Asian-Aust. J. Anim. Sci.
Vol. 22, No. 7 : 977 - 983
July 2009

www.ajas.info

Bacterial Inoculant Effects on Corn Silage Fermentation and Nutrient Composition

D. Jalč, A. Laukova*, M. Pogany Simonova, Z. Varadyova and P. Homolka[1]
Institute of Animal Physiology, Slovak Academy of Sciences, 040 01 Kosice, Slovak Republic

ABSTRACT: The survival and effect of three new probiotic inoculants (*Lactobacillus plantarum* CCM 4000, *L. fermentum* LF2, and *Enterococcus faecium* CCM 4231) on the nutritive value and fermentation parameters of corn silage was studied under laboratory conditions. Whole corn plants (288.3 g/kg DM) were cut and ensiled at 21°C for 105 days. The inoculants were applied at a concentration of 1.0×10^9 cfu/ml. Uninoculated silage was used as the control. The chopped corn was ensiled in 40 plastic jars (1 L) divided into four groups (4×10 per treatment). All corn silages had a low pH (below 3.55) and 83-85% of total silage acids comprised lactic acid after 105 days of ensiling. The probiotic inoculants in the corn silages affected corn silage characteristics in terms of significantly ($p<0.05$-0.001) higher pH, numerically lower crude protein content and ratio of lactic to acetic acid compared to control silage. However, the inoculants did not affect the concentration of total silage acids (acetic, propionic, lactic acids) as well as dry matter digestibility (IVDMD) of corn silages *in vitro*. In the corn silages with three probiotic inoculants, significantly (CCM 4231, CCM 4000) lower n-6/n-3 ratio of fatty acids was detected than in control silage. Significant decrease in the concentration of $C_{18:1}$, and significant increase in the concentration of $C_{18:2}$ and $C_{18:3}$ was mainly found in the corn silages inoculated with the strains *E. faecium* CCM 4231 and *L. plantarum* CCM 4000. At the end of ensiling, the inoculants were found at counts of less than 1.0 log10 cfu/g in corn silages. (**Key Words :** Corn Silage, Probiotics, Composition, Quality)

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 feed intake, nutrient utilization and milk production of ruminants (Huhtanen et al., 2002, 2003). Fresh whole corn (*Zea mays*, L.) dry matter (DM) which generally contains 30-40% grains is rich in linoleic acid (LA, $C_{18:2}$, 55-62%) and oleic acid ($C_{18:1}$, 24-32%) and poor in α-linolenic acid (ALA, $C_{18:3}$, <2%; Chilliard et al., 2007). Concentrations of ALA vary with plant and environmental factors such as stage of maturity, genetic differences, as well as season and light intensity (Elgersma et al., 2006). When forages are conserved as silage, they maintain the same concentration of long chain-FA as when harvested fresh (Chilliard et al., 2001). Lactic acid bacteria (LAB) are often used as silage additives to enhance lactic acid fermentation, hence to better preserve the ensiled material. Bacterial inoculants have advantages over chemical additives because they are easy to use, safe, do not pollute the environment and are regarded as natural products. Most commercially available inoculants contain homofermentative LABs, which improve the silage fermentation. Among the homofermentative LAB most frequently used are *Lactobacillus plantarum, L. acidophilus, Enterococcus faecium* and *Pediococcus acidilactici*. Numerous papers have reported the ensiling of corn without inoculants (Abdehadia et al., 2005; Kozakai et al., 2007), with the inoculants *Lactobacillus plantarum* (Filya, 2003), *Lactobacillus buchneri*, and *Propionibacterium acidipropionici* (Filya and Sucu, 2007), commercial inoculants containing lactobacilli, enterococci, pediococci (Weinberg et al., 2004) and with mixtures of inoculants, such as *L. plantarum*+*L. buchneri* (Filya, 2003), *L. plantarum*+*Enterococcus faecium*; and *L. plantarum*+*Pediococcus acidilactici* (Sucu and Filya, 2006; Koc et al., 2008). Some *in vitro* experiments showed that certain microorganisms e.g., lactobacilli, lactococci, propionibacteriae,

* Corresponding Author: Andrea Laukova. Tel: +421-55-72 92964, Fax: +421-55-7287842, E-mail: laukova@saske.sk
[1] Research Institute of Animal Production, 104 01 Praha 114 - Uhříněves, Czech, Republic.
Received May 15, 2008; Accepted February 23, 2009

Table 1. Nutrient composition of corn before ensiling (n = 3)

	Composition
Dry matter (g/kg)	288.3
Crude protein (g/kg DM)	57.2
Crude fibre	203.6
NDF	513.3
ADF	216.4
Lignin	30.1
Fat	22.8
Ash	53.6
Organic matter	272.9

NDF = Neutral detergent fibre; ADF = Acid detergent fibre.

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). However, Bessa et al. (2000) revealed the possibility of an alternative pathway in the production of CLA from linolenic acid ($C_{18:3}$) due to extreme microbial diversity in the reticulo-rumen. Also, our screening of microorganisms showed that some lactobacilli and enterococci isolated from rumen fluid and silages were able to convert linoleic acid (LA) to CLA in special growth medium *in vitro* (Marciňáková, 2006). The objective of this study was to evaluate the survival and effect of three new probiotic inoculants (isolates of our Institute and *L. plantarum* CCM 4000 which was kindly supplied by Dr. Nemcová, University of Veterinary Medicine, Košice, Slovakia) on corn silage fermentation and chemical composition (including mainly polyunsaturated fatty acids, PUFA).

MATERIAL AND METHODS

Treatments, material and ensiling

The silages were made from fresh cut corn plants (*Zea mays*, L.) wilted for 16h. Whole plants were chopped to a length of 20 mm with a forage chopper and pressed into 1 L sealed polyethylene jars and 0.7 L glass jars equipped with a lid that enabled gas release only. The corn dry matter (DM) was 288.3 g/kg DM and it contained crude protein at 57.2, and NDF at 513.3 g/kg DM (Table 1). The ensiling of corn was carried out in 40 jars that were divided into four groups. Four treatments (each with 10 jars) were used: i) the untreated corn (control) without inoculant (CS); ii) corn inoculated by the strain *Enterococcus faecium* CCM 4231 (CS+EF); iii) corn inoculated by the strain *Lactobacillus fermentum* LF2 (CS+LF); iv) corn inoculated by the strain *Lactobacillus plantarum* CCM 4000 (CS+LP), respectively. For the ensiling experiments a fresh culture of each inoculant was prepared in Ringer solution to a concentration of 10^9 cfu/ml. Then the cultures were applied at 10 ml per kg of fresh corn. The ensiling of corn was carried out at 21°C for 105 days. Representative samples of the raw material (untreated chopped corn) were taken for microbiological and chemical analyses before division into jars. In addition, two jars per treatment were opened on days 7, 21, 40 and four jars per treatment were opened on day 105 of ensiling for microbiological and chemical analyses.

Characterization of the silage inoculants and microbial analysis

Enterococcus faecium CCM 4231 (Lauková et al., 1993) is the first described bacteriocin-producing isolate of rumen origin possessing probiotic properties and which is able to transform linoleic acid into CLA (Marciňáková, 2006). *Lactobacillus plantarum* CCM 4000, is a ruminal isolate (Nemcová, 1989) and *L. fermentum* LF2 is isolated from a commercial canine feed mixture Propesko, Marciňáková, 2006). To enumerate the counts of inoculants as well as the other enterococci and lactic acid bacteria, 10 g of silage was sampled and mixed with 90 ml of Ringer solution (pH 7.0, Basingstoke, England); 100 µl aliquots of serial dilutions were plated onto M-Enterococcus agar (in duplicate) to enumerate enterococci, and onto MRS (De Man-Rogosa-Sharpe) agar to enumerate lactic acid bacteria (Becton and Dickinson, Cockeysville, USA; Merck, Darmstadt, Germany). To differentiate *E. faecium* CCM 4231 strain from other enterococci, its rifampicin marked variant was prepared by the subsequent cultivation of the strain using Todd-Hewitt agar (Becton and Dickinson) with rifampicin (100 µg/ml) at 37°C (Strompfová, 2004). *L. plantarum* CCM 4000 as well as *L. fermentum* LF2 were also marked by rifampicin to differentiate them among the counts of lactic acid bacteria. Moreover, the identity of the inoculants (*E. faecium*, *L. fermentum* and *L. plantarum)* during ensiling and in the silage was also confirmed by the PCR method according to Woodford et al. (1997), Chagnaud et al. (2001) and Bethier and Ehrlich (1998). Bacterial counts were expressed in colony forming units (log10 cfu) per g±SD.

Chemical analyses

Corn dry matter was determined by oven drying at 103°C for 16 h. Dried (60°C, 48 h) samples were analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (Goering and Van Soest, 1970) using a Fibertec 2010 (Tecator Comp., Sweden). Standard methods were used for determining ash (AOAC, 1990, No. 942 05), nitrogen (AOAC, 1990, No. 968 06), fat (AOAC, 1990, No. 983.23) and crude fibre (AOAC, 1990, No. 962 09). *In vitro* dry matter digestibility (IVDMD) of grass and grass silages was determined by the *in vitro* fermentation gas production method (Váradyová et al., 2005). A water extract of silage was prepared by adding deionized water to 20 g of silage to achieve a total of 300 g. The values of pH, organic acids,

Table 2. Nutrient composition and fermentation parameters in corn ensiled for 105 days (n = 3)

Parameter	CS	CS+LP	CS+LF	CS+EF	Pooled SEM
DM (g/kg)	279.5	280.2	271.0*	277.6	1.8
OM (g/kg)	264.9	265.7	257.2*	263.3*	1.8
Ash (g/kg DM)	52.1	51.4	51.0	51.7	1.1
Crude protein	68.9	63.2	64.4	63.6	7.6
Crude fibre	213.9	209.0	210.7	220.0	9.1
NDF	526.1	540.5	486.7***	523.4	5.1
ADF	243.3	244.8	244.1	250.8	3.2
Fat	22.9	21.6	23.5	22.3	0.7
IVDMD (%)	76.1	76.5	74.9	78.2	3.8
pH	3.44	3.48*	3.50**	3.54***	0.01
Lactic acid (g/kg DM)	11.13	11.41	10.3	9.76	0.7
Acetic acid	1.68	1.85	1.60	1.82	0.1
Propionic acid	0.17	0.15.	0.11*	0.14	0.01
n-butyric acid	-	-	-	-	-
NH_3- N	0.34	0.29	0.43*	0.32	0.02

CS = Corn silage; DM = Dry matter; IVDMD = *In vitro* dry matter digestibility; NDF = Neutral detergent fibre; ADF = Acid detergent fibre.
EF = *Enterococcus faecium* CCM 4231; LF = *Lactobacillus fermentum* LF2; LP = *Lactobacillus plantarum* CCM 4000.
* p<0.05; ** p<0.01; *** p<0.001 (comparing to CS).

and ammonia nitrogen were measured in the water extracts of the samples (AOAC, 1990, No. 920 03). Organic acids - lactic acid and volatile fatty acids (VFA: acetic, propionic, *n*-butyric acids) - were analysed by the method of Naumann and Bassler (1997). The fatty acids in corn plants and corn silages were determined in lyophilized samples. Lipids from freeze-dried corn and corn silages were extracted using an extraction-transesterification procedure described by Sukhija and Palmquist (1988). A mixture of chloroform and methanol (2:1) was chosen as the extraction solvent. The extracted lipids were dissolved in 1mL hexane with internal standard $C_{13:0}$ and the esterification of lipids was carried out with 2 ml N sodium metoxide (30 min, 50°C) and 3 ml 3 N methanolic HCl (60 min, 50°C). After centrifugation (5 min, 2,500 rpm), samples of the upper hexane layers were used for gas chromatographic analyses. GC analysis of the methyl esters was performed using a GC 6890N Agilent Technology gas chromatograph equipped with a programmed 60 m HP-Innowa capillary column (180-240°C) and a flame ionization detector.

Statistical analyses

Statistical analysis used analysis of variance (Graphpad Instat, Graphpad Software Inc., San Diego, CA, USA). The model included the effect of uninoculated (CS) and three inoculated corn silages (CS+LP, CS+LF and CS+EF, respectively) on nutrient composition and fermentation parameters. Differences between the control (CS) and experimental silages (CS+LP, CS+LF and CS+EF, respectively) were analysed by one-way analysis of variance using the Student-Newman-Keuls post-test. The counts of the inoculants (Table 4) were analysed using Student *t*- tests.

RESULTS AND DISCUSSIONS

Effect of inoculants on chemical composition in corn silage

The nutrient composition of whole corn plants before ensiling is shown in Table 1. Silage samples were subjected to analyses that included determination of dry matter, nitrogen, ash, NDF, ADF, lignin. In addition to these components lactic, propionic, *n*-butyric and fatty acids were quantified in this experiment. Mean DM content in corn before ensiling was 288.3 g/kg (Table 1) and ensiling decreased DM concentration (Table 2). Control silage had a lower DM content (g/kg) of about 8.8 units and inoculated corn silages (CS+LP, CS+LF, CS+EF) of about 8.1, 17.2, and 10.6 units, respectively, than corn before ensiling (Tables 1 and 2). However, the differences in DM and OM (organic matter) content were significantly less ($p<0.05$) between the control silage and inoculated corn silages (Table 2). Similar results were presented by Koc et al. (2008) when a mixture of inoculants (*L. plantarum*+ *Pediococcus acidilactici*) and enzyme (amylase) at a concentration of 5×10^5 and 1×10^6 cfu/g in corn silage was used. IVDMD was similar in all corn silages (Table 2). In addition, 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 48 h 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 DM) was numerically lower in inoculated silages compared to control silage (Table 2). In addition, Koc et al. (2008) found a decrease ($p<0.05$) of CP content in inoculated corn silages when the concentration of

5×10^5 and 1×10^6 cfu/g was used. However, Baytok et al. (2005) found that a higher concentration (1×10^{11} cfu/g) of the same inoculant did not affect CP concentration in corn silage. The crude fiber content was higher in all corn silages compared to corn before ensiling, but significantly ($p<0.01$) only in CS+EF. Crude fiber content in all silages was similar and the differences between corn silages were not significant. Detergent fibre analyses showed that NDF and ADF content in corn silages was increased ($p<0.01$) in comparison to their content in corn before ensiling (Tables 1, 2), except for CS+LF. However, the differences in NDF and ADF content in corn silages were not significant (Table 2), except for CS+LF. These results are in the agreement with those reported by Filya (2003). Nevertheless, other authors (Baytok et al., 2005; Koc et al., 2008) found a significant decrease in NDF and ADF content in corn silages after inoculation with previously described inoculants.

Effect of inoculants on fermentation parameters in corn silage

The inoculation of ensiled corn silage influenced the fermentation parameters of corn silage.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 2). The pH of all corn silages tended to be under the value of 3.55 considered 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% to 70% of the total silage acids in well-fermented silage (Shaver, 2003). Lactic acid concentration in control silage (CS) and inoculated silages (CS+LP, CS+LF, CS+EF) represented 85.6%, 84.6%, 85.9%, and 83% of total silage acids (Table 2). The concentrations of acetic and propionic acids were low in all corn silages (below 1.85% and 0.17%); the concentrations of n-butyric acid 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 were presented by Sucu and Filya (2006), and Koc et al. (2008) when bacterial inoculants (*L. plantarum*+*E. faecium*) or (*L. plantarum*+ amylase+*Pediococcus acidilactici*) 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 higher 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). Total acid concentration, which indicates the extent of fermentation during ensiling, was higher (+0.42%) in CS+LP, and lower (-0.97% CS+LF; -1.26% CS+EF) than in control silage (CS, Table 2). In other experiments, the inoculants *L. plantarum*, *L. plantarum*+*L. buchneri* (Filya, 2003), or *L. plantarum*+ *Pediococcus acidilacti*+amylase (Baytok et al., 2005; Koc et al., 2008) significantly increased lactic acid production. In contrast, the inoculants *L. plantarum*+*Pediococcus acidilacti*+amylase (Baytok et al., 2005; Koc et al., 2008) and *L. plantarum* alone significantly decreased and the inoculants *L. plantarum*+*L. buchneri* or *L. buchneri* alone significantly increased acetic acid production in corn silages. The use of 10 different commercial lactic acid bacteria as the inoculants showed different effects on LA and AA production in corn silages (Weinberg et al., 2007). The ash, fat and crude protein contents were unchanged in all corn silages after ensiling compared to the control (Table 2). Ammonia concentration can be expressed as a percentage of the crude protein. Ideally, ammonia should be <7% of the protein content. Our results showed that the proportion of ammonia nitrogen in crude protein (g/kg DM) content constituted 0.49%, 0.46%, 0.84%, and 0.5% for the control and inoculated (CS+LP, CS+LF and CS+EF) corn silages, respectively. In inoculated corn silages, the ammonia concentration significantly ($p<0.05$, CS+LF) or numerically decreased (CS+LP, CS+EF) in comparison to control silage. McDonald et al. (1991) reported that lower pH values inhibited protein degradation in silages. Therefore, the concentrations of ammonia-N of all corn silages were low (0.29-0.43 g/kg DM) in this experiment.

Effect of inoculants on fatty acid composition in corn silage

Knowledge regarding the fat content and composition of forages is limited. The alterations in FA composition of corn during ensiling may be due to microbial intervention during the ensiling process, but 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 3). 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 significantly ($p<0.05$-0.01) higher in inoculated silages by about 3-5% (SFA) or 3-7% (PUFA), mostly in CS+EF (Table 3) 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. As expected, the isomers of fatty acids - conjugated linoleic acid (CLA, *cis* 9, *trans* 11 $C_{18:2}$) and *trans* - vaccenic acid (TVA, *trans* 11 $C_{18:1}$) - were not

Table 3. The fatty acid composition of corn silage after 105 days of ensiling (n = 3)

FA (g/100 g)	CS	CS+LP	CS+LF	CS+EF	SEM
$C_{8:0}$	0.08	0.10**	0.19***	0.16***	0.004
$C_{10:0}$	0.09	0.12	0.16**	0.09	0.01
$C_{12:0}$	0.34	0.39**	0.56***	0.52***	0.01
$C_{14:0}$	0.98	1.23***	1.42***	1.02*	0.01
$C_{16:0}$	17.1	17.9**	17.1	16.8	0.14
$C_{17:0}$	0.33	0.44***	0.35	0.33	0.01
$C_{18:0}$	7.42	8.19**	7.86*	8.44***	0.09
$C_{18:1}$ n-9	23.2	17.7***	17.3***	15.0***	0.30
$C_{18:1}$ n-7	1.19	1.03*	0.77***	0.64***	0.04
$C_{18:2}$ n-6	37.6	38.5	41.0***	41.4***	0.37
$C_{18:3}$ n-6	0.23	0.12	0.28	0.34	0.04
$C_{18:3}$ n-3	6.32	8.83***	7.71**	8.89***	0.22
SFA (%)	26.4	31.13**	29.6*	31.5**	0.73
MUFA	25.9	20.02***	19.0***	16.4***	0.69
PUFA	45.3	48.9*	50.1*	52.2**	0.99
SCFA	1.09	1.60***	2.0***	1.99***	0.01
MCFA	19.7	20.9	19.8	18.8	0.51
LCFA	79.2	77.5	78.2	79.2	1.0

CS = Corn silage; SFA = Saturated fatty acids; UFA = Unsaturated fatty acids; SCFA = ($C_{6:0}$- $C_{12:0}$); MCFA = $C_{14:0}$- $C_{17:0}$; LCFA>$C_{18:0}$.
EF = *Enterococcus faecium* CCM 4231; LF = *Lactobacillus fermentum* LF2; LP = *Lactobacillus plantarum* CCM 4000.
* p<0.05; ** p<0.01; *** p<0.001 (comparing to CS).

Table 4. The counts of the inoculants, enterococci and lactic acid bacteria in corn silages

Silages	ME/MRS-rif	Enterococci	LAB
Day 0-1			
Control	nd	3.57±0.47	4.91±1.23
EF CCM 4231	nd	6.36±0.89	4.12±1.94
LF2	nd	3.38±0.28	6.04±0.00
LP CCM 4000	nd	5.94±0.17	5.43±1.21
Day 7			
Control	nd	4.77±0.00	7.03±0.08
EF CCM 4231	4.11±0.00	6.17±1.87	7.10±0.15
LF2	4.60±0.00	5.62±1.52	7.27±0.04
LP CCM 4000	5.23±0.00	5.32±0.07	6.69±0.22
Day 22			
Control	nd	3.70±0.40	6.40±0.30
EF CCM 4231	2.20±0.90	2.62±1.52	6.10±0.00
LF2	4.37±0.77	3.93±0.03	6.30±0.00
LP CCM 4000	4.33±0.23	3.65±1.05	6.60±0.50
Day 40			
Control	nd	1.00±0.10	3.90±0.80
EF CCM 4231	<1.00	<1.00	5.39±0.85
LF2	3.20±0.10	1.10±0.00	3.39±0.09
LP CCM 4000	2.90±0.00	1.35±0.25	4.35±0.05
Day 105			
Control	nd	<1.00	1.10±0.00
EF CCM 4231	<1.00	<1.00	<1.00
LF2	<1.00	<1.00	<1.00
LP CCM 4000	<1.00	<1.00	<1.00

Nd = Not determined, ME/MRS-rif = M-Enterococcus medium and MRS medium enriched with rifampicin were used to differ the inoculants from the other bacteria, LAB = Lactic acid bacteria.
The counts are expressed in log 10 cfu/g±SD; EF CCM 4231 = *Enterococcus faecium*; LF2 = *Lactobacillus fermentum*; LP CCM 4000 = *Lactobacillus plantarum*.

detected in the corn silages. They are produced as the intermediary products from rumen biohydrogenation of $C_{18:2}$ and $C_{18:3}$ fatty acids (Jenkins, 1993). Of the three main fatty acids, the concentrations (g/100 g of total FA) of oleic acid ($C_{18:1}$) in inoculated silages were significantly (p<0.001) lower, mostly in CS+EF; linoleic acid ($C_{18:2}$) was 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. 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 decreased $C_{18:3}$ concentrations as compared to the control (Lee et al., 2006).

Microbial status

The three probiotic silage inoculants multiplied sufficiently during the ensiling process (Table 4). On day 7 (1 week of ensiling) their counts reached 4.1, 4.60 and 5.23 log10 cfu/g and the counts were well balanced; despite this, the ruminal strain *L. plantarum* CCM 4000 was enumerated in the highest amounts (5.23 cfu/g). By decreasing the pH value in corn ensiling (Table 2), the counts of inoculants were also decreased; especially the counts of *E. faecium* CCM 4231 strain on day 22 (2.20 cfu/g). Lactobacilli can survive well in a pH of around 3 (Nemcová, 1997); this is a

possible explanation for their enumeration in the count 4.37 and 4.33 cfu/g, respectively 3.20 and 2.90 cfu/g. However, on day 105, all three inoculants were counted at less than 1.00 log10 cfu/g. At the beginning of the ensiling process, the inoculants formed the dominant portion among enterococci and LAB, (Table 4); near the end of ensiling process (105 day) their counts continually decreased. In grass silages produced under the same conditions (the same inoculants), the probiotic inoculants were determined in higher counts (even at the end in silage up to 6.0 cfu/g; Jalč et al., 2008; 2009). The pH values were more acceptable for their growth than in corn ensiling. However, in both grass and corn silages, the inoculants form the main part of the counted enterococci and LAB.

CONCLUSION

The microbial inoculants did not affect IVDMD, as well as the concentration of total silage acids in inoculated corn silages. Three probiotic inoculants significantly or numerically lowered n-6/n-3 ratio of fatty acids in the inoculated silages and significantly decreased the concentration of $C_{18:1}$, and/or significantly increased the concentration of $C_{18:2}$ and $C_{18:3}$, in the corn silages. Finally, 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 probably, due to lower pH (3.44-3.54). In future, these inoculated corn silages will be used to study their effect on lipid metabolism in experiments *in vitro* (artificial rumen) and *in vivo* (cows).

ACKNOWLEDGMENTS

The study was supported by the project of Slovak Research and Development Agency (APVV- No. 0043-07) and the project of Ministry of Agriculture of the Czech Republic (MZE - No. 00 270 1404), partially also by funds from the Grant Agency of the Ministry of Education of Slovak Republic and Slovak Academy of Sciences VEGA (No. 2/0008/08).

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