The effect of silage quality on gross energy losses

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Summary

Silage is a feed for ruminants resulting in the preservation of fresh forage crops by acidification, which is achieved under anaerobic environment. The process of ensiling is mainly depends upon the chemical and microbial composition of forage. During the fermentation process, changes in chemical composition of forage occur mainly due to bacterial activities. These changes are always accompanied with losses commonly expressed as DM losses. Another way to express these losses is in form of gross energy losses. The aim of the study was to investigate the effect of quality of silage fermentation influenced by silage additive application on the gross energy value of silages after the fermentation process and after aerobic stability test. Clover-grass mixture in 22 % DM treated with silage additive at the rate 1L, 2L, 3L and 5 L per ton fresh forage was compared with untreated control. Forage was ensiled in 1.7 glass jars with water lock for 90 days. Chemical and microbiological analyses were performed to determine fermentation quality of silages. In addition, silages were weighed to determine DM losses as well as aerobic stability test was performed on silages. The gross energy was analyzed by bomb calorimetric method. The pH of control silages was significantly higher ($P<0.001$) than the additive treated silages. The production of propionic acid ($P<0.001$), butyric acid ($P<0.001$), 2,3-butanediol ($P<0.001$) and ethanol ($P<0.001$) was significantly reduced in all additive treated silages in comparison with the control silage. The concentration of lactic acid ($P<0.001$) and acetic acid ($P<0.001$) was higher in additive treated silages than in control. The concentration of ammonia-N was found lower ($P<0.04$) in S2 and S3 silages in comparison with the rest of silages. Clostridia spore count was significantly reduced ($P<0.001$) in all additive treated silages in comparison with the control silage. The DM losses in control silages were found to be higher ($P<0.001$) during the whole storage time in contrast with treated silages. No differences in aerobic stability were found between additive treated and control silages. There were no statistical differences between gross energy of all silages and gross energy of fresh forage in both after fermentation and after the stability test. Energy losses expressed in % of initial energy concentration in the silo showed no significant variations among both silages, after fermentation ($P=0.5$) and after the stability test ($P=0.2$). The improved the silage fermentation by the application of silage additives was reflected in reduced DM losses. However, the improvement in silage fermentation had no effect on energy losses formation in silages.

Introduction

Conserved forage is essential component of ruminant's diets in many countries of the world where growing seasons are restricted. In Sweden a considerable proportion of nutrient demands of ruminants are fulfilled through forages in the winter, therefore large proportion must be preserved in form of hay or silage. The production of silage in Sweden is increased over the past years, while the production of hay decreased substantially. Silage is a feed for ruminants resulting in the preservation of fresh forage crops by acidification, which is achieved under anaerobic environment. Achievement of high nutritional value and good hygienic quality of ensiled forage is important in terms of quality and economy of animal products.

Ensiling process can be influenced by many factors. One of the features, which influence the ensiling process, is the nature of the crop. Chemical and microbial composition of the fresh crop determines its ability to be successfully ensiled. During the fermentation process, changes in chemical composition of forage occur mainly due to bacterial activities. These changes are always accompanied with losses. The extent of the losses during fermentation depends on the type of bacteria that dominated the fermentation process. It has been shown

that silages display lower losses when lactic acid bacteria carry out fermentation whereas silages where bacteria such as clostridia or yeast dominated show higher losses (Lindgren et al., 1987).This is due to various efficiency of conversion of ensiling substrate to fermentation products. These losses are commonly expressed as weight loss of forage dry matter (DM). These losses are assumed to originate from the silage DM where substrate (e.g. water soluble carbohydrates (WSC), lactic acid, nitrate), as the part of DM, is transformed to the volatile fermentation products such as carbon dioxide, hydrogen, nitrous oxide and ammonia etc. Eventually, the weight of ensiled forage is reduced due to loss of DM in form of volatilities. Another form of presentation silage quality is to determine the total energy in silage, namely gross energy. This method of assessment of silage quality seems to be more precise since it can better express the relation between fermentation quality and potential animal performance. The better expression is due to the fact that all fermentation products including these of less effective microorganisms (clostridia, enterobacteria and fungi, etc.) contribute to the energy value of silage. According to (Derwhust et al., 1986; McDonald et al., 1973) fermentation end products (organic acids) can increase the gross energy of silage up 10 to 14 %. Therefore large differences in silage quality and DM losses might not be reflected in significant variation of gross energy value.
The main objectives of the present study were; to investigate the effect of quality of silage fermentation influenced by silage additive application on the gross energy value of silages, compare the difference between the expressions of silage losses in common form of DM with gross energy losses, and quantify the effect of improved aerobic stability on gross energy losses in silages.

Literature Study

Expression of losses during the ensiling process

Ensiling is a method used for the preservation of wet forage crops. It is based on a spontaneous lactic acid fermentation under anaerobic conditions, whereby lactic acid bacteria (LAB) convert WSC in the crop to lactic acid and to lesser extend to the acetic acid (Oude Elferink et al., 1999).The conservation is caused by the pH drop of ensiled material due to organic acid formation which inhibits the growth spoilage microorganisms in silage. The speed in which pH is lowered and then maintained during the storage period is important for the efficiency of the ensiling. The ensiling efficiency then can be express in form of losses. Silages where LAB fermentation did not dominated resulted in increased butyric acid and ammonia concentration and reduced palatability of the silage (Seglar, 2003). The whole ensiling process can be divided into four stages (Pahlow et al., 2003)**.** All these phases have different length, various biochemical processes with different intensity taking place and accordingly different losses can occur.

Phase 1: Aerobic Phase

The first stage begins when plant is harvested and continues until the oxygen in the silage mass is depleted. This stage mainly involves continue respiration, enzymatic processes in the forage (McDonald et al., 1991). All these processes are undesirable in regards to ensiling process. Plant respiration reduces WSC concentration in fresh forage, which is the main substrate for LAB fermentation. Prolonged respiration thus increases DM losses. Another important chemical change is proteolysis producing non-protein nitrogen (NPN), peptides, amino acids, and ammonia due to clostridia. The protein decomposition, which additionally causes inhibition of silage acidification, reduces nitrogen utilization of silage (Slotner, 2004). Likewise the respiratory and enzymatic processes, microbial activities take place during this stage. The existence of oxygen stimulates the growth of facultative and obligate aerobic

microorganisms such as moulds, yeasts, and enterobacteria (McDonald et al., 1991; Oude Elferink et al., 1999). Aerobic micro-organisms activity is undesirable in terms of quality of silage fermentation, since they also utilize WSC. As respiration of plant forage in silo continues, the oxygen is depleted and microbial population is gradually converted from aerobic to anaerobic microorganisms. Aerobic phase should be as short as possible to eliminate not only the growth of aerobic microbes but also respiration and enzymatic activity that cause detrimental effect to the silage quality and increase silage losses.

Phase 2: Fermentation phase

This phase starts with the achievement of anaerobic conditions in the silo. Under the anaerobic conditions the LAB should ferment available plant nutrients, namely WSC to mainly lactic acid. The activity of LAB is fundamental for successful ensiling process. Between LAB groups belong *pediococcus, enterococcus, lactococcus,* and *leuconostoc*. These groups of bacteria can be divided into three groups; homofermentative, and facultative and obligate heterofermentative LAB. Homofermentative LAB (*streptococci, pediococci* and *lactobacilli*) use two pyruvate molecules either from glucose or fructose produce two molecules of lactate without loss of DM, and slight energy losses (McDonald et al., 1973). Heterofermentative (*leuconostocs* and *lactobacilli)* utilize one mole glucose with two moles fructose and produce two moles of mannitol with one mole each of lactate, acetate and carbon dioxide. The energy losses from hetrofermentative LAB are small but DM losses varied between 5% and 24% (McDonald et al., 1973). Clostridia are other bacteria that occur in fermentation stage. Their sporulating and anaerobic growing ability make them the most detrimental microorganisms involved during fermentation process. Clostridia possess also the proteolytic activity. They utilize alanine and glycine for the production of ammonia, acetate, carbondioxide and also produces isovaleric and isobutyric acid from the catabolism of amino acids (Ohyama and McDonald, 1975).The increase in population of clostridia in the silage causes more butyric acid and ammonia rather than lactic acid, resulting in the silage of bad quality with higher DM losses and low feeding value (Oude Elferink et al., 1999). Clostridia contaminated silage shows 51.4% of DM losses and energy losses of 18.4% (McDonald et al., 1991). Enterobacteria are other bacteria, which compete with LAB for the substrate. Their growth is restricted as the development of lactic acid bacteria proceeds (E.Ostling and Lindgren, 1993). These bacteria use glucose as substrate and produce the acetate and ethanol, 2,3-butanediol and carbon dioxide. In addition, enterobacteria show activity towards nitrate as they convert the nitrate to either ammonia via nitrite or to produce nitrous oxide. The rate of reduction of nitrate depends on the rate of acidification. The DM losses and energy losses from these bacteria are 41.1% and 16.6% respectively (McDonald et al., 1991). The rapid dominance of LAB in the fermentation is required for achievement of low ensiling losses. Slow development of LAB cause slow fermentation rate and results in slow pH drop allowing activity of undesirable microorganisms (Scudamore and Livesey, 1998) which might increase DM and energy losses and also reduce the silage intake due to presence of increase amount of butyric acid or ethanol (Seglar, 2003). As fermentation progress and lactic acid concentration increases, activity of LAB are eventually reduced due to low pH (Moon et al., 1981). After the completion of fermentation stage, ensiling process should enter into stable phase.

Phase 3: Stable Phase

The low pH from lactic acid production inhibits the growth of other microbes such as yeast, bacilli, enterobacteria and clostridia and eventually even lactic acid bacteria themselves. Under these conditions the ensiled forage can be stored for long period of time. However, the process of deterioration called secondary fermentation may be initiated by endospores of bacilli and clostridia (Jonsson, 1991). The secondary fermentation is related either to a

deficiency of available substrate or slow production of lactic acid leading to ineffective inhibition of spoilage flora such as clostridia. Clostridia use lactate, acetate and glucose as a substrate in order to produce the butyrate, carbon dioxide and hydrogen. Not all the clostridia produce butyrate. Some such as *C. spenoides* utilize glucose and produce ethanol, acetate the carbondioxide and hydrogen (McDonald et al., 1991).The sign of clostridia activity during this process is associated with the increase in silage pH (Jonsson, 1991).

Phase 4: Feed-out phase

This phase starts as the silage is exposed to the air, which is previously kept in oxygen free conditions. This allows the undesirable microbes (yeasts and moulds, listeria, bacilli,) to degrade lactic acid and residual WSC to produce carbon dioxide, ethanol and water with evolution of heat (Merry and Davies, 1999). Particularly yeast is microorganism considered to be mainly responsible for the initiation of deterioration in silages (Pahlow et al., 2003; Woolford, 1990) . Their ability to utilize lactic acid as substrate causes raising the silage pH, which promotes the growth of other microorganisms, such as mould or bacilli, even clostridia. In addition, there is a great risk of hygienic quality problem of silages due to the ability of moulds to produce mycotoxins (Adesogan, 2009). Proteins in silage are also very sensitive to excessive heat accumulation. New linkages within and between the peptides are formed and they resist against the digestive proteases (Ford, 1975). All these changes reduce the silage quality and cause additional DM losses.

Factors influencing the quality of silage fermentation

Silage losses always occur even in well-managed conserve system use for silage making. The level of losses and the quality of the ensiling process can be affected by many factors. In general, two primary features can be distinguished. The first is the chemical and microbiological composition of the crop. This includes buffering capacity (BC), WSC and DM content. Crops having more WSC content and low BC are more favorable for LAB fermentation resulting in less DM loss. On the other hand forages like legumes having low WSC content and high BC are more prone to clostridial fermentation than grasses (Pahlow et al., 2003; Dinić et al., 2010). The second feature is associated with ensiling condition imposed by the silage-maker, such as wilting, chopping of forage, packing density, and use of additives. Silage losses originated from the crops harvesting and continue until the completion of fermentation. The management in the field and mechanical treatments of forage during the silage making affect the silage fermentation in terms of microbial activity. Wilting is an important pre-ensiling treatment of forage. The reason of witling is to avoid effluent losses and eliminate activity of undesirable microorganisms during the ensiling process, particularly clostridia. (Jonsson, 1990) found that wilting of crop to 35-40 % considerably eliminate the growth of clostridia in silages. Precise chopping results in more available substrate and water released from damaged forage cells, which in turn increases fermentation rate of silage (Pauly and Lingvall, 1999). Packing density has also great importance in the ensiling process because silage porosity is determined from silage density and DM content i.e. important during the feed-out phase to eliminate aerobic deterioration processes and thereby DM losses (Muck and and Holmes, 2000). The use of different silage additives has been an efficient way to improve silage fermentation and thereby reduce ensiling losses. The lactic acid bacteria inoculants are used to reduce DM losses by enhancing the homolactic fermentation (Weinberg and Muck, 1996).Using the formic acid and propionic acid was reported also to reduce the microbial activity of clostridia in wet silages (Knický, 2005). The combination of sodium benzoate, potassium sorbate and sodium nitrite used as an effective silage additive against clostridia spores in low DM silages and also successful against the yeast in high DM silages

(Knicky and Sporndly, 2011)

Material and methods

The mixture of timothy, meadow fescue and red clover were harvested (1st cut) in the first week of June 2010. The crop consists of 57 % of timothy (heads visible), 31% of meadow fescue (heads visible) and 11 % of red clover (before flowering). The Crop was chopped to 5cm length in stationary cutter. After the chopping the crop was mixed and was divided into fractions of 3kg fresh matter in each. All fractions were inoculated with spore suspension of *Clostridium tyrobutyricum* at a rate of 10^3 per g fresh matter (FM). The control fraction remains untreated. The other forage fractions named S1, S2, S3 and S5 were treated with silage additive solution (Safesil™). The composition of silage additive was a water solution containing on weight basis, 20% of sodium benzoate, 10% of potassium sorbate and 5% of sodium nitrite. The treatment rate was 1, 2, 3 and 5 liter per ton FM receptively. The last treatment S5 was treated with additive having all same ratios of chemicals except sodium nitrite, which was 2% instead of 5 %. Clostridia and additive treatments were applied by hand with a spray bottle. Each forage fraction was ensiled in lab-silos (1.7 liter volume with a fermentation lock on lid) in 3 replicates in each treatment. Finally, silos were stored at room temperature (21 °C) for 120 days.

Fresh Forage analyses

Microbial analyses

Two samples of fresh forages (without additive treatment) were taken for the microbiological analyses. The lactic acid bacteria growth (LAB) and clostridia spores were measured by spread plate methods described by (Jonsson, 1990) and (Pahlow, 1990) respectively.

Chemical Analyses

The chemical analyses consisted of determination of DM, ash, CP, WSC, buffering capacity and nitrite. The content of dry matter of the forages was measured in two steps. In the first step the samples were weighted and dried in oven for 18 hours at 60 °C, and then samples were milled through hammer milled to 1.0 mm in size. In the second step, milled samples were placed in the oven at 103 °C or overnight. The ash content of the samples was measured in muffle furnace through combustion at 550 °C for 3 hours. The crude protein of the samples was analyzed through Kjeldahl technique according to (Bremner and Breitenbeck, 1983). The concentration of WSC was analyzed from dried and milled sample diluted with 250 ml distilled water, boiled for 10 minutes and drained through H-602 filter paper (Watman GmbH., Germany) and then analyzed using enzyme-based acid hydrolysis by (Larsson and Bengtsson, 1983), buffering capacity was analyzed according to (McDonald and Henderson, 1962), nitrate were assessed according to method ASN 110-01/92 in the system from FOSS-Tecator (1992). The silos were weighed at the time of filling and then during the storage until the end of storage to calculate the weight losses.Weight losses were assumed to originate from the silage DM (loss of CO_2) and losses were therefore expressed in % of DM in the silo after silo filling. No correction was made for the CO_2 bound in the silage liquid.

Silage analyses

Microbial analyses

Clostridia spores, yeasts and moulds were determined in silage samples using the same techniques as in the fresh forage.

Chemical Analyses

The DM concentrations of samples were determined the same way as in the fresh forages except that a 1.4 % unit as a constant correction for silage volatiles was added to the final calculation. The pH of the silages was measured by pH electrode in 654 pH-meter Methrom AG, Herisau, Switzerland. In the silage extract, fatty acids, ethanol and 2.3-butanediol was measured by using HPLC according to (Andersson and Hedlund, 1983), ammonia-N (ASN 50-01/92 in FIA-system from FOSS-Tecator, 1992). Aerobic stability at the end of the storage period was calculated by measuring temperature increase in 1300 ml PVC tubes covered at the bottom with a polyurethane-fiber net. Packing density was decided in relation to DM concentration according to the equation: filling weight (g FM) = (-205.57 x ln (% DM)) + 1061) based on DLG (2006) recommendations. Tubes were placed in an insulating Styrofoam block and kept at room temperature for 5-7 days. The number of days it took for a silage to increase 3 °C was used to express aerobic stability (Hönig, 1990).

Gross Energy Analyses

Two Samples from forage prior to ensiling, 15 samples from silages after the storage period, and 15 silage samples extracted from silages after storage stability test were used for gross energy determination. Duplicate samples were analyzed yielding in total 64 samples. Before the gross energy determination, an approximately 30g of each sample was homogenized by using hand processor (Braun Minipimer 3). Grinding procedure was performed on frozen samples and after grinding the samples were kept into freezer for further use. A quantity between 1g and 1.5g of sample was weighed in metal crucible. Then the pellet of benzoic acid (Parr Instrument Company USA) was added to the metal crucible and recorded the weight. The open mouth of metal crucible was closed with tape and weight of tape was recorded. Finally metal crucible was fixed in the bomb and bomb was placed in the calorimeter. The bomb number, sample name and sample weight and used energy were recorded in the digital system of bomb calorimeter (Parr Instrument company, USA).
The calculation for the gross heat of combustion:
(Observed temperature rise*energy equivalent of the calorimeter and bomb in use) - sum of corrections (tape, benzoic acid) / mass of sample.

Statistical analyses

Three replicate silos were used in each treatment. Statistical analyses using R 2.13.1(R Development Core Team, 2011). The analysis of variance (ANOVA) were used to evaluate the effect of additive treatment on silage quality and gross energy. Least significance difference (LSD) test was employed to compare the means of treatments at probability of <0.05.
Statistical model was as follows:

$$Y_{ij} = \mu + Treatments_i + error_{ij}$$

Where: μ = overall mean, i = different additive treatments of silage,
j = replications.

Results

The chemical and microbiological composition of forage prior ensiling is presented in Table 1. Forage was mainly characterized a high quantity of yeasts but low amount of LAB and clostridia, even after addition.

Table 1. Chemical and microbiological compositions of fresh forage (n=2)

Analyses	Unit	
DM	%	22
Ash	% of DM	8.6
CP	% of DM	13.8
WSC	% of DM	12.1
NDF	% of DM	45.2
ME	MJ/kg DM	11.1
Buffering capacity	g LA/100 g DM	6.3
LAB	log cfu/g FM	1.2
Yeast	log cfu/g FM	6.3
Mould	log cfu/g FM	3.2
Clostridia spores		
before inoculation	log cfu/g FM	<1.7
after inoculation	log cfu/g FM	2.6
pH of forage mass		5.8
Fermentation coefficient		37

DM=Dry matter.
CP = Crude protein.
WSC= Water soluble carbohydrates.
NDF =Neutral detergent fiber.
ME =Metabolize energy.
LAB=Lactic acid bacteria.
Cfu=Colony forming unit.

The comparison of biochemical changes between additive treated silages and untreated (control) silages is presented in Table 2. The pH of control silages was significantly higher ($P<0.001$) than the additive treated silages. The lowest pH was found in S2 and S3 silages Silage pH of additive treated silages showed the increasing trend ($P<0.001$) with increasing additive dosage. The similar pattern as in silages after the fermentation was obtained in pH silages after the aerobic stability test ($P<0.001$). The production of propionic acid ($P<0.001$), butyric acid ($P<0.001$), 2.3-butanediol ($P<0.001$) and ethanol ($P<0.001$) was significantly reduced in all additive treated silages in comparison with the control silage. In treated silages (S1, S2, S3) as the application dosage of additive was increased the formation of ethanol was significantly decreased ($P<0.001$). The concentration of lactic acid ($P<0.001$) and acetic acid ($P<0.001$) was higher in additive treated silages than in control. Concentration of lactic acid declined as silage additive dosage increased resulting in the highest concentration in S1 silages and the lowest in S5 silages ($P<0.001$). The concentration of ammonia-N was found lower ($P<0.04$) in S2 and S3 silages in comparison with the rest of silages.

Table 2. Biochemical composition in the silages at the end of storage (n=3, values within columns with different superscripts are significantly different at $P < 0.05$).

Treatment	DM	pH	pH after stability	Am-N	Propionic acid	Lactic acid	Acetic acid	Butyric acid	2.3-Butanediol	Ethanol	Aerobic Stability
				% of TN			% of DM				days
Control	21.6	4.8^{a}	4.8^{a}	3.5^{ab}	0.4^{a}	4.0^{d}	0.5^{b}	2.9^{a}	0.63^{a}	1.8^{a}	6.8
S1	21.7	4.3^{d}	4.4^{d}	3.7^{ab}	0.04^{b}	7.2^{a}	1.8^{a}	0.04^{b}	0.04^{b}	1.6^{b}	6.8
S2	21.9	4.4^{c}	4.4^{cd}	2.4^{c}	0.04^{b}	6.3^{b}	1.7^{a}	0.04^{b}	0.04^{b}	1.3^{c}	6.8
S3	22.0	4.4^{c}	4.4^{c}	2.8^{bc}	0.04^{b}	6.0^{bc}	1.8^{a}	0.04^{b}	0.04^{b}	0.9^{d}	6.8
S5	22.2	4.5^{b}	4.5^{b}	3.9^{a}	0.04^{b}	5.6^{c}	1.6^{a}	0.04^{b}	0.04^{b}	0.83^{d}	6.8
SEM		0.04	0.04	0.18	0.04	0.29	0.14	0.31	0.06	0.10	0
p value		<0.001	<0.001	0.038	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns

DM-dry matter
TN-total nitrogen
Am-N-ammonia nitrogen (the value is corrected for N added with the additive in form of $NaNO_2$).

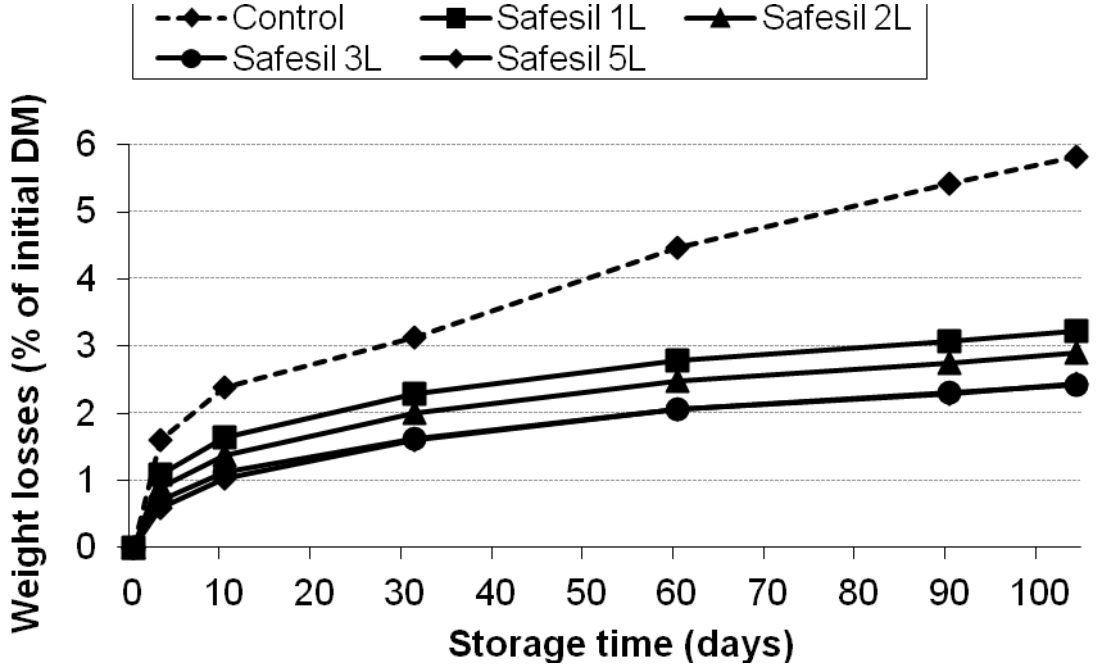
Table 3. Microbiological composition in the silages at the end of storage (n=3; values within columns with different superscripts are significantly different at $P < 0.05$.)

Treatment	Clostridium spores	Total yeast	Moulds	LAB
		log cfu/g		
Control	5.9[a]	1.7	1.7	7.0
S1	2.1[b]	2.1	1.7	7.0
S2	1.7[b]	1.7	1.7	7.0
S3	1.7[b]	1.7	1.7	7.0
S5	1.7[b]	1.7	1.7	7.0
SEM	0.44	0.07	0	0.05
p value	<0.001	ns	ns	ns

LAB-lactic acid bacteria
cfu-colony forming units.

The microbial composition of treated and untreated silages is shown in Table 3. The variation in quantity of yeast, moulds and LAB between silage treatments was statistically insignificant. Clostridia spore count was significantly reduced ($P<0.001$) in all additive treated silages in comparison with the control silage.
The development of DM losses of silages during storage time is presented in Figure 1. The DM losses in control silages were found to be higher ($P<0.001$) during the whole storage time in contrast with treated silages. The losses in treated silages decrease as concentration of additive was increased resulting in highest in S1silages and lowest in S5 silages ($P<0.001$).

Figure 1. Comparison of dry-matter losses in silages during the storage time (n=3).



No differences in aerobic stability were found between additive treated and control silages.
The measurements of gross energy of silages before storage and after the aerobic stability test are demonstrated in Table 4. There were found no statistical differences in gross energy concentrations between fresh forage and silages after fermentation ($P=0.7$) as well as between fresh forage and silages after the stability test ($P=0.5$). Energy losses (Table 5) expressed in % of initial energy concentration in the silo showed no significant variations among both silages, after fermentation ($P=0.5$) and after the stability test ($P=0.2$).

Discussion

The ensiling capability of fresh forages primarily depends upon their microbial and chemical composition. Weissbach et al. (1974) used DM, WSC and buffering capacity of the fresh forage to predict its ensilability. The influence of these parameters on forage conservation has been expressed in a fermentation coefficient (FC). Forages having FC value below 35 are not easy to successfully ensile while forages having FC value more than 45 are easily. In the present study used forage displayed FC value which classified the used forage rather among difficult ensilable crop and potentially higher DM losses. Moreover, silages having low DM are prone to clostridial fermentation (Pahlow et al., 2003). Their activities in silage are associated with increased butyric acid production and high DM losses. In present study control silages showed higher DM losses in comparison with treated silages. These losses are probably due to the clostridia activity in these silages as it can be manifested by presence of

Table 4.Gross energy concentration in fresh forage and silages analyzed after the storage time and after aerobic stability test. (values within columns with different superscripts are significantly different at $P < 0.05$).

Treatment	Gross energy in silages (MJ/kg FM)	
	after fermentation	after stability test
Fresh forage	4.0[a]	4.0[a]
Control	4.2[a]	4.2[a]
S1	4.1[a]	3.9[a]
S2	3.9[a]	4.1[a]
S3	4.0[a]	4.2[a]
S5	4.0[a]	4.2[a]
SEM	0.12	0.13
p value	0.7	0.5

Table 5. Energy losses in silages after the fermentation and after the aerobic stability test. (values within columns with different superscripts are significantly different at $P < 0.05$).

Treatment	Energy losses in silages (% of initial energy content)	
	after fermentation	after stability test
Control	+1.5[a]	4.0[a]
S1	0.1[a]	7.4[a]
S2	4.7[a]	+2.3[a]
S3	1.0[a]	+1.2[a]
S5	2.5[a]	+3.9[a]
SEM	2.41	3.65
p value	0.5	0.2

butyric acid and clostridia spores. Ammonia-N formation is another feature of clostridial activity in silages, particularly of those proteolotical. However, the level of ammonia-N formation in all silages in the present experiment was far below the threshold level of 8 % of total nitrogen for good quality silages (Spörndly, 2003). Knicky and Sporndly (2011) used the mixture of sodium benzoate, potassium sorbate, and sodium nitrate to significantly decrease clostridial growth in low DM forages, as it was demonstrated on low butyric acid and ammonia-N formation. In the present work the clostridial activity in additive treated silages were also significantly reduce as it can be demonstrated on reduced clostridia spore count and lower concentration of butyric acid in additive treated silages. However, result with the ammonia-N was inconsistent. Ammonia-N was significantly reduced only in silages with 2 and 3 L additive application but not in silages with 5L application dosage. These results might be due to decreased proportion of sodium nitrate in the silages additive. As the consequence of reduced activity of clostridia, DM losses of additive treated silages were lower in comparison with control silages. As the growth of clostridia was inhibited by the additives, so more WSC were available to lactic acid bacteria which hence produced more of lactic acid. Aerobic stability is common problem of high DM forages associated with yeasts growth (Woolford, 1990). In our study silages were all silages aerobically stable because of less

activity of yeast. Reduced ethanol production can give evidence of reduced yeast activity in silages (Pahlow et al., 2003). This might be as a result of the antimycotic acitivites of additive particularly sodium benzoate and potassium sorbate (Woolford, 1975). On the other hand, long stability of untreated control silages was due to presence of butyric acid (Weissbach and Haacker, 1988).

During the silage fermentation DM losses are higher than the energy losses (Zimmer, 1967). This is because of energy recovery is higher than the DM recovery during the chemical transformations of substrate in silages (McDonald et al., 1973). In present study, however, the relation between DM losses and energy losses was not completely confirmed. Another unexpected result of the present study was that there were no statistically significant differences in energy losses between treatments neither after fermentation nor after aerobic stability test. This result could be explained by combination of tree reasons. First, there was a large variation in energy concentrations within the silage treatments resulting in large variation of energy losses within treatments. Second, differences in DM losses between silage treatments had not such extent to be reflected in significant variation of energy losses. Third, energy concentration of silages did not differ from energy concentration of fresh forage. This is contrary to McDonald et al. (1973) that energy concentration of silages was regularly higher in comparison with fresh forage. The increase in silage energy concentration is caused by formation of fermentation end products, organic acids in particular. These fermentation end products can increase the gross energy of silage up 10 to 14 % (Derwhust et al., 1986). It is common that clostridia activity in silages causes higher DM losses (Pahlow et al., 2003). However, energy losses in these silages might not be greater in comparison with silages with LAB domination since butyric acid possesses energy value of 24.93 MJ/kg DM whereas lactic acid 15.16 MJ/kg DM (McDonald et al., 1973). Such example can be seen in the present study where control silages had the highest DM losses but they gained energy after the fermentation process.

Conclusion

Improved silage fermentation by the application of silage additives was reflected in reduced DM losses. However, the improvement in silage fermentation had no effect on energy losses formation in silages.

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