EFFECT OF *LACTOBACILLUS RHAMNOSUS* AND *PROPIONIBACTERIUM FREUDENREICHII* INOCULATED SILAGE ON NUTRIENT UTILIZATION BY DAIRY COWS

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Summary. First cut red clover-grass mixture sward were ensiled in two pits with inoculant (*Lactobacillus rhamnosus + Propionibacterium freudenreichii ssp. Shermanii*) and without any additives. Fermentation quality, nutrient losses and aerobic stability of silages were determined. The inoculated silage had no butyric acid, nutrient losses were lowered by 19.4%. In inoculated silage organic matter digestibility and energy values were significantly higher compared to the ordinary one. In addition, a feeding study to compare influence of inoculated and non-inoculated silage on milk yield, yield of energy corrected milk (ECM), milk fat and milk protein was conducted. Ten lactating dairy cows were divided randomly into 2 groups each of 5 cows. First group of cows were fed inoculated silage (experimental) and second group was on non-inoculated silage (control). The lactating dairy cows in experimental group consumed on average 0.89 kg^{-1}DM more compared to controls. Further, inoculated silage fed cows tended to increase the milk yield and ECM was by 2.1 $kg^{-1}day^{-1}$ cow higher compared to non-inoculated silage fed cows. In cows on inoculated silage the output of milk fat and milk protein was by 84.8 and by 58.6 g $day^{-1}cow^{-1}$ higher compared to cows on non-inoculated silage ($P<0.05$).
Key words: silage, *L. Rhamnosus*, *Propionibacterium Freudenreichii,* fermentation quality, milk yield, ECM.

MITYBINIŲ MEDŽIAGŲ PANAUDOJIMAS ŠERIANT MELŽIAMAS KARVES SILOSU, PAGAMINTU SU *LACTOBACILLUS RHAMNOSUS* IR *PROPIONIBACTERIUM FREUDENREICHII* PRIEDU

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Santrauka. Raudonųjų dobilų ir varpinių žolių mišinio silosas buvo pagamintas dviejose tranšėjose su bakterinio inokulianto (*Lactobacillus rhamnosus +Propionibacterium freudenreichii* ssp. *Shermanii*) priedu ir be jokių priedų. Buvo tirta siloso fermentacijos rodikliai, mitybinių medžiagų nuostoliai ir pašaro aerobinis stabilumas. Silose su inokulianto priedu nerasta sviesto rūgšties, o mitybinių medžiagų nuostoliai jame buvo 19,4 proc. mažesni nei įprastai užraugto siloso. Biologiškai apdoroto siloso mitybinės medžiagos buvo virškinamos geriau, dėl to jo mitybinė vertė buvo didesnė negu siloso be priedų. Melžiamos karvės inokuliuoto siloso sausųjų medžiagų vidutiniškai per parą suėdė 0,89 kg daugiau, nei siloso be priedų ir iš jų primelžta 2,1 kg 4 proc. riebumo pieno daugiau, nei iš kontrolinių karvių. Taigi iš karvių, šertų inokuliuotu silosu, gauta vidutiniškai per parą 84,8 g pieno riebalų ir 58,6 g baltymų daugiau negu iš karvių, gavusių įprastai užraugtą silosą.
Raktažodžiai: silosas, *L. Rhamnosus, Propionibacterium Freudenreichii,* fermentacijos rodikliai, karvės pienas.

Introduction. Cattle production in Lithuania generally involves indoor feeding seven month per year. Due to the short grazing period and long indoor feeding period in Lithuania, conserved forages play an important role in ruminant feeding. Over 70% of feed used is roughage, of which 60-70% is preserved as grass and legume grass 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). Silage fermentation is an exceedingly complex process involving interactions among the forage, microbial populations and the ensiling environment (Muck and Shinners, 2001). The quality and feeding value of the silages depend on the crop characteristics on the one side and the run of in silo fermentation, on the other. Successful silage production depends upon the promotion of the fermentation brought by beneficial bacteria (Ziggers, 2003). Compared with grass, pure lucerne and red clover, as well as grass-legume mixtures, have a high feeding value because of high intake characteristics, high digestibility and high concentration of protein, Ca and Mg (Campling, 1984). The advantages of legumes as one of the main nitrogen sources and valuable winter forage are still underused. In particular, legume-based systems are known to contribute to sustainable, environmentally-friendly and energy-efficient agriculture (Porqueddu et al., 2003). Opportunities for promoting grassland utilisation are related to the positive health characteristics it gives to animal products. 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. Studies (Playne et al., 1966) have confirmed that clovers have approximately twice the buffering capacity of the ryegrass and this is clearly an important factor associated with the difficulties encountered in the ensilage of leguminous crop. A variety of silage additives have been developed to assist the producer in making high quality silage. Silage additives can be divided into two major groups, namely, fermentation inhibitors (e.g. organic acids) and fermentation stimulators (e.g. strains of *lactobacillus* and enzymes) (Mc Donald et al., 1991, Additives). 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 of silages (Fychan et al., 2002; Winters et al., 2002; Wrobel et al., 2004). An experiment was conducted to compare the effects of ensiling red clover-grass mixture untreated and treated with biological additive on silage quality and to examine the nutritive value of these silages.

Materials and methods

A legume-grass mixture was used in the experiment (64% to red clover (*Trifolium pratense* L.) cv. Arimaiciai, 12% - timothy (*Phleum pretense* L.) cv. Gintaras, 16% - meadow fescue (*Festuca pratensis* Huds.) cv. Kaita and 8% - others) on second (2) year's use. The sward was cut at the flowering stage of red clover on 20 June, 2004 with the mower conditioner *Kverneland 347*. The crops were allowed to prewilt for approximately 30 h before they were chopped with a chop harvester (E-281). The inoculant (based on two patented bacterial strains: *Lactobacillus rhamnosus* LC 705 (DSM 7061) and *Propionibacterium freudenreichii* ssp. *Shermanii* JS (DSM 7067), Finland) dosage 10^6 cfu g^{-1} was applied using a commercial pump "HP-20" in the chopper. Dry inoculant was mixed with water according to the instruction and applied at the rate 5 l/t. The grass was ensiled in the period, when the weather was good (temperature 18-21^{0}C), without rain. The herbages was ensiled in two ferro-concrete pits 100 t each (one – inoculant free, another – with inoculant). During the ensilage, samples of chopped grass were collected to determine its chemical composition (AOAC, 1995). Five control bags of 1 kg weight each were put into each pit to determine dry matter (silage fermentation) losses. Ensiling was finished in a 2 days time and the pressed mass was covered tightly with plastic.

The feed from the pits was offered to animals on 10 December, 2004. After withdrawal and weighing of the control bags, the chemical composition, fermentation quality and dry matter losses of silages were determined. Aerobic stability was measured by changes in silage temperature following exposure to air for 10 days. A representative sample (200 g) of each silage was placed in an open plastic bag that was subsequently placed into a polystyrene box (volume about 1.5 l, and 10 mm wall thickness). There was a 25 mm round opening in the lid of the box through which the rest of the plastic bag was pulled and opened so that air could freely pass. A thermal probe was inserted into the mid point of silage through the opening. Boxes were kept in a room with constant temperature (≈ 20^{0}C). The temperature of the samples was measured once daily, following exposure to air for 10 days.

Ten dairy cows of the Lithuanian Black-and-White breed were used in the experiment. A three-week pre-experimental period was used in which untreated silage was offered *ad libitum* together with the compound feed. Compound feed (consisting at 75% barley, 10% wheat, 15% soybean meal and vitamin-mineral concentrate *4923 Optima Dairy Extra)* to cows was fed individually according to the milk yield (310g for 1 kg 4% milk). In experimental period (100 days) each group consisting of five cows was fed its respective silage *ad libitum* offered in two meals per day. The weight of the offered silage was determined once weekly on two consecutive days and refusals were weighed back and subtracted when calculating daily intake. The amount of compound feed was recorded at each meal. Milking of cows was performed twice daily in the stable. Milk yield was recorded for two consecutive days every two weeks and aliquat milk samples from morning and evening milk were bulked and content of fat, protein was analysed. The data were analysed by one-way ANOVA, and a mean comparison by Fisher'PLSD.

The feeding trial was performed in pursuance with the Lithuanian animal care, management and operation legislation (No 8-500, 6 November 1997).

Results and discussion

The chemical compositions of the herbages and the silages are given in Table 1. The activity of the inoculant was evidenced in this experiment by higher water soluble carbohydrates (WSC) by 18.5g kg^{-1} ($P<0.05$), total acids by 9.81 g kg^{-1} ($P<0.05$) and lactic acid by 21.23 g kg^{-1} ($P<0.01$) and lower acetic acid by 10.41 g kg^{-1}($P<0.05$), butyric acid by 1.03 g kg^{-1}($P<0.01$) contents of the inoculated silage compared with the untreated silage. There were found (Driehuis et al., 2001) that silages inoculated with *L. buschneri* plus *Pediococcus pantosaceus* and *L. plantarium* had significantly higher concentrations of lactic acid and lower concentrations of acetic acid. As compared with the untreated silage, the inoculant reduced proteolysis. Lower protein breakdown occurred in the inoculated silage as indicated by the lower ($P<0.05$) ammonia –N content. Winters et. al., 2002 found that inoculation with *L. plantarium* improved silage quality and reduced the extent of protein breakdown during ensilage of red clover. Due to the higher fermentation quality the nutrient (DM) losses were lower by 19.4% ($P<0.01$) in the inoculated silage compared with the untreated one. *In vitro* organic matter digestibility of inoculated silage was 760 g kg^{-1}DM and that of the untreated silage 748 g kg^{-1}DM. The inoculation had a positive effect on the nutritional value of silages, however, the digestible energy of the inoculated silage was higher by 0.67 MJ g kg^{-1}DM ($P<0.01$) compared with untreated. The results of the study Olt A. et al., 2005; Rajčáková L. et al., 2005 showed that inoculation with *Lactobacillus* sp. and *Propionic bacterium* ensiling material, rich in red clover, improved fermentation and silage quality, also decreased DM losses.

Table1. **Chemical composition of herbage and silages and fermentation quality of silages**

Treatment	Herbage	Silages C	Silages I	$LSD_{0.05}$	$LSD_{0.01}$	$S_{\bar{x}}$
Dry matter, (DM) g kg^{-1}	327.8	318.6	331.5	27.377	39.835	2.582
In dry matter g kg^{-1}:						
organic matter	938.5	933.8	937.5	5.303	7.716	0.174
crude protein	124.4	125.8	130.1	14.872	21.64	3.564
crude fibre	214.2	227.3	221.1	9.03	13.139	1.235
WSC	111.5	28.5	47.0*	17.066	24.833	13.85
NDF	490.8	513.6	502.6	14.352	20.883	0.866
ADF	294.1	335.9	322.0	17.813	25.918	1.66
total acids		67.68	77.49*	6.948	10.109	2.935
lactic acid		40.01	61.24**	8.681	12.631	5.258
acetic acid		26.25	15.84*	9.103	13.245	13.26
butyric acid		1.30	0.27**	0.700	1.018	27.38
Ammonia N, g kg^{-1} total N		39.96	33.18*	4.81	6.999	4.033
pH		4.39	4.23*	0.133	0.193	0.946
ME, MJ kg^{-1} DM		8.21	8.88**	0.233	0.339	0.836
DM losses, g kg^{-1} DM		101.81	82.02**	8.302	12.08	2.77

* and ** denotes significant at level 0.05 and 0.01 respectively.

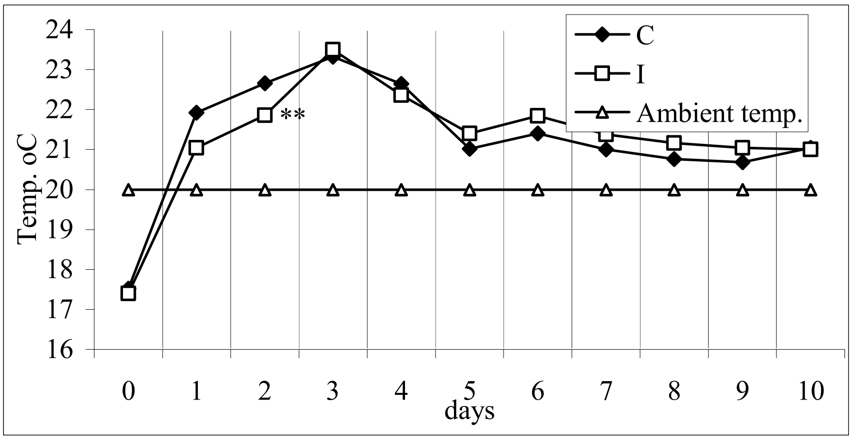

Fig.1. **Changes of temperature in silages (* denotes significant at level 0.05.)**

The inoculant was not found to have a negative influence on air stability of the silage (Fig.1). Both inoculated and untreated samples increased in temperature by more than 3^0C after 3 days from the start. The temperature of inoculated and untreated silages rose above the ambient temperature within 1 day, and the untreated silage had a temperature rise of more than 2^0C within 1 day while the inoculated silage had a temperature rise of more than 2^0C in more than 2 days. Other authors found that some inoculants can improve the aerobic stability in silages by inhibiting the growth of both yeast and molds in silages (Driehuis et al., 2001).

As shown in Table 2 inoculation gave the higher (by 0.89 kg cow^{-1} day^{-1}) dry matter intake than the untreated silage. Milk yield was affected due to the higher intake and the higher nutritive value of the inoculated silage. Average milk yield was higher by 13.2% for the inoculated silage diet compared with the untreated silage. Higher silage dry matter intake and better performance of animals were found by Winters *et al.,* 2001. The results showed that milk fat and milk protein did not differ markedly between the treatments. Due to higher milk yield the output of milk fat and milk protein were higher respectively by 84.8 and by 58.6 g $day^{-1}cow^{-1}$ for cows fed the inoculated silage compared with the untreated silage.

Table 2. **Intakes, yields and composition of milk of dairy cows fed inoculated and untreated silages**

Treatment	Silages		$LSD_{0.05}$	$LSD_{0.01}$	$S_{\bar{x}}$
	C	I			
Silage intake, kg DM cow^{-1} day^{-1}	13.34	14.23	3.769	6.25	6.963
Total DMI, kg cow^{-1} day^{-1}	18.58	19.97	5.30	8.789	7.003
Total ME intake, MJ	175.94	200.31	50.663	84.012	6.859
Daily milk production					
Milk, kg cow^{-1} day^{-1}	15.57	17.67	2.854	4.732	4.373
ECM, kg cow^{-1} day^{-1}	15.92	18.03	4.579	7.594	6.869
Milk composition					
Fat, g kg^{-1}	41.06	41.30	7.782	12.904	4.813
Protein, g kg^{-1}	33.44	32.92	1.902	3.154	1.46
Milk constituent output					
Fat, g day^{-1}	646.2	731.0	232.34	385.28	8.593
Protein, g day^{-1}	521.4	580.0	104.299	172.954	4.819

Conclusions

1. The use of biological additive in ensiling prewilted material, rich in red clover, improved fermentation and silage quality: increase total fermentation acids ($P<0.05$) and lactic acid ($P<0.01$) contents and decrease acetic acid ($P<0.05$) and butyric acid ($P<0.01$) contents. Lower protein breakdown occurred in the inoculated silage as indicated by the lower ($P<0.05$) ammonia –N content.
2. Due to the higher fermentation quality the nutrient (DM) losses were lower by 19.4% ($P<0.01$) in the inoculated silage compared with the untreated one.
3. The digestible energy of the inoculated silage was higher by 0.67 MJ g kg^{-1}DM ($P<0.01$) compared with untreated.
4. Feeding silage with *L. rhamnosus* and *Propionibacterium freudenreichii* ssp. *Shermanii* JS was beneficial to productivity of dairy cows - i.e. the yield of energy corrected milk (ECM) was higher by 2.1 $kg^{-1}day^{-1}$, and output of milk fat and milk protein were higher respectively by 84.8 and by 58.6 g $day^{-1}cow^{-1}$ for cows fed the inoculated silage compared with the untreated silage.

However, the current data are not yet sufficient to identify a silage inoculant efficacy. More research is needed to study the interactions between feeding status of the animal and the effect of LAB silage inoculants on animal performance, and the interactions between LAB from silage and rumen microorganisms and fiber digestibility.

Acknowledgements. This work is supported by Lithuanian State Science and Studies Foundation (Research Project Nr P-30/06).

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