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The Effect of a Silage Inoculant on Silage Quality, Aerobic Stability and Milk Production

Research Article

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ABSTRACT

A silage inoculant [Biomin® BioStabil Plus, BSP, a blend of *Enterococcus faecium* (DSM 3530), *Lactobacillus brevis* (DSM 19456) and *Lactobacillus plantarum* (DSM 19457)], was used on legume-grass silage [32% of dry matter (DM)] *vs.* an untreated control silage (CT). The material had mean crude protein (CP) and water soluble carbohydrate (WSC) concentrations of 174 and 88 g/kg, respectively. BSP resulted in significantly higher CP (159 *vs.* 149 g/kg DM; P<0.05) and digestible protein concentrations (117.8 *vs.* 108.9 g/kg DM; P<0.01). Inoculant increased fermentation rates, resulting in a significant decrease in pH (P<0.05) and a significant increase of total fermentation acids concentration (P<0.05), as well as higher quantity of lactic acid (P<0.01) and higher content of acetic acid compared to CT. Butyric acid and Ammonia N concentrations were significantly decreased (P<0.01) through the use of BSP. Dry matter (DM) losses were significantly lower (P<0.01) using BSP treated grass-legume silages. The digestible energy (P<0.01) and net energy lactation (P<0.05) were higher in the inoculated silage compared to the CT (2.1 and 1.25%, respectively). The inoculation of silage with BSP also improved stability. Twenty-four dairy cows were fed with both type of silages and their productivity response was evaluated over a 92-days feeding period. Animals were assigned to two treatments in a randomized-block design experiment. Silage was offered *ad libitum* to animals in both treatments and cows were supplemented with 4 kg DM of a commercial compound feed per day. The silage DM feed intake was higher in BSP (+6.5%), whereas the energy corrected milk (ECM) production for treated silages increased by 1.4 kg per cow per day. The milk fat and protein content were numerically higher in animals under the treatment with BSP and the efficiency of the conversion of feed-NEL into milk was significantly higher (P<0.05) in cows which were fed with the silage treated using BSP.

KEY WORDS aerobic stability, inoculant, milk, production, silage.

INTRODUCTION

The production of feed is seasonal. This means there are seasons in which the farmer has an overproduction and other seasons in which there is a lack of feed. To balance this deficit of feed, the farmer must preserve part of the feed produced in favorable seasons in order to guarantee stable animal production throughout the whole year.

Ensiling is an old method of preserving feed from deterioration. It is an anaerobic process in which water soluble carbohydrates (WSC) are converted, mainly into lactic acid, by lactic acid bacteria. The importance of ensiling process is increasing day to day by farmers, because the process is not as dependent on the climate conditions as, for example, hay making. In order to better understand the fermentation and to achieve other collateral effects, such as longer aerobic stability, many farmers are currently using silage inoculants (Weddell *et al*. 2002; Wilkins *et al*. 1999; Ziggers, 2003). Their use is an expanding market in which silage quality, and no longer just quantity, is the main focus of interest for dairy farmers (Kramer, 2002; Merry *et al*. 2000; Weinberg *et al*. 2004).

The aim of this trial was to study the effect of silage additive Biomin® BioStabil Plus (BSP) based on the fermentation, aerobic stability and nutritive value of first cut grass-legume silage, as well as the effect on the feed intake and dairy cows milking productivity in Lithuanian farming conditions.

MATERIALS AND METHODS

In the trial mixed grass-legume swards (35% *Lolium perenne*, 15% *Phleum pratense*, 45% *Trifolium pratense* and 5% others species in a second cut) were wilted for 6-8 hours to 320 g DM/kg and then were ensiled. The chemical composition of the material before ensiling process is presented in Table 1. The concentrations of WSC and crude protein (CP) were moderate and the concentration of nitrate was low. The calculated fermentation coefficient was 49, >45 indicating a good fermentability according to results reported by Pahlow *et al*. (2002).

The swards were cut and picked with a precision chop forage harvester (theoretical particle length of 30 mm) and wilted for 6-8 hours. The grass-legume swards were treated during the collection from field after wilting with BSP [Biomin® BioStabil Plus, a blend of *Enterococcus faecium* (DSM 3530), *Lactobacillus brevis* (DSM 19456) and *Lactobacillus plantarum* (DSM 19457)], guaranteeing a level of at least $2x10^5$ colony forming units per gram of forage (1 gram in 1 liters of water/ton) *vs*. a control treatment (CT) without inoculation. After weighing the material, it was transferred to one of two ferro-concrete trenches (100 t each). Five control bags, each filled with 1 kg of ensiling mass were put into each silo to determine DM losses. The silos were then filled within 12 hours and were covered with a polyethylene sheet which was weighted with a 20 cm layer of chopped straw.

Representative samples of harvested and wilted grass-legume mixtures were taken throughout harvesting. Silages were sampled several weeks during the feeding experiment (from 20/11/2008 to 11/03/2009), which began 90 days after ensiling. During each sampling, two samples (approximately 500 g each) were taken 40-50 cm deep from the cut surface, by coring vertically to the full depth of the silo using a 50 mm silage corer.

The following parameters were determined in the original material and in the silage samples: dry matter (DM) and chemical composition, buffer capacity and nitrate, volatile fatty acids, lactic acid and ethanol content, as well as the ammonia-N content. DM losses were calculated by measuring differences in weight of the silo control bags before and after ensiling.

Aerobic stability was also measured using data loggers which recorded the temperature once every six hours from thermocouple wires placed in three replicate 200-g silage representative samples, which were aerated in open plastic bags and placed into open-top polystyrene boxes (volume about 1.5 L and 10 mm wall thickness). The boxes were kept at a constant room temperature (21˚C). Aerobic deterioration was denoted by hours in which the temperature of the silage did not surpass the ambient temperature by more than 2 ˚C.

Twenty-four Lithuanian black-and-white dairy cows were selected for the experiment from a larger group (from a herd of 120 dairy cows) according to parity, lactation stage, date of calving, present milk yield, last year's milk yield, and live weight using a multi-criteria method. During a three week period of housing, prior to the experimental period, selected animals were fed with untreated (control) silage, similar to that which was fed during the experiment. At the start of the trial, the animals were randomly allocated to two groups of twelve cows. Animals were group-fed, bedded on straw and had access to water *ad libitum*. During the experiment, fresh silages were provided two times a day. The daily silage intake was calculated once a week over two consecutive days. Common commercial compound feed was individually fed by cows twice a day and intake was recorded.

Cows were milked twice a day and their total milk yield was registered once a day. Once a week milk samples were taken from the morning and evening milking and the fat, protein, lactose contents and the count of somatic cells were analyzed in milk from each animal.

Data was analyzed using variance analysis to test the effect of silage treatments. For the feed intake and feed conversion rates, a group of 12 cows were selected as the experimental unit. For milk yield, milk protein yield and milk fat yield each cow, within a group, was considered as the experimental unit. The Fisher's least significant difference (LSD) procedure at the 5% significance level was used to determine differences in treatment means. A probability of $0.05<P<0.10$ was considered a near-significant trend.

RESULTS AND DISCUSSION

I. Ensiling trials

There were not significant differences in DM, crude fiber, nitrogen free extract (NFE), acid and neutral detergent fiber (ADF and NDF) content (Table 2) between the untreated and treated silages.

Table 1 Chemical composition, buffering capacity and nitrate content of material at ensiling

Parameter	Unit	n	Average	Standard deviation	SEM*
Dry matter (DM)	g/kg	5	320.2	3.147	1.407
Crude protein (CP)	g/kg DM	5	174.3	3.458	1.546
Digestible protein		5	132.1	3.227	1.443
Crude fat		5	17.2	1.356	0.606
Crude fiber		5	254.9	10.834	4.845
Nitrogen free extract		5	508.0	14.683	6.566
Crude ash		5	45.6	5.009	2.240
Water soluble carbohydrates (WSC)		5	88.34	8.684	3.884
Buffering capacity	mEq100/g DM	3	39.8	1.127	0.651
Nitrate	g/kg DM	3	0.405	0.1005	0.058
Digestible energy (DE)	MJ/kg DM	5	13.5	0.074	0.033
Net energy for lactation (NEL)		5	6.68	0.068	0.031

* SEM: standard error of the mean.

Table 2 Effect of Biomin® BioStabil Plus treatment on the chemical composition of ensiled grass-legume swards

Parameters	Unit	Treatments		Standard error	P-value
		Control X±SD	BSP X±SD		
Dry matter (DM)	g/kg	315.4±3.12	319.2±5.96	1.072	0.079
DM losses	g/kg DM	106.2±6.30	88.3±6.75	3.565	**
Crude protein (CP)		149.4±6.37	159.0±6.91	1.732	*
Digestible protein		108.9±5.92	117.8±6.42	1.611	**
Crude fat		25.3±2.45	23.6±1.55	0.469	0.057
Crude fiber		284.4±10.09	276.5±7.64	2.049	0.053
Nitrogen free extract (NFE)		470.1±8.82	469.7±6.94	1.652	0.907
Crude ash		70.7±5.04	71.2±4.51	0.997	0.826
Water soluble carbohydrates (WSC)		9.4±1.43	10.7±2.39	0.433	0.139
Neutral Detergent Fiber (NDF)		456.3±32.72	455.6±30.17	6.548	0.955
Acid Detergent Fiber (ADF)		331.9±35.55	326.2±17.03	5.833	0.635
Digestible Energy (DE)	MJ/kg DM	13.05±0.10	13.32±0.09	0.035	**
Net Energy Lactation (NEL)		6.42±0.09	6.50±0.07	0.019	*

* and ** denote statistical significance at level 0.05 and 0.01, respectively.

However, treatment with BSP resulted in significantly lower DM losses (+17.9 g/kg of DM, P<0.01), significantly higher CP (159 *vs.* 149.4 g/kg of DM; P<0.05) and digestible protein concentrations (117.8 *vs.* 108.9 g/kg of DM; P<0.01). Kramer (2002) found fermentations differences in the ensiled material related to higher DM losses in control treatment by presence of homofermentative bacteria than in BSP by presence of heterofermentative bacteria. Higher CP content was also found in silages treated with an inoculant according to results presented previously by Jatkauskas and Vrotniakiene (2006) for grass-legume mixtures and Winters *et al.* (2002) for red clover swards. A quick reduction in the silage pH limits the breakdown of protein due to inactivate plant proteases (Kung, 2000). The digestible energy (DE) and net energy lactation (NEL) were also significantly higher in the treatment with BSP compared to the control (+0.27 and +0.08 MJ/kg DM, respectively). The treatment with BSP increased fermentation rates, resulting in a significant pH decrease (P<0.05) and a significant increase in the concentration of total fermentation acids (P<0.05) compared to the control silage (Table 3).

The inoculant produced more lactic acid (P<0.01), which reflected the results obtained by Filya *et al.* (2000), Muck *et al.* (2007), and Muck and Kung (1997), and numerically higher acetic acid content compared to that of the control silage. Kramer (2002) gave a reference value of 1% for acetic acid in fresh matter for a proper aerobic stability and good silage intake whereas gave an average value of 2-3% in DM.

The butyric acid content and the ammonia nitrogen were significantly 10 times lower when BSP was used (P<0.01, in both cases) compared to control. Butyric acid is the main product of the *Clostridia* metabolism, which can be controlled by a quick and deep acidification (Kramer, 2002 and Kung, 2000).

Ohmomo *et al.* (2002) found no butyric acid in well fermented inoculated silages (pH of 4.1-4.2) while silages which were not well inoculated contained certain amounts of that acid. In more than 60% of reviewed literature, Muck and Kung (1997) reported lower ammonia nitrogen contents in silages treated with inoculants compared to those untreated.

The non-inoculated control silage was already heated after 54 hours and after 108 hours and it reached a temperature higher than the ambient temperature by 2 ˚C (Figure 1). The maximum temperature (23.5 ˚C) in the control silage was reached within 120 hours from the start of exposure to air. Increased concentrations of acetic acid in silage treated with BSP had a positive effect on the aerobic stability of the silage (Danner *et al.* 2003 and Weinberg *et al.* 2004). The temperature rise in inoculated silage was lower and first heated after 102 hours. However, no temperature rise of 2 ˚C over the ambient temperature was observed during the 10 day of exposure to air. This is related to a higher acetic acid content, which stops yeast growth. Classical microbial inoculants, containing only homolactic bacteria, have no effect and can even deteriorate the aerobic stability of the silage (Muck and Kung, 1997; Weinberg *et al.* 2002).

For example, Inglis *et al.* (1999) found no positive effect on the aerobic stability when using a blend of homolactic lactic acid bacteria. Several authors (as Ranjit and Kung, 2000; Danner *et al.* 2003) have discovered that heterolactic lactic acid bacteria positively improve the aerobic stability of silage.

II. Feeding trials

Silages and DM intake are presented in Table 4. Even though these parameters are not statistically significant in most cases, there are tendencies which show the advantage of using a silage inoculant. On the basis of the data recorded during the experimental period (92 days), the feed intake of silage DM was higher by 6.5% for treated silage than that of the untreated silage being this in relation with the results pointed by Winters *et al.* (2001).

Table 3 Effect of Biomin® BioStabil Plus treatment on the fermentation characteristics of ensiled grass- legume swards

Parameters	Unit	Treatments		Standard error	P-value
		Control X±SD	BSP X±SD		
pH	-	4.38±0.09	4.25±0.08	0.023	*
Total organic acids	g/kg DM	67.16±7.49	76.62±8.60	1.970	*
Lactic acid	g/kg DM	36.74±5.26	44.15±5.93	1.419	**
Acetic acid	g/kg DM	28.23±3.18	32.17±5.43	1.021	0.051
Butyric acid	g/kg DM	2.15±1.98	0.23±0.36	0.362	**
Ethanol	g/kg DM	7.87±1.16	7.06±0.69	0.217	0.059
Ammonia N	g/kg total N	57.5±7.24	46.0±4.03	1.746	**

* and ** denote statistical significance at level 0.05 and 0.01, respectively.

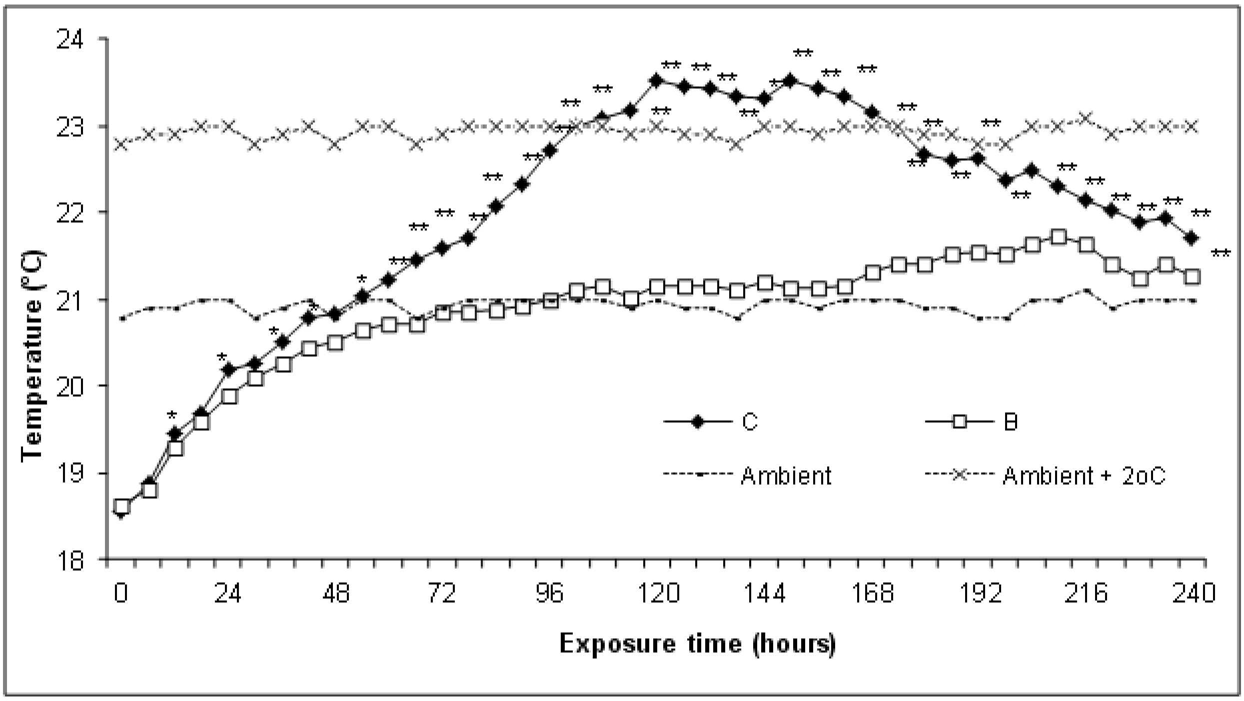

Figure 1 Stability of grass-legume silages, untreated (C) or treated with Biomin® BioStabil Plus (B)

* and ** denote statistical significance of means at 0.05 and 0.01 levels, respectively

The intake of compound feed did not differ as it was restricted to a certain amount for both treatments. The energy intake (DE and NET) was also higher for the silage treated with BSP (+6.1 and 5.3%, respectively) compared to the untreated control treatment (CT). Milk production was also higher for animals fed with treated silage than for those fed with control treatment (+1.1 L/cow/d). The Energy Corrected Milk (ECM) production was also higher in the BSP treatment (+1.4 kg/cow/d). Weinberg *et al.* (2004) reported a milk production increase of 3-5%. Kung and Muck (1997) reported increased milk production in approximately 50% of the reviewed studies, with a statistically significant average improvement of +1.41 kg/cow/d. The feed conversion, calculated as the quotient between the NEL intake and the ECM production, denoted better efficiency in the conversion of energy into milk in the treatment with the BSP inoculant due to cows fed with the treated silage needed less energy (5.77 MJ NEL/1 kg of ECM) than others fed with an untreated silage (5.93 MJ NEL/1 kg of ECM). This difference of 0.16 MJ was of high statistical significance (P<0.01), in spite of the fact that the differences in the parameters silage intake and milk production were not statistical significant. This could be explained by the high individual internal variation reflected in the standard errors for those parameters (1.26-1.51 and 2.40-2.69 for silage intake and ECM production, respectively). However, the standard errors in feed conversion were relatively low (0.08-0.09) since the parameters silage intake and ECM production apparently varied proportionally. According to Weinberg *et al.* (2004), the feed efficiency can be increased by up to 9%. The milk composition and the somatic cell count are shown in Table 5.

Table 4 The effect of inoculant Biomin® BioStabil Plus on silage intake, milk yield and feed conversion

Parameters	Unit	Treatments		Standard error	P-value
		Control X±SD	BSP X±SD		
Silage intake	kg DM/cow/d	10.7±1.51	11.4±1.26	0.288	0.225
Compound feed	kg DM/cow/d	4.0±0.61	4.0±0.49	0.110	0.988
Total dry matter intake	kg DM/cow/d	14.7±2.12	15.4±1.74	0.394	0.382
Total digestible energy intake	MJ/cow/d	200.9±29.06	213.2±24.16	5.487	0.272
Total net energy lactation intake	MJ	103.0±14.94	108.5±12.33	2.792	0.341
Daily milk production	kg/cow/d	16.6±2.21	17.7±2.33	0.467	0.254
Daily energy corrected milk (ECM) production	kg/cow/d	17.4±2.69	18.8±2.40	0.531	0.183
Feed conversion (FC)	MJ NEL /1 kg ECM	5.93±0.08	5.77±0.09	0.024	**

** denote statistical significance at level 0.01.

Table 5 The effect of inoculant Biomin® BioStabil Plus on milk constituents and the somatic cell count

Parameters	Unit	Treatments		Standard error	P-value
		Control X±SD	BSP X±SD		
Fat	%	4.30±0.40	4.43±0.28	0.07	0.376
Protein	%	3.36±0.15	3.42±0.22	0.037	0.451
Lactose	%	4.80±0.15	4.87±0.19	0.035	0.317
Somatic cell count	x1000	222.3±152.13	125.1±30.98	24.144	*

* denote statistical significance at level 0.05.

The protein, fat and lactose contents were higher in the BSP treatment, but not statistically significant (P>0.05), compared to the control treatment. The somatic cell count of milk produced from cows fed with the treated silage was of statistically lower significance (P<0.05) than that of the control treatment (125000 *vs.* 222000).

This correlates to a higher hygiene status in the treated silage compared to that untreated. This parameter of milk quality should be considered as a consequential effect of better silage hygiene. It is well known that the somatic cell count is a polyfactorial parameter (Pennington, 2011; Schukken *et al.* 2003) very important for characterizing milk production quality.

CONCLUSION

The biological silage inoculant Biomin® BioStabil Plus (BSP) had a significant effect on the quality characteristics of grass-legume- silage in terms of lower pH due to a higher lactic acid fermentation caused by the homofermentative lactic acid bacteria. As a consequence of better fermentation, inoculated silage had higher digestible energy (P<0.01) and (P<0.05) net energy lactation concentrations compared to untreated silage by 2.1 and 1.25%, respectively. Inoculant treatment significantly decreased butyric acid content, N-NH_3 fraction and dry matter losses. The heterofermentative lactic acid bacterium *Lactobacillus bre-*

vis added in microbial mix had a tendency to shift fermentation towards acetic acid. The inoculation of silage improved aerobic stability of ensiled grass-legume swards and reduced feed waste in circumstances in which heating and molded feeds could be a major problem. Improved silage fermentation was achieved by using BSP in the ensiling process and this was related to an increased silage intake and milk production response in dairy cows. The utilization of feed energy was better, which was reflected in a significantly higher efficiency of the conversion of feed-net energy lactation into milk. Significantly lower somatic cell counts were observed in milk produced by cows fed with the treated silage, BSP indicating higher hygienic milk quality in comparison to milk produced by cows fed with the control treatment. The use of BSP is a promising silage inoculant for grass-legume silages due to its capacity to improve silage chemical composition and fermentation characteristics and dairy cows' milk performance.

REFERENCES

Danner H., Holzer M., Mayrhuber E. and Braun R. (2003). Acetic acid increases stability of silage under aerobic conditions. *App. Environ. Microb.* **69(1),** 562-567.

Filya I., Ashbell G., Hen Y. and Weinberg Z.G. (2000). The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Anim. Feed Sci. Technol.* **88,** 39-46.

Inglis G.D., Yanke L.J., Kawchuk L.M. and Mc Allister T.A. (1999). The influence of bacterial inoculants on the microbial ecology of aerobic spoilage of barley silage. *Canadian J. Microbiol. / Rev. Canadian Microbiol.* **45(1),** 77-87.

Jatkauskas J. and Vrotniakiene V. (2006). Effect of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* inoculated silage on nutrient utilization by dairy cows. *Veterinarija ir Zootechnika*. **36(58),** 17-20.

Kramer W. (2002). Neue Entwicklungen und Strategien im Bereich der Silierzusätze. 8. Alpenländisches Expertenforum, 9-10 April, Bundsanstalt für alpenländische Landwirtschaft, Österreich.

Kung L.Jr. (2000). Use of forage additives in silage fermentation. 2000-2001. Pp. 39-44 in Direct-fed Microbial, Enzyme and Forage Additive Compendium. The Miller Publishing Company, Minnesota, USA.

Kung L.Jr. and Muck R.E. (1997). Animal responses to silage additives. Pp. 200-210 in Proc. From the Silage: Field to Feedbunk North American Conference, Hershey, Pennsylvania, USA.

Merry R.J., Jones R. and Theodorou M.K. (2000). The conservation of grass. Pp. 196-228 in Grass, Its Production and Utilisation, Hopkins A. (ed.), 3rd Ed., Oxford: UK, Blackwel Science Ltd. Site of Publication.

Muck R.E. and Kung L.Jr. (1997). Effects of silage additives on ensiling. Pp. 187-199 in Silage: Field to Feedbunk. NRAES-99. Northeast Reg. Agric. Eng. Serv., Ithaca, New Yprk.

Muck R.E., Filya I. and Contreras-Govea F.E. (2007). Inoculant effects on alfalfa silage: *In vitro* gas and volatile fatty acid production. *J. Dairy Sci.* **90,** 5115-5125.

Ohmomo S., Tanaka O., Kitamoto H.K. and Cai Y. (2002). Silage and microbial performance, old story but new problems. *Jap. Agric. Res.* **36(2),** 59-71.

Pahlow G., Rammer C., Slottner D. and Tuori M. (2002). Ensiling of legumes. *Landbauforschung Voelkenrode Sonderheft*. **234**, 27-32.

Pennington J. (2011). Reducing somatic cell count in dairy cattle. Cooperative Extension Service, Division of Agriculture, University of Arkansas, FSA 4002. http://www.uaex.edu/Other_Areas/publications/PDF/FSA4002.pdf Accessed 01.11.2011.

Ranjit N.K. and Kung L.Jr. (2000). The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or chemical preservative on the fermentation and aerobic stability of corn silage. *J. Dairy Sci.* **83,** 526-535.

Schukken Y.H., Wilson D.J., Welcome F., Garrison-Tikofsky L. and Gonzalez R.N. (2003). Monitoring udder health and milk quality using somatic cell counts. *Vet. Res.* **34,** 579-596.

Weddell J.R., Agnew R. and Cottrill B. (2002). The UK Forage Additives Approval Scheme - Developments and Products Approvals. Pp. 230-231 in Proc. 8th International Silage Conference. Auchincruive, Scotland.

Weinberg Z.G., Ashbell G., Hen Y., Azrieli A., Szakacs G. and Filya I. (2002). Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri*. *J. Ind. Microbiol. Biotechnol.* **28,** 7-11.

Weinberg Z.G., Muck R.E., Weimer P.J., Chen Y. and Gamburg M. (2004). Lactic acid bacteria used in inoculants for silage as probiotics for ruminants. *App. Biochem. Biotechnol.* **118,** 202-209.

Wilkins R.J., Syrjala-Qvist L. and Bolsen K.K. (1999). The future role of silage in sustainable animal production. Pp. 23-40 in Proc. 7th International Silage Conference. Uppsala, Sweden.

Winters A.L., Fychan R. and Jones R. (2001). Effect of formic acid and a bacterial inoculant on the amino acid composition of grass silage and on animal performance. *Grass. Forage. Sci.* **56,** 181-192.

Winters A.L., Lloyd J., Leemans D., Lowes K. and Merry R. (2002). Effect of inoculation with *Lactobacillus plantarum* on protein degradation during ensilage of red clover. Pp. 108-109 in Proc. 8th International Silage Conference, Auchincruive, Scotland.

Ziggers D. (2003). Good or bad guys determine silage quality. *Dairy beef.* **2,** 27-29.