactobacillus brevis was common in all silages (Figure 1). In untreated direct-cut silage, both desirable (*Pediococcus pentosaceus* and *Lactococcus lactis*) and undesirable (*Clostridium tyrobutyricum*) bacteria were detected. *Lactobacillus buchneri* distinctively appeared upon molasses addition regardless of wilting. The bacterium was detectable after the 7-day aerobic spoilage test in direct-cut silages, except for silage to which only *L. rhamnosus* was added. In wilted silage to which *L. rhamnosus* and molasses were added, bacteria other than *L. brevis* and *L. rhamnosus* appeared to have been elimi-

nated. Although this study suggested an association of *L. buchneri* with spoilage inhibition in direct-cut lucerne silage, the data are insufficient to account for the high stability of wilted silage, particularly silage to which *L. rhamnosus* was added. Further experiments are needed to understand the microbial community associated with lucerne ensiling.

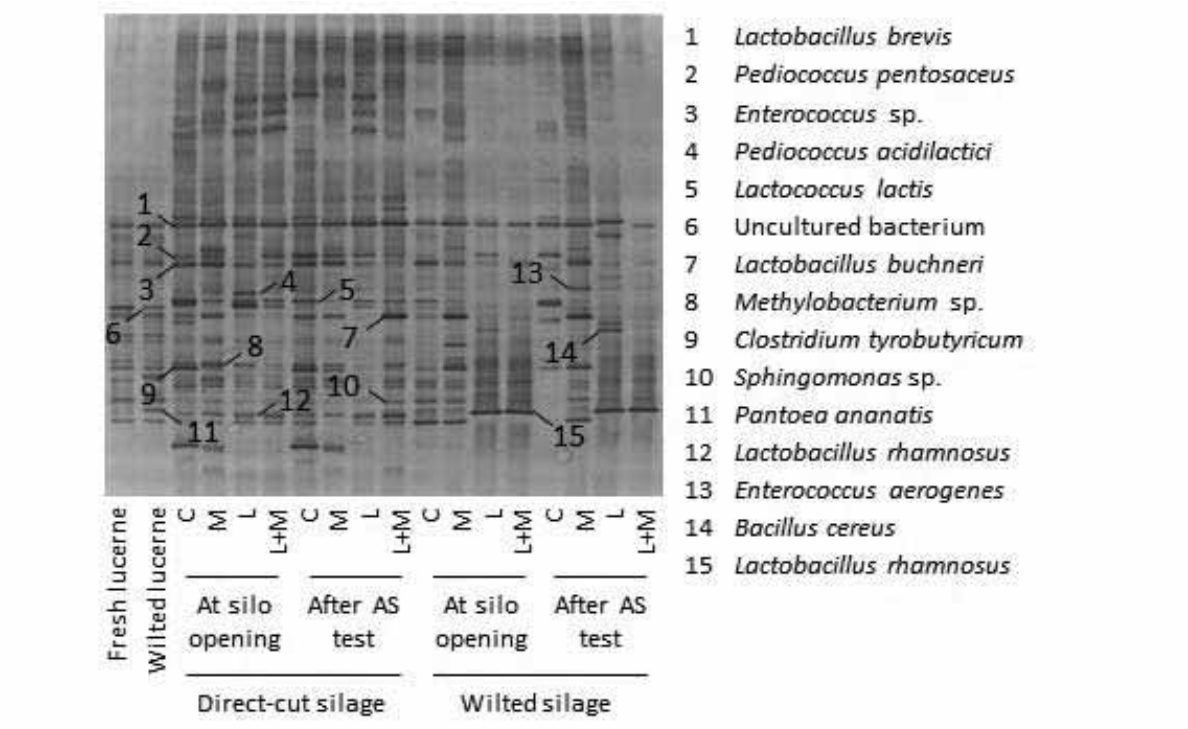

Figure 1. Bacteria communities of direct-cut and wilted lucerne silage added with and without *Lactobacillus rhamnosus* and molasses.

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Ensiling of forage legumes in Finland

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Keywords: Red clover, lucerne, fodder galega, lotus, silage quality

Introduction Red clover is the most extensively grown and persistent forage legume in Finland (Nissinen et al. 2002). Other legume species are investigated for improved persistency and yield. In some locations lucerne has given high yields and fodder galega has shown good persistency. In this study ensilability characters of four forage legumes, red clover (RC), lucerne (LU), fodder galega (FG) and lotus (LO) were studied on laboratory scale silos over two years.

Material and methods Lucerne (*Medicago sativa* L., *cv*. Vertus), red clover (*Trifolium pratense* L., *cv*. Björn) and fodder galega (goat's rue) (*Galega orientalis* Lam., *cv*. Gale) were studied in 1998 and red clover and lotus (bird's foot trefoil) (*Lotus corniculatus* L., *cv*. Leo) in 1999 at the University of Helsinki, Finland. Factorially arranged treatments were two growth stages, two wilting levels and three additives (see Tuori et. al. 2012).

Results and discussion In 1998 the time span between budding (BUD) and flowering (FLO) was 12, 11 and 14 days for LU, RC and FG, respectively, and in 1999 13 and 12 days for RC and LO. The contents of the herbage at BUD stage at ensiling (kg^{-1} DM) for LU, RC and FG were 196, 191 and 216 g crude protein (CP); 51, 62 and 38 g water soluble carbohydrates (WSC); 406, 417 and 536 g NDF and buffering capacity (BC) was 79, 88 and 67 g lactic acid kg^{-1} DM. In 1999 the respective composition (kg^{-1} DM) for RC and LO was 180 and 156 g CP; 84 and 82 g WSC; 303 and 312 g NDF; BC was 62 and 71 g lactic acid. The average counts of epiphytic lactic acid bacteria (log cfu g^{-1} FM) in 1998 were 6.2, 5.3 and 6.5 for LU, RC and FG, and in 1999 the counts were 3.3 and 3.1 for RC and LO, respectively. In 1998 the content of CP decreased from BUD to FLO stage by 2.3, 3.3 and 2.4 g day^{-1}, but NDF increased by 5.2, 4.3 and 2.6 g day^{-1} for LU, RC and FG. For RC and LO in 1999 the respective changes were -1.5 and -0.5 g for CP, and 2.8 and 3.3 g for NDF.

Lucerne was very difficult to ensile. At budding stage with LW (wilting to 250 g DM kg^{-1}) the quality of CO (control without additive) and LAB (*Lactobacillus plantarum* Ecocyl®) silages was very poor (DLG-points being 2 and 3) and at HW (wilting to 400 g DM kg^{-1}) only acceptable (DLG 61 for both CO and LAB). At flowering stage the quality was better, at LW resulted 82 and 74 and at HW 92 and 98 with CO and LAB. The quality of FA (formic acid) silages was very good (DLG>89) for all LU treatments. Also RC was difficult to ensile at budding stage after LW with CO and LAB. In 1998 DLG-score for CO and LAB was 34 and 29 and in 1999 40 and 95. With HW at budding stage the quality of RC silage was good or very good with CO and LAB likewise with the flowering stage at both wilting levels. Exception was RC in 1999 with LW at flowering stage when the quality of CO was -3 containing 25.5 g kg^{-1} DM butyric acid and ammonia-N was 268 g kg^{-1} N. Same RC treatment ensiled with FA was also poor, DLG only 42 with high butyric acid content. All other FA treatments for RC gave good or very good quality silages. FG silages at budding stage were good quality, at LW 74 and at HW 78 for both CO and LAB. However, ammonia-N was high (139, 114, 122 and 123 g N kg^{-1}N, respectively) with increased pH indicating extensive proteolysis. At flowering stage and LW the quality of LAB silage was good (78) and CO silage acceptable (68). After HW at flowering stage both CO and LAB gave very good quality silage for FG as well as FA for all FG treatments. Quality of lotus silage was good or very good even with LW at budding stage with CO and LAB (DLG 85 and 94). Noteworthy was the low ammonia-N in all lotus treatments.

Conclusions For successful ensiling of forage legumes, delayed harvest and higher wilting level can improve the silage quality and are important when using Lactobacillus inoculants. If these conditions cannot be achieved, the acid additives are preferable. The present DLG silage evaluation scheme does not include ammonia-N giving high DLG-values even for silages with high ammonia content. Lotus was easier to ensile with LW at BUD with an inoculant treatment compared to other legumes.

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Table 1. Fermentation quality parameters of the forage legume silages in 1998 and 1999.

		BUD-LW CO	BUD-LW FA	BUD-LW LAB	BUD-HW CO	BUD-HW FA	BUD-HW LAB	FLO-LW CO	FLO-LW FA	FLO-LW LAB	FLO-HW CO	FLO-HW FA	FLO-HW LAB	SEM	Growth stage	Wilting	Ensiling	CO vs. FA	CO vs.LAB	FA vs.LAB
	DM	149	154	144	468	476	472	266	257	256	417	413	402	4.3	<0.001	<0.001	0.06	1.00	0.09	<0.10
	pH	4.91	4.23	4.88	4.86	4.70	4.89	4.25	4.14	4.20	4.52	4.53	4.30	0.013	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	LA	100	75	92	83	58	85	127	43	114	96	59	91	5.0	0.04	<0.001	<0.001	<0.001	0.27	<0.001
RC98	AC	69	22	75	37	16	32	35	15	22	28	17	16	1.6	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	BUT	0.19	2.72	0.18	0.16	0.16	0.16	0.21	0.17	0.26	0.22	0.20	0.18	0.739	0.37	0.31	0.41	0.48	1.00	0.48
	NH_3N	124	53	128	94	79	91	73	44	61	77	64	53	3.0	<0.001	0.02	<0.001	<0.001	<0.01	<0.001
	DLG	34	84	29	86	100	94	83	96	95	99	99	100	3.1	<0.001	<0.001	<0.001	<0.001	0.20	<0.001
	DM	223	235	224	348	347	332	307	307	298	435	425	430	2.2	<0.001	<0.001	<0.001	0.95	<0.001	<0.001
	pH	6.54	4.25	6.67	5.56	4.60	5.63	4.66	4.21	4.70	4.74	4.52	4.57	0.102	<0.001	<0.001	<0.001	<0.001	0.97	<0.001
	LA	11	74	28	53	72	62	90	53	92	74	59	72	3.8	<0.001	<0.01	0.03	0.03	0.06	0.96
LU98	AC	47	19	49	48	27	48	36	12	37	28	14	21	1.4	<0.001	<0.01	<0.001	<0.001	0.71	<0.001
	BUT	18.85	0.07	16.97	0.10	0.11	0.13	0.12	0.12	0.10	0.09	0.10	0.17	1.291	<0.001	<0.001	<0.001	<0.001	0.88	<0.001
	NH_3N	208	28	220	150	53	151	108	20	111	96	51	65	2.4	<0.001	<0.001	<0.001	<0.001	0.10	<0.001
	DLG	2	94	3	61	97	61	82	100	74	92	99	98	2.5	<0.001	<0.001	<0.001	<0.001	0.96	<0.001
	DM	247	252	248	289	293	291	293	285	282	454	456	459	1.8	<0.001	<0.001	0.36	0.78	0.71	0.33
	pH	4.90	4.35	4.84	4.85	4.38	4.82	4.56	4.19	4.57	4.42	4.32	4.32	0.016	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001
	LA	63	22	61	55	62	55	70	34	79	65	48	71	2.3	<0.001	<0.01	<0.001	<0.001	<0.10	<0.001
FG98	AC	35	6	35	32	14	33	40	11	33	17	9	14	1.3	<0.001	<0.001	<0.001	<0.001	0.06	<0.001
	BUT	0.05	0.17	0.20	0.08	0.18	0.37	0.56	0.15	0.19	0.11	0.13	0.13	0.093	0.51	0.32	0.60	0.79	0.94	0.58
	NH_3N	139	33	114	122	70	123	116	48	101	72	43	65	3.8	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	DLG	74	91	74	78	91	78	68	95	78	100	100	100	1.6	<0.001	<0.001	<0.001	<0.001	0.12	<0.001
	DM	263	275	283	415	409	412	244	263	266	415	424	428	2.9	0.12	<0.001	<0.001	<0.01	<0.001	0.08
	pH	5.34	4.55	4.18	5.38	4.94	4.06	5.84	4.70	3.87	5.06	4.73	4.05	0.044	0.19	0.12	<0.001	<0.001	<0.001	<0.001
	LA	46	3	110	13	4	94	7	3	78	23	1	77	3.3	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001
RC99	AC	26	5	16	8	5	14	19	10	17	16	9	14	2.8	0.29	<0.01	<0.001	<0.001	0.66	<0.01
	BUT	14.50	0.13	0.20	0.65	0.14	0.16	25.52	6.22	0.29	1.80	0.22	0.32	3.896	0.18	<0.01	<0.01	<0.01	<0.01	0.86
	NH_3N	215	26	21	55	29	27	268	58	19	68	40	27	7.3	<0.001	<0.001	<0.001	<0.001	<0.001	0.02
	DLG	40	87	95	90	90	100	-3	42	83	71	93	95	13.3	0.01	<0.001	<0.001	0.02	<0.001	0.26
	DM	255	260	263	469	465	471	282	283	284	410	415	415	1.5	<0.001	<0.001	<0.01	0.16	<0.01	0.11
	pH	4.84	4.57	4.22	5.52	4.84	4.33	4.49	4.37	4.04	4.99	4.82	4.20	0.068	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	LA	47	5	99	2	1	85	57	2	110	17	0	77	8.5	0.40	<0.001	<0.001	<0.001	<0.001	<0.001
LO99	AC	14	6	15	5	6	10	13	5	15	9	6	12	1.9	0.61	<0.01	<0.001	<0.01	0.16	<0.001
	BUT	0.51	0.11	1.02	0.22	0.11	0.09	0.39	0.11	0.19	0.17	0.17	0.37	0.099	0.07	<0.01	<0.01	0.03	0.39	<0.001
	NH_3N	77	22	36	37	21	21	65	23	34	68	40	33	2.5	<0.001	<0.001	<0.001	<0.001	<0.001	0.07
	DLG	85	86	94	90	100	100	90	91	99	90	91	87	2.2	0.30	0.11	<0.01	0.10	<0.01	0.19

RC = red clover; LU = Lucerne; FG = fodder galega; LO = lotus; BUD = budding stage; FLO = flowering stage; LW = low wilting; HL= high wilting; DM = dry matter, kg DM kg^{-1};LA = lactic acid, g kg^{-1}DM; AC = acetic acid, g kg^{-1}DM; BUT = butyric acid, g kg^{-1}DM; NH_3N, g N kg^{-1}N; DLG = fermentation quality, (Optimum = 100 points) ; CO = control without additive; FA = formic acid; LAB = *lactobacillus* inoculant (Ecosyl®); number of observations: 3, Lotus-flowering-HW-CO 2 obs.; SEM for 2 obs. 1.73 x SEM)

Ensiling of red clover in Finland

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Keywords: Red clover, silage, formic acid, *Lactobacillus* inoculants, wilting, growth stage

Introduction Red clover (RC) is an important forage crop especially in organic farming due to its nitrogen fixing ability by *Rhizobium* bacteria. Crude protein (CP) content and buffering capacity (BC) are high and content of water soluble carbohydrates (WSC) is low making RC difficult to ensile. To secure good silage quality acid based preservatives are usually used. However, acids are corrosive and hazardous at work and therefore biological additives are preferred over acids. In this study a *Lactobacillus* containing inoculant was compared with formic acid and an untreated control for the ensiling of RC, harvested at two growth stages and wilted to two dry matter (DM) levels.

Material and methods Ensiling of red clover (*Trifolium pratense* L., *cv*. Björn) was studied in laboratory scale silos in 1998 and 1999 at the University of Helsinki, Finland. Factorially arranged treatments were two growth stages, two wilting levels and three additives (Pahlow et al. 2001). The primary growth herbages were cut either at budding (BUD) or flowering stage (FLO), wilted to 250 (LW) or 400 (HW) g DM^{-1}kg and ensiled either without additive (CO), with formic acid (FA 850 g kg^{-1}, applied at a rate of 6 or 3.5 l^{-t}) or with lactic acid bacteria (LAB at the inoculation rate of 10^6 colony forming units (cfu) g^{-1}) for the LW and HW crop. The inoculant (Ecosyl®) contained a single strain of *Lactobacillus plantarum* MTD/1. Three replicate silos were stored at room temperature (25°C) until opening after 104 – 172 days. A fourth silo was opened after 3 days for measuring pH. The silos were weighed to determine fermentation losses on days 1, 2, 3, 5, 7, 12, 21, 28, 56 and 84 after filling. After opening of the silos the pH was measured and the samples were taken for fermentation quality determinations. Herbage samples before ensiling were analyzed for DM, CP, WSC and BC. Silage quality was evaluated according to standard methods of VDLUFA, listed in DLG-Handbook (DLG, 2011). In the statistical model growth stage, wilting level, additives and their interactions were fixed factors and year and year x treatments random factors. Treatment means were tested by Tukey's test.

Results and discussion CP-content of RC at BUD stage was 191 and 180 g kg^{-1} DM, and it decreased by 3.3 and 1.5 g CP day^{-1} in years 1998 and 1999. BC was 88 and 62 g lactic acid kg^{-1} DM and decreased by 1.1 and 0.9 g day^{-l}, respectively. Content of WSC was 76 and 81 g kg^{-1}DM and epiphytic lactic acid bacteria count was 5.4 and 3.3 log cfu g^{-1} fresh matter in years 1998 and 1999. Growth stage had no significant effect on silage fermentation or fermentation losses, thus results in Table 1 are means over growth stages. The pH three days after ensiling was higher at HW than at LW silage, but after opening the silos differences in pH were not significant. Higher wilting reduced fermentation intensity, especially acetic acid and ethanol contents were lower than in the LW silage. Higher wilting reduced solubility of nitrogen by 12% and fermentation losses by 28%. Formic acid reduced fermentation. WSC content was highest in FA silages. Compared to CO acetic acid, ethanol, VFA, ammonia-N content and fermentation losses were lower in FA silage. Compared to CO inoculant reduced pH, tended to decrease ammonia-N content and to increase lactic acid (LA) content. At the low wilting level quality of CO silages was poor (DLG points <51) but FA and LAB gave good (DLG>71) quality silage. Wilting up to 400 g DM^{-1} kg gave good or very good (DLG>89) fermentation quality with all additives. Quality differed greatly between two years. In 1999 the LAB count was lower and fermentation was reduced but content of butyric and higher acids was higher especially at flowering stage compared to the previous year. This is reflected in the ratio of lactic acid to total fermentation products in silage, 0.71 in 1998 and 0.45 in 1999.

Conclusions Wilting is a more important factor than growth stage for good quality ensilage. Inoculant and FA produced good quality silage at both wilting levels. However, wilting to higher DM concentration is recommended. For reliable results on the effects of additives in red clover silage fermentation, ensiling at least on two different years is advisable.

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Table 1. Effect of wilting and silage additive on fermentation quality of red clover silage.

	Low wilting (W1)			High wilting (W2)				Significance (P-value)				
Silage additive	CO	FA	LAB	CO	FA	LAB	SEM	W1 vs W2	CO vs FA	CO vs LAB	FA vs LAB	Wilting × silage additive
DM, g/kg	231	237	237	429	430	428	24.8	<0.001	0.99	0.99	1.00	0.99
pH, after 3 days ensiling	5.35	4.56	4.52	5.70	5.10	4.95	0.234	0.04	0.03	0.02	0.91	0.92
pH	5.08	4.40	4.28	4.95	4.73	4.32	0.231	0.69	0.17	0.03	0.52	0.63
Lactic acid, g kg^{-1} DM	70	31	99	54	31	87	26.5	0.41	<0.10	<0.10	<0.01	0.84
WSC, g kg^{-1} DM	8	65	15	46	73	22	18.2	0.07	<0.01	0.74	<0.01	0.32
Acetic acid, g kg^{-1} DM	37	13	33	22	12	19	10.9	0.07	0.04	0.80	0.11	0.47
Propionic acid, g kg^{-1} DM	1.4	0.1	0.9	0.1	0.0	0.0	0.43	0.07	0.31	0.79	0.66	0.40
Butyric acid, g kg^{-1} DM	10.1	2.3	0.2	0.7	0.2	0.2	3.22	0.13	0.35	0.21	0.93	0.27
Total VFA, g kg^{-1} DM	57	16	34	23	12	20	9.8	0.03	0.02	0.26	0.33	0.23
Ethanol, g kg^{-1} DM	13.3	3.8	7.0	4.8	2.2	3.0	3.15	0.02	0.04	0.20	0.62	0.30
Lactic acid:ferm. products	0.45	0.45	0.72	0.59	0.48	0.79	0.164	0.40	0.88	0.12	0.05	0.86
NH3N, g kg^{-1} N	170	45	58	73	53	49	27.0	0.17	0.05	0.07	0.98	0.16
Soluble N, g kg^{-1} N	673	479	589	543	478	514	35.4	<0.01	<0.01	0.13	0.04	0.09
DLG-points	38	77	76	86	95	97	12.9	0.02	0.19	0.20	0.99	0.46
Fermentation losses, g kg^{-1} DM	86	36	55	47	34	45	7.7	0.02	<0.01	0.12	0.19	0.09

CO = control; FA = formic acid; LAB = *Lactobacillus* inoculants; DLG-points according to DLG (2011).

The aerobic stability of total mixed ration can be managed by silage additive

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Keywords: aerobic stability, fermentation quality, grass silage, heterofermentative, total mixed ration

Introduction There is plenty of research on aerobic stability of silages (Pahlow and Muck 2009). However, the aerobic stability of the total mixed ration (TMR) prepared from grass silages has gained less attention. A comparative experiment was conducted to explore the effects of silage additives on silage quality and on the aerobic stabilities of the silage and the subsequent TMRs.

Material and methods Eight commercial silage additives (Table 1) including lactic acid bacteria (LAB) inoculants (L1 – L5), salt (S6) and acid based products (A7 and A8) were dosed to the prewilted precision chopped grass material (timothy-meadow fescue, dry matter (DM) 220 or 540 g/kg) according to the dosage recommendations of the manufacturer. In addition, control treatments without additive were prepared. The grass was ensiled in 12 l laboratory silos in triplicate for three months. Silages were analysed for fermentation quality (Kuoppala et al. 2008), yeast and mould counts (Seppälä et al. 2012) and ethanol (enzymatic kit, Cat No.981680, analyser Pro 981489, KONE Instruments). TMRs were prepared using the silages as the main component (grass silage 500, pelleted concentrate 400 and brewer's grain 100 g/kg). Aerobic stability of the silages and TMRs were measured as described by Seppälä et al. (2012) with the exception that the sample size was 174 g DM. Treatment effects on silage quality and aerobic stability were tested by variance analysis using the GLM-procedure of the SAS system and Tukey test.

Table 1. Silage additives and application levels.

Code	Composition	Dosage
0	no additive	0
L1	*Lactobacillus plantarum* (VTT E-78076), Lactofast	1 000 000 cfu/g
L2	*Enterococcus faecium* BIO 34 (DSM 3530), *Lactobacillus brevis* IFA 92 (DSM 19456), *Lactobacillus plantarum* IFA 96 (DSM 19457), Stabil Plus	200 000 cfu/g
L3	*Lactobacillus plantarum* (NCIB 30083, 30084), *Pediococcus acidilactici* (NCIB 30085, 30086), cellulose, Feedtech 18	1 000 000 cfu/g
L4	*Lactobacillus plantarum* (DSM No 11672), *Pediococcus acidilactici* (DSM No 11673), cellulase, Josilac	1 000 000 cfu/g
L5	*Lactobacillus plantarum* (LSI ja L-256), *Pediococcus acidilactici* (P6 ja P11) *Lactococcus lactis* (SR3.54 NCIMB 30117), *Enterococcus faecium* (M74 NCIMB 11181), cellulase, sodium bentsoate, Feedtech 22	200 000 cfu/g + 300 g/t sodium bentsoate
S6	sodiumbentsoate, potassiumsorbate, sodium nitrite, Safesil	3 l/t*)
A7	formic acid 440, sodium formiate 200, propionic acid 120, bentsoic acid 15, glycerol 10, water 215 g/kg, AIV Nova	5 l/t
A8	formic acid 600,ammonium formate 200, water 200 g/kg, Prima (Agrimarket)	5 l/t

*) When ensiling the grass material with the dry matter level 220 g/kg the application level of additive was 2.62 l/t.

Results and discussion Spontaneous fermentation in the control treatment of moist grass material produced high concentrations of fermentation acids and used almost all sugars available in the grass material without pH drop under the critical level (pH 4) to ensure good quality (Table 2). The other silages had good or very good quality according to DLG silage quality grading (Kaiser et al. 2006). The silages with lower DM had strong lactic acid fermentation with all the LAB-treatments. Chemical additives (S6, A7 and A8) produced silages with unexpected low sugar content and high ethanol content on the lower DM level. The LAB-treatments were capable to produce lactic acid (> 35 g/kg DM) still when the DM of the raw material was 540 g/kg. The product with heterofermentative strain (L2, *L. brevis*) produced also acetic acid and was able to prevent growth of yeasts.

There is a general assumption that silages with good aerobic stability will provide TMR with good aerobic stability (Kung 2005). We detected that the moist grass without any additive turned to silage that had a good aerobic stability. However, the aerobic stability of the TMR prepared from the same silage was not improved compared to the aerobic stability of the other TMRs. In contrast chemical additives (salts and organic acids) were capable to improve aerobic stability of both the silage and TMR.We were not able to detect differences in aerobic stability of the silages with high DM content. However we could see differences between different additive treatments when respective TMRs were compared in aerobic stability. When a product with heterofermentative lactic acid bacteria (L2) was used as silage additive the aerobic stability of respective TMR was superior.

Table 2. Quality of the grass silages and aerobic stabilities of the silages and respective TMRs.

	Additive treatments[2]								
	0	L1	L2	L3	L4	L5	S6	A7	A8
Silage dry matter 220 g/kg									
pH	4.26^{A}	3.71^{C}	3.86^{BC}	3.75^{C}	3.72^{C}	3.73^{C}	3.98^{B}	4.01^{B}	4.01^{B}
Sugar[1]	6^{C}	40^{B}	13^{C}	50^{A}	48^{A}	50^{A}	8^{C}	13^{C}	12^{C}
Lactic acid [1]	89^{B}	135^{A}	125^{A}	123^{A}	135^{A}	135^{A}	92^{B}	70^{B}	69^{B}
Acetic acid [1]	46^{A}	8^{D}	24^{C}	10^{D}	11^{D}	11^{D}	41^{B}	25^{C}	21^{C}
VFA[1]	48^{A}	9^{E}	26^{CD}	11^{E}	12^{E}	12^{E}	41^{B}	27^{C}	21^{D}
Ethanol [1]	16^{A}	4^{C}	9^{B}	7^{BC}	4^{C}	5^{BC}	16^{A}	14^{A}	17^{A}
NH_4 N g/kg N	61^{A}	23^{B}	65^{A}	37^{AB}	23^{B}	38^{AB}	49^{AB}	39^{AB}	51^{AB}
Yeasts log cfu/g	< 2.0	3.9-5.4	< 2.0	4.4-5.0	3.3-4.3	< 3.8	< 2.0	< 2.0	< 2.0
Moulds log cfu/g	< 2.0	3.8-4.5	< 4.3	2.9-3.6	3.8-4.9	4.4-4.9	< 2.0	< 2.3	< 2.3
Stability silage[3]	330^{A}	35^{D}	133^{C}	29^{D}	35^{D}	62^{CD}	336^{A}	292^{AB}	220^{B}
Stability TMR[3]	10^{BCD}	4^{D}	8^{CD}	5^{D}	5^{D}	8^{CD}	34^{A}	24^{AB}	21^{ABC}
Silage dry matter 540g/kg									
pH	5.41^{A}	4.10^{F}	4.31^{E}	4.05^{F}	4.45^{D}	4.25^{E}	5.25^{B}	4.87^{C}	4.45^{D}
Sugar[1]	120^{AB}	99^{B}	115^{B}	126^{AB}	116^{B}	146^{AB}	130^{AB}	156^{A}	115^{B}
Lactic acid [1]	6^{F}	54^{B}	41^{D}	58^{A}	36^{E}	49^{C}	9^{F}	2^{G}	12^{F}
Acetic acid [1]	7^{C}	6^{C}	13^{A}	7^{BC}	6^{C}	7^{BC}	8^{B}	6^{C}	9^{B}
VFA[1]	7^{C}	7^{C}	14^{A}	8^{BC}	7^{C}	8^{C}	9^{B}	8^{C}	9^{B}
Ethanol [1]	11^{A}	7^{B}	8^{B}	5^{BC}	7^{B}	6^{BC}	7^{B}	4^{C}	7^{B}
NH_4 N g/kg N	25^{BC}	19^{CD}	27^{B}	27^{BC}	22^{CD}	20^{D}	26^{BC}	24^{C}	34^{A}
Yeasts log cfu/g	3.5 - 4.7	< 3.2	< 2.0	< 2.7	< 3.9	< 2.0	< 3.8	< 2.9	< 2.0
Moulds log cfu/g	< 2.3	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
Stability silage[3]	> 232	> 232	> 232	> 232	> 232	> 232	> 232	> 232	> 232
Stability TMR[3]	62^{B}	63^{B}	141^{A}	80^{B}	83^{B}	110^{AB}	71^{B}	74^{B}	59^{B}

Differences between values within same row without same superscript are statistically significant ($p < 0.05$).

[1]Unit of the values is g/kg DM, VFA= volatile fatty acids g/kg DM

[2]Additive treatments explained in Table 1.

[3]Aerobic stabilities of silages and total mixed rations (TMR), hours.

Conclusions When DM concentration of the grass material is relatively low additives were needed to ensure good fermentation quality of the silage. In addition, chemical additives were able to improve aerobic stability of both the silage and respective TMR. The LAB product with heterofermentative *Lactobacillus brevis* strain was capable to give better aerobic stability to silage or TMR than the homofermentative strains alone.

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The effect of different types of silage additives on dry matter losses, fermentation pattern, volatile organic compounds and aerobic stability of sorghum silage

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Keywords: chemical additives, ethanol, ethyl esters, sorghum, silage, volatile organic compounds (VOC)

Introduction Volatile organic compounds (VOC), e.g. alcohols, organic acids and esters thereof, which can be detected in silages, may detrimentally affect feed intake by dairy cattle (Weiss *et al*., 2009). As data on the formation of VOC in sorghum silages is still lacking, this study aimed at testing the effects of different silage additives on dry matter (DM) losses, fermentation pattern, VOC production and aerobic stability of this type of silages. Sorghum was chosen as silage type because it represents an important forage source for ruminants in semi-arid regions, and its production often bears the risk of excessive ethanol fermentation so that high concentrations of VOC are to be expected.

Material and methods Two varieties of *Sorghum bicolor* (Maya: 261 g DM/kg, 271 g DM/kg water-soluble carbohydrates; Goliath: 216 g/kg DM, 235 g/kg DM water-soluble carbohydrates) were chopped to a theoretical particle size of 20 mm, treated with silage additives, filled into 1.5 l glass jars and subsequently anaerobically stored at 25 °C for 105 days. Three replicates per treatment were prepared. The following treatments were tested: CON - Control, LP - *L. plantarum* DSM 3676/*L. plantarum* DSM 3677 (50%/50%, $1x10^5$ cfu/g forage; trade name: KOFASIL LAC), *Lactobacillus buchneri* DSM 13573 (LB)1 - $1x10^5$ cfu/g forage (trade name: KOFASIL S), LB2 - $2.5x10^5$ cfu/g forage, LB3 - $5x10^5$ cfu/g forage, LP+LB1 ($2x10^5$ cfu/g forage; trade name: KOFASIL DUO) and BS – 500 g/t sodium benzoate+300 g/t potassium sorbate (applied in 2 l/t aqueous solution; trade name: KOFASIL STABIL). All additives were manufactured by ADDCON EUROPE GmbH and diluted with tap water to give an application rate of 10 ml/kg fresh forage. The control treatment received 10 ml of tap water per kg fresh forage.

Chemical analysis of the fresh crop was performed according to official German standards for feed evaluation. DM of silages was measured and corrected for the loss of volatiles during drying according to Weissbach and Strubelt (2008). Determination of pH was done potentiometrically using a calibrated pH electrode. Lactic acid was analyzed by HPLC (Weiss and Kaiser, 1995); volatile fatty acids, alcohols and VOC were determined by GC as described by Weiss (2001). Losses of DM during fermentation were calculated according to Weissbach (2005), and aerobic stability was measured for 14 days by using the temperature method by Honig (1990).

Data were subjected to statistical analysis (2-way ANOVA) by employing the procedure MIXED of SAS. Differences among means were tested by the Tukey test, and significance declared at $P \leq 0.05$.

Results and discussion DM losses during fermentation were affected by variety and treatment, and interactions were observed between the two factors (table 1). However, application of BS to both varieties of sorghum always resulted in the lowest losses. Regarding the final pH after 105 days of fermentation, also significant differences were found among means, which were influenced by variety and treatment. The sole use of the heterofermentative *L. buchneri* strain consistently resulted in the highest pH of sorghum silages.

Table 2 summarizes the results of fermentation products, which play a crucial role in the formation of the determined VOC. Lactic and acetic acids were affected by variety and treatment, and an interaction was determined between the two factors for lactic acid. Ethanol was reduced by LB at all inoculation rates, and the lowest levels were consistently found if BS were used. The use of LP alone or in combination with LB1 did not affect ethanol production when compared with control silages. The concentrations of reaction products of ethanol and organic acids – ethyl lactate and ethyl acetate – were affected by variety and treatment. Application of BS and LB (regardless of inoculation rate) caused the lowest ester contents, and no differences between CON, LP and LP+LB1 were found. These observations are in line with results by Weiss and Auerbach (2012) on whole-crop maize silages, where the use of the combination of sodium benzoate and potassium sorbate showed the lowest VOC contents and outperformed the other tested chemical silage additives (mixtures of formic and propionic acids and their sodium salts).

Table 1. Effects of silage additives on DM losses and pH of sorghum silages.

Treatment	DM loss (%)		pH	
Variety	Goliath	Maya	Goliath	Maya
CON	8.4^{bA}	9.0^{dB}	3.70^{bcA}	3.77^{bcB}
LP	8.3^{bA}	8.8^{bcB}	3.68^{bA}	3.75^{bB}
LB1	9.2^{dB}	8.7^{bA}	3.88^{dB}	3.79^{cA}
LB2	9.3^{cdA}	9.0^{bdA}	3.89^{deB}	3.82^{dA}
LB3	9.4^{cdA}	9.2^{cdeA}	3.91^{eB}	3.83^{dA}
LP+LB1	9.0^{cA}	9.5^{eB}	3.71^{cA}	3.72^{aA}
BS	5.3^{aA}	6.1^{aB}	3.63^{aA}	3.72^{aB}
SEM	0.30	0.24	0.024	0.010
Significance level				
Variety	***		*	
Treatment	***		***	
Variety × Treatment	***		***	

means in columns with unlike superscripts and means within rows bearing unlike capital superscripts differ significantly at P≤0.05 (Tukey test)

Table 2. Effects of silage additives on selected fermentation products and volatile organic compounds of sorghum silages.

Treatment	Lactic acid (g /kg DM)		Acetic acid (g /kg DM)		Ethanol (g /kg DM)		Ethyl esters[1)] (mg/kg DM)	
Variety	Goliath	Maya	Goliath	Maya	Goliath	Maya	Goliath	Maya
CON	92.0^{bA}	40.3^{cB}	24.9^{abA}	27.3^{bcA}	31.7^{cA}	34.2^{cdA}	381^{dA}	587^{dB}
LP	90.2^{bA}	38.3^{bcB}	20.2^{aA}	22.0^{aA}	34.9^{cA}	28.8^{cA}	506^{dA}	586^{cdA}
LB1	24.3^{aA}	22.8^{bA}	47.4^{bcB}	37.0^{abdA}	19.9^{bA}	19.5^{bA}	251^{cA}	414^{bcB}
LB2	22.3^{aA}	24.6^{bA}	51.6^{cB}	45.5^{cdA}	18.9^{bA}	18.3^{bA}	245^{bcA}	365^{abcB}
LB3	19.9^{aA}	17.8^{aA}	53.5^{cA}	51.8^{dA}	17.3^{bA}	19.6^{bA}	214^{abA}	299^{aB}
LP+LB1	103.6^{bA}	26.9^{abcB}	24.3^{abA}	22.8^{abA}	34.2^{cA}	39.5^{dA}	460^{dA}	559^{dB}
BS	95.4^{bA}	24.4^{bB}	27.6^{abA}	27.9^{bA}	6.9^{aA}	7.7^{aA}	131^{aA}	239^{abA}
SEM	8.18	1.83	3.05	2.46	2.23	2.31	31.1	33.2
Significance level								
Variety	***		*		ns		***	
Treatment	***		***		***		***	
Variety × Treatment	***		ns		ns		ns	

[1)] sum of ethyl acetate and ethyl lactate; means in columns with unlike superscripts and means within rows bearing unlike capital superscripts differ significantly at P≤0.05 (Tukey test)

As all silages were aerobically stable over the entire experimental period of 14 days of exposure to air, no effect of silage additive and variety could be demonstrated (data not given).

Conclusions Silage additives affected DM losses, pH and fermentation pattern, but no effect was found on aerobic stability. Regardless of sorghum variety, VOC formation solely depended on the level of ethanol, lactic and acetic acids, and thus can be modified by silage additive type. The mixture of sodium benzoate and potassium sorbate was superior to all other treatments in reducing VOC, and is therefore highly recommended to prevent the detrimental effects of VOC on feed intake by ruminants.

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Effect of additives on fermentation quality of sorghum-sudangrass hybrids silage

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Keywords: additive, fermentation quality, Sorghum-Sudangrass hybrids, silage

Introduction Sorghum-Sudangrass hybrids (*Sorghum bicolor* (L.)×*Sorghum sudanense* (Piper) Stapf.), which is an annual grass family forage crop, has high palatability, high nutritional value and good agronomic characters of lodging and drought resistance. It could be used for grazing or silage. At present, research on Sorghum-Sudangrass hybrids are few, so this study was designed to determine proper silage conditions to improve Sorghum-Sudangrass hybrids fermentation quality and nutritional value. Additives are used to lower the risk of poor fermentation quality, high losses and reduced nutritive value that can occur when ensiling problem or 'at risk' forage (Kaiser et al. 2004). So, this study selected two varieties, domestic breeded one and foreign one, which were respectively added acetic acid (AA), propionic acid (PA), propionic acid plus urea (PA+U), two different LAB inoculants after mowed to analysis the effect of different silage additives on Sorghum-Sudangrass hybrids silage.

Material and methods JiCao No.2 (JC2), which is breeded by Chinese, is a distant hybrid of type A sorghum as female parent and sudangrass as male parent. Everlush (EL) is breeded by Australian. They were both cultivated in Hengshui of Hebei Province (37.44°N, 115.42°E).

The two varieties were mowed at heading stage, chopped, respectively mixed with 0.2% AA, 0.5%PA, 0.5%(PA+U), lactic acid bacteria(LAB) inoculant snow LACT-JL (SL, 0.0005%), LAB inoculant acremo conc.(AC, 0.0017%) and without additives (Control). After that 200g materials were packed into polyethylene bags and air was extracted by vacuum seal packaging machine in September 2009. Silages were stored at 25 centigrade for 270 days, and then sampled for analysis of fermentation quality of pH value, lactic acid (LA), acetic acid (AA), propionic acid (PA), butyric acid (BA) and ammonia nitrogen to total nitrogen content (AN/TN) and chemical composition of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Data were analysed using variance and Duncan multiple comparison.

Table 1. Fermentation quality and chemical compositions of JC2 silage.

Treatment	Fermentation quality						Chemical compositions			
	pH	LA/% DM	AA/% DM	PA/% DM	BA/% DM	AN/% TN	DM	CP/% DM	NDF/% DM	ADF/% DM
Control	4.95^{a}	2.12^{a}	3.65^{a}	0.21^{bc}	0.06^{a}	18.19^{a}	11.11^{a}	9.61^{f}	53.79^{c}	35.56^{a}
AA	3.79^{bc}	4.49^{a}	1.45^{b}	0.03^{c}	0.06^{a}	3.02^{c}	11.83^{a}	11.71^{a}	51.29^{e}	32.56^{f}
PA	3.69^{c}	4.73^{a}	0.63^{b}	0.91^{a}	0.12^{a}	2.08^{c}	10.61^{a}	11.47^{b}	51.74^{d}	33.66^{d}
PA+U	3.94^{b}	4.33^{a}	0.86^{b}	0.82^{a}	0.12^{a}	10.48^{b}	11.69^{a}	10.44^{d}	51.25^{f}	32.92^{e}
SL	4.84^{a}	3.58^{a}	1.18^{b}	0.61^{ab}	0.00^{a}	10.91^{b}	10.97^{a}	10.64^{c}	54.62^{b}	34.64^{c}
AC	3.82^{bc}	3.95^{a}	2.51^{ab}	0.18^{bc}	0.14^{a}	3.19^{c}	10.90^{a}	9.89^{e}	55.82^{a}	35.18^{b}

Note: LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid, AN/TN: ammonia nitrogen to total nitrogen, DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber. Different letters in the same column indicate significant differences (P<0.05), the same as below

Table 2. Fermentation quality and chemical compositions of LS silage.

Treatment	Fermentation quality						Chemical compositions			
	pH	LA/% DM	AA/% DM	PA/% DM	BA/% DM	AN/% TN	DM	CP/% DM	NDF/% DM	ADF/% DM
Control	4.55^{a}	3.27^{b}	3.24^{a}	0.09^{b}	0.06^{b}	14.88^{a}	11.61^{b}	9.15^{f}	61.27^{a}	37.68^{a}
AA	3.76^{b}	4.34^{ab}	1.73^{b}	0.00^{b}	0.00^{b}	3.12^{c}	12.78^{ab}	11.95^{b}	59.02^{b}	36.59^{b}
PA	3.72^{b}	4.84^{ab}	0.27^{c}	0.80^{a}	0.12^{b}	1.31^{c}	13.52^{a}	11.75^{c}	56.52^{d}	35.18^{c}
PA+U	4.00^{b}	4.36^{ab}	0.71^{c}	0.73^{a}	0.13^{b}	10.08^{ab}	13.14^{ab}	14.95^{a}	53.51^{f}	32.23^{f}
SL	4.39^{a}	3.36^{b}	1.54^{b}	0.10^{b}	0.15^{b}	11.31^{ab}	12.79^{ab}	10.64^{e}	56.74^{c}	34.76^{d}
AC	3.78^{b}	5.93^{a}	0.46^{c}	0.03^{b}	0.41^{a}	5.95^{bc}	13.01^{ab}	11.43^{d}	56.02^{e}	33.50^{e}

Results and discussion Except for SL treatment, other additives decreased pH value both of JC and EL comparing with the control ($P<0.05$). LA concentration in JC2 treated with additives was higher than the control, however, there was no significant difference ($P>0.05$) between additives treatments and the control, and LA concentration in EL treated with AC was significantly higher than the other treatments of EL ($P<0.05$). AN/TN concentration in JC2 control was higher than JC2 treatments with additives ($P<0.05$). Except for PA+U and SL treatments, AN/TN concentration in EL control was higher than EL other treatments ($P<0.05$).

Comparing JC2 silage alone with EL, pH value of JC2 was higher than that of EL, LA concentration in JC2 was lower than that in EL and AN/TN concentration in JC2 was higher than EL, which means the fermentation quality of EL was better than JC2. For JC2 and EL, these additives could significantly increase CP value and decrease ADF value comparing with the control ($P<0.05$), which means these additives could improve nutrition value.

CP value of JC2 was higher than EL, however, the difference was small. NDF and ADF value of JC2 were higher than EL.

Conclusions The fermentation quality of EL was better than JC2, however, effects of the two Sorghum-Sudangrass hybrids varieties silage without additives were not satisfactory. Using additives could decrease pH value, increase CP and decrease ADF value of JC2 and EL silages, improving fermentation quality and nutrition concentration in JC2 and EL silages.

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Effect of applying molasses and bacterial inoculants on fermentation and aerobic stability of whole crop triticale silage

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Keywords: aerobic stability, bacterial inoculants, molasses, triticale silage

Introduction Ensiling is a preservation method for moist forage crops. In order to improve the ensiling process, various chemical and biological additives have been developed. The biological additives are advantageous because they are safe and easy to use, non-corrosive to machinery, do not pollute the environment, and are regarded as natural products. Bacterial inoculants are added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH, and thus improving silage preservation (Filya et al. 2000). Usually bacterial inoculants have a positive or no effect on aerobic stability of whole crop silage (Nadeau, 2007).

Molasses in numerous silage experiments has been proven to be an effective silage additive in terms of promoting lactic fermentation, reducing silage pH, discouraging a clostridial fermentation and proteolysis, and generally decreasing organic matter losses. It is of particular benefit when applied to forage crops low in fermentable carbohydrates for lactobacilli. Keady (1996) concluded that molasses treatment improved silage preservation, but did not significantly alter the silage digestibility or animal performance although silage dry matter (DM) intake was improved. The objective of the present study was to determine the effect of molasses and microbial inoculation on ensiling characteristics, chemical composition and aerobic stability of whole crop tritcale silage (WCTS).

Material and methods This trial was conducted in dairy farm of high education center of Jihad-e- Agriculture of Khorasan Razavi Province, Iran. Whole crop triticale ensiled in 3.0 liter special anaerobic buckets (40 cm height and 30 cm diameter), equipped with a lid that enables gas release only. Each bucket filled with about 3 kg (wet weight) of chopped forage and opened after 42 days. The experimental silages were: 1) WCTS without additive, 2) WCTS treated with molasses (50 g/kg DM), and 3) WCTS treated with bacterial inoculants (Ecosyl®, applied at 1×10^5 cfu/g). There were 8 buckets per treatments and they were stored at ambient temperature (22-26°C). Aerobic stability was determined by returning 3 kg of silage to its respective silo and exposing it to air at 22 to 25°C. The temperature of experimental silages was recorded every 10 min. Each silo was covered with a double layer of sterile cheesecloth to avoid contamination and drying out of the forage. Aerobic stability was defined as the number of hours that silage was exposed to air before a 2°C increase in temperature above ambient temperature.

The ruminal degradable parameter of dry matter and crude protein of silages were determined using *in situ* procedure (Fathi Nasri et al. 2006). The bags (16×10 cm) were made of polyester nylon cloth with a pore size of 48 µm. Three Brown Swiss heifers fitted with the rumen fistula were used in this experiment. Approximately 5 g DM of the samples, that ground to pass a 2 mm screen, was placed in each bag and incubated in the rumen for 24 h (6 observations per treatment). After removal from the rumen, bags were washed in cold running water and dried at 60°C in a forced-air oven for 48 h. Then weighed to determine the DM disappearance. Statistical analysis was carried out using the GLM procedure of SAS 9.1 (SAS institute 1989).

Results and discussion Chemical composition of experimental silages are shown in Table 1. Results showed that WCTS treated with bacterial inoculants had the highest contents of chemical composition. Also the lowest contents of chemical contents were related to WCTS without additive. The bacterial inoculants had a desired effect on crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) which can be due to the fast decrease of silage pH (Moshtaghi Nia and Wittenberg 1999).
Experimental silages had significant effect ($p<0.05$) on pH and aerobic stability (Table 2). WCTS treated with bacterial inoculants and WCTS without additive had the highest and lowest pH, respectively (4.33 *vs*. 4.09). Stockes (1992) concluded that pH can be decreased in corn silage treated with bacterial inoculants. In the present study, molasses treated silages decreased significantly aerobic stability ($p<0.05$) compared with WCTS without additive and WCTS treated with bacterial inoculants.

The results of the incubation of the experimental silages using nylon bag technique showed that

there were significant differences (p<0.05) between the experimental silages (Table 2). WCTS treated with bacterial inoculants had the highest *in situ* dry matter and crude protein degradability 24h (61.02 and 71.15, respectively). The higher dry matter and crude protein degradability 24h in silages 2 and 3 can be due to higher concentration of quickly degradation carbohydrates and then better fermentation in these silages (McDonald et al. 1990).

Table 1. Chemical composition of experimental silages (on DM basis).

Item (%)	Experimental silages†		
	1	2	3
Dry matter	29.82	30.10	33.15
Crude protein	10.82	11.30	11.30
NDF	36.45	37.10	37.57
ADF	20.32	22.30	22.80
Ash	9.77	9.68	10.17
Ether extract	2.42	2.45	2.67
Cacium	0.57	0.61	0.64
Phosphorous	0.42	0.37	0.39

† The experimental silages were: 1) WCTS without additive, 2) WCTS treated with molasses (50 g/kg DM), and 3) WCTS treated with bacterial inoculants (Ecosyl®, applied at 1×10^5 cfu/g).

Table 2. pH, aerobic stability and ruminal degradable parameters of experimental silages.

Item	Experimental silages†			SEM
	1	2	3	
pH	4.09[b]	4.27[a]	4.33[a]	0.07
Aerobic stability (h)	23.75[a]	21.00[b]	22.75[a]	1.48
Degradability 24h (%)				
Dry matter	56.91[c]	57.61[b]	61.02[a]	0.23
Crude protein	69.81[c]	70.49[b]	71.15[a]	0.10

† The experimental silages were: 1) WCTS without additive, 2) WCTS treated with molasses (50 g/kg DM), and 3) WCTS treated with bacterial inoculants (Ecosyl®, applied at 1×10^5 cfu/g).
Data with different letters in the same row are significantly different (P<0.05).

Conclusions Results of this experiment showed that WCTS treated with molasses and WCTS treated with bacterial inoculants had higher dry matter and crude protein degradable coefficients compared with WCTS without additive, therefore must be regarded in feeding of fattening calves for utilization of protein and energy.

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Applying of lactic acid bacteria for wheat straw silage preparation

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Keywords: lactic acid bacteria, silage, wheat straw

Introduction Wheat (*Triticum aestivum* L.) straw still contains a good source of nutrients suitable for ruminant feeding after wheat ears have been harvested, however, a major drawback of wheat straw is that it yields low quality silage, due to poor digestibility of nutrients, mostly crude fiber. Silage fermentation and preservation are considered to be the most effective techniques for fresh wheat straw resources, and the lactic acid bacteria (LAB) may play an important role to effectively promote fermentation quality of silage.

In the he present study, the fermentation characteristics and chemical compositions of fresh wheat straw silage treated with a selected strain and a commercial inoculant were studied.

Material and methods Strain ZZ 1 was isolated by using MRS medium and its 16S rDNA sequence was determined. Fresh wheat straw was obtained after the harvesting of wheat ears in a farm, Henan, China. The silage was made by small-scale fermentation method (Figure 1), and a commercial inoculant Chikuso-1 (*Lactobacillus casei*) and a selected strain ZZU 1 were used as additives at 1.0 x 10^5 colony forming units (cfu)/g of fresh matter to wheat straw. The silage treatments were designed as untreated control, Chikuso-1 and strain ZZU 1 inoculation. The chemical compositions of silages were determined by conventional methods, and the organic acid contents were measured by high-performance liquid chromatography (Pang et al. 2011).

Results and Discussion The strain ZZU 1 isolated from corn was Gram-positive and catalase-negative rod that did not produce gas from glucose (Figure 2). This strain could grow at 15°C and weakly grow at pH 3.0, and produced acid from glucose, fructose and sucrose. According to the analysis of 16S rDNA sequence, the strain ZZU 1 was positioned within a cluster in the genus *Lactobacillus* and was assigned to be the species of *Lactobacillus plantarum* (Figure 3).
Compared to the control silage (Table 1), the Chikuso-1 and strain ZZU 1-inoculated silage were well preserved; had lower pH values, butyric acid, propionic acid and crude fiber (CF) content, and higher contents of lactic acid. Compared with the Chikuso-1 silage, the strain ZZU 1-inoculated silage had a little higher content of lactic acid (2.55% FM) and crude protein (CP) (5.4% DM). The results showed that the LAB inoculants could improve the fermentation quality of silage.

Conclusions The selected strain ZZU 1 may have potential to develop into an excellent silage inoculant, With the wheat straw silage, most of the processes used to date still rely on heavy chemical treatments with ammonia and sodium hydroxide which are reported to reduce the palatability to ruminants. This could be improved by the LAB inoculants.

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Fig. 1. Small-scale fermentation method. **Fig. 2.** Gram-stain of the strain ZZU 1.

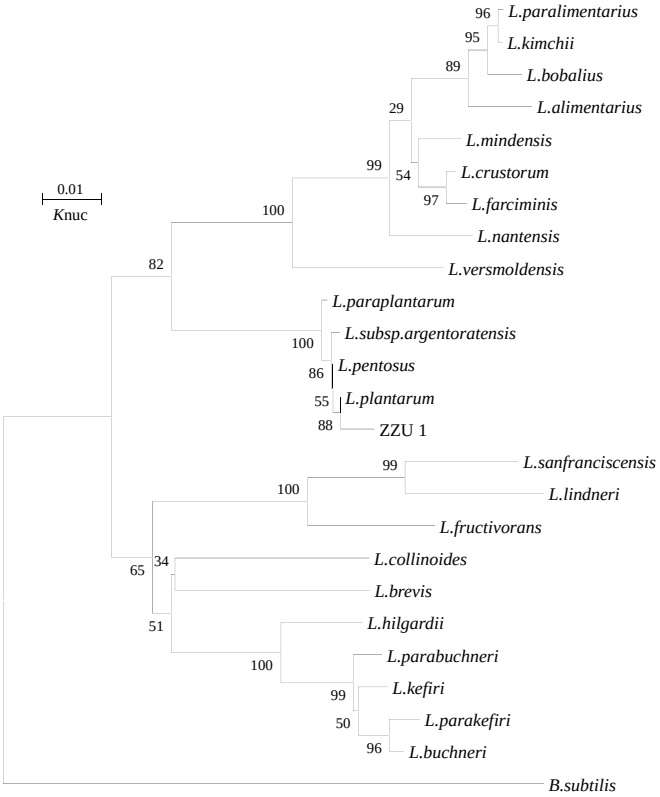

Fig. 3. Phylogenetic tree of strain ZZU 1.

Table 1. Fermentation quality and chemical composition of silages

Table 1. Fermentation quality and chemical composition of silages

Silages	pH	Moisture (%)	Organic acid (% FM)				Ammonia-N (g/kg of FM)	Chemical composition (% DM)			
			Lactic	Acetic	Propionic	n-Butytic		CP	EE	CF	OM
0 day	5.6	53.8	0.92	0.85	0.41	0.21	11.0	4.3	2.7	22.2	91.3
30 days:											
Control	4.2^{a}	52.9^{b}	1.98^{a}	0.54^{ab}	0.40^{a}	0.20^{a}	11.0	4.6^{a}	3.2	26.3^{a}	92.6
FG 1	3.6^{b}	53.4^{b}	2.39^{b}	0.65^{a}	0.24^{b}	0.11^{b}	10.9	5.1^{b}	3.4	23.4^{b}	93.5
ZZU 1	3.5^{b}	51.1^{a}	2.55^{b}	0.45^{b}	0.19^{b}	ND^{c}	10.8	5.4^{b}	3.3	23.0^{b}	93.3

^{a,b,c}Means in the same column within a silage type with different superscripts differ ($P < 0.05$).

Values are means of three silage samples.

FM, fresh matter; DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; OM, organic matter.

ND, not detected.

Effects of different additives on fermentation quality of fodder ramie silage (*Boehmeria nivea L.*)

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Keywords: fermentation quality, fodder ramie silage, lactic acid bacteria, molasses

Introduction Ramie (*Boehmeria nivea L.*) is widely cultivated in the high humidity and temperature areas of southern China. "Zhongsizhu No.1" was a new variety of fodder ramie cultivated by Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences. Xiong et al. (2005) reported the contents of crude protein (CP), crude fiber, crude ash, crude fat and calcium of "Zhongsizhu No.1" were 22.0%, 16.7%, 15.4%, 2.3% and 4.1% on dry matter (DM) basis, respectively. Ensiling may be an appropriate method to preserve its high nutritive value. Thus, fodder ramie seems to be a good alternative feed source for animals and has a great potential to be widely used in southern China to relieve the pressure on the protein feed deficiency. The purpose of this study was to assess the effects of lactic acid bacteria, molasses and their mixture during ensiling on the fermentation quality of fodder ramie silages.

Material and methods Fodder ramie (Zhongsizhu No.1) for ensiling was harvested from Hunan province in October. The forage was cut to a 2 cm theoretical length then divided into four equal portions. The treatments were designed as follows: treated with distilled water (Control); addition of molasses at a rate of 50 g/kg for fresh forage (M); addition of *Lactobacillus plantarum* Chikuso-1(Snow Brand Seed Co., Ltd, Japan) at a rate of 5 mg/kg to supply 1.0×10^5 colony forming units (CFU) of lactic acid bacteria per gram of fresh forage (LAB); addition of mixtures of LAB and M (LABM). The fresh fodder ramie was sampled before the additives were applied. Approximately 100 g treated forages were packed into plastic film bags, which were sealed with a vacuum sealer and stored at room temperature (20 to 25°C). Triplicate silos were randomly opened on days 0, 3, 7, 14, 28 and 60 of ensiling for sampling and determining the ensiling characteristics.

Results and discussion The fresh fodder ramie had a low DM and water soluble carbohydrate (WSC) content of approximately 11.2% and 6.2% on DM basis, respectively, and a high CP concentration and buffering capacity of 18.8% DM and 490.2 mE/kg DM (Table 1). Both the low WSC content and high buffering capacity made fodder ramie difficult to be ensiled. Furthermore, the population of lactic acid bacteria of pre-ensiled fodder ramie was as low as 6.5×10^3 cfu g^{-1} which is also a disadvantage to its ensiling.

As shown in Figure 1, there was a rapid decline in pH during the first 14 days for all additive treatments, then followed by a slow decrease until day 60 except for LAB treatment where the pH declined slowly at initial days then kept relatively stable at about 5.39 ($P < 0.05$). However, it was found that the pH of control silage showed an irregular rise at the later stage because of the abnormal deterioration of sample ($P < 0.05$). Silage pH is one of main criteria reflecting the fermentation quality of ensiled forages. The lactic acid fermentation resulted in faster accumulation of lactic acid, lower pH and improved forage conservation by inhibiting some harmful and disadvantage microbiological activities.

Figure 2 shows the changes in lactic acid concentration of silages during the ensiling period. The lactic acid concentration increased rapidly during ensiling for all additive treatments except for LAB treatment where the content of lactic acid increased to 2.60% for the first 7 days then kept relatively stable ($P < 0.05$). The control silage increased quickly for the first 7 days then decreased significantly until day 60. Overall, M and LABM treatments had higher lactic acid concentration than the control silage and LAB treatment during almost all ensiling periods ($P < 0.05$). All these results based on lactic acid fermentation in which the lactic acid bacteria convert WSC into lactic acid under anaerobic conditions. Thus, suitable population of lactic acid bacteria and sufficient WSC are the main factors influencing the extent of fermentation and silage quality of fodder ramie. In our present study, however, the LAB treatment didn't reach the anticipated effects on fermentation quality of silage. The results of adding LAB seemed contrary to some previous studies (Kung et al. 2003, Wang et al. 2011), thus, it still needs further research.

Conclusions Addition of molasses and combination of molasses and lactic acid bacteria could improve the fermentation quality of fodder ramie silage to some extent, but adding lactic acid bacteria didn't reach the anticipated effect of improving ensiling characteristics and fermentation quality which needs further research.

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Table 1. Chemical compositions (% dry matter) of fresh fodder ramie.

Item	Leaves	Stems	Whole crop
DM (% fresh material)	17.8 ± 0.06	7.7 ± 0.09	11.2 ± 0.25
OM	81.5 ± 0.03	86.4 ± 0.19	83.7 ± 0.15
CP	24.5 ± 0.05	10.8 ± 0.02	18.8 ± 0.04
WSC	3.3 ± 0.02	10.0 ± 0.42	6.2 ± 0.08
ADF	26.1 ± 1.19	35. 8 ± 0.66	30.1 ± 0.45
NDF	47.0 ± 5.06	49.6 ± 2.23	48.0 ± 4.99
BC (mE/kg DM)	515.9 ± 1.58	454.5 ± 2.44	490.2 ± 1.78

DM - dry matter; OM - organic matter; CP - crude protein; WSC - water soluble carbohydrate; NDF - neutral detergent fiber; ADF - acid detergent fiber; BC - buffering capacity.
Means ± SD, n=3

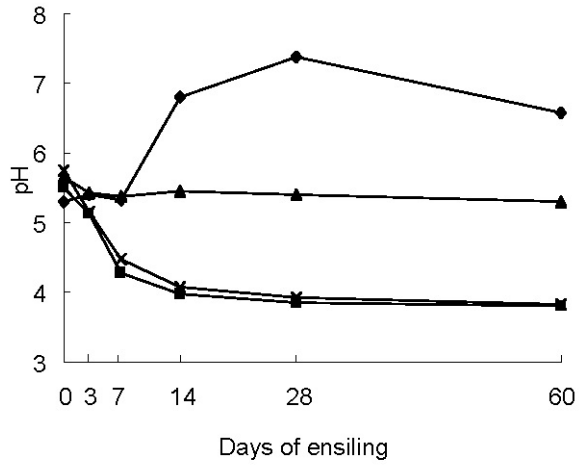

Figure. 1 Changes in pH of silages treated with molasses (M), lactic acid bacteria (LAB), lactic acid bacteria + molasses (LABM) and the control during ensiling. Control (♦); LAB (▲); Molasses (■); LABM (×).

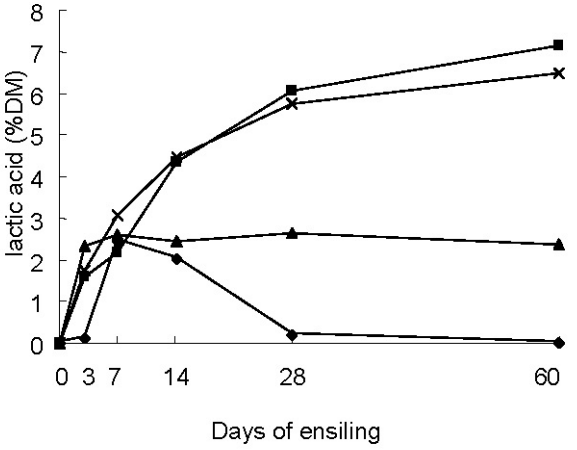

Figure. **2** Changes in lactic acid concentration of silages treated with molasses (M), lactic acid bacteria (LAB), lactic acid bacteria + molasses (LABM) and the control during ensiling. Control (♦); LAB (▲); Molasses (■); LABM (×).

The effect of silage additives on quality of silage made from sugar beet and shrubs

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Keywords: *Caragana intermedia, Lespedeza hedysaroides*, mixed silage, sugar beet

Introduction Sugar beet and two shrubs (*Caragana intermedia* and *Lespedeza hedysaroides*) can be mixed and ensiled the nutrient and moisture content of each component being complementary to each other in mixed silage, because *Caragana intermedia* and *Lespedeza hedysaroides* could supply the shortage of protein and absorb excess moisture of sugar beet pulp in mixed silage, see Alli (1984). The aim of this study was to survey the silage additives' effect on silage quality of mixed silage.

Material and Methods The raw materials of *Caragana intermedia*, *Lespedeza hedysaroides* and sugar beet were collected from Linxi (43.62 N, 118.02 E; altitude, 900 m) located in Inner Mongolia of China. There were five additives in silage treatments (Table 1). Each treatment had three replicates. Chopping length of *Caragana intermedia* and *Lespedeza hedysaroides* were about 2 cm. Silage raw materials were mixed as follows: 1/4 *Caragana intermedia* and 3/4 sugar beet pulp mixed silage; 1/4 *Lespedeza hedysaroides* and 3/4 sugar beet pulp mixed silage. Five additives were sprayed into mixed silage material using equal amount of water, and the ensilage was put into 2.5-liter plastic fermentation tank, see McDonald (1991). The fermentation quality of the mixed silage was determined after 35 days of the ensilaging. The data were processed by Microsoft Office Excel 2003 and analyzed with Tukey test of ANOVA by SAS.

Results and discussion It was showed that in the *Caragana intermedia* and sugar beet treatments, concentrations of lactic acid, acetic acid and propionic acid were significantly lower for LF than for the CK; while with other additive treatments the concentrations of lactic acid, acetic acid and propionic acid were outstandingly higher than those for CK ($P<0.05$). In the *Lespedeza hedysaroides* and sugar beet treatments, concentrations of lactic acid, acetic acid and propionic acid for additive treatments were lower than those for CK ($P<0.05$) (Table 2).

In the *Caragana intermedia* and sugar beet treatments, the mixed silage treatments with additives increased the crude protein (CP) content and reduced the neutral detergent fiber (NDF) content, as compared with the CK silage. In the *Lespedeza hedysaroides* and sugar beet treatments, the crude ash (CA) content of silage with additive treatments was lower than that of CK (Table 3).

Conclusions Ensiling sugar beet and two shrubs as mixed silage resulted in very good silage fermentation quality. Lactic acid concentrations of the silages in total acids were more than 67% (Lactic Acid / Total acids) and no butyric acid was detected in any of the mixed silages.

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Table 1. Five additives and their dosage used in mixed silages.

Five additives	Dosage
Water (CK)	Used as control with qual amount
Lalsia Fresh (LF)	0.5×10^{10}cfu·g^{-1} fresh matter (FM)
Cellulase	1.0 g/kg fresh matter (FM)
Sugar beet silage-specific	0.5×10^{10}cfu·g^{-1} fresh matter (FM)
Cellulase + Lalsia Fresh (LF)	1.0 g/kg + 0.5×10^{10}cfu·g^{-1} fresh matter (FM)

Table 2. Effect of different additives on organic acid[1] concentrations (% in dry matter) in mixed silages.

Treatment		pH value	Lactic Acid	Acetic Acid	Propionic Acid	Total acids
Caragana intermedia + Sugar beet	Control	3.86	9.12±0.02e[2]	0.15±0.01e	0.13±0.03bc	9.41
	Lalsia Fresh	3.66	7.96±0.01d	0.36±0.02c	0.10±0.00c	8.42
	Cellulase	3.45	13.94±0.02a	0.50±0.01b	0.21±0.02a	14.65
	Sugar beet silage-specific	3.6	10.72±0.03b	0.22±0.06d	0.18±0.01ab	11.12
	Cellulase + Lalsia Fresh	3.61	9.88±0.01c	0.79±0.03a	0.24±0.01a	10.91
Lespedeza hedysaroides + Sugar beet	Control	3.83	12.84±0.01a	0.28±0.01c	0.21±0.03b	13.33
	Lalsia Fresh	3.71	9.53±0.01e	0.14±0.03d	0.31±0.01a	9.98
	Cellulase	3.36	11.27±0.03b	0.56±0.00a	0.21±0.02b	12.04
	Sugar beet silage-specific	3.66	9.89±0.07d	0.23±0.04c	0.18±0.01bc	10.31
	Cellulase + Lalsia Fresh	3.44	10.24±0.02c	0.40±0.01b	0.12±0.04c	10.76

[1]There were no butyric acid in all experimental silages.

[2]Means within columns with different superscripts are significantly different (P<0.05) between the treatments.

Table 3. Effect of different additives on the chemical composition of mixed silages.

Treatment		Crude protein	Neutral detergent fiber	Acid detergent fiber	Ether extract	Crude ash
Caragana intermedia+ Sugar beet	Control	13.09±0.04d[1]	58.31±0.14a	37.13±0.18b	2.87±0.13a	3.89±0.09a
	Lalsia Fresh	13.60±0.06c	57.27±0.20b	40.44±0.09a	2.51±0.11b	3.50±0.03b
	Cellulase	13.91±0.01a	55.99±0.22c	38.16±0.39b	2.27±0.07bc	3.79±0.04a
	silage-specific	13.58±0.04c	55.65±0.19c	38.49±0.48ab	2.00±0.16c	3.50±0.07b
	Cellulase+ Lalsia Fresh	13.81±0.03b	54.51±0.18d	36.80±0.04b	2.01±0.09c	3.44±0.08b
Lespedeza hedysaroides+ Sugar beet	Control	13.99±0.04b	60.14±0.01bc	33.18±0.43b	1.31± 0.07a	4.90±0.03a
	Lalsia Fresh	14.89±0.05a	61.43±0.26a	33.75±0.01a	1.04± 0.10b	4.74±0.01b
	Cellulase	13.75±0.08c	59.77±0.14c	31.43±0.17c	1.26± 0.11a	4.52±0.04c
	Sugar beet silage-specific	14.09±0.03b	60.35±0.39b	34.11±0.03a	1.27±0.06a	4.65±0.06bc
	Cellulase+ Lalsia Fresh	14.04±0.01b	58.40±0.11d	30.37±0.09d	1.27± 0.03a	4.60±0.04c

[1]Means within columns with different superscripts are significantly different (P<0.05) between the treatments.

The effect of dose of chemical additive and temperature of sugar beet pulp on the quality of silage

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Keywords: additive, silage, sugar beet pulp, temperature

Introduction Sugar beet pulp silages (SBPS) are characterized by a high feeding value. They represent a convenient energy fodder (6.3 MJ NEL/kg dry matter and higher) and have specific effects on rumen fermentation leading to an improved digestibility of the organic mass in the feeding ration. SBPS have higher (up to 35%) enzymatic digestibility then fresh sugar beet pulps (SBP) with positive influence on nutritive value indicators (Zheng 2011, Kilic and Saricicek 2010, Weber et al. 2006ab).

Correctly preserved SBP usually have light greyish colour and pleasant smell, but they are very quickly perishable. The speed and the type of microbial processes depend on the moisture content, the composition of SBP, on their microbial cleanness, and on the humidity and temperature of the environment. In agricultural practice, mostly for economic reasons, the SBP are often ensiled without additives and sometimes even with a substantial delay after their dispatch from the sugar factory while stored anaerobicaly in silage bags (Weber et al. 2006ab).

In similar experiments with biological inoculants the researchers (Dolezal et al. 2005) found that such practice reduced losses significantly and improved the indicators of fermentation quality (pH and higher contents of lactic acid). Zheng 2011, Kilic and Saricicek 2010 and Weber et al. 2006ab recommended preservation using chemical additives aimed at strengthening of the aerobic stability of SBPS.

Material and methods The goal of the fermentation experiment with the preservation of SBP was to asses what dose of a chemical preservative is necessary to add and treat them just as fresh and ensiled with a 3 days delay while preserving quality and stability.

In the experiment, the original mass of fresh SBP, still warm at about 48°C (W = warm) was taken for laboratory analyses. The SBP were stuffed into the glass vessels of 5-litre volume and stored for 90 days in a dark environment at a temperature of about 20-25°C. The other SBPs were left on a heap for three days to cool down to a temperature of about 22°C (C = cold). The cooled SBP were processed in the same manner as the fresh SBP. Contents of molds and yeast cells (CFU/g of SBP) were monitored at the fresh SBP in addition to that. Additionally to control silages without preservatives (marked with D0), the SBP were experimentally ensiled with doses of 1, 3 and 6 l/t ensilaging matter (D1, D3 and D6). A chemical preservative (containing sodium benzoate and sodium propionate) were applied.

Each product was created in six repetitions. Samples were analyzed according to ČSN 46 7092. The neutrally detergent fibre (NDF) was determined according to ČSN EN ISO 16472 after the sample had been modified with amylase. Pectin was determined by cleaning the pectin substances by calcium chloride into insoluble calcium pectan, whose weight was determined. The result is expressed in % of calcium pectan. The CFU numbers of yeast cells and molds were determined by cultivation on an agar substrates. The results were evaluated by Statistica 9.01 program (StatSoft, Inc., Tulsa, Oklahoma, 2010).

Results and discussion Dry matter (DM) of W was 205.6 g, but DM of C 223.7 g. The three-day delay in ensilaging was not enough to fully demonstrate negative influences on the contents of nutrients, values C were convincingly ($P<0.05$) worse (NDF was 597 g/kg DM of W, vs. 631 g/kg DM of C; resp. netto energy of lactation NEL was 6.31 MJ/kg DM of W, vs. 6.28 MJ/kg DM of C). The content of pectin was significantly reduced ($P<0.05$) in C as compared to W. The ensiling delay of three days resulted also in an increased number of live colonies of molds (from $0.70x10^2$ to $3.88x10^2$ CFU/g) and yeast cells (from $9.3x10^2$ to $1.97x10^3$ CFU/g). Zheng (2011) and Weber et al. (2006ab) also warned about the danger of growth of molds and particularly yeast cells that are able to decompose completely the already low quantity of water-soluble sugars present in SBP. Then the sugar is not left for bacteria of lactic acid fermentation creating a negative impact on the progress of fermentation.

The fermentation process in silages without preservative ensiled with warm condition (WD0) was not as successful as that of the experimental silages with chemical preservatives. The pH values of WD0 were quite high (4,99). The pH value was significantly ($P<0.05$) lower in silages with extra preservative (WD1-WD6) and proteolysis was lower too. For the evaluation of the result of fermentation process, the level of proteolysis (protein decomposition) was significant while it should not exceed 8 %. The proteolysis values exceeding that value were found only at the WD0 silages. The fermentations with preservative (at a dose of 1 l/t already) had more favourable development than the control fermentations without preservative.
In silages ensiled in cold condition without the additives (CD0) the fermentation had significantly more

alcoholic character. The difference in the fermentation process between silages with different aditive doses was substantial (P>0.05). In the experiment with preservation of cooled pulps the best results were achieved under use of the chemical additive at the highest dose, 6 l/t. The difference between the additive doses were not statistically significant (P>0.05).

Our results are in compliance with the results of Weber et al. (2006ab), however they had evaluated experimentally preserved silages in big plastic tubes and only with one-day delay.

Table 1. The effect of the dose of chemical additive and the temperature of SBP on the quality of their silage

Index	Units	WD0	WD1	WD3	WD6	CD0	CD1	CD3	CD6
DM	g/kg	184.4 a	195.7 ab	190.9 ab	198.0 ab	203.8 b	199.3 b	205.6 b	204.0 b
NDF	g/kg DM	633 bcd	648 d	642 cd	620 bcd	569 a	617 bc	617 bc	608 b
Protein	g/kg DM	100.9 ab	100.3 ab	99.7 ab	97.9 a	101.0 ab	102.0 ab	102.0 ab	103.2 b
Ash	g/kg DM	63.1 a	67.7 ab	66.3 a	66.1 a	66.6 a	67.9 ab	72.3 b	72.3 b
pH		4.99 e	4.60 cd	4.50 abc	4.69 d	4.22 a	4.27 ab	4.34 abc	4.63 cd
MA	%	0.71 ab	0.78 ab	0.77 ab	0.77 ab	0.94 b	1.03 b	0.88 b	0.62 a
VFA	%	0.69 ab	0.68 ab	0.65 a	0.66 a	0.63 a	0.74 b	0.78 b	0,76 b
MA/VFA	%	1.03 ab	1.15 ab	1.22 ab	1.07 ab	1.48 b	1.42 ab	1.17 ab	0.95 a
Alcohol	%	0.05 a	0.04 a	0.03 a	0.04 a	0.13 b	0.08 ab	0.09 ab	0.05 a
Proteolyses	%	9.6 c	6.4 ab	6.4 ab	7.4 bc	6.9 b	5.4 ab	5.5 ab	5.1 a

Explanation: mean values with different letters in rows (a,b,c, ..) are significant at P<0.05. SBP = sugar beet pulp, W = warm, C = cold, D = dose, WD0 = SBP ensiled in warm condition and with 0 dose of additive per 1 tone of matter, WD1 = SBP ensiled in warm condition and with 1 litre of additive (WD3 = with 3 litre, WD6 with 6 litre, resp.), CD0, CD1, CD3, and CD6 = SBP ensiled in cold condition, with dose 0, 1, 3 and 6 litre of additive, resp. DM = dry matter, NDF = neutral detergent fibre, MA = milk acid, VFA = volatile fatty acid.

Conclusions The experiments bring the following recommendations: The sugar beet pulps intended for ensiling should be preserved right away, preferably when it is still warm, with the maximum delay of three days. The dose of additive 1 l/t is recommended, from the economic perspective, considering the experiment with preservation of warm pulps ensiled fresh. In the experiment with the preservation of cooled pulps ensiled with three-day, the best results were achieved under the use of the chemical additive 6 l/t (highest dose). It must be considered from the practical perspectives whether the differences in the results of the fermentation process are economically favorable considering substantial dose of additive required.

Acknowledgements Supported by project MZe 0002701404.

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Additives for sugar cane silage

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Keywords: Dairy cattle, milk production, sugar cane, silage, calcium oxide

Introduction Sugar cane might be a good replacer to corn silage, as it has high productivity (between 80 and 120 tons/ha), low costs, and it maintain good quality during the dry season in tropical conditions. Therefore, some work has been being conducted with good results both in dairy and beef cattle (Santos et al. 2006; Fernandes et al. 2007). However, it needs to be harvested daily for feeding animals, and this is becoming a problem in most farms, especially because of cost with labor. Ensiling the sugar cane could be a good alternative for this problem, but most of research shows that the sugar cane silage has great amount of ethanol, and high dry matter lost. Therefore, a large amount of additives is available to improve sugar cane silage quality. This study was conducted to evaluate effect of additives on sugar cane silage quality.

Material and methods To study the sugar cane silage, a database with 59 papers was build. A descriptive analysis was conducted to obtain the general improvement in the silage using 10 chemical additives, 5 biological additives, or 9 by-products (including coffee hulls, citrus pulp, ground corn with cobs and straw, wheat meal, soybean meal, cottonseed meal, cassava meal, residue of soybean harvest, and soybean plant. Afterwards a meta-analysis was conducted to study the effect *Lactobacillus plantarum, Lactobacillus buchneri*, urea, CaO, and NaOH on sugar cane silage. Biological additives were evaluated as qualitative variables, and chemical additives were evaluated as quantitative variables. We used only papers where a control treatment was present and the additive was the only addition in the silage. A random coefficient model was used to identify significant fixed and random effects using generalized least-square (GLS) regression. The MIXED procedure was used to conduct the analysis, using the Restricted Maximum Likelihood method to estimate variance compounds. Critical level of significance was assumed to be $P < 0.05$ for fixed effects and $P < 0.20$ for random effects (study). Four variance-(co) variance matrix structures were tested in the random coefficient model: compound symmetry, heterogeneous compound symmetry, unstructured, and autoregressive. The Akaike's Information Criterion (AIC) was used to select the statistical model with the best fit.

Results and discussion NaOH, CaO, $CaCO_3$, and urea decreased the concentration of forage NDF, and increased *in vitro* digestibility of dry matter. It is likely that NaOH, CaO and CaCO3 might promote a hydrolysis in the fiber carbohydrates, and that urea increases the amount of nitrogen available for microbial growth during the fermentation in the silo. These four additives were also efficient in controlling ethanol production. *Lactobacillus buchneri* and *Lactobacillus plantarum* were the inoculants most used in the data base evaluated. *L buchneri* controlled 8% of ethanol production, but increased in 41% the amount of NH_3–N in the silage. *L. plantarum* did not control ethanol production once its amount was increased by 67%. The NH_3-N was also high using this inoculant (+13%). Residue of soybean harvest was the best byproduct for ensiling sugar cane (data not shown). It reduced ethanol production in 66%, and increased dry matter in vitro digestibility in 17%.

The meta-analysis has shown that *L. plantarum* cannot improve chemical composition ($P > 0.05$), and it has increased ethanol production in sugar cane silage ($P = 0.043$, Table 1). *L. buchneri* had a positive effect on dry matter ($P = 0.005$), increasing non fiber carbohydrates ($P = 0.012$), and dry matter in vitro digestibility ($P = 0.032$), without any effect on NH_3-N production ($P = 0.401$).

As expected, urea increased protein content on sugar cane silage ($P < 0.0001$), however it also increased NH_3-N ($P = 0.010$). The CaO and NaOH were effective in reducing lignin content in the silage ($P = 0.070$), suggesting once more a hydrolysis during ensiling (Table 2). All additives (urea, CaO, and NaOH) reduced NDF ($P < 0.0001$), and increased dry matter in vitro digestibility ($P < 0.061$). Only CaO was efficient in reducing ethanol production ($P < 0.001$).

Table 1. Effect of Lactobacillus plantarum, and Lactobacillus buchneri on chemical composition, and fermentative parameters of sugar cane silage.

Item	*Lactobacillus plantarum* (n = 14)			*Lactobacillus buchneri* (n = 20)		
	Control	Additive	*P value*	Control	Additive	*P value*
Dry matter (% fresh matter)	26.0	25.4	0.397	26.2	27.7	0.005
Organic matter (%DM)	94.1	93.6	0.414	94.6	94.4	0.784
Crude protein (%DM)	2.95	3.35	0.054	3.09	3.53	0.371
Neutral detergent fiber (%DM)	68.3	62.4	0.084	66.2	63.7	0.177
Acid detergent fiber (%DM)	43.4	43.7	0.688	44.2	42.8	0.091
Lignin (%DM)	7.68	8.35	0.104	7.67	7.94	0.346
Non fiber carbohydrates (%DM)	-	-	-	15.0	25.2	0.012
NH_3-N(%DM)	6.69	6.30	0.694	6.00	14.32	0.401
In vitro DM-digestibility (%DM)	44.7	44.9	0.925	43.4	49.4	0.032
pH	3.51	3.49	0.752	3.58	3.60	0.838
Ethanol (%DM)	7.61	10.3	0.043	5.90	5.79	0.920
Gas production (%DM)	17.3	18.9	0.459	21.4	19.3	0.076
Effluents (%DM)	69.7	52.9	0.687	53.4	46.6	0.438

Table 2. Effect of urea, calcium oxide (CaO), and sodium hydroxide (NaOH) on chemical composition, and fermentative parameters of sugar cane silage.

Item	Urea (n = 23)			CaO (n = 14)			NaOH (n = 11)		
	β_0	β_1	*P value* (β_1)	β_0	β_1	*P value* (β_1)	β_0	β_1	*P value* (β_1)
Dry matter (% fresh matter)	25.2	1.17	<0.001	24.3	3.70	<0.001	25.2	1.20	0.007
Organic matter (%DM)	95.2	-	0.767	95.6	-4.68	<0.001	92.7	-	0.069
Crude protein (%DM)	3.18	8.11	<0.001	3.77	-0.71	<0.001	2.84	-	0.242
Neutral detergent fiber (%DM)	69.4	-3.65	<0.001	64.2	-8.94	<0.001	64.2	-6.19	<0.001
Acid detergent fiber (%DM)	44.7	-	0.112	40.5	-5.15	<0.001	43.6	-2.80	<0.001
Lignin (%DM)	8.58	-	0.569	7.36	-1.44	0.007	9.20	-0.77	0.007
Non fiber carbohydrates (%DM)	16.9	-4.84	0.036	30.0	-	0.327	17.5	12.63	<0.001
NH_3-N (%DM)	3.80	15.28	0.010	-	-	-	4.81	-	0.409
In vitro digestibility (%DM)	42.4	2.28	0.061	55.0	13.39	<0.001	45.7	16.80	<0.001
pH	3.58	0.56	<0.001	3.50	0.73	<0.001	3.75	0.34	0.001
Ethanol (%DM)	8.21	-	0.537	5.83	-4.45	<0.001	8.30	-	0.188
Gas production (%DM)	16.8	-	0.230	21.1	-10.58	0.001	14.2	-2.65	0.006
Effluents (%DM)	66.0	-	0.606	44.8	-	0.517	59.9	-	0.655

Conclusions In conclusion, Urea, CaO, NaOH and *L. buchneri* are recommended as additives for sugar cane silage.

Acknowledgements Supported by Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Fundação Arthur Bernardes (FUNARBE), and Conselho Nacional de Pesquisa (CNPq)

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The effect of *Lactobacillus buchneri* alone or in association with *Lactobacillus plantarum* on the fermentation and aerobic stability of high moist corn ensiled as whole grain or ground grain

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Keywords: High Moisture Corn, *Lactobacillus buchneri* NCIMB 40788, *Lactobacillus plantarum* MA18/5U

Introduction High Moist Corn (HMC) refers to corn kernel harvested at 26 percent or greater moisture, stored and allowed to ferment in a silo or other storage structure, and used as feed for livestock (Lary and Anderson 2010). HMC offers many advantages for producers who feed beef, dairy cattle or pigs. However, successful use of HMC requires that attention is paid to harvest timing, processing, storage conditions and feeding management (Lary and Anderson 2010). Dry matter losses occur during storage but also during desiling. HMC is often prone to aerobic deterioration (Kung et al. 2006), because it is high in starch, low in moisture, and is less extensively fermented relative to typical forage crops (Taylor and Kung 2002). Additives such as organic acids or microbial inoculants can be beneficial in reducing fermentation and feedout losses in HMC (Kung et al. 2006).The objective of this trial was to study the effect of addition of a single strain or a double strains microbial additive on the fermentation, aerobic stability and nutritional value of HMC.

Material and methods The trial was designed as a 3x2x2 factorial with the following factors: (i) no additive (C) vs *Lactobacillus buchneri* NCIMB 40788 (LB) (300 000 cfu/g fresh HMC) vs *L.buchneri* + *Lactobacillus plantarum* MA18/5U (LBLP) (LB 150,000 cfu/g fresh HMC + LP 100,000 cfu/g fresh HMC), (ii) whole (WG) vs ground grain (G) (2 mm) and (iii) 36 vs 32g moisture / 100g of fresh corn. Each experimental group had 6 replicates (microsilos). The anaerobic and aerobic phases lasted 144 and 13 days respectively. Dry matter (DM) losses were determined by weighing all silos individually at the start and the end of each phase. Combining this data with the DM analysis allowed calculating the DM losses. Samples were taken for dry matter, pH and chemical composition determinations at 11 and 20 days of the anaerobic phase (by opening 1 of the replicates), at the opening of the silos and after 13 days of the aerobic phase. Table 1 shows the chemical composition of the different HMC at harvest. Data were compared using the student-t- test. Significance was declared for $P<0.05$.

Table 1. Average composition of silages at harvest.

	GHMC Control 36	GHMC Control 32	WGHMC Control 36	WGHMC Control 34
Moisture g/100g fresh matter	34.5	30.1	31.7	29.7
pH	4.5	4.7	5.9	5.2
DM, g/100 g fresh matter	65.5	69.9	68.3	70.3
Protein (Nx6.25), g/100g DM	8.0	8.0	7.0	7.7
$N\text{-}NH_3$, g/100g DM	1	1	0	0
Starch g/100g DM	77.4	76.6	74.9	75.0
Lactic acid, g/100g DM	6.00	8.28	5.88	6.16

Results and discussion Table 2 summarizes the DM losses observed during both phases of the trial. It also shows the % of DM finally available for the animals. In the case of the 36 % moisture HMC, lower losses in DM ($P=0.05$) were observed with the combination of inoculants - LBLP. For GHMC as well as WGHMC, a higher fraction of the total DM was conserved at the end of the aerobic phase, compared to the Control (*i.e*. +5.3 % vs + 2.4% respectively, $P= 0.07$). The use of LB alone conserved 2.6% more DM in GHMC at the end of the aerobic phase. For WGHMC, little differences were seen in DM losses between C and LB alone. Taylor and Kung (2002) reported that the aerobic stability (number of h prior to a 2°C rise in temperature after exposure to air) was markedly improved by the addition of the same strain of LB. Contrary to our findings these authors however did not see an additional benefit of the combination LBLP. Amyot and Couture (2008) noted a significant decrease in aerobic losses for *L.buchneri* treated high moisture ear corn (HMEC), compared to a non treated control.

Table 2. HMC 36% moisture – DM losses during the different phases.

	Anaerobic losses (% of total DM at ensiling)	Aerobic losses (% of total DM at ensiling)	DM available for animals (% of total DM at ensiling)
GHMC			
Control	2.2± 0.5[a]	8.8 ± 0.4	89.0 ± 0.4
LB	3.3± 1.4[b]	5.1 ± 1.3	91.6 ± 1.3
LBLP	2.5± 0.2[a]	3.3 ± 0.2	94.3 ± 0.2
WGHMC			
Control	1.8± 0.8[a]	6.4 ± 0.8	91.8 ± 0.8
LB	4.1± 2.0[b]	4.0 ± 2.0	91.9 ± 1.9
LBLP	3.4± 1.3[a]	2.4 ± 1.3	94.2 ± 1.3

a,b: mean values within a row with no common superscripts differ significantly (p<0.05)

In terms of variations in pH (Table 3), the values for LB and LBLP treated GHMC were higher (P<0.01) than C at the end of the anaerobic phase (4.5 ± 0.03 and 4.2 ± 0.10 vs 4.0 ± 0.03 resp.). A moderately higher pH at desiling in silages treated with *L. buchneri* was also reported by Kung *et al.* (2006). However at opening, the pH of LB and LBLP treated silos stayed stable, whereas the pH of the C increased (P<0.05) rapidly (4.6 ± 0.05 and 4.3 ± 0.06 vs 5.5 ± 0.37). These results are in line with the observations of Amyot and Couture (2008) who noted a 1.6 rise in pH for non treated HMEC, versus a stable pH for *L.buchneri* treated product. In WGHMC the pH measured at opening was 4.5 for all experimental groups and rose by 0.5 units during the aerobic phase. These pH values are a little lower than the values found by Kung *et al.* (2006) in their experiment with WGHMC (*i.e.* 4.91 for C and 4.80 for LB).

Table 3. GHMC 36% moisture - pH values of silos at the end of anaerobic and aerobic phase.

	pH end anaerobic	pH end aerobic
Control	4.0 ± 0.03	5.5 ± 0.37
LB	4.5 ± 0.03	4.6 ± 0.05
LBLP	4.2 ± 0.10	4.3 ± 0.06
Prob.	<0.01	< 0.05

In general, the chemical composition of HMC (*i.e.* whole or ground) seemed not to be affected by the presence of inoculants when compared to the Control.

Similar trends were observed with regards to the parameters tested concerning HMC with 32% moisture content; however differences observed seemed less pronounced in comparison to the HMC with 36% moisture.

Conclusions Results from this trial show that it is beneficial to add a single additive or a combination of silage inoculants to HMC. The best results were obtained with the combination LBLP. For ground corn, the pH of Treatment groups remained stable after opening in contrast to Control groups. Moreover, the significant reduced DM loss at feed out with the additives compensates the slight increased DM loss during fermentation. The results also show that the effect on DM losses is more pronounced for 36% moisture than for 32/34% moisture corn.

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Ensiling crimped barley grain at farm scale in plastic tube bag with formic and propionic acid based additives

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Keywords: aerobic stability, *Hordeum vulgare,* hygienic quality, total mixed ration, yeast,

Introduction In Nordic conditions feed grain needs to be artificially dried, preserved (aerobic) or ensiled (anaerobic) in order to preserve grain quality after harvest until feeding. No drying cost, less dependence on weather and extended harvest season are the main arguments that contribute to the increasing popularity of storage of high moisture grain. The moist grain is combined (dry matter (DM) content of 550 – 650 g/kg, Palva et al. 2005) and run through the crimper machine, which will break and flatten the grains. Typical storages have been clamp silos sealed with plastic sheets. However, a recent trend on farms has been to store dryer grain in plastic tube bags. Grain with around 750 g/kg DM content is crimped and pressed with a bagging machine into plastic bags. Advantage of the higher dry matter is that the grain does not freeze, if ambient temperature falls below zero. However dryer grain may pose an increased risk of mould growth (Olstorpe et al. 2010). In this experiment formic acid or propionic acid based additives were applied to crimped barley grain at the time of ensiling to explore their effect on fermentation quality, microbial quality and aerobic stability of the ensiled product and total mixed ration (TMR) prepared using the grain as one component.

Material and methods Fully ripe spring barley (varieties Justiina and Triple) was combine harvested 10th – 12th August 2010 at Loimaa, Southern Finland. Each load of barley (2700-8300 kg) was weighed, crimped and bagged in a plastic tube bag (2 m diameter) using a Murska 1400 s2x2 roller mill. Additives were applied immediately after crimping in the discharge auger of the mill in random order. The applied chemical additives (Kemira Oyj) were a formic acid based additive (FA: 590 formic acid, 200 propionic acid, 45 ammonium formate, 25 benzoic acid/sorbate and 140 water g/kg) or a propionic acid based additive (PA: 726 propionic acid, 214 ammonium propionate and 60 water g/kg). The doses were 0, 3, 6 and 9 litres per ton of grain (wet basis) of both additives. Elho Pro Flow 6000 applicator with a membrane (diaphragm) pump was used for the doses 6 and 9 l/t and a Tuhti applicator with an impeller pump was used for the dose 3 l/t. Two applicators were used to achieve the broad flow range needed in the experiment. Three replicates were made of each treatment.

Ensiled barley was analysed for volatile fatty acids according to Huhtanen et al. (1998) and lactic acid according to Haacker et al. (1983). Ethanol concentration was measured using an enzymatic kit (Cat No.981680) and the analyser Pro 981489 (KONE Instruments). Yeasts and moulds were determined on Dichloran Rose Bengal Chloramphenicol Agar medium (DRBC, Difco 258710) which was supplemented with 50 µg/ml of oxytetracycline hydrochloride (AppliChem BioChemica A5257). The petri dishes were incubated at 25 ± 1ºC. The colonies were counted after 5 d. Total number of aerobic bacteria was determined on Plate Count Agar (PCA, Difco 247940) dishes incubated at 30 ˚C for 72 hours. Aerobic stability of the ensiled crimped barley was determined by monitoring temperature changes of the feeds when exposed to air. Triplicate samples of barley (800 g) were weighed and placed in 2.5 dm^3 containers made of expanded polystyrene. The temperature was automatically recorded at 10 minute intervals using a thermocouple wire connected to a data logger for 320 h. Aerobic stability was defined as the time taken for the temperature of the feed to rise 2.0 °C above the ambient temperature. TMR was prepared by mixing the barley and grass silage (barley 400 g/kg DM, grass silage 600 g/kg DM). Separate TMRs were prepared from each of the treated barley. TMRs had an average DM concentration of 390 g/kg. Aerobic stability of each TMR was measured from 500 g samples as described above. Statistical analysis were performed using GLM-procedure of the SAS system to test the treatment effects on the aerobic stability of barley and TMR. Contrasts were performed to test the linear effect of dosage level of each preservative.

Results and discussion Dry matter content of the ensiled barley was so high that the amount of lactic acid fermentation was negligible in most of the samples. Two control samples having the lowest DM concentration (720 g/kg) had some fermentation, as some lactic acid, acetic acid and ethanol were detected. The amount of lactic acid is however low compared to the lactic acid concentrations detected in other experiments (40 g/kg DM). The number of aerobic bacteria was high (> 6 log cfu/g) in all control samples. Two out of four control samples also had high numbers (> 6 log cfu/g) of yeasts and one control sample had a high number of moulds (Figure 1). One FA 3l/t sample had high numbers of bacteria, yeasts and moulds, and one PA 9 l/t sample had elevated numbers of bacteria and yeasts. Rest of the

additive treated samples (16) had only low levels of aerobic bacteria and the number of yeasts and moulds were under the detection limit (< 2 log cfu/g). Barley samples having high numbers of yeasts had poor aerobic stability.

Both additives linearly improved aerobic stability of TMR (P<0.01). Additives increased aerobic stability by 20 to 60 hours compared to 90 hours aerobic stability of the control treatment (Figure 2). According to Kung (2005) it is better to control yeasts at the time of ensiling rather than in TMR. These results confirm this, as the application levels of acids were low (0.6-1.8 l/t TMR) compared to effective application levels of TMR stabilisers (> 2 l/t TMR, Seppälä et al. 2010). Thus additive application to the crimped barley prior ensiling would be a cost-effective way to control heating of TMR.

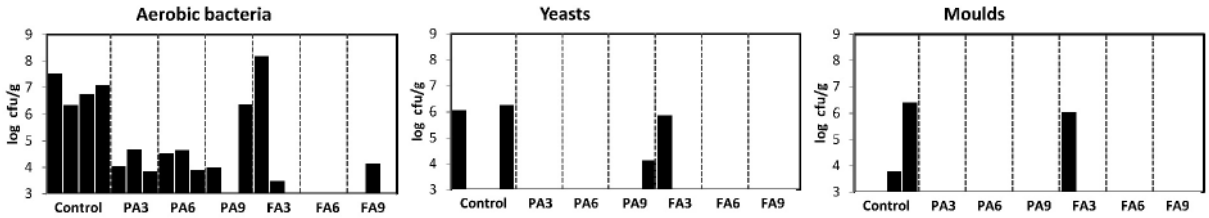

Figure 1. Microbial counts of the crimped barley after ensiling. Each bar within a column represents one replicate.

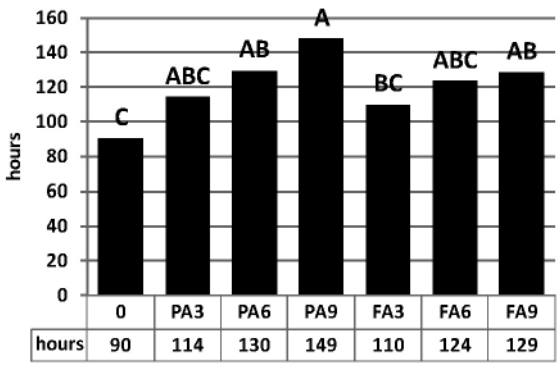

Figure 2. Aerobic stability of TMR prepared from grass silage and crimped ensiled barley. Additive treatments of crimped barley prior to ensiling improved aerobic stability of the TMR. Treatments PA (726 propionic acid, 214 ammonium propionate and 60 water g/kg) and FA (590 formic acid, 200 propionic acid, 45 ammonium formate, 25 benzoic acid/sorbate and 140 water g/kg). Numbers refer to the dosage level l/ton crimped grain. Differences between columns without the same superscript are statistically significant (p<0.05, Tukey test).

Conclusions There is only little fermentation when ensiling crimped barley with DM between 720 and 840 g/kg. The acid-based additives tested in this experiment were able to reduce the numbers of aerobic bacteria, yeasts and moulds to a low level in all the other samples except two out of 18. All the samples without additive had high numbers of aerobic bacteria and half of them had high numbers of yeasts. The samples having high numbers of yeasts had poor aerobic stability. The additives linearly improved the aerobic stability of the mixture of the barley and grass silage. Thus additive application to the crimped ensiled barley would be a cost effective way to control heating of TMR.

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Silage quality of whole and crushed *Vigna unguiculata* beans inoculated with lactic acid bacteria strains from sow milk

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Keywords: crushing**,** inoculants, lactic acid bacteria, sow milk, *Vigna unguiculata* grain

Introduction In the post-weaning phase, piglets often suffer from diarrhea and weight loss caused by the abrupt change of the diet from milk to solid feed. Weaning between the 21st and 35th day post natum is nowadays also practiced in small and medium scale farms in Colombia. In this project, it is considered to profit from the beneficial effects of organic acids and probiotics on the intestinal tract by ensiled weaner diet in combination with a local high-quality feed resource (cowpea grain, *Vigna unguiculata*) using lactic acid bacteria (LAB) from sow milk on the one hand. On the other hand, it is assumed that piglets learn from the sow. Thus, sows shall be supplemented with fermented cowpeas during lactation to increase feed acceptance. In the first phase of the project, our objective was to identify LAB strains from sow milk, which are suitable to ensile cowpea grains. Since not all farmers may have access to a proper chopper it was assessed additionally, whether for the ease of handling whole grains were ensilable, as for the sows' feed supplement.

Material and methods For the experiment, *Vigna unguiculata* CIAT 4555 whole grains were soaked overnight in abundant tap water as one base material and the remaining water was decanted the next day. The other base material was dry cowpea grain which was crushed in a forage chopper (Sertaneja) before adding 8.26 l tap water per kg grain and soaked for 17 h. Three common treatments were applied in both, the whole and the crushed grain: control, LAB 605, LAB 628. Strains had been isolated from two different sows and pre-selected based on an *in-vitro* test with minced *Vigna* grains (Rostock Fermentation Test). Prior to the inoculation at 10^6 cfu/g fresh matter (FM), the LAB strains were grown for 24h in MRS broth at 37 ºC. The material was ensiled for 30 d (± 3) at 27 C° (±3) in quadruplicate in plastic bags which were vacuum sealed. The dry matter (DM) content before ensiling was about 400 g/kg for the whole beans and about 600 g/kg for the ground material. When opened, silages were evaluated for their fermentation quality, i.e. ammonia and organic acids, and DM losses were calculated. The number of epiphytic LAB before ensiling was $3.5*10^3$ cfu/g FM for the whole beans, and $2.0*10^5$ cfu/g FM in the ground material.

Results and discussion The fermentation quality is presented in Table 1. All silages were low in acetic acid, while lactic acid was remarkably lower than butyric acid in whole grain silages.The competitivity of the lactic acid bacteria against clostridia obviously decreased at lower DM (400 g/kg vs. 600 g/kg) and probably reduced nutrient availability from intact beans at the same time.Crushing beans led to significantly higher lactic acid concentration in the corresponding silages, to lower DM losses, pH and butyric and acetic acid amount as well as ammonia-N compared to whole grain silages. Resuming this, the overall fermentation quality was clearly improved by the kibbling treatment.

The use of inoculants affected all parameters except for butyric acid, which was generally absent in crushed grains, where higher osmotic pressure probably favoured lactic acid bacteria.

While there was no significant difference in fermentation products for the silages inoculated with the two strains from sow milk in whole grains, LAB 605 showed a significantly higher lactic acid production and less proteolysis in terms of ammonia-N than LAB 628. The former might be due to an amylase inherence. However, overall fermentation quality was good in all silages of parted beans. The interaction between inoculants and grain treatment (Table 1), which was significant for lactic acid, NH_3-N of N total and the pH, indicate that the efficiency of inoculants depends on the texture and condition of the plant material.

Conclusions It is recommended to crush the dry cowpeas prior to soaking in a defined volume of water. This clearly contributed to better fermentation characteristics of the silages, probably together with the higher DM content. Under this condition, LAB 605 and 628 are both suitable to promote fermentation and generate desirable ensiling products, represented e.g. in a significantly lower pH compared to the control, with slightly better attributes by LAB 605. Both strains seem to be suitable to ensile pig feeds. In further steps, the bacteria will be evaluated for their probiotic characteristics together with other strains, and their phylogenetic relations.

Acknowledgement The financial support by the Federal Ministry for Economic Cooperation and Development, Germany (BMZ), is gratefully acknowledged.

Table 1. Dry matter (DM) losses, lactic acid (LA), acetic acid (AA), butyric acid (BA), NH_3-N of N total and pH of the whole and crushed *Vigna* bean silages.

	Whole beans			SD	Crushed beans			SD	Stat. signific.[1]		
	Control	LAB 605	LAB 628		Control	LAB 605	LAB 628		INOC	GRAIN	GRAIN × INOC
DM losses (g/kg DM)	89.0	77.0	84.4	9.25	42.3	18.0	20.8	14.0	**	***	
LA (g/kg DM)	13.6	10.0	11.7	1.87	32.3	61.7	48.6	12.6	***	***	***
AA (g/kg DM)	7.1	9.0	10.0	1.59	5.4	6.9	6.6	0.64	***	**	
BA (g/kg DM)	28.4	48.3	39.1	19.5	n.d.	n.d.	n.d.	0.00		***	
NH_3-N of N total (g/kg N)	52.8	55.0	57.9	4.27	23.7	12.5	29.0	7.37	***	***	***
pH	6.20	5.94	6.08	0.12	5.01	4.30	4.61	0.30	***	***	***

[1]Statistical significance: Comparison of inoculants, whole vs. crushed grains, interaction between inoculant (INOC) and grain (GRAIN) treatment, SD = standard deviation, LAB = lactic acid bacteria

Ensiling of tomato pulp: initial steps

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Keywords: fermentation, silage, tomato pulp

Introduction Hadjipanayiotou (1994) found that ensiled tomato pulp could be a potential protein- and energy source in animal nutrition. According to these results, the aim of the present study was to determine the nutrient content, fermentation quality and microbial status of wet tomato pulp silage after applying different treatments. Dried whole grain wheat (20% based FM) was applied in order to reduce the risks of effluent production, un-desirable fermentation processes, and moreover to increase the energy content of tomato pulp, as a winter feed for game (deer and wild boar).

Material and methods Ensiling of tomato pulp was carried out in metal barrels with a capacity of 150-180 kg/barrel. Treatments were designed as follows: (T1) tomato pulp as control (T2) tomato pulp covered with 1 kg/barrel of salt (NaCl) in order to reduce aerobic spoilage on the top surface, (T3) mixture of tomato pulp and dried whole wheat grain (20%) covered with 1 kg/barrel salt , (T4) mixture of tomato pulp and dried whole wheat grain (20%) covered with 1 kg/barrel salt and treated with a silage inoculant (*Lactobacillus acidophilus* and *Enterococcus faecium*; dose: 10 kg/ton, 10^5 CFU/g fresh material). Samples (2 kg silage/sample) were taken from the barrels (5 barrels/treatment) removing the upper 50 cm. Samples were transported to the laboratory in cooled boxes within 2 hours. Chemical composition, starch, total sugar, total carotene, pH, lactic and volatile fatty acid composition, aerobic mesophyl bacteria (AEMB) and moulds were analysed (n=5) on the 100th day of fermentation according to the Hungarian National Standards (Hungarian Feed Codex, 2004). The chemical compositional data and microbial counts were analyzed for their statistical significance (ANOVA and Tukey post test) with SPSS (version PASW Statistics 18). All microbial counts were log10 transformed to obtain log-normal distributed data.

Results and discussion Nutrient contents can be seen in *Table 1.*In the case of fresh tomato pulp an adequate fermentation was found with a good hygienic status after 100 days of ensilage (Table2). Treatment T2 (salt on the top) had no significant effect on fermentation or microbial status of the tomato pulp silage either on the top, or in the core. Presumably due to the high packing density of the tomato pulp (208.7 kg DM/ m^3) such that aerobic spoilage of the surface (3-5 cm on the top) had no effect on the fermentation in the core (50 cm depth). However, the salt was ineffective on the top layer, and a similar spoiled layer was observed in both treatments T1 and T2 (3-5 cm). Mixing of 20% dried whole wheat grain significantly reduced the acetic acid ($P \leq 0.05$), and the volatile fatty acid ($P \leq 0.05$) concentration, but increased the lactic:acetic acid ratio in the core of the silages as compared to T2 (T2: 1.72±0.07 *vs* T3: 3.25±0.09). According to the results, in treatment T3 a lower fermentation intensity was found in combination with a better volatile fatty acid profile, presumably due to a higher DM content in this treatment T3 (375.8 g/kg DM), than in T2 (288.8 g/kg DM). However, aerobic spoilage was found in the top 20 cm of the mixed silages compared to T2, where the spoiled layer was only 3-5 cm. Therefore it is not recommended to add whole grains to the wet by-product due to the negative effects on the top 1-20 cm layer (aeration). It is therefore proposed to use dried ground cereal as fine structural and hygroscopic additive. The applied microbial additive had a negative effect on fermentation in the case of mixed tomato pulp silage (significantly higher acetic acid ration and mould $P \leq 0.05$, and higher propionic acid, with a lower LA:AA ration as compared to T3 treatment,) Dried whole grain wheat (used at 20%) increased the net energy content for maintenance of tomato pulp (NEm: 4.88 MJ/kgDM; NEg: 2.53MJ/kgDM; NEl: 4.46MJ/kgDM) by 38.7% (NEm: 6.77MJ/kgDM; NEg:4.20MJ/kgDM; NEl: 6.18MJ/kgDM), which has an important role in game feeding during the winter . The calculated lactation net energy content is similar to a maize silage harvested with approx. 25-30% starch content.

Conclusions This study showed that wet tomato pulp had a limited fermentation capacity, but under anaerobic conditions it was possible to store for a long period (minimum of 100 days) with a good microbial status. It is recommended to use dried ground cereal as an additive (20%) to increase dry matter and energy content, moreover to improve volatile fatty acid composition of the wet tomato pulp silage.

Table 1. Nutrient content of tomato pulp silage according to the different treatments (n=1).

Content		Treatment T1	Treatment T2	Treatment T3	Treatment T4
DM	g/kg	253.2	288.8	375.8	362.5
Crude protein	g/kg DM	191.1	199.2	168.7	169.1
Crude fat	g/kg DM	154.5	174.2	112.0	117.5
Crude fiber	g/kg DM	431.9	412.4	216.6	229.9
NDF	g/kg DM	574.5	541.7	332.7	336.4
ADL	g/kg DM	323	308.8	156.1	166.6
Total carotene	g/kg DM	430.2	505.7	215.3	216.1

Table 2. Fermentation profile of the different tomato pulp silages (n=5).

Treatments			Treatment T1	Treatment T2	Treatment T3	Treatment T4
pH		Mean	4.35	4.30	4.20	4.29
		Std. dev.	0.22	0.11	0.04	0.03
Lactic acid	g/kg DM	Mean	35.96	33.20	31.16	33.40
		Std. dev.	7.17	3.00	3.28	3.64
Acetic acid	g/kg DM	Mean	18.95a	19.28a	9.61b	12.46c
		Std. dev.	2.15	1.19	1.13	0.38
Propionic acid	g/kg DM	Mean	0.35	0.18	0.06	0.18
		Std. dev.	0.33	0.06	0.04	0.06
Butyric acid	g/kg DM	Mean	0.64	1.59	0.22	0.23
		Std. dev.	0.17	1.10	0.11	0.16
Volatile fatty acids	g/kg DM	Mean	19.95a	21.05a	9.89b	12.87b
		Std. dev.	1.94	1.23	1.15	0.38
Organic acids	g/kg DM	Mean	55.91a	54.26a	41.05b	46.28a
		Std. dev.	8.54	4.04	4.40	3.61
LA/AA ratio	g/g	Mean	1.89a	1.72a	3.25b	2.68b
		Std. dev.	0.28	0.07	0.09	0.31
AEMB	log10 CFU/g FM	Mean	4.03	4.00	3.47	3.98
		Std. dev.	0.56	0.44	0.15	0.69
Moulds	log10 CFU/g FM	Mean	3.81a	3.76a	4.07a	4.63b
		Std. dev.	0.07	0.23	0.28	0.23
Total sugar	g/kg DM	Mean	4,95a	4,95a	10,30b	12,52b
		Std. dev.	1,32	1,61	3,26	1,88

Different letters show significant differences at level of $P \leq 0.05$

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A new solution for ensiling of wet by-products: tomato pulp baled silage for feeding game

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Keywords: bale, biological additive, fermentation, silage, tomato pulp

Introduction Hadjipanayiotou (1994) has found that the ensiled tomato pulp is a potential protein- and energy source in animal nutrition. Deneka and Canb (2006) showed that 4 and 6% wheat grain addition increased in vitro dry matter digestiblity of tomato pulp silage. The appropriate large-scale ensiling technology is a problem in the case of wet by-products. Description of a recent development the 'special baling technology' as described by Orosz et al. (2008), could solve this problem. The aim of this study was to produce baled tomato pulp silage mixed with dried ground corn (20%) before ensiling on the nutrient content, fermentation quality and microbiological status of the baled silage. In addition a 0,5% salt application was used to assess the potential of this approach as an antibacterial and antifungal agent on the quality of the wet by-product silage.

Material and methods Baling was carried out by a Göweil LT Master fixed-chamber baler-wrapper machine, applying a pressure of 130 bar during the baling process. Nominal size of the bales was: 1.20 x 1.22 m. Film wrap (25 µm thick) was applied 70% pre-stretched and with 6 layers (by 28 turns) . Experimental treatments were as follows: (1) mixture of tomato pulp and dried ground corn (20%), (2) mixture of tomato pulp and dried ground corn (20%) treated with 0.5 % salt, (3) mixture of tomato pulp and dried ground corn (20%) treated with Sil All 4x4 silage inoculant (Enterococcus *feacium, Pediococcus acidilactici, Lactobacillus plantarum, Lactobacillus salivarius,* and amylase, hemicellulase, cellulase, pentosanase; dose: 5g/ton, 10^5 CFU/g fresh material, sprayed in 2 litre water/ton). Chemical composition, starch, total carotene, pH, lactic and volatile fatty acid composition, aerobic mesophyl bacteria and moulds were analysed on the 70th day of fermentation according to the Hungarian National Standards (Hungarian Feed Codex, 2004). The chemical compositional data and microbial counts were analyzed for their statistical significance (ANOVA and Tukey post test) with SPSS (version PASW Statistics 18).

Table 1: Nutrient content of fresh tomato pulp, dried ground corn and baled tomato pulp silages according to the different treatments.

	Fresh tomato pulp	Treatment 1	Treatment 2	Treatment 3
DM (g/kg)	269.3	408.3	409.2	375.5
Crude protein (g/kg DM)	197.9	146.1	147.3	147.4
Crude fiber (g/kg DM)	400.9	209.2	216.0	217.4
Total starch (g/kg DM)	24.0	283.1	313.9	290.2
Total carotene (mg/kg DM)	167.8	144.1	147.2	146.0
Aerobic bacteria (log_{10} CFU/g)	5.00			
Moulds and yeasts (log_{10} CFU/g)	1.90			

Results and discussion Fresh tomato pulp was mixed with 20% hygroscopic dried ground corn in order to reduce the risks of effluent production,an un-desirable fermentation processes, and moreover to increase nutritive value of the by-product (baled tomato pulp silage ensiled with 20% dried ground corn: 6.84 MJ/kg DM NE_m; 6.33 MJ/kg DM NE_l and 4.26 MJ/kg DM NE_g). Dried ground corn (used in 20%) increased the net energy content for maintenance of tomato pulp by 40% (tomato pulp 4.88 MJ/kg DM NE_m; 2.53MJ/kgDM NE_g; 4.46MJ/kgDM NE_l), which has an important role in game feeding (roe deer and red deer, wild boar) in the winter time. The calculated lactation net energy content is similar to a maize silage harvested with approx. 30-35% starch content.

It was confirmed that the new baling system was able to form well-shaped and stable bales with such a wet by-product as fresh tomato pulp with a small particle size (initial dry matter range of the mix was 362.6-375.7 g/kg). High 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. Effluent production ranged between 6-10 liter per bale. High density, quick

wrapping (within 120 sec after bale-formation), has a beneficial effect on fermentation quality. However, low fermentation intensity was found in the control tomato pulp (20% corn) baled silage (Table 2). An undesirable fermentation process (high butyric acid concentration, P≤0.05) was found in the case of 0,5% salt treatment in the mixed tomato pulp baled silage (Table 2), therefore application of salt is not recommended. Inoculation effectively inhibited the production of butyric acid, and reduced the protein loss by 6 % as compared to the control, therefore it is highly recommended to apply as silage inoculant during the ensilage of the wet by-product.

Table 2: Fermentation profile of the different baled tomato pulp silages (n=3).

			Treatment 1	Treatment T2	Treatment T3
pH		Mean	4.97a	5.13b	4.57a
		Std. dev.	0.16	0.14	0.05
Lactic acid	g/kg DM	Mean	17.85	16.32	19.82
		Std. dev.	5.11	1.87	3.88
Acetic acid	g/kg DM	Mean	9.16	9.63	10.39
		Std. dev.	1.21	0.10	0.90
Propionic acid	g/kg DM	Mean	1.46	6.24	0.85
		Std. dev.	0.25	8.14	0.00
Butyric acid	g/kg DM	Mean	1.48a	3.70b	0.00a
		Std. dev.	0.80	0.77	0.00
Volatile fatty acids	g/kg DM	Mean	12.10	19.57	11.24
		Std. dev.	1.95	8.01	0.90
Organic acids	g/kg DM	Mean	42.05	55.45	42.29
		Std. dev.	1.93	15.19	2.07
Lactic acid:acetic acid ratio	g/g	Mean	2.02	1.70	1.93
		Std. dev.	0.80	0.20	0.54

Different letters show significant differences at level of P≤0.05

Conclusions Based on the experimental results, it can be concluded, that the new bale-forming technology provides stable wet tomato pulp silage (20% ground corn) for long term storage, moreover, the transportable baled silage with considerable energy and protein concentration and as carotene source can have beneficial effects for game feeding during the winter time. A high concentration of butyric acid (>5 g/kg DM) indicates that silages have undergone clostridial fermentation, which is undesirable and can affect nutrient quality and palatability.Prolonged fermentation in uninoculated silage allows proteolytic Clostridia to degrade plant protein and convert it to ammonia, increasing the pH. Homofermentative lactic acid bacteria inoculation has generally a beneficial effect on lowering the butyric acid concentration even in low dry matter silages (Davies et al. 2005; Mayne and O'Kiely 2005).In this study the butyric acid concentration was under the above mentioned threshold, however the homofermentative bacteria inoculation was effective at inhibiting butyric acid fermentation processes in the tomato pulp (+20% dried ground corn) mixed baled silages. Thus application of a biological additive is recommended in order to improve silage fermentation quality in baled tomato pulp silages.

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Silages of sweet potato vines treated with bacterial inoculant

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Keywords: acid detergent insoluble nitrogen, lactic acid bacterial, molds, neutral detergent fiber, pH

Introduction The variation in forage availability during the year in tropical regions combined with the need to use lower-cost ruminant feed has contributed to increase the demand for new feed alternatives. The use of unconventional feeds for ruminants is a promising alternative for meeting the challenges of small farmers for whom the cost of feeding is often an impediment to raise animals. Sweet potato can be used both in human and animal feeds, as well as for industrial purposes. Its cultivation is relatively easy and cheap, which, together with its adaptability to various climatic conditions, enables its production by family farmers. Besides the storage roots, the vines serve as feed for animals, which may be fed fresh or preserved as silage. The information available in scientific literature on the potential of sweet potato vines as silage and the silage quality is not adequate to make the silage technology available to farmers. The objective of this study was to evaluate the chemical composition, pH and populations of lactic acid bacteria, fungi, yeasts and enterobacteria in vine silage of five genotypes of sweet potato, with and without the use of a microbial inoculants as silage additive.

Material and methods Silages were produced from sweet potato vines of genotypes BD-25, BD-08, BD-23, BD-31 TO and BD-43 from the UFVJM, and the dry matter yields were respectively, 7.9; 7.8; 7.2; 6.9 and 6.6 t/ha. The experiment was arranged in a 5x2 factorial (five genotypes x with and without microbial inoculant), in a completely randomized design with three replications. After wilting in the field for 48 h, the material was chopped in a stationary forage chopper. The inoculant All Sil (Alltech, Brazil) was diluted in distilled water and applied at a ratio of 5 g per tonne of fresh forage. The forage was ensiled in 10 L buckets fitted with a Bunsen-type valve, in a density of 650 kg/m^3 silo. The silos were opened 90 days after ensiling and two samples were collected: one sample was used fresh for determining the pH and microbiological analysis and the other was pre-dried in a forced air oven at 55° C, ground in a Wiley mill with 1 mm sieve for determining dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG) and acid detergent insoluble nitrogen (ADIN), according to the methods described by AOAC (1990), as well as water soluble carbohydrates. The isolation and count of microorganisms in the silage were carried out by plating on selective medium. Samples of silage (25 g) were removed at the time of the silo opening and homogenized for one minute in a blender containing 225 mL of Ringer's solution. The solution obtained was diluted in series (10^{-1} to 10^{-7}), using screw-capped tubes containing the buffer solution. The microbial populations were quantified using selective culture media for each group: Rogosa agar for lactic acid bacteria (LAB) count, pour-overlay-method using Violet Red Bile for enterobacteria count, and Potato Dextrose Agar for fungi and yeasts count. The enterobacteria count was performed after 18 hours of incubation, while the LAB, yeasts and fungi counts were carried out after 36 hours incubation at 32 °C. Plates were considered countable when containing 30 to 300 cfu. The data were examined by the analysis of variance and comparison of means by the Tukey test.

Results and discussion In the vine silages, the genotypes differed in the pH and DM and ADF contents, the inoculant affected only the pH (Table 1) and the interaction genotype x inoculant was not significant for any parameters evaluated. The pH of the silage for the genotypes ranged from 3.9 to 4.1, and although the difference between means was significant, all values were within the range from 3.8 to 4.2, which is considered ideal by McDonald (1981), indicating a proper fermentation. The silages differed in DM, and the genotype BD-31 TO (20.4%) had similar content to that of BD-43 and lower than the others. DM greater than 25% were obtained with DB-08, DB-23 and DB-25. There was no difference in CP, with an average content of 12.7% recorded. The average content of NDF in the DM was 43.9%, well below the 60% limit considered by Van Soest (1994) as responsible for the reduction in consumption of the feed. The genotypes differed in contents of ADF in the silages. BD-43 (34.6% DM) showed contents lower than those recorded for BD-31 TO (37.6% DM) and BD-23 (39.1% DM). There was no significant difference in the lignin content in the silage among the genotypes, with an average value of 15.3%. High lignin content tends to limit digestibility, because its content is highly correlated with the digestibility of

the cellulose and hemicellulose (Van Soest 1994). The levels of SC in the silage were much lower than in the wilted vines, indicating the consumption during the fermentation process. The average value of ADIN was 7.0% of Total-N. There was no effect of the factors studied on the LAB, fungi and yeasts populations, with averages of 7.0, 4.5 and 4.4 log cfu/g silage, respectively. Enterobacteria were not observed, which can be attributed to the low pH of the silage, inhibiting the growth of these microorganisms. The use of inoculant reduced the pH of the silage from 4.1 to 4.0 ($P<0.05$). The use of bacterial additives did not alter the chemical composition, providing average levels of DM, CP, NDF, ADF, lignin, soluble carbohydrates and ADIN of 24.8, 12.7, 43.9, 37.3, 15.3, 4.1 and 7.0% respectively. It is likely that the good conditions of vine fermentability have prevented positive responses to the inoculant.

Table 1. Average values for pH, dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG), soluble carbohydrates (SC) and insoluble nitrogen acid detergent (ADIN), and lactic acid bacteria (LAB), fungi and yeast populations in the silages of sweet potato genotypes, with and without microbial inoculants.

	Genotypes						Inoculant		
Parameters	BD-08	BD-23	BD-25	BD-31TO	BD-43	SEM[1]	with	without	SEM[1]
pH	4.1 a	4.0 ab	4.1 a	3.9 b	4.0 ab	0,04	4.0 b	4.1 a	0.03
DM %	26.8 a	25.4 a	27.4 a	20.4 b	24.1ab	1,27	24.7 a	24.9 a	0.81
CP (%DM)	12.7 a	12.8 a	12.1 a	12.9 a	12.8 a	0,34	13.0 a	12.4 a	0.22
NDF (%DM)	45.0 a	44.8 a	43.3 a	42.4 a	44.1 a	1,39	43.2 a	44.6 a	0.88
ADF (%DM)	35,2 bc	39,1 a	35,1 bc	37,6 ab	34,6 c	0,64	36.0 a	36.7 a	0.41
LIG (%DM)	15,2 a	16,2 a	15,1 a	15,0 a	15,1 a	0,78	15.5 a	15.2 a	0.50
SC (% DM)	4,4 a	4,0 a	4.0 a	3,9 a	4,3 a	1,16	4.0 a	4.2 a	0.73
ADIN (%Nt)	6.9 a	7.2 a	7.3 a	7.1 a	6.6 a	0,48	7.1 a	6.9 a	0.30
LAB (log cfu/g)	7,3 a	7.0 a	7,1 a	6,7 a	6,9 a	0,19	6.9 a	7.1 a	0.13
Fungi (log cfu/g)	4,9 a	4,7 a	3,96 a	4,62 a	4,39 a	0,49	4.9 a	4.1 a	0.31
Yeasts (log cfu/g)	4,9 a	4,8 a	4,8 a	3,8 a	4.0 a	0,34	4.5 a	4.4 a	0.21

[1]SEM - standart error of the mean

Means followed by the same letter on rows did not differ by the Tukey test ($P>0.05$)

Conclusions The CP, NDF, ADF and ADIN contents in the silages of all genotypes can be considered adequate, indicating that the silages of sweet potato vines have good nutritional value. The application of inoculant did not affect the evaluated parameters in the silages.

Acknowledgements Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, funding agency of the project

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In vitro measurement of methane production from Finnish farm silage samples

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Keywords: digestion rate, gas production, *in vitro*, methane production, ruminant production systems, silage composition

Introduction After carbon dioxide (CO_2) methane (CH_4) is the most important contributor to the greenhouse effect trapping about 20 times more heat than CO_2. Enteric fermentation in the fore-stomachs of domestic ruminants contributes to approximately one quarter of anthropogenic CH_4 production. Strategies to reduce enteric CH_4 production have been subjected to intensive research during the last decade. Most research has focused on specific feed additives or fat supplements that can be expensive and/or negatively affect feed intake and production. However, factors such as forage quality can have much greater effects since improved forage quality is associated with increased intake and production, and consequently CH_4 emissions per unit of milk or beef can be reduced with practically feasible strategies. The objective of the present study was to determine the variation in CH_4 production of silage samples from practical farms differing in digestibility.

Material and methods Silage samples (n = 17) used in this study were samples sent by Finnish farmers to Valio Ltd. for routine analysis of feed value. The samples consisted of three silage types (ST); 8 grass silages (G), 5 grass + legume silages (G + L) and 4 whole crop (WC) silages. Automated *in vitro* gas production system (Hetta et al. 2003) was used and methane production (ml per g DM) and other *in vitro* gas parameters were predicted as outlined by Ramin and Huhtanen (2012). Two-pool Gompertz model was fitted to gas production data. Derived kinetic parameters were used in a mechanistic, dynamic rumen model to predict methane. Indigestible neutral detergent fibre (iNDF) free of residual ash was determined by a 12-d ruminal *in situ* incubation in Swedish Red cows. Potentially digestible organic matter (pdOM) was calculated as: 1000 – iNDF - ash. Organic matter digestibility (OMD) was determined by pepsin-cellulase method (Nousiainen et al. 2003). The effects of forage type and silage composition on methane production were subjected to general linear model of the SAS program.

Results and discussion Chemical composition and fermentation quality of the three types of silages are given in Table1. There was significant difference in methane production (ml/g dry matter (DM)) between the three different types of silages (P=0.04).Total gas production (ml/g DM) was significantly (P<0.01) different between the treatments, and it tended to be more gas produced for G + L silage mixture (Table 1). No significant differences were observed on kinetic parameters (Table 1). Of single silage variables pdOM was the best predictor of methane production as follows:

CH_4 = 2.1 (±8.12) + ST + 0.042 (±0.011) × pdOM (R^2=0.61, RMSE=2.35).

The ST effects were: G -4.9 (P<0.01), G + L -2.2 (P=0.17) and WC 0.0, respectively. The model indicates that WC silages produced more methane when pdOM digestibility was taken into account. The effects of crude protein, neutral detergent fibre and fermentation products were not significant. However, including silage DM concentration improved the model as follows:

CH_4 = 0.00 (±7.17) + ST + 0.039 (±0.0094) × pdOM + 0.010 (±0.0046) × DM (R^2=0.72, RMSE=2.06). The ST effects were -4.5 (P<0.01), -1.7 (P=0.24) and 0.0 for G, G + L and WC silage, respectively.

Conclusions It can be concluded that relative to pdOM, WC silages produced more methane than G silages with G + L being an intermediate. Predicted methane production per g DM increased with the increasing concentrations of pdOM and DM in silages.

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Table 1. Chemical composition (g/kg DM if not otherwise stated) of three different silages used and their least square means of *in vitro* gas production parameters.

Parameters	GS	G + L	WC	SEM[a]	P
Chemical composition					
Ash	70.9	69.0	62.1	-	-
CP[1]	143	137	119	-	-
NDF[2]	535	535	459	-	-
iNDF[3]	94.0	119	166	-	-
OMD[4]	721	688	673	-	-
pdOM[5]	787	771	747	-	-
DM[6]	374	349	396	-	-
Ammonia N (g/kg total N)	42.1	31.3	27.2	-	-
Lactic acid	35.2	42.7	22.0	-	-
VFA[7]	9.2	15.1	10.8	-	-
In vitro gas production					
Methane production (ml/g DM)	30.3	32.0	33.3	0.49	0.04
Methane production rate (/h)	0.057	0.057	0.058	0.0010	0.32
Asymptotic CH_4 production (ml/g DM)	36.7	38.7	40.0	0.72	0.06
Total gas production (ml/g DM)	230	236	231	0.3	<0.01
Gas production rate (/h)	0.089	0.084	0.086	0.0040	0.44
Asymptotic gas (ml/g DM)	260	265	257	2.2	0.10
CH_4 / gas	0.132	0.135	0.144	0.0021	0.05

[1]CP = crude protein.
[2]NDF = neutral detergent fibre.
[3]iNDF = indigestible NDF.
[4]OMD = organic matter digestibility.
[5]pdOM = potentially digestible organic matter.
[6]DM = dry matter.
[7]VFA = volatile fatty acids.
G: grass silage, L: legume, WC: whole crop silage.
[a]Standard error of mean, 1.32 higher for WC and 1.07 higher for G + L.

Greenhouse gas emissions from fermentation of corn silage

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Keywords: carbon dioxide, CO_2-eq, GHG, maize silage

Introduction Warming of the climate system is unequivocal, as evident from observations of increases in global average air and ocean temperatures (IPCC, 2001). Agricultural systems have been presented as an important source of greenhouse gas (GHG) emissions, mainly due to the use of fertilizers, deforestation and enteric fermentation from ruminants. The Brazilian inventory of Greenhouse Gases Emissions estimated that 71% of all methane produced in this country comes from agriculture. Emissions of methane (CH_4) and nitrous oxide (N_2O) contribute considerably to global warming with potential 23 and 296 times higher than carbon dioxide (CO_2) (IPCC, 2001). Despite of the importance of silage as a feed ingredient all over the world, its global warming potential is poorly understood. Natamycin is a bacteriocin that inhibits the growth of yeasts, and it is usually used in human food such as wines and cheeses. The aim of this trial was to evaluate the GHG emission during the fermentation of corn silages and the potential for mitigation by natamycin as an additive.

Material and methods The trial was carried out at the Centro de Pesquisa em Forragicultura (CPFOR) of Federal University of Paraná, in Curitiba, PR, Brazil. Whole corn plant was harvested at 310 g kg^{-1} of dry matter (DM) and the following treatments were applied: CONTROL – no additives; LOW (4 g t^{-1}) or HIGH (8 g t^{-1}) dosage of Natamycin (Patent request 0000221109471488) (wet basis), with five replicates for each treatment. The treated forage was ensiled in plastic buckets (20 L), equipped with a mobile apparatus to recover and measure the volume of gas produced during fermentation. Silos were packed at 600 kg m^{-3}, sealed with plastic glue. The bulk density of the silos was 600 kg m^{-3}, and they were stored for 63 days. The room temperature was monitored twice a day.

Using a pipeline and a graduated collection chamber made of low density polyethylene, the total amount of gas of each silo was registered daily. In the first 3 days it was taken every 3 hours. Samples of gas were collected for determination of the concentration of CO_2, N_2O and CH_4 at days 5 and 15. Polypropylene syringes (20 mL) equipped with a valve were used for sampling. After collection syringes were kept in a styrofoam box with ice (below 10 °C) and sent to Soil Science Laboratory of Federal University of Rio Grande do Sul for analysis using Gas Chromatography (GC Shimadzu 14-A). Data were analyzed as a completely randomized design using PROC GLM of SAS (9.2).

Results and discussion No treatment effect was detected on the production of CO_2, N_2O or CH_4, probably due to the high coefficient of variation (Table 1). Same results were observed in a previous trial with sugarcane silages (Schmidt et al., 2011). Gases were only produced in the first 21 days of ensiling and the peak happened in the second day (Figure 1). The average gas production was 416 L per ton of ensiled forage, and carbon dioxide was the main gas produced (19458 ppmv – 99.9%), with low levels of methane (7 ppmv) and nitrous oxide (1 ppbv). Probably, the high concentration of CO_2 is related to the metabolism of plant cells and aerobic microorganisms capable to convert glucose to CO_2 (McDonald et al., 1991), while oxygen is still present in the silo. The corn silage produced significantly less gases compared to sugarcane silages, which showed 2,080 L per ton of ensiled forage, during 66-day trial (Schmidt et al., 2011).

The GHG emissions were 15.7; 15.8 and 12.0 g CO_2-eq per ton of forage (wet basis) for treatments CONTROL, LOW and HIGH respectively. These values are lower than the mean value of 36.4 g CO_2-eq ton^{-1} verified for sugarcane silage (Schmidt et al., 2011). However, these values are much lower than the estimates of GHG emission from feedlot cattle (5.6 kg CO_2-eq per kg of live weight gain) and dairy (1.1 kg CO_2-eq per kg of milk) production (Phetteplace et al., 2008).

The silages showed an adequate pattern of fermentation, and the fermentative losses were quite low (8.8 g kg^{-1} DM). Although no treatment effect was detected, the high dosage of natamycin seems to slightly reduce the gas production during storage period, probably due to the inhibition of undesirable microorganisms, such as yeasts and molds. This additive has a strong potential for improving the quality of silages with high fungal activity, such as sugarcane silages, or silages exposed to aerobic deterioration.

ConclusionsThe variability of the data suggests that new trials must be done. Carbon dioxide is the main gas produced during fermentation. The ensiling process does not generate GHG as intensively as other agricultural sources.

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Table 1. Greenhouse gas emissions during ensiling of corn silage.

Variable	Treatment[1]			Mean	SEM[2]
	Control	Low	High		
Gas production, L per ton of forage	424	493	332	416	55.8
GHG, g CO_2-eq t^{-1} forage	15.7	15.8	12.0	14.5	2.0
Gas production, g kg^{-1} of DM	7.4	4.6	3.8	5.3	2.2
Total DM losses, g kg^{-1}	12.2	9.5	4.6	8.8	2.4

[1]Control, no additives; Low, 4 g of natamycin per ton; High, 8 g of natamycin per ton (wet basis).
[2]Standard error of mean

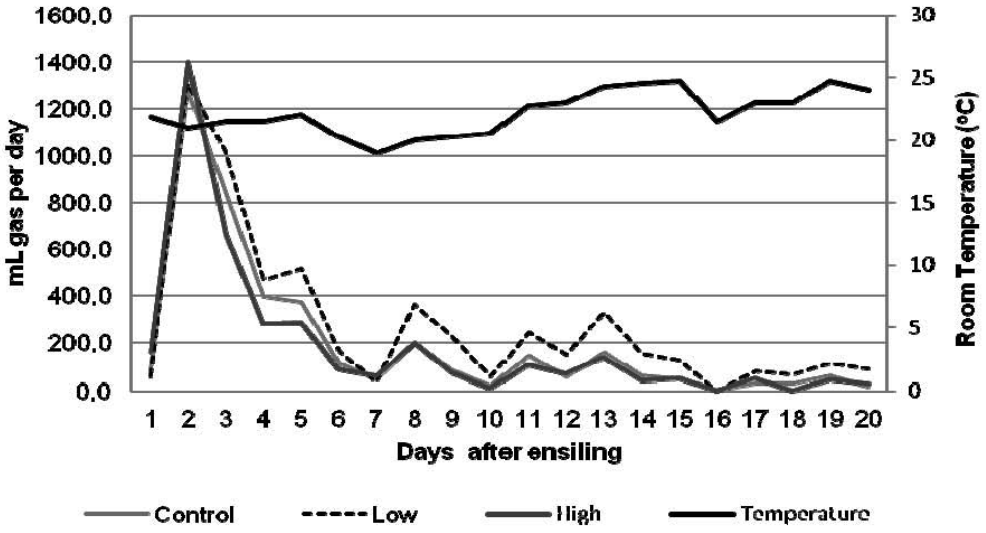

Figure 1. Daily gas production of corn silage and room temperature.

Methane yield - a new DLG-test scheme for silage additives

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Keywords: additive, biogas yield, DLG quality seal, fermentation pathway, silage, test scheme

Introduction Biogas production based on energy crops is very common in Germany. The degradation of organic matter in the biogas fermenters ("concrete cows") is often compared with the processes in the rumen, but there are important differences. In practice silage additives are offered to improve the biogas yield (Banemann et al. 2010). The question is whether ensiling with silage additives and producing different fermentation pathways (homo- or heterofermentative) affects fermentation losses, aerobic stability, and therefore the specific and absolute methane yield and chemical kinetics in the biogas fermenter. The DLG seal of quality for silage additives has been providing impartial advice on using such additives for feed crops for about 30 years now. The new possibilities of using silage additives in biogas production make it expedient to develop a reasonable test scheme that can verify their promised "improvements in biogas yield". The new test scheme should focus on all processes – from harvesting energy crops, through silage fermentation, including all losses (McDonald et al. 1991) with or without air stress (Honig 1990), to the processes in the biogas reactor. Similarly, the test scheme should be able to work with small quantities of silage in order to run the silage tests on a laboratory scale.

Material and methods In biogas fermenters acetic acid is one of the precursors of methane. Therefore it was first tested whether the specific methane yields produced from the main fermentation products of silage differ significantly. In that case, the biogas potential of silages could in principle be estimated from their contents of fermentation products. A possibly different biogas potential of lactic acid compared with acetic acid would be most relevant, as Nussbaum (2009) found that significant differences exist between most fermentation products with the exception of lactic acid and acetic acid. A repetition of these investigations (Nussbaum, 2010, unpublished), revealed the same ranking of the biogas potential, for instance lactic and acetic acid< butyric acid< 1,2-propanediol <<ethanol. Similar results were published by Pieper and Korn (2010). From these findings it can be concluded that a testing system for silage additives must in principle measure the respective yields of methane from the individual silages, which have received different treatments. The evaluation of additives with respect to gas yield must include complete accounting with all losses, from the crop at ensiling up to the silages prepared under optimal as well as suboptimal conditions.

The Hohenheim Biogas Yield Test (HBT) was developed at the University of Hohenheim (Stuttgart, Germany) for measuring biogas yield at different steps of the process. Typically 400 mg dried silage are incubated together with 30 grams of biogas slurry over 35 days at 37 °C (Helffrich and Oechsner 2003). However, this approach of analyzing dry substrates does not consider the losses of volatile substances occurring during the drying procedure of silage samples. The accompanying losses must be taken into account because considerable amounts of alcohols, (e.g. 1,2-propanediole, ethanol) are produced during ensiling, especially if heterofermentative lactic acid bacteria are used. Consequently it was tested whether the HBT method is suitable for non-dried silages also. This required homogenizing of the silage without heating up the sample. A hand-operated meat mincer was used for this purpose and 1200 mg of the resulting homogenates were incubated in triplicate for the HBT. The results (Nussbaum, 2011, unpublished) showed that the HBT also works successfully with fresh silage and mirrors the effects of silage treatments. However, only some of them were statistically significant. This was attributed to the insufficient homogeneity of the sample, which caused too large variations in gas yield. This problem was overcome by increasing the initial size of the sample from 50 to 100 g FM and homogenizing in a special blender (Thermomix TM 31, www.thermomix.de). This equipment disintegrates all types of deep-frozen silages (-18 °C) within 20 seconds without increasing their temperature above 0 °C. Four replicates of the thoroughly mixed homogenate were incubated for the HBT. The results of the test are presented in a separate contribution (Nussbaum, 2012).

Results and discussion Figure 1 shows the newly developed testing scheme. It allows testing of quite different silage additives under different ensiling conditions (with or without air challenge treatment), which can be completed by determining aerobic instability. Silages can be prepared routinely on laboratory scale (Pflaum et al. 1996). Losses are recorded by weighing. Stability tests require periodic temperature measurements. The correction of the dry matter content for volatile substances is highly important (Weissbach, 2008). Methane yields can be recorded by batch tests such as the HBT. This re-

quires proper homogenization of the silages without excessive heating of the previously frozen material, which can be reliably achieved with the Thermomix TM31.

The accounting for the evaluation of silage additives includes losses as well as specific gas yields. The benchmark is the methane yield of the material prior to ensiling.

Test scheme on a laboratory scale:

No.	Target parameter	Method
1	Losses (fermentation)	Weighing
2	Losses (aerobic stability)	Weighing and temperature rise
3	Biogas yield	Biogas batch test (HBT)

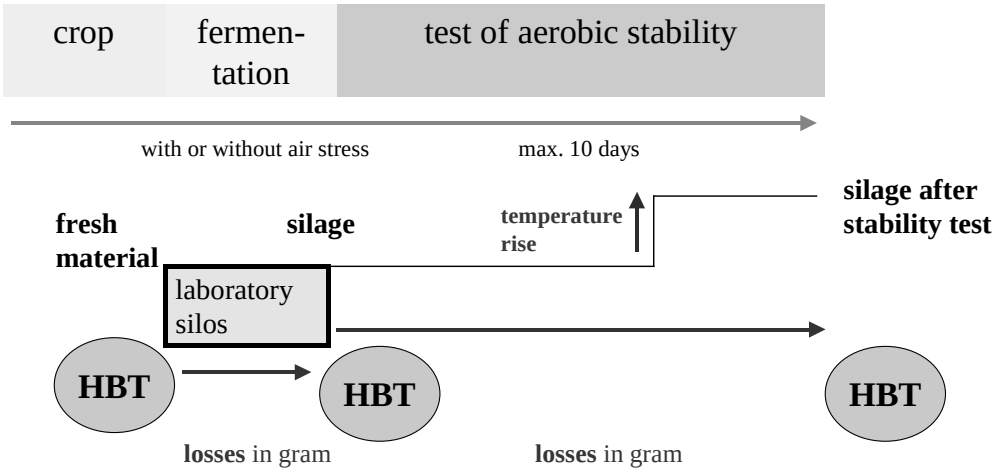

Figure 1. Test scheme over time from harvest to feed out period, showing the target parameters and test methods.

Conclusions A new testing scheme to predict methane yield of silages has been established. The new test scheme includes a procedure working directly with fresh silage to avoid the neglect of volatile fatty acids and alcohols during the drying process of samples. Novel methods for processing and homogenizing small quantities of frozen silage samples (50 to 100 g) were developed. The new test will be operated from 2012.

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Effects of silage additives based on homo- or heterofermentative lactic acid bacteria on methane yields in the biogas processing

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Keywords: biogas yield, DLG quality seal, fermentation pathway, grass silage, silage additive, test

Introduction Silage additives containing homofermentative lactic acid bacteria (LAB) accelerate and intensify the lactic acid fermentation (McDonald et al. 1991). Consequently, due to low levels of acetic acid, the fermentation losses are usually lower, but the risk of aerobic instability at the feed out period increases noticeably. Additives containing heterofermentative LAB (*Lactobacillus buchneri*) produce more acetic acid and may thus prevent the risk of heating (Oude Elfering et al. 1997). However, heterofermentative dominated fermentation often adds to fermentation losses. So far, the effect of homo- or heterofermentative LAB on the yield of biogas has been discussed controversially. For evaluation, the consideration of the potential biogas yield of fermentation acids is not sufficient on its own (Nussbaum 2009). Therefore, a test procedure was developed to determine both the fermentation losses (with or without air stress) and the yield of biogas directly to the original silage (Nussbaum and Staudacher 2012). The effects of silage additives on the yield of methane gas were accounted for and were set in relation to the amount of methane gas in the original material prior to ensiling.

Material and methods The experiments (control versus homo- or heterofermentative LAB) were performed on a laboratory scale (1.5 l, 3 replications) without (optimal ensiling, 90 days) or with (suboptimal, 49 days) air stress (stress at day 28 and 42). The harvested crop was *Lolium perenne* (32 % DM) with best ensilability (ratio between water soluble carbohydrates and buffering capacity 4.9, fermentation coefficient 68.2). We recorded all losses (weighing), fermentation acids, alcohols (ethanol, 1,2-propanediol) and aerobic stability (Honig 1990) and used the Hohenheim Biogas Yield Test (HBT) to measure the yield of biogas (Helffrich and Oechsner 2003). 1200 mg homogenized and macerated silage was incubated over 35 days at 37 °C, together with 30 grams of biogas slurry (Nussbaum and Staudacher 2012). Methane concentration and methane yield (litres methane per kg organic dry matter (oDMc)) were determined several times. The HBT was conducted with four replications per silage (Schwarz 2012). DM was corrected over the content of fermentation acids.

Results and discussion The use of homo- or heterofermentative LAB influenced the pattern of fermentation in the typical manner. The effects were along the same lines with and without air stress (Table 1). Compared to the silage without additive, the additive containing homofermentative LAB induced a significant increase in the content of lactic acid (4.5 to 7.5 % in DM without stress, 4.6 to 7.5 % in DM with stress), and thus lowered the pH. In addition, the concentration of acetic acid and ethanol were reduced, as were the fermentation losses (2.5 to 1.4 % without stress, 3.0 to 2.2 with stress). In contrast, however, aerobic stability impaired (12.3 to 6.5 days without stress, 8.0 to 2.4 days with stress). With the inoculation of heterofermentative LAB the content of lactic acid was reduced. In contrast, the content of acetic acid increased (2.1 to 5.3 % in DM without stress, 2.0 to 4.9 % in DM with stress), as did ethanol and 1,2-propanediol. The fermentation losses were about twice as high compared to the untreated control silage (2.5 to 5.3 % without stress, 3.0 to 5.4 % with stress). The higher level of acetic acid, however, improved the aerobic stability (12.3 to 14.1 days without stress; 8.0 to 10.0 days with stress). It should be noted, that the results for aerobic stability were determined after 14.1 days in the test without stress, and 10 days in the test with air stress. The improved aerobic stability reduced the DM losses significantly during the stress test, from 12.6 % (Control) to 2.9 % (heterofermentative inoculants). In contrast, losses were high as a result of rapid and sustained deterioration of those silages treated with homofermentative inoculants. At the same time the content of DM was reduced (33.6 % to 29.3 %). The use of different silage additives showed little effect on the methane content in the biogas (48.4 to 50.2 %). This is consistent with literature data (Herrmann et al. 2011). Despite the small differences in the methane content, those silages treated with heterofermentative inoculants consistently showed the highest methane content in the biogas by a significant margin. This is due to the content of ethanol and 1,2-propanediol. The experiment without air stress showed no differences regarding the specific methane yield. In contrast, after the test of aerobic stability a significant positive effect of the heterofermentative LAB was detected (317.0 to 306.8 l methane/kg oDMc). In this test (49 days, with air stress), comparison between “Control” and “Homofermentative LAB” test variant did not show differing results. The most obvious differences are seen after the test of aerobic stability. When exposed to heat, carbon compounds are lost in the form of carbon dioxide. Therefore, both “Control” (minus 30.1 l/kg oDMc) as well as “Homofermentative LAB”

(minus 58.7 l/kg oDMc) showed significantly lower specific methane yields. The differences between "Control" and "Homofermentative LAB" (minus 28.6 l/kg oDMc) are significant too.

When comparing with material prior to ensiling (litres total) at optimum fermentation, only minor effects can be detected between "Control" and "Homofermentative LAB". At suboptimal fermentation with air stress, there were no differences. The negative methane gas losses are presumed to relate to processes, which improve the digestibility of structural carbohydrates during the fermentation, and to the low specific yield (294 l/kg oDM) in the original grass prior to ensiling. They did not affect calculations substantially, because this effect was observed in all test variants. The most obvious differences are seen again after the ten day test of aerobic stability. By preventing aerobic deterioration the heterofermentative LABs caused the lowest methane gas losses (minus 1.3 %). "Control" (-17.1 %) and "Homofermentative LAB" (- 21.1 %) only differ marginally from each other.

Table 1. Effect of different silage additives on fermentation quality, losses and specific methane yield

Var	n	% DMc	pH	% DMc LA	% DMc AA	% DMc E	% DMc 1.2PD	NH_3N to N_t %	DM losses %	Aerobic stability Days	content vol.-%	Methane spec.yield l/kg oDMc	losses %
90 days fermentation without air stress													
Control	3	33.19b	4.33b	4.46b	2.05b	0.40b	0.04b	5.48b	2.54b	12.30b	48.4b	296.0	2.59a
LAB hetero	3	32.91c	4.47a	2.96c	5.13a	0.72a	2.87a	6.35a	5.33°	14.08a	49.3a	311.4	0.24ab
LAB homo	3	33.62a	4.10c	7.47a	0.96c	0.30b	0.12b	3.39c	1.40c	6.50c	48.4b	312.0	-3.84b
LSD 5%		0.20	0.01	0.38	0.29	0.15	0.10	0.32	0.14	1.82	0.73	n.s.	5.29
49 days fermentation with air stress (day 28 and 42)													
Control	3	33.43ab	4.35b	4.61b	1.98b	0.24c	0.00b	6.64b	2.98b	7.97b	48.9b	306.8b	-0.71
LAB hetero	3	33.33b	4.41a	3.48c	4.85a	0.67a	2.07a	8.13a	5.35a	10.00a	49.6a	317.0a	-1.94
LAB homo	3	33.56a	4.09c	7.50a	0.84c	0.45b	0.12b	5.07c	2.17c	2.36c	48.4c	305.2b	-1.02
LSD 5%		0.22	0.01	0.60	0.37	0.19	0.15	1.20	0.14	1.71	0.50	7.48	n.s.
49 days fermentation with air stress after test of aerobic stability (10 days)													
Control	3	33.60a							12.63a		49.3b	292.8b	17.08a
LAB hetero	3	32.44a							2.93c		50.2a	322.9a	1.31b
LAB homo	3	29.31b							7.22b		50.8a	264.2c	21.11a
LSD 5%		1.32							1.35		0.60	28.35	10.69

LA: lactic acid , AA: acetic acid, E: ethanol, 1,2PD: 1.2-propanediol, DMc: DM corrected, oDMc: organic DMc
Differing letters (e.g. a, b, c) in the same column indicate significant differences ($P \leq 0.05$)

Conclusions Depending on the conditions of ensiling, silage additives (homo- or heterofermentative lactic acid bacteria) have an impact on fermentation pattern, aerobic stability and fermentation losses, as well as on the content and specific yield of methane gas. The best effects in terms of yield of methane gas can be achieved, where use of heterofermentative inoculants improves the aerobic stability, and thus aerobic deterioration losses can be prevented. The new test procedure is able to demonstrate these effects well and makes them statistically detectable on a laboratory scale.

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The influence of ensiling on substrate specific methane yield and methane yield per hectare

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Keywords: batch fermentation test, biogas yield, dry matter losses, maize, silage, wheat

Introduction Biogas production has expanded substantially in Germany within the last years. In addition to manure, especially energy crops are used as substrates. These energy crops have to be preserved to ensure a year-round feeding of biogas plants. The ensiling leads both to a loss of mass dry matter and a change in the composition of substrate. The minimization of the dry matter losses and the exact pre-estimation of the substrate specific biogas and methane yield are essential for the economic assessment of practical biogas plants as well as for the calculation of their optimal dimensions/capacities.

Material and methods The current study was based on a 2-year (2007-2008) field trial within the framework of the Biogas-Expert project of Kiel University, Northern Germany. Commonly grown energy crops maize and wheat were cultivated with different amounts of fertilizer (0, 240, 360 kg N ha^{-1}). Substrate samples of energy crops - prepared for fermentation tests - were taken at several growth stages (Tab. 1) and for the different fertilizer treatments. Samples were chopped (< 1 cm) and either frozen immediately (fresh samples) or ensiled under laboratory conditions (three replicates, preserving jars, storage temperature 25°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 Weende-analysis. The silages were examined for pH, dry matter and organic dry matter, fermentation acids and ammonia (external laboratory). The silage quality was assessed in accordance to the DLG (German Agricultural Society) guidelines (DLG 2011).

To determine the biogas and methane yield of fresh substrates and chosen silages under laboratory conditions a common fermentation batch test (five lab replicates; anaerobic fermentation for 28 days at 38 °C, max. of 36 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). The methane yield per hectare results from the combination of the specific methane production with the dry matter yield. The dry matter loss which occurred during the ensiling is taken into account.

Table 1. List of selected substrates - Date of sampling and growth stages of substrates (+: substrate was also ensiled).

Energy crop	Date	Growth stage	Silage
Maize	mid-August	EC 71	
	early September	EC 79	+
	early October	EC 85	+
Wheat	early June	EC 69	
	mid-June	EC 73	+
	end of June	EC 77	+
	early July	EC 85	+

Results and discussion Depending on the early growth stages of wheat (EC 73 and EC 77) ensiling process was not working optimally and therefore dry matter losses of 15 to 23% could be observed. The corresponding silage quality was poor to very poor, which is mainly due to the presence of butyric acid and pH-values of 4 to 5. Only at a later harvest (EC 85), the dry matter losses were in single digits, the silage quality was very good without butyric acid. The use of a silage additive (homofermentative lactic acid bacteria) in the second year of the trial also allowed an optimal ensiling process for the earlier stages of wheat, the dry matter losses could be reduced to 5 to 11%. The corresponding pH-values were always lower than 4, butyric acid was not detected. Maize could be ensiled optimally without silage additives, the losses amounted to 1 to 19%, the pH-values were in most cases lower than 4. Butyric acid was detected only in exceptional cases, at very low concentrations.

Fresh substrates achieve in most cases a lower specific methane production than silages (Fig. 1). Especially the poor silages of wheat (EC73 and 77) reach high methane yields, which is caused by the high gas yields of the fermentation acids, in particular of butyric acid. Thus, the stoichiometric methane yield of butyric acid is almost twice as high as acetic acid or lactic acid (Weißbach, 2009).

The methane yields per hectare of silage are in a similar range to that of fresh substrates, despite some high dry matter losses. Due to the increased gas production of silages dry matter losses are compensated under lab conditions. Plöchl et al. (2009) reported similar observations. Opposite to this under full scale and field conditions, mass loss is usually much higher and therefore it will be not possible to compensate these losses by the higher methane yield of the ensiled substrates.

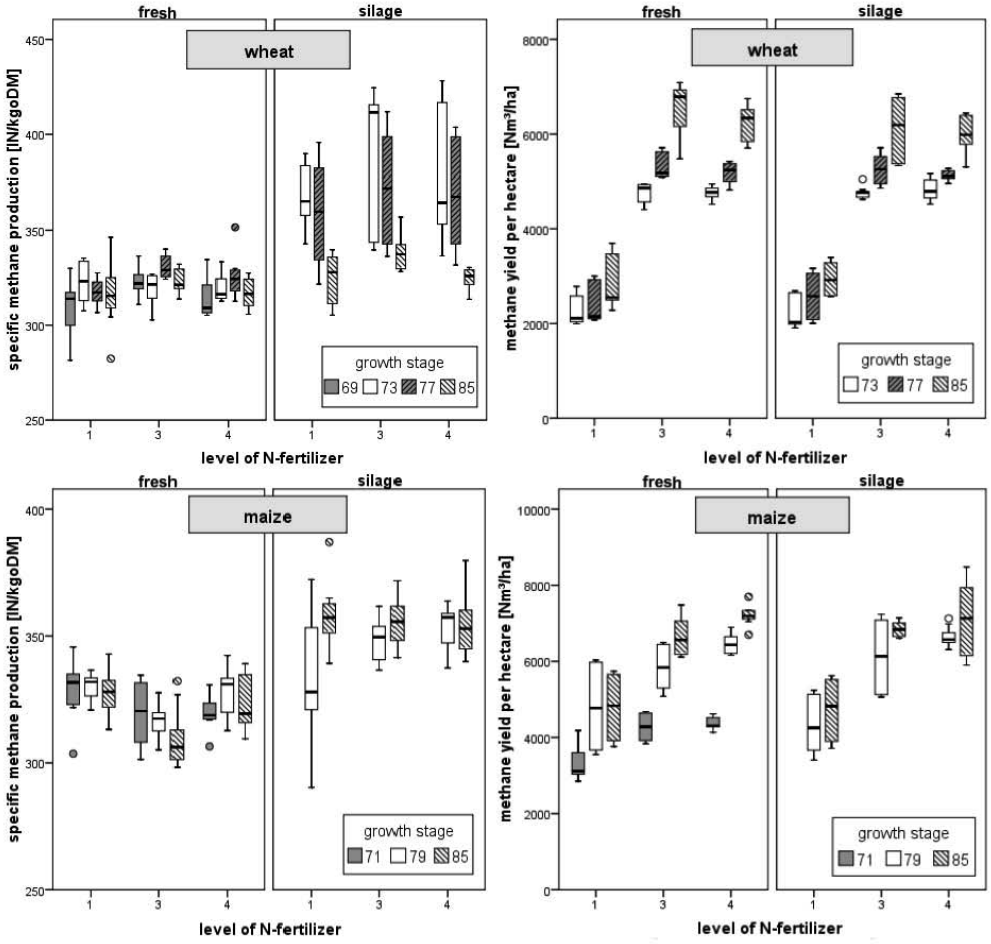

Figure 1. Specific methane production and methane yield per hectare of fresh and ensiled wheat and maize with level of N-fertilizer 1: 0 kg N ha^{-1}, 3: 240 kg N ha^{-1}, 4: 360 kg N ha^{-1}

Conclusions The specific methane production of silage is often higher than that of fresh substrates, due to increased methane production of fermentation acids. Therefore it is important to consider the dry matter losses, because it is assumed that under practical conditions the amount of dry matter losses due to ensiling can not be compensated by the higher methane production of silages.

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Degradation kinetics of fibre components of grass silage in the fermentation process and effects of enzyme application

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Keywords: biogas, degradation kinetic, enzyme application, fibre components

Introduction The gas yield of energy crops is mainly estimated in batch-tests or calculated on the chemical composition. The profitability of biogas plants mostly depends on a maximized gas yield and short retention times. Also the quality of silage plays an important role during the biogas process. Further research is especially needed for energy rich and cellulose containing renewable raw materials as grass silage to ensure an efficient and stable operation of biogas plants. In an "In-Sacco-Batch-Fermentation-Test" the kinetic of the decomposition of structural substances of grass silage with and without adding of enzymes is investigated. Therefore freshly harvested grass was ensiled in lab scale and after 90 days of ensiling the grass silage was fermented in an "In-Sacco-Batch-Fermentation-Test". First results show a significant higher decomposition of the structural substances NDF and ADF during the first 10 trial days at the variant with enzyme application on the grass silage before starting the fermentation. As a result it can be highlighted that the space-time-ratio is influenced. The structural substances of energy rich substrates will be degraded faster and the hydraulic retention time in biogas plants becomes shorter.

Material and methods The harvested material is taken from a ryegrass (Zarastro, diploid, fourth cut) trial and was ensiled in 1.5 liter preserving jars for 90 days and a storage temperature of 21 degrees. The grass silage was fermented in 60 litre barrels which were stored during the trial period of 42 days in a heating chamber with a temperature of 40 degrees. As inoculum for the batch trials sewage sludge from a public waste water treatment plant was used. Two hundred and fifty grams fresh weight of grass silage was weighed into each permeable nylon bag (pore size of 53+10 µm). As a variant in half of the nylon bags an enzyme mixture (amylase, cellulose, esterase, pentosanase and pectinase) was added on the grass silage. The application rate was 500 g/t fresh material. Each bag was sealed and connected with a ring at the barrel lid of the fermenter. The barrels filled with sewage sludge were closed and the air in the headspace of the fermenter was displaced with nitrogen (inert).

With the help of a ball valve and a plastic sleeve the biogas is collected in gas sampling bags. The biogas quality and quantity is measured during the whole trial period. After an incubation time of 4, 7, 10, 15 and 42 days the nylon bags (triple determination per day and variant) were given a cursory wash in ice water and then the bags were washed in a washing machine to remove the rest of the inoculum and debris. At the next step the bags were dried and weight before the rest of the silage in the bags is grounded. The following parameters were analysed: dry matter, crude fibre, ADF and NDF.

Results The application of an enzyme mixture improved the degradation of crude fibre, ADF (Acid Detergent Fibre) and NDF (Neutral Detergent Fibre). First results show a higher degradation of fibre components up to day 10 of an In-Sacco-Batch-Test compared to the untreated alternative: crude fibre +14.4 %, ADF +15.3 % (Figure 1) and NDF +15.2 % (Figure 2). The improvements are negligible after 42 days of fermentation. The biogas production of both variants is nearly the same.

Conclusions Kinetics of fibre degradation is estimated by the In-Sacco-Batch-Fermentation. In this trial enzyme application showed an increased degradation of fibre components within the first days. These results can be used by existing biogas plants to optimize their biogas production rate by applying enzymes. Especially for energy rich and cellulose containing renewable raw materials the application of enzymes is usefully because of extending the retention time artificially by accelerating the degradation. Seed production companies can use these data to focus on highly digestible energy plants.

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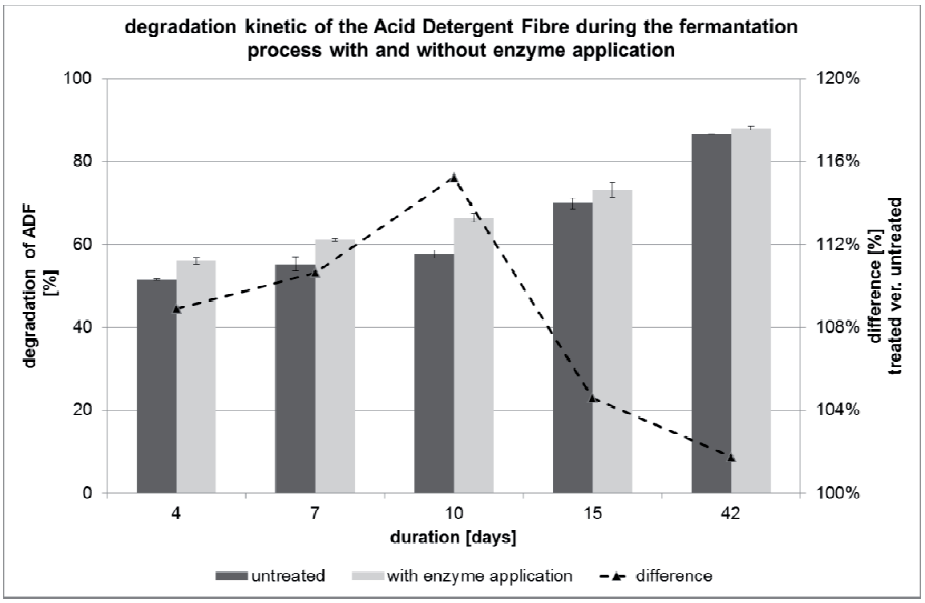

Figure 1. Degradation of grass silage (ADF) during an In-Sacco-Batch-Test with and without enzyme application

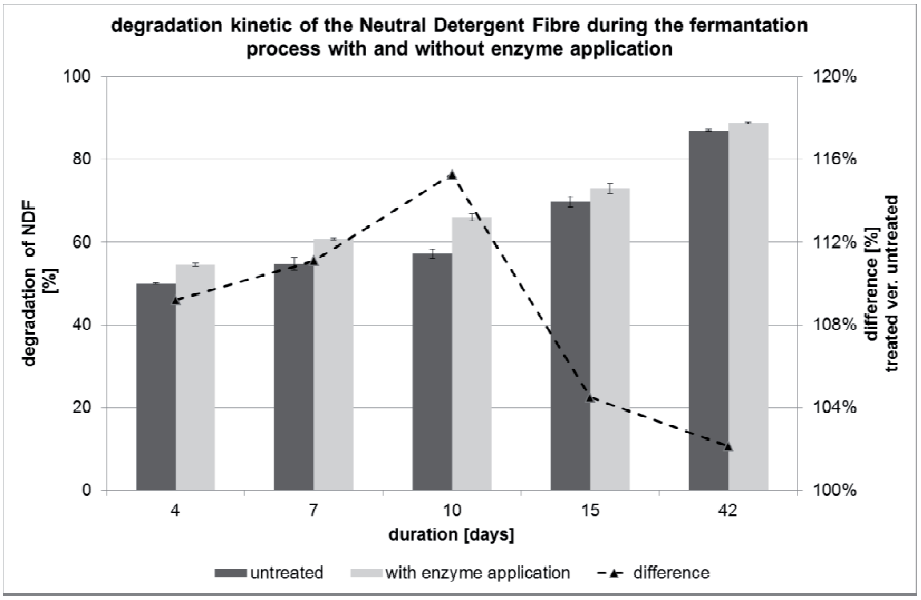

Figure 2. Degradation of grass silage (NDF) during an In-Sacco-Batch-Test with and without enzyme application

Grass for biogas – the effect of advancing plant maturity and ensiling on methane production

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Keywords: grass silage, harvest date, methane production

Introduction Grassland represents the most significant biomass resource in Ireland, accounting for approximately 0.91 of the 4.3 million hectares of agricultural land. Consequently, grass will be a dominant feedstock for anaerobic digestion on Irish farms. Grass can be an excellent energy crop and can be classified as a high yielding (up to 20 t dry matter (DM)/ha/a), low input perennial crop. However, in order to ensure a predictable quality and a constant supply of feedstock to an anaerobic digestion facility, grass will need to be harvested and stored as silage. This study investigated the effect of advancing plant maturity and ensiling on the methane production. Methane production was determined using small-scale, high-throughput batch digestion tests.

Material and methods Tall fescue (*Festuca arundinacea* Schreb var. Fuego) was grown in field plots (each 20 m^2; with three replicate blocks) under a fertiliser nitrogen input of 125 kg N/ha and harvested at three dates (Harvests 1 to 3 on 12 May, 9 June and 7 July, respectively; n = 9) in the primary growth. At each harvest date, appropriate plots were precision-chopped and representative 6 kg samples were ensiled in laboratory silos (O'Kiely and Wilson 1991). After 100 days ensilage, representative silage samples were taken for further analyses. Samples pre- and post-ensiling were oven dried (98 and 85ºC for 16 h, respectively) to estimate DM concentration. Dried (40ºC for 48 h), milled samples were used for the determination of dry matter digestibility (DMD), neutral detergent fibre (NDF) and water soluble carbohydrate (WSC) concentrations, while silage aqueous extracts were used for the determination of pH and the concentration of fermentation products (lactic acid, acetic acid, propionic acid, butyric acid and ethanol) and ammonia-N, using methods described previously (McEniry et al. 2006).

Representative dry, milled herbage samples pre- and post-ensiling were also used to determine methane production in 160 ml batch digestion tests, according to VDI guideline 4630 (2006). Briefly, substrate and inoculum were added to the bottles at a volatile solids (VS) inoculum to substrate ratio of 2:1 and at a final VS concentration of 10 g/kg. The inoculum was sourced from a cattle slurry digester at the Agri-Food and Biosciences Institute in Hillsborough, Northern Ireland. Micro- and macro- mineral solutions were added to ensure that nutrient conditions in the bottles were not limiting and sodium hydrogen carbonate was added as a buffer system (3.5 g/L). Water was added to each bottle to adjust the final volume to 70 ml, the pH was adjusted to 7.2 and the bottles were flushed with N_2 and sealed with butyl rubber stoppers. Six replicate bottles with inoculum only (blanks) were also included. Bottles were incubated at 38°C and mixed daily. The gas headspace pressure inside each bottle was recorded after 2, 5, 8, 13, 19, 26 and 36 days incubation using a detachable pressure transducer and the total amount of gas produced was estimated. A 0.8 ml sample of this gas was used to determine CH_4 concentration by gas chromatography (Purcell et al. 2011).

Means and standard deviations were calculated for silage pH and chemical composition. Other data were analysed as a split-plot design using the Proc MIXED procedure of SAS, Version 9.1.2 with harvest date as the main plot and herbage type (i.e. pre- or post-ensiling) as the sub-plot, and accounting for replicate blocks (all data) and the repeated measures effect of sampling day (batch digestion data only).

Results and discussion On average, herbage DMD decreased ($P<0.001$) with advancing harvest date (Table 1), while herbage DM ($P<0.05$) and NDF ($P<0.001$) concentrations were lowest and WSC ($P<0.001$) concentration highest at the early (Harvest 1) compared with the later harvest dates (Harvests 2 and 3). This reflects the general decrease in the plant leaf to stem ratio and the increasing cell wall content within the stems with advancing plant maturity (Buxton 1996). Since this process is accompanied by increasing lignification within the cell wall fraction there is an overall reduction in digestibility. This resulted in a lower ($P<0.05$) volume of total CH_4 being produced at the later harvest dates (Harvests 2 and 3) and an apparent slower ($P<0.001$; data not shown) rate of digestion in the batch digestion tests.

On average, a small decrease ($P<0.05$) in herbage DMD was observed during ensiling. This is a result of the loss of organic matter during ensiling as sugars are not utilised with complete efficiency by lactic acid bacteria (Table 1). The proportion of lactic acid in fermentation products was numerically higher for the later harvest dates indicating a more dominant lactic acid bacterial fermentation. However, the concentrations of both ammonia-N (< 100 g/kg N) and butyric acid (< 5g/kg DM) were generally low

in all silages and were thus indicative of a successful fermentation. The relatively small differences between the herbages pre-and post-ensiling were also reflected in total CH_4 production. Although ensiling resulted in a small numerical increase in total CH_4 production (235 and 243 L CH_4/kg VS_{added} for herbage pre- and post-ensiling, respectively), this difference was not significant ($P>0.05$). The specific CH_4 yield of some silages has been reported to be higher than the original parent material due to the formation of fermentation products (e.g. ethanol, 1,2-propanediol) with a higher potential CH_4 yield than the original fermentation substrates (Herrmann et al. 2011). It has also been suggested that ensiling increases the rate of CH_4 formation (Heiermann et al. 2002) as some of the fermentation products produced act as precursors to CH_4 formation. However, for the oven-dried samples used in this study, where some of the volatile fermentation products may have been lost during thermal drying, little difference in total CH_4 production was observed between herbages pre- and post-ensiling.

Conclusions The increase in fibre concentration and decrease in digestibility with advancing plant maturity has a negative effect on total CH_4 production. Storage of grass as silage under good management conditions has no effect on total CH_4 production.

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Table 1. Herbage chemical composition (g/kg dry matter, unless otherwise stated and excluding pH) pre- and post-ensiling (means (s.d.) presented for silage variables) and total CH_4 production (L CH_4/kg volatile solids added) in 36 day batch digestion tests.

Harvest[1]	Herbage type	Chemical composition[2]								Total CH_4 production
		DM	DMD	NDF	WSC	pH	LA	LA/FP	NH_3-N	
1	Pre-ensiling	184	788	529	161	-	-	-	-	268
1	Post-ensiling	167	784	536	4	4.24 (0.095)	57 (28.3)	0.38 (0.129)	62 (31.0)	273
2	Pre-ensiling	213	680	623	92	-	-	-	-	220
2	Post-ensiling	209	641	642	8	3.93 (0.170)	74 (10.4)	0.67 (0.111)	64 (24.5)	231
3	Pre-ensiling	217	582	653	88	-	-	-	-	216
3	Post-ensiling	218	530	667	10	3.76 (0.295)	66 (27.3)	0.55 (0.209)	63 (2.6)	224
	s.e.m.									
Harvest (main-plot)		5.0	10.5	5.9	2.3	-	-	-	-	7.1
Herbage type (sub-plot)		3.3	7.9	4.6	1.9	-	-	-	-	4.7
Harvest × herbage type		5.8	13.7	8.0	3.2	-	-	-	-	8.2
Levels of significance										
Harvest		*	***	***	***	-	-	-	-	*
Herbage type		NS	*	NS	***	-	-	-	-	NS
Harvest x herbage type		NS	NS	NS	***	-	-	-	-	NS

[1] Harvest 1 = 12 May, Harvest 2 = 9 June, Harvest 3 = 7 July

[2] DM = dry matter (g/kg), DMD = dry matter digestibility (g/kg), NDF = neutral detergent fibre, WSC = water soluble carbohydrate, LA = lactic acid, LA/FP = lactic acid as a proportion of total fermentation products (lactic acid + acetic acid + propionic acid + butyric acid + ethanol), NH_3-N = ammonia-N (g/kg N)

Fermentation losses during ensiling of sugar beets as substrate for biogas production

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Keywords: biogas production, ensiling, fermentation losses, sugar beet

Introduction Sugar beets have attracted increasing attention as substrate for biogas production. In order to make them available throughout the year, preservation is needed. Results of previous trials have shown that it is possible to ensile crushed sugar beets in water-proof silos and of whole sugar beets in plastic tubes (Wagner et al. 2009). During ensiling, sugar is fermented mainly to ethanol by yeasts. Fermentation losses and possibilities to influence them in sugar beet ensiling are not yet well established. Therefore, the aim of this study was to determine the losses of dry matter (DM) and of methane-forming potential (MFP) during the fermentation process.

Material and methods Whole as well as crushed sugar beets were ensiled, either untreated or treated with an antimycotic chemical silage additive (KOFASIL® STABIL, 2 L/t, containing sodium benzoate and potassium sorbate). These ensiling trials were carried out using air-tightly closed 120 L plastic drums, which were stored at room temperature for 90 days. Fresh beets, silages and silage effluents were sampled and analyzed for chemical composition.

Volatile organic compounds (VOC) were determined by gas-chromatography. Dry matter (DM) content of silages as well as that of effluent was corrected for the loss of VOC during drying (Weissbach and Strubelt 2008):

$$DM_c = DM_n + 0.95\ VFA + 0.08\ LA + 1.0\ AL \quad [g/kg\ FM],$$

where DM_c is the content of corrected and DM_n the content of non-corrected dry matter (measured by oven drying after 3 hours at 105 °C), VFA is the sum of volatile fatty acids, LA is lactic acid and AL is the sum of alcohols. Based on the chemical composition of fresh sugar beets, sugar beet silages and their effluents, the respective contents of fermentable organic matter (FOM) were estimated according to Weissbach (2009):

$$FOM = 991 - ash - 0.50\ ADF_{org} \quad [g/kg\ DM_c].$$

By using FOM and the content of alcohols, the methane-forming potential (MFP) of fresh beets, silages and their effluents could be calculated (Weissbach 2009):

$$MFP = 375\ FOM + 0.32\ AL \quad [L/kg\ DM_c].$$

Fermentation losses were determined by balancing input and output of DM_c and MFP, respectively (Weissbach 2011, Weissbach et al. 2011). Data were subjected to statistical analysis (one-way ANOVA) by employing SPSS Statistics of IBM. Differences among means were tested by Student-Newman-Keuls test, and significance declared at $P \leq 0.05$.

Results and discussion In untreated sugar beet silages the vast proportion of sugar was converted into ethanol (Table 1), thereby generating DM losses during fermentation of more than 20% (Table 2). On the contrary, microbiological activity caused by yeasts was almost completely suppressed in the sugar beet silages treated with the antimycotic silage additive. As a consequence, most of the initial sugar content was preserved in the silage. As during ethanol formation most of the energy of the sugar is retained in the fermentation end-product ethanol, the energy content of untreated sugar beet silages and, thus, also the MFP of the silage DM was elevated when compared with fresh sugar beets or with treated silage. MFP increased by approximately 50 litres per kg DM. In this way, the high DM losses of untreated silage were compensated for, so that the MFP losses were less than 10%. The use of the antimycotic silage additive reduced DM losses significantly, whereas MFP losses were not significantly affected.

Conclusions Ensiled sugar beets can sustain high DM losses by excessive ethanol formation due to intensive yeast activity. However, because of the high energy content of ethanol, the losses of MFP are much lower than those of DM in this case. Thus, DM losses do not reflect energy losses in sugar beet silages.

The antimycotic silage additive KOFASIL® STABIL suppresses ethanol fermentation, thereby leading to decreased losses of both, DM and MFP. Mechanical processing of sugar beets prior to ensiling has only a minor effect on fermentation losses as long as air contact to silage is excluded.

Table 1. Effects of a chemical additive on dry matter concentration, sugar content, fermentation products, fermentable organic matter and methane-forming potential in sugar beet silages.

Treatment	DM	Sugar	Lactic acid	Acetic acid	Ethanol	FOM	MFP
	(%)	(g/kg DM)				(g/kg DM)	(L/kg DM)
Fresh beets	25.0	732	n.d.	n.d.	n.d.	938	352
Ensiled beets							
Whole beets							
Untreated	20.7[a]	289[a]	77	28[b]	162[b]	920	403[b]
Treated[1)]	24.0[b]	641[c]	24	21[a]	12[a]	927	352[a]
Crushed beets							
Untreated	21.2[a]	271[a]	78	31[b]	171[b]	912	402[b]
Treated[1)]	24.2[b]	455[b]	66	40[c]	9[a]	924	350[a]
Significance[2)]	*	**	n.s.	***	***	n.s.	***

FOM = fermentable organic matter; MFP = methane-forming potential, volume under standard temperature and pressure; n.d. = not determined; n.s. = not significant; [1)]KOFASIL STABIL (2 L/t); [2)]means in columns with unlike superscripts differ significantly at P ≤ 0.05 (Student-Newman-Keuls test).

Table 2. Effects of a chemical additive on losses of dry matter and methane-forming potential during fermentation of sugar beets.

Treatment	Fermentation losses (%)			
	DM		MFP	
	Mean	SEM	Mean	SEM
Ensiling whole beets				
Untreated	21.0[a]	4.7	9.0	4.3
Treated[1)]	7.6[b]	2.5	7.1	2.8
Ensiling crushed beets				
Untreated	20.3[a]	2.0	8.3	1.7
Treated[1)]	6.4[b]	0.8	5.7	0.5
Significance	***		n.s.	

DM = dry matter; MFP = methane-forming potential, volume under standard temperature and pressure; SEM = standard error of mean; n.s. = not significant; [1)]KOFASIL STABIL (2 L/t); [2)]means in columns with unlike superscripts differ significantly at P ≤ 0.05 (Student-Newman-Keuls test).

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Production cost of excess silage for bioenergy in Finnish cattle farms

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Keywords: bioenergy, biogas, grass yield variation, production costs,

Introduction Grass growth is highly dependent on weather conditions. Standard deviation of grass yield in Finnish variety trials (Kangas et al. 2011) has been about 11 % of the yearly average yield. Grass silage is difficult to compensate in cattle feeding in Finland. Therefore a risk-averse farmer may choose to grow grass on bigger field area than needed on average. By this strategy the farmer will have adequate silage stock for his cattle in most of the years. Due to this risk avoidance behavior of farmers, there is potential to increase silage production in cattle farms during good years with relatively small additional inputs needed. For dairy cow feeding this excess silage may be considered second class, but for bio-energy use (e.g. anaerobic digesters) the quality is most likely adequate. Variation in grass yield between years in variety trials and crop production statistics was compared. Further calculations were made to estimate production cost for excess silage that would be available for bio-energy use in good grass years in cattle farms.

Material and methods The grass yield variation was determined from results of Finnish variety trials (Kangas et al. 2011). Years between 1983 and 2010 and four varieties (timothies Tammisto II and Grinstad, meadow fescue Kasper and tall fescue Retu) were included to the analysis due to the long time series available of those varieties. The deviation of yield difference was analyzed with SAS univariate procedure. This data was compared to the grass yields reported from Finnish farms (Tike 2012).

Silage production cost was calculated in two cases. In both cases the model farm had 50 hectares cultivated grass area, silage was harvested twice a year and the target yield was 240 ton dry matter (DM) /year. Yield from this area would be adequate in 95 % of years. Variation of grass yield potential was assumed to be equal to yield variation in variety trials. In case 1, the farmer harvests in maximum the needed 240 ton dry matter. As there is no demand for the excess silage, in case of good year the second harvest is replaced by mowing using a flail-mower. In case 2, there is effective market for silage and the farmer always fertilizes and harvests all the fields and sells the excess silage.

In both cases grass was harvested using mower-conditioner followed by integrated baler wrapper. The bales were transported from field to farm by tractor and wagon. Fields were fertilized to replace the phosphorus and potassium contained in harvested grass. Further 100 kg nitrogen was applied for each harvest. Variable costs from crop husbandry as well as fixed costs caused by machinery are included in the cost calculation. Silage production costs were calculated for both cases for five different yield potential alternatives 63 %, 75 %, 100 %, 125 %, 137 % of mean yield (7560 kg DM/ ha /year).

Results and discussion During the last 15 years yield potential of grass varieties measured in the variety trials has increased. But in the same time yields reported from farms have decreased (Tike 2012). In the first decade of 21st century the positive yield variation in Finnish farms seems nearly non-existent (Figure 1). This together with smaller variation in farm yield data (standard deviation 8.6 %) and clearly smaller average DM yield in farm data (5500 vs. 9300, assuming 300 g DM/kg) suggest risk avoidance behavior of the farmers related to silage production. Further this reveals some potential to increase silage production on Finnish cattle farms in case of future demand.

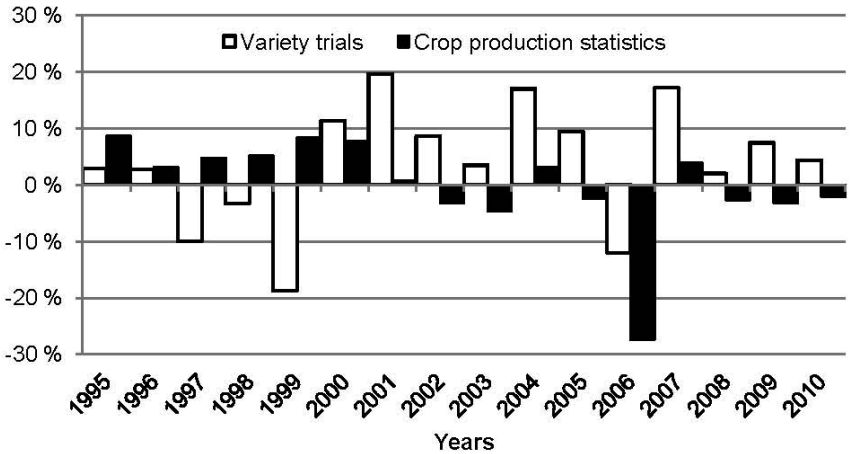

Figure 1. Grass yield variation (as percentage of mean) in variety trials and crop production statistics.

Due to high moisture content of silage and rapid spoiling of opened silos, typical distance between silage buyer and seller is relatively short. After unfavorable growing season the shortage of silage is thus not easily alleviated or it will be expensive. A farmer who is not willing to face the problems due to inadequate silage yield more than once in a decade will lose grass production potential of 25 %, but if the farmer is willing to face the problem only once in twenty years the amount of lost potential is 37 %.

In case 1, the silage production cost slightly decreases with higher yield due to spared fertilizing, machinery and labor costs (Figure 2). Although the harvested amount of silage was the same between years, the harvesting big yield from smaller area is more effective than harvesting the same amount from larger area.

In case 2, the production costs per produced DM ton decrease with higher yield, but total costs increase heavily due to up to 117 % increase in total harvested DM. Taking the total costs of case 1 as the necessary minimum costs to reach the target DM yield, the marginal production cost of excess silage was calculated as difference between total costs of the two cases divided by the produced excess silage. In high yield years the marginal production cost for excess silage can go down to 64 % of respective production costs of case 1.

Harvesting system used in this calculation (round baler and wrapping) has high variable costs compared to eg. field chopper and bunker silo. In a system where the share of fixed costs is high the increase in utilization rate might reduce the production costs even more. In practise, the only way to increase the utilization rate of the machinery is to harvest second and third cut as well, as during first cut most of the machinery is efficiently used already.

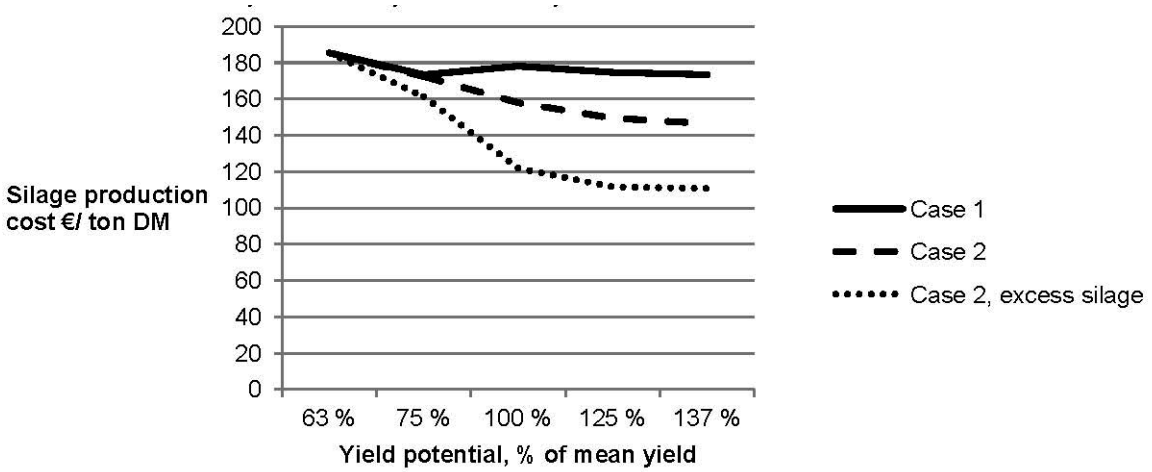

Figure 2. Grass silage production costs (€ / ton dry matter (DM)) for two grass production strategies. Case 1 is only producing silage for constant yearly consumption without any reserve storages or any selling of the product. Case 2 is producing grass on the market and utilises whole yield potential of his fields. Marginal production cost for excess silage is calculated as difference between the total costs of the two strategies.

Conclusions Grass yield varies greatly between years due to weather conditions. A farmer that wants to ensure sufficient silage ensiled each year has to scale up his grass area to be sufficient in unfavourable years as well. During average or good years the surplus grass production may not be harvested if there is no demand for it. The farmer that is willing to scale up his grass area by 33 % will suffer for shortage of silage only once in decade, and by 59 % scaling only once in twenty years. Respectively the same amount of silage production potential is lost if reserve silage is not ensiled in good years. However, scaling up the grass area and harvesting silage only for the next winter's use will keep silage production cost quite stable from year to year.

Having some demand for extra silage from second cut, the farmer would be able to reduce production costs of silage by 10 %, the excess silage produced by 70 % of the costs compared to the alternative strategy, not harvesting the excess grass. Market for this excess silage would benefit silage producing cattle farm in two ways: Lowering the production cost of the silage consumed on the farm and offering a new source of income through selling the excess silage.

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Silage quality of biomass harvested from semi-natural grassland communities

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Keywords: biodiversity, biogas, chemical composition, nature conservation, nutritional characteristics, semi-natural grassland

Introduction Grasses from extensively used and increasingly abandoned grasslands are not satisfying as feed for high producing cows. The replacement of intensively managed grass with forages from semi-natural grasslands reduces nutritional values and fermentation quality of grass and grass silages. Additionally, semi-natural grasslands are not utilised sufficiently by grazing animals, which results in the decay of flora and eventual abandonment of the grassland. Thus, the production of bioenergy from biomass, whilst reserving the biodiversity of NATURA 2000 grassland habits according to the mandatory rules of their agricultural and ecological utilisation within the PROGRASS Life+ Project (2009-2012), was investigated.The major goal of this study was to estimate the effect of location and treatment, i.e., applying silage additives, on silage quality as estimated from chemical composition of a larger set of samples. The specific objectives were to evaluate the effects of different silage additives on the silage quality to produce a better silage as feedstuff for ruminants, as well as improving biogas production.

Material and methods Grass silage samples originated from three different semi-natural grassland sites mainly from Natura 2000 grassland territories of Estonia (EE), Germany (DE) and Wales (UK) in 2009. Each site had six different experimental areas and each area consisted six 10 x 10 m sub-plots. Most plant species were blooming when harvested. Biomass for ensiling was harvested from the unfertilised sub-plots as first cut, and chopped to approximately 50 mm length. The cut grass was subdivided into 4 homogenous batches prior to treatment with one of three different silage additives. The four different treatment were: a control without additive (CON); a chemical additive (CHEM), i.e., a combination of calcium formate, calcium propionate and 10% sodium benzoate (Anta-Sil BZ®, Dr. Eckel GmbH, Niederzissen, Germany), applied at 1 g/kg fresh matter (FM); a combination of *Pediococcus acidilactici* and *Lactobacillus plantarum* (Anta-Sil LA®, Dr. Eckel GmbH) at 2.56 x 10^8 colony forming units (CFU)/kg FM plus 35 granulated sugar g/kg FM (SUG); and a silage inoculant of *Lactobacillus buchneri* (Pioneer 11CH4®, Pioneer Hi-Bred Northern Europe, Buxtehude, Germany) at 1.00 x 10^8 CFU/kg FM (INO). Each treatment consisted of three replicates on a total of 18 experimental areas (3 sites x 6 areas). Plastic (PVC) tubes were used as mini-silos (0.002915 m^3), the endings were sealed with rubber caps to prevent oxygen inflow, locked by pipe clamps and a gas valve was provided for gas release.The duration of ensilage was 252 to 266 days and silos were stored at 15±2°C and then subdivided after opening. Homogeneous sub-samples were taken and stored anaerobically in vacuum-sealed polyethylene bags. Dry matter (DM) content was determined by lyophilisation to a constant weight (72 h) and further drying at 103°C overnight to determine the residual moisture content. The DM was corrected for losses of volatiles during drying according to Weissbach and Kuhla (1995).The dried silage samples were successively ground to pass a 5-mm screen and a 1-mm screen. The proximate constituents of freeze-dried, milled samples were analyzed in duplicate according to the official German methods (VDLUFA, 2007), and method numbers are given. The ash content was determined as the gravimetric residue after incineration at 550°C overnight (8.1). Total nitrogen (N) was estimated by the Dumas method, and crude protein (CP) was calculated as total N x 6.25 (4.1.2). The contents of neutral detergent fibre (6.5.1) assayed with a heat stable amylase and expressed exclusive residual ash (aNDFom), acid detergent fibre (6.5.2) expressed exclusive residual ash (ADFom) and lignin (6.5.3) determined by solubilisation of cellulose with sulfuric acid (lignin(sa)) were determined sequentially using an ANKOM^{2000} Automated Fiber Analyzer (ANKOM Technology, Macedon, NY, USA).

Results were analysed statistically using SAS (2008) using a completely randomized factorial design considering treatment, area and the interaction between treatment and area. Least squares means were compared and adjusted for multiple comparisons by Tukey`s Honestly Significant Difference (HSD) test and differences were regarded statistically significant if $P<0.05$.

Results and discussion At all three sites, a significant area effect was observed on all measured chemical composition variables (Table 1). The highest DM occurred in the SUG treatment, but this difference was significant ($p<0.001$) only in Wales. Generally, the SUG treatment had the lowest concentrations of ash (DE, $p<0.05$; UK, $p<0.001$), CP (DE, $p<0.05$, UK, $p<0.001$; EE, $p<0.05$), aNDFom (DE, $p<0.001$; UK, $p<0.001$; EE, $p<0.001$) and ADFom (DE, $p<0.001$; UK, $p<0.001$; EE, $p<0.001$) across all treatments and sites. Lignin(sa) content of SUG was only lower at the German site ($p<0.05$). All silages from Wales had very high lignin(sa) concentrations (> 145 g/kg DM). Interactions between area and treatment were observed for all variables except lignin(sa) in Wales, and in Germany only for aNDFom ($p<0.05$). Fermentation acid analysis that is currently undertaken will provide additional information on the quality of the silages as a raw material for non-feed utilisation of silages.

Table 1. Chemical composition (g/kg dry matter unless stated), standard error of the means (SEM) and statistical significance of different treatments at the different sites in 2009.

Variables	Treatment[1]				SEM	Statistical significance[2]		
	CON	CHEM	SUG	INO		Area	Treatment	Area × Treatment
Germany (DE)								
Dry matter (g/kg)	308	306	320	300	6.69	***		
Ash	109	105	100	107	2.42	***	*	
Crude protein	96.5	98.9	89.5	98.6	1.20	***	*	
aNDFom	576	568	542	595	7.05	***	***	*
ADFom	377	371	352	389	4.07	***	***	
Lignin(sa)	93.9	92.9	87	101	3.10	***	*	
Wales (UK)								
Dry matter (g/kg)	345	349	360	342	3.37	***	***	***
Ash	43.1	44.4	41	43.7	2.19	***	***	*
Crude protein	103	104	96.6	103	1.43	***	***	***
aNDFom	726	721	686	736	5.33	***	***	
ADFom	466	435	438	468	6.28	***	***	***
Lignin(sa)	156	155	147	159	7.58	***		***
Estonia (EE)								
Dry matter (g/kg)	306	308	327	312	5.15	***		
Ash	64.6	67.8	67	65.1	1.52	***		
Crude protein	110	109	96.5	111	2.26	***	*	
aNDFom	612	604	551	608	6.50	**	***	
ADFom	399	395	353	401	3.50	**	***	
Lignin(sa)	89.1	86.6	92	90.1	2.06	**		

[1]Each value represents the mean of 18 observations (6 areas x 3 replicates). CON: control, CHEM: calcium formate, calcium propionate and 10% sodium benzoate, SUG: granulated sugar (35 g kg^{-1}) plus *L. plantarum, P. acidilactici* (2.56×10^5 CFU g^{-1}), INO: *L. buchneri* (1.00×10^5 CFU g^{-1}). [2] *p<0.05, **p<0.01, ***p<0.001.

Conclusions Based on the preliminary results of the first year, it appeared that the added sugar slightly improved the DM concentration in the silages. Whether the lower fibre and CP values in the SUG silages were the results of partial degradation of plant cell walls and higher degree of proteolysis of plant protein during long-term ensiling, respectively, or simply a dilution effect due to the addition of sugar, need further clarification.

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Harvesting and storage alternatives for biomass feedstock from green fallow and nature management fields in Finland

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Keywords: bioenergy, biogas, conservation, fallows, harvesting technology, storage

Introduction Nearly 200 000 hectares of arable land in Finland is used in green fallow and nature management field programmes. Management guidelines of those fields restrict use of fertilizer (principally fertilizing is not allowed) and choice of crops. Most of the area is used by perennial swards. Utilizing the yield is allowed. Cutting the swards is required and in most cases farmers' cut the swards and leave the mulch on the field. The biomass would be suitable feedstock material for biogas plants and studies are underway to assess the amount of biomass available and its suitability for biogas production (Niemeläinen et al. 2012). In this study the harvesting and storing alternatives were studied and their costs were compared. Harvesting and storage alternatives are similar to silage harvesting and the results can be utilized assessing costs of silage making chains.

Material and methods In all five contractor scale harvesting & storage (h&s) chains were studied. Two of chains were based on self-propelled forage harvester and three are based on baling. The selected chains were following: 1) Forage harvester harvesting and storage in clamp silo, 2) Forage harvester harvesting and storage in tube, 3) Big square bale harvesting and storage in tube, 4) Round bale harvesting (combi system) and round bale storage and 5) Round bale harvesting and storage in tube.

The h&s chains consist of mowing, raking, harvesting, transporting to the yard of biogas plant (distance 6 km) and storing. Baling chains (3-5) include also bale crushing, hence every chain produces suitable chaff for biogas process. Also silage additive (formic acid) and plastics for storages were included to the calculations. In the base case machinery was expected to use 400 ha/a (chains 1-3) or 200 ha/a (chains 4-5). The grass yield was assumed to be 4 ton/ha DM per harvest and only one harvest was assumed to be done per year.

The costs of harvest chains were calculated by using TTS-Kone-program (www.tts.fi). The program uses average price of machinery on the Finnish market, straight-line depreciation and 5 % interest. Salary costs of driver (22 eur/h), fuel costs, maintenance costs, insurances, storage costs and financial risk cost (5 %) were included to calculation. The results were validated by comparing obtained values to average contractor charges (Palva 2011) and to Swedish silage cost study (Gunnarsson et al. 2009).

Results and discussion Harvesting and storage costs ranged from 67 to 77 eur/ton DM (figure 1). The plastic costs were the highest in chain 4. Differences were surprisingly small between the harvesting chains, but they are not meaningless. If we have 400 kW biogas plant using only grass, it would consume about 1100 ton DM grass per year, which means that plant would save 11 000 eur per year by choosing chain 2 instead of chain 4. The results showed that loose chaff chains are cheaper than round bale chains up to 20 km transport distances, if the field size is large enough.

There are also other things than total costs, which has to be taken into account when choosing h&s method for biogas plant or for cattle farm. For example, all h&s chains are not available everywhere as contractor service, which can naturally affect on choice of chain. An effective self-propelled forage harvester + clamp silo chain requires 6 – 8 workers to work simultaneously. If adequate amount of labour is not available, choosing the baling chain, which can work effectively with 3 – 4 workers could be more feasible.

We also studied, how higher utilization of the capacity of the harvesting chain would affect harvest costs (figure 2). This could be situation of a silage contractor planning to expand business to biogas silage harvesting. According to our calculation, if harvested area increased from 400 ha/a to 600 ha/a, this would decrease h&s costs about 10 eur/ton DM. If biogas silage harvest takes place at different time than cattle silage harvest, expanding to biogas silage could be a good opportunity for the contractors. Higher yield per ha would also decrease h&s costs, because costs of mowing and raking do not depend on the yield. Most of contractors have fixed price also for silage chopping (eur/ha) but bales are normally charged as eur per bale (Palva 2011).

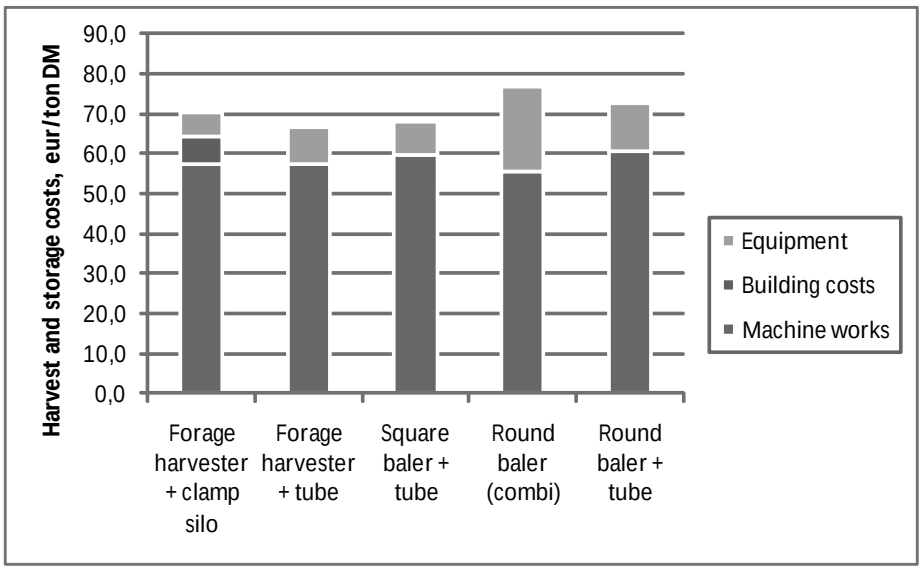

Figure 1. Harvest and storage costs of biogas grass in Finland (yield 4 ton/ha DM). Building costs of clamp silo and costs of plastic, baling nets and silage additives (Equipment) were included.

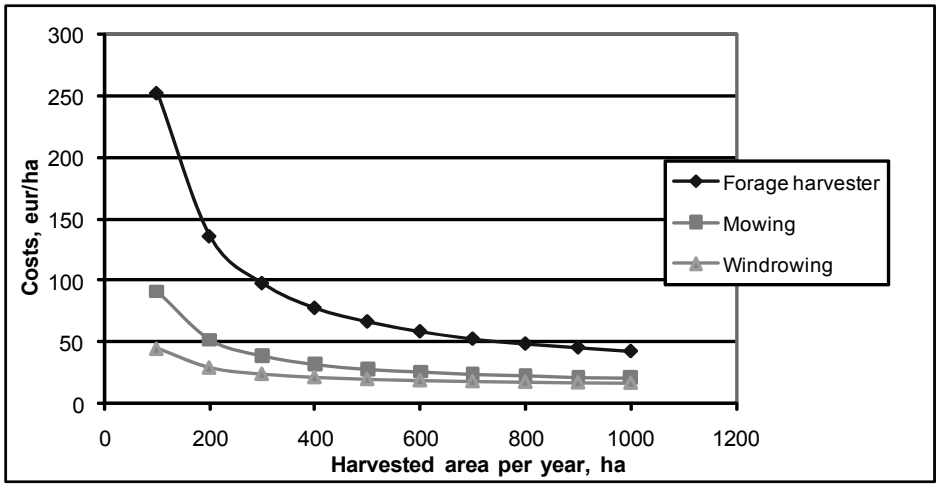

Figure 2. Harvest costs of different work stages when harvested area is increasing.

Conclusions If grass from green fallow and nature management field was used for biogas production, it would produce biogas about 3 MWh/ton DM (Lehtomäki 2006). If this was converted to electricity according to Finnish tariff (8–12 cents/kWh), value of grass would be 80–120 eur/ton DM before taking off costs of h&s and biogas plant. Thus it is clear that electricity production from grass via biogas is not profitable. But if biogas was processed to transport fuel, economy could be better.

Harvesting grass for biogas plant feedstock could improve utilization of the silage harvesting chain capacity and thereby decrease harvesting costs also in silage harvesting.

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Survival of silage lactic acid bacteria in the gastrointestinal tract of ruminants as determined by PCR-DGGE with *Lactobacillus*-specific primers

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Keywords: denaturing gradient gel electrophoresis, gut, lactic acid bacteria, silage

Introduction Silage can be a good vehicle to increase and convey probiotic functions for animals, but the criteria for screening and functions to be implemented have not been well defined. Survival in the gastrointestinal tract does not necessarily imply probiotic potential, but it is not clear whether silage lactic acid bacteria (LAB) can affect the population and community of gut bacteria in ruminant animals. In this study, *Lactobacillus*-specific PCR–denaturing gradient gel electrophoresis (DGGE) was performed to evaluate survival of silage LAB in the goat gastrointestinal tract.

Material and methods First-growth Italian ryegrass was harvested at full heading stage and wilted for 4 days to prepare low-moisture silage (DM 661 g/kg). The wilted herbage was ensiled without additives in a polyethylene bag for 6 months and then fed to 3 castrated male goats fitted with rumen cannulae. Silage was given alone (period 1) or with concentrates at 50:50 on a DM basis (period 2). Each 11 day experimental period comprised 7 day adaptation, 3 day faecal collection followed by rumen fluid sampling on the last day. Feeds were offered at 0900 and 1700, each providing 1.1 times more feed than was recorded on the previous day. Rumen fluid was taken prior to and at 2, 4, and 8 h after the 0900 feeding. Bacterial DNA was purified with a commercial kit (QIAamp DNA Stool Mini Kit; QIAGEN, Germantown, MD, USA). The *Lactobacillus* 16S rRNA gene was PCR amplified (Heilig *et al.*, 2002), and the amplicons were subjected to second-round PCR targeting a variable (V3) region. DGGE was used to separate bacterial DNA according to sequence (Nishino *et al.*, 2012). BLAST searches were made against the GenBank database to determine the closest relatives of these partial 16S rRNA gene sequences.

Results and discussion Concentrations of lactic acid, acetic acid and ethanol (3.10, 3.02, and 7.79 g/kg DM, respectively) were low, whereas numbers of LAB and yeasts (1.9×10^6 and 2.8×10^5 cfu/g, respectively) were moderate in the wilted Italian ryegrass silage. Goats fed silage and concentrates consumed more DM than those fed silage alone (36.1 vs. 26.6 g/kg body weight/day), which increased the LAB counts and VFA concentration and decreased the pH of rumen fluid across the time of sampling (Figure 1). The acetic acid/propionic acid ratio in the rumen fluid remained high with or without concentrates.

The LAB detected in wilted Italian ryegrass silage included *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus murinus* and *Lactobacillus sakei* (Figure 2). Bands indicative of these LAB species, except for *L. murinus*, were not found in rumen fluid or faeces, suggesting that it is difficult for silage LAB to survive the digestion process in the gut. The LAB community in rumen fluid showed few changes among sampling times; however, bands detectable in rumen fluid were not necessarily the same as those seen in faeces. The LAB community in rumen fluid and faeces appeared unaffected by feeding concentrates.

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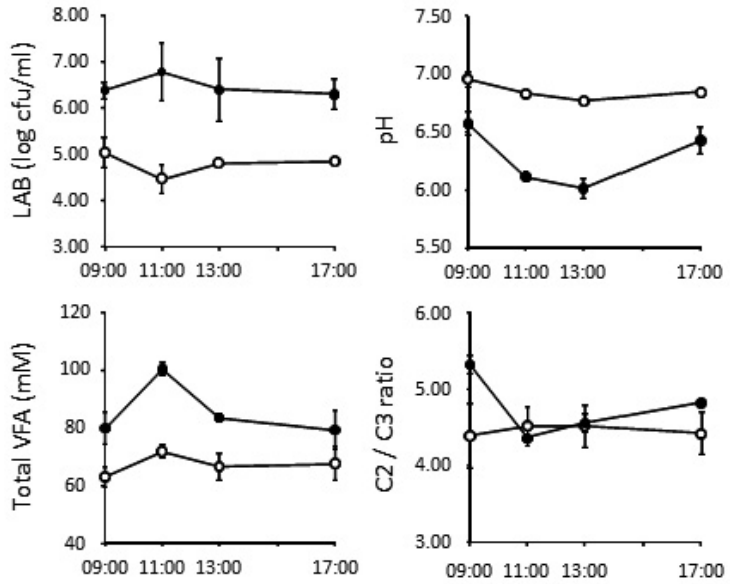

Figure 1. Changes in lactic acid bacteria (LAB) counts, pH value, total volatile fatty acid concentrations and acetic acid/propionic acid ratio in the rumen fluid of goats fed wilted Italian ryegrass silage alone (○) or with concentrates at 50:50 on a DM basis (●).

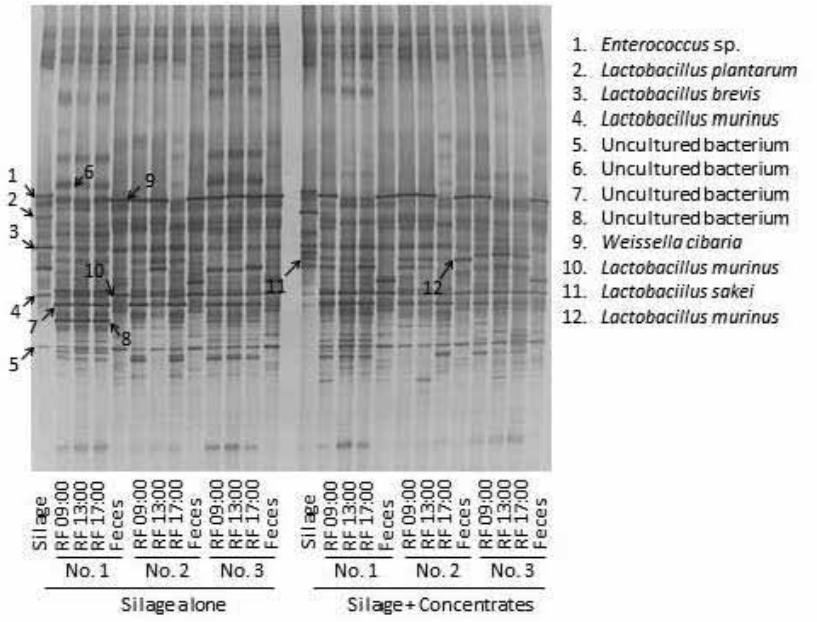

Figure 2. Survival of silage lactic acid bacteria (LAB) in the gastrointestinal tract as determined by PCR-DGGE with *Lactobacillus*-specific primers. Goats were given wilted Italian ryegrass silage alone or with concentrates at 50:50 on a DM basis. Rumen fluid (RF) was taken prior to and at 2, 4, and 8 h after the 0900 feeding. Because few changes were seen in rumen fluid LAB community due to sampling times, data for 11:00 h samples were omitted.

Performance of Holstein cows fed diets containing maize silage from silos with different covering methods

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Keywords: aerobic deterioration, aflatoxin B1, diet digestibility, maize silage, milk yield

Introduction Achieving and maintaining anaerobic conditions is critical to successful ensilage (McDonald et al. 1991; Borreani et al. 2008). The plastic film used to cover silage has oxygen permeability and small amounts of air will penetrate the silage. The objectives of this trial were to evaluate different plastic films to seal horizontal silos on the silage quality and performance of high yielding dairy cows.

Material and methods The trial was carried out in Piracicaba, Brazil, during 14 weeks. Forty high producing Holstein cows (24 multiparous and 16 primiparous) were randomly assigned to ten complete blocks and individually fed *ad libitum* twice daily (6:30 h and 17:30 h). Treatments were defined according to silo covering method: OB+BW - oxygen barrier film 45 µm thick + black-on-white polyethylene film 200 µm thick over the OB film; BW - black-on-white polyethylene film 200 µm thick; B - black polyethylene film 200 µm thick; RB+SB - recycled black polyethylene film 200 µm thick + sugarcane bagasse over the RB film. Diets were balanced to reach isonitrogenous content and contained 52.8% of maize silage (% DM) to meet NRC (2001) requirements. The deteriorated inedible silage was discarded every day and only edible silage was used to prepare the total mixed rations (TMR). Offered and refused TMR were recorded daily and consumed ration composition was calculated based on orts chemical analyse. Dry matter intake (DMI) and milk yield were recorded every day and milk was sampled once weekly to determine the composition. On 11th week, total faeces were collected from 16 cows (4 per treatment) during three days to measure the diet digestibility. Data was analyzed as repeated measures using the MIXED procedure of SAS and treatment means were declared significant at $P<0.05$ using the Tukey test.

Results and discussion The diet containing silage from RB+SB treatment showed extra protection resulting in improved milk yield, while the lowest mean was found in the B film. The DMI was similar across treatments, thus the differences observed in milk yield was due to diet digestibility induced by sealing strategies. In this way, cows fed OB+BW and RB+SB silages showed higher DM and OM digestibilities when compared to BW and B (Table 1). Probably, the higher digestibilities values might be attributed to the effect of low oxygen permeability of the OB film and/or the gas transmission rate would be reduced by the presence of sugarcane bagasse over the film preventing the losses of nutritive value. The aflatoxin B1 concentration was lower in edible silage than in inedible silage (Table 2). Although there were no statistic differences across treatments, the silage covered by the B film revealed seven times more aflatoxin B1 than RB+SB one. Table 3 showed the mean values of silage quality and chemical composition of maize silage collected monthly during lactation trial.

Conclusions The protection over the plastic films was effective on controlling losses and maximizing the animal performance. The black colour film is not recommended for covering silos in relation to risk of aflatoxin contamination and the important decrease in milk production.

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Table 1. Responses of dairy cows fed maize silage stored under different sealing strategies.

Parameter	Treatments[1]				SE	*P*[2]		
	OB+BW	BW	B	RB+SB		T	W	T*W
DMI, kg/d	21.70	22.68	21.30	21.88	0.70	NS	**	**
DM apparent digestibility, %	64.98^{ab}	58.94^{b}	59.24^{b}	67.48^{a}	1.64	*	-	-
OM apparent digestibility, %	72.86^{ab}	67.84^{b}	69.31^{b}	74.10^{a}	1.49	*	-	-
Milk yield, kg/d	32.31^{ab}	32.91^{ab}	30.43^{b}	34.42^{a}	1.58	*	**	NS
4%FCM, kg/d	30.28^{ab}	30.44^{ab}	29.53^{b}	32.47^{a}	1.11	*	**	NS
Fat,%	3.65	3.57	3.83	3.61	0.14	NS	**	NS
Protein, %	3.29^{ab}	3.34^{ab}	3.44^{a}	3.18^{b}	0.07	*	**	NS
Lactose, %	4.65	4.67	4.64	4.65	0.06	NS	**	NS
Total solids, %	12.51	12.50	12.82	12.35	0.21	NS	**	NS
Milk/DMI	1.49^{ab}	1.44^{b}	1.44^{b}	1.59^{a}	0.05	*	**	**

[1]OB+BW - oxygen barrier film 45 µm thick + black-on-white polyethylene film 200 µm thick over the OB film; BW - black-on-white polyethylene film 200 µm thick; B - black polyethylene film 200 µm thick; RB+SB - recycled black polyethylene film 200 µm thick + sugarcane bagasse over the RB film. [2]T: treatment effect, W: week effect, T*W: interaction between treatment and week effect.

Table 2. Mycotoxin concentration in maize silage stored under different sealing strategies.

Treatments[1]	Aflatoxin B1 (ppb)	
	Edible silage	Inedible silage
OB+BW	1.35	1.70
BW	1.30	1.20
B	0.50	3.50
RB+SB	0.55	0.50
Mean	0.93	1.72
Standard deviation	0.61	2.00
Minimum	0.00	0.00
Maximum	2.30	7.00

[1]OB+BW - oxygen barrier film 45 µm thick + black-on-white polyethylene film 200 µm thick over the OB film; BW - black-on-white polyethylene film 200 µm thick; B - black polyethylene film 200 µm thick; RB+SB - recycled black polyethylene film 200 µm thick + sugarcane bagasse over the RB film.

Table 3. Chemical composition and fermentation profile of maize silage stored under different sealing strategies.

Parameter	Treatments[1]				SE	P
	OB+BW	BW	B	RB+SB		
WSC, % of DM	3.70	3.01	3.44	3.58	0.29	NS
pH	4.17	4.23	4.35	4.27	0.05	NS
Lactic acid, % of DM	2.91	3.08	3.35	3.78	0.44	NS
Acetic acid, % of DM	1.31	1.44	1.08	1.14	0.10	NS
Butyric acid, % of DM	0.05	0.07	0.08	0.03	<0.01	NS
DM, %	33.30	35.80	33.70	33.10	1.48	NS
Ash, % of DM	4.10	4.15	4.54	4.39	0.16	NS
CP, % of DM	7.20	6.98	7.06	6.98	0.16	NS
NDF, % of DM	50.60	51.54	52.78	52.61	0.91	NS

[1]OB+BW - oxygen barrier film 45 µm thick + black-on-white polyethylene film 200 µm thick over the OB film; BW - black-on-white polyethylene film 200 µm thick; B - black polyethylene film 200 µm thick; RB+SB - recycled black polyethylene film 200 µm thick + sugarcane bagasse over the RB film.

Frosted corn silage with or without a bacterial inoculant in dairy cattle ration

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Keywords: bacterial inoculant, dairy cattle performance, frost damaged corn, Lactisil Maize, *Lactobacillus buchneri*

Introduction Late planting dates and early falling in ambient temperature may result in situations where corn is killed by frost. Frost reduces the level of beneficial silage bacteria -primarily lactic acid bacteria (LAB)- on the standing plant and elevates numbers of spoilage organisms such as yeasts and moulds. Lactisil Maize (Medipharm, Kågeröd, Sweden) –a new multi purpose LAB inoculant- contains both homofermentative (*Enterococcus faecium M74*, *Lactobacillus plantarum LS1*, *Lactobacillus casei* and *Pediococcus pentosaceus*) and heterofermentative (*Lactobacillus buchneri*) LAB. The aims of the current study were to investigate the effects of Lactisil Maize on fermentation characteristics and aerobic stability of frosted corn silage and also the effects of these silages on dairy cattle performance at early lactation.

Material and methods The corn crops were harvested at 298 g DM/kg with a killing frost and were chopped at 2.5 cm of theoretical cut length. Crops were inoculated by spraying the inoculants (1.5×10^5 cfu per g of fresh forage) over the crops (500 g of the inoculant were diluted into 200 L of water and applied for 50 t of fresh forages). For the control treatment, same amount of inoculant-free water was applied. After 120 days of ensiling, six sites on each silo were chosen and about 15 kg silages were sampled from each site. All subsamples were thoroughly well mixed to obtain representative 3-kg samples. The TMR diets were fed to eight Holstein dairy cattle (65± 18 d in milk) in a replicated 2 × 2 Latin square designed experiment. The experiment had two periods of 21 d, with the first 14 d for adaptation and the last 7 d for sampling and data collection. Cows were housed in individual stalls and were fed twice and milked three times daily. Diets were different in silage source (treated or untreated corn silage). The Diets were formulated based on the NRC recommendations (2001) to contain 183 g DM/kg crude protein and 1.72 NE_L (Mcal/kg DM). Aerobic stability was defined as the number of hours that it took the silage to rise 2 °C above the ambient temperature. Acid detergent insoluble ash was used as an internal marker to calculate the digestibility of the nutrients. Milk samples were analyzed by Milk-O-Scan. Volatile fatty acids and lactic acid were measured by gas chromatography. Data for corn silage composition and fermentation characteristics were analyzed as a completely randomized design experiment using GLM procedure of SAS (2003). In vivo data were analyzed using the MIXED procedure of SAS (2003) with the model including the fixed effects of treatment and period, and random effects of square and cow within square. For all variables that sampling was repeated over time, the effect of time was included in the REPEATED statement of the model.

Results and discussion Concentrations of DM and CP and value of pH were not affected in response to inoculation (Table 1). However, lactate and acetate concentration was greater in silages with inoculants and in result, aerobic stability was not influenced by inoculation. Our finding is according to Filya (2003) who reported higher concentrations of lactic and acetic acid in L. buchneri + L. plantarum-inoculated silages.

Table 1. Chemical composition and fermentation characteristics of pre-ensiled and ensiled crops.

Item	Fresh crops	Corn silage		SE	Significance
		Control	Lactisil Maize		
DM (%)	29.76	24.01	24.16	0.34	NS
CP (%)	8.10	7.02	6.78	0.18	NS
NDF (%)	59.33	56.93	57.81	0.40	**
pH	6.38	3.72	3.78	0.03	NS
Lactic acid (%)	ND	5.30	6.20	0.31	**
Acetic acid (%)	ND	1.36	1.87	0.12	**
Aerobic stability (h)	ND	86	90	2.40	NS

*=significant difference at $P<0.05$; **=significant difference at $P<0.01$; NS=no significant differencet; ND=not determined

Animals receiving inoculants-treated silage had lower dry matter intake (DMI), 4% FCM and milk protein and fat yield (Table 2). Higher concentration of NDF in inoculated silages and respective diets may limit DMI. Also, it is widely accepted that extensive fermentation in silages which distinct by greater organic acids and volatile compounds such as lactic acid, VFA and ammonia-N limits DM intake (Buxton et al. 2003). Moreover, previously it has been shown that greater concentrations of acetic acid esters (Kristensen et al. 2010) and some biogenic amines (Nishino et al. 2007) in *L. buchneri* inoculated silages negatively affect DMI. Total tract apparent digestibility of nutrients and molar proportion of VFA in rumen of cattle was similar between treatments and in result milk fat and milk protein percentages were not affected in response to inoculation.

Table 2. Dry matter intake (DMI), milk production and apparent total tract digestibility of organic matter (OM), crude protein (CP) and neutral detergent fiber (NDF).

	Treatments			
	Control	Lactisil Maize	SE	Significance
DMI (kg/d)	28.32	27.23	0.84	**
Milk (kg/d)	38.53	37.17	0.79	**
Fat yield (kg/d)	1.24	1.20	0.05	**
Fat %	3.22	3.22	0.09	NS
4% FCM (kg/d)	34.07	32.91	1.04	**
Protein yield (kg/d)	1.19	1.13	0.03	**
Protein %	3.08	3.05	0.05	NS
Body weight (kg)	649.15	641.47	22.48	NS
Ruminal pH	6.33	6.35	0.10	NS
Acetate (mol/100 mol)	71.77	73.26	2.19	NS
Propionate (mol/100 mol)	18.31	16.44	2.04	NS
Butyrate (mol/100 mol)	9.86	10.26	1.14	NS
OM digestibility (%)	63.20	63.71	2.86	NS
CP digestibility (%)	64.32	64.47	2.40	NS
NDF digestibility (%)	52.43	54.42	2.81	NS

*=significant difference at $P<0.05$; **=significant difference at $P<0.01$; NS=no significant difference

Conclusions Frost damaged corn had a good potential to be ensiled. Lactisil Maize slightly affected chemical characteristics of silages but it failed to improve aerobic stability. Greater concentration of NDF and volatile compounds in inoculated silages led to decrease in DMI and milk yield.

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Influence of extreme high and low temperature on the quality of maize silage and milk yield of dairy cows

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Keywords: aerobic stability, Holstein cows, milk yield, silage, temperature

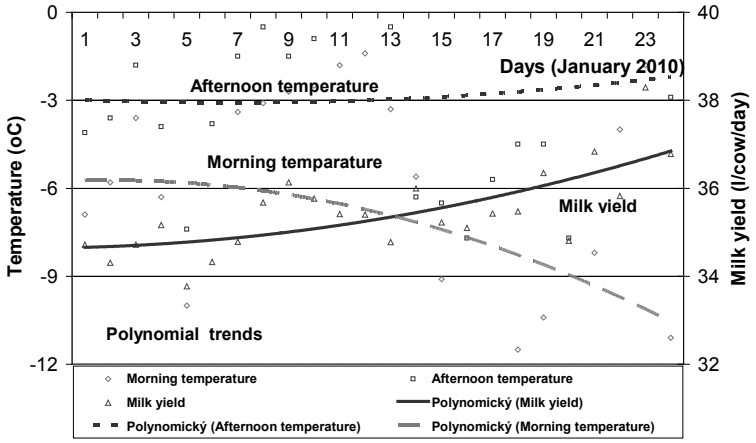
Introduction From the dietetic point of view, it is not recommended to feed dairy cows (especially the high-producing ones) either with frozen food in winter or with aerobic deteriorated maize silage in summer. Schnier et al. (2003) relate freezing weather with the influence on milk production. Their retrospective study followed the curve of milk yield of cows bred in so called "cold" system of free stabling as compared to classical system. The cows in "cold" system produced one litre less milk daily but the difference was not statistically significant. The influence of changes of daily temperatures on milk production is usually related to breed of beef cattle in summer, in arid areas, or in breeding in insufficiently ventilated stables. That results from the survey processed by Kadzere et al. (2002). When strong heat and humidity last long time, high milk yield, good health and welfare of dairy cows must be preserved by combination of all available possibilities, i.e. cooling down (particularly by evaporation), correct defining and meeting of the needs of the animals from the perspective of nutrition and feeding (West 2003). High temperatures at feeding deteriorate the aerobic stability of the fodder (Koca et al. 2009, O'Kiely 1993).The first objective of this study was to ascertain the maximum depth of frozen silage in silos and to describe the response of dairy cows to frosts and frozen food. The second objective of the work was to ascertain how dairy cows respond by milk production to extreme high temperatures and how the temperature influences the aerobic stability of maize silage.

Material and Methods Daily temperatures were measured in the dairy farm of Institute of Animal Science. During the monitoring, the temperature of a sample of maize silage taken in a depth of 0 to 5 cm and in a depth of 30 to 45 cm under the surface was measured. We used a special contact probe thermometer TPT 64+ for the measurement. The temperatures of the whole silage face were measured by thermographic camera Flir P45.

The values of outside temperatures were taken from a small meteorological station situated near the stables. Daily temperatures were recorded at 7 AM and at 2 PM, when the fodder was being prepared. The feeding dose contained maize silage, CCM (corn cob mix), alfalfa silage and grain-mineral mix. The dairy cows were bred in airy stables, in summer with evaporation cooling. They were milked twice a day.

Results and discussion Experiment 1 in winter. The lowest air temperatures were on January 27th (–21.4°C and –7.7°C, in 7 and 14 o'clock, respectively). During the experimental period (January 8.-31.) the average temperatures in the morning were -7.3 ± 4.9°C, at the afternoon -2.8 ± 2.9°C. The average daily milk yield of 120 Holstein cows was 35.5±0.9 litre per cow per day. In the course of January, the records of milk production on very cold (i.e. frost) days were comparable with those recorded on days with milder days. The overall correlation was relatively low (i.e. only 0.35). Although the dairy cows reacted to decreased temperatures of air and temperatures of feed mixture TMR by a decrease in daily milk production, this response was not significant (P>0.05).

Figure 1: Influence of low ambient temperatures on milk yield



The influence of very low daily temperatures on milk production is shown by the polynomial trend in figure 1.

The milk production grew with increasing differences between afternoon and morning outside temperature (the morning temperature was near -10°C zero and the afternoon temperature was near ±0°C).

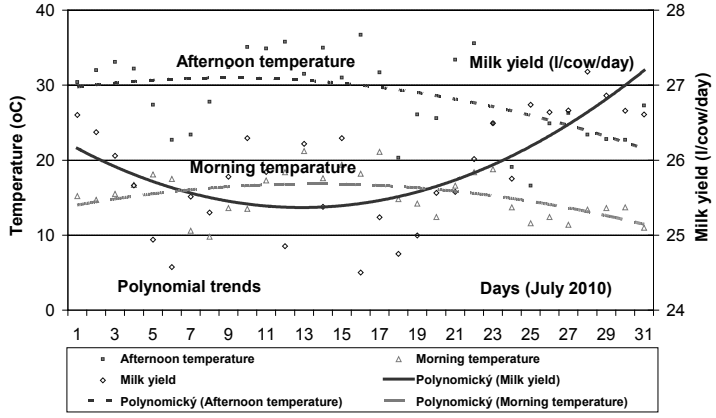
Although several days before the measurement, the frost dropped from -10°C to -20°C and at the time of measurement to -7.7°C in the morning hours, the frozen layer of maize silage was nowhere higher than 4 cm. The silage, shortly after

being taken by the milling cutter of the feeding vehicle, had a very different temperature in a depth of 30 cm below surface; it oscillated from zero to 26°C (measured by thermographic camera). The temperature of the mixed feeding dose (TMR) was only several degrees above freezing point after being mixed in the feeding vehicle and it cooled down very quickly. The dairy cows obviously did not mind the low temperature of TMR; the fodder intake was comparable to other, less frosty days.

The results of the studies of Kadzere et al. (2002), Schnier et al. (2003) were confirmed – if the dairy cows are fed with qualitatively balanced feeding doses, the temperatures below freezing point have no significant effect on milk yield.

Experiment 2 in summer. The following daily temperatures were measured in the same dairy farm of our Institute of Animal Science in July: at the morning 15.3±3.0°C, at the afternoon 28.5±5.4°C; Fifteen days were tropical, with temperatures above 30°C. The lowest milk yield of all 194 Holstein cows in a farm (24.5 l/cow/day) was registered on the day when the outside temperature was the highest (36.7°C). The average daily milk yield amounted to 25.9±0.7 l/cow/day in that month. When it got colder to about 20°C after three weeks, the milk yield significantly (P≤0.05) increased from 25.8 to 27.2 litres of milk per dairy cow and per day within four days.

Figure 2: Influence of high ambient temperatures on milk yield



The influence of very high daily temperatures on milk production is shown by the polynomic trend in figure 2.

The milk production decreased when the afternoon and morning outside temperatures increased (the morning temperature was near +20 °C and the afternoon temperature was above +30 °C). As soon as the temperatures started decreasing, the yield started increasing quickly.

More detailed measurement was performed on July 20 and 21 when the average air temperature in the silage chute was of 26.2°C. The silage samples taken after 24 hours of aeration from a depth of 0 to 15 cm had average temperatures of 56.3±3.4°C, from a depth of 16 to 30 cm 42.0±2.1°C, from a depth of 31 to 45 cm 29.8±4.1°C. The results of Koca et al. (2009) and O'Kiely (1993) were confirmed – high outside temperatures have negative impact on the aerobic stability of the ensilages.

Conclusions In the frosty period and in the periods of summer heat, the extreme oscillations of outside temperatures had only minimal impact on the yield of the milk cows; the impact was bigger in periods of heat than in those of frost. The silages have relatively stable temperature in depths below 30 cm under the surface, regardless of freezing or tropical weather.

Acknowledgements Supported by project NAZV QI91A240.

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Effect of replacing corn silage with sweet sorghum silage on nutrient digestibility and performance of dairy cows

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Keywords: corn silage, dairy cows, digestibility, sweet sorghum silage, performance

Introduction Sweet sorghum is one of the important varieties of sorghum plant that is adapted for hot and arid condition. The mature plant has about 2 m height and is resistant to pests and salinity of soil. Its growing is halted in restriction of water and would be resumed following to irrigation. In Iran as in many other countries corn silage is dominant silage fed to dairy cow and mostly cultivated in the areas where sufficient water is available. However, successive drought and economical conditions oblige farmers to find alternatives.

Oliver et al. (2004) revealed that in almost all comparisons between conventional sorghum silage and corn silages, dry matter intake (DMI) and milk yield decreased in sorghum treatments. They also indicated that low digestibility of neutral detergent fiber (NDF) of sorghum is a determinant factor in lowering the performance of animals. Nevertheless, other studies showed that there is a significant difference among sorghum varieties in NDF digestibility. The *in vivo* digestibility was greater in sweet sorghum when compared with grain and brown mid-rib sorghum varieties (Di marco et al. 2009). It seems that sweet sorghum with high potential of yield (20 to 40 dry Mg ha^{-1}) would be a good option for finding alternative for corn silage. Therefore, this study was conducted to evaluate the effects of replacing corn silage with sweet sorghum silage on nutrient digestibility, milk production, rumen parameters and feeding behavior of dairy cows.

Material and methods Eight Holstein dairy cows (146 ± 14 days in milk and 35.5 ± 3.5 kg/d mean milk production) were allocated in a replicated 4 × 4 Latin square design. Cows were paired and randomly assigned to four treatments where corn silage (*KSC704* hybrid) was substituted by sweet sorghum silage (*Italian* variety) at levels of 0 (control group), 33.3, 66.6 and 100 percent of dry matter (DM). Experimental periods were 21 d, with 14 d for adaptation and 7 d for data collection. Ratio of forage to concentrate in diets was 54.5:45.5. Diets included 43% silage and 11.5% beet pulp. The chemical composition of diets and forages were nearly similar, except acid detergent lignin (ADL) that in sweet sorghum silage was higher than corn silage (3.9 *vs.* 2.2 %DM). Milk production was recorded during the last two consecutive days of each period and analyzed for compositions by milk-o-scan apparatus (134 BN Foss Electric, Hillerød, Denmark). Acid detergent insoluble ash was used as an internal marker to determine apparent total tract nutrient digestibility. Samples of ruminal fluid were taken on 21th d in each experimental period at 3 h after feeding using a stomach tube; After measuring pH value, ruminal volatile fatty acids (VFAs) concentration was determined by gas chromatography (CP-9002, 259 a.m, Chrompack, Netherlands). On 19th d of each period, chewing activity parameters were monitored visually for each cow over a 24 h period. All laboratory analyses were done according to standard methods. Data on all variables were analyzed using the mixed procedure of SAS. Period and treatment were the fixed effects in the model and square and cow within square statement included as random factors. Linear, quadratic and cubic contrasts were included in the model to test the effect of replacing. Significance was declared at $P \leq 0.05$ and trend was considered to exist if $0.05 < P \leq 0.15$. All reported values are Least Squares Means.

Results and discussion Increasing levels of sorghum silage had not any significant effect on DM, NDF, acid detergent fiber (ADF) and crude protein intake (Table1). This is may be due to equal NDF levels across the diets which determine DMI of dairy cattle. Nevertheless, replacing corn with sweet sorghum silage decreased digestibility of DM and other nutrients ($P < 0.05$). As previously mentioned, sorghum silage had higher content of ADL, which is a determinant factor for suppressing digestibility. Milk yield and compositions were not affected by increasing levels of sweet sorghum in diets. However, body weight in cows fed 100% sweet sorghum silage tended to be significantly lower than control diet ($P = 0.12$). It might be contributed to higher digestibility of corn silage which consequently affects net energy of diet

for animal. Ruminal pH was not affected by the treatments and only control treatment had a tendency (P = 0.08) to have lower pH compared to 100% sweet sorghum silage treatment. Total VFA concentration as well as acetate, propionate and butyrate were linearly decreased by increasing sorghum levels in the diets which also might be related lower digestibility in those diets. Eating and total chewing activity time was similar among treatments, but cattle fed 100% sweet sorghum silage significantly spent more time for rumination than animals in control diet. This finding shows that low particle fragility (due to higher ADL) of sorghum silage increase rumen retention time for indigestible particles and consequently motivates more rumination.

Conclusions Overall, despite the decreased digestibility of nutrients by replacing corn silage with sweet sorghum silage, milk yield and dry matter intake were not affected. Sweet sorghum silage can fairly successfully be used in dairy cow nutrition in areas where corn production is uncertain.

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Table1. Effect of replacing corn silage (CS) with sweet sorghum silage (SS) on DMI, nutrient digestibility and performance of dairy cow.

	Proportion of SS (%)					
	0	33.3	66.6	100	SEM	contrast[1]
Dry matter and nutrient intake (kg/d)						
Dry matter	23.19	21.99	22.96	22.90	0.71	NS
Organic matter	21.31	20.17	20.99	21.07	0.65	NS
Neutral detergent fiber	9.53	9.03	9.44	9.41	0.32	NS
Acid detergent fiber	4.82	4.55	4.75	4.72	0.18	NS
Crude protein	3.62	3.44	3.59	3.60	0.71	NS
Total tract nutrient digestibility (%)						
Dry matter	58.95^{a}	56.27^{ab}	52.15^{d}	54.99^{cb}	0.63	L*,Q*,C*
Organic matter	61.85^{a}	58.57^{b}	53.65^{d}	57.73^{cb}	0.57	L*,Q*,C*
Neutral detergent fiber	58.61^{a}	49.90^{b}	40.27^{d}	44.49^{c}	0.66	L*,Q*,C*
Acid detergent fiber	30.74^{a}	28.90^{ab}	20.40^{d}	24.46^{c}	1.08	L*,Q*,C*
Crude protein	56.94^{a}	52.22^{b}	47.01^{c}	46.11^{cd}	0.95	L*,Q*
Milk production and composition						
Yield (kg/d)	31.95	31.10	31.00	31.00	1.30	NS
Fat (%)	3.05	2.29	3.12	3.21	0.3	NS
Protein (%)	2.95	3.00	3.05	3.04	0.07	NS
Lactose (%)	5.52	5.47	5.48	5.42	0.1	NS
Ruminal fermentative characteristic						
pH	6.47	6.57	6.61	6.68	0.09	NS
Ammonia nitrogen (mg/l)	107	103	100	110	1.81	NS
Total volatile fatty acid (mM)	123.1^{a}	119.6^{ab}	113.3^{cd}	112.2^{d}	1.5	L*
Acetate (mM)	77.8^{a}	76.4^{ab}	73.5^{d}	74.5^{cd}	0.87	L*
Propionate (mM)	23.9^{a}	23.0^{ab}	21.1^{cd}	20.4^{d}	0.82	L*
Butyrate (mM)	14.8^{a}	14.5^{a}	13.2^{a}	12.1^{b}	0.59	L*
Feeding behavior (min/d)						
Eating	220.0	213.5	217.7	212.5	5	NS
Ruminating	360.2^{c}	389.8^{bc}	382.5^{bc}	405.6^{ab}	8.31	L*
Total chewing activity	580.2	603.3	600.2	618.1	15.45	NS
Body weight (kg)	622.9	618.9	616.8	614.5	12.70	NS

$^{a, b, c, d}$ Means in the same row with different superscripts letters are significantly different (P < 0.05); [1]linear (L), quadratic (Q) and cubic (C), $^{*}P$ < 0.05; NS = not significant.

The effect of feeding grass silage treated with Powerstart on dairy herd fertility

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Keywords: Commercial dairy herds, fertility, inoculant, silage quality

Introduction Many scientific studies are published showing the effects of the addition of silage additives on fermentation and silage quality. Fewer but still a large number show the benefits of homo-fermentative inoculants on the feeding qualities of silage in relation to animal performance measured in terms of intake and live-weight gain or milk production under controlled scientific experiments in research establishments. However, studies looking at the effects that silage inoculants may have on indicators of herd health are not common place, possibly due to the large numbers of animals required to undertake such studies. In addition studies are normally executed on research farms and do not assess the effects of silage additives across commercial farms.

Material and methods The aim of the current study was to assess the effect of using a silage inoculant (Powerstart; containing homo-fermentative lactic acid bacteria) on grass silage and subsequent dairy cow fertility on farms across the United Kingdom.

A survey was undertaken on 103 dairy herds to compare the effect of feeding grass silage made with the application of Powerstart with grass silage made without Powerstart hitherto referred to as the control group on dairy herd fertility. The herds were initially selected to take part in the survey based on their participation in a reproductive management program for recording fertility data (RMS Genus). Subsequent selection criteria were used to ensure that the herds were balanced as far as possible in terms of herd size. The control group contained silages that had a range of other additives or no additive treatment on the grass silage. In total the reproductive records of 25036 animals were examined, 11621 animals in 49 herds were fed silage made with Powerstart while 13415 animals in 54 herds were not. The average herd size in the Powerstart group was 237 cows compared to 248 cows in the control group.

Results and discussion There was a significant difference in mean calving to conception interval for cows fed Powerstart treated silage (125 days versus 135 days $P<0.05$). Further analysis of the data set indicated that the effects of farm, lactation number and RMS technician were also found to be significant. However, when taking into account these potential confounders within the statistical analysis the effect of Powerstart treated silage was still found to have a positive effect on fertility (Odds Ratio for pregnancy 1.15, 95% CI 1.08-1.22, $P<0.05$).

The results shown here indicate that factors affecting silage quality can have effects on herd health. O'Kiely et al.(2002) showed that the use of homolactic lactic acid bacteria as an inoculant for grass silage can result in lower concentrations of blood urea-N when fed to growing steers. It is likely that this is brought about by improved silage protein quality (Winters et al.,2001) resulting in better rumen microbial capture of forage nitrogen (Davies et al. 1999) resulting in lower ruminal ammonia-N production and recycling through the blood as blood urea-N. Blood urea-N can have multiple effects on fertility through factors such as early embryo mortality and effects on progesterone levels.

Conclusions In conclusion the data presented here indicate the importance of silage quality on commercial dairy farms to dairy cow fertility. It also shows that reliable farm datasets have the potential to be used to assess the effects of a range of management factors and elucidate what impact they may have on the efficiency of livestock production. Such data sets should be used by researchers and practitioners alike to provide data generated from much larger numbers of animals than could ever be conducted on a research farm.

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Lactating cow response to lucerne silage inoculated with *Lactobacillus plantarum*

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Keywords: silage inoculant, milk production, lucerne, rumen microbial biomass production

Introduction Inoculants, applying lactic acid bacteria to crops at ensiling, are commonly used to ensure a good fermentation and improve animal performance. However, it is unclear why these products would improve milk production in lactating dairy cattle. Recently we reported that silages in mini-silo trials treated with a *Lactobacillus plantarum* inoculant (Ecosyl, Yorkshire, UK) had increased *in vitro* rumen microbial biomass production (Contreras-Govea et al. 2011). These results suggest that inoculant effects on milk production may be tied to rumen fermentation. Our objective was to determine if lucerne silage treated with this inoculant could produce a milk production response commensurate with the *in vitro* responses.

Material and methods Lucerne [240 g crude protein (CP)/kg dry matter (DM), 300 g neutral detergent fibre (NDF)/kg DM, 500 g DM/kg] was ensiled with (LP) and without (C) Ecosyl inoculant. Twenty-eight multiparous Holstein cows in early lactation (8 ruminally-cannulated) were blocked by days in milk and randomly assigned to two diets (C or LP-treated silage) in a double crossover design with four 28-d periods. Diets were formulated to contain 165 g CP and 280 g NDF/kg DM, consisting of (g/kg DM): lucerne silage (500), maize silage (200), high moisture maize grain (226), soy hulls (50) and vitamin/mineral mix (24). Milk production and DM intake were recorded for the last 14 d of each period. Milk samples were collected from each cow at both milkings on days 20, 21, 27 and 28 for analysis of milk composition. Body weights were taken on the last two days of each period. In the fourth week, omasal samples were taken over 3 d according to Reynal et al. (2005) to estimate microbial protein flow from the rumen. Means for LP were compared against means for C before and after LP using a paired t-test in Proc MIXED of SAS.

Results and discussion The two lucerne silages were of similar DM, CP, fibre and ash concentrations, but the inoculant was effective in reducing silage pH compared to that of the control (Table 1) and in shifting fermentation toward lactic acid (data not shown). Composition was similar between the two diets (Table 2). Compared to control, the LP diet increased milk production but had no effect on fat-corrected or energy-corrected milk production (Table 3). There was a trend for increased DM intake with the LP diet, but efficiency of milk production was not affected by treatment. The milk protein content was reduced in the LP diet, and lactose content was increased. Daily yield of milk fat, protein and lactose was unaffected by treatment. Milk urea nitrogen was reduced by LP, suggesting that more of the degraded protein on the LP diet was being converted to microbial protein and less to ammonia in the rumen. Analysis of the omasal samples is not complete. These samples may provide more direct evidence as to whether the diet with the inoculated lucerne silage produced more rumen microbial protein.

Table 1. Characteristics of the lucerne silages (untreated control and treated with *Lactobacillus plantarum* MTD/1, LP) at feeding.

Silage characteristics	Control	LP	s.e.
DM, %	49.3	48.3	1.38
pH	4.86	4.56	0.046
CP, % DM	25.1	25.1	0.16
NDF, % DM	31.7	31.5	0.26
ADF, % DM	24.1	23.8	0.32
Ash, % DM	10.4	9.9	0.19

Table 2. Average composition (g/kg DM) of the untreated (Control) and inoculated (LP) lucerne silage diets as fed.

Ration components	Control	LP
Lucerne silage	502	497
Maize silage	204	216
High moisture maize grain	220	216
Soy hulls	47	45
Vitamin/mineral mix	28	26
Crude protein	162	162
Neutral detergent fibre	273	274

Table 3. Lactating cow response to diets containing lucerne silage, either untreated (Control) or inoculated with *Lactobacillus plantarum* MTD/1 (LP).

Response	Control	LP	s.e.	*P*
DMI[1], kg/d	25.4	25.8	0.17	0.072
BW change, kg/d	-0.19	-0.17	0.058	0.842
Milk, kg/d	39.6	40.4	0.26	0.027
Milk/DMI	1.56	1.57	0.009	0.379
3.5% FCM, kg/d	40.8	41.2	0.45	0.587
FCM/DMI	1.60	1.60	0.014	0.774
ECM, kg/d	36.4	36.8	0.38	0.470
ECM/DMI	1.43	1.43	0.012	0.847
Fat, %	3.80	3.79	0.038	0.984
Fat, kg/d	1.47	1.48	0.023	0.725
Protein, %	2.81	2.78	0.009	0.048
Protein, kg/d	1.09	1.09	0.007	0.918
Lactose, %	4.82	4.89	0.013	<0.001
Lactose, kg/d	1.89	1.93	0.017	0.097
MUN, mg/dL	12.7	11.6	0.17	<0.001

[1]DMI=dry matter intake; BW=body weight; FCM=fat-corrected milk; ECM=energy-corrected milk; MUN=milk urea nitrogen.

Conclusions Silage inoculated with *Lactobacillus plantarum* MTD/1 increased milk production and reduced milk urea nitrogen, supporting the hypothesis that the inoculated silage is increasing formation of microbial biomass in the rumen.

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Effects of feeding red clover versus lucerne silage to lactating dairy cattle

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Keywords: red clover, N utilization, milk production

Introduction Research has shown that the reduced soluble non-protein nitrogen (NPN) content of red clover (*Trifolium pratense*) silage (RCS) was related to a greater N efficiency in lactating dairy cows relative to lucerne (*Medicago sativa*) silage (LS) (Broderick et al. 2001). Red clover has a polyphenol oxidase enzyme that is responsible for lower NPN compared to alfalfa (Jones et al. 1995). The objective of this study was to compare feeding RCS to LS for milk production and N efficiency in lactating dairy cows as indicated by milk urea N (MUN).

Material and methods Twelve multiparous lactating Holstein cows were fed total mixed rations that contained either 50% RCS or 50% LS (dry matter (DM) basis). The remainder of the ration consisted of maize silage, high moisture maize and soybean meal; both diets contained about 17% crude protein (CP) and 27% neutral detergent fibre (NDF). Cows were randomly assigned in a replicated switch-back design with three, 4-wk periods to assess effects of forage source on milk production and milk characteristics including MUN. Means for LS were compared against means for RCS by the MIXED procedures of SAS.

Results and discussion The LS had a higher CP (24% versus 20%) and lower NDF (37% versus 39%) compared to RCS (Table 1). Feeding the LS diet gave rise to better performance than the RCS ration: DM intake on LS was significantly higher as was production of milk and 3.5% fat-corrected milk (FCM), but MUN concentration was also higher (Table 2). There were no differences between LS and RCS in yield of milk fat or milk protein.

This trial and six others comparing RCS versus LS support our hypothesis that production results generally favor LS but nitrogen efficiency is greater on RCS. The lower DM content, as was observed with LS, was the most powerful indicator of silage quality for this cow trial (Table 1). There was a significantly higher lactate content in RCS indicating that RCS had a good fermentation (Table 1). The better fermentation of RCS could not overcome the difference in silage quality CP and NDF of the LS.

Conclusions The differences between LS and RC silage quality determined DM intake. Feeding the RCS diet resulted in 3.3 kg/d lower DM intake, which in turn resulted in substantially lower yield of milk and FCM. The significantly higher milk production from LS, establishes LS as the most advantageous forage. However the analysis of NPN is not complete. These samples may show whether RCS has qualities that make it convert more feed protein to microbial protein.

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Table 1. Chemical composition and fermentation characteristics of lucerne silage (LS) and red clover silage (RCS).

Item	LS	RCS	SE	P > F
DM[1], g/kg	358	438	12.1	0.01
pH	5.07	4.56	0.05	0.01
CP, g/kg DM	242	204	8.4	0.02
NDF, g/kg DM	372	394	4.4	0.01
ADF, g/kg DM	281	288	2.9	0.26
Ash, g/kg DM	122	88	4.7	0.01
NH3, mM	1.87	0.95	0.09	0.01
TAA, mM	0.01	0.005	0.001	0.01
WSC, g/kg DM	7.86	33.9	2.98	0.01
Fermentation products, g/kg DM				
Succinate	6.6	3.8	0.03	0.01
Lactate	45.5	60.0	2.53	< 0.01
Acetate	51.9	14.8	0.42	0.01
1,2-Propanediol	0.38	1.47	0.01	0.01
Propionate	0.06	0.41	0.008	0.04
2,3-Butanediol	7.49	5.43	0.025	0.001
Ethanol	0.41	0.13	0.013	0.37
Butyrate	3.40	0	0.038	0.01
Formate	0.25	0	0.008	0.13

[1]Dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), ammonia N (NH_3), total amino acids (TAA), water soluble carbohydrates (WSC).

Table 2. Effect of feeding 50% of dietary DM as lucerne silage (LS) or red clover silage (RCS) on performance of lactating dairy cows.

Item	LS	RCS	SE	P > F
DMI, kg/d	23.0	19.7	0.44	< 0.01
Milk, kg/d	30.5	26.4	1.20	0.03
3.5% FCM[1], kg/d	32.5	26.9	1.84	0.09
Milk fat yield, kg/d	1.18	1.02	0.08	0.30
Milk protein yield, kg/d	0.93	0.83	0.04	0.15
MUN, mg/dl	15.0	14.5	0.84	0.02

[1]Fat corrected milk (FCM), milk urea nitrogen (MUN)

Rapeseed expeller is a better protein supplement than soybean expeller in dairy cow diets based on grass-clover silage

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Keywords: amino acid, milk production, protein supplementation, organic production, red clover

Introduction Soybean based feeds are widely used as protein supplements for dairy cows, but rapeseed provides an alternative source of high quality plant protein into ruminant diets. There is increasing evidence that rapeseed based feeds are excellent protein supplements for dairy cows fed grass silage based diets (Huhtanen et al. 2011). Our objective was to evaluate the dairy cow responses particularly in N metabolism to increasing levels of rapeseed and soybean expellers. The diet characteristics (expeller rather than meal, 0.5 red clover in the silage on dry matter (DM) basis and concentrate proportion 0.36 of DM intake) were chosen to agree with requirements set for organic milk production.

Material and methods The experiment was conducted at MTT Agrifood Research Finland according to an incomplete 5×4 Latin Square design using five ruminally cannulated Finnish Ayrshire cows and four experimental periods of 21 d each. The rapeseed expeller (RSE) and soybean expeller (SBE) were produced by Mildola Ltd. (Kirkkonummi, Finland) with crude protein (CP) concentrations of 371 and 480 g/kg DM and ether extract concentrations of 94 and 76 g/kg DM, respectively. They were fed at two isonitrogenous levels (Table 1), and replaced the basal concentrate which was a 1:1 mixture of pelleted barley and oats. The amount of concentrate fed was 9 kg/d for all diets. The basal silage was a 1:1 mixture of pure red clover silage and pure grass silage. The in vitro digestible organic matter (OM) concentration (D-value) of the silage mixture was 668 g/kg DM and the CP and neutral detergent fibre concentrations were 157 and 498 g/kg DM, respectively. Both parent silages had rather low DM concentrations (198 and 262 g/kg for red clover and grass, respectively) but they were prepared using formic acid based additives and were well preserved [pH 3.79 and 4.20 and ammonia N in total N 48 and 54 g/kg for red clover and grass, respectively].

Standard feed intake and milk production records were taken. The flow of nutrients to omasum was measured using omasal sampling over four days and triple-marker method. Diet digestibility was measured by conducting total faecal collection during 4 days. Blood samples were taken from the tail vein at 0, 3 and 6 h after the morning feeding on the last day of each period. For more details regarding the experimental procedures and analytical methods, see Vanhatalo et al. (2009). The feed values were calculated according to MTT (2012). The results were calculated using SAS GLM by including the effects of period, cow and diet in the model. The effect of diet was split into effects of RSE *vs.* SBE, linear effect of protein supplementation and their interaction by using contrasts.

Results and discussion Increasing protein supplementation increased linearly DM, energy and protein intake, supply of N to the omasum, and milk production (Table 1). The increase in milk production was greater when RSE rather than SBE was fed. Protein supplementation also increased diet OM and CP digestibility, and the increase was greater when SBE rather than RSE was fed. The true protein digestibility calculated using the Lucas equation was 0.848 for RSE and 0.955 for SBE. The effective degradability of rapeseed and soybean expeller protein in the rumen is 0.60 and 0.75 (MTT 2012), respectively, resulting in metabolizable protein (MP) values of 174 and 167 g/kg DM for them. When the protein efficiency was expressed as regression between CP intake and milk protein output, RSM was superior compared to SBE (slopes 102 vs. 55). When MP was used as input, the difference declined (slopes 0.297 vs. 0.230) showing that Finnish MP was rather good in predicting the production responses of these two protein supplements.

The N or individual AA (data not shown) flows to the omasum did not differ between the supplements, and the differences in the plasma AA concentrations were minor. There was however a tendency for a lower plasma methionine concentration on SBE diets compared to RSE diets.

Conclusions The N use efficiency was higher when RSE based diets compared to the SBE diets were fed, but increasing the level of protein intake from both supplements decreased the N use efficiency. Although protein supplementation elicited significant milk production responses, the level of milk production was rather high even when the control diet was fed.

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Table 1. Feed and nutrient intake, milk production, diet digestion and plasma amino acid concentrations of dairy cows supplemented with rapeseed or soybean expeller.

	Control	Rapeseed exp.		Soybean exp.		SEM	Stat. signific.[1]		
		Low	High	Low	High		R vs S	Lin	1×L
Feed and nutrient intake per day									
Dry matter (DM, kg)	19.6	21.4	21.7	20.3	20.7	0.45	*	*	
Silage (kg DM)	12.7	13.3	13.6	12.3	13	0.38	o		
Protein suppl. (kg DM)	0	1.78	3.34	1.34	2.51	---			
Crude protein (kg)	2.89	3.62	4.15	3.47	4.07	0.064		***	
Metab. energy (MJ)	221	242	245	233	239	5.5		*	
Metab. protein (g)	1735	2061	2245	1907	2053	41.2	**	***	*
N flow to omasum (g/d)	549	625	662	617	631	17.6		***	
Milk production									
Milk (kg/day)	32.7	35.3	35.8	33.7	34.1	0.91		o	*
ECM[2] (kg/day)	31.1	34.0	34.5	32.2	32.4	0.57	**	*	*
Fat (g/kg)	37.6	37.7	38.2	38.0	37.0	1.59			
Protein (g/kg)	29.8	30.9	30.7	29.5	30.4	0.48			
Urea (mg/100 ml)	18.1	25.8	29.9	26.1	34.0	1.91		***	
Diet digestibility from total faecal collection									
Organic matter	0.710	0.708	0.712	0.727	0.733	0.0046	**	o	*
Crude protein	0.618	0.642	0.672	0.663	0.704	0.0076	**	***	*
Amino acid (AA) concentration in plasma (µmol/l)									
Methionine	15.3	15.9	18.8	17.3	12.5	1.16	o		**
Histidine	34.9	44.2	46.9	45.5	45.4	2.89		*	
Branched-chain AA	598	723	801	709	778	69.9		o	
Essential AA	974	1135	1247	1157	1163	93.5		o	
Non-essential AA	1112	1107	1139	1165	974	34.4			**
Total AA	2087	2242	2385	2321	2137	124.6			

[1]R vs S = Rapeseed expeller vs. soybean expeller, Lin = linear effect of protein supplementation, 1×L = interaction between protein source and Lin [2]Energy corrected milk (Sjaunja et al. 1990)

Sugarcane silage replacing corn silage in lactating dairy cows rations

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Keywords: effective NDF, fibre, forage, intake, milk production

Introduction Sugarcane is broadly used as a supplement forage in Brazil during the dry winter season. Traditionally, small plots are daily harvested and offered green chopped to the animals but the need for more efficiency in farm management has led to the increase in its use as silage. The use of sugarcane, fresh or ensiled, for ruminants is increasing due to its high biomass yield (50-60 t DM/ha) and nutritive value (60% TDN). The possibility of totally or partially replacing corn silage by sugarcane, fresh or ensiled, can increase efficiency in farms that raise high-yielding dairy cattle. According to Queiroz et al. (2008) fresh or ensiled sugarcane can match the nutrient requirements of high milk producing dairy cows. The fibre content of rations for lactating dairy cows must provide a minimum NDF value of 25%, being 19% from forage (NRC 2001). The effective NDF (eNDF) is related to the sum total ability of a feed to replace forage or roughage in a ration so that the percentage of fat in milk produced by cows eating the ration is effectively maintained (Mertens 1997). The objective of this trial was to evaluate sugarcane silage potential to replace corn silage in lactating dairy cows' rations based on eNDF substitution.

Material and methods The experiment was carried out in Piracicaba, SP, Brazil at the Department of Animal Science of the University of São Paulo – College of Agriculture "Luiz de Queiroz". Twenty four mid-lactating Holstein cows were grouped and randomly assigned to a 3x3 Latin Square, with 8 cows per treatment, and 21 days period. Effective NDF represents the total replacement value of a feed for an equivalent of amount of NDF from forage in its ability to maintain milk fat production (Mertens 1997). For the rations formulation it was considered a fiber effectiveness factor of 1.0 for corn silage and 1.2 for sugarcane silage (Goulart et al. 2010). Cows were fed with the following iso-effective NDF rations: 1) CS - Corn silage (100% eNDF from corn silage); 2) SS - Sugarcane silage (100% eNDF from sugarcane silage); and 3) CSSS - Corn silage and Sugarcane silage (50% eNDF from corn silage + 50% eNDF from sugarcane silage). Concentrate was composed of finely ground corn grain, soybean meal, pelleted citrus pulp and mineral premix (Table 1). The effective NDF content (% DM) for rations were 25.61, 25.85 and 25.88%, for CS, SS and CSSS, respectively. Cows were housed in a tie-stall barn, which allowed individual intake control. Milk was recorded daily at the last week of each period and analyzed for fat content (%), protein content (%), casein content (%), lactose content (%), urea nitrogen (mg/dL) and somatic cell count (SCC – 1,000/mL). Data were analyzed using the MIXED procedure of SAS program, and means were compared by Tukey test with 5% significance.

Results and discussion There was no difference ($P>0.05$) for dry matter intake across treatments: 22.2, 22.6 and 22.7 kg/day for CS, SS and CSSS, respectively. Milk production (3.5% fat-corrected) ranged from 28.2 to 29.6 kg/day, and did not differ ($P>0.05$) across treatments. Mertens (1995) evaluated five forage sources (corn silage, alfalfa silage, wheat silage, orchardgrass silage and sorghum x sudan silage) in a iso-NDF (30.3 to 31.4 % DM) ration for lactating dairy cows and also observed no difference ($P>0.05$) in DMI, milk production and milk fat content between rations. Queiroz et al. (2008) observed lower ($P<0.01$) DMI for cows fed corn silage in comparison with sugarcane silage in iso-NDF rations, however there was no difference ($P>0.05$) for milk production. Corrêa et al. (2003) fed 200 g of forage neutral detergent fiber per kg of dry matter as either corn silage or fresh sugarcane and observed that sugarcane depressed DMI by 6.9% and milk yield by 7.3% in cows yielding 34 kg of milk per day. These two trials did not consider the effectiveness factor of 1.2 for sugarcane in the ration formulation, which may explain the differences observed. The use of iso-effective rations seems to equalize the production potential of different forage sources. Feed and energy efficiency did not differ ($P>0.05$) across treatments. Mean fat, protein, casein and lactose content was 3.92, 3.72, 2.86 and 4.5%, respectively, and did not differ ($P>0.05$). Urea nitrogen content was 14.3, 14.7 and 13.8 for CS, SS and CSSS, respectively. SCC ranged between 123,130 and 178,860 cells/mL.

Conclusions By using the effectiveness factor of 1.2 for sugarcane silage the replacement for corn silage does not affect intake, and milk production and composition, when iso-effective NDF rations are considered at ration formulation for lactating dairy cows.

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Table 1. Total mixed rations composition (%).

	Corn silage	Sugarcane silage	Corn silage + Sugarcane silage
Corn silage	50	-	25
Sugarcane silage	-	39.2	19.6
Finely ground corn grain	13.8	22.1	17.9
Soybean meal	20.9	23.4	22.2
Pelleted citrus pulp	13.0	13.0	13.0
Mineral premix	2.3	2.3	2.3
Forage eNDF factor	1.0	1.2	-
eNDF, % DM[1]	25.6	25.9	25.9

[1]effective neutral detergent fibre

Table 2. Dry matter intake, milk production and composition of the evaluated silages.

Variables	Corn silage	Sugarcane silage	Corn silage + Sugarcane silage	SEM	*P*
DMI (kg/day)	22.2	22.6	22.7	0.63	0.74
Feed efficiency (kg milk/kg DM)	1.23	1.16	1.18	0.30	0.12
Energy efficiency (Mcal/kg DM)	0.91	0.86	0.88	0.03	0.25
Milk production (kg/day)	27.6	26.5	26.7	0.98	0.38
Milk production, 3.5% fat-corrected (kg/day)	29.5	28.2	28.6	1.13	0.56
Milk composition					
Fat (%)	3.92	3.90	3.94	0.14	0.96
Protein (%)	3.73	3.72	3.72	0.08	0.95
Casein (%)	2.90	2.89	2.84	0.07	0.66
Lactose (%)	4.49	4.55	4.46	0.04	0.09
Urea nitrogen (mg/100 mL)	14.3	14.7	13.8	1.07	0.54
SCC (1,000/mL)[1]	178.86	145.68	123.13	-	-
LnSCC[2]	4.84	4.65	4.50	0.18	0.32

[1]SCC-somatic cell count; [2]Log-transformed somatic cell count

Effects of TMR distribution twice a week on lactating cows performance: efficacy of a silage additive on TMR stability.

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Key words: silage, TMR, stability, working time, bacterial additive

Introduction Reducing feeding frequency is a major concern for dairy farmers willing to better control their working time and to improve equipment management. If this is easily done with dry forages, it still remains difficult to achieve for farmers feeding silages.

Farrié et al. (2004) have evaluated the possibility to reduce the frequency of forage distribution to nursing cows. Feeding silage three times a week instead of daily, and allowing self-serviced hay in the meanwhile, seemed to have no effect on breeding performance of neither cows nor calves during the winter period. However, Bonnefoy et al. (2009) studied the kinetics of stability of corn or grass silages at 10°C, 20°C and 30°C. They showed that silage stability was maintained at 10°C but fermentation restart, pH increase, and development of yeasts and moulds were rapidly observed from 20°C. To be able to recommend simplified feeding systems, an optimal preservation of silage is necessary, otherwise, silage degradation may rapidly occur with subsequent reduction in intake and productivity, and an increased risk of undesirable or even pathogenic microorganisms development.

In this context, the aim of this study was to evaluate the impact of a reduced frequency of total mixed ration distribution and the efficacy of a microbial silage additive on diet stability and performance of lactating cows.

Material and methods The trial was performed with 3 groups of cows (n=7 per group, mid-lactation) during 8 weeks (spring period). During the two first weeks cows were adapting to their diet. The animals were fed with a total mixed ration (TMR) containing at least 70% of corn silage (33% DM), concentrate (cereal concentrate 2kg/head/day and soybean meal 3kg/ head/day), urea (150g/ head/day), minerals and vitamins supplement (230g/ head/day) and meadow hay (1 kg/ head/day).

Group 1 was fed daily and the corn silage was not treated with any additive. Groups 2 and 3 were fed only twice a week (Mondays and Thursdays). In group 2, the corn silage was not treated whereas in group 3, the corn silage had been treated with a silage additive containing *Lactobacillus buchneri* NCIMB 40788 -LB- (Lalsil Fresh, Lallemand Animal Nutrition, 3 x10^5 cfu/g of silage).

Yeasts and moulds concentration was determined at silo opening by enumeration on malt agar plates. Three samples (100 g each) were taken at silo opening and brought back to the laboratory; yeast colonies were counted after 2 days of incubation at 25°C, and mould colonies were detected after 5 days of incubation.

Evolution of the TMR temperature was recorded every 5 min in the feed bunks using individual automatic probes (Tomkey AES Chemunex). Ambient temperature was also recorded. TMR offered and refusals were individually weighted in order to calculate mean individual feed intake per week. Milk yield and composition were weekly recorded. Somatic cell counts (SCC) and butyric spores were also analyzed. Statistical analysis was performed using Proc Mixed procedure of SAS, with repeated measures.

Results and discussion At silo feedout, yeasts and moulds concentrations were lower (P<0.01) in corn silage treated with LB than in non treated silage (Table 1).

Table 1: Yeast and mould counts (Log10 CFU/g of silage, n=3) in corn silages treated or not with *Lactobacillus buchneri* (LB), at silo feedout.

	Control silage	LB-treated silage
Total yeast counts	6.88±0.02	3.38±0.12
Total mould counts	3.44±0.33	Not Detected

A significant increase (P<0.05) in temperature in TMR left for 3 or 4 days in the feed bunks was measured as compared with the TMR distributed daily.

When comparing the two TMRs distributed only twice a week, the TMR containing the LB-treated silage heated significantly less than the TMR containing non treated silage (table 2; P<0.001). As shown on figure 1, temperature exceeded 25°C from the second week of the trial in the group 2 and only from week 4 in the group 3. Also, in group 2 maximum temperatures were greater than in group 2. During the last two weeks of experimental period, temperatures as high as 47°C were recorded in TMR prepared with non treated silage, whereas 40°C was never reached in TMR prepared with LB-treated silage.

Table 2. Differences between TMR temperature and ambient temperature for TMR prepared with LB-treated or non treated corn silage.

	[TMR temperature in bunk – Ambient temperature]	
	After 3 days in bunk	After 4 days in bunk
TMR prepared with control silage	13.3	18.7
TMR prepared with LB-treated silage	10.2	14.1

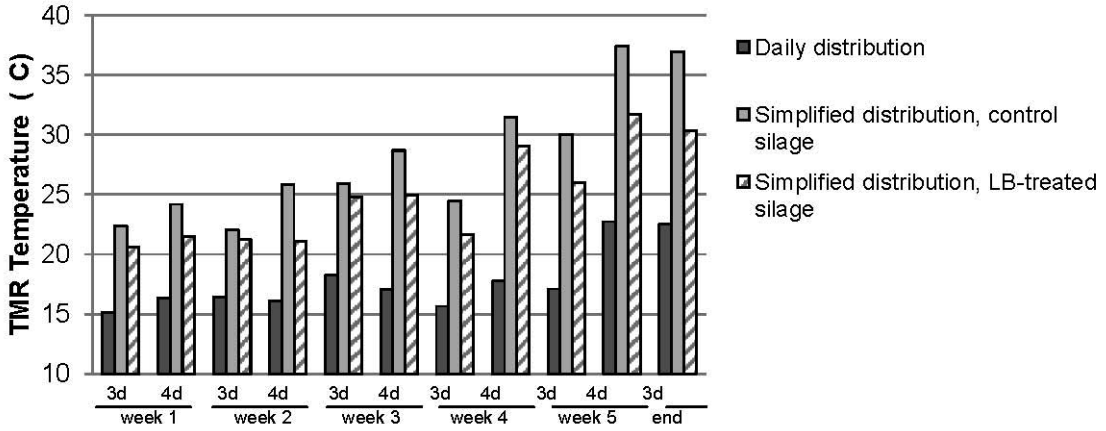

Figure 1. Mean temperatures (°C) recorded in the TMR left in the feed bunk after 3 days or 4 days (simplified distribution) or each day (daily distribution) of each experimental week.

Dry matter intake and milk performance of the cows were neither significantly modified by the frequency of TMR distribution nor by LB treatment of the corn silage.

During the last week of the experiment, a higher SCC and butyric spores concentration was noticed in group 2 receiving twice a week TMR prepared with non treated silage as compared to group 3 receiving TMR prepared with LB-treated silage.

These data suggest that reducing the frequency of distribution of TMR could be performed without significant modification of dairy cow perfomance. However, TMR distribution twice a week may induce, in particular during increasing temperatures, a higher risk of degradation of silage and a decrease in TMR feed value and hygienic quality.

Our results suggest that the use of *L. buchneri* NCIMB 40788 could be useful to prevent such a deterioration. Indeed, this silage additive has demonstrated its efficacy to improve aerobic stability of corn silages and avoid the growth of undesirable microorganisms.

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Effect of diet composition during the dry period on insulin resistance in dairy cows

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Keywords: dry cow, energy restriction, NDF content, insulin sensitivity, plasma glucose, plasma NEFA

Introduction Voluntary dry matter intake (DMI) of dairy cows decreases during the final 2 to 3 weeks before parturition (Ingvartsen and Andersen 2000). The DMI decrease is accompanied by a gradual increase in lipid mobilisation from the adipose tissue and elevation of plasma non-esterified fatty acid (NEFA) concentration. In order to reduce lipid mobilisation and the consequent hepatic lipid accumulation, there has been considerable interest to maximize prepartum feed intake (Grummer et al. 2004). However, high-energy intake increases plasma insulin concentration and may increase insulin resistance in peripheral tissues during late pregnancy, thus promoting tissue mobilisation during the periparturient period. The clearance rate of glucose during the intravenous glucose tolerance test (IVGTT) performed after calving, was decreased in cows that were over conditioned and had higher plasma NEFA at calving compared with leaner cows (Holtenius et al. 2003).

Insulin resistance is distinguished by an abnormal response to standard levels of circulating insulin that is accompanied by glucose intolerance and decreased glucose uptake by peripheral insulin sensitive tissues (Kahn 1978). Elements that are involved in the development of insulin resistance are related to those participating in the development of metabolic diseases in periparturient ruminant. These factors include e.g. obesity, hyperinsulinaemia and hyperlipidaemia (Hayirli 2006).

The current study was carried out to test the hypothesis that high energy allowance during the dry period, based on ad libitum feeding of grass silage, induces increased whole-body insulin resistance in dairy cows, and this effect remains during early lactation. Further, we hypothesized that restriction of energy intake by increasing the NDF content of the diet would decrease insulin resistance.

Material and methods Sixteen multiparous, pregnant, dry Ayrshire cows were used in a randomized complete block design. The cows were paired according to the expected calving date, parity and body condition score (BCS). Within pairs, cows were randomly allocated to treatments eight weeks prior to the expected parturition: 1) Ad libitum feeding of wilted grass silage (SILAGE) or 2) Ad libitum feeding of total mixed ration consisting of 55%, 40% and 5% of wilted grass silage, wheat straw and rapeseed meal, respectively (TMR). The crude protein content of the diets was balanced by adjusting the proportion of rapeseed meal in the TMR. In addition to the forage, the cows received 1 and 2 kg/d of commercial concentrate during the last 10−6 and 5−0 days before the expected calving date, respectively.

After calving, all cows were offered wilted grass silage ad libitum and an increasing amount of commercial concentrate reaching maximum amount of 16 kg/d at 32 d postpartum. The cows were kept in tie stalls until lactation day 10, and thereafter in the loose house. Feeds offered and feeds refused were recorded daily. Milk yield was recorded for every milking during the lactation weeks 1−8. Milk samples were collected at 1, 2, 4, 6 and 8 weeks after parturition. Live weights and BCS were recorded every other week during the dry period, and also 5 d before the expected calving date. After calving live weights were measured on d 1, 2, 6, 7, 14, 28, 42 and 56. BCS was recorded on d 1, 7, 14, 28, 42 and 56.

Intravenous (i.v.) glucose tolerance test (IVGTT) was performed at 11 ± 1 d before the expected calving date and 8 ± 1 d postpartum by administering 0.25 g of glucose i.v./kg of body weight. Blood samples were collected at -10, -5, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210 and 240 min relative to glucose infusion. Plasma glucose, insulin and NEFA responses to IVGTT were calculated as clearance rates (CR) and net incremental areas under the response curve (AUC). Data were analysed by mixed procedure of SAS (version 9.2) with a model that included the random effect of cow and fixed effect of treatment. The time between IVGTT and actual calving date was included in the model as fixed effect when analysing the prepartum data.

Results and discussion The average DMI of SILAGE and TMR from 8 weeks to 11 days prepartum was 13.1 and 11.0 kg/d, respectively. The daily intake of ME (MJ/d) in SILAGE group was approximately 35 percent higher than in TMR. The energy content of the grass silage and TMR was in average 10.4 and 9.2 MJ ME/kg DM, respectively. The NDF-content of TMR was approximately 100 g/kg DM higher than that of SILAGE.

During the first six weeks of the dry period, the cows in SILAGE group gained 0.4 kg/d more body

weight than cows in TMR (1.4 vs. 1.0 kg/d; P<0.05). According to the Finnish nutrient requirements (MTT 2012) the greater energy intake of SILAGE group should have resulted in a more pronounced daily weight gain (approximately 1 kg/d). The change of BCS during the experiment was not affected by the dry period feeding. In the beginning of the experiment the BCS of SILAGE and TMR cows were 3.4 and 3.5, and in the end 3.1 and 2.9, respectively

Ad libitum feeding of grass silage during the eight weeks dry period increased plasma insulin concentration (24.3 vs. 16.1 µIU/ml; P<0.10) and glucose concentration (4.0 vs. 3.8 mmol/l; P<0.05) compared to TMR. Glucose concentration of SILAGE group remained higher than that of TMR group postpartum (3.4 vs. 3.1 mmol/l, P<0.10). Plasma NEFA was not affected by prepartum feeding, which indicates that moderate oversupply of energy during the dry period did not accelerate tissue mobilization near calving and during early lactation.

Parameters from intravenous glucose tolerance test that was done 11 d before expected calving date indicate that the insulin response to glucose load (insulin AUC) was increased in SILAGE compared to TMR (Table 1). Simultaneously, the glucose AUC in SILAGE group was decreased compared to TMR. These results suggest that the lower glucose AUC in silage fed cows resulted primarily from the higher insulin concentration, which was caused by a more intensive dry period feeding. The treatments had no effect on plasma NEFA responses in IVGTT 11 d prepartum. No differences were observed between SILAGE and TMR groups in IVGTT 8 d after calving.

Table 1. Effect of dry period ad libitum feeding of silage or TMR[1] on plasma glucose, insulin and NEFA[2] responses to intravenous glucose tolerance test (IVGTT; 0.25 g of glucose/kg of BW) performed 11 d before the expected date of calving and 8 d postpartum.

	-11 d				+8 d			
	Silage	TMR	SEM	Stat. signific.	Silage	TMR	SEM	Stat. signific.
Plasma glucose								
Basal[3] (mmol/l)	4.0	3.9	0.12		3.1	3.0	0.16	
CR_{60}[4] (%/min)	1.4	1.3	0.12		2.0	1.7	0.17	
AUC_{240}[5] (mmol/l x 240 min)	413	525	47.9	*	322	374	30.1	
Plasma insulin								
Basal[3] (µIU/ml)	15.7	13.8	2.03		5.7	6.2	0.75	
Peak (µIU/ml)	382	225	66.9	*	125	110	17.5	
AUC_{240}[5] (µIU/ml x 240 min)	17762	10064	3520.3	o	3884	3063	560.7	
Plasma NEFA								
Basal[3] (mmol/l)	0.25	0.36	0.059		0.62	0.53	0.069	
CR_{60}[4](%/min)	0.9	1.2	0.16		1.4	1.2	0.24	
AUC_{60}[6] (mmol/l x 60 min)	-2.26	-4.46	1.70		-7.88	-5.16	2.05	

[1]TMR = Total mixed ration consisting of wilted grass silage, straw and rapeseed meal (55%:40%:5%); [2]NEFA = Non-esterified fatty acids; [3]Basal = Average concentration at 10 and 5 min before IVGTT; [4]CR_{60} = Clearance rate during the first 60 min of IVGTT; [5] AUC_{240} = Area under the response curve during IVGTT; [6]AUC_{60} = Area under the response curve during the first 60 min of IVGTT.

Conclusions High energy allowance, based on ad libitum feeding of grass silage during the dry period increased plasma glucose and insulin concentrations. Energy intake during late pregnancy had no effect on the degree of whole-body insulin resistance of dairy cows during the transition period.

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Effect of fatty acids supplementation on performance and milk fatty acid composition in goats fed grass silage based diet

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Keywords: fatty acids, flax seed, goat milk, megalac, sunflower.

Introduction The optimization of milk production can be decisive by dietary supplementation, goat milk has been identified as a viable alternative for consumers that are sensitive or develop allergic reactions to bovine milk. Intake of fatty acids (FA) by ruminants, such as vegetable oils or seeds can affect the quality of milk (Bernard et al. 2009). The objective of this study was to determine the production and chemical composition of goat milk supplemented with three different sources of FA, sunflower seed (18:2 n-6), flax seed (18:3 n-3) and Megalac-R (C16:0).

Material and methods Six multiparous Alpine goats (52.5 ± 5 kg LW), in mid lactation (70 ± 3 d) offered three diets according to a replicated 3 x 3 Latin square design with 21 d experimental periods using two animals per group. Each experimental period was comprised of 15 d adaptation and 6 d sampling periods. Goats were housed in individual cages, with free access to water and milked at 08.00 h, which were fed with Megalac-R (MG) 7%, sunflower seeds (SF) 14%, flax seed (FS) 12%, supplemented with grass silage, grass hay, and a concentrate based on sorghum grain, canola meal, and mineral salts (12% CP; ME, 18.3 Mj/kg DM). Intakes, digestibility and milk yield were recorded daily, to determine its chemical and milk composition, milk samples were analyzed using the Eco-Milk (Milk Analyzer. Milkana Kam 98-2 [a], Hillerød, Denmark) and FA profile was determined by gas chromatography.

Results and discussion DM, OM and NDF intake was not affected by the treatments (Table 1), ADF intake was lower (P=0.08) for MG diet compared with SF and FS diet. Total digestible DM, OM, NDF and ADF were not improved between treatments. N intake and N in urine was higher for MG (P=0.06) compared with SF and FS, N balance was not different among treatments. FA sources had no effect in milk yield, protein and non fatty solids concentration. Fat concentration (g/kg) was higher (P=0.01) for MG with respect to FS diet. The effect of the physico-chemical nature of the feed intake on the milk fat content in the ruminant depends basically on the quantity and quality of the fiber fraction of the diet (Sanz Sampelayo et al. 2007). There were not significant differences in the milk fatty acids C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C18:0 and C18:3 n-3 between treatments. In milk fat, the highest concentration of C16:0 (P<0.01) was for MG than SF and FS treatment, C18:2 n-6 (P<0.01) was higher for MG than FS treatment. Palmitic acid, main FA in MG, had relationship with their high content in milk fat. Feeding of palmitic acid increased goat milk C16:0 % considerably at the expense of C10:0 to C14:0 and of C18:1 (Chilliard et al. 2003), in fact milk content of C18:1 was lower (P<0.01) in MG compared with FS. C18:2 n-6 was higher (P<0.01) for MG than FS diet. This was probably due to the extensive hydrogenation of this acid, abundant in FS and SF, increasing C18:0 in milk. Chilliard and Ferlay (2004) comparing SF oil and seeds in goats revealed that seed C18:2 n-6 was more strongly hydrogenated to C18:0 than oil C18:2 n-6.

Conclusions Supplementation with different sources of fatty acids in the diet of dairy goats had no effect on intake, digestibility, milk yield production, and components, except the fat content. Supplementation with MG showed a higher concentration of C16:0 and C18:2 n-6, but the flax seed supplementation showed a higher concentration of C18:1.

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Table 1. Intake (g $LW^{0.75}$/day**),** digestibility (g/kg), N balance (g/d), milk yield (kg/d) and fatty acids profile in dairy goats fed with different fat sources (n=18).

Ingredients	Megalac-R	Sunflower	Flax seed	SEM	*P-value*
Intake, g kg $LW^{0.75}$/ day					
DM	62.92	63.22	64.91	3.04	0.88
OM	61.63	62.36	63.39	1.82	0.79
NDF	24.09	25.80	27.04	1.01	0.15
ADF	12.04	12.99	13.60	0.44	0.08
Digestibility (g/kg)					
DM	630.66	609.03	596.32	27.26	0.67
OM	685.98	658.11	648.10	11.93	0.09
NDF	585.90	551.08	565.95	23.66	0.59
ADF	547.51	492.32	528.95	28.07	0.39
N balance (g/d)					
N intake	28.99	27.53	27.09	0.64	0.06
N excretion					
Urine	8.50	7.15	7.13	0.17	0.06
Feces	10.83	11.18	12.18	0.45	0.12
N Balance	9.66	9.18	7.78	0.57	0.22
Milk					
Milk Yield (g/d)	743	782	772	0.2	0.55
Fat	39.91	40.49	38.51	1.68	0.69
Protein	33.56	35.61	35.25	1.24	0.46
Non Fatty Solids	71.84	76.01	75.15	2.58	0.48
Concentration (g/kg)					
Fat	53.5^{a}	51.4^{ab}	49.8^{b}	1.1	0.01
Protein	45.0	45.5	45.7	0.4	0.60
Non Fatty Solids	96.5	97.2	97.3	0.5	0.54
Fatty Acids (% total FA)					
∑SFA	72.23	65.94	68.39	1.92	0.08
C4:0	1.78	1.27	1.56	0.21	0.27
C6:0	2.70	2.09	2.35	0.25	0.25
C8:0	3.54	2.84	3.07	0.37	0.40
C10:0	11.72	10.10	10.67	1.01	0.52
C12:0	5.09	4.65	4.81	0.35	0.68
C14:0	9.58	11.28	11.46	0.75	0.16
C16:0	28.24^{a}	23.07^{b}	22.42^{b}	0.80	0.01
C18:0	9.56	10.61	12.02	0.89	0.16
MUFA					
C18:1	24.11^{b}	27.57^{ab}	28.39^{a}	1.03	0.01
∑PUFA	4.06^{a}	3.61^{ab}	3.21^{b}	0.22	0.03
C18:2	3.38^{a}	3.02^{ab}	2.45^{b}	0.20	0.01
C18:3	0.68	0.59	0.75	0.06	0.17
MUFA/PUFA	6.25^{c}	7.76^{b}	8.92^{a}	0.32	0.01
PUFA/SFA	0.05	0.05	0.04	0.003	0.17

^{abc} Different letters in the same row, P<0.05

The effects of untreated and urea-treated whole crop barley silage on performance of young Holstein dairy calves

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Keywords: performance, urea, whole crop barley silage, young dairy calves

Introduction There is an increased range of alternative forages available to Iranian dairy and beef cows farms. Whole crop cereals are an attractive alternative to grass or corn silage in areas of restricted rainfall or climatic conditions where the likelihood that harvestable yields of forage are low (Hill and Leaver 1999). The environmental conditions in eastern Iran are not suit for corn silage production. However, consistent yields of small grain cereal crops can be achieved. In situations, where water is a limiting factor for growing corn, triticale and barely may be a better alternative fodder crops (Hill and Leaver 1999).

The main method of conservation of whole-crop cereals harvested at dry matter (DM) contents of higher than 500 g/kg is ensiling with urea (40 kg/t DM; Hill and Leaver 1999). The use of urea has been demonstrated as a cost-effective method of supplementation (Preston and Leng 1987). Excessive use of urea in diets of cattle can, however, have detrimental effects on ruminant production, leading to hyperammoniation and possibly death, if it is not fed with a source of readily available carbohydrates (Huntington and Archibeque 1999). The objective of this experiment was to evaluate the performance of young dairy calves offered untreated and urea-treated whole crop barley silage in warm climate.

Material and methods This experiment was conducted at an industrial dairy farm, Gonabad, Khorasan-Razavi province, Iran. Whole crop barley was harvested at milk stage (about 35% DM), chopped to about 20 mm using a single chop forage harvester and ensiled in 2 trench silos (with and without urea), sealed with two layers of plastic sheets and then allowed to ferment for 45 days.

Based on the mean of two consecutive daily live weights at the start of the experiment, twenty-eight Holstein young dairy calves (mean initial live weight, 197.51±30.18 kg) were used in the experiment. During the fattening period, the average temperature and relative humidity were 33.8 °C and 29.5%, respectively. The calves were housed individually in stalls equipped with feeding troughs and had free access to clean, fresh water. The calves were fed the experimental diets at 105–110% of their ad libitum intake twice daily at 08.00 and 20.00 h during the whole experiment. The adaptation period to the experimental diets was 15 days and the experimental period was 30 days.

Diets were formulated based on beef NRC (1996) requirements (Table 1). The total mixed rations were consisted of 35% forage (silage) and 65% concentrate (DM basis). Chemical composition of all diets was similar (Table 1). The experimental diets were: 1) untreated WCBS, and 2) WCBS treated with urea (20 g/kg DM). Voluntary dry matter intake (DMI) of experimental diets by calves was calculated as the difference between amounts of feed offered and refused daily. Animals were weighed before morning feeding once a week throughout the duration of the trial. Blood samples were collected from the jugular vein on day 30 immediately 2h after morning feeding. Rumen liquid samples were taken approximately 3h after morning feeding by stomach tube, and then pH was determined immediately. Also, rumen liquid samples were strained through two layers of cheese cloth and acidified with 10cc of HCl solution (50% vol/vol) for ammonia-N analysis.

Comparisons between the experimental diets were made using complete randomized design. Weight at the start of the experimental period was used as a covariate in analyses of experimental period performance. Statistical analysis was conducted using SAS 9.1 (SAS Institute 1989).

Results and discussion The voluntary dry matter intake (DMI) and daily weight gain (DWG) of the calves were not significantly affected ($P>0.05$) by the experimental diets (Table 2). These results were inconsistent with Phipps et al. (1993) who reported that addition of urea-treated forages into diets containing grass silage tended to maintain or to increase DMI of growing cattle. Results showed that the experimental diets had no significant effect ($P>0.05$) on feed conversion ratio (FCR) and feed efficiency (FE).

There were no significant treatment effects on blood urea nitrogen (BUN) and rumen liquid pH of the calves ($P>0.05$) The concentration of rumen liquid ammonia-N increased significantly ($P<0.05$) in the diet containing urea-treated WCBS compared to untreated WCBS diet. Increased supply of non-protein nitrogen from urea-treated WCBS could be converted to microbial protein (Huntington and Archibeque 1999).

Table 1. Ingredients of experimental diets fed to the animals.

	Experimental diets†	
	1	2
Ingredients (% DM)		
Untreated WCBS	35	0
Urea-treated WCBS	0	35
Barley grain	26	26
Corn grain	13	13
Cottonseed meal	8.06	8.38
Soybean meal	11.05	11.05
Beet molasses	3.25	3.25
Salt	0.32	0.32
Urea	0.97	0.65
Calcium carbonate	0.72	0.72
Magnesium oxide	0.33	0.33
Sodium bicarbonate	0.65	0.65
Vitamin-mineral mix	0.65	0.65
Chemical composition		
Metabolizable energy (Mcal/kg DM)	2.74	2.74
Non fibre carbohydrate (%)	39.9	39.9
Ether extract (%)	2.78	2.78
Crude protein (%)	15.2	15.2
Calcium (%)	0.48	0.48
Phosphorus (%)	0.39	0.39

† The experimental diets were: 1) untreated WCBS, and 2) WCBS treated with urea (20 g/kg DM).

Table 2. Effects of experimental diets on performance of the calves.

Item†	Experimental diets†		SEM	p-Value
	1	2		
DMI (kg/d)	7.07	6.93	0.15	0.539
DWG (g/d)	1628.6	1633.3	79.75	0.963
FCR (g DMI/g DWG)	4.28	4.38	0.26	0.775
FE (g DWG/g DMI)	0.24	0.25	0.01	0.429
BUN (mg/dl)	13.75	14.50	0.78	0.478
Rumen liquid pH	6.36	6.41	0.02	0.205
NH_3-N (mg/100 ml)	13.02^b	17.93^a	0.78	0.011

† The experimental diets were: 1) untreated WCBS, and 2) WCBS treated with urea (20 g/kg DM).
Data with different letters in the same row are significantly different (P<0.05).

ConclusionsThe results suggested that urea-treated WCBS had no effect on DMI and performance of Holstein male calves in warm climate. Also, DMI and performance of the calves fed with experimental diets were similar to other studies in Iran.

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The replacement of corn silage by treated and untreated whole crop triticale silage in diets of fattening male calves

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Keywords: Bacterial inoculants, molasses, performance, whole crop triticale silage

Introduction In Iran, there are many alternative types of forage which can be used in animal nutrition. Corn silage is a major component of diets fed to dairy and beef cows because of the high energy yield, relatively high palatability, the ease of mechanization and storage, the uniformly high feeding value, and incorporating easily into total mixed rations (Cherney et al. 2004). However, on drought prone sandy soils, and in years with insufficient rainfall the yield of maize is very low (Van Duinkerken et al. 1999). In situations where water is a limiting factor for growing corn, triticale may be an alternative fodder crop (Hill and Leaver 1999).

Triticale grows mainly during the early spring when there usually is a precipitation surplus and water is not a limiting factor for growth. When triticale is harvested as whole crop silage the dry matter (DM) yield ranges between 9000 and 11000 kg/ha. Therefore, under water limiting conditions it may be attractive to replace corn silage by whole crop triticale silage (Van Duinkerken et al. 1999). There is limited study about the effects of inoculants and molasses on fermentation and nutritive value of triticale silage. Thus the aim of this study was to determine the effects of replacing corn silage (CS) with whole crop triticale silage (WCTS) treated with bacterial inoculants and molasses on performance of fattening male calves.

Material and methods Corn plants and whole crop triticale at milk stage (about 30% DM) were harvested, chopped (20 mm theoretical length of cut) and ensiled in trench silos, sealed with two layers of plastic sheets and then allowed to ferment for 45 days. Thirty two (8 calves per treatment) Brown Swiss male calves (mean initial live weight, 215±15.23 kg) were used in a complete randomized design for 100 days. Calves were randomly assigned to 1 of 4 experimental diets: 1) CS, 2) WCTS without additive, 3) WCTS treated with molasses (50 g/kg DM) and 4) WCTS treated with bacterial inoculants (Ecosyl®, applied at 1×10^5 cfu/g). Animals were kept in individual stalls and had free access to water. Diets were formulated based on beef NRC (1996) requirements. The diets used in the present experiment included (% from DM) experimental silage (30), barley grain (34), corn grain (12), cottonseed meal (3), canola meal (3), wheat bran (4), beet sugar pulp (6), beet molasses (5.5), urea (0.5), calcium carbonate (0.5), sodium bicarbonate (0.5), salt (0.3), di-calcium phosphate (0.2) and mineral-vitamin premix (0.5). Diets were isocaleric (Metabolizable energy: 2.58 Mcal/kg DM) and isonitrogenous (crude protein (CP): 14.4%). Other chemical composition of diets were: neutral detergent fiber (NDF): 32.1%, acid detergent fiber: 16.8%, ether extract: 2.6%, calsium: 0.71 and phosphorus: 0.56%. The total mixed rations were consisted of 30% forage (silage) and 70% concentrate (DM basis). Calves were fed enough total mixed rations in two separate feeding at 0700 and 1900 h to allow a daily refusal of approximately 50 g/kg intake.

To determine the dry matter intake (DMI), the a.m. and p.m. feed offered was recorded and refusal was collected and weighed daily. The calves were weighed every 3 weeks throughout the trial (After a 12 h fast by removing feed only) and amount of diet offered was adjusted based upon their live weights. Then average daily weight gain (DWG) and feed conversion ratio (FCR) were calculated. To determine nutrients apparent digestibility, feeds, refusals and feces of each calf were collected for 5 days and sampled on days 96 to 100 of the fattening period. These samples were frozen at -20°C for future analysis. In this trial, nutrients apparent digestibility was measured using fecal collection. Rumen liquid samples were taken approximately 3h after morning feeding by stomach tube, then pH was determined immediately. During the trial ruminating activity of calves for 24 h was measured by method of direct observation every 5 minutes for 24 h.

Comparisons between the experimental diets were made using complete randomized design. Weight at the start of the experimental period was used as a covariate in analysis of experimental period performance. Statistical analysis was conducted using SAS 9.1 (SAS Institute 1989). Planned contrasts included diet 1 *vs.* 2, 3 and 4, 1 *vs.* 2, 2 *vs.* 3, 2 *vs.* 4 and 3 *vs.* 4.

Results and discussion There were no significant differences (P>0.05) in DMI, DWG or FCR among treatments (Table 1). The lower DMI of WCTS can be related to high NDF content of this silage compared with corn silage, digestibility, palatability, time spent chewing during eating and ruminating (Khorasani et al. 1996). Rumen liquid pH and ruminating activity were significantly (P<0.05) affected by the diets (Table 1). Rumen liquid pH was highest for calves fed WCTS treated with bacterial inoculants and lowest for calves fed CS without additive (diets 1 *vs.* 2,3,4 and 1 vs. 2). This result can be due to the larger particle size in diets containing CS compared with those containing CS. The effect of particle size on rumen liquid pH in the present study contrasts with the commonly accepted principle that increasing particle length promotes chewing activity, and thus increases buffering capacity within the rumen due to increased saliva secretion. The experimental diets had significant effect (P<0.05) on nutrients digestibility coefficients (Table 2). The addition of molasses to whole crop triticale at ensiling had significantly higher nutrients digestibility than other diets (2 *vs.* 3 and 2 *vs.* 4). These findings are in agreement with the observations of Carpintero et al. (1969) who reported that nutrients digestibilities of whole crop corn silage treated with molasses were higher than the corn silage.

Table 1. Effects of experimental diets on performance, rumen pH and chewing activity of the calves.

Item	Experimental diets†				SEM	Contrasts (*P*-value)				
	1	2	3	4		1 vs. 2, 3, 4	1 vs. 2	2 vs. 3	2 vs. 4	3 vs. 4
DMI (kg/d)	9.00	8.50	8.49	8.56	0.829	0.689	0.729	0.970	0.942	0.916
DWG (kg)	1.33	1.24	1.22	1.19	0.091	0.229	0.388	0.963	0.651	0.696
FCR	7.13	6.77	6.70	7.24	0.284	0.322	0.259	0.988	0.334	0.346
Rumen pH	6.24	6.54	6.38	6.56	0.095	0.037	0.043	0.258	0.849	0.190
Ruminating activity (min/d)	297	398	344	316	30.21	0.130	0.031	0.224	0.071	0.522

† Experimental diets were: 1) CS, 2) WCTS without additive, 3) WCTS treated with molasses (50 g/kg DM) and 4) WCTS treated with bacterial inoculants (Ecosyl®, applied at 1×10^5 cfu/g).

Table 2. Nutrients digestibility coefficients of the experimental diets.

Item	Experimental diets†				SEM	Contrasts (*P*-value)				
	1	2	3	4		1 vs. 2, 3, 4	1 vs. 2	2 vs. 3	2 vs. 4	3 vs. 4
DM	0.71	0.73	0.75	0.70	0.010	0.125	0.109	0.221	0.028	0.002
Organic matter	0.74	0.73	0.75	0.71	0.006	0.974	0.214	0.070	0.030	0.001
CP	0.69	0.68	0.71	0.65	0.008	0.325	0.601	0.034	0.005	<0.0001
NDF	0.45	0.42	0.46	0.40	0.005	0.001	0.001	0.000	0.011	<0.0001

† Experimental diets were: 1) CS, 2) WCTS without additive, 3) WCTS treated with molasses (50 g/kg DM) and 4) WCTS treated with bacterial inoculants (Ecosyl®, applied at 1×10^5 cfu/g).

Conclusions Replacement of corn silage with triticale silage had no significant differences on performance of fattening male calves. Addition of molasses and microbial inoculants can improve nutrients digestibility of triticale silage.

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Intake and productive performance of Nellore steers fed diets containing different proportions of *Stylosantes cv* Campo Grande and corn silages

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Keywords: beef production, dry matter intake, growth performance, silages, tropical legume

Introduction Low animal productivity is attributed to the seasonality in feed supply from pastures, in response to climate change, resulting in crop and intercrop periods of animal products. In this case, an adequate dietary plan is required to adjust between forage demand and supply, aiming to alleviate dry season forage shortages, and enabling animal production uniformly throughout the year.

The use of forage conservation in the form of silage is a feasible alternative to ensure high quality forage supply during the food storage period. In this context, corn and sorghum cultures have been highlighted as the most used species in the ensiling process due to their ease of cultivation, high yields and, especially due to the quality of silage produced (Pereira et al. 2009). However, there is recent interest in studies on the use of legume silage for animal feeding in Brazil.

Among the legumes, alfalfa is recognized worldwide as one of the best options in terms of nutritional value. However, its use is limited in the tropics due the high production cost and cultivation difficulties. Therefore, the Campo Grande *Stylosanthes,* a physical mixture of seeds of the species *S. capitata* (80%) and *S. macrocephala* (20%) is a good forage alternative because it increases the protein content of the animal diet, especially in periods of low forage availability. Thus, the high DM yield and nutritional value enhance the farmers, increasing the animal production per unit area. The objective of this study was to evaluate the dry matter intake and performance of beef cattle fed diets with different ratios of Campo Grande *Stylosanthes* and corn silages.

Material and methods The experiment was conducted at Experimentation Center, Research Extension of the "Triângulo Mineiro" (CEPET) at the Universidade Federal de Viçosa (UFV). A total of 40 Nellore steers, 386.50 ± 31.00 kg body weight (BW) at the beginning of the experiment, were distributed in a randomized block design with eight replications. The treatments consisted of different ratios of Campo Grande *Stylosanthes* silage (StS): corn silage (CS): 0:100, 25:75, 50:50, 75:25 and 100:0, with a forage:concentrate ratio of 50:50 in dry matter (DM) basis. Diets were formulated to allow daily gain of 1.1 kg live body weight. The experiment lasted 99 days, divided into three periods of 28 days each and fifteen days of adaptation. The animals were housed in individual stalls with protected feeders and waterers. The feed was provided ad libitum twice daily at 7 AM and 3 PM, with orts of 10% of the total feed supply. Samples from the supplied and orts feed were collected daily. The animals were slaughtered, the carcass yields (CY) were evaluated and expressed by dividing the hot carcass weight (HCW) by the respective final live weight (FLW) of each animal after 16 hours of fasting. The variables were analyzed using the GLIMMIX procedure of the SAS as a complete block design. The body weight at the beginning of the experiment was considered as block. The block was included as a random effect. The LSMEANS was used to obtain individual treatment means. Orthogonal polynomials contrasts were determined for partition of the effects into control versus replacing CS by StS, linear, quadratic, and cubic.

Results and discussion The chemical composition of the feeds used in the diets is presented in Table 1. The values of pH and NH_3-N in this study indicated that both silages were well preserved, once the lactic acid concentration was greater than the other acids. The DM intake (kg/d) did not differ ($P > 0.05$) among treatments (Table 2). Weight gain, carcass yield and feed conversion were also not affected by the treatments and the mean values were 1.25 kg/d, 55.6% and 8.46, respectively. Several nutritional strategies have been reported to maximize the efficiency of nitrogen utilization in legume silage based diets, such as the combination with higher starch content silages. Since Campo Grande *Stylosanthes* is relatively newly developed forage legume, trials evaluating the nutritive value of this forage in beef production are limited. Voluntary ingestion of DM was closely related to the neutral detergent fiber (NDF) concentration of the feed because fermentation and passage of the NDF through the reticulum-rumen are slower than other dietary constituents, with variations in filling and retention time, compared to non-fibrous components of the feed (Van Soest 1994). However, although the NDF concentration in the corn silage (52.30%) was less than that of Campo Grande *Stylosanthes* silage (66.37%), this was not reflected in a greater DM intake for this diet.The absence of diet effects on dry matter intake reflected in similar animal performance among the evaluated diets.

Therefore, although the StS has a greater lignin level (12.32%) than the CS (4.02%), the quality of the fiber content may have affected the digestibility and resulted in the similar response among the treat-

ments, since the lignin composition apparently has a more important role in the cellular wall digestibility than the lignin amount. In addition, the ruminal filling effect caused by the legume NDF is apparently lesser than the grasses, as the legumes generally have a greater weakness of diet particles and lesser rumen retention time (Oba & Allen, 1999). Thus, is indicated that only the knowledge of diet NDF level does not give enough information about the potential of use of insoluble fiber in the ruminant gastrointestinal tract, which consequently affects the animal performance (Detmann, 2010).

Conclusion It can be concluded that all *Stylosanthes*:corn silage ratios evaluated can be fed to Nellore steers because it resulted in similar intake and body weight gain. However, the utilization of Stylosanthes silage in feedlots for beef cattle depends on economic factors.

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Table 1. Chemical composition of silages and concentrate (%).

	Chemical composition									
	DM	CP	NDF	Lignin	pH	NH_3-N	Lactic acid	Acetic acid	Propionic acid	Butyric acid
StS*	30.02	11.18	66.37	12.32	4.27	6.74	5.48	2.54	1.43	0.15
CS†	35.85	6.92	52.30	4.02	3.74	4.26	5.04	3.63	1.85	0.18
Concentrate	89.13	14.7	15.89	--	--	--	--	--	--	--

* Campo Grande *Stylosanthes* silage; †Corn silage
DM = dry matter; CP = crude protein; NDF = neutral detergent fiber

Table 2. Dry matter intake and animal productive performance of beef cattle.

Item	Level of Campo Grande *Stylosanthes* silage (%)						P-value[1]			
	0	25	50	75	100	SEM	Control	L	Q	C
DMI* (kg/d)	9.89	10.20	9.66	9.51	9.63	0.58	0.77	0.53	0.53	0.86
ADG†(kg/d)	1.21	1.35	1.22	1.33	1.16	0.08	0.52	0.20	0.88	0.15
CY‡ (%)	56.44	54.89	56.14	55.40	55.15	0.60	0.06	0.97	0.22	0.42
FC§	8.33	7.75	8.00	8.00	8.34	0.54	0.57	0.42	0.91	0.83

* Dry matter intake; †Average daily gain; ‡Carcass yield; §Feed conversion
[1]Control vs replacing StS, L, Q and C = Linear, quadratic and cubic effects respectively

Performance and ingestive behaviour of young Nellore bulls fed with maize silage inoculated with *L. buchneri* and two roughage: concentrate ratio

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Keywords: average daily gain, feed: gain ratio, idle, rumination

Introduction *Lactobacillus buchneri* increases the silage aerobic stability due to the higher acetic acid production compared to the homolatic lactic acid bacteria (LAB) (Kleinschmit and Kung Jr. 2006). Acetic acid preserves the silage after silo opening, because yeasts and moulds are controlled. According to Weinberg et al. (2003), the LAB can survive in ruminal fluid, it changes the pH values and the rumen volatility fatty acids composition, affecting the animal performance. However, Muck (2010) reported that there are few studies evaluating the effect of the silage inoculants on the animal performance. The aim of this research was to evaluate the effect of the maize silage inoculated or not with *Lactobacillus buchneri* associate to two roughage: concentrate ratio on performance and ingestive behaviour of the Nellore young bulls.

Material and methods The maize studied was the 2B688kx hybrid. The maize plant was harvested with dry matter content between 30 to 35%. Treatments evaluated were: control silage (untreated) and maize silage inoculated with *Lactobacillus buchneri* "strain NCIMB 40788" ($1x10^5$ cfu/g of forage) associate to two roughage: concentrate ratio (60:40 and 40:60). The inoculum was diluted in distilled water and sprayed on the forage before filling the silos (bunker with a 60 tons capacity). Twenty eight Nellore young bulls, with average initial body weight of 320 kg were used. Animals remained in adaptation period for 18 days (until stabilization of dry matter intake), starting the experiment after this period. Dry matter (DM) intake was measured subtracting the orts from the offered. Diets offered were composed by maize silage inoculated with *L. buchneri* or not and concentrate (soybean meal, urea, ground maize grain and mineral salt) in two silage roughage: concentrate ratio (60:40 and 40:60). The diet was offered once a day (7:00 hours) to allow *ad libitum* intake (orts 10% of the supplied quantity). Animal behavior was evaluated for 2 days (12 hours per day) at intervals of 10 minutes between observations, measuring the feeding, rumination and idle time. The animals were weighed after fasting (16 hours) at the beginning and the end of the experiment to obtain the average daily gain. Feed: gain ratio was calculated as the amount of feed required for gain of 1.0 kg of body weight (NRC, 2000). The animals were slaughter with 500 kg after 116 days of experimental period. Experimental design used was completely randomized in factorial 2x2 (two silages and two roughage: concentrate ratios) with seven replications. The data were submitted to ANOVA and means were compared by Tukey test at 5% significance level evaluating the effects of silage, roughage: concentrate ratio and their interactions.

Results and discussion The DM intake increased by maize silages inoculation with *L. buchneri*, and also by utilization of the 60% of concentrate in diet (Table 1). Observed interaction between the variables studied to DM intake (Table 2). There was higher intake (9.52 kg/day) when the young bulls were fed with maize silage inoculated, associated with 40:60 roughage: concentrate ratio, compared to the silage control in the same roughage: concentrate ratio, and also in the maize silage inoculated and the 60:40 ratio. This result probably is due to *L. buchneri* produce ferulate-esterase, which is responsible by the increase in the fiber digestibility (Nsereko et al. 2008). The average daily gain (ADG) was higher in young bulls fed with maize silages inoculated. There was interaction between silage and roughage: concentrate ratio to ADG. Observed higher ADG (1.63 kg/day) when the young bulls were fed with maize silage inoculated, associated to 40:60 roughage: concentrate ratio. This event occurred because of the highest DM intake observed in the same treatment. Weinberg et al. (2003) also reported that the animal performance can be maximized because to the improved of the rumen microorganisms performance in response to the possible probiotic effect, resulting in higher DM intake, and digestibility. In general, the animals fed with maize silages inoculated and diet with 40% of roughage presented less time feeding. suggesting higher DM digestibility. Not observed effect of silages, roughage: concentrate ratio and interaction between these variables to rumination time. The idle time was not affected by silages and roughage: concentrate ratio, however, there was interaction between the factors in these variable. Thus, the animals fed with maize silage inoculated associated to 60% of concentrate remained greater idle time compared to the animals that received control silage and the 40% of roughage, and compared to the young bulls that were fed with maize silage inoculated, associated with 60:40 roughage: concentrate

ratio. Although the DM intake and ADG have been changed by silages and roughage: concentrate ratio, there was not effect on the feed: gain ratio. The overall average of feed: gain ratio observed was 5.99 kg: 1.00 kg of body weight.

Conclusions Young bulls fed with maize silage inoculated present higher dry matter intake and average daily gain. The diet containing 60% of concentrate results in higher dry matter intake. The maize silage inoculated with *Lactobacillus buchneri* associated to roughage: concentrate ratio of 40:60 results in higher dry matter intake, lower ingestion time, and increasing the average daily gain.

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Table 1. Performance and ingestive behaviour of Nellore young bulls fed with maize silage inoculated or not with *L. buchneri* associate to two roughage: concentrate ratio.

Item	Silage (S)		R:C[1]		SEM	P-value		
	Control	*L. buchneri*	60:40	40:60		S	R:C	S×R:C
Dry matter intake, kg/d	8.42	8.98	8.40	9.00	0.199	0.0002	<0.0001	0.0007
Average daily gain, kg/d	1.40	1.51	1.42	1.49	0.203	0.0318	0.1593	0.0005
Feeding, minutes	177.14	174.79	189.07	162.86	0.199	0.0024	0.7552	0.0024
Rumination, minutes	136.43	129.28	138.93	126.78	0.198	0.1971	0.4410	0.0644
Idle, minutes	410.00	413.86	398.93	424.93	0.198	0.0976	0.7984	0.0177
Feed: gain ratio	6.06	5.84	5.79	6.11	0.197	0.2139	0.0844	0.1402

[1]Roughage: concentrate ratio.

Table 2. Deployment of the interaction between silages and roughage: concentrate ratio to dry matter intake, average daily gain and feeding time.

Silage (S)	Control		*L. buchneri*	
Roughage: concentrate ratio (R:C)	60:40	40:60	60:40	40:60
Dry matter intake, kg/d	8.36	8.47	8.43	9.52
Average daily gain, kg/d	1.46	1.34	1.38	1.63
Feeding time, minutes	177.14	177.14	201.00	148.57

Can volatile compounds from sugarcane silage alter the digestion pattern?

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Keywords: beef steer, ethanol, intake, volatile organic compounds

Introduction Ensiling sugarcane leads to the conversion of water soluble carbohydrates to fermentation end-products, which are well characterized by high levels of volatile organic compounds, such as ethanol. The effects of silage fermentation products on ingestion and digestion had been previously studied for maize (Phillip et al., 1980) and sorghum (Senel & Owen, 1966). Nevertheless, there is a poor understanding if these chemical compounds might affect the voluntary feed intake and the digestion process of animals fed sugarcane silage. The objective of this trial was to determine whether the volatile fraction from sugarcane silage and forage proportion would affect dry matter intake and digestion in cattle.

Material and methods Six rumen-cannulated Nellore steers were randomly assigned to a replicated 3x3 Latin square design with 14-d period. Steers were housed in a tie-stall barn, and individually fed ad libitum daily at 0800 h. Dietary treatments were balanced to reach isonitrogen content: 75D – 75% sugarcane silage without volatile fraction (dried at 60°C and re-hydrated) and 25% concentrate, 75W – 75% wet sugarcane silage and 25% concentrate, and 40W – 40% wet sugarcane silage and 60% concentrate (DM basis). Voluntary feed intake was recorded from d 11 to d 14 by the difference between the amount of offered and refused feeds. The DM contents were determined by toluene distillation (Dewar and McDonald, 1961). To determine in situ DM degradation, samples of sugarcane silage were dried in a forced air oven (at 60°C), ground at 5 mm, and sealed in dacron bags, in triplicate. On d 11 of each period, at feeding time, all bags were positioned into the ventral sac of the rumen during 24 h. Urine samples were collected on d 12, four to six hours after feeding, and concentrations of allantoin, uric acid, and creatinine were determined by liquid chromatography. The (allantoin+uric acid)/creatinine ratio was considered as marker of microbial protein synthesis. On d 13, rumen fluid was collected every two hours for 24 hours to determine pH and redox potential (E_h). For that, anaerobic atmosphere was kept by nitrogen gas. As the redox was measured with a platinum electrode (E_0) values were corrected by the equation (Marden et al., 2005): $E_h = E_0 + 199$ mV. Aliquots of ruminal fluid were immediately frozen in liquid nitrogen and stored at -20°C for determination of VFA and NH_3. On d 14 of each period, rumen was evacuated and washed with saline solution (NaCl 0.9%, at 37°C). Fifteen liter of solution containing CrEDTA, 100 mL of valeric acid, and 65 mL of ethanol (pH = 6.5) was infused and sampled at 0, 0.5, 1, 1.5, and 2 hours after infusion. Rumen content was weighed and samples were dried in an oven at 60°C for determination of dry matter and indigestible NDF contents. Valerate was determined by gas chromatography, Cr concentration by ICP-plasma and ethanol by enzymatic method (Sigma procedure No 332 – UV). Fractional absorption rate of valerate and ethanol were determined by the decay rates of valerate/Cr and ethanol/Cr ratios over time. Turnover rate of rumen DM (%/h) was calculated as (Voelker and Allen, 2003): 100*(intake of DM/ruminal pool of DM)/24. Passage rate of solids was estimated as (Krizsan et al., 2010): kp (%/h) = 100*(intake of iNDF/ruminal pool of iNDF)/24. Apparent digestibility of nutrients in total tract (%) was determined from d 11 to d 13 by total feces collecting and used to calculating diet TDN. Data were analyzed using the Mixed procedure of SAS including effects of Latin square, animal nested within Latin square, period, and treatment. Orthogonal contrasts were used for specific comparisons: 75D versus 75W to test the volatile fraction effect, and 75W vs 40W to test the forage:concentrate ratio effect.

Results and discussion About 21% of dry matter of sugarcane silage consisted of volatile compounds. The fate of these compounds did not alter the dry matter intake but increased ruminal acetate/propionate ratio and the fractional absorption rates of valerate and ethanol. The lower forage content of 40W treatment led to a higher dry matter intake and, in turn, changed most of the rumen parameters and digestibility traditionally associated with high concentrate diets. The higher ruminal turnover rate of DM observed for the higher 40W was caused by the higher degradation rate of DM, whereas passage rate was similar across treatments. Each percentage unit of sugarcane silage replaced with concentrate resulted in an increase in diet TDN of 0.16. When the volatile compounds were taking into account, diet TDN values were 1.1 to 3.8 percentage units higher than that generated by the traditional oven dried samples.

Conclusions The volatile fraction did not alter dry matter intake and digestion process of the animal, but represented an important contribution to the energy content of sugarcane silage. Decreasing of forage to concentrate ratio is a tool to promote nutrient intake of sugarcane silage based diets.

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Table 1. Intake and digestion of steers fed sugarcane silage.

	Treatments[1]				75D vs	75W vs
Item	75D	75W	40W	SE[2]	75W	40W
DM intake, kg/d	6.54	6.39	9.96	0.41	0.71	<0.01
Rumen						
pH	6.65	6.71	6.33	0.07	0.29	<0.01
Redox, mV	-97.88	-96.72	-64.17	12.28	0.88	<0.01
NH_3, mg/dL	9.25	9.67	12.84	0.79	0.71	<0.01
VFA, mM	81.40	80.38	94.03	4.40	0.57	<0.01
Acetate/Propionate	3.10	3.74	3.84	0.16	<0.01	0.43
Valerate absorption, %/h	36.68	55.16	56.38	6.83	<0.01	0.78
Ethanol absorption, %/h	115.21	208.75	157.70	28.94	0.02	0.13
Turnover rate of DM_{oven}[3] in vivo, %/h	4.61	5.46	7.82	0.64	0.14	<0.01
Passage rate of DM_{oven} in vivo, %/h	2.84	3.03	2.84	0.42	0.48	0.44
Degradation of DM_{oven} in vivo, %	42.97	42.82	64.87	1.77	0.91	<0.01
Degradation of silage DM_{oven} in situ, %/24h	40.05	39.27	36.96	1.37	0.67	0.21
Urine						
(Allantoin+uric acid)/creatinine	0.77	0.86	1.20	0.16	0.44	0.04
Total tract						
NDF digestibility, %	60.08	57.31	54.40	2.22	0.42	0.37
Diet TDN_{oven}[4], %	71.94	69.99	77.41	1.45	0.36	<0.01
Diet TDN[5], %	73.08	73.74	78.96	1.37	0.74	0.02

[1]75D: 75% sugarcane silage without volatile fraction and 25% concentrate, 75W: 75% wet sugarcane silage and 25% concentrate, and 40W: 40% wet sugarcane silage and 60% concentrate (DM basis).
[2]SE: standard error of the mean.
[3]DM_{oven}: dry matter determined in a forced air oven.
[4]TDN_{oven}: total digestible nutrients excluding volatile compounds.
[5]TDN: total digestible nutrients including volatile compounds.

Effect of forage silage species and beef sire breed on steer performance, carcass and meat quality using a forage-based beef production system

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Keywords: Beef, breed, red clover, silage, timothy.

Introduction Production of hormone- and antibiotic-free, forage finished beef appeals to consumers on the issues of animal welfare and the environment. Past studies in northern Ontario (Berthiaume et al., 2009a; 2009b) found that grass silage can be used to produce 400 kg body weight beef cattle at 365 days of age without feeding grain or using growth promotants. However, these beef production systems need to be designed to ensure acceptable levels of productivity and financial returns for the producer, while ensuring high quality beef for consumers. Our objectives were to determine the effect of forage silage (red clover-grass mixture vs. grass) and beef cattle sire breed (Angus vs. Simmental) on yearling performance, carcass and meat quality.

Material and methods The experimental work was conducted at the Kapuskasing Beef Research Farm Canada in 2010. A total of 40 crossbred steer calves [20 Simmental cross (SM) and 20 Angus cross (AN)] were used. The 40 animals were then assigned to ten blocks based on breed type and date of birth. The two forage silages (red clover-grass mixture vs. grass) were fed to AN and SM cross steer calves, with equal numbers of calves for each forage silage/breed subclass. The silages were fed *ad libitum* from 240 to 365 days of age. Both silages were made from the primary growth. The red clover-grass silage (RCS) contained a mixture of red clover (*Trifolium repens*), timothy (*Phleum pratense*) and weeds (60:30:10), whereas the grass silage (GS) contained a mixture of tall fescue (*Festuca arundinacea*) and weeds (70:30). The RCS was harvested at 28.9% DM and treated with 4 L formic acid (85%) tonne^{-1} whereas the GS was harvested at 29.5% DM and treated with 2 L formic acid tonne^{-1}. Steers were slaughtered according to industry procedures with the carcasses federally graded. Meat quality was measured according to standard methods. The data were statistically analyzed as a 2 x 2 factorial arrangement within a randomized complete block design to evaluate the main effects of forage silage type (RCS vs GS) and breed (SM vs AN) and the interaction between silage type and breed.

Results and discussion Both silages produced were of good nutritional quality and well conserved (pH < 4.2; ammonia concentrations < 7.3% of total nitrogen content). There were no silage by breed interactions for any growth performance, carcass or meat quality trait. Feed intakes were not affected ($P \leq 0.13$) by forage silage or breed (Table 1). However, average daily gain (ADG) was greater ($P = 0.0002$) in steers fed RCS as compared to GS (Table 1). This may be related to the higher crude protein content of RCS (13.8%) versus GS (11.7%), and lower soluble nitrogen of RCS (46% vs. 53%) as compared to GS. ADG was similar ($P > 0.49$) between breeds. The gain:feed ratio was significantly lower ($P < 0.0001$) for steers fed GS as compared to cattle fed RCS, but cattle breed did not affect ($P=0.32$) gain:feed ratio (Table 1).

Hot carcass weights tended ($P= 0.07$) to be affected by the forage silage fed (Table 1). The tendency for heavier carcass weights with RCS- vs. GS-fed cattle is most likely due to faster growth rates in cattle fed RCS, as initial body weights were similar for cattle allocated to both forage silages (Table 1). Hot carcass weights for SM also tended ($P= 0.07$) to be heavier than AN. While these results are not surprising given the literature comparing British vs. Continental cattle, the differences observed in the present study are due to heavier body weights at the start of the trial for SM vs. AN sired cattle (Table 1). One of the concerns with forage finishing is the risk for production of inferior carcasses based on the low energy values for forages relative to feed grains, which may limit fat deposition that can impact beef quality. The Canadian Beef Grading Agency (CBGA 2010) requires a minimum of two mm backfat for individual beef carcasses to be classified as high quality beef. In this trial, only two out of the 40 animals were downgraded as their carcasses had less than the required two mm backfat. There were no differences ($P= 0.10$) in backfat tickness between the two forage silages, whereas backfat deposition was greater ($P = 0.02$) in AN versus SM sired cattle (Table 1). CBGA graders assigned a higher ($P= 0.01$) quality grade to AN carcasses which is supported by higher ($P = 0.03$) marbling scores and greater ($P= 0.002$) intramuscular fat (IMF) values for AN vs. SM carcasses. Forage silage did not have any effect ($P > 0.05$) on marbling, quality grade and IMF content (Table 1).

The pH values for *longissimus* muscle were similar ($P \geq 0.20$) across both forage silages and

sire breeds (Table 1). Hue values were lower (P ≤ 0.05) for GS vs. RCS, and SM vs. AN. Lower values for hue are associated with a redder product that customers often prefer. Shear force values for grilled *longissimus* muscle steaks were lower (P < 0.0001) in beef steers fed RCS vs. steers fed GS, whereas AN beef was more tender (lower shear force values) (P= 0.0003) than SM beef (Table 1).

Conclusions Forage finishing can be used to produce high quality yearling beef for niche markets. However, forage silage and breed differences in tenderness were found in this study.

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Table 1. Effects of forage silage species and breed [Angus (AN) vs. Simmental (SM)] on steer performance, carcass and meat quality.

	Forage Silage				Breed			
	Red clover (RCS)	Grass (GS)	SEM	P-value	AN breed	SM breed	SEM	P-value
Performance traits								
Initial weight (kg)	338	344	6.2	0.53	332	350	6.2	0.05
Final weight (kg)	465	450	4.2	0.02	451	464	4.2	0.03
Dry matter intake								
Total intake (kg d^{-1})	7.91	8.30	0.251	0.28	8.09	8.11	0.251	0.96
g kg^{-1} body weight	19.7	21.0	0.59	0.13	20.6	20.0	0.59	0.44
Average daily gain (kg)	1.08	0.92	0.034	0.0002	1.01	0.99	0.034	0.49
Gain:Feed	0.14	0.11	0.004	< 0.0001	0.13	0.12	0.004	0.32
Carcass traits								
Hot carcass weight (kg)	231.8	225.3	2.47	0.07	225.3	231.8	2.47	0.07
Backfat, mm[z]	2.6	3.2	0.26	0.10	3.4	2.4	0.26	0.02
Marbling[y]	4.28	4.43	0.170	0.54	4.63	4.08	0.170	0.03
Quality grade[x]	1.75	1.90	0.119	0.38	2.05	1.60	0.119	0.01
Intramuscular fat (IMF) content (%)	2.64	2.61	0.115	0.88	2.89	2.35	0.115	0.002
Meat traits of *Longissimus dorsi*								
pH	5.50	5.52	0.011	0.22	5.50	5.52	0.011	0.20
Hue	17.0	15.9	0.39	0.05	17.3	15.6	0.39	0.002
Shear force (kg)	4.47	5.46	0.137	< 0.0001	4.61	5.32	0.137	0.0003

[z] Grade fat is the minimum depth of subcutaneous fat in the last quadrant over the *longissimus* muscle at the $12^{th}/13^{th}$ rib interface.

[y] Marbling was assessed subjectively using a 10 point scale (1 = devoid, 2 = practically devoid, 3 = traces, 4 = slight, 5 = small, 6 = modest, 7 = moderate, 8 = slightly abundant, 9 = moderately abundant, 10 = abundant).

[x] Quality Grade data were coded before statistical analysis was conducted as follows: A (trace marbling) = 1, AA (slight marbling) = 2, AAA (small to moderate marbling) = 3.

Effects of concentrate level and rapeseed meal supplementation on animal performance and fatty acid composition of *Longissimus dorsi* muscle of Hereford and Charolais bulls offered grass silage-barley -based rations

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Keywords: beef production, bulls, concentrate supplementation, supplementary protein, fatty acids

Introduction In intensive beef production in Finland grass silage is typically supplemented with grain to increase the energy and nutrient intake of the growing bulls. Rapeseed meal (RSM) is the most important protein feed used in concentrates for cattle. The objectives of the present study with growing Hereford (Hf) and Charolais (Ch) bulls were to determine the effects on animal performance and fatty acid composition of *Longissimus dorsi* muscle of (1) the proportion of concentrate in the diet, and (2) the inclusion of RSM in the barley-based concentrate in total mixed ration (TMR) feeding.

Material and methods A 2×2×2 factorial design was used to study the effects of breed, concentrate level in the diet, and inclusion of RSM. Two feeding experiments comprised in total of 28 Hf-bulls and 24 Ch-bulls. The bulls were fed TMR *ad libitum*. The two concentrate proportions were 200 (L) and 500 (M) g/kg dry matter (DM), fed without RSM (RSM−) or with RSM (RSM+). Rapeseed meal was given so that the crude protein (CP) content of the concentrate was raised to 160 g/kg DM in the RSM+ diets. In the RSM− diets the CP content of the concentrate was 128 g/kg DM, so the content increased 25% with RSM supplementation. The grass silage used in the experiment included digestible organic matter 666 g/kg DM and the CP and neutral detergent fibre concentrations were 146 and 566 g/kg DM, respectively. The DM concentration of the silage was 276 g/kg and it was prepared using formic acid based additive and was well preserved (pH 4.05 and 71 g ammonia N in total N).

Dressing proportion, carcass conformation and the carcass fat score of the bulls were determined according to the EUROP classification. After slaughter, the carcasses were cooled for 24 h at 2 °C. *Longissimus dorsi* muscle (LM) samples were taken by complete cross-section between the 12th and 13th ribs. The fatty acid composition in intramuscular fat of LM was analyzed using gas chromatography method. The data was subjected to analysis of variance using the SAS general linear models procedure. The statistical model used was $y_{ijkl} = \mu + \delta_l + \alpha_i + \beta_j + \gamma_k + (\alpha\times\beta)_{ij} + (\alpha\times\gamma)_{ik} + (\beta\times\gamma)_{jk} + (\alpha\times\beta\times\gamma)_{ijk} + (\delta\times\alpha)_{li} + (\delta\times\beta)_{lj} + (\delta\times\gamma)_{lk} + e_{ijkl}$, where μ is the overall mean and e_{ijkl} is the random error term. α, β, γ and δ are the effects of breed, concentrate level, RSM supplementation and experiment, respectively. In this paper the results are presented for the main effects of breed, concentrate level and RSM supplementation.

Results and discussion Dry matter intake (kg/$W^{0.75}$) of the Hf-bulls was 5% higher than that of the Ch-bulls ($p<0.01$) but carcass gain of the Ch-bulls was 20% higher than that of the Hf-bulls ($p<0.001$) (Table 1). The carcass weight and carcass conformation score of the Ch-bulls were also higher than the corresponding values of the Hf-bulls ($p<0.001$). The carcass fat score of the Hf-bulls was 64% higher than that of the Ch-bulls ($p<0.001$). The *n*-6/*n*-3 fatty acid ratio of the LM of the Ch-bulls was 22% higher than the corresponding value of the Hf-bulls ($p<0.01$). The LM of the Hf-bulls contained a higher proportion of 10:0 ($p<0.05$) and 18:1 *cis*-9 ($p<0.001$) fatty acids compared to that of the Ch-bulls. On the contrary the LM of the Ch-bulls contained a higher proportion of 16:0 ($p<0.05$), 16:1 *cis*-9 ($p<0.001$), 18:1 *cis*-11 ($p<0.001$), 18:2 *cis*-9,*cis*-12 ($p<0.001$), 18:3 *cis*-9,*cis*-12,*cis*-15 ($p<0.001$) and 18:3 *cis*-6,*cis*-9,*cis*-12 ($p<0.001$) fatty acids compared to that of the Hf-bulls. It is possible that the differences in carcass fat score between breeds affected also to differences in fatty acid composition of LM because according to de Smet et al. (2004) carcass fat score affect to meat fatty acid profile.

Consistently with Huuskonen et al. (2007) increasing the level of concentrate led to an improvement of carcass gain ($p<0.001$) (Table 1). The carcass weight was higher with the increased concentrate level ($p<0.05$) and carcass conformation score of the M-bulls was 16% higher than the corresponding value of the L-bulls ($p<0.001$). However, there was no significant effect of concentrate level on the carcass fat score. The *n*-6/*n*-3 fatty acid ratio of the LM increased with higher concentrate level ($p<0.001$). The increasing concentrate level decreased significantly the relative proportion of 15:0 ($p<0.001$), 17:0 ($p<0.001$), 18:1 *cis*-11 ($p<0.05$) and 18:3 *cis*-9,*cis*-12,*cis*-15 ($p<0.001$) fatty acids of the LM and in-

creased the relative proportion of 18:1 *cis*-9 ($p<0.05$) and 18:2 *cis*-9,*cis*-12 ($p<0.01$) fatty acids of the LM. These results are mainly in accordance with Daley et al. (2010) who concluded that the increasing concentrate level generally increases the *n*-6/*n*-3 fatty acid ratio and decreases 18:3 *cis*-9,*cis*-12,*cis*-15 fatty acid proportion of the muscle.

The RSM supplement had no effects on the DM intake or carcass gain of the bulls which is consistent with earlier studies with growing bulls (e.g. Huuskonen et al. 2007). Furthermore, there were no effects of RSM supplementation on carcass weight, carcass conformation score, carcass fat score or *n*-6/*n*-3 fatty acid ratio of the LM. Rapeseed meal supplementation decreased significantly the relative proportion of 14:0 ($p<0.05$), 16:0 ($p<0.001$) and 16:1 *cis*-9 ($p<0.05$) fatty acids of the LM.

Conclusions In conclusion, the carcass gain of the bulls increased with increasing concentrate level and increasing the concentrate allowance also improved carcass conformation. Rapeseed meal did not affect animal performance. According to this study, the choice of breed and feeding can affect intramuscular fat composition.

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Table 1. Effects of breed (B), concentrate level (C) and rapeseed meal supplementation (R) on feed dry matter intake (DMI), carcass gain (g/d), carcass characteristics and fatty acid composition of *Longissimus dorsi* muscle of growing beef bulls.

	B		C		R		SEM	Statistical significance						
	HF	CH	200	500	-	+		B	C	R	B×C	B×R	C×R	B×C×R
Duration, d	385	347	382	350	364	368	24.3	**	*					
Intake														
DMI, kg/d	8.9	9.1	8.6	9.4	9.0	9.0	0.34		***		*			
DMI, kg/W$^{0.75}$	86	82	82	87	85	84	2.1	**	***					
Energy, MJ/d	103	105	97	112	104	104	4.1		***		*			
Live weight, kg														
Initial	253	298	274	276	276	274	10.0	***						
Final	725	763	730	759	737	751	11.1	*	o		o			
Carcass gain	693	831	703	822	751	774	42.0	***	***					
Carcass measurements														
Weight, kg	389	431	400	420	405	415	6.9	***	*		o			
Dressing, g/kg	536	564	548	553	549	552	2.8	***	o					
Conformation[1]	6.2	8.5	6.8	7.9	7.5	7.2	0.19	***	***		**			
Fat score [2]	4.6	2.8	3.6	3.8	3.6	3.8	0.12	***						
Fatty acid composition of *Longissimus dorsi* muscle, % of total fatty acids														
14:0	2.9	3.0	3.0	2.9	3.0	2.8	0.19			*				
16:0	28.8	29.7	29.5	29.1	30.0	28.5	0.70	*		***				
16:1 *cis*-9	3.5	3.9	3.7	3.7	3.8	3.6	0.21	***		*				
17:0	1.0	1.0	1.0	0.9	1.0	1.0	0.06		***					
18:0	18.1	17.4	18.1	17.5	17.3	18.2	0.90			o				o
18:1 *cis*-11	2.0	2.3	2.3	2.0	2.1	2.2	0.16	***	*		*	*		
18:1 *cis*-9	38.6	35.6	36.4	38.1	36.8	37.6	1.13	***	*					
18:2 [3]	1.5	2.0	1.6	1.9	1.7	1.8	0.16	***	**			o	*	*
18:3 [4]	0.6	0.7	0.8	0.5	0.6	0.7	0.08	***	***					
18:3 [5]	0.5	0.9	0.7	0.7	0.6	0.7	0.11	***				o	**	*
SFA[6]	51.4	52.4	52.9	50.9	52.0	51.8	1.77		o					
MUFA[7]	45.4	42.9	43.0	45.2	44.2	44.1	1.69	**	o					
PUFA[8]	3.2	4.7	4.0	3.9	3.9	4.1	0.51	***						*
n-6/*n*-3	3.7	4.5	3.2	5.0	4.1	4.1	0.56	**	***					

[1] Conformation: (1=poorest, 15=excellent). [2] Fat score: (1=leanest, 5=fattest). [3] 18:2 *cis*-9, *cis*-12. [4] 18:3 *cis*-9, *cis*-12, *cis*-15. [5] 18:3 *cis*-6, *cis*-9, *cis*-12. [6] Saturated fatty acids. [7] Monounsaturated fatty acids. [8] Polyunsaturated fatty acids.

A comparison of feeding whole crop barley mixed with Italian ryegrass silage versus tall fescue hay for Holstein growing cattle

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Keywords: Italian ryegrass/barley silage, tall fescue hay, growing cattle, body gain

Introduction Korea imports forage, timothy, tall fescue (TF) and alfalfa hay, from foreign countries such as America, Canada and etc. The cost of imported forage is not cheap, compared to rye silage which is domestic silage (Seo and Yook 2002). Also, the quality, quantity and cost of imported forage are unstable (Sung 2000). Since 2000s, as the price of imported forage increased due to international oil price rise, the interest in production of domestic forage has had a high profile. Moreover, the production of domestic forage is important in aspects of contributing to environment-friendly livestock industry by the efficient use of animal manure and high quality and safe livestock products. In terms of efficient utilization of land, growing whole crop annual forages using rice paddies is the most practical way to secure domestic forage. Forage production in paddy fields has been promoted since the early 1990s, using whole crop barley and Italian ryegrass (IRG) silage in southern part of Korea. Particularly, whole crop barley and IRG may possibly be fed to Holstein growing cattle since it shares similar nutritional value with Timothy and TF hay in crude protein, NDF and TDN contents. Therefore, this study examines the effect of feeding domestic whole crop barley and IRG (BIRG) silage instead of imported TF hay on feed intake and daily body gain of Holstein growing cattle.

Material and methods The experiment was carried out at Naju during June and November, 2011 in Republic of Korea. Fifty two female Holstein growing cattle (av. BW 218±78kg), based on completely randomized design, were assigned to two treatments, TF HAY group fed with *ad-libitum* feeding of TF hay and 4kg/d (as-fed) of concentrate, BIRG SILAGE group with 7kg/d of BIRG silage and 3kg/d of concentrate. TF hay was imported from abroad in the form of first cut hay. Whole crop barley and IRG were seed-mixed in autumn after rice-harvest season, harvested in late heading stage and dispensed in round bale BIRG silage (moisture content 57%) after wilting process. Data were analyzed using ANOVA procedure of SAS (SAS Institute, 1999) and the means were compared for significance by Duncan multiple range test at $p<0.05$. The pH of BIRG silage was 5.15 and was in prime condition for fermentation (Table 1). The content of crude protein and NDF between TF hay and BIRG silage had no significant difference, whereas the content of TDN was higher in BIRG silage. The animals were housed in sheltered drylot facilities with *ad libitum* access to water. Body weight measured at the beginning, midterm and end of experiment. Chemical composition of feed measured by AOAC (1990) procedure.

Results and discussion The forage:concentrate ratio of the TF HAY and BIRG SILAGE group was 52:48 and 60:40 respectively, the BIRG SILAGE group being higher in the forage. No significant difference was shown in forage dry matter intake (kg/d) between the TF HAY and BIRG SILAGE group, while concentrate dry matter intake (kg/d) was 0.83kg higher in the TF HAY group (table 1). Crude protein and TDN intake of concentrate was higher in the TF HAY group than the BIRG SILAGE group ($p<0.05$). However, both CP and TDN intake satisfied the nutritional requirement for maintenance and growth of growing cattle (NIAS 2007). These results are in accordance with those of Mir et al. (1993), reporting that the nutritional requirements for optimum performance of growing steers could be met by feeding intercropped barley/annual ryegrass with minimum amounts of barley grain. Average daily body gain was significantly higher ($p<0.05$) in the BIRG SILAGE group compare to the TF HAY group, although dry matter intake was lower in the BIRG SILAGE group. Further research efforts need to be also focused on basic rumen parameters and digestibility of nutrients for the two forage type in Holstein growing cattle.

Conclusions The results of this study suggest that reduction of daily gain was not shown in Holstein growing cattle fed with domestic whole crop barley and Italian ryegrass silage instead of imported tall fescue hay, saving partial amount of concentrate.

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Table 1. Chemical composition of feed, feed intake and average daily body gain of Holstein growing cattle fed with whole crop barley and Italian rye grass silage versus tall fescue hay based diets.

	BIRG[1)] SILAGE group		TF[2)] HAY group	
	Silage	Concentrate	Hay	Concentrate
Forage/concentrate rate (%)	60.2	39.8	52.2	47.8
Chemical composition of feed				
DM (%)	43.0	90.7	88.1	89.1
CP (DM,%)	9.5	16.4	9.0	18.0
NDF (DM,%)	73.7	32.3	79.0	35.9
TDN (DM,%)	55.2	70.0	50.9	70.0
BIRG Silage pH	5.15	-	-	-
Feed intake (kg/d, DM)	4.0±1.1	2.6[b]	3.9±3.3	3.6[a]
Crude protein intake	0.38	0.43[b]	0.35	0.64[a]
TDN intake	2.20	1.85[b]	1.98	2.52[a]
Body gain (kg/d)	0.8±0.3[a]		0.6±0.2[b]	

[1)] Italian ryegrass silage mixed with whole crop barley silage
[2)] Tall fescue hay
[ab)] Values with different superscripts in the same row differs significantly ($p<0.05$)
[±)] Means ± standard deviation where n = 26

Grass silage can replace concentrate feeds in dairy bull fattening

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Keywords: beef production, carcass quality, forage, meat quality, concentrate supplementation

Introduction Good quality silage can support high levels of performance of growing cattle with moderate or even with no concentrate supplementation (Randby et al. 2010), but high levels of concentrates are typically used in intensive beef production. With good quality (high digestibility and preservation quality) silage, marginal responses to increased concentrate supplementation in animal performance decline. In this work the effects of concentrate level combined with *ad libitum* silage feeding on performance and meat quality of dairy bulls were studied.

Material and methods Fifty-three Finnish Ayrshire bulls were used in a 2×3 factorial arrangement of dietary treatments. The concentrate was given at low (L) and high (H) levels, and the respective concentrate dry matter (DM) allocations were 47 or 89 g/kg live weight (LW)$^{0.60}$, respectively. The other factor was periodic allocation of concentrate, i.e. steady, increasing or decreasing amount of concentrate during the growing period, but these results are not presented. The total amount of concentrate consumed within the concentrate level during the whole experimental period was same for the concentrate allocation treatments. Grass silage was offered *ad libitum*. The silage was prepared from timothy-meadow fescue sward, slightly pre-wilted and ensiled with a formic acid based additive. The concentrate used was rolled barley.

Total urine collection was conducted using 24 bulls to estimate microbial protein synthesis in the rumen based on urinary excretion of purine derivatives (Chen and Gomes, 1992). During the urine collection, the bulls received four different concentrate levels, which were proportionally 0, 0.203, 0.402 and 0.573 of total DM intake (DMI).

Bulls were taken into the experiment at an average age of 82 (s.e. 1.0) days and an average LW of 94 (s.e. 1.9) kg. Bulls were slaughtered when they reached the target LW of 550 kg. The carcasses were classified for conformation and fatness using the EUROP quality classification. The meat quality was measured from *Musculus longissimus dorsi.* It included objectively measured pH, colour, drip loss, length of sarcomeres, tenderness and chemical composition including DM, crude protein (CP) and HCl-fat, and sensory assessment including tenderness, succulence and taste.

The experiment was set up according to a complete randomized block design with animal as an experimental unit. The results were subjected to analysis of variance using SAS general linear models procedure. The concentrate feeding regime imposed at the moment of microbial protein synthesis measurements was evaluated using polynomial contrasts. When the dressing proportion, carcass conformation and fat score were tested, slaughter group (n=6) was included in the model, and warm carcass weight was used as a covariate.

Results The silage quality was good both in terms of feed values and preservation quality (pH 4.25, DM 287 g/kg, 127 g CP, 555 g NDF, 45 g lactic acid, 24.9 g volatile fatty acids, 95 g water soluble carbohydrates and 10.8 MJ metabolizable energy (ME) per kg DM, and 47 g ammonia N per kg N). The barley had typical chemical composition and feed values (13.0 MJ ME and 119 g CP per kg DM).

Decreased concentrate intake increased silage intake ($P<0.01$) but the total DMI decreased from 6.76 to 6.50 kg/d ($P<0.05$). The substitution rate (decrease in silage DMI / increase of concentrate DMI) was 0.81. There was no effect of the level of concentrate on the efficiency of microbial protein synthesis in the rumen.

When concentrate allowance decreased from 2.88 to 1.52 kg DM/d, LW gain (LWG) decreased from 1158 to 1059 g/d ($P<0.01$). Growth response to 1 kg additional concentrate DMI was 73 g/d. The LWG of the bulls consuming silage alone diet in the early part of growing period (up to 360 kg LW) was as high as 1016 g/d. Increased concentrate level tended to decrease DMI per kg LWG ($P<0.10$) because of higher energy density of the diet, but it did not affect ME consumption per kg LWG. Decreasing level of concentrate increased the number of growing days by 29 days ($P<0.01$) because of slower growth rate of the bulls.

Decreased concentrate level decreased carcass weight by 10 kg ($P<0.01$) but it did not affect dressing proportion, carcass conformation or fat score. Decreased concentrate level decreased fat ($P<0.05$) and tended to decrease DM ($P<0.10$) concentrations of *Musculus longissimus dorsi*, but there were no other effects on meat quality.

Discussion It is well established that increasing concentrate allowance decreases silage intake and increases DM and ME intakes (Steen and Kilpatrick 2000). It may also lead to only limited or no increases in total DMI and subsequently ME intake (Steen et al. 2002). Substitution rate usually increases with increasing silage digestibility (Randby et al. 2010). In the current experiment, digestibility and fermentation quality of grass silage was good and as a consequence substitution rate was rather high, 0.81.

The current increase in growth of 73 g/d per 1 kg increase in concentrate DMI is consistent with other experiments (e.g. Martinsson 1990) although sometimes responses have been even smaller showing that responses to concentrate feeding can be rather limited. With good quality silage, a reasonable LWG can be achieved even when the silage is given as a sole feed (Randby et al. 2010). Increasing allowance of concentrate tended to improve feed DM conversion rate but there was no effect in ME conversion rate. This result shows that silage can successfully replace concentrate in growing bull rations. In contrary to many other experiments, increasing the allowance of concentrate did not increase carcass fat score or carcass conformation.

In the present experiment concentrate level did not affect eating quality of meat, although meat fat concentration increased. Fatness has been connected with a better eating quality of meat, but in this experiment all bulls were low-fat, which may explain the lack of effect.

Conclusions Good quality grass silage can replace concentrate feed without decreasing feed energy conversion rate or carcass and meat quality. However, when concentrate intake decreases, growth rate decreases and the length of growing period increases.

Table 1. Effects of concentrate level on feed intake, performance and meat quality of growing bulls.

Level of concentrate:	Low	High	SEM[1)]	P-value
Number of observations	27	26		
Dry matter (DM) intake (kg/day)				
Concentrate	1.52	2.88		
Silage	4.87	3.77	0.077	<0.01
Total	6.50	6.76	0.079	0.02
Barley intake (DM during whole experiment)	661	1165		
Silage intake (DM during whole experiment)	2105	1520	32.8	<0.01
Metabolizable energy intake (MJ/day)	73.9	79.6	0.90	<0.01
Live weight gain (g/day)	1059	1158	22.7	<0.01
Growing days	434	405	7.5	<0.01
Kg DM/kg weight gain	6.17	5.89	0.098	0.05
MJ metabolizable energy/kg weight gain	70.1	69.4	1.13	0.66
Warm carcass weight (kg)	285	295	2.7	0.01
Dressing proportion (g/kg)	519	525	2.4	0.13
EUROP conformation (scale 1-11)	3.86	4.16	0.143	0.16
EUROP fat score (scale 1-5)	2.05	2.30	0.100	0.11
Meat quality (*M. longissimus dorsi*)				
pH	5.53	5.57	0.018	0.16
DM (g/kg)	266	274	2.9	0.06
Crude fat (g/kg DM)	43.4	53.1	2.9	0.03
Sensory assessment (scale 1-7)				
Tenderness	4.71	4.70	0.151	0.95
Succulence	4.86	4.99	0.086	0.30
Taste	4.94	4.92	0.090	0.89

[1)]SEM = standard error of the mean.

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Serum biochemical profile of sheep fed olive-pulp silage for extended period

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Keywords: olive pulp silage, serum biochemistry, sheep

Introduction Invaluable studies carried out in the recent years have indicated the potential use of olive by-products (e.g. olive-pulp silage; OPS) as an animal feed. However, the performance characteristics may be adversely affected by antinutritional compounds, including tannins (Makkar 2003). Despite numerous studies on the mostly various nutritional effects of short-term feeding of olive by-products in sheep, longer term feeding trials, especially in breeding animals, are scarce. On the other hand, in many experiments, olive by-products did not constitute a significant proportion of the daily ration, which may have masked any detrimental effect of anti-nutritional factors in this by-product. Therefore, the present experiment was conducted to determine any deleterious effect of feeding 70% OPS in the daily ration for 120 days on serum biochemical profile of rams.

Material and methods Sixteen rams of two fat-tailed breeds (Ghezel and Mehraban) were allocated to two diets (four rams per breed per diet), consisting of 700 g/kg corn silage or 700 g/kg OPS in addition to 300 g/kg Lucerne hay. Blood samples were collected by jugular venipuncture on days 0, 60, 120 and one month after the experimental diets were replaced with the pre-experimental ration. After the centrifugation, the serum samples were analyzed for glucose, blood urea nitrogen (BUN), creatinine, globulin, albumin, total protein, alanine aminotransferase (ALT), alkaline phosphatase (ALP), calcium, phosphorus, and iron, by spectrophotometric analysis, using Cobas Mira Chemistry Analyzer (Roche, Germany). Experimental data were subjected to the Proc Mixed (SAS 2002) for repeated measure data after data transformation where appropriate. Body weight was included as a covariate for analysis of variance and the treatment means were compared by the least squares means adjusted for the Tukey's test.

Results and discussion The diet containing OPS resulted in lower serum total protein, ALT, calcium and phosphorus levels and higher glucose concentration compared to the corn silage diet (Table 1). The type of silage fed did not affect the serum levels of BUN, creatinine, and ALP. Time of sampling significantly influenced the values for BUN, creatinine, ALT, phosphorus, calcium and iron. The phosphorus concentrations of Ghezel rams were significantly higher than those of Mehraban rams. However, the effect of time of sampling on BUN and ALT was significant. The interactions between diet and breed, breed and time, as well as breed, time and diet, were not significant, except for diet by time interaction, which significantly affected the serum creatinine levels.

The lower serum levels of total protein might be due to lower degradability of crude protein in olive by-products or a decrease in ruminal microbial activity. Decreases in calcium and phosphorus levels could be a consequence of lower nutrient availability due to low digestibility and/or an imbalanced mineral intake. In addition, hypoproteinemia, as was observed in our work, might result in lower serum levels of calcium, as protein-bound form of calcium constitutes 40 to 50 percent of total plasma calcium (Coles 1986). Serum levels of ALT were significantly, of course within physiological range, decreased according to which no dysfunction in hepatic activity would be occurred. Serum levels of glucose in rams fed OPS were increased compared to those fed on corn silage, indicating that OPS was not a feed of inferior nutritional value in this respect. Increased glucose levels might be due to high oil content in olive-pulp, thus sparing the blood glucose. A diet × time interaction was found for creatinine level in our study, whereby creatinine levels decreased as the duration of OPS feeding increased. Blood creatinine level is normally constant and is not influenced by the diet. A decrease in muscle mass accompanied by a decrease in metabolism of creatine and phosphocreatine would result in decreased level of blood creatinine (Coles 1986). The precise cause of decreased creatinine level in the present work is not known, as body weight of rams remained almost constant during the experiment.

Conclusions The results of the present study along with those indicating no adverse effect on the seminal characteristics (unpublished data) led us to conclude that a dietcontaining 700 g/kg OPS and 300g/kg Lucerne hay did not have a deleterious effect on blood biochemical and reproductive parameters of mature rams when fed up to 120 days. Being a cheap source of by-product feedstuff, olive-pulp can be an economical feed for mature rams for extended periods.

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Table 1. Least square means of serum biochemical attributes in Ghezel and Mehraban rams fed olive-pulp (OPS) or corn silage for 120 days.

Trait	Diet		Breed			Level of significance[1]		
	OPS	Corn silage	Ghezel	Mehraban	SE	Diet	Breed	Time
Glucose (mg/dL)	68.0	61.4	63.4	65.9	1.50	**	NS	NS
BUN (mg/dL)	16.1	15.4	16.0	15.5	0.77	NS	NS	***
Creatinine (mg/dL)	1.27	1.35	1.35	1.30	0.06	NS	NS	***
Globulin (g/dL)	3.90	4.26	3.92	4.20	0.20	NS	NS	NS
Albumin (g/dL)	3.79	3.69	3.88	3.67	0.10	NS	NS	*
Total protein (g/dL)	7.69	8.23	7.87	8.07	0.09	***	NS	*
ALT (U/L)	17.2	21.1	19.5	18.8	0.90	**	NS	***
ALP (U/L)	389.3	391.1	341.2	439.2	40.6	NS	NS	NS
Calcium (mg/dL)	9.80	10.20	10.06	10.02	0.11	*	NS	NS
Phosphorus (mg/dL)	6.32	7.36	7.24	6.44	0.17	***	**	**
Iron (µg/dL)	200.0	196.0	200.20	195.8	11.3	NS	NS	**

[1]Interaction effects were not significant except for the effect of diet × time interaction on creatinine ($P < 0.05$). ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; BUN: Blood urea nitrogen.

Performance of lambs fed maize silage inoculated or not with *L. buchneri* and two roughage: concentrate ratio

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Keywords: average daily gain, dry matter intake, feed: gain ratio

Introduction The lactic acid bacteria, as *Lactobacillus buchneri*, are used in maize silage to improve the aerobic stability. However, these bacteria, probably may increase the fibre digestibility and improve the animal performance, because ferulate esterase could be produced and there is an interaction between the microorganisms populations of the silage and rumen. Thus, the aim of this research was to evaluate the effect of inoculation of maize silages with *Lactobacillus buchneri* associated with two roughage:concentrate ratios on dry matter intake, average daily gain and feed conversion ratio of lambs.

Material and methods The maize studied was hybrid 2B688KX (Dow Agroceres), harvested with dry matter content between 33 to 35%. Treatments evaluated were: control silage (untreated) and maize silage inoculated with *Lactobacillus buchneri* (LB) "strain NCIMB 40788" ($1x10^5$ cfu/g of forage) associated with two roughage:concentrate ratios (60:40 and 40:60). The inoculum was diluted in distilled water and sprayed on the forage during filling of silos (bunker with a capacity of 60 tons). Twenty eight non-castrated male lambs (Santa Inês x Dorper), with average initial body weight of 25 kg were used. Animals remained in adaptation for 14 days, starting the experiment after this period. Dry matter intake was measured subtracting the orts from the offered. The diets offered were composed by maize silage inoculated with or without *L. buchneri* and concentrate (soybean meal, wheat bran, maize ground grain and mineral salt). The diet was balanced to maintain daily gain of 300 g/ day (NRC 2007). Diet was offered twice a day (7 am and 5 pm 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 feed conversion was calculated. Animals were slaughtered at 38 kg weight. The data were analyzed according a randomized block design in factorial 2x2 (two silages and two roughage: concentrate) with seven replicates. The data were submitted to ANOVA and means were compared by Tukey test at 5% significance level.

Results and discussion The DM intake was not affected by the silages (DMI untreated= 1190 g/ day; LB= 1270 g/day) and by concentrate levels (60:40= 1150 g/day; 40:60= 1310 g/day) (Table 1). However, there was interaction between silages and concentrate levels ($P<0.05$). Lambs fed with 60% of untreated silage and 40% of concentrate showed lower intake than untreated (40:60) and LB silage (60:40) (Table 2). Although silages inoculated with *L. buchneri* showed higher acetic acid content, and this volatile fat acid has been reported to affect negatively the intake (Charmley 2000), in this study this fact was not observed. The average daily gain (ADG) were affected ($P<0.05$) by the inoculation of maize silages with *L. buchneri* (ADG untreated= 251 g/day; LB= 261 g/day) and by roughage: concentrate ratio (60:40= 251 g/day; 40:60= 261 g/day) (Table 1). This was in accordance with the results of Ranjit et al. (2002) who also found higher weight gain in sheep fed with maize silage treated with *L. buchneri*. The highest weight gain in lambs fed maize silage inoculated with *L. buchneri* can be explained by increase in the fibre digestibility of this silage. This fact probably was associated to the ferulate esterase production by the *L. buchneri* (Kang et al. 2009). There was an interaction between silages and roughage:concentrate ratio in the feed conversion ($P<0.0001$) (Table 2). Animals fed with 60% of untreated silage and 40% concentrate (4.45) and with 40% of silage LB and 60% concentrate (4.73) had lower feed conversion compared with other treatments (LB 60:40 = 4.87 and 40:60 untreated= 5.52).

Conclusions The inoculation of maize silage with *L. buchneri* and the concentrate levels affect the performance of lambs.

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Table 1. Dry matter intake (DMI), average daily gain (ADG) and feed conversion (FC) of lambs fed maize silage without inoculant (untreated) and inoculated with *L. buchneri* (LB) and two concentrate levels (40 and 60%).

Variables	Silages		RO:CO Ratio[1]		P value			VC
	Untreated	LB	60:40	40:60	Silages	RO:CO	interaction	(%)[2]
DMI (g/day)	1190	1270	1150	1310	0.0953	0.0024	0.0118*	9.66
ADG (g/day)	251[B]	261[A]	251[B]	261[A]	0.0249	0.0284	0.3677	4.54
FC (g intake/ADG)	4.82	4.80	4.66	4.96	0.8524	0.0038	0.0001**	4.99

*Means followed by same letter do not differ by Tukey test (P>0.05).
[1]Roughage:Concentrate Ratio. [2]Coefficient of variation (%).

Table 2. Interaction between silage and roughage:concentrate ratio to dry matter intake (DMI) and feed conversion (FC).

RO:CO Ratio[1]	DMI (g/day)		FC (g intake/ADG)	
	Silages		Silages	
	Untreated	LB	Untreated	LB
60:40	1050[bB]	1250[aA]	4,45[bB]	4,87[aA]
40:60	1330[aA]	1290[aA]	5,19[aA]	4,73[aB]

*Means followed by same letter (lowercase in the column and uppercase on the line) do not differ by Tukey test.
[1]Roughage:Concentrate Ratio.

Preference of horses for haylage ensiled with propionic acid based additive

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Keywords: aerobic stability, forage, horse, palatability, quality, wrapping

Introduction In Nordic conditions, obtaining good forage quality is very weather-dependent. Haylage, with its higher moisture content compared to dry hay, has smaller weather risk and often therefore better feed quality. However, haylage still contains enough moisture for microbial growth, especially moulds and yeasts. The use of additives can reduce the hygienic risk, but there still are many doubts for example of feed palatability. The aim of this trial was to study the effect of different application rates of propionic acid based additive on the hygienic quality and feed preference by horses of haylage with different numbers of layers of plastic wrapping.

Material and methods Primary growth of timothy-meadow fescue (*Phleum pratense* L./*Festuca pratensis* Huds.) grass was cut on June 21, prewilted for 48 hours (to dry matter (DM) content of >720 g/kg) and ensiled as haylage with different application rates (0, 3 or 9 l/tn) of propionic acid based (propionic acid 730 g/kg and ammonium propionate 210 g/kg) additive. The grass was baled in big square bales and wrapped with 6 or 12 layers of plastic with four replicates. After 141 – 158 days, the bales were opened and feed samples were taken. Conventional feed analysis, neutral detergent fibre (NDF; Van Soest et al. 1991) and water-soluble carbohydrates (WSC; Somogyi 1945) were determined. Microbiological analyses of yeasts, moulds and aerobic bacteria were determined as described by Seppälä et al. (2012). Aerobic stability was determined from triplicate samples (220 g) as described by Seppälä et al. (2012). Dry matter losses after 320 hours aerobic exposure were measured as weight change multiplied by dry matter content.

Preference of the haylages was studied with six Finnhorse mares in 'cafeteria' –trials, where horses were allowed to make a choice between three different feeds offered at the same time. The preference was determined in two periods, each consisting of five days and 14 feeding times. During the first test period, haylages with six layers of wrapping and 0, 3 or 9 l of additive were tested. On the second test period, the additive levels were the same but the bales were wrapped with 12 layers. During the experimental periods, the forages were offered three times a day for 1.5 hours. Horses had simultaneous access to all haylages (1.4 kg each) in plastic containers and the placement of the haylages was rotated for each feeding. Horses were monitored during eating and activities were registered according to a protocol which was applied with slight changes from Müller and Udén (2007). The obtained parameters were: 'first choice', total observations per feed, 'smell/taste', feed entirely eaten and feed consumption.

Statistical analysis were performed using GLM-procedure of the SAS system (SAS 2008) to test the treatment effects (level of additive, number of wrapping layers) on the characteristics of haylages. The results from the preference test are presented descriptively.

Results and discussion The DM content of the haylages varied between 675 and 742 g/kg (Table 1). The DM content was lowest in the bales without additive and highest in the bales with 9 l of additive. There were differences in the content of WSC, varying from 136 to 154 g/kg DM, and the lowest concentrations were found in bales with no additive. Adding number of the wrapping layers from 6 to 12 reduced the visual observations of surface moulds from 88 to 19 % of the bales. Microbiological analysis from the drilled feed samples detected mould counts only in some bales with very small amounts. Yeast and aerobic bacteria counts were more commonly detected. In general, increasing the application rate of additive and plastic wrapping layers reduced the yeast counts in bales. Inclusion of additive reduced aerobic bacteria counts. Increasing additive application and wrapping layers reduced DM losses during ensiling in a dose dependent manner. Aerobic stability of the haylages was improved by using the additive.

Table 1. Characteristics of experimental haylages.

Number of wrapping layers	6 layers			12 layers				Probabilities of fixed effects[a]	
Dose level, l/t	0	3	9	0	3	9	SEM	Dose level	Wrapping
Dry matter (DM), g/kg	692	716	739	675	727	742	7.4	P<0.0001	NS
Ash, g/kg DM	56.7	57.5	55.2	59.9	51.8	55.3	2.91	NS	NS
WSC[b], g/kg DM	136	155	155	145	166	154	3.8	P=0.0002	P=0.0479
Yeasts, log cfu/g[c]	6.26	4.81	2.56	4.67	3.05	<2.00	0.418	P<0.0001	P=0.0013
Moulds, log cfu/g[c]	<2.00	<2.00	2.04	< 2.00	< 2.00	2.22	0.055	P<0.0407	NS
Aerobic bacteria, log cfu/g	6.33	4.96	4.13	6.41	3.86	3.35	0.314	P<0.0001	NS
Aerobic stability, h	212	326	326	197	326	326	24.1	P<0.0001	NS
Dry matter losses, %	7.33	3.15	-0.57	8.75	1.64	-0.40	1.197	P<0.0001	NS

[a] Interaction between dose level and number of wrapping layers was not significant for any of the tested effects.
[b] Water soluble carbohydrates.
[c] Observations under detection limit (100 cfu/g) have been set to 99 before statistical analysis.

In the preference test, all parameters supported the result obtained from 'First Choice', so only results for this parameter are presented here. According to the preference test, horses favoured haylages where additive was used (Figure 1), the haylage without additive was always the least preferred. The level of additive inclusion did not have any effect on the preference of horses. The amount of plastic layers did not have a clear effect on the preference. When the preference test results were compared to the quality of the haylages, it seemed that the horses favoured feeds with the best hygienic quality in terms of yeasts, moulds and aerobic bacteria counts.

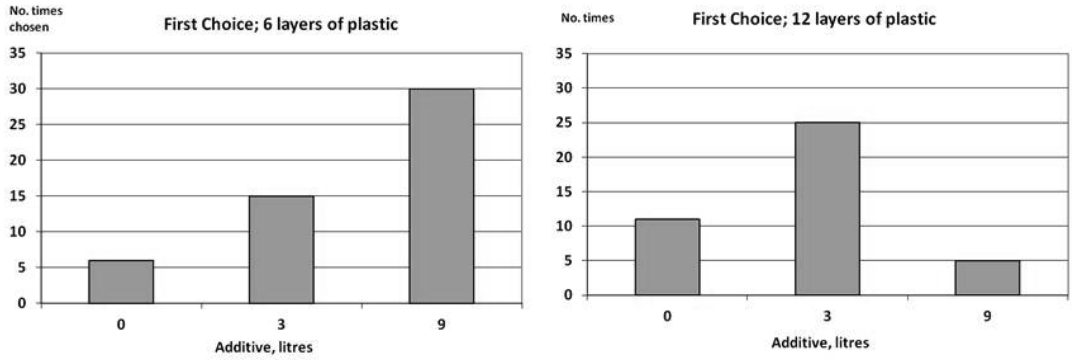

Figure 1. Number of times the feed was chosen as First Choice, the first forage the horse ate during at least five consecutive minutes.

Conclusions The use of propionic acid based additive enhanced the hygienic quality and aerobic stability of the haylage and preference of the feed by horses. The overall conservation quality seemed to be the key element in the preference of the forage by horses. Based on the experiences from this trial, the preference of forage can be determined as 'First Choice' of the horses.

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Consumption pattern of pigs supplemented with ensiled tropical forages

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Keywords: palatability, pigs, protein supplementation, tropical forages

Introduction In different tropical regions, forages may offer an alternative feeding option for pigs because of their high protein content, especially with regard to legumes, and good biomass yield and other ecological and economic advantages. Ensiling forages allows to harvest the crop at the optimal point of time and to preserve its nutritional quality, moreover smell and taste might be more appetizing than fresh herbage and wilting helps to reduce volume and concentrate nutrients. In contrast to producing herbage meal with ≥90% dry matter (DM), silage making requires less energy and time with a target DM of around 35% DM only. In a smallholder context in the subhumid tropics these factors and also the lack of a powerful mill can make ensiling the method of choice. In a framework project from 2009 to 2012, different tropical forage species were assessed as protein supplement for pigs and chicken in Colombia, Nicaragua and DR Congo.The objective of our study presented here was to assess the palatability of different silages in fattening pigs and hence the inclusion potential in their diet.

Material and methods The herbaceous legumes *Clitoria ternatea* CIAT 20692 (12 weeks growth, flowering), *Centrosema brasilianum* CIAT 5234 (17 weeks, pre-flowering) from CIAT Palmira, Valle del Cauca, the shrub legume *Cratylia argentea* CIAT 18516/18668 from Santander de Quilichao and the *Brachiaria* grass hybrid Mulato II CIAT 36087 (regrowth with high percentage of dead material) from Popayan, Cauca, Colombia, were ensiled during April to May 2010. The forages were wilted to > 350 g dry matter (DM)/kg fresh matter (FM) and chopped before applying sucrose at 20 g/kg FM and 10^5 cfu/g FM of a tropical *Lactobacillus plantarum* strain (CIAT S66.7). The material was compacted in 18.9 l plastic buckets which were closed tightly with lids with rubber gasket and stored roofed at ambient temperature.

In March 2011, 30 commercial pigs (47.0 ± 4.7 kg live weight (LW)), were housed individually on the experimental farm of the National University in Palmira, to evaluate the consumption of forage silages. A crossover design with double Latin square including five treatments, three replicates and two periods of 14 days each was applied. The 5 treatments were: Control, *Cratylia argentea, Centrosema brasilianum, Clitoria ternatea* and Mulato II silage supplement, respectively.

The Control consisted of 593 g maize, 150 g wheat bran, 230 g soybean meal, 2.5 g L-lysine HCl, 3.5 g DL-methionine and 21 g mineral and vitamin supplements per kg total diet. The diets were offered in five portions a day, starting with 80 g DM/kg $LW^{0.75}$. Composition of the control diet and silages is showed in the Table 1. In the silage supplemented diets, 50 g DM/kg $LW^{0.75}$ of the Control diet was offered and the silages ad libitum, starting with 30 g DM/kg $LW^{0.75}$. Silage and control diet were mixed before being offered to the animals. Pigs were weighed each week to adjust the amount of food to provide.

The statistical differences in consumption and initial live weights were determined by means of the GLM procedure and multiple range test of Duncan, using SAS statistical software.

Results and discussion The pH of all 4 silages ranged between 4.0 and 4.3 with DM contents > 370 g/kg FM. The ammonia-N of total silage nitrogen was lowest in *Centrosema* and *Clitoria* silages (44 and 45 g/kg N resp.) and a bit higher in *Cratylia* and Mulato II silages (60 and 74 g/kg N resp.). All silages were butyric acid free and the sum of acetic and propionic acid ranged between 4 and 11 g/kg DM.

Consumption of diets and silages is shown in Table 1. Pigs receiving *Cratylia* or *Clitoria* silage consumed the same amount of diet compared to those fed only on control diet. In both feeding regiments, *Cratylia* and *Clitoria* silage corresponded to 46.7% of total DM consumption on average, the rest (53.3%) was control diet. Mulato II and *Centrosema* silage were less consumed by pigs than *Cratylia* and *Clitoria*, possibly due to their lower nutritional quality. Neutral detergent and acid detergent fiber as well as lignin contents of Mulato II were higher than of *Cratylia*, *Clitoria* and *Centrosema* silages (Table 2).

Another important factor is the water-holding capacity (WHC), which explained satisfactorily the effects on intake of feeds such as grass which appeared to limit intake through their bulk (Kyriasakis and Emmans 1995). Here, WHC was higher in Mulato II silage (5.7 g/g) than in *Cratylia* (5.1 g/g), *Centrosema* (4.5 g/g) and *Clitoria* silage (4.1 g/g).

Dry matter content was lower in the silages of Mulato II and *Centrosema* in comparison to the other two forage legume silages (*Cratylia* and *Clitoria*). Some models which attempt to predict the voluntary feed intake of pigs use dry matter of the feed as a measure of bulk (Whittemore 1983). Indeed, Leterme et al. (2005) registered the higher DM intake in sows fed dry leaf meals (900 g DM/kg FM),

compared to fresh leaves (160-200g DM/kg FM). In the present experiment, basal diet (890 g DM/kg FM) was similarly consumed as *Cratylia* and *Clitoria* silages, with half the DM content. Possibly growing pigs (45 kg LW), can ingest bulk food with more than 440 g DM/kg FM without presenting physiological constraints for the animal. The dry matter content in the forage silages was the factor that best explained the consumption by pigs.

Conclusions It is concluded that *Cratylia* and *Clitoria* silages of high DM and good quality have the potential to serve as feed supplement in growing pig diets. Inclusion rates between 300 and 400 g/kg DM does not affect dry matter intake. Growth performance studies have to reveal the effect on live weight gain.

Acknowledgements The present work was supported by the Federal Ministry for Economic Cooperation and Development, Germany.

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Table 1. Palatability of diets including tropical legume silages or grass silage respectively, for growing pigs.

Parameter	[1]Control	[1]Cratylia	[1]Clitoria	[1]Centrosema	[1]Mulato II	VC%	SE	Sig
Initial live weight (kg)	48.78	45.70	48.40	45.35	45.17	10.1	4.7	
Consumption (g DM/pig*day)	1752[a]	1642[a]	1710[a]	1395[b]	1358[b]	9.70	152	***
Consumption (g DM/kg $LW^{0.75}$)[2]	94.74[a]	93.84[a]	93.78[a]	80.19[b]	78.18[b]	3.90	3.4	***

[1]Control supplemented with the respective forage silage; [2]50g DM/kg $LW^{0.75}$ corresponded to Control diet and the rest to each silage respectively. Different letters within rows mean significant differences among treatments

Table 2. Composition of control diet, three legume silages and one grass silage (g/kg DM).

Parameter	Control diet	*Cratylia argentea*	*Clitoria ternatea*	*Centrosema brasiliensis*	Mulato II
Dry matter	887	438	526	370	379
Crude protein	202	192	198	129	58.5
Neutral detergent fiber	188	476	490	463	732
Acid detergent fiber	73.5	349	380	349	468
Acid detergent lignin	29	157	109	113	200

Growth response of pigs supplemented with two contrasting tropical legume silages in Colombia

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Keywords: *Canavalia brasiliensis*, growth performance, pigs, silage quality, *Vigna unguiculata*

Introduction In a framework project from 2009 to 2012, the suitability of several tropical forage legumes in fresh and processed form was evaluated as feed supplement for pigs. As in selected forages crude protein (CP) concentration can be above 200 g/kg DM and acid detergent fiber (ADF) below 400 g/kg DM, they were tested as candidates to reduce purchasing costs of farmers in the tropics for commercial concentrates. The objective of the study presented here was to assess the growth potential of pigs when part of the soybean meal of the diet is replaced by *Vigna unguiculata* or *Canavalia brasiliensis* silage respectively.

Material and methods The annual herbaceous legume *Vigna unguiculata* CIAT 4555 was harvested in December 2009 at around 7 weeks at CIAT Palmira, Valle del Cauca, Colombia, and wilted for 2 days to achieve a dry matter (DM) of 300 g/kg fresh matter (FM). It was chopped, 20 g sucrose per kg FM were added, and inoculated with 10^5 cfu/g FM of a tropical *Lactobacillus plantarum* strain (CIAT S66.7). The material was compacted manually in 18.9 l plastic buckets to about 10 kg FM/bucket. They were tightly closed by a lid with rubber gasket. The annual to biannual creeping *Canavalia brasiliensis* CIAT 17009 was harvested with 3 months regrowth on two different days in February 2011 on-farm in the Cauca department. The material was chopped and wilted overnight to approx. 300 g DM/kg FM before preparing the silages in buckets with the mentioned inoculant in an open cattle stable which was temporarily disengaged.

In June 2011, 12 commercial pigs with an initial live weight (LW) of 43.0 ±1.6 kg were housed individually on the experimental farm of the National University in Palmira to evaluate growth performance using forage legume silages as one protein source in balanced diets. A crossover design with duplicated Latin squares was applied, for a total of four squares with three treatments and six orders. The treatments were: Control, *Vigna unguiculata* and *Canavalia brasiliensis* silage supplement resp., replacing 200 g/kg CP soybean meal in the diet. The main components of the diets and chemical composition are shown in the Table 1. *Vigna* silage made up 150 g/kg of the total diet (DM base) and *Canavalia* silage 190 g/kg DM resp., as a lower CP concentration was assumed in the latter basing on analysis of dried fresh plant material. The silage was mixed with the basal diets before feeding. The diet for each animal changed every three weeks, for a total of 9 weeks. Food was offered ad libitum twice a day and quantity adjusted every week, starting with 80 g DM/kg $LW^{0.75}$*d.

The differences in behavior productive variables and initial live weights were determined by means of the GLM procedure and multiple range test of Duncan, using SAS statistical software.

Results and discussion Table 2 shows live performance parameters of growing pigs fed with control or including the forage silages. Performance on Canavalia was lower than on the other two treatments ($P<0.001$). Although the Canavalia diet contained a higher percentage of silage because of assumed less CP concentration, the difference of final protein consumption could have caused less live weight gain. While animals receiving Control diet consumed a total of 300 g CP/day, it was 234 g with Canavalia and 276 with Vigna, whereby part of it was ammonia-N, with higher concentrations in Canavalia (see below).

The lower consumption of pigs given *Canavalia brasiliensis* silage in the diet could be due to the larger silage volume in the feed, which could have surpassed the physical capacity of ingestion. Water-holding capacity (WHC), which explained satisfactorily the effects on intake of feeds in studies of Kyriasakis and Emmans (1995), was higher in *Canavalia brasiliensis* (3.95 g water/g DM), compared to *Vigna unguiculata* silage (2.89) in our experiment.

The complete Vigna silage diet had around thirty five percent less acid detergent fiber than the Canavalia treatment. Sarria et al. (unpublished data) found no effect ($P>0.05$) of increasing *Canavalia brasiliensis* meal inclusion (0, 100, 200 or 300 g/kg basal diet) on consumption by pigs. Yet, it decreased digestibility of DM, CP and energy, when more than 200 g/kg DM was included in the diet ($P<0.05$), while *Vigna unguiculata* meal had a medium digestibility coefficient (534 g/kg crude protein) (Sarria et al., 2010). Silage of *V. unguiculata* was highly degradable *in-vitro* compared to other forages (Heinritz et al., this volume).

Also, the fermentation quality of the two silages was very distinct: while the *Vigna* silage (pH 4.4)

was butyric acid free, *Canavalia* silage (pH 5.3) contained on average 15 g/kg DM butyric acid, which might have depressed intake. Acetic and propionic acid summed up to 24 and 45 g/kg DM for *Vigna* and *Canavalia* respectively. While the total nitrogen content was similar in both silages (crude protein 224-228 g/kg DM, calculated as N x 6.25), ammonia-N accounted for 67 g/kg of the nitrogen in *Vigna* and 122 g/kg N in *Canavalia*. It is assumed that *Canavalia* was contaminated by clostridial spores while ensiling under farm conditions.

Conclusions Good quality forage silage of *Vigna unguiculata* offers a promising option to be included in balanced diets for growing-finishing pigs, while live weight gain with *Canavalia brasiliensis* silage was still within an acceptable range.

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Table 1. Composition of the experimental diets for growing pigs using silage of forage legumes as partial source of protein.

	Control	Canavalia	Vigna
Ingredients (g/kg)			
Yellow corn	586.7	555	596
Wheat bran	153	0	0
Soybean meal	240	200	200
Palm oil	---	30	30
L-Lysine HCL 78%	0.3	3	2
Silage	---	190	150
Vitamin and mineral	20	22	22
Composition g/kg dry matter			
Dry matter	877	765	792
Crude protein	168	160	163
Neutral detergent fiber	271	240	207
Acid detergent fiber	75	101	65
Acid detergent lignin	23	28	22
Crude Energy, kcal/kg	3781	3836	3698

Table 2. Performance parameters of growing pigs fed with diets containing or not tropical legume silages.

Parameter	Control	Canavalia	Vigna	CV%	SE	Sig.
Daily gain (g/pig)	877.6[a]	618.2[b]	831.6[a]	15.6	120.8	***
Daily consumption (kg DM/pig)	1.79[a]	1.51[b]	1,70[a]	7.3	121.8	***
Daily consumption (g DM/kg $LW^{0.75}$)	100.83[a]	87.26[b]	95.97[a]	6.0	5.73	***
Daily Feed : LW gain	2.00[a]	2.47[b]	2.06[a]	14.3	0.31	**

CV coefficient of variation, SE standard error
Values with different letters within a row differ significantly

In-vitro digestibility of *Vigna unguiculata, Centrosema brasilianum* and *Flemingia macrophylla* before and after ensiling for pigs

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Keywords: *Centrosema brasilianum*, *Flemingia macrophylla*, *in-vitro* digestibility, legume herbages, pigs, *Vigna unguiculata*

Introduction Within a framework project from 2009 to 2012, the suitability of several tropical forage species to serve as alternative feed supplement in pig and poultry nutrition was assessed. Fresh forage, forage meal and silage were regarded as options to include the locally grown feeds in pig diets. The objective of the study presented hereafter was to evaluate three contrasting forages for their *in-vitro* degradability and gas production before and after ensiling.

Material and methods The two herbaceous annual legumes *Vigna unguiculata* CIAT 4555 and *Centrosema brasilianum* CIAT 5234 were harvested with 7 and 8 weeks each in CIAT Palmira, Valle del Cauca, and on-farm in Patía, Cauca, Colombia, respectively. *Fleminigia macrophylla* CIAT 21087 branches were cut from 20 months old shrubs in Quilichao, Cauca, and leaves chopped at 300 g dry matter (DM)/ kg fresh matter (FM). *Vigna,* which was wilted to about 300 g DM/kg FM, *Centrosema* (260 g DM/kg FM) and *Flemingia* were lyophilized to assess their digestibility before ensiling and to determine the bromatological composition (DM, neutral detergent fibre (NDF), acid detergent fibre (ADF), non fibre-bound protein (Heinritz et al. 2012), water soluble carbohydrates (WSC)) and if so, anti-nutritional factors (ANF) such as condensed tannins (Barahona et al. 1997) and trypsin inhibitory activity (Smith et al. 1980). For ensilage, all of the materials were inoculated with a tropical *Lactobacillus plantarum* strain (CIAT S66.7) at 10^5 cfu/g FM, and 20 g sucrose/kg FM was added, before vacuum sealing them in small plastic bags in quadruplicates and storing them for three months at ambient temperature. Lyophilized ground (1 mm) fresh and ensiled forages underwent an enzymatic hydrolysis, incubating the samples at 39 C° for 2 hours with porcine pepsin (2000 FIP-U g^{-1}, Roth) and 4 hours with pancreatin (Pancreatin 8 x USP specifications, Sigma n°P-7545) as triplicates in three test runs (Boisen and Fernandez 1997). The predigested material was then used in a gas test (Bindelle et al. 2007), where it was incubated with pig faeces at 39 °C for 72 hours. Each gas production (GP) test included three replicates per fresh forage sample and each of the four silage replicates per specie was run in duplicate simultaneously. The volume (V) of the produced gas was read from the syringes at the following times: 0, 2, 5, 8, 12, 16, 20, 24, 30, 46, 52, 58 and 72 h.

Ground maize was included in the digestion studies as easily digestible feedstuff, solely and in combination with *Vigna* (maize:*Vigna* 60:40), as a simplified ration for pigs in practice.

Results and discussion *Vigna* before ensiling showed significantly ($P<0.05$) highest enzymatic degradability (D) of DM, followed by *Centrosema* and *Flemingia* (Figure 1). Similar to D, the gas production (GP) after 72 h was highest in *Vigna*. *Centrosema* showed a GP of 30.0 ml/g DM and *Flemingia* was lowest (Figure 2). The good results with *Vigna* might be explained by the lowest amounts in NDF and ADF (365 g/kg DM and 235 g/kg DM, resp.) and highest values in WSC (111 g/kg DM) and non fibre-bound protein (185 g/kg DM), whereas *Flemingia* and *Centrosema* contained ANF such as condensed tannins (207 g/kg and 79 g/kg DM, resp.) and trypsin inhibitors (199 mg and 8.9 mg trypsin inhibited per g DM). There was no significant difference between the D of the silage and the original material of *Vigna* ($P>0.05$) (Figure 1). *Centrosema* silage was significantly better degradable than the fresh plant material, *Flemingia* as well had a slightly but significantly higher D in silage (both $P<0.05$), which in both cases might be due to a reduction of condensed tannins during ensiling by 55% and 84% respectively. GP of all ensiled forages decreased compared to the not ensiled material (Figure 2). The D of maize alone was 814 g/kg DM, and when combined with *Vigna*, the calculated D of *Vigna* increased to 608 g/kg DM. Equally, maize showed the highest GP, followed by maize+*Vigna.*

Conclusions The chemical composition of the feed materials was reflected in their *in-vitro* digestibility, where *Flemingia macrophylla* appeared as the least suitable legume to be included in monogastric diets. Nevertheless, through ensilage anti-nutritional factors were reduced, which improved the enzymatic digestibility. On the other hand, ensiling reduced the gas production, which might be due to a decreased availability of WSC in silages for the bacteria in the colon. *Vigna unguiculata* showed the highest degradability of the tested forages. The combination of *Vigna* with cereals such as maize has potential as

alternative feedstuff in tropical countries, which is assessed in *in vivo* studies (Artiles Ortega et al., Sarria et al. this volume; Sarria et al. 2010).

Acknowledgements The financial support by the Federal Ministry for Economic Cooperation and Development, Germany (BMZ), is gratefully acknowledged.

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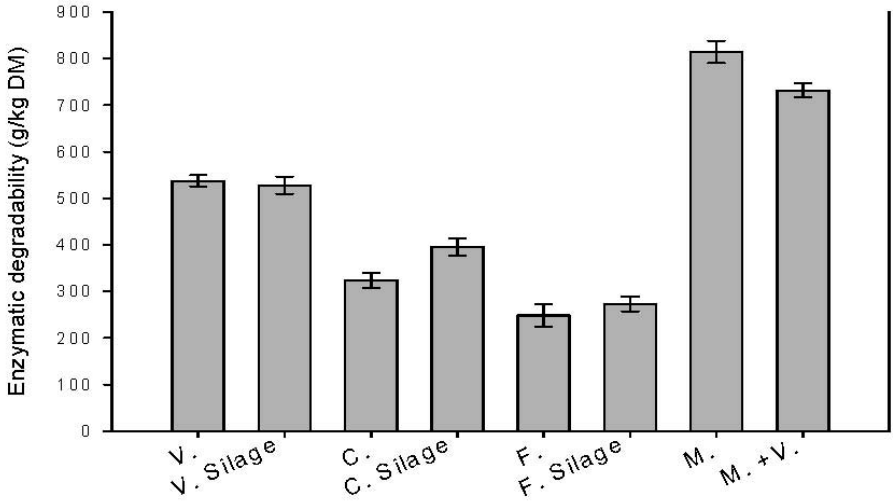

Figure 1. Enzymatic degradability of *Vigna (V.), Centrosema (C.), Flemingia (F.)* and their silages, maize (M.) and maize+*Vigna* (60/40) (error bars represent standard deviation).

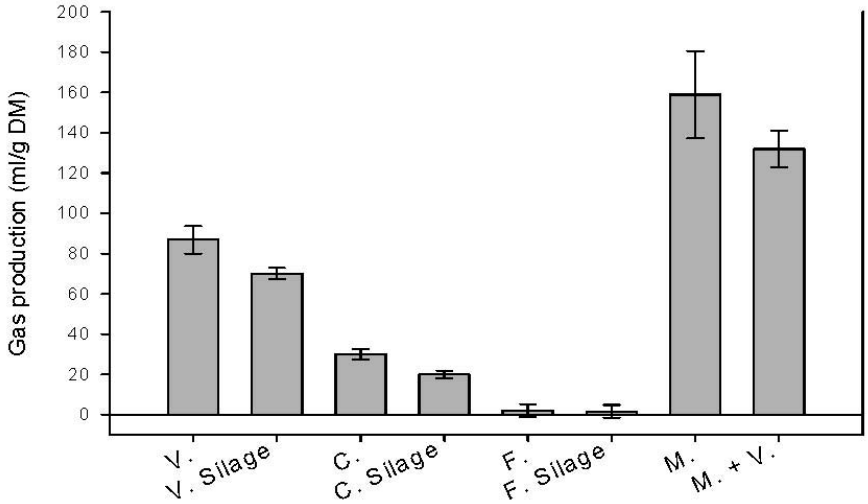

Figure 2. Gas production of *Vigna (V.), Centrosema (C.), Flemingia (F.)* and their silages, maize (M.) and maize+*Vigna* (60/40) (error bars represent standard deviation).

Effects of ensiling soaked cowpea (*Vigna unguiculata*) grains mixed with sorghum (*Sorghum bicolor*) grains on fermentation quality, selected anti-nutritional factors and precaecal digestibility of amino acids in pigs

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Keywords: amino acids, precaecal digestibility, silage, soaking, *Sorghum bicolor*, *Vigna unguiculata*

Introduction Tropical native legumes are an alternative to the cost-intensive conventional ones used in animal feeding. However, due to the tropical weather conditions pest and diseases can occur during storage, affecting the nutritional quality of the feedstuff. Ensiling is seen as a possibility to face these problems. Furthermore, enhanced digestibility or reduced contents of anti-nutritional factors (ANFs) are welcome side effects of fermentation processes (Deshpande and Salunkhe 2000). The objective of the study was to evaluate effects of ensiling a mixture of soaked cowpea and sorghum grains on fermentation quality, contents of individual ANFs and the precaecal digestibility (pcD) of selected amino acids (AA) in pigs.

Material and methods Cowpea (*Vigna unguiculata*) grains were soaked (24 h) at a grain:water ratio of 1:4 (w:v), drained and milled. Coarsely ground sorghum (*Sorghum bicolor*) grains (4 mm mesh size) were mixed with soaked cowpea grains to achieve 18% of crude protein in the dry matter (DM). Molasses (4%) and lactic acid bacteria (LAB, *Lactobacillus plantarum,* DSM 8862 and 8866, $3x10^5$ cfu/g) were applied as silage additives. The thoroughly homogenized mixture was ensiled in plastic tons (120 L) for 60 d. Representative samples were taken for chemical analysis and evaluation of fermentation quality.

Standardized pcD of selected AA was determined in ensiled and not ensiled mixes of cowpea and sorghum grains. For the latter air-dry cowpea and sorghum grains were coarsely ground (4 mm mesh size) and mixed in the same proportion like silages to achieve a crude protein content of 18%. Neither molasses nor LAB was applied. Eight adult castrated minipigs with end-to-end ileo-rectal anastomosis (Hennig et al. 1986, Laplace et al. 1994) were placed in two 4x4 Latin Square designs (4 pigs each). The pcD of the raw mixture (RM) and the silage (SL) was determined by a regression method (GfE 2005) restricting the cowpea inclusion to 0, 10, 20 and 30% of dietary DM. Regression characteristics were compared using Sachs (2006). The impact of ensiling on fermentation patterns and contents of ANFs was investigated by ANOVA (SPSS 13.0) with a level of significance pre-set at $P<0.05$.

Results and discussion Silage evaluation revealed good fermentation quality. Only marginal differences were observed in proximate nutrient composition between SL and RM. However, ensiling caused a remarkable reduction of starch from 620 to 312 g/kg DM. This agrees with findings in ensiled triticale, barley and wheat grains (Hackl et al. 2010, Pieper et al. 2010) and ensiled lupine grains (Gefrom et al. 2009). During ensiling, condensed tannins (CT) were reduced ($P<0.05$) from 0.24 to 0.15% of DM. Likewise trypsin inhibition activity decreased from 39.6 to 31.1 mg trypsin inhibited/g DM ($P<0.05$). Cyanide on the other hand increased from 41.9 to 98.5 mg/100 g DM ($P<0.05$). Among most interesting AA, pcD of Lys and Cys tended to decrease whereas pcD of Thr and Met increased. This effect was significant ($P<0.05$) for Met only (57.7% in RM and 70.4% in SL). The reduction of tannins and trypsin inhibitors, which are known to decrease digestibility of protein and AA, may be the main reason for the pcD increase through ensiling, but there is no explanation for the different effects in individual AA. Actually, ensiling increased the content of precaecal digestible AA in the DM to a moderate extent for Met, but in case of Lys it decreased remarkably (Tab. 1).

Conclusions Ensiling soaked cowpea grains and sorghum grains resulted in well fermented silages. Starch was apparently fermented to a large extend with implications for the energy content. The fermentation process caused a decrease in the content of ANFs, particularly of those contributing to reduced digestibility of AA such as condensed tannins and trypsin inhibitors. This, however, actually resulted in elevated pcD of Met only. Nevertheless, ensiling gave rise to an elevated content of cyanide which should be investigated in further studies.

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Table 1. Contents of nitrogen and amino acids as well as of standardized precaecal digestible (pcd) nitrogen and amino acids in raw and ensiled cowpea-sorghum mixtures.

	N and amino acids [g/kg DM]		pcd N and amino acids [g/kg DM]	
	Raw mixture	Silage	Raw mixture	Silage
N	27.8	28.4	18.0	20.0
Threonine	6.6	6.4	4.7	4.8
Valine	9.0	8.6	6.0	6.2
Isoleucine	8.2	6.8	5.5	4.6
Leucine	16.5	15.7	12.0	12.1
Tyrosine	4.1	4.2	2.9	3.1
Phenylalanine	10.0	9.5	7.1	7.0
Histidine	5.5	5.8	3.7	4.2
Lysine	9.1	8.3	6.6	6.0
Arginine	10.1	10.0	7.5	7.6
Cystine	2.2	2.0	1.4	1.3
Methionine	2.8	2.5	1.6	1.8