IMPROVEMENT OF GRASS SILAGE QUALITY BY INOCULANT WITH LACTIC BACTERIA AND ENZYMES

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Abstract. The effect of applying lactic acid bacteria (LAB) and enzymes at ensiling of legume-grass and performance of fattening bulls was studied under farm conditions in experimental department of Lithuanian Institute of Animal Science. The silages were made from second cut legume-grass (72% - red clover, 20% - timothy, 8% - other, DM content – 180 gkg^{-1}). Legume-grass was harvested on 24-25 August 2002 and ensiled in bales. The inoculant "Feedtech" (2 Pediococcus acidilactici, 2 Lactobacillus plantarum and Cellulase) improved the quality of fermentation of the legume-grass (with low DM content) big bale silage. The water-soluble carbohydrates in both silages decreased with pH, more in the control, than the in inoculated. The application of an inoculant significantly enhanced the lactic acid content.

However, acetic acid and ammonia-N concentration were lower with inoculated silage and inoculation was effective in reducing butyric acid fermentation and dry matter losses, and increasing silage intake and energy content in 1 kg DM of silage. Fattening bulls fed inoculated silage showed 8.3% higher live weight gains than bulls on untreated silage.

Keywords: silage, big bales, inoculant, fermentation, feeding value, fattening bulls.

ŽOLĖS SILOSO KOKYBĖS GERINIMAS PIENO RŪGŠTIES BAKTERIJOMIS IR FERMENTŲ PRIEDU

Santrauka. Bandymo metu buvo tiriama siloso, pagaminto iš ankštinių–varpinių žolių mišinio turinčio mažą sausųjų medžiagų kiekį, ritiniuose pridedant pieno rūgšties bakterijų ir fermento celulazės cheminė sudėtis, fermentacijos kokybė ir jo įtaka penimų buliukų augimui. Silosas buvo pagamintas iš antros pjūties ankštinių–varpinių žolės mišinio (72% – raudonieji dobilai, 20% – motiejukai ir 8% – kitos žolės), 1 kg turinčio 180 g sausųjų medžiagų. Žolė į pradalges nupjauta 2002 m. rugpjūčio 24–25 d. Iš jos pagaminti siloso ritiniai, kurie apvynioti 6 sluoksniais specialios plėvelės. Dėl lietaus silosuojamos masės nebuvo galima pavytinti. Inokuliantas ”Feedtech“ (2 štamai *Pediococcus acidilactici*, 2 štamai *Lactobacillus plantarum* ir fermentas celulazė) pagerino siloso fermentacijos kokybę. Inokulianto priedas padidino silose pieno rūgšties kiekį ir sumažino acto rūgšties, sviesto rūgšties ir amoniakinio azoto kiekį. Silose su ”Feedtech“ priedu pH buvo optimalesnis – jame liko daugiau cukraus, lyginant su kontroliniu silosu. Dėl kryptingo fermentacijos proceso silose su inokulianto priedu sausųjų medžiagų (rūgimo) nuostoliai buvo mažesni ir 1 kg siloso sausųjų medžiagų buvo daugiau apykaitos energijos bei baltymų.

Penimi buliukai, šerti silosu su inokulianto priedu, suėdė daugiau ir augo 8,3% intensyviau, nei gavę įprastai užraugtą silosą.

Raktažodžiai: silosas, ritiniai, inokuliantas, fermentacija, mitybinė vertė, penimi buliukai.

Introduction. Today, silage is the world's largest fermentation process, with an estimated 287 million tonnes being produced in the EU alone (Wilkins et al., 1999). The quality and feeding value of the silages depending of the crop characteristics on one side, and on run of in-silo fermentation, on the other Successful silage production depends upon the promotion of the fermentation brought by beneficial bacteria (Ziggers, 2003). Good management practices such as cutting the forage at the correct stage of maturity, careful fertiliser usage, sufficient chopping, ensiling in good weather, wilting if possible, avoiding soil contamination, rapid ensiling, well maintained silo structures, adequate compaction, efficient sealing with weighed down good quality plastic and care removal silage from silo during feeding are critical (Seale, 2002). Common practice in many countries to use silage additive to control the silage fermentation. Traditionally, organic acids, mostly formic, have been used to achieve preservation by acidification (McDonald et al., 1991). There have been a number of advances in silage inoculants over the past 20 years, probably the most significant recent development being the on-farm freshly cultured approach (Merry et al., 1995).

Inoculants have a major advantage over acids for the silage makers in that they are safe to handle and environment and do not corrode expensive harvesting machine. The inoculants control the natural fermentation and they are now the main type of additive used in many countries (Weddell et al., 2002). The ensiling process may also be improved by the addition of cellulolytic enzymes (Filya et al., 2002). The research outlined in this paper describes how the inoculant "Feedtech" ("Medipharm", Sweden) improve legume-grass, with low dry matter content, silage quality in big bales and resulted in measurable higher fattening bulls performance on farm scale.

Materials and Methods. The experiment run at the experimental department of Lithuanian Institute of Animal Science on 2002-2003. Second cut legume-grass

grass (72% - red clover, 20% - timothy, 8% - other, DM content – 180 gkg^{-1}) was cut in swath with self-propelled harvester E-281 on 23-24 of August 2002. Round big bales were made using baler GREENLAND-RF-130 and wrapped with 6 layers of stretch film (width 70 cm) using equipment "Elko 1410". 50 round big bale silages were made without any additives and 50 – treated with inoculant "Feedtech" (2 Pediococcus acidilactici, 2 Lactobacillus plantarum and Cellulase). The inoculant was applied about 10^6 cfu g^{-1} grass. Baler was equipped with a HP-20 additives applicator and inoculant "Feedtech" were applied by spraying on swath. Due to the rain DM content was low. During the ensilage, samples of herbage were collected to determine its chemical composition. Five additive free bales and five inoculated bales were weight after wrapping and after 70 days storage for measuring DM losses. Bales were opened and samples collected after 70 days storage and silages fermentation quality and chemical composition were measured.

Grass and silage samples were chopped and subsampled prior to analysis. Grass and silage dry matter (DM) content was determined by drying at 67°C for 24 h, equilibrated to 100 m humidity overnight, milled and further dried at 104^0C to the constant weight. The conventional feed analysis was performed by standard methods. Crude protein was analysed according to the Kjeldahl method with the apparatus *Kjeltec System 1002* (Foss Tecator, Sweden). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed using the *Ancom* (USA) filter bag technology with the *Ancom* fibre analyzer (according to the instruction given by the company *Ancom).* Volatile fatty acids, lactic acid, ammonia-N and sugar were determined on an aqueous extract for fresh silage. Fatty acids were determined by the Vigner method (1969), ammonia N by the AOAC 920.03 method, using *Kjeltec system 1002* (Foss Tecator, Sweden). Silage pH was determined electrometrically.

Feeding trial (126 days) with fattening bulls was carried out. Fourteen Lithuanian Black-and-White bulls divided into two groups were used. The average weights of the bulls in the control and in the experimental group were 312.0 kg. In order to become accustomed to the feed, the bulls underwent a 24-days adaptation period before the start of the trial. During the adaptation period the bulls in both groups were fed *ad libitum* with same silage without additives and concentrate.

All bulls were weighed at the end of the adaptation period in order to determine the weight gain of bulls fed with identical silage, which was one of the criteria for selecting bulls for the trial in two groups. The average weight and weight gain in one group were almost identical, with those in the other group. Each bulls was placed in separate pens.

During the entire trial bulls of control group (C) were offered *ad libitum* silage without additives and bulls of experimental group (F) were offered *ad libitum* inoculated silage in two meals per day. The concentrates used to feed the experimental as well as the control bulls had the same composition and nutritional value and were offered individually, according to weight in two meals per day. A watering-trough placed in each pen ensured unlimited access to drinking water for all bulls.

Silages offered was recorded once per two weeks on two consecutive days and refusals were weighed back in morning and subtracted to give daily intakes. Compound feed offered was recorded during every meal. Animals were weighed on two consecutive days once per month of trial and on the last day of the experiment.

Results and discussions. Due to the rain legume grass used for big bale silage had a law dry matter of 178.9 g kg fresh material.

Table 1. **Chemical composition of the second cut legume-grass and fermentation quality of silages**

	Herbage	Silages		sed	sig
		C	F		
Dry matter (DM; g kg^{-1})	178.9	214.4	237.3	6.21	NS
In DM (g kg^{-1} DM):					
Organic matter	927.0	910.0	915.0		
Crude protein	166.3	146.4	165.6	3.70	*
Crude fibre	250.0	270.4	231.2	4.21	NS
WSC	97.1	43.9	45.9	1.5	NS
NDF	510.2	517.6	504.3	2.1	NS
ADF	401.2	400.4	393.9	1.9	NS
D-value		622.0	661.0	3.4	NS
Total organic acids		60.60	63.74		
Lactic acid		32.94	49.79	2.7	*
Acetic acid		27.16	13.85	0.7	**
Butyric acid		0.50	0.10	1.2	NS
Ammonia N (g kg^{-1} N)		9.67	8.81	0.45	NS
pH		4.90	4.52	0.03	***
ME MJ kg^{-1} DM		8.67	9.59		
FU kg^{-1} DM		0.79	0.96		
DM losses, %		11.41	9.28	1.2	NS

Statistical significance of probably levels: *P < 0.05; **P < 0.01; ***P < 0.001.

Crude protein and water soluble carbohydrates contents of 166.3 and 97.1 g kg $^{-1}$ DM respectively. For the legume-grass of second cut and low dry matter content inoculant "Feedtech" was efficient and improved the quality of fermentation by enhancing the production rate and concentration of lactic acid (Table 1).

Well fermentation of inoculated silage reflected by pH value and lactic acid concentration. Inoculated silage show lower by 0.38 unit pH value and higher by 3.14 g kg^{-1} DM total organic acid concentration and higher by 16.85 g kg^{-1} DM ($P < 0.05$) lactic acid content.

However, acetic acid concentration was by 13.31 g kg^{-1} DM ($P < 0.01$) lower with inoculant treated silage compared to these made without other treatment. Positive was also the effect of inoculation decreasing the proteolysis which achieved ammonia-nitrogen concentration in the non-treated silage 9.67 g kg^{-1} N and in biologically treated silage – 8.81 g kg^{-1} ($P < 0.001$). These results indicate the value of "Feedtech" in improving the quantity and quality of protein in legume-grass silage (Davies et al., 2002). Inoculation was effective in reducing butyric acid fermentation by 0.4 g kg^{-1} DM in comparison with control silage. Inoculant "Feedtech" contains enzyme cellulase, which degrade cellulose and hemicellulose. Due to the enzyme activity the content of crude fibre in inoculated silage was by 39.2 g kg^{-1} DM lower and content of NDF by 13.3 g kg^{-1} DM lower compared to untreated silage. Nutrient (dry matter) losses were lowered by 2.13%, and digestible value by 6.2% and energy value by 10.6% were higher for the inoculated silage in comparison with the ordinary silage.

Seale (2002) claims fermentation benefits observed include a higher lactic acid and residual sugar content and lower contents undesirable fermentation products such as acetic and butyric acids, ammonia-nitrogen and reduction in dry matter losses in silage, when inoculants were been used.

The results of feeding trials indicate that silage dry matter intakes were consistently higher feeding inoculated silage than the untreated (8.47 *vs* 7.8 kg DM per animal per day) (Table 2).

Table 2. **Voluntary intake of silages and diet composition**

	Group of bulls		
	C	F	sed
Fed intake kg $^{-1}$ DM day:			
Untreated silage	7.86	-	0.07
Inoculated silage	-	8.47	0.07
Hay	0.85	0.85	0.00
Molasses	0.52	0.52	0.00
Concentrate	1.86	1.86	0.00
Nutritive value of diet:			
Dry matter, kg	11.09	11.70	-
Metabolizable energy, MJ	106.6	111.5	-
Fed units	9.28	9.83	-
Crude protein, g	1357.2	1427.8	-
Digestible protein, g	950.1	1028.0	-
Crude fibre, g	2482.5	2315.5	-
Crude fat	261.5	266.2	-
Sugar, g	923.8	967.7	-
Ca, g	102.0	115.5	-
P, g	80.3	91.4	-

The higher intake of inoculated silage can be explained by better silage quality, because "Feedtech" treated silage contain more less butyric acid, acetic acid and ammonia-nitrogen then ordinary silage. Animal performance benefits were closely linked with improvement in silages fermentation quality and energy contents (Rook et al., 1990) and may be the key factor explain why inoculant "Feedtech" improved fattening bulls performance (Fig. 1).

Due to the higher value of metabolizable energy and protein and silage intake growth rate of bulls fed inoculated silage was by 8.39% higher than of those fed untreated silage. Animals in both groups consumed the some daily amount of concentrated feed (1.86 kg^{-1} DM), molasses (0.52 kg^{-1} DM) and hay (0.85 kg^{-1} DM) per animal per day.

Conclusions. The "Feedtech" inoculant improved fermentation patterns of legume-grass with low dry matter content silage in bales: there were higher lactic acid content and lower acetic acid, butyric acid and ammonia-nitrogen concentration compared to these made without other treatment.

Containing cellulose enzyme in inoculant reduced crude fibre content and improved organic mater digestibility.

Higher preservation of nutrients in inoculated silage, due to a better fermentation, reduce dry matter losses and increase energy concentration. Trial consistently show that the intake of fattening bulls fed inoculant treated silage was higher, they receive more energy content silage and show better performance.

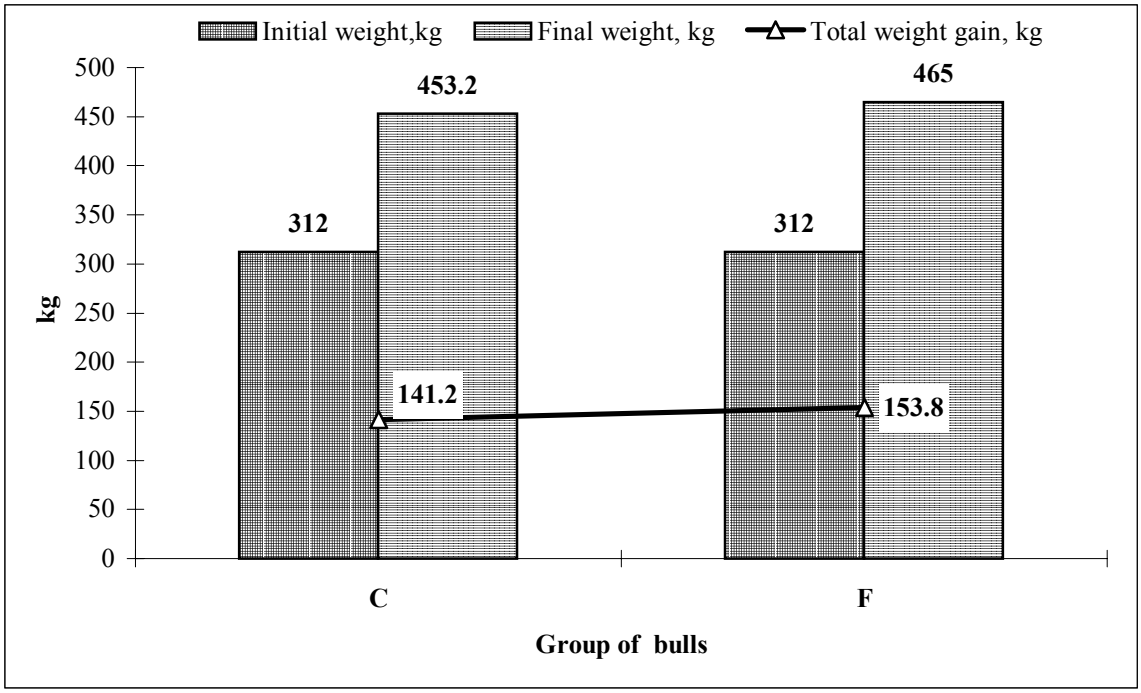

Fig. 1. **Fattening bulls performance**

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