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EFFECT OF INOCULANTS ON FERMENTATION PARAMETERS AND CHEMICAL COMPOSITION OF GRASS AND CORN SILAGES

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ABSTRACT

The effect of three microbial inoculants (*Lactobacillus plantarum* CCM 4000, *L. fermentum* LF2, and *Enterococcus faecium* CCM 4231) on the fermentation and nutritive value of grass and corn silage was studied under laboratory conditions. The chopped corn and orchardgrass were ensiled in 40 plastic jars (1L) divided into four groups (4× 10 per treatment) each. The orchardgrass and whole corn plants (280.0 and 288.3 of DM.kg^{-1}) were cut and ensiled at 21ºC for 105 days. All inoculants were applied at 1.0× 10(9) cfu.mL^{-1}. Non-inoculated silage served as control. Overall, microbial inoculants generally had a positive effect on grass silage characteristics in terms of lower pH and higher lactic acid concentration, and significantly increased the lactic to acetic acid ratio in inoculated silages. The bacterial inoculants were established in the grass silage very well. All corn silages had a low pH (below 3.55) and 83-85% of total silage acids represented by lactic acid after 105 days of ensiling. The inoculants in the corn silages affected corn silage characteristics in terms of significantly higher pH, numerically lower crude protein content and ratio of lactic to acetic acid comparing to control silage. At the end of ensiling, the inoculants were found in the counts less than 1.0 log10 cfu.g^{-1} in corn silages.

Key words: corn; orchardgrass; silage; bacterial inoculants; quality; PUFA

INTRODUCTION

The main aim of silage making is to conserve the plants with minimal loss of nutritive value by fermentation of soluble carbohydrates in an anaerobic environment into organic acids, preferably lactic acid, which reduce pH (Saarisalo et al., 2007). The fermentation quality of silages has a major effect on the feed intake, nutrient utilization and milk production in ruminants (Huhtanen et al., 2002, 2003). Inoculants that include lactic acid bacteria (LAB) are often used as silage additives to enhance lactic acid fermentation, hence, to better preserve the ensiled material. Numerous papers published the ensiling of grass with inoculants *Lactobacillus plantarum* (Muck et al., 2007), *Lactobacillus buechneri* (Driehuis et al., 2001) *Lactococcus lactis, Lactobacillus pentosus* (Muck et al., 2007), *Lactobacillus buchneri* (Tyrolová and Výborná, 2008), *Enterococcus faecium* EF9296 (Marciňáková et al., 2008); and corn without inoculants (Kozakai et al., 2007), with the inoculants, *Lactobacillus plantarum* (Filya, 2003), *Lactobacillus buchneri, Propionibacterium acidipropionici* (Filya and Sucu, 2007), and with the mixtures of the inoculants, respectively; such as *L. plantarum* + *L. buchneri* (Filya, 2003), *L. plantarum* + *Enterococcus faecium*; *L. plantarum*+ *Pediococcus acidilactici* (Sucu and Filya, 2006; Koc et al. 2008) or *L. plantarum* + *Enterococcus faecium* + *Lactococcus lactis* + *Pediococcus pentosaceus* (Gálik et al., 2007) . Bacterial inoculants have advantages over chemical additives because easy to use, they are safe, they do not pollute the environment and are regarded as natural products. Some *in vitro* experiments showed that certain microorganisms –

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lactobacilli, lactococci, propionibacteriae, bifidobacteriae and *enterococci* are able to form conjugated linoleic acid (CLA- *cis* 9, *trans* 11 $C_{18:2}$) from linoleic acid in special growth medium (Coakley et al., 2003; Sieber et al., 2004). CLA originates mainly from bacterial isomerization and/or biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen and the desaturation of *trans* –fatty acids in the adipose tissue and mammary gland (Griinari and Bauman, 1999). Also our screening of microorganisms showed that some *lactobacilli* and *enterococci* isolated from rumen fluid and silages were able to convert linoleic acid to CLA in special growth medium *in vitro* (Marciňáková 2006). Therefore, the objectives of this study were to evaluate the effect of three different probiotic inoculants (*Enterococcus faecium* CCM 4231, *Lactobacillus fermentum* LF2, *Lactobacillus plantarum* CCM 4000) on fermentation parameters, chemical composition and the concentration of polyunsaturated fatty acids (PUFA) in grass and corn silages. Also, the population of inoculants and selected microbiota in grass and corn silages was studied.

MATERIAL AND METHODS

Treatments, material and ensiling

The silages were made from first cut orchardgrass (*Dactylis glomerata)* and whole corn plants (*Zea mays*, L.) wilted for 16 h. The grass (growth stage) or corn (milk maturity stage) was chopped to a length of cut of 20 mm with a forage chopper and about 900 g of fresh grass or corn was pressed into 1L sealed polyethylene jars. The grass dry matter (DM) was 279.7 $g.kg^{-1}$ DM and it contained crude protein (CP)150, and neutral detergent fibre (NDF) 515.5 $g.kg^{-1}$ DM; and corn dry matter (DM) was 288.3 $g.kg^{-1}$ DM and it contained CP 57.2, and NDF 513.3 $g.kg^{-1}$ DM. The ensiling of grass and corn was carried out in 40 PET jars which were divided into four groups. The four treatments (each with 10 jars) were used: (1) the untreated grass (GS) and corn (CS) without inoculant; (2) GS and CS inoculated by the strain *Enterococcus faecium* CCM 4231; (3) GS and CS inoculated by the strain *Lactobacillus fermentum* LF2; (4) GS and CS inoculated by the strain *L. plantarum* CCM 4000, respectively. For the ensiling experiments a fresh culture of each inoculant strain was diluted with Ringer solution to a population density of 10^9 $cfu.mL^{-1}$. The diluted culture was applied at 10 mL per kg grass or corn. The ensiling was carried out at 21 ºC for 105 days. Representative samples of the raw material (corn, grass) were taken for microbiological and chemical analyses before the division into jars. In addition, two jars per treatment were opened on days 7, 21, 40 and and four jars on 105 day of ensiling for microbiological and chemical analyses.

Microbiological analyses

To enumerate the counts of the inoculants as well as of the other enterococci and lactic acid bacteria, 10 g of ensiling grass and/or silage was sampled and mixed with 90 ml of Ringer solution (pH 7.0, Basingstoke, England); 100 µl aliquots of serial dilutions were plated into the following media (in duplicate): M-Enterococcus agar to enumerate enterococci, MRS (De Man-Rogosa-Sharpe) agar to enumerate LAB (Becton and Dickinson, Cockeysville, USA; Merck, Darmstadt, Germany). Bacterial counts were expressed as colony forming units (log10 cfu) per ml.

Chemical analyses

After drying at 60 ºC, the grass or corn dry matter was determined by oven drying at 103 ºC for 16 hours. The samples dried at the temperature less than 60 ºC were analysed for NDF, acid detergent fibre-ADF (Goering and Van Soest, 1970) using Fibertec 2010 (Tecator Comp., Sweden). Standard method was used for determining nitrogen (AOAC,1990 No. 968 06). *In vitro* dry matter digestibility (IVDMD) of grass, grass silages and corn or corn silages were determined by the *in vitro* fermentation gas production method (Váradyová et al., 2005). A water extracts of silages were prepared by adding deionized water to 20 g of silage to achieve a total of 300 g. The water extract was measured for pH and organic acids. Organic acids - lactic acid and volatile fatty acids (VFA: acetic, propionic, n-butyric acids) were analysed on Ionosep 2003. The fatty acids (FA) were determined in lyophilized samples. Lipids from freeze-dried samples were extracted using an extraction - transesterification procedure described by Sukhija and Palmquist (1988). The analysis of methyl esters was performed using a GC 6890N Agilent Technology gas chromatograph equipped with a programmed 60 m HP-Innowa capillary column (180-240 ºC) and a FI detector.

The means of results from treatments were statistically analyzed with one-way ANOVA using the Student-Newman-Keuls test (Graphpad Instat, Graphpad Software Inc., San Diego, CA, USA). Differences between the treatment means were considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Mean DM content in grass before ensiling was 279.7 $g.kg^{-1}$ DM and ensiling caused a significant decrease ($P< 0.001$) in the DM concentration of grass. Control silages had 5% and inoculated silages 2-11% lower IVDMD than wilted grass. The effect of 14 microbial inoculants in alfalfa silage showed that 48 h *in vitro* true DM digestibility was not improved by inoculation

with lactic acid bacteria (Filya and Sucu, 2007). Crude protein content in all grass silages was significantly decreased compared to grass before ensiling. Also, crude protein content in control silage was numerically (NS) decreased in comparison to inoculated grass silages. CP content varied from 126 to 146 g.kg^{-1} DM. The optimum mean concentration of grass silage is approximately 160 g.kg^{-1} DM, although it can range from 39 to 282 g.kg^{-1} DM (Merry et al., 2000). The acid detergent fibre (ADF) was significantly or numerically (NDF) lower in all inoculated silages, mostly in CCM 4000 compared to control (Table 1.).

The mean pH values (about pH 6.0) before ensiling decreased during ensiling and treated silages had significantly lower pH than untreated silage (Table 1). The pH of inoculated silages after 105 days of ensiling tended to be under 4.5, considered acceptable for grass silages (Cherney et al., 2006). Lactic acid and other organic acids - acetic, propionic and n - butyric acids are usually responsible for most of the drop in silage pH. Lactic acid should be at least 65-70 % of the total silage acids in good silage (Shaver, 2003). Lactic acid concentration in inoculated silages with *Enterococcus faecium* CCM 4231, *Lactobacillus fermentum* LF2, *L. plantarum* CCM 4000 was 2.0, 2.9 and 3.2 times higher ($P < 0.001$) as in untreated silage (Table 1.).The concentrations of acetic and propionic acids were low in all grass silages, even the concentrations of n - butyric acids were not detectable. Similar results were presented by McAllister et al. (1998), where two bacterial inoculants (*L. plantarum* +*E. faecium*) or *L. plantarum* alone were used as bacterial inoculants for ensiling of chopped lucerne. The ratio of lactic acid to acetic acid is an indicator of the efficiency of the silage fermentation. The ideal ratio of lactic acid to acetic acid should not be less than 3:1 and higher is better. The inoculants significantly increased the lactic: acetic acid ratio in control silage (9.4) compared to inoculant treated silages with CCM 4231 (36.6) , LF2 (12.2) and CCM 4000 (13.6; Table 1.). Nadeau et al. (2000) presented similar effect of cellulase alone or combined with a bacterial inoculant (*Lactobacillus plantarum* and *Pediococcus cerevisiae*) on orchardgrass silage.

The percentual proportion of saturated FA-SFA, short chain FA –SCFA ($C_{8:0}$ – $C_{12:0}$), medium chain FA - MCFA ($C_{14:0}$- $C_{17:0}$) as well as long FA –LCFA (> $C_{18:0}$) was similar in all grass silages (Table 2.). Only, percentual proportion of PUFA-polyunsaturated FA was significantly (CCM 4231, LF2) lower or significantly (CCM 4000) higher in comparison to control silage. As expected, the conjugated linoleic acid (CLA, *cis* 9,*trans* 11 $C_{18:2}$) and *trans* –vaccenic acid (TVA, *trans* 11 $C_{18:1}$) were not detected in the grass silages. They are produced as the intermediary products from rumen biohydrogenation of $C_{18:2}$ and $C_{18:3}$ fatty acids (Jenkins, 1993). From the three main fatty acids, the concentrations (g.100g^{-1} FA) of palmitic acid ($C_{16:0}$) in inoculated silages were significantly ($P < 0.001$) lower (CCM4231, CCM4000) or higher ($P < 0.001$) in LF2 and, linoleic acid ($C_{18:2}$) significantly ($P< 0.001$) higher in all inoculated silages. The concentration of α-linolenic acid ($C_{18:3}$) was lower ($P < 0.001$) in inoculated grass silages (CCM 4231; LF2) or higher ($P< 0.001$) in CCM 4000 compared to control silage. Elgersma et al. (2003) found that ensiling of grass lowered the contents of most FA, especially of $C_{18:1}$ and $C_{18:3}$ and ensiling of grass with the addition of *L. plantarum* inoculant declined $C_{18:3}$ concentrations as compared to control (Lee et al., 2006). The inoculant bacteria were established sufficiently during ensiling and

Table 1: Nutrient composition (g.kg^{-1} DM) and fermentation parameters in grass and corn silages after 105 days of ensiling (n-4)

	GS	GS+EF	GS+LF	GS+LP	CS	CS+EF	CS+LF	CS+LP
DM (g.kg^{-1})	222.8	241.5***	229.0*	246.6***	279.5	277.6	271.0*	280.2
Crude protein	126.4	141.5	146.6	139.9	68.9	63.6	64.4	63.2
NDF	698.4	686.8	691.3	664.4**	526.1	523.4	486.7***	540.5
ADF	407.5	392.2**	385.3**	380.5**	243.3	250.8	244.1	244.8
IVDMD (%)	66.4	59.9**	60.4**	69.5*	76.1	78.2	74.9	76.5
pH	5.26	4.49***	4.26***	4.35***	3.44	3.54***	3.50**	3.48*
Lactic acid	29.6	60.4***	84.9***	94.1***	11.13	9.76	10.3	11.41
Acetate	3.14	1.65***	6.98***	6.89***	1.68	1.82	1.60	1.85
Propionate	-	3.72	4.80	7.70	0.17	0.14	0.11*	0.15

DM-dry matter; NDF-neutral detergent fibre; ADF-acid detergent fibre; IVDMD-*in vitro* dry matter degradability;GS-grass silage; CS-corn silage; EF-*Enterococcoccus faecium* CCM 4231;LF-*Lactobacillus fermentum* LF2; LP-*Lactobacillus plantarum* CCM 4000;

*p< 0.05, **p < 0.01, ***p< 0.001 (comparisons were made between CS to CS+EF, CS+LF, CS+LP and GS to GS+EF, GS+LF, GS+LP)

on day 105 (the end of ensiling) were recorded at the highest counts (7-8 log 10 cfu.g^{-1}).

The ensiling decrease in DM concentration of corn silages (Table 1). Control silage had lower DM content (g.kg^{-1}) - about 8.8 units and inoculated corn silages (CS+LP, CS+LF, CS+EF) - about 8.1, 17.2, and 10.6 units, respectively than corn before ensiling (Table 1). Similar results were presented by Koc et al. (2008), where the mixture of inoculants and enzyme (amylase) with the concentration 5×10^5 and 1×10^6 cfu.g^{-1} in corn silage were used. IVDMD was similar in all corn silages (Table 1.). Also, Filya (2003) found that inoculation of corn silage with inoculants (*L. buchneri, L. plantarum* or their mixture) did not affect *in situ* dry matter and organic matter degradability after 48h of fermentation. Crude protein content in all corn silages significantly increased ($p< 0.001$, CS; $p< 0.05$, CS+LP; $p< 0.01$, CS+LF; $p< 0.05$, CS+ EF) compared to corn before ensiling. Nevertheless, crude protein content (g.kg^{-1} DM) was numerically lower in inoculated silages compared to control silage (Table 1.). Also, Koc et al. (2008) found a decrease ($p<0.05$) in CP content in inoculated corn silages when the concentrations 5 x10^5 and 1 x10^6 cfu. g^{-1} were used. But, Baytok et al. (2005) found that higher concentration (1x10^{11} cfu.g^{-1}) of the same inoculant did not affect CP concentration in corn silage. The detergent fiber analyses showed, that NDF and ADF content in corn silages was increased ($p< 0.01$) in comparison to NDF and ADF content in corn before ensiling (Table 1), except for CS+LF. However, the differences in NDF and ADF content in corn silages were not significant (Table 1), except for CS+LF.

The mean pH values of corn (about pH 5.3) before ensiling decreased during ensiling and all treated silages had higher pH (3.48-3.54) than untreated silage (3.44) after 105 days of ensiling (Table 1). The pH of all corn silages tended to be under 3.55, which is considered to be acceptable for corn silages (Kung and Shaver, 2001). Lactic acid and other organic acids: acetic, propionic and *n*-butyric acids are usually responsible for most of the drop in silage pH. Lactic acid should be at least 65-70% of the total silage acids in well-fermented silage (Shaver, 2003). Lactic acid concentration in CS and inoculated CS (CS+LP, CS+LF, CS+EF) represented 85.6%, 84.6%, 85.9%, and 83% of total silage acids (Table 1). The concentrations of acetic and propionic acids were low in all corn silages (below 1.85% and 0.17%), even the concentrations of n- butyric acids were not detectable. High moisture (about 25% DM) corn silage usually has a low acetic acid content (less than 1%, Kung and Shaver, 2001). Similar results have been presented by Sucu and Filya (2006) and Koc et al. (2008), where bacterial inoculants were used in corn silages. The ratio of lactic acid (LA) to acetic acid (AA) is a good indicator of the efficiency of the silage fermentation. Ideally, the ratio of lactic acid to acetic acid should not be less than 3:1, and a higher one is better (Kung and Shaver, 2001). Addition of inoculants slightly decreased LA:AA ratio from control silage (6.61:1) to inoculant treated corn silage (CS+LP, 6.16:1; CS+ LF, 6.43:1; CS+EF, 5.36:1).The use of 10 different commercial lactic acid bacteria as the inoculants showed the different effect on LA and AA production in corn silages (Weinberg et al., 2007).

The alterations in FA composition of corn during ensiling may be due to microbial intervention during the ensiling process, also enzymes of vegetable origin might be active during ensiling. The proportion of medium chain fatty acids-MCFA ($C_{14:0}$- $C_{17:0}$), as well as long chain fatty acids - LCFA (> $C_{18:0}$) was similar in all corn silages (Table 2). However, the proportion of short chain fatty acids –SCFA ($C_{6:0}$- $C_{12:0}$) was significantly ($p< 0.001$) higher in inoculated corn silages in comparison to control silage. The proportion of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) was

Table 2: The fatty acid composition of grass and corn silages after 105 days of ensiling (n-3)

g.100g^{-1} FA	GS	GS+EF	GS+LF	GS+LP	CS	CS+EF	CS+LF	CS+LP
$C_{16:0}$	21.9	19.3***	23.2***	19.7***	17.1	16.8	17.1	17.9**
$C_{18:0}$	3.10	3.33***	3.94***	2.53***	7.42	8.44***	7.86*	8.19**
$C_{18:1}$ n-9	3.50	6.93***	7.39***	4.29	23.2	15.0***	17.3***	17.7***
$C_{18:2}$ n-6	17.8	20.3***	18.3**	22.5***	37.6	41.4***	41.0***	38.5
$C_{18:3}$ n-3	39.7	34.3***	29.8***	40.8***	6.32	8.89***	7.71**	8.83***
SFA (%)	30.6	28.6	34.8	26.1*	26.4	31.5**	29.6*	31.13**
PUFA (%)	65.4	63.4***	56.5***	69.2***	45.3	52.2**	50.1*	48.9*
SCFA (%)	1.30	0.92	1.25	1.01	1.09	1.99***	2.0***	1.60***
MCFA(%)	25.8	23.8	28.9	22.3	19.7	18.8	19.8	20.9
LCFA (%)	72.9	71.4	65.2	73.9	79.2	79.2	78.2	77.5

SFA-saturated fatty acids; PUFA-polyunsaturated fatty acids; SCFA ($C_{6:0}$-$C_{12:0}$)-short chain fatty acids; MCFA; ($C_{14:0}$ –$C_{17:0}$)-medium chain fatty acids; LCFA (> $C_{18:0}$)- long chain fatty acids

significantly ($p< 0.05$-0.01) higher in inoculated silages about 3-5% (SFA) or 3-7% (PUFA), mostly in CS+EF (Table 2) compared to control silage. On the contrary, the proportion of monounsaturated fatty acids (MUFA, %) was significantly ($p< 0.001$) lower in inoculated silages, mostly in CS+EF. From the three main fatty acids, the concentrations ($g.100g^{-1}$ of total FA) of oleic acid ($C_{18:1}$) in inoculated silages were significantly ($p<0.001$) lower, mostly in CS+EF; linoleic acid ($C_{18:2}$) significantly ($p< 0.001$, CS+LF, CS+EF)) or numerically higher (CS+LP) than in control silage. The concentration of α-linolenic acid ($C_{18:3}$) was significantly higher ($p< 0.001$ for CS+LP and CS+EF, respectively; $p< 0.01$ for CS+LF) compared to control silage. The inoculants (LP CCM 4000, EF CCM 4231) in corn silages significantly lowered ($p<0.01$, about 1.5 and 1.3 units) or numerically lowered (LF, about 0.7 units) the n-6/n-3 ratio compared to control silage.The inoculant bacteria were poorly established during ensiling and on 105 day reached the concentration less than 1.log 10 $cfu.g^{-1}$ in inoculated corn silages. Also, the other results with inoculated grass and corn silages were presented by Jalč et al. (2009 a,b).

CONCLUSION

The microbial inoculants generally had a positive effect on grass silage characteristics in terms of lower pH and higher lactic acid concentration, and were established in the grass silage very well. In corn silages, the microbial inoculants did not affect IVDMD, as well as the concentration of total silage acids. The counts of inoculants decreased during ensiling of corn, and at the end of ensiling (105 days), the counts of inoculants were less than 1.0 log10 cfu/g in corn silages probably for lower pH (3.44-3.54). In future, these inoculated corn and grass silages will be used to study their effect on lipid metabolism in the experiments *in vitro* (artificial rumen) and *in vivo* (cows), respectively.

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REFERENCES

AOAC, 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, (ed. K. Herlick), Arlington, VA, USA, 1990, p.1230.

BAYTOK, E. – AKSU, T. – KARSLI, M. A. – MURUZ, H. 2005. The effects of formic acid, molasses and inoculant as silage additives on corn silage composition and ruminal fermentation characteristics in sheep. *Turk. J. Vet. Anim. Sci.*, vol. 29, 2005, p. 469-474.

CHERNEY, D. R. J. – ALESSI, M. A. – CHERNEY, J. H. 2006. Influence of grass species and sample preparation on ensiling characteristics. *Crop Science,* vol. 46, 2006, p.256-263.

COAKLEY, M. – ROSS, R. P. – NORDGREN, M. – FITZGERALD, G. – DEVERY, R. – STANTON, C. 2003. Conjugated linoleic acid biosynthesis by human- derived *Bifidobacterium* species. *Journal of Applied Microbiology,* vol. 94, 2003, p.138-145.

DRIEHUIS, F. – OUDE ELFERINK, S. J. W. H. – VAN WIKSELAAR, P. G. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri* with or without homofermentative lactic acid bacteria. *Grass and Forage Science,* vol. 56, 2001, p. 330-343.

ELGERSMA, A. – ELLEN, G. – VAN DER HORST, H. – MUUSE, B.G. – BOER, H. – TAMMINGA, S. 2003. Comparison of the fatty acid composition of fresh and ensiled perennial ryegrass (*Lolium perenne,* L.) affected by cultivar and regrowth interval. *Anim. Feed Sci. Technol.*, vol. 108, 2003, p.191-205.

FILYA, I. 2003. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. *J. Dairy Sci.* vol. 86, 2003, p.3575-3581.

FILYA, I. – SUCU, E. 2007. The effect of bacterial inoculants and a chemical preservative on the fermentation and aerobic stability of whole-crop cereal silages. *Asian-Australasian J. Anim. Sci*., vol. 20, p.378-384.

GÁLIK, B. – BÍRO, D. – JURÁČEK, M. – ŠIMKO, M. – MICHÁLKOVÁ, J. 2007. Effect of combined biochemical additives on nutritive value and fermentation process of ensiled crimped high moisture corn. *Slovak J. Anim.Sci.*, vol. 40, p.30-33.

GOERING, H. K. – VAN SOEST, P. J. 1970. Forage Fiber Analyses. Agriculture Handbook No. 379, USDA, Washington D. C, USA, 1970, p.1-20

GRIINARI, J. M. – BAUMAN, D. E. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: YURAWECZ, M. P. – MOSSOBA, M. M. – KRAMER, J. K. G. – PARIZA, M. W. – NELSON, G.: *Advances in conjugated linoleic acid research*. Champaign, IL, USA: AOCS Press, 1999, p.180-200. ISBN 189 399 7286

HUHTANEN, P. – KHALILI, H. – NOUSIAINEN, J. I. – RINNE, M. – JAAKKOLA, S. – HEIKKILA, T. –NOUSIAINEN, J. 2002. Prediction on the relative intake potential of grass silage by dairy cows. *Livestock Production Science,* vol. 73, 2002, p. 111-130.

HUHTANEN, P. – NOUSIAINEN, J. I. – KHALILI, H. –JAAKKOLA, S. – HEIKKILÄ, T. 2003. Relationship between silage fermentation characteristics and milk production parameters: analyses of literature data. *Livestock Production Science,* vol. 81, 2003, p.57-73.

JALČ, D. – LAUKOVÁ, A. – SIMONOVÁ, M. – VÁRADYOVÁ, Z. – HOMOLKA, P. 2009. The use of bacterial inoculants for grass silage: their effects on nutrient composition and fermentation parameters in grass silages. *Czech J. Anim. Sci.,* vol. 54, 2009, p. 83-90.

JALČ, D. – LAUKOVÁ, A. – POGÁNY SIMONOVÁ, M.

– VÁRADYOVÁ, Z. – HOMOLKA, P. 2009. Bacterial inoculant effects in corn silage fermentation and nutrient composition. *Asian Australasian J. Anim. Sci.,* vol. 22, 2009, p. 977-983.

JENKINS, T. C. 1993. Lipid metabolism in the rumen. *Journal of. Dairy Science,* vol. 76, 1993, p. 3851-3863.

KOC, F. – COSCUNTUNA, L. – ORDUVEN, L. 2008. The effect of bacteria + enzyme mixture silage inoculant on the fermentation characteristics,cell wall contents and aerobic stabilities of maize silage. *Pakistan J. Anim. Sci.,* vol. 7, 2008, p. 222-226.

KOZAKAI, K. – NAKAMURA, T. – KOBAYASHI, I. – TANIGAWA, T. – OSAKA, I. – KAWAMOTO, S. – HARA, S. 2007. Effect of mechanical processing of corn silage on *in vitro* ruminal fermentation, and in situ bacterial colonization and dry matter degradation. *Can. J. Anim. Sci,* vol. 87, p. 259-267.

KUNG, L. Jr. – SHAVER, R. 2001. Interpretation and use of silage fermentation analysis reports. *Focus on Forage,* vol. 3, 2001, p. 1-5.

LEE, S. W. – CHOUINARD, Y. – VAN, B. N. 2006. Effect of some factors on the concentration of linolec acid in forages. *Asian-Aust. J. Anim. Sci.,* vol. 20, 2006, p. 1525-1538.

MARCIŇÁKOVÁ, M. 2006. Probiotic microorganisms in the feed and the digestive tract of animals and their role in prevention. In: PhD thesis, Košice, 2006, p. 1-145.

MARCIŇÁKOVÁ, M. – LAUKOVÁ, A. – SIMONOVÁ, M. – STROMPFOVÁ, V. – KORÉNEKOVÁ, B. – NAĎ, P. 2008. Probiotic properties of *Enterococcus faecium* EF9296 strain isolated from silage. *Czech Journal of Animal Science,* vol. 53, 2008 , p. 336-345.

McALLISTER, T. A. – FENIUK, R. – MIR, Z. – MIR, P. –SELINGER, L. B. – CHENG, K. J. 1998. Inoculants for alfalfa silage: Effect on aerobic stability, digestibility and the growth performance of feedlot steers. *Livestock Production Science,* vol. 53, 1998, p.171-181.

MERRY, R. J. – JONES, R. – THEODOROU, M. K. 2000. The conservation of grass. In: HOPKINS, A.: *Grass- its production and utilization*: Oxford, UK, 3rd.ed., Blackwell Science, p. 130-156. ISBN 0-632-050179

MUCK, E. – CONTRERAS, F. E. – MERTENS, D. R. 2007. Silage inoculant effects on *in vitro* rumen fermentation. *Journal of Animal Science,* vol. 85, 2007, suppl. 1, p. 284.

NADEAU, E. M. G. – RUSSELL, J. R. – BUXTON, D. R. 2000. Intake,digestibility and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. *Journal of Animal Science,* vol. 78, 2000, p. 2980-2989.

SAARISALO, E. – SKYTTÄ, E. – HAIKARA, A. – JALAVA, T. – JAAKKOLA, S.2007. Screening and selection of lactic acid bacteria strains suitable for ensiling grass. *Journal of Applied Microbiology,* vol. 102, 2007, p. 327-336.

SHAVER, R. D. 2003. Practical application of new forage quality tests. Proceedings of the 6th Western Dairy Management Conference, Reno, NV, USA, p. 22-25.

SIEBER, R. – COLLOMB, M. – AESCHLIEMANN, A. – JELEN, P. – EYER, H. 2004. Impact of microbial cultures on conjugated linoleic acid in dairy products- a review. *International Dairy Journal,* vol. 14, 2004, p.1-15.

SUCU, E. – FILYA, Y. 2006. Effects of homofermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of low dry matter corn silages. *Turk. J. Vet. Anim. Sci.,* vol. 30, p. 83-88.

SUKHIJA, P. S. – PALMQUIST, D. L. 1988. Rapid method for the determination of total fatty acid content and composition of feedstuffs and faeces. *Journal of Agricultural and Food Chemistry,* vol. 36, 1988, p. 1202-1206.

TYROLOVÁ, Y. – VÝBORNÁ, A. 2008. Effect of the stage of maturity on the leaf percentage of lucerne and the effect of additives on silage characteristics. *Czech Journal of Animal Science,* vol. 53, 2008, p. 330-335.

VÁRADYOVÁ, Z. – BARAN, M. – ZELEŇÁK, I. 2005. Comparison of two *in vitro* fermentation gas production methods using both rumen fluid and faecal inoculum from sheep. *Animal Feed Science and Technology,* vol. 123-124, 2005, p. 81-94.

WEINBERG, Z. G. – SHATZ, O. – CHEN, Y. – YOSEF, E. – NIKBAHAT, M. – BEN-GHEDALIA, D. – MIRON, J. 2007. Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. *J. Dairy Sci.,* vol. 90, 2007, p. 4754-4762.