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# EFFECTS OF A BACTERIAL MIX INOCULANT ON GRASS-LEGUME SILAGE FERMENTATION AND NUTRITION VALUE FOR THE DAIRY COWS

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*ABSTRACT*

*Two silages were prepared from the first cut grass-legume sward consisting of ryegrass, red clover and fescue and wilted for up to 10 h. The control silage had no additives and the experimental silage was prepared with a mix of bacterial inoculant. Both silages were stored in round-bales wrapped with 6 layers of stretch film for 82 days, and silage pH, organic acids, ammonia N and nutrient content were investigated. In control and inoculated silage pH were 4.6 and 4.2, amount of lactic acid 27.6 and 46.1 gkg$^{-1}$ DM (P<0.05), acetic acid 12.3 and 13.7gkg$^{-1}$ DM, and butyric acid 4.7 and 2.2 gkg$^{-1}$ DM, respectively. DM losses were reduced (P<0.01) and DM metabolisable energy concentration was increased (P< 0.01) by the bacterial inoculation. The inoculated silage was more (P<0.05) aerobically stable than the control silage. Ten third lactation Lithuanian Black-and-White cows, in the fifth month of the lactation stage, were used to measure intake of silages and animal performance. Treatment of the silage with inoculant showed a trend towards increased silage dry matter intakes and an improvement in average milk yield of 1.2 kg/day, whereas these differences were not significant. Milk composition was not affected by inoculation, but the output of milk constituents (butterfat and protein) was improved. It is concluded that the increase in milk production with inoculant-treated forage is the result of improvements in both intake of the forage and increasing efficiency of utilisation.*

***Key words:*** *silage, inoculant, fermentation, aerobic stability, dairy cows, milk production*

## INTRODUCTION

Silage is the acid fermented product of anaerobic fodder fermentation, the aim being to preserve summer crops for winter feeding. Silage fermentation is an exceedingly complex process involving interactions among the forage, microbial populations and the ensiling environment [10]. Quality of feeds is characterized via their nutritional value and hygienic quality. Bacterial inoculants are useful means of improving both nutritional value and hygienic quality of silages [5, 6, 12, 13]. Inoculants can increase the rate of acidification and reduce the final pH and protein degradation in silages [4]. The inoculants can increase the rate of acidification and reduce the final pH and protein degradation in silages [5]. Inoculants containing lactic acid bacteria (LAB) can improve the fermentation quality and reduce dry matter (DM) losses of grass silages, providing that the herbage contains sufficient fermentable carbohydrates and the inoculant bacteria dominate the epiphytic population of LAB [8, 11]. 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 workers, and it is clear from the results that inoculants have a beneficial effect on the improvement of the fermentation quality [6, 12, 13] and hygienic quality of silages [5]. While an increasing number of studies have reported positive benefits from using some bacterial inoculants as silage additives, relatively few have reported production responses measured through dairy cows. Gordon (1989) has reported that dairy cattle offered inoculant treated silage achieved 9% higher silage DM intakes and a 10% higher milk yield than those offered untreated silage. However, Chamberlain *et al.* (1987) using the same inoculant found no such responses. According to the research made by foreign experts separate elements of silaging technology such as like technique, storage, supplements of conservation, packing material, etc, influence a lot the rates of fermentation, nutritional materials as well as the level of energy and hygienic conditions of feed. The research also noticed that these separate elements should be applied differently accordingly to geographical situation and climate conditions.

Therefore, this study was aimed at determining the effectiveness of homo-fermentative lactic acid bacteria mix at improving the fermentation and aerobic stability of red clover/grass silage. A further objective was to evaluate feed intake and milk production with diets based on grass-legume silages prepared using new technologies of biological inoculants and big bales.

## MATERIALS AND METHODS

Grass/legume sward (50% *Lolium perenne,* 20% *Festuca pretense and* 30% *Trifolium pretense* (fresh matter basis) was harvested and wilted for up to 10 h. Round-bales (1.2 m diameter) were wrapped with 6 layers of stretch film. Forty-five bales were made without additive (hereafter named Control silage, C) and other 45 bales were inoculated with a mix of bacterial inoculant (*Lactobacillus plantarum* Milab 393, *Pediococcus acidilactici* P6 and P11, *Enterococcus faecium* M74, and *Lactococcus lactis* SR3.54), (hereafter named inoculated silage, L). The inoculant (dosage $5x10^5$ cfu/g fresh herbage; manufacture's advice) was applied using a commercial pump HP-20 (Junkkari, Finland). During the ensiling, samples of green forage were collected to determine the chemical composition. Five control bales and five inoculated bales were weighed inmediately after wrapping and also at day 82 of storage to measure dry matter (DM) losses.These bales were opened at day 82 and samples taken for analyses of fermentation pattern (fermentation acids, ammonia N and pH), chemical composition (standard methods of the AOAC (1995)) and aerobic stability of the silage material.

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 liters 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 days (or hours) until the start of a sustained increase in temperature of more than 2˚C above the ambient temperature.

Ten third lactation Lithuanian Black-and-White cows, in the fifth month of the lactation stage, were used to measure intake of silages and animal performance. During a 3-week pre-experimental period cows were offered *C silage ad libitum* together with the compound feed (75% barley, 10% wheat, 15% soybean meal (DM basis) plus vitamin-mineral premix). The compound feed was fed individually according to the cows' milk yield (280g per kg milk yield). At the end of the pre-experimental period dairy cows were assigned into two groups of 5 cows each. Five pairs were made according to their milk production during the previous lactation, days in milk and milk yield, milk fat, milk protein during pre-experimental period. During the experimental period, which consisted of 100 days, two groups of cows (5 animals each) were offered *ad libitum* either C or L silages in two meals per day. During the experiment, the amounts of silages offered and refused were recorded daily for each cow. Forages were sampled once per collection period and pooled across collection periods, whereas the amount of compound feed was recorded at each meal. ME concentrations of dairy cows diets were estimated using the measured silage data, predicted silage methane energy output and tabulated energy values of concentrates.

Milk yield was recorded every two weeks and aliquot samples from morning and evening milking were bulked and contents of fat and protein analysed. The data were analysed by one-way ANOVA and means compared by Fisher's protected least significance difference (Fisher'PLSD).

## RESULTS AND DISCUSSION

The total fermentation acids and lactic acid concentrations in the control silage (C) were lower by 41% ($P<0.05$) and by 70% ($P<0.05$) respectively than those that were additive-treated (L) Butyric acid and and ammonia-N concentrations were numerically higher in the C treatment than in the inoculant treatment but there were lower concentrations (by 30%, $P<0.05$) of water soluble carbohydrates (WSC) (Fig. 1; Table 1).

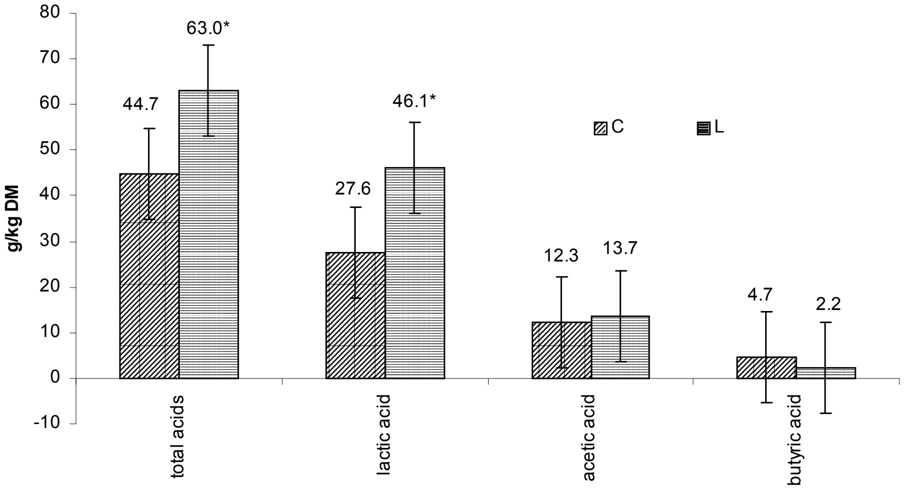


Fig. 1. **Effect of additive treatment on the fermentation characteristics of control (C) and inoculated (L) silages; (asterisk denotes significant differences of means at 0.05 level)**

Table1. **Effect of inoculant treatment on the chemical composition of grass-legume silage**

| Treatment | Green forage | Silages | | SD | Level of significance |
|---|---|---|---|---|---|
| | | C | L | | |
| Dry matter,(DM) g $kg^{-1}$ | 348.3 | 336.8 | 328.4 | 14.70 | ns |
| In dry matter g $kg^{-1}$: | | | | | |
| crude protein | 132.8 | 137.9 | 139.5 | 9.19 | ns |
| crude fibre | 298.6 | 292.4 | 287.1 | 12.58 | ns |
| WSC | 115.3 | 52.3 | 36.9 | 11.23 | * |
| Ammonia N, g $kg^{-1}$ total N | | 45.6 | 35.2 | 8.65 | ns |
| pH | | 4.6 | 4.2 | 0.29 | ns |
| ME, MJ $kg^{-1}$ DM | | 9.9 | 10.6 | 0.36 | ** |
| DM losses, g $kg^{-1}$ DM | | 104.7 | 86.2 | 10.90 | ** |

*Superscripts * and ** denote statistical differences of means at 0.05 and 0.01levels, respectively.*

Driehuis *et al.* (1997) working with silages inoculated with four strains of *L. plantarum* and two strains of *E. faecium* reported lower proportion of ammonia-N and pH and significantly higher concentrations of lactic acid compared to untreated silages and the present study confirms the above. In fact, as compared to the C silage, the L silage had numerically lower ammonia-N indicating reduced proteolysis. Winters *et al., (*2002) found that inoculation with *L. plantarum* improved silage quality and reduced the extent of protein breakdown during ensiling of red clover. Due to the higher fermentation quality the nutrient losses (indicated by DM) were reduced by 18.5% ($P<0.01$) in the L silage compared with the C silage (Table 1). Thus, the metabolisable energy (ME) content of the L silage was 0.65 MJ $kg^{-1}$DM higher ($P<0.01$) than that of the C silage.

The inoculant also increased ($P<0.05$) the aerobic stability (decrease of secondary fermentation, d3 and d4) of the silage (Fig.2). Moreover, the temperature rise (2˚C) during the first two days of ensiling was similar in both silages. Yeast and molds activity in the air-exposed material increase the silage temperature and Driehuis *et al.,* (2001) found that some inoculants can improve the aerobic stability in silages by inhibiting the growth of these organisms.

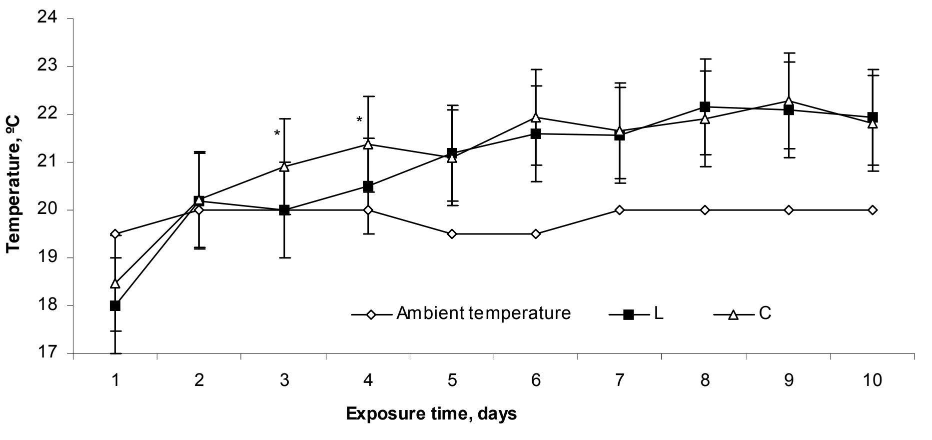


Fig. 2. **Effect of additive treatment on aerobic stability of grass-legume silage (asterisk denotes significant differences of means at 0.05 level)**

Table 2 shows the dairy cows feeding experiment results. The animals receiving the inoculant-treated silage consumed 0.3 $kgd^{-1}$ more silage dry matter and produced 1.2 kg $d^{-1}$ more milk than those given the control silage, whereas, these differences were not significant. Martinsson (1992) reported that the inoculant-treated silage increased silage DM intake by 7% during weeks 4–12 of lactation and attributed the effect to the higher digestibility of the inoculated silage. Castle and Watson (1973) concluded that an increase of one percentage unit in digestible organic matter in the DM would result in an increase in silage DM intake of 0.25 $kgd^{-1}$. Higher silage dry matter intake and better performance of animals were found by Gordon (1989).

The contents of milk-fat and milk-protein did not differ between the silages. Nevertheless, due to higher milk yields the output of milk fat and milk protein for cows offered the L silage were slightly higher than outputs of cows offered the C silage, but differences were statistically not significant (Table 2).

Table 2. **Effects of different treated grass-legume silages on feed intake and milk production**

| Treatment | Silages | | SD | Level of significance |
|---|---|---|---|---|
| | C | L | | |
| Silage intake, kg DM $cow^{-1}$ $day^{-1}$ | 11.8 | 12.1 | 0.87 | ns |
| Total DMI, kg $cow^{-1}$ $day^{-1}$ | 16.4 | 17,0 | 1.59 | ns |
| Total ME intake, MJ | 172 | 186 | 20.15 | ns |
| Daily milk production | | | | |
| Milk, kg $cow^{-1}$ $day^{-1}$ | 18.4 | 19.7 | 2.01 | ns |
| ECM, kg $cow^{-1}$ $day^{-1}$ | 17.9 | 19.1 | 2.09 | ns |
| Milk composition | | | | |
| Fat, g $kg^{-1}$ | 38.3 | 38.0 | 11.02 | ns |
| Protein, g $kg^{-1}$ | 34.1 | 34.0 | 9.12 | ns |
| Milk constituent output | | | | |
| Fat, g $day^{-1}$ | 706 | 747 | 39.51 | ns |
| Protein, g $day^{-1}$ | 627 | 668 | 7.94 | ns |

## CONCLUSIONS

The homo-fermentative lactic acid bacteria mix additive was effective at increasing lactic acid concentration in silage, at preventing butyric acid fermentation and DM losses, also it was the effective treatment for curtailing proteolysis and enhancing aerobic stability. These studies confirmed the higher intake potential and milk production from inoculated grass- legume silage compared with spontaneous fermented silage. Inoculated silage led to a 1.2 kg/d increase in milk yield in comparison with spontaneous fermented silage.

It is, therefore, concluded that homo-fermentative lactic acid bacteria mix, which contained *Lactobacillus plantarum* Milab, *Pediococcus acidilactici*, *Enterococcus faecium* M74, and *Lactococcus lactis*, is a promising inoculant for grass-legume silage.

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# BAKTERIJŲ MIŠINIO PRIEDO ĮTAKA VARPINIŲ-ANKŠTINIŲ ŽOLIŲ SILOSO FERMENTACIJAI IR MAISTINEI VERTEI BEI MELŽIAMŲ KARVIŲ PRODUKTYVUMUI

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Santrauka

Buvo tiriamas pieno rūgštį produkuojančių homofermentatyvinių bakterijų mišinio (*Lactobacillus plantarum* Milab, *Pediococcus acidilactici*, *Enterococcus faecium* M74, and *Lactococcus lactis*) priedo efektyvumas 10 val. pavytintų varpinių-ankštinių žolių siloso, pagaminto ritiniuose, fermentacijos rodikliams, pašaro aerobiniam stabilumui ir melžiamų karvių produktyvumui. Kontrolinio siloso ir siloso su inokulianto priedu pH rodiklis buvo atitinkamai 4,6 ir 4,2, pieno rūgšties – 27,6 ir 46,1 gkg$^{-1}$ SM, acto rūgšties – 12,3 ir 13,7gkg$^{-1}$ SM, sviesto rūgšties – 4,7 ir 2,2 gkg$^{-1}$ SM. Inokulianto priedas sumažino (P<0,01) SM nuostolius ir padidino (P<0,01) energijos koncentraciją pašaro sausojoje medžiagoje bei pagerino siloso (P<0,05) aerobinį stabilumą. Šėrimo bandymas atliktas su dešimčia trečios laktacijos ir penkto laktacijos mėnesio Lietuvos juodmargių veislės melžiamų karvių. Inokulianto priedas pagerino siloso ėdamumą ir 1,2 kg per dieną padidino pieno primilžius, tačiau šie skirtumai statistiškai nepatikimi. Silosas su inokulianto priedu neturėjo esminės įtakos melžiamų karvių pieno sudėčiai, tačiau vidutiniškai iš vienos karvės buvo gauta daugiau pieno riebalų ir pieno baltymų. Galima teigti, kad karvių produktyvumas padidėjo dėl geresnio inokuliuoto siloso suėdimo ir geresnio jo maisto medžiagų įsisavinimo, lyginant su įprastai užraugtu silosu.

**Raktažodžiai:** silosas, inokuliantas, fermentacija, aerobinis stabilumas, karvė, pieno produkcija

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# ВЛИЯНИЕ БАКТЕРИАЛЬНОЙ СМЕСИ НА ФЕРМЕНТАЦИЮ СИЛОСА ИЗ ЗЛАКОВО-БОБОВОЙ ТРАВЫ И ПРОДУКТИВНОСТЬ ДОЙНЫХ КОРОВ

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Резюме

В опыте изучали влияние смеси гомоферментативных бактерий (*Lactobacillus plantarum* Milab, *Pediococcus acidilactici*, *Enterococcus faecium* M74 и *Lactococcus lactis*) на ферментацию силоса, его аэробную стабильность и продуктивность дойных коров. Силос был приготовлен в рулонах из 10 часов провяленной злаково-бобовой травы без добавок или с добавкой смеси гомоферментативных молочнокислых бактерий (*Lactobacillus plantarum* Milab, *Pediococcus acidilactici*, *Enterococcus faecium* M74 и *Lactococcus lactis*). В контрольном силосе и в силосе с добавкой инокулянта показатель pH составил соответственно 4,6 и 4,2, молочной кислоты – 27,6 и 46,1 г/кг$^{-1}$ СВ, уксусной кислоты – 12,3 и 13,7 г/кг$^{-1}$ СВ, масляной кислоты – 4,7 и 2,2 г/кг$^{-1}$ СВ. Добавление инокулянта снизило ($P < 0,01$) потери СВ и повысило ($P < 0,01$) энергетическую ценность СВ и аэробную стабильность силоса по сравнению с контрольным силосом. Опыт по кормлению силоса проведен с десятью дойными коровами на третьем и на пятом месяцах лактации. Дойные коровы поедали больше силоса, приготовленного с инокулянтом, и их среднесуточная продуктивность была на 1,2 кг больше по сравнению с коровами, получавшими контрольный силос. В итоге от одной опытной коровы получено в среднем больше молочного жира и молочного белка по сравнению с коровами контрольной группы. Результаты опытов показали, что продуктивность дойных коров повысилась из-за лучшей поедаемости и усвояемости инокулированного силоса по сравнению с контрольными.

**Ключевые слова:** силос, инокулянт, ферментация, аэробная стабильность, корова, молочная продукция

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