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The influence of application of a biological additive on the fermentation and nutritive value of lucerne silage

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Abstract

Studies were conducted in 2008 at the Institute of Animal Science of LVA to examine the influence of combined microbial inoculant (*Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Pediococcus pentosaceus*) on the fermentation parameters, nutrient content and aerobic stability of wilted lucerne silages. The silages were made from difficult to ensile, second growth cut lucerne wilted 7–8 h (up to 340 g kg^{-1} DM fresh weight). The lucerne was harvested from five different fields and was ensiled in 0.7-litre laboratory silos when determining pH after 2 days or in 3-litre silos when determining the remaining parameters after 100 days. The lucerne was ensiled without additives (C) or treated with bacterial mix applied at 150 000 cfu g^{-1} fresh weight (I). Three replications without additive and three replications with the added inoculant from each field were evaluated. The addition of the microbial mix resulted in more rapid fall in pH ($P < 0.01$), in a significant ($P < 0.01$) increase in lactic acid concentration and in a significant ($P < 0.05$) decrease in the concentration of undesirable fermentation products such as butyric acid. Inoculation reduced proteolysis of plant proteins, because ammonia-N concentration was lower ($P < 0.01$) in the inoculated silage compared to the control. The treatment with the bacterial mix significantly ($P < 0.01$) reduced dry matter losses; therefore, the inoculated silage had higher preservation of nutrients. However, inoculation had a marginal increase on aerobic stability of silages.

Key words: *Medicago sativa* L., silage, microbial inoculant, fermentation products, aerobic stability.

Introduction

Opportunities for promoting grassland utilisation are related to the positive health characteristics it gives to animal products. Silage is the world's largest fermentation process, with estimated 287 million tons being produced in the EU alone /Wilkins et al., 1999/ It is purpose for dairy and beef cattle farmers to produce more good quality, energy and protein rich silage. The cheapest and highest-value forages involving the lowest energy inputs can be produced from legume and legume-grass swards /Halling et al., 2002/. Obtaining good fermentation quality, digestibility of nutrients and high energy and protein value in silages, requires the regulation of the ensilage process, particularly for herbages with the higher values of buffering capacity /McDonald et al., 1991/.

The key factors influencing the feeding value of silages for dairy cattle include the crop characteristics, stage of development of the crop at ensiling and the extent and type of fermentation achieved within the silo. Silage additives have elicited much interest through the years. It is widely accepted that silage additives can increase animal intake and animal performance through their effect on silage quality /Merry et al., 2000/. However, the market became resistant to acid additives which were considered corrosive to machinery and concrete trench and dangerous to farmers who had to use them. Microbial inoculants are added to silages to direct and promote the fermentation /Weddell et al., 2002; Ziggers, 2003/. For example, classical microbial inoculants containing homofermentative lactic acid bacteria (e.g. *Lactobacillus plantarum*) are often added to silage because they produce large quantities of lactic acid very rapidly, which lowers the pH of silage /Muck, Kung, 1997; Muck et al., 2007/. The advantages of the use of biological inoculants, recently obtained bacterial additives, thanks to the suitable selection of lactic acid bacteria, have been stressed by many authors, and it is clear from the results that inoculants have a beneficial effect on the improvement of the fermentation quality of silages /Wrobel, Zastawny, 2004/. However, classical microbial inoculants can often have no effect or even make the aerobic stability of silages worse /Muck, Kung, 1997; Weinberg et al., 2002/ because high levels of lactic acid alone are not very antifungal. Recently, the aerobic stability of a variety of silage crops has been markedly improved by inoculation with a heterolactic acid bacterium, *Lactobacillus buchneri*. For example, improvements in aerobic stability brought about by this organism have been reported in corn silage /Ranjit, Kung, 2000/. *Lactobacillus buchneri* has been shown to inhibit the proliferation of yeasts in silage via the production of acetic acid, because, aerobic deterioration can be initiated in silage by yeasts and by acetic acid bacteria. Prevention of growth of these organisms is crucial to restrict aerobic deterioration. As the other heterofermentative group, *Lactobacilus brevis* strains were investigated by Danner et al. (2003).

Hereby, the selection of the best strains is essential and based on their ability to rapidly reduce the numbers of contaminant bacteria and fungi and prevent secondary fermentation (increasing aerobic stability).

Lactic acid bacteria that are regularly associated with silage are members of the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus*, *Lactococcus* and *Streptococcus*. They are able to decrease the silage pH to pH 4–5, depending on the species and the type of forage crop /Devriese et al., 1992; Hammes et al., 1992; Holzapfel, Schillinger, 1992; Weiss, 1992/. Based on their sugar metabolism, lactic acid bacteria can be classified as obligate homofermenters, facultative heterofermenters or obligate heterofermenters. Obligate homofermenters produce more than 85% lactic acid from hexoses such as glucose, but cannot degrade pentoses such as xylose. Facultative heterofermenters also produce mainly lactic acid from hexoses, but in addition they also at least degrade some pentoses to lactic acid, and acetic acid and/or ethanol. Obligate heterofermenters degrade both hexoses and pentoses, but unlike homofermenters they degrade hexoses to equimolar mounts of lactic acid, CO_2, and acetic acid and/or ethanol /Hammes et al., 1992; Schleifer, Ludwig, 1995/. Facultative heterofermenters are, for example, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *Enterococcus faecium*. Obligate heterofermenters include

the members of the genus *Leuconostoc*, and some *Lactobacillus* spp. such as *Lactobacillus brevis* and *Lactobacillus buchneri* /Devriese et al., 1992; Hammes et al., 1992; Holzapfel, Schillinger, 1992; Weiss, 1992/.

Selection of microbes for inclusion in a silage inoculant is the principal factor that will influence the impact of the product on silage fermentation and subsequently, animal performance. *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Enterococcus faecium*, *Pediococcus acidilactici* and *Pediococcus pentosaceus* are the most frequently used species, but other species such as *Lactobacillus buchneri* have been considered /Weinberg, Muck, 1996/. Many recently developed inoculants contain multiple species, building on evidence that growth of one bacterial species may facilitate growth of another /Fitzsimons et al., 1992/. For example, *Pedicococcus* spp. and *Enterococcus* spp. grow more rapidly and are more tolerant of high DM conditions than are *Lactobacillus* spp. Rapid growth of *Pedicococcus* and *Enterococcus* increases the rate of acid production in recently ensiled forage, and the rapid decline in pH facilitates the establishment of lactobacilli as predominant microorganisms in the fermentation process. This synergism has only been demonstrated experimentally with combinations of two or three species, however.

Selecting particular strains of a single species for inclusion in an inoculant is likely just as important as deciding what combination of species to include. Several studies have shown that fermentation responses differ widely among strains of the same species /Fitzsimons et al., 1992/. Thus, two products containing identical bacterial species, but different strains, could exert different effects on silage fermentation and, consequently, animal performance.

The trial was performed with difficult to ensile lucerne (*Medicago sativa* L.) forage with the aim of determining the efficacy of a combined premix of micro-organisms and containing bacterial strains (*Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Pediococcus pentosaceus*) on dry matter losses, pH-decrease, concentration of volatile fatty acids (e.g. acetic, butyric and propionic acids), lactic acid, ethanol and ammonia N compared to a negative control after 90 days ensiling at +20ºC.

Materials and methods

Experiment was conducted according to the DLG (Deutsche Landwirtschafts-Gesellschaft e. V. / internationally acknowledged German Agricultural Society) Guidelines for the testing of silage additives and the Guidelines on the assessment of safety and efficacy of silage additives, on a request from the Commission under Article 7 (5) of Regulation (EC) No 1831/2003 (EFSA-Q-2004-088), adopted on 20 April 2006.

Plant material. Harvesting and ensilage. Lucerne (*Medicago sativa* L.) was harvested on 11–12 August from five different fields: field 1 – cv. ‘Europa’, a 2-year-old, second cut; field 2 – cv. ‘Birute’, a 1-year-old, second cut, beginning of the flowering stage maturity; field 3 – cv. ‘Zydrune’, a 3-year-old, second cut, beginning of the flowering stage maturity; field 4 – cv. ‘Verko’, a 2-year-old, second cut, of the flowering stage maturity; field 5 – cv. ‘Birute’, a 4-year-old, second cut, of the flowering stage maturity.

The herbage was cut with a disk mower 'Taarup 2016' and wilted 7–8 h. Fine weather prevailed on the day before cutting and on cutting and wilting day. Pre-wilted lucerne was delivered to the laboratory and chopped. Cut length of herbage was 1.5–2.0 cm.

Preparing of the silages. An exact amount of chopped crop was weighed in a plastic container. Chopped lucerne was ensiled in laboratory 0.7-litre silos when determining only pH after 3 days or in 3-litre silos when determining the chemical composition and the remaining fermentation parameters (lactic, acetic, butyric acid and ammonia-N). The herbage was ensiled without additive (control) or with combined microbial inoculant (*Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Pediococcus pentosaceus*). The additive was sprayed at the same time as the chopped lucerne was mixed and transferred to a new container. The dose of the inoculant was 10 mg bacteria mix per kg of lucerne fresh matter, corresponding to a dose of 150 000 cfu g^{-1} treated forage. The bacteria mix was dissolved and added in water at 4 ml per kg. The control silage was prepared the same way as the additive-treated silage, except that only 4 ml of water kg^{-1} herbage fresh matter was added. Samples of the herbage were collected directly after spraying and at the time of filling the silos.

The chopped, sprayed and mixed lucerne was hand-packed in silos to a volume weight of ca. 400–500 kg m^{-3}. The silos were sealed with a cover and placed in a room with a temperature of +20ºC. Approximately the same amount, 1.3 kg of fresh crop, was filled in each 3-litre silo and 0.3 kg in 0.7-litre silo.

Three replications (of 3- and 0.7-litres silos) without additive and three replications (of 3- and 0.7-litres silos) with the bacterial mix from each field were evaluated.

Sampling and analytical methods. Three forage (herbage) samples were collected from each field immediately after spraying and at the time of filling the silos for analyses. The silos with a volume of 0.7 litres were opened after 3 days (of ensilage) for determining the reductions in pH-value. The silos with a volume of 3 litres were opened after 97 days of ensilage and the silage from each silo was divided into two parts for determination of the chemical composition, fermentation quality (Table 1), and aerobic stability.

Table 1. Analytical methods
1 lentelė. *Analitinių tyrimų metodai*

Quality parameters *Kokybės rodikliai*	Object *Objektas*	Short description, reference *Trumpas metodo aprašymas arba šaltinis*
1	2	3
Dry matter (DM) *Sausosios medžiagos (SM)*	Herbage* *Augalas* Silage** *Silosas*	Oven drying at +67°C for 24 h, equilibrated to room humidity overnight, milled through a 1 mm sieve and further dried at +105°C to constant weight. *Džiovinama 24 val. esant +67 °C temperatūrai, laikoma per naktį kambario temperatūroje, malama naudojant 1 mm sietą ir toliau džiovinama +105 °C temperatūroje iki pastovaus svorio.*

Table 1 continued
1 lentelės tęsinys

1	2	3
Crude protein *Žali baltymai*	* **	Kjeldahl-AOAC 984.13. With block digestion and 'Tecator Kjeltec system 1002' distilling unit. *Kjeldalio metodas AOAC 984.13. Mineralizacija atliekama bloke, naudojant distiliavimo įrenginį „Tecator Kjeltec system 1002".*
Crude fat / *Žali riebalai*	* **	Extraction by 'Soxtec System' using petrol ether +40–60°C. Crude fat residue determined gravimetricaly after drying. *Ekstrakcija su „Soxtec System" įranga, naudojant petrolio eterį (+40–60 °C frakcija). Riebalų ekstraktas nustatomas gravimetriškai po džiovinimo.*
Crude fibre *Žalia ląsteliena*	* **	With Fibercap ('Foss Tecator') using sulphuric acid and Na hydroxide treatment. *Naudojama „Foss Tecator" įranga, ėminys apdorojamas sieros rūgšties ir Na hidroksido tirpalu.*
Acid detergent fibre (ADF) *Rūgštaus detergento tirpalo netirpi ląsteliena*	* **	ANKOM A200 Filter Bag Technique (FBT). *Metodas ANKOM A200, naudojant sieros rūgšties tirpalą.*
Neutral detergent fibre (NDF) *Neutralaus detergento tirpalo netirpi ląsteliena*	* **	ANKOM A200 Filter Bag Technique (FBT). *Metodas ANKOM A200, naudojant neutralų tirpalą.*
Water soluble carbohydrates (WSC) *Vandenyje tirpūs angliavandeniai (VTA)*	* **	Using the anthrone reaction assay from the herbage or silage extracts obtained from steeping fresh herbage or silage in water. *Nustatoma vandens ištraukoje, naudojant antrono reagentą.*
Crude ash / *Žali pelenai*	* **	AOAC Method 942.05. *Metodas AOAC 942.05.*
Buffering capacity *Buferinio tirpalo kiekis*	*	According to Playne and McDonald (1966), expressed as mequiv of alkali required to change the pH from 4 to 6 per 100 g of dry matter. *Nustatomas titruojant vandens ištrauką NaOH (pagal Playne, McDonald, 1966), išreiškiamas mekv NaOH, kurio reikia 100 g sausosios medžiagos pH rodiklį padidinti nuo 4 iki 6.*
Nitrate / *Nitratai*	*	Herbage extracts obtained from steeping fresh herbage in water analyzed using the nitrate ion selective electrode. *Nustatoma vandens ištraukoje, naudojant potenciometrą.*
Lactic acid / *Pieno rūgštis* Acetic acid / *Acto rūgštis* Butyric acid / *Sviesto rūgštis*	** ** **	On an aqueous extract from fresh silage according to the standard methods /Naumann, Bassler, 1997/. *Nustatoma vandens ištraukoje, taikant standartinius metodus /Naumann, Bassler, 1997/.*
Ammonia N *Amoniakinis N*	**	Distillation – AOAC 941.04. *Distiliavimas – AOAC 941.04.*

Table 1 continued
1 lentelės tęsinys

1	2	3
pH after 3 and 97 days *pH po 3 ir 97 dienų*	**	Silage extracts obtained from steeping fresh forage in water analyzed using 'ThermoOrion Posi-pHlo SympHony' electrode and 'Thermo Orion 410' meter. *Nustatomas vandens ištraukoje, naudojant elektrodą „ThermoOrion Posi-pHlo SympHony" ir potenciometrą „Thermo Orion 410".*
DM losses / *SM nuostoliai*	**	Were estimated by measuring differences in silo DM weights before and after ensiling. *Nustatomi pagal silosuojamos medžiagos ir siloso sausųjų medžiagų svorio skirtumą.*

Note. * – three herbage samples for analyses were collected from each field immediately after spraying and at the time of filling the silos, ** – silages from three silos in each treatment (including control) were sampled after 97 days of storage (for pH – after 3 days, additionally).
*Pastaba. * – iš kiekvieno lauko buvo paimta po tris silosuojamos žaliavos ėminius, silosuojamą masę apipurškus darbiniu tirpalu ir pripildant siloso talpas. ** – siloso ėminiai (įskaitant kontrolinį) buvo paimti praėjus 97 dienoms nuo silosavimo pradžios, paimant po tris kiekvieno apdorojimo ėminius (nuo silosavimo pradžios praėjus trims dienoms, nustatyti pH rodikliui buvo papildomai paimta po tris kiekvieno apdorojimo ėminius).*

Determining of aerobic stability. Aerobic stability was measured using data loggers that recorded every four hours temperature readings from thermocouple wires placed in three replicate 200 g silage representative samples aerated in open plastic bags placed into open-top polystyrene boxes (volume about 1.5 litres and wall thickness of 10 mm). There was an opening (diameter 25 mm) in the lid of the box through which the remainder of the plastic bag was pulled and opened so that air could freely pass. Thermocouple wires were inserted into the mid point of silage through the opening. The boxes were kept in constant room temperature (≈ +21°C). Aerobic deterioration was denoted by hours until the start of a sustained increase in temperature of more than +3°C above the ambient temperature.

Statistical analysis. The SAS statistical package was used to analyze the data. Separation of untreated and microbial inoculant-treated means was done in a collected analysis in which the fields were used as one factor (over fields). Three replications (silos) were used per additive treatment. Silos was analyzed as a randomized complete block.

Results and discussion

Description of ensiled herbage. The chemical composition of fresh lucerne before ensiling is summarized in Table 2. Mean (s.e.) DM, crude protein, WSC, nitrate concentration and buffering capacity for wilted lucerne were 340 (2.4) g kg^{-1}, 203 (2.3) g kg^{-1} DM, 49 (2.3) g kg^{-1} DM, 1.3 (0.2) g kg^{-1} DM and 570 (1.6) mEq kg^{-1} DM, respectively. Consequently, the concentration of WSC of wilted lucerne was low and that of crude protein high. Pahlow et al. (2003) presented a figure of 75 g WSC kg^{-1} DM as a lower threshold to establish a good fermentation. Buffering capacity (BC) was

high, but at normal level for second cut lucerne. WSC/BC ratio was 0.8. The lucerne, therefore, was difficult to ferment. Forages with insufficient fermentable substrate or too low dry matter content have WSC/BC ratio <1.0 and a fermentation coefficient <35 /Weissbach, Honig, 1996/. The herbage had a low concentration of nitrate.

Table 2. Chemical composition, buffering capacity and nitrate of lucerne at ensiling
2 lentelė. *Liucernų cheminė sudėtis, buferinio tirpalo ir nitratų kiekis*

Quality indicator of herbage at ensiling and silages *Silosuojamos žaliavos ir siloso kokybės rodikliai*	*n*	Mean value of indicator *Rodiklio vidutinė vertė*	Standard error of the difference between means *Vidurkio paklaida*
Dry matter (DM) g kg^{-1} *Sausosios medžiagos (SM) g kg^{-1}*	30	340	2.36
Crude protein g kg^{-1} DM *Žali baltymai g kg^{-1} SM*	30	203	2.29
Crude fat g kg^{-1} DM *Žali riebalai g kg^{-1} SM*	30	32	1.66
Crude fibre g kg^{-1} DM *Žalia ląsteliena g kg^{-1} SM*	30	305	4.30
Crude ash g kg^{-1} DM *Žali pelenai g kg^{-1} SM*	30	74	1.38
WSC g kg^{-1} DM / *VTA g kg^{-1} SM*	30	49	2.31
NDF g kg^{-1} DM / *SM*	30	410	2.96
ADF g kg^{-1} DM / *SM*	30	322	2.60
Buffering capacity mequiv 100 kg^{-1} DM *Buferinio tirpalo kiekis mekv 100 kg^{-1} SM*	15	570	2.03
Nitrate g kg^{-1} DM / *Nitratai g kg^{-1} SM*	15	1.3	1.60

The nutrient content and fermentation parameters of lucerne silages. Application of the combined microbial inoculant resulted in a significantly higher ($P < 0.01$) dry matter content. Crude fat, crude ash and water soluble carbohydrate (WSC) concentrations did not differ between the treatments. The treatment with the combined microbial inoculant resulted in a significantly higher ($P < 0.05$) crude protein concentration (Table 3).

Compared with the control, inoculated silages had a significantly lower ($P < 0.01$) concentration of the crude fibre, ADF and NDF. These results show that the digestibility and energy value of the inoculated lucerne silage may be higher compared to untreated silage /Ziggers, 2003/. There was no significant treatment effect on the WSC content in the silages.

Good silage depends upon a rapid drop in pH to prevent the growth of clostridia and enterobacteria, which in turn depends upon a rapid and effective fermentation. Obviously, selection of microbes for inclusion in an inoculant is the principal factor that will determine the impact of the product on silage fermentation and subsequent animal performance /Davies et al., 2005/. The criteria which the ideal silage inoculant would

meet were formulated by McDonald et al. (1991), but the core features are generally considered to be the following: rapid growth and successful competition with the natural microflora; homofermentation of sugars, quick production of lactic acid and drop of pH.

Table 3. Chemical composition and fermentation parameters of inoculated and untreated lucerne silages

3 lentelė. *Inokuliuoto ir be priedų užraugto liucernų siloso cheminė sudėtis ir fermentacijos rodikliai*

Measured parameters *Rodikliai*	Untreated control *Kontrolinis variantas (be priedų)*	Combined microbial inoculant treated *Inokuliuotas silosas*	Average *Vidurkis*	Fisher's $LSD_{0.05}$ $R_{0,05}$	Significance *Patikimumo lygis*
Dry matter (DM) g kg^{-1} *Sausosios medžiagos (SM) g* kg^{-1}	326	332	329.0	2.3	**
Crude protein g kg^{-1} DM *Žali baltymai g* kg^{-1} *SM*	207	214	210.6	5.4	*
Crude fat g kg^{-1} DM *Žali riebalai g* kg^{-1} *SM*	50	53	51.3	5.8	NS
Crude fibre g kg^{-1} DM *Žalia ląsteliena g* kg^{-1} *SM*	347	322	334.5	10.4	**
Crude ash g kg^{-1} DM *Žali pelenai g* kg^{-1} *SM*	82	80	81.1	2.7	NS
WSC g kg^{-1} DM / *VTA g* kg^{-1} *SM*	10	10	10.2	1.3	NS
ADF g kg^{-1} DM / *SM*	354	336	345.0	7.4	**
NDF g kg^{-1} DM / *SM*	448	426	436.6	7.0	**
Lactic acid g kg^{-1} DM *Pieno rūgštis g* kg^{-1} *SM*	20	26	23.2	2.7	**
Acetic acid g kg^{-1} DM *Acto rūgštis g* kg^{-1} *SM*	24	23	23.3	3.3	NS
Butyric acid g kg^{-1} DM *Sviesto rūgštis g* kg^{-1} *SM*	5.1	1.0	3.1	3.75	*
Ammonia N g kg^{-1} total N *Amoniakinis N g* kg^{-1} *suminio N*	63	41	52.3	2.1	**
pH after 97 days / *pH po 97 dienų*	4.97	4.53	4.75	0.083	**
pH after 3 days / *pH po 3 dienų*	5.28	4.79	5.03	0.054	**
DM losses g kg^{-1} DM *SM nuostoliai g* kg^{-1} *SM*	85	61.4	73.4	13.2	**

Note / *Pastaba*. * – $P < 0.05$, ** – $P < 0.01$.

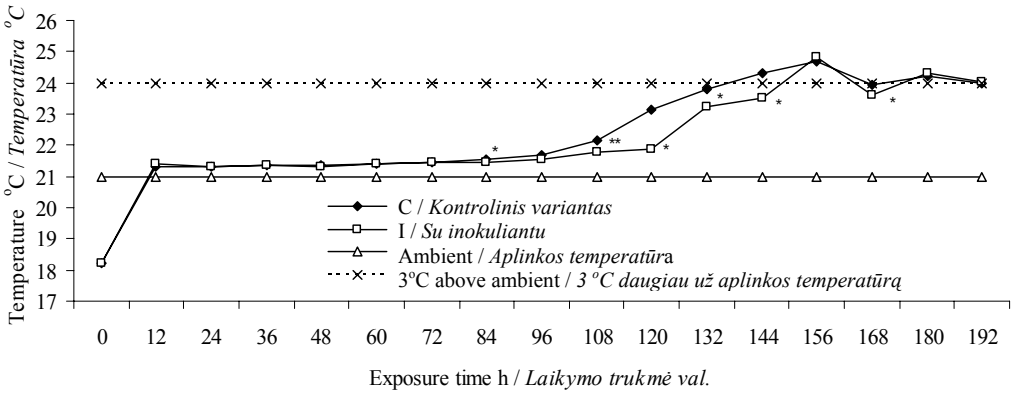
The combined microbial inoculant treatment resulted in the lower ($P < 0.01$) pH value after 3 days of ensilage. The beneficial effect of the inoculant was related to a more rapid acidification that probably reduced the activity of plant enzymes and proteolysis. For the inoculated silage the pH value was significantly lower ($P < 0.01$)

after 97 days of ensilage. There were no significant additive differences with respect to acetic acid concentration. However, compared with the untreated control, lactic acid concentration was higher ($P < 0.01$) in the silage treated with the bacterial mixture. In the four experiments carried out in the Netherlands /Driehuis et al., 1997/ the inoculant treated silages showed significantly lower pH and significantly higher lactic acid concentration than control silages. Butyric acid concentrations were significantly ($P < 0.05$) decreased by application of the combined microbial inoculant. In our experiment ammonia-N concentration was significantly lower ($P < 0.01$) in the inoculated silage compared with the untreated silage. Faster acidification of the silage leads to a reduction in proteolysis /Cussen et al., 1995/. The fermentation processes probably continued during a longer period in the untreated silages than in the inoculated silages possibly resulting in more excessive fermentation of readily degradable sugars and proteolysis of plant proteins. High levels of ammonia-N may depress voluntary intake of silage. The reduced proteolysis of forage protein in bacterial inoculated silage may be beneficial for animal production as well as reduced losses of nitrogen to the environment.

When compared to the untreated silage, dry matter losses were also reduced for the silage treated with the combined microbial inoculant. The treatment with the bacterial mix lowered dry matter losses by 23.6 g kg^{-1} DM ($P < 0.01$) compared with the untreated silage. Lower DM losses for the inoculated silage may be explained by a better fermentation process compared to the untreated silage. Comparable effects have been found for inoculant treatment of grass silages with high DM content /Driehuis et al., 1997/ and lucerne silages /Jones et al., 1992/. When the inoculant bacteria improve fermentation, dry matter losses from the silo decrease 2–3 percentage units on average /Muck, 2000/.

Aerobic stability of lucerne silages. Aerobic stability is one of the major problems of the silages. When the silo is opened to remove the stored material, the ingress of air is inevitable. The pH and temperature of the silo rises as organic acids and residual WSC are degraded, and there is an increase in volatile basic nitrogen. There is also a loss of dry matter content. This aerobic growth rapidly degrades the energy content of the silage, and will often decrease palatability and reduce voluntary intake /McDonald et al., 1991/.

In our study the aerobic stability of the silages, represented as the changes in temperature upon exposure to ambient temperature is presented in Figure. Both the inoculated and the untreated silage were aerobically stable. However, the untreated silage started heating after 78 h, while the inoculated silage started heating after 96 h. The untreated silage had a temperature rise of more than 3ºC above the ambient temperature after 138 h (5.5 days). The temperature of the bacterial mixture treated silages rose by more than 3ºC above the ambient temperature within 150 h (6.5 days). However, inoculating silages with lactic acid bacteria has not always resulted in silage with good aerobic stability /Kung et al., 1991/. In review of the literature, Muck and Kung (1997) reported than inoculation improved aerobic stability in less than 30% of the studies.



Note / *Pastaba*. * – $P < 0.05$; ** – $P < 0.01$.

Figure. Aerobic stability of combined microbial inoculant (I) treated and untreaded (C) liucerne silages

Paveikslas. *Liucernų siloso, pagaminto su bakterijų mišinio priedu ir be jo, aerobinis stabilumas*

Conclusions

The results of the study showed that the addition of combined microbial inoculant (*Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* and *Pediococcus pentosaceus*) improved the fermentation quality and nutrient levels in difficult to ensile lucerne silages:

1. A better fermentation was caused by more rapid fall in pH. The treatment significantly decreased butyric acid content, N-NH_3 fraction and dry matter loss.

2. The results of the study showed that inoculation improved the nutritive value of silages (higher preservation of the DM, crude protein and other nutrients), due to a significantly better fermentation and lower DM losses compared to the untreated silages.

3. Both treated and untreated silages were aerobically stable, however, the treatment with the bacterial mixture had a marginal increase in the aerobic stability of silage.

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Biologinio priedo įtaka liucernų siloso fermentacijai ir maistinei vertei

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Santrauka

Tyrimai atlikti LVA Gyvulininkystės institute 2008 m., siekiant nustatyti inokulianto, sudaryto iš bakterijų mišinio (*Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus buchneri* bei *Pediococcus pentosaceus*), įtaką vidutiniškai pavytintų liucernų siloso fermentacijos rodikliams, maisto medžiagų sudėčiai ir aerobiniam stabilumui. Silosas buvo pagamintas iš sunkiai silosuojamų antrosios pjūties liucernų, nupjautų iš penkių skirtingų laukų žydėjimo pradžioje arba jo metu ir pavytintų 7–8 valandas (iki 340 g kg^{-1} SM). Susmulkintos liucernos buvo silosuotos 0,7 litro laboratorinėse talpose, siekiant siloso pH nustatyti nuo silosavimo pradžios praėjus 2 dienoms, ir 3,0 litrų laboratorinėse talpose, siekiant siloso pH, fermentinių rūgščių, amoniakinio N ir siloso cheminę sudėtį nustatyti nuo silosavimo pradžios praėjus 100 dienų. Liucernos buvo silosuotos be priedų (C) ir su bakterijų mišinio priedu (I), įterpus 150 000 ksv g^{-1} silosuojamos masės. Iš kiekvieno lauko po tris laboratorines talpas (0,7 litro ir 3,0 litrų) silosuota su inokulianto priedu ir po tiek pat – be priedų. Bakterijų mišinio priedas pagreitino siloso rūgimą, nes po silosavimo praėjus 2 dienoms pH rodiklis buvo iš esmės mažesnis ($P < 0,01$), palyginti su be priedų užraugtu silosu. Inokulianto priedas iš esmės padidino ($P < 0,01$) pieno rūgšties kiekį ir iš esmės sumažino ($P < 0,05$) nepageidautinos sviesto rūgšties kiekį. Inokulianto, sudaryto iš pieno ir acto rūgštis gaminančių bakterijų, priedas sumažino baltymų skilimą. Nustatyta, kad amoniakinio N koncentracija buvo iš esmės mažesnė ($P < 0,01$) silose su inokulianto priedu, palyginti su įprastai užraugtu silosu. Bakterijų mišinio priedas liucernų silose iš esmės sumažino ($P < 0,01$) sausųjų medžiagų nuostolius. Bakterinio inokulianto priedas pagerino liucernų siloso aerobinį stabilumą.

Reikšminiai žodžiai: *Medicago sativa* L., silosas, bakterinis inokuliantas, fermentacijos produktai, aerobinis stabilumas.