Effects of homo-fermentative bacterial inoculants on fermentation characteristics and nutritive value of low dry matter corn silage

Sadeghi, K.[1]; Khorvash, M.[1*]; Ghorbani, G. R.[1]; Forouzmand, M. A.[2]; Boroumand, M.[3] and Hashemzadeh-Cigari, F.[1]

[1]Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran; [2]Department of Animal Sciences, College of Agriculture, Yasouj University, Yasouj, Iran; [3]Isfahan Agricultural Jahad Education Center, Isfahan, Iran

[*]**Correspondence:** M. Khorvash, Department of Animal Sciences, College of Agriculture, Isfahan University of Technology, Isfahan, Iran. E-mail: khorvash@cc.iut.ac.ir

(Received 17 Apr 2011; revised version 23 May 2012; accepted 30 May 2012)

Summary

This study was conducted to evaluate the effects of inoculation of homo-fermentative lactic acid bacteria (LAB) on ensiling characteristics and nutritive value of low dry matter corn silage (LDMCS). Corn forage was harvested at milk stage (22.8 ± 0.9% DM), chopped at theoretical length of cut (TLC) 2.5 cm, and stored in eighteen 3.8 L mini silos for each treatment. The following treatments were used, 1) control (uninoculated), 2) ecosyl (treated with ecosyl^{TM} corn silage inoculants containing *Lactobacillus plantarum*), and 3) biotal (treated with biotal^{TM} corn silage inoculants containing *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Propionibacter freudenreichii*). Triplicate silos for each treatment were opened and sampled for chemical analyses after 3, 6, 12, 16, 21 and 90 days of ensiling. Neither ecosyl nor biotal improved fermentation characteristics of LDMCS compared to the control silage. Neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), water soluble carbohydrate (WSC) contents and lactic acid (LA) concentration were not affected significantly by inoculants ($P>0.05$). Acetic acid concentration of control silages was higher; however, ethanol concentration was lower than the other silages. Biotal treated silages had the highest ammonia-N (NH3-N) concentrations compared to the control ($P<0.05$). *In vitro* dry matter disappearance (IVDMD) of control silage was higher than treated silages ($P<0.05$). In conclusion, the results showed that homo-fermentative LAB inoculants used in this experiment did not improve the fermentation characteristics and nutritive value of LDMCS.

Key words: Low dry matter corn silage, Fermentation characteristics, Inoculants, Nutritive value

Introduction

Ensiling is a preservation method for wet forage crops based on converting WSC into organic acids, mainly lactic acid, (Filya *et al*., 2000) and keeping low ammonia nitrogen levels (Hristov and McAllister, 2002; Rowghani and Zamiri, 2009) in anaerobic condition by LAB (McDonald *et al*., 1991). To improve the ensiling process, biological additives have been developed to obtain lactic acid fermentation (Filya *et al*., 2000). While there are different objectives in using silage additives, the main objective is to prevent secondary fermentation and to decrease butyric acid production (Chamberlain, 1982; Alçiçek and Özdoğan, 1997; Rowghani *et al*., 2008). As a result, pH decreases and the wet forage is preserved from spoilage microorganisms, leading to minimum dry matter (DM) losses associated with increased nutritive value of silage (Filya *et al*., 2000; Rizk *et al*., 2005). The biological additives like bacterial inoculants are beneficial relative to chemical additives, because of safety, ease of use, they are non-corrosive to machinery and do not contaminate the environment (Filya *et al*., 2000). Most commercially available inoculants consist of selected strains of homo-fermentative LAB, such as *Lactobacillus plantarum*, *Pediococcus*, and

Enterococcus species (Weinberg and Muck, 1996).

LAB proliferation yield adequate amounts of lactic acid to reduce the original pH (6.0) of the forage mass to a stable pH range of 3.8-4.5. Quantitatively, the amount of acid required to drop the original pH = 6 to a stable pH = 4 is dependant on the silages DM content and storage system. Forages with high DM content are fermented at a slower rate than forages with low DM because of low water activity (Whiter and Kung, 2001; Hristov and McAllister, 2002; Rizk *et al*., 2005). Filya (2004) reported that silage inoculants should have a positive effect on fermentation quality and aerobic stability of low dry matter corn silage; however, others reported that homo-fermentative LAB inoculants did not improve the fermentation parameters of high moisture corn silages (Kung *et al*., 1993; Meeske and Basson, 1998; Sucu and Filya, 2006).

In Iran, most dairy farms use corn silage for about 50% of forage source and it is harvested as a second crop containing high moisture content, about 80% (Khorvash *et al*., 2006); hence low quality silage, susceptible to loose nutrients is produced from silo as an effluent. So, the objective of this study was to evaluate the effects of lactic acid bacteria inoculants on ensiling characteristics and nutritive value of LDMCS in Iran.

Materials and Methods

Silages were produced at the Dairy Research Facilities of Lavark Research Station (Isfahan University of Technology, Isfahan, Iran) as mentioned below and silos were transferred to the Animal Nutrition Laboratory (Isfahan University of Technology, Isfahan, Iran) until opened for sampling. Corn forage (hybrid S.C.700 recently planted extensively in Iran) was harvested at the milk stage (22.8 ± 0.9% DM) and chopped for 2.5 cm theoretical length. Treatments were 1) control (uninoculated corn forage), 2) ecosyl (corn forage treated with Ecosyl®, corn silage inoculants containing *Lactobacillus plantarum*, Ecosyl Products Ltd. Stokesley, UK), and 3) biotal (corn silage treated with biotal®, corn silage inoculants containing *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Propionibacter freudenreichii*, Animal Nutrition, France). Inoculants were applied at 1×10^5 CFU/g of fresh material. Experimental silages ensiled in 54 mini-silos made up PVC tube (10 cm diameter and 60 cm height) equipped with a lid enabling gas release for 90 days. To calculate DM recovery of the silages, weights of the empty and full silos were recorded and then silos were stored at ambient temperature (20 to 27°C) in an enclosed place. Representative samples of each treatment were obtained prior to and after application of the additives to silages.

Analytical procedures

Triplicate silos for each treatment were opened and sampled for chemical analysis after 3, 6, 12, 16, 21 and 90 days of ensiling. Silage samples were stored at -20°C for further analysis. DM was determined by oven drying for 48 h at 55°C and ash was obtained after 3 h at 550°C. Crude protein (CP) was determined by a Kjeldahl method (AOAC, 1980). To determine lactic acid and volatile fatty acids (VFA) including acetate, butyrate and propionate, wet samples were mixed with distilled water (1:9 ratio) and extracted for 60 seconds using electrical blender, then one ml of each filtered experimental silage extract was combined with 200 µl of 25% meta-phosphoric acid and 200 µl crotonic acid (1 mM) as an internal standard. Samples were centrifuged for 15 min at 8050 g and analysed by gas chromatography (Chrompack CP9002) using a capillary column over a temperature range 40 to 240°C (Khorvash *et al*., 2006). Water soluble carbohydrate (WSC) was determined by the phenol-sulphuric acid method (Dubois *et al*., 1956). Silage ammonia N (NH_3-N) was determined by extraction of 40 g of frozen sample with 360 ml of distilled water for 3 min in a stomacher blender (IUL, Barcelona, Spain). The extract was filtered through Whatman filter paper No. 1 (Whatman, Maidstone, UK), and 25 ml of the extract was used for distillation in a micro Kjeltech auto analyser (tecator, kjeltec auto analyser, 1030) without digestion step (Filya, 2003). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were

determined according to the method of Van Soest *et al*. (1991). *In vitro* DM disappearance (IVDMD) was determined using the method of Tilley and Terry (1963).

Statistical analysis

Silage data were analysed using the GLM procedure of SAS (2002), based on the following model:

$Y_{ijk} = \mu + I_i + D_j + K_{ij} + e_{ijk}$

Where,
Yijk: Parameters
I_i: Effect of inoculants
D_j: Effect of length of ensiling
K_{ij}: Interaction between inoculants and days
e_{ijk}: Random error
Differences among means were tested for significance (P<0.05) by least square means test.

Results

The chemical composition of the fresh corn forages are given in Table 1. The results showed that forages were similar in DM, CP, NDF, water soluble carbohydrate (WSC) and *in vitro* DM disappearance.

In this experiment, inoculation had a minor effect on fermentation characteristics of the experimental corn silages (Table 2). Inoculation did not significantly affect NDF, lactic acid, ethanol concentrations, effluent and DM recovery but decreased DM, acetic acid and WSC concentrations and *in vitro* DM disappearance significantly (P<0.05).

The control silages had lower pH and ammonia-N concentration and higher DM, residual WSC and IVDMD compared to biotal inoculated silages (P<0.05). Both inoculants decreased acetate content compared to control (P<0.05). Between microbial inoculated silages biotal treatment had higher pH and ammonia-N concentration and lower DM, WSC and IVDMD than ecosyl treated silages (P>0.05). The pH and WSC of all silages decreased, and concentrations of lactic and acetic acid, ethanol, and ammonia-N increased during fermentation in all silages.

In this experiment, acetate and lactate concentrations tended to be similar between inoculated and control silage at the majority

Table 1: Chemical composition of the pre-ensiled corn forages (g/kg DM)

Treatment	pH	DM	NDF	CP	IVDMD	WSC
Control	5.70	227.00	515.7	76.6	582.9	101.1
Ecosyl	5.87	229.33	517.3	78.2	590.6	101.8
Biotal	5.61	228.00	518.8	78.2	586.8	101.8
SEM	0.091	1.504	0.889	0.483	6.332	2.258

SEM = Standard error of the means

Table 2: Effect of microbial inoculation on fermentation quality and nutritive value of experimental corn silages

Parameters	Treatments			SEM	Treatment	(D×T)[1]
	Control	Ecosyl	Biotal			
DM (g/kg DM)	214.5[a]	208.5[ab]	206.4[b]	1.28	0.0294	NS
pH	3.79[b]	3.85[b]	3.94[a]	0.038	0.0001	0.0001
CP (g/kg DM)	76.6[b]	78.2[ab]	78.8[a]	0.41	0.0298	NS
NDF (g/kg DM)	487	506.2	497.2	0.4	NS	NS
ADF (g/kg DM)	32.1	33.4	32.8	0.38	NS	NS
Lactic acid (g/kg DM)	52.1	50.6	50.5	3.3	NS	NS
Acetic acid (g/kg DM)	8.3[a]	4.7[b]	5.5[b]	0.48	0.0002	NS
NH_3-N (g/kg DM)	2.5[b]	2.5[b]	3.2[a]	0.13	0.0080	NS
WSC (g/kg DM)	54.5[a]	44.9[ab]	40.5[b]	2.3	0.0235	NS
Ethanol (g/kg DM)	5.2	5.3	5.0	3.2	NS	NS
IVDMD (g/kg DM)	549.0[a]	522.6[ab]	510.6[b]	0.62	0.0233	NS
Effluents (ml)	314	321	312	2.29	NS	NS
DM recovery (g/kg DM)	938	934	929	0.0028	NS	NS

[a, b] means with different superscripts in the same row differ (P<0.05). [1](D×I) = Interaction effects (ensiling duration × inoculation). NS = Not significant (P<0.05), and SEM = Standard error of means

Table 3: Fermentation characteristics of the experimental corn silages during ensiling process (g/kg DM)

Item	Ensiling duration	Treatments			SEM
		Control	Ecosyl	Biotal	
pH	3	3.93[b]	3.98[ab]	4.21[a]	0.038
	6	3.93	3.98	3.98	
	12	3.87	3.86	3.86	
	16	3.72[b]	3.76[ab]	3.88[a]	
	21	3.57	3.72	3.64	
	90	3.76[b]	3.78[b]	4.09[a]	
Lactic acid	3	26.30	28.71	35.10	3.3
	6	46.57	33.96	41.12	
	12	49.12	45.94	45.69	
	16	55.56[ab]	69.33[a]	46.21[b]	
	21	60.20[b]	85.62[a]	59.10[b]	
	90	74.81	74.95	75.74	
Acetic acid	3	3.86	2.85	3.30	0.48
	6	5.27	3.88	4.07	
	12	5.84	4.10	5.25	
	16	10.72[a]	4.42[b]	4.00[b]	
	21	10.94[a]	6.68[b]	6.64[b]	
	90	13.21[a]	6.60[b]	9.83[ab]	
Ethanol	3	1.81	2.28	1.96	3.2
	6	4.65[b]	2.72[a]	2.69[a]	
	12	5.13	4.82	3.90	
	16	6.31	6.10	5.90	
	21	6.01	7.40	6.42	
	90	7.30[b]	8.40[ab]	9.34[a]	
NH3-N	3	1.63	1.66	1.80	0.13
	6	1.82	2.10	2.48	
	12	2.00	2.50	2.70	
	16	3.00	2.53	3.33	
	21	3.06[ab]	3.00[b]	4.12[a]	
	90	3.60[ab]	3.23[b]	4.49[a]	
WSC	3	62.45	75.48	61.50	2.3
	6	58.44	43.34	39.34	
	12	54.92	44.86	32.10	
	16	48.04	33.92	30.21	
	21	48.02	34.64	46.87	
	90	55.38	37.53	33.07	
IVDMD	3	513.90	559.33	500.60	0.62
	6	548.40	530.60	501.63	
	12	550.80	521.63	528.83	
	16	559.63	538.13	533.83	
	21	550.50	503.23	511.40	
	90	571.66[a]	482.63[b]	487.30[ab]	

[a, b] means with different superscripts in the same row differ (P<0.05). NS = Not significant (P<0.05), and SEM = Standard error of means

of the time-points (Table 3). After 3 days of ensiling the pH decreased to below 4.0 and the final pH values were 3.76 to 4.09 for all silages. Data showed decreasing WSC content of all silages was associated with the reduction of pH. Although pH of control silage was lower compared to inoculated silages, this treatment had a greater amount

of acetate than the two LAB inoculated silages. As shown in Table 3, there was no consistent trend among treatments for decreasing pH. Results showed that biotal was less effective in decreasing pH and preventing production of NH_3-N and ethanol, so after 90 days of ensiling, biotal inoculated silages had higher pH, NH_3-N and ethanol than the control and ecosyl inoculated silages ($P<0.05$). Propionate and butyrate concentrations were below detectable concentrations.

Discussion

The main objective of using microbial additives is to enhance lactic acid fermentation that results in well-preserved silage with lower final pH values, raised lactate:acetate ratios, lower ethanol and ammonia nitrogen concentrations, and improved DM recovery (McDonald *et al*., 1991). To attain greater lactic acid fermentation, special consideration must be given to controlling the homo-fermentation or hetero-fermentation. The homo-fermentation converts glucose almost exclusively to lactic acid with a high molar conversion ratio, whereas the hetero-fermentation converts glucose to acetic acid, ethanol and carbon dioxide gas as well as lactic acid. This latter pathway results in lower fermentation efficiency because of a low molar conversion of sugars to lactic acid and production of greater wasteful end products such as carbon dioxide gas (Ohmomo *et al*., 2002). So, in most cases, adding suitable additives that encourage homo-fermentation is necessary and the helpfulness of additives depends on the degree of preventing such fermentation in silages (Chamberlain, 1982; Alçiçek and Özdoğan, 1997).

In contrast to many researches that observed positive results due to addition of homo-fermentative LAB inoculants, in this study, none of the two homo-fermentative LAB inoculants improved fermentation parameter and IVDMD of low DM corn silages. However, it is necessary to keep in mind that the success of microbial additives depends on many factors, such as the type and properties of the crops to be ensiled, epiphytic micro flora, ensiling skill, the properties of the inoculants, climatic circumstances (Henderson and McDonald, 1984) and moisture content (McDonald *et al*., 1991). So observing inconsistent results due to the addition of microbial inoculants is not surprising and highlights the importance of developing new microbial inoculants from native LAB.

After 3 days of ensiling, the WSC and pH of control silages immediately decreased and lactate and acetate increased quickly as well as inoculated silages, indicating the number of epiphytic LAB on fresh forage was sufficient to be dominant during ensiling process. Inoculants did not affect lactic acid levels of silages compared to control silage. In agreement with our study, Bolsen *et al*. (1992) reported that whole corn crop fermented rapidly and bacterial inoculants had little effect on the rate and efficiency of silage fermentation. Also, observations reported by Sucu and Filya (2006) were similar to the results of our experiment. Filya (2004) concluded that extensive fermentation in low DM corn silages led to higher fermentation losses. The same trend was shown in this experiment. In the present study, all silages had lower pH values at an earlier stage of ensiling. The sugar compounds are a great source of energy for lactic acid bacteria (Johnson *et al*., 2002), so the lower pH of high moisture corn silage was due to high levels of WSC in immature corn silage (McDonald *et al*., 1991) which could promote rapid and extensive fermentation. After 6 days of ensiling, both inoculants increased the weight losses of silages. Similar to this result, Sucu and Filya (2006) reported that inoculants after 8 days of ensiling increased weight losses of silages. This result showed high fermentation losses of silages, particularly at the beginning of ensiling process, therefore, high level of DM losses of immature corn silage is common because of a greater level of available rapidly fermentable substrate.

The lower IVDMD and WSC concentration of inoculated silages were attributed to the higher activity of commercial LAB inoculants in lower pH compared to epiphytic LAB existing on fresh forage. Although concentrations of ammonia-N of all corn silages were low in

the experiment, biotal inoculated silages had higher levels of ammonia-N due to higher pH value. McDonald *et al*. (1991) reported that lower pH values inhibited protein degradation in silages.

In conclusion, in the present study, tested commercial homo-fermentative LAB inoculants did not improve the fermentation characteristics and IVDMD of low DM corn silages, therefore, they were not able to improve nutritive value and quality of low DM corn silage more than what epiphytic lactic acid bacteria are able to do.

References

Alçiçek, A and Özdoğan, M (1997). A research on the determination of quality of corn and barley silages prepared in farmer conditions. J. Anim. Product., 37: 94-102.

AOAC (1980). *Official methods of analysis*. 13th Edn., Washington, D.C., Association of Official Analytical Chemists.

Bolsen, CKK; Lin, CJ; Brent, BE; Feyrherm, AM; Urban, JE and Aimutis, WR (1992). Effect of silage additives on the microbial succession and fermentation process of alfalfa and corn silages. J. Dairy Sci., 75: 3066-3083.

Chamberlain, DG (1982). Effect of added glucose and xylase on the fermentation of perennial ryegrass silage inoculated with *Lactobacillus plantarum*. J. Sci. Food Agric., 46: 129-138.

Dubois, M; Gilles, KA; Hamilton, JK; Rebers, PA and Smith, FF (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

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., 86: 3575-3581.

Filya, I (2004). Nutritive value and aerobic stability of whole crop corn silage harvested at four stages of maturity. Anim. Feed Sci. Technol., 116: 141-150.

Filya, I; Ashbellb, G; Henb, Y and Weinberg, ZG (2000). The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. Anim. Feed Sci. Technol., 88: 39-46.

Henderson, AR and McDonald, P (1984). The effect of a range of commercial inoculants on the biochemical changes during the ensilage of grass in laboratory studies. Res. Dev. Agric., 3: 167-171.

Hristov, V and McAllister, TA (2002). Effect of inoculants on whole-crop barley silage fermentation and dry matter disappearance in situ. J. Anim. Sci., 80: 510-516.

Johnson, LM; Harrison, JH; Davidson, D; Mahanna, WC; Shinners, K and Linder, D (2002). Corn silage management: effects of maturity, inoculation, and mechanical processing on pack density and aerobic stability. J. Dairy Sci., 85: 434-444.

Khorvash, M; Colombatto, D; Beauchemin, KA; Ghorbani, GR and Samei, A (2006). Use of absorbants and inoculants to enhance the quality of corn silage. Can. J. Anim. Sci., 86: 97-107.

Kung, L; Chen, JH; Kreck, EM and Knutsen, K (1993). Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows. J. Dairy Sci., 76: 3763-3770.

McDonald, P; Henderson, AR and Heron, SJE (1991). *The biochemistry of silage*. 2nd Edn., UK, Chalcombe Publications.

Meeske, R and Basson, HM (1998). The effect of a lactic acid bacterial inoculants on corn silage. Anim. Feed Sci. Technol., 70: 239-247.

Ohmomo, S; Tanaka, O; Kitamoto, HK and Cai, Y (2002). Silage and microbial performance, old story but new problems. JARQ. 36: 59-72.

Rizk, C; Mustafa, AF and Phillip, LE (2005). Effects of inoculation of high dry matter alfalfa silage on ensiling characteristics, ruminal nutrient degradability and dairy cow performance. J. Sci. Food Agric., 85: 743-750.

Rowghani, E and Zamiri, M (2009). The effects of a microbial inoculant and formic acid as silage additives on chemical composition, ruminal degradability and nutrient digestibility of corn silage in sheep. Iranian J. Vet. Res., 10: 110-118.

Rowghani, E; Zamiri, M; Khorvash, M and Abdollahipanah, A (2008). The effects of *Lactobacillus plantarum* and *Propioni-bacterium acidipropionici* on corn silage fermentation, ruminal degradability and nutrient digestibility in sheep. Iranian J. Vet. Res., 9: 308-315.

SAS (2002). *SAS User's Guide: Statistics*. Version 9.1, SAS Institute Inc., Cary, NC, USA.

Sucu, E and Filya, I (2006). Effects of homo-fermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of LDMCS. Turk. J. Vet. Anim. Sci., 30: 83-88.

Tilley, JMA and Terry, RA (1963). A two stage technique for the *in vitro* digestion of forage crops. J. Br. Grassland Soc., 18: 104-111.

Van Soest, PJ; Robertson, JB and Lewis, BA (1991). Methods of dietary fiber, neutral detergent fiber and non-starch poly-saccharides in relation of animal nutrition. J. Dairy Sci., 74: 3583-3597.

Weinberg, ZG and Muck, RE (1996). New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol. Rev., 19: 53-68.

Whiter, AG and Kung, L (2001). The effect of a dry or liquid application of *Lactobacillus plantarum* MTD1 on the fermentation of alfalfa silage. J. Dairy Sci., 84: 2195-2202.