EFFECT OF ENSILING RED CLOVER-RYEGRASS USING BLENDS OF HOMO-AND HETEROFERMENTATIVE LACTIC ACID BACTERIA ON FERMENTATION CHARACTERISTICS, AEROBIC STABILITY AND HYGIENIC PARAMETERS

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Abstract. The second cut of a second-year red clover (*Trifolium pretense L.*) and perennial ryegrass (*Lolium perenne L.*) mixture (50:50 on fresh weight basis; 265 g dry matter kg $^{-1}$) was ensiled in 3.0 litre laboratory bottles. The forage was ensiled (I) without additive, and (I1) with homo-and heterofermentative lactic acid bacteria (LAB) blend (*L. plantarum*, *E. faecium* and *L. buchneri*) or (I2) homofermentative LAB blend (*E. faecium*, *L. lactis*, *L. plantarum*). Application rates of the additives I1 and I2 were 250 000 cfu g $^{-1}$ and 150 000 cfu g $^{-1}$ fresh forage weight respectively. Both inoculants significantly increased dry matter content and retained significantly higher water-soluble carbohydrates compared to the uninoculated controls. Bacterial blends I1 and I2 had a positive effect on red clover-ryegrass silages with significantly lower pH and shifting fermentation toward lactic acid with homofermentative LAB (I2) or toward acetic acid with homo-and heterofermentative LAB (I1). Both inoculated silages had significantly lower ammonia-N, butyric acid, alcohols concentrations and in-silo DM loss. The application of both the additives was sufficient to inhibit clostridia, yeast and moulds growth and increased silages aerobic stability.

Keywords: ryegrass/clover swards, silage, homo-and heterofermentative LAB, forage quality, fermentation, aerobic stability.

HOMOFERMENTATYVINIŲ IR HETEROFERMENTATYVINIŲ PIENO RŪGŠTIES BAKTERIJŲ MIŠINIO ĮTAKA RAUDONŲJŲ DOBILŲ IR DAUGIAMETĖS SVIDRĖS SILOSO FERMENTACIJOS SAVYBĖMS, AEROBINIAM STABILUMUI BEI HIGIENINIAMS RODIKLIAMS

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Santrauka. Raudonųjų dobilų (*Trifolium pretense* L.) ir daugiametės svidrės (*Lolium perenne* L.) antrų metų, antros pjūties mišinys (natūralaus drėgnumo žolių santykis 50:50; 265 g kg^{-1} sausųjų medžiagų) buvo silosuotas laboratorinėmis sąlygomis į 3 litrų talpos indus. Silosas gamintas be priedų (I), su homofermentatyvinių ir heterofermentatyvinių pieno rūgšties bakterijų (PRB) mišinio (*L. plantarum*, *E. faecium, L. buchneri*) priedu (I1) ir su homofermentatyvinių PRB mišinio (*E. faecium*, *L. lactis*, *L. plantarum*) priedu (I2). Į silosuojamą žaliavą įdėta priedų I1 ir I2, atitinkamai 250 000 ksv g^{-1} ir 150 000 ksv g^{-1} silosuojamos žaliavos svorio. Abu silosavimui naudoti inokuliantai padidino sausųjų medžiagų kiekį silose ir išsaugojo daugiau vandenyje tirpių angliavandenių palyginti su silosu be priedų. I1 ir I2 bakterijų mišiniai darė teigiamą įtaką raudonųjų dobilų ir daugiametės svidrės siloso kokybei, kuri pasireiškė patikimai mažesniu pH rodikliu. Homofermentatyvinių PRB mišinys (I2) skatino siloso pienarūgščio rūgimo fermentaciją, o homofermentatyvinių ir heterofermentatyvinių pieno rūgšties bakterijų (PRB) mišinys skatino acto rūgšties fermentaciją. Silose su abiejų inokuliantų priedu buvo patikimai mažiau amoniakinio azoto, sviesto rūgšties ir alkoholių palyginti su silosu be priedų. Abu priedai sumažino pašaro maisto medžiagų nuostolius. Bakteriniai priedai patikimai slopino klostridijų, mielių ir pelėsių vystymąsį ir pagerino siloso aerobinį stabilumą.

Raktažodžiai: svidrės/dobilų žolynas, silosas, homo ir heterofermentatyvinės PRB, pašaro kokybė, fermentacija, aerobinis stabilumas.

Introduction. Silage additives have elicited much interest through the years. Inoculants containing lactic acid bacteria (LAB) can improve the fermentation quality and reduce dry matter (DM) losses of silages, providing that the herbage contains sufficient fermentable carbohydrates and the inoculant bacteria dominate the epiphytic population of LAB. Many inoculants have selected strains of homofermentative LAB, such as *L. plantarum*, *E. faecium*, *L. lactis,* and *Pediococcus* spp. LAB can result in a faster decrease in pH, lower final pH values, higher lactate/acetate ratios, lower ethanol and ammonia nitrogen, and a 1 to 2% improvement in DM recovery (Weinberg and Muck, 1996). 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). Driehuis et al. (1999) showed that LAB affected activity of yeasts in two ways, first during anaerobic conditions, the survival of yeasts is reduced and second, during the aerobic exposure, yeast

growth is reduced. Inoculating forages at harvest with a heterofermentative LAB species *L. buchneri* has improved the aerobic stability of the silages (Muck, 1996; Weinberg et al., 1999; Taylor et al., 2002), because under anaerobic conditions this organism converts lactic acid to acetic acid (Oude Elfernik et al., 2001) and that inhibits fungi and thus preserves silages susceptible to spoilage upon exposure to air (Filya, 2003). Recently, *L. buchneri* has been marketed in combination with homolactic acid bacteria, which are commonly added into silages to increase lactic acid production, rapidly drop pH, and decrease DM losses (Kung et al., 2003, Jaakkola et al., 2010).

Thus, the objectives of this study were to determine the effects of inoculants containing new combination of lactic acid-producing bacteria strains (homo-and heterofermentative LAB) on fermentation end-products, hygienic parameters and the aerobic stability of legume-grass silages.

Materials and methods

Test material

Lactic acid bacteria strains rather than formulations were registered as silage additives in the EU. Therefore, trade names are not provided in Table 1. Inoculants were supplied by Chr. Hansen A/S (Hørsholm, Denmark) as freeze-dried powders, sealed aluminium pouches clearly labelled. The test materials were stored at temperatures below 5 ^{0}C until used. Opened pouches were discarded after use.

Crop material and laboratory-silo preparation

The second cut of a second-year red clover (*Trifolium pretense L.*) and perennial ryegrass (*Lolium perenne L.*) mixture (50:50 on fresh weight basis) was harvested with standard field equipment on mid September. The crop was wilted to a dry matter (DM) concentration of 265 g kg $^{-1}$ and chopped by a forage harvester under farm conditions to 2–3 cm length. Chopped forage was transported in a polyethylene bag to the laboratory and ensiled in 3.0 litre laboratory silos. Laboratory experiments started within 2 h from crop preparation. Two silage inoculants were used in the experiment and there were ensiled 30 glass jars (laboratory-silos), 10 untreated and 20 inoculated.

The application rates of the inoculants were in accordance with the level of LAB (lactic acid bacteria) in the inoculant as determined by manufacturer and in accordance with Table 1, column 5. The test materials were suspended in distilled water immediately prior to application. 2.00 g of each inoculant preparation were diluted in 1000 ml of water targeting in a concentration of cells of 2.5 x 10^8 cfu ml^{-1} suspension (I1) and 1.5 x 10^8 cfu ml^{-1} suspension (I2). 1.00 ml of each suspension was used per 1 kg forage (added 3ml water for uniform spraying). The same volume (4 ml per 1 kg forage) of water was used instead of the suspension in the control treatment (for spontaneous fermentation). Subsequently, the additives and water were sprayed into the fresh forage using a spray bottle and the forage was thoroughly mixed. The number of viable bacteria in the water and in the inoculants was determined by enumeration of water and diluted suspension used for inoculating the micro-silos on the day of laboratory-silo filing for enumeration of the added microorganism in order to document correct inclusion (target +/-30%) on DeMan-Rogosa- Sharpe (MRS) agar and Petri dishes incubated anaerobically at 37°C for 48 h. (ISO 15214, Leuchner et al., 2003).

Table 1. **Blends of the bacterial strains which were used as test material**

LAB blend	Bacterial strains	Bacterial proportion, %	Concentration, cfu g^{-1} product	Application rate, cfu g^{-1} fresh forage
I1	*Lactobacillus plantarum* DSM 16568 *Entrococcus faecium* NCIMB 11181 *Lactobacillus buchneri* (CCM 1819)	20 30 50	1.3 x 10^{11}	250000
I2	*Entrococcus faecium* NCIMB 11181 *Lactococcus lactis* NCIMB 30117 *Lactobacillus plantarum* DSM 16568	30 30 40	7.6 x 10^{10}	150000

cfu-colony-forming units; I1- *Lactobacillus plantarum*, *Entrococcus faecium*, *L. buchneri*; I2- *Entrococcus faecium*, *Lactococcus lactis*, *Lactobacillus plantarum*

The density of forage in the silage was in compliance with DLG recommendations, 1 kg DM per 5 litre volume. Hereby, micro-silos (volume 3 l) were filled with 2.11 – 2.22 kg of fresh crop at DM concentration of 265 g/kg. Silos were closed immediately with caps, with a potential to vent gas, 30 min after being filled. Ensiling lasted for 90 days at a constant temperature of 20 °C. At the end of the ensiling period, silages were subjected to chemical analysis and to aerobic stability test.

Sampling and analysis

Five representative samples (>500g each) of fresh chopped forage were collected for subsequent chemical analysis. At the sampling time of silages on day 90 of the ensiling period, five laboratory-silos per treatment (15 jars) were weighed for determination of DM loss and subsequently opened and sampled to analyze the DM content, pH, fermentation products and ammonia. The remaining 15 micro-silos (5 from each treatment) were used for aerobic stability measurement. The DM content of forage and silage was determined by oven-drying at 105°C for 24 h. For the analysis of chemical composition of herbage, samples were oven-dried (1 h at 102°C and

48 h at 50°C) and then ground to pass a 1-mm sieve. Silage DM content was corrected for volatile alcohols and fatty acids during oven drying as described by Weissbach (2009). The total nitrogen was determined by Kjeldahl-AOAC 984.13. A 10.5 g of catalyst was used with Block Digestion and Tecator Kjeltec system 1002 Distilling Unit. Crude protein (CP) content was calculated by multiplying the total nitrogen content by a factor of 6.25. The NDF and ADF concentrations were determined according to Van Soest et al. (1991) by using an Ankom^{200} fibre analyzer (Ankom Technology, Fairport, NY). Water soluble carbohydrates (WSC) were determined by using the anthrone reaction assay (MAFF, 1986), from the herbage or silage extracts obtained from steeping fresh herbage or silage in water. Ash concentration was determined by ashing the samples in a furnace at 600°C for 15 h. Buffer capacity of the forage was determined according to Playne and McDonald (1966), expressed as mequiv of alkali required to change the pH from 4 to 6 per 1 kg of DM. Lactic acid, volatile fatty acid, alcohol, and ammonia N concentrations and pH were determined in silage extracts, prepared by adding 270 g of demineralized, deionized water to 30 g of silage and homogenizing for 5 min in a laboratory blender. Lactic acid, volatile fatty acids and alcohol concentrations were determined by gas-liquid chromatography. Demonized water (3ml) of an internal standard solution (0.5g 3-methyl-n-valeric acid in 1000 ml o.15mol/l oxalic acid) were added to 1ml of filtrate from the above, and the solution filtered through a 0.45 µm polyethersulphone membrane into a chromatographic sample vial for analysis. Gas - liquid chromatograph GC -2010 SHIMADZU were used wide-bore capillary column (Stabilwax®-DA 30m, 0.53mm, ID, 0.5µm) according to Gas Chromatography and Biochemistry Analyzer official methods Ammonia-N concentration was determined by direct distillation using the Kjeltec Auto System 1030 (AOAC 941.04). The pH of silage was measured by using ThermoOrion Posi-pHlo SympHony Electrode and Thermo Orion 410 meter. Weight loss (DM losses) were estimated by measuring differences in silo weights after ensiling (on day 0 after ensiling) and at the end of the ensiling period (on day 90 after ensiling). Presence of *Clostridium perfringens* was quantified on reinforced clostridium agar Petri dishes incubated anaerobically at 37°C for 72 h. (Horizontal method for the enumeration of *Clostridium perfringens* - Colony-count technique (ISO 7937:2004). Amount of lactate-reducing yeasts and moulds at the time of silage unloading was tested on extract-dextrose-chloramphenicol-agar-medium Petri dishes incubated aerobically at 25°C for 3 to 5 days. (Microbiology – General guidance for enumeration of yeasts and moulds – Colony-count technique at 25°C (ISO 7954:1987(E).

After opening the laboratory-silos, all silages were subjected to a 19-day aerobic stability test. A 1000 ± 10 g sample from each silo (five micro-silos from each treatment) was loosely placed into a polystyrene box according to recommendations from DLG and allowed to aerobically deteriorate at constant room temperature (≈20^{0}C). The top and bottom of the boxes contained a 2-cm-diameter hole to allow air to enter and CO_2 to leave. A transducer was placed in the centre of the silage mass through a hole in the cover of the box, which exposed the silage to air. These silages were not disturbed during the period of recording the temperatures. Ambient temperature and the temperature of each silage was recorded every 6 h by a data logger. Ambient room temperature was measured by using an empty control box. Aerobic stability of silages was examined by calculating the differences between silage temperature and ambient room temperature adjusted for base ambient temperature. Aerobic stability was defined as the number of hours the silage remained stable before rising more than 3°C above the ambient temperature (Moran et al., 1996).

Statistical analyses

Five replications were used per additive treatment. Silage composition data were subjected to one-way analysis of variance for a 3 (additive) factorial arrangement of treatments within a randomised complete block design by using Proc GLM of SAS (SAS, 2000). Aerobic stability data for each herbage type were analyzed separately by one-way analysis of variance in a randomised complete block design and where temperatures were treated as repeated measurements. Significance was declared at $P < 0.05$.

Results

The same forage was used for all treatments. The chemical and microbiological composition of the fresh forage is presented in Table 2. Analyses from the fresh crop revealed medium concentration of water-soluble carbohydrates and high concentration of crude protein. The buffering capacity of forages was medium. The crop had a medium count of yeast and moulds.

Enumeration test of the water and diluted suspension with 2 different products as listed under test (Table 1) and used for inoculating indicates that the correct inclusion (target +/-30%) was for the all suspensions. Actual bacteria counts cfu ml^{-1} were: of the control water < $1.0x10^2$, of the suspension I1- 2.5×10^8 (± 0 %), and of the suspension I2- 1.6×10^8 (± 5.3%).

The results of the chemical analyses of the silages after 90 days of ensiling are presented in Table 3. Inoculation resulted in higher ($P < 0.05$) DM concentrations in both examined silages if compared with the control silages. Silage made without additive (I) was poorly fermented, as indicated by its relatively high pH, ammonia-N and butyric concentration and DM loss and low lactic acid concentration. Upon examination of pooled experiment data from both treatments (I1 and I2), strong effects of the inoculants on the fermentation indicators were detected. Silages inoculated with both inoculants showed a lower ($P < 0.05$) final pH as well as lower ($P < 0.05$) DM loss then uninoculated silages. The lowest pH values were observed in silages treated with additive I2.

Table 2. **Chemical composition of a mixture of red clover and perennial ryegrass (50:50) at ensiling**

	n	Mean	Sd
DM, g kg^{-1}	5	265.1	0.553
Crude protein, g kg^{-1} DM	5	174.4	3.061
Crude ash, g kg^{-1} DM	5	112.2	2.129
WSC, g kg^{-1} DM	5	89.5	4.592
ADF, g kg^{-1} DM	5	286.8	6.723
NDF, g kg^{-1} DM	5	360.6	11.908
Buffer capacity, (mEq kg^{-1} DM	5	341.9	46.313
pH	5	6.6	0.031
Clostridia spores, log cfu g^{-1} FM	5	<1.0	0
Yeast, log cfu g^{-1} FM	5	3.8	1.228
Moulds, log cfu g^{-1} FM	5	4.9	0.352

DM- dry matter; FM, fresh matter; WSC- water-soluble carbohydrate; OMD- organic matter digestibility; ADF- acid detergent fibre; NDF- neutral detergent fibre; cfu- colony forming units.

Table 3. **Comparison of the composition and DM* loss of silages after 90 days of ensilage with added blends of bacterial strains or/and other components as silage inoculants**

	I	I1	I2	Average	SE
DM, g kg^{-1}	238^{b}	249^{a}	249^{a}	246.01	1.561
WSC, g kg^{-1}	2.7^{b}	10.7^{a}	6.1^{a}	6.51	1.322
DM loss, %	12.3^{a}	6.7^{b}	6.4^{b}	8.47	0.822
Ammonia-N, % of total N	9.2^{a}	5.1^{b}	4.6^{b}	6.33	0.565
pH	5.55^{a}	4.71^{b}	4.32^{c}	4.86	0.138
Lactic acid, g kg^{-1} DM	13.9^{c}	37.7^{b}	62.6^{a}	38.08	5.542
Acetic acid, g kg^{-1} DM	18.2^{b}	32.1^{a}	23.7^{b}	24.66	1.747
Butyric acid, g kg^{-1} DM	37.5^{a}	1.8^{b}	0.9^{b}	13.42	4.592
Propionic acid, g kg^{-1} DM	0.7^{b}	1.9^{a}	0.4^{b}	1.00	0.207
Alcohols, g kg^{-1} DM	14.7^{a}	5.7^{b}	5.0^{b}	8.50	1.260
Clostridia spores, log cfu g^{-1} FM	1.28^{a}	0.99^{b}	0.99^{b}	1.09	0.052
Yeast, log cfu g^{-1} FM	3.18^{a}	1.31^{b}	1.51^{b}	2.00	0.258
Moulds, log cfu g^{-1} FM	3.00^{a}	1.30^{b}	1.44^{b}	1.91	0.221
Aerobic stability, h	192^{c}	$>450^{a}$	258^{b}	300	29.301

DM and calculated DM losses are corrected for volatiles

$^{a, b, c}$ – Means with different superscript letters in a line indicate significant differences of $P < 0.05$

DM- dry matter; FM- fresh matter; cfu- colony forming units; I1- *Lactobacillus plantarum, Enterococcus faecium, L. buchneri;* I2- *Enterococcus faecium, Lactococcus lactis, Lactobacillus plantarum.*

Inoculation significantly increased lactic acid, and significantly decreased butyric acid, alcohols and ammonia-N concentrations compared with untreated silages. Among inoculated silages the largest ($P < 0.05$) increase of lactic acid was observed in silages treated with product I2, and the highest ($P < 0.05$) increase in acetic acid was detected in silages treated with product I1. Silages treated with product I1 had significantly higher concentration of propionic acid and numerically higher concentration of butyric acid than silages treated with product I2.

There was a significant effect of additives I1 and I2 on clostridial as well as on yeast and moulds growth. In comparison with control (I) silages, inoculated (I1 and I2) silages had significantly lower ($P < 0.05$) clostridia, yeast and moulds count, whereas no differences were observed between products used for silages inoculation.

Irrespective of treatment, all of the laboratory-silo silages were stable for 156 h (6.5 days) after the silos were opened and aerated (Figure 1).

During aerobic exposure, the untreated silage had a temperature rise of more than 3^{0}C above the ambient after 192 h and reached temperatures of more than 7 ^{0}C above ambient temperature after 318 h. Silages treated with product I1 remained stable during the full aerobic stability measurement period and had temperature rise only 2^{0}C above the ambient. Silages inoculated with product I2 had a temperature rise of more than 3^{0}C above the ambient after 258 h. Consequently, inoculant I1 increased silage aerobic stability dramatically and inoculant I2 increased ($P < 0.05$) aerobic stability by 66 h (2.7 days) in comparison with untreated silages.

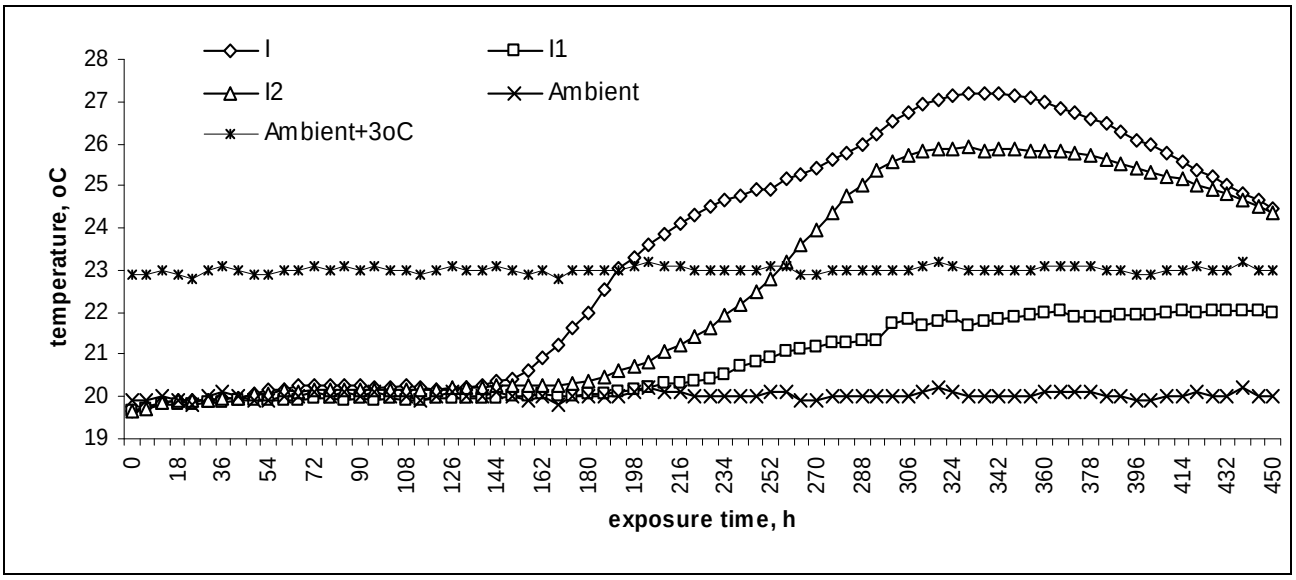

Figure 1. **Aerobic stability of silages after 90 days of ensilage in micro-silos untreated or inoculated with bacteria blends**

I1- *Lactobacillus plantarum, Enterococcus faecium, L. buchneri*; I2- *Enterococcus faecium, Lactococcus lactis, Lactobacillus plantarum*

Discussion

The quality of silage is of great importance because it benefits animal production, animal health and food quality. This study examined the impact of blends of homo-and heterofermentative LAB in low-wilted red clover-ryegrass mixtures. The low DM content (265 g kg^{-1}) in combination with medium concentration (89 g kg^{-1} DM) of WSC and high concentration (174 g kg^{-1} DM) of crude protein of the forage means that the ensiling properties were not ideal as suggested also by McDonald et al. (1991) and Buxton and O'Kiely (2003). Therefore, the trial provided a great challenge for the inoculants to improve silage fermentation. Silages treated with examined inoculations were characterized by higher concentrations of DM compared with the untreated silages and may be explained by the addition of DM to the silages with additives as well as delays in the fermentation processes in the untreated silages, which caused DM losses (McDonald et al., 1991). This also finds conformation in literature (Driehuis et al., 2001).

In the present study, untreated silages were poorly preserved as evidenced by a high pH and high butyric acid and ammonia-N concentrations, all typical of clostridial silages. A lower pH value should be reached for moist forage to prevent clostridial activity (Pahlow et al., 2003). It is probable that fermentable carbohydrates might have been depleted by that time and lactic acid fermentation ceased. Consequently, the pH of the silage increased as a result of butyric acid fermentation (Pursiainen and Tuori, 2008). At the end of the ensiling period, 90 days, inoculation with tested products significantly reduced pH, butyric acid, ammonia-N, alcohols concentrations and DM loss and significantly increased lactic acid concentrations, relative to the untreated silages. The beneficial effect of the inoculants was related to a more rapid and higher acidification that probably reduced the activity of plant enzymes and proteolysis. Lower values of pH in the inoculated silages compared to those of the untreated silages indicate that fermentation was initiated effectively by added LAB. Filya et al. (2007) concluded that main effect of silage inoculants were the increased production of lactic acid connected with significant reduction of pH value and minimised dry matter losses. Weinberg and Muck (1996) stated that LAB can result in a faster decrease in pH, lower final pH values, higher lactate/acetate ratios, lower ethanol and ammonia nitrogen, and a 1 to 2 percentage units improvement in DM recovery. Due to an improved fermentation and conservation effect a significantly lower DM loss was achieved in treated silages in our experiment. Comparable effects have been found for inoculant treatment of grass silages with low DM content (Jones et al., 1992). When the inoculant bacteria improve fermentation, dry matter losses from the silo decrease by 2–3 percentage units on average (Muck, 1996). The applied preparations reduced ($P < 0.05$) the numbers of clostridia, yeast cells and mould fungi in comparison with the control. Nishino et al. (2004) and Kleinschmit et al. (2005) found reduced fungal populations in silages treated with LAB. Mixtures used our experiment suppressed yeast growth was reflected in the concentration of alcohols, generally correlated to yeast activity in silages.

Among the two products used for inoculation there were significant differences in fermentation products. Silages inoculated with heterofermentative LAB *L. buchneri* plus homofermentative LAB (I1) had significantly higher pH and propionic acid concentration, significantly lower lactic acid concentration and lactic to acetic acid ratio when compare with silages inoculated with Homofermentative LAB (I2) used in present experiment. The reduction in the lactic acid with *L. buchneri* was reported by Oude Elferink et al. (2001).

Driehuis et al. (1996, 2001) also reported a marked reduction in lactic acid concentration and an increase in propionic acid concentration in silages treated with *L. buchneri*.

One key factor that influences proteolysis in the silo is the speed of pH decline (Kung et al*.,* 2003). The faster acidification of the silage and higher lactic acid concentration lead to a reduction in proteolysis (Cussen et al., 1995). Multiplication of clostridia and enterobacteria, which are responsible for protein degradation resulting in the formation of ammonia, stops at pH 4.5 (Pahlow et al., 2003). The positive effects of LAB inoculants on nitrogen fractions can be explained either by domination of the fermentation resulting in the rapid achievement of a low pH or via the low proteolytic activity of the strains (Winters et al., 2000).

Irrespective of treatment, all silages were stable for 156 h (6.5 d) after the silos were opened and aerated. Certain plant characteristics could make a positive contribution to wards limiting aerobic spoilage in the silo and legume silages (red clover and lucerne) are more stable than maize or grass silage (Davies et al., 2005). The data presented in this paper show that inoculation with *L. buchneri* in combination with homofermentative LAB (I1) increases the fastness of slight wilted red clover-ryegrass to aerobic deterioration. The amount of undissociated acetic acid has been identified as the most important factor to consider when attempting inhibiting yeast growth in silages (Weissbach, 1996). Weinberg et al. (1999) investigated several homofermentative lactic acid bacteria for their potential on aerobic stability when used as silage inoculants. However, inoculating silages with lactic acid bacteria has not always resulted in silage with good aerobic stability (Kung et al., 2003). Other studies (Danner et al., 2003) provide more definitive evidence for the existence of certain LAB strains with the power to inhibit yeast and moulds growth and to improve aerobic stability.

Conclusions

Blends of homofermentative LAB and blends of homofermentative LAB in combination with heterofermentative LAB *L. buchneri* generally had a positive effect on red clover-ryegrass silages in terms of significantly lower pH and shifting fermentation toward lactic acid with blend homofermentative LAB or toward acetic acid with blend of homo-and heterofermentative LAB.

Both LAB blends used for silages inoculation significantly lowered ammonia-N, butyric acid, alcohols concentrations and in-silo DM loss.

The application of both the additives was sufficient to inhibit clostridia, yeast and moulds growth and increase silages aerobic stability.

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