The impact of a bacterial inoculant on chemical composition, aerobic stability and *in sacco* degradability of corn silage and the subsequent performance of dairy cows

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Abstract

The aim of this study was to investigate the effect of bacterial inoculation on the fermentation and aerobic stability of corn silage and on the dry matter degradability and milk composition when fed to sheep or cows. Four male sheep were selected to measure dry matter degradability of uninoculated (UCS) and inoculated (ICS) corn silage. Milk composition was measured for 22 Holstein cows, separated into two treatment groups and fed with either UCS or ICS diets. Similar lactic acid concentrations but higher propionic and lower acetic acid concentrations were recorded for ICS diets compared to UCS diets ($p < 0.05$). Aerobic stability was 12 h and 32 h for UCS and ICS, respectively. Values of *in sacco* dry matter loss were higher for ICS than for UCS ($p < 0.05$). Lactose and solid non-fat content was higher in the milk of cows fed with ICS than UCS ($p < 0.05$). We conclude that the Lalsil bacterial inoculant containing *propioni bacterium acidipropionici* and *Lactobacillus plantarum* can be used as an additive due to its positive effect on fermentation, aerobic stability and dry matter degradability of corn silage. The slight positive effect of inoculcation on the nutritional value of silage appears to be limited to milk production improvement in dairy cows.

Introduction

Corn (*Zea mays*) is the most popular cereal crop and is conserved as silage in many parts of the world (McDonald *et al.,* 1991). Corn (*Zea* maize) silage has a relatively high dry matter content, low buffering capacity and adequate water soluble carbohydrates for fermentation to lactic acid (Meeske and Basson, 1998) and is regarded as an ideal silage crop. Corn silage is the main silage fed to dairy cattle in Iran and plays a vital role in supplying the required digestible fiber and energy. Both the quantity of corn forage produced and the proportion that is preserved as silage have increased world wild in recent years (Abido *et al*., 2007).

The establishment of anaerobic conditions during ensiling is required for the production of high quality corn silage. These conditions restrict plant respiration by retarding the growth of oxidative microbes. Failure to achieve such conditions may lower the protection of feed nutrients, leading to lower quality silage, which may result in reduced feed intake and performance in animals (Cleale *et al.*, 1990).

In order to improve the ensiling process, various chemical and biological additives have been developed. The biological additives are advantageous because they are safe and easy to use, non-corrosive to the machinery, do not pollute the environment, and are regarded as natural products (Filya *et al.*, 2000). Microbial inoculants are the most common biological additives applied to forages at the time of ensiling to accelerate the decline of pH during the initial stage of silage fermentation, to preserve plant carbohydrates through homofermentation, and to preserve plant proteins by decreasing proteolysis and deamination (Hristov and McAllister, 2002).

Several studies have demonstrated the effects of inoculation on the corn silage fermentation characteristics (Harrison *et al.*, 1996; Higginbotham *et al*., 1998; Johnson *et al.*, 2003; Aksu *et al.*, 2004). A review of 17 studies demonstrated an increase in lactic acid levels, and a significant reduction in the pH of fermented corn silage, following the addition of an inoculant (Harrison *et al.*, 1996). In some cases, an appreciable effect of silage inoculation on animal performance has also been observed. For example, feed intake, live-weight gain, feed efficiency and milk

production were improved in the Harrison *et al*. (1996) study. However, improvements in milk production have been reported far less frequently than alterations in fermentation (Zahiroddini *et al.*, 2004).

The objective of this research was to determine the effects of a bacterial inoculant on the fermentation characteristics**,** dry matter degradability and nutritive value of corn silage. The effects on feed intake, milk yield and milk composition of lactating Holstein dairy cows fed with bacterial inoculated corn silage were also investigated.

Materials and Methods

Forage preparation and ensiling

Whole-plant corn from Tarek 644 hybrid was harvested during the summer from the grounds of the Amin-Abad Animal Research Unit of the Faculty of Veterinary Medicine, Iran. The forage was in an early stage of maturity, with 22 % dry matter (DM) content. The crop was harvested to a length of 0.95 cm, using a conventional forage harvester. Approximately 10 kg of fresh corn forage (FCF) was sampled and frozen at -20°C for further analysis. Forage was ensiled as either uninoculated corn silage (UCS), as a control, or inoculated corn silage (ICS). For the ICS treatment, a bacterial inoculant was used (Lalsil® MS01, containing *propioni bacterium acidipropionici* MA126/4U and *Lactobacillus plantarum* MA18/5U; Lallemand Animal Nutrition, France). A 200 g measure of inoculant was dissolved in 40 l of water and the solution was sprayed on top of each 20 cm thickness of corn forage at ensiling. For both UCS and ICS, one bunker silo 40-ton capacity was filled to follow the fermentation dynamics during ensiling. Both the UCS and ICS bunkers were covered by thick polyethylene nylon for isolating environment in order to establish anaerobic conditions. Sixty days after ensiling, a 10 kg sample was collected from the UCS and ICS silos and frozen at -20°C for chemical analysis and the evaluation of fermentation characteristics, aerobic stability determination, and *in sacco* degradability.

Chemical composition

Four replicate samples (10 g each) of FCF, UCS and ICS samples were homogenized with 100 ml of distilled water for 1 min. The pH of the water extract was determined immediately, using a digital pH meter (Corning, UK). Silage buffering capacity (miliequivalents of NaOH/100g of DM required to raise the silage pH from four to six) was also determined on homogenates of fresh forage and silage composites by titration (Playne and McDonald, 1966).

A portion of the extract was stored at -20°C prior to further analysis. One millilitre of water extract was combined with 200 µl of 250 g/kg metaphosphoric acid containing 2-ethyl butyric acid, as an internal standard for volatile fatty acids (VFA) measurements. Samples were centrifuged for 15 mins at 10,000 × g and analyzed for acetic, propionic, butyric and other volatile fatty acids by gas chromatography (Hewlett Packard model 5890 series II), equipped with flame ionization detector and model 7673 auto injector (Hewlett Packard, Palo Alto, CA, USA) fitted with a 15 m Nukol fused capillary column (Supelco, Inc., Bellefonte, PA, USA). Column temperature was fixed at 150°C for a run time of eight mins. Injector and detector temperatures were 180°C and 200°C, respectively. Gas flows were 30, 300, and 30 ml/min for He, air, and H_2, respectively (Hassanat *et al.*, 2007).

Samples of FCF, UCS and ICS were extracted for 3 mins in a blender with water. The water-soluble carbohydrate (WSC) content of the sample was determined using the phenol sulfuric acid method (Dubois *et al.*, 1956). The ammonia-N (NH_3-N) content of FCF, UCS and ICS was determined by extraction of 40 g frozen samples with 360 ml of distilled water in a Stomacher blender (IUL, Barcelona, Spain) for 3 mins. The extract was filtered through Whatman No.1 paper (Whatman, Maidstone, UK), and 100 ml of the extract was used for distillation with a Kjeltech auto analyzer (Gerhardt, Bonn, Germany), without a digestion step. Gas losses were then evaluated by weight comparison (Filya, 2003).

Parts of the FCF, UCS and ICS samples were dried and used for the subsequent chemical analysis. The DM content of samples was determined by drying in a fan-assisted oven at 60°C for 48 hrs. The ash content was assayed following burning in an electric furnace at 600°C for 24 hrs. The Macro-Kjeldahl method (AOAC, 1990) was used to determine the crude protein (CP) content of samples. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were assayed, according to the method of Van-Soest *et al.* (1991).

Aerobic stability

The aerobic stabilities of UCS and ICS silages were determined. Approximately 10 kg of representative samples from each treatment were loosely packed into plastic bags and allowed to aerobically deteriorate at room temperature (24°C). In each set of silos, thermocouple probes were placed in the geometric-center of the silage samples. These silages were not disturbed throughout temperature recording. The ambient temperature and the temperatures of the silages were recorded every 2 hrs. Aerobic stability was defined as the number of hours the silage remained stable before rising 2°C above the ambient temperature (Moran *et al.*, 1996).

Animals and experiments

Two separate experiments were performed involving fistulating sheep and lactating dairy cows as described below.

a) Degradability measurements (Experiment 1)

Four mature Iranian Chall × Zandi male sheep with 40 kg (± 3 kg) of live weight were selected for the 4 × 4

Latin square design experiment. The degradability rates of DM from the FCF, UCS and ICS feed samples were determined using the nylon bag technique as described by Ørskov and McDonald (1979). The size of nylon bags was 12 × 5 cm with 40 µm pore size. The animals were kept individually in metabolic cages with free access to water and mineral blocks. Sheep were fed twice daily at 8:00 and 17:00. Alfalfa hay, wheat straw, barley grain, cottonseed meal and wheat bran were used as the feed ingredients of the diets. The forage to concentrate ratio of the total mixed ration (TMR) was 75:25. Prior to commencing the *in-situ* trial, the FCF, UCS and ICS samples were all dried and milled through a 2-mm sieve. Then 4 g of each sample was incubated in nylon bags in the rumen for 4, 8, 16, 24, 48, 72 and 96 hrs. After each incubation, nylon bags were removed from the rumen and immediately washed in cold tap water until all the water soluble content had been removed. All nylon bags were then dried for 24 h at 60°C and their DM losses were determined. The value of degradability at time zero was obtained by washing the bags containing the relevant materials in a washing machine (cold water) for 1 h. Data with respect to *in situ* DM losses for FCF, UCS and ICS samples were adjusted using the exponential equation described by Ørskov and McDonald (1979). The respective DM degradability parameters were then calculated.

b) Dairy cattle performances (Experiment 2)

Twenty-two multiparous Holstein cows with an average body weight (BW) of 600 kg (±30 kg), a milk yield of 35 kg (±6 kg) per day (MY/day) and 90 (±30) days in milk (DIM) were separated into two treatment groups, such that the groups were identical in terms of mean BW, MY/day, DIM and parity of the cows. The treatment groups differed only in the type of corn silage used (UCS or ICS). All cows consumed a similar total mixed ration (TMR) with a concentrate to forage ratio of 65:35. Diets were formulated according to the NRC recommendations for dairy cows (2001; Table 1). The allocation of cows for each dietary group was by random selection. The cows were fed ad-libitum and the amount of feed consumption for each group was measured daily by monitoring refusals and spillages from the offered feeds. Fresh water was available at all times and animal care standards. The trial was eight weeks in duration, with a two-week adoption period and a six-week experimental period. Milk yield was recorded three times daily, at 05:00, 13:00, and 21:00. Milk was sampled from each cow once weekly, at morning milking, and then preserved with potassium dichromate until analysis. Samples were analyzed for fat content, total protein, milk urinary nitrogen (MUN), lactose and solid non-fat (SNF) content, using a Milko-Scan (Ithaca, NY infrared analysis Foss 605B, Foss Electric, Hillelrod, Denmark).

Table 1. Dry matter, ingredients, chemical composition and energy content of uninoculated (UCS) and inoculated (ICS) corn silage constituents of the total mixed rations.

	Total mixed rations	
Items	UCS	ICS
Dry matter (DM, g/kg diet)	608.00	619.00
Ingredients (g/kg DM)		
Uninoculated corn silage	146.80	-
Inoculated corn silage	-	146.80
Shelled corn (ground)	62.60	62.60
Cottonseed meal	60.00	60.00
Soy bean meal	80.30	80.30
Rape seed meal	60.90	60.90
Barley grain (ground)	170.50	170.50
Wheat bran	126.50	126.50
Sugar beet pulp (dehydrated)	69.00	69.00
NaCl	1.70	1.70
Sodium bicarbonate	7.80	7.80
$CaCl_2.2H_2O$	3.00	3.00
Mineral-vitamin mix*	6.50	6.50
Chemical composition (g/kg DM)		
Total digestible nutrients	673.00	673.00
Crude protein	154.00	154.00
Rumen degradable protein	102.56	102.56
Rumen non-degradable protein	41.44	41.44
Neutral detergent fiber	384.00	384.00
Acid detergent fiber	218.00	215.00
Non-fibrous carbohydrates (NFC) †	366.00	366.00
Ether extract	24.00	24.00
Calcium	11.00	11.00
Phosphorus	5.70	5.70
Potassium	11.40	11.40
Energy content (Mcal/kg DM) ‡		
Net energy for lactation (NE_L)	1.64	1.64

* Containing 2,000 mg Mn, 3,000 mg Zn, 3,000 mg Fe, 280 mg Cu, 100 mg I, 1mg Se, 100 mg Co, 55,000 mg Na, 20,000 mg Mg, 195,000 mg Ca, 90,000 mg P, 500,000 IU of vitamin A, 100,000 IU of vitamin D and 100 IU of vitamin E. † Calculated based on NRC (2001), following: NFC = 100 − (FA % + NDF % + CP % + Ash %). ‡ Calculated based on the NRC (2001) values for individual feedstuffs.

Calculations and statistical analysis

Chemical analysis data, fermentation parameters and degradability were subject to analysis of variance tests using the GLM procedure in the SAS software package (2005) for each set of four replicate samples. The mean values were compared using Duncan's test (Duncan, 1955). The milk production and milk composition data were analyzed using the SAS PROC MIXED model. Time (in days of milk production and weeks for milk composition) was used as repeated measurements with compound symmetry covariance structure (Littell, 1998). Comparisons of mean values were made using the least significant difference method and were considered significant for $p < 0.05$.

Results

Corn forage and silage characteristics

The DM content of FCF was 21.8% at harvest. Sixty days after ensiling, UCS and ICS had DM contents of 20.9% and 21.3%, respectively. Similarly, CP, ADF, NDF and total ash content were measured in the fresh forage and in both inoculated and uninoculated corn silages (Table 2).

Table 2: The chemical composition and fermentation characteristics of fresh corn forage (FCF), uninoculated (UCS) and inoculated corn silage (ICS).

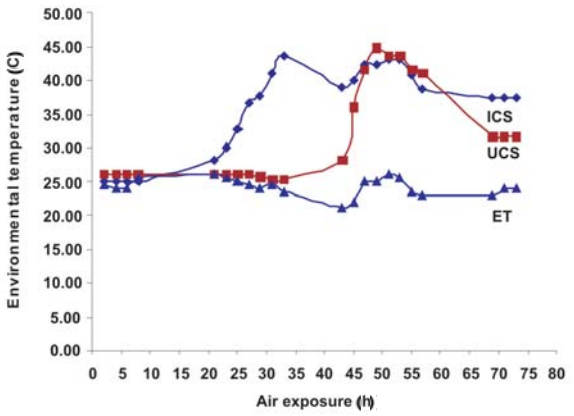
Items	Treatments			
	FCF	UCS	ICS	S.E.M
Dry matter (g/kg)	218	209	213	8.60
Crude protein (g/kg)	74.50	75.50	75.00	1.46
Ash (g/kg)	80.00	83.50	83.00	17.81
Neutral detergent fiber (g/kg)	543.00	563.00	559.00	18.81
Acid detergent fiber (g/kg)	327.00	357.00	332.00	5.07
pH	6.83^{a}	3.75^{b}	3.67^{c}	0.012
Buffering capacity (milieq. NaOH/100 g DM)	14.01	91.00	98.20	3.36
Water-soluble carbohydrates (g/kg)	119.60^{a}	14.20^{b}	34.30^{c}	1.73
Ammonia-nitrogen (g/kg)	-	0.68^{a}	0.39^{b}	0.023
Organic acids (g/kg)				
Lactic acid	4.50^{a}	72.00^{bc}	77.00^{bc}	0.29
Acetic acid	5.32^{a}	38.70^{b}	22.50^{c}	1.59
Propionic acid	4.07^{c}	6.80^{b}	8.50^{a}	0.24
Butyric acid	-	0.47	-	-
Iso-butyric acid		0.37^{a}	0.26^{c}	0.034^{b}
Valeric acid		0.37		
Iso-valeric acid	0.75^{c}	1.09^{ab}	1.12^{ab}	0.036

$^{a, b, c}$ Different superscripts within a row indicate a significant difference between mean values ($p < 0.05$).

Both silages were found to be well preserved. The pH values of both silages decreased to below four after ensiling. However, the pH of ICS was lower ($p < 0.05$) than that of UCS. No differences were observed between the buffering capacity and lactic acid content of UCS and ICS. The concentration of acetic acid was found to be higher in UCS than that in ICS ($p < 0.05$). In contrast, propionic acid concentration was found to be higher ($p < 0.05$) in ICS compared to UCS. No butyric acid or valeric acid was observed in ICS. The concentration of iso-butyric acid was found to be higher in UCS than that in ICS ($p < 0.05$). Iso-valeric acid content was found to be similar for UCS and ICS.

The ICS contained higher ($p < 0.05$) residual WSC and lower ($p < 0.05$) NH_3-N concentration compared to UCS. The inner temperature of UCS and ICS declined very soon after air exposure, due to the very low DM content of both silages. However, upon aerobic exposure, the UCS spoiled faster than the ICS. The aerobic stability of UCS was approximately 12 h compared to 32 h for ICS (Figure 1).

Figure 1. Changes in environmental temperature (ET) for uninoculated (UCS) and inoculated corn (ICS) silage during a 73-h period of exposure to air.



Degradability parameters

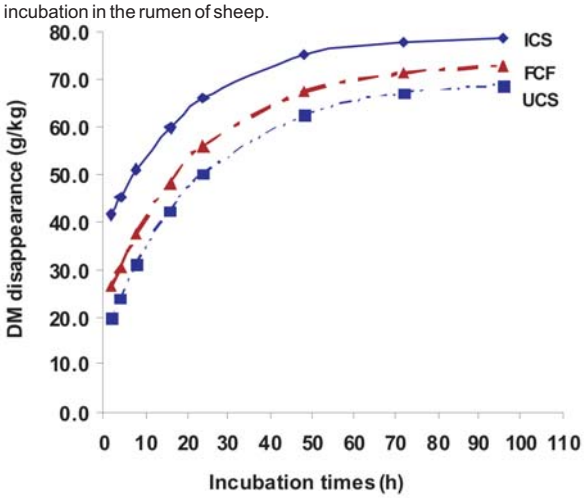
The mean values of *in situ* DM loss for FCF, UCS and ICS after various incubation times and their respective degradability parameters are shown in Table 3. All parameters (*i.e.*, A, B and C) were higher ($p < 0.05$) in FCF compared to UCS and ICS. The soluble fraction (A) was higher ($P < 0.05$) in ICS than UCS. No difference was found between the potentially degradable fractions of silages (B). The rate of DM degradation (C), lag time (L) and the effective dry matter degradability (EDDM) were all found to be higher ($P < 0.05$) in ICS compared UCS. As shown in Figure 2, FCF exhibited the highest *in situ* DM loss at all incubation times, followed by ICS and then UCS.

Table 3. Dry matter loss and degradability characteristics of fresh corn forage (FCF), uninoculated (UCS) and inoculated corn (ICS) silage.

Items	Treatments			
Loss (g/kg DM)	FCF	UCS	ICS	S.E.M
0 h	433^{a}	238^{c}	269^{b}	13.50
4 h	449^{a}	249^{c}	304^{b}	1.07
8 h	509^{a}	264^{c}	374^{b}	13.10
16 h	598^{a}	408^{c}	492^{b}	8.80
24 h	659^{a}	522^{c}	557^{b}	11.50
48 h	749^{a}	638^{c}	673^{b}	11.40
72 h	778^{a}	677^{bc}	713^{bc}	14.50
96 h	788^{a}	680^{c}	727^{b}	11.50
Degradability parameters				
A* (g/kg)	377^{a}	153^{c}	220^{b}	10.60
B* (g/kg)	416^{c}	544^{ab}	455^{ab}	19.30
C* (h)	0.048^{abc}	0.042^{abc}	0.045^{abc}	0.0031
L* (h)	2.97^{ab}	4.05^{a}	2.27^{ab}	0.44
EDDM (g/kg) †	583^{a}	410^{c}	464^{b}	6.40

*A and B = Rapidly and slowly-degrading fractions, C = Rate of degradation and L = Lag time. † EDDM = Effective dry matter degradability. $^{a, b, c}$ Different superscripts within a row indicate a significant difference between mean values ($p < 0.05$).

Figure 2. *In sacco* dry matter (DM) loss for fresh corn forage (FCF), uninoculated (UCS) and inoculated (ICS) corn silage during a 96-h incubation in the rumen of sheep.



Dairy cattle performances

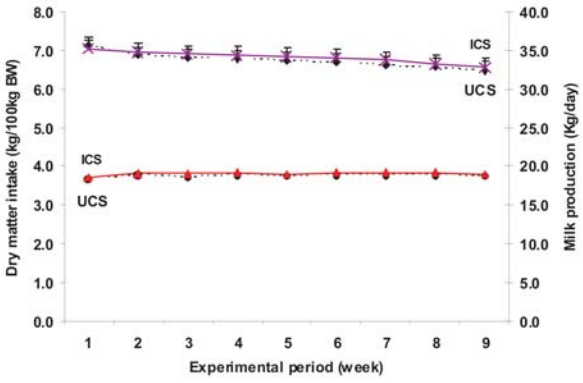
Results relating to the DMI, MY/day and milk composition of cows fed with UCS and ICS are presented in Table 4. Although DMI was not affected by the different treatments, cows in the ICS group consumed slightly larger amounts of feed during experiment 2 (3.79 and 3.75 kg/100kg BW for ICS and UCS, respectively; Figure 3). No difference was found between the MY/day of cows in the two groups. Furthermore, milk composition was independent of the silage production method. However, the lactose and SNF contents of milk were found to be higher ($P < 0.05$) in cows fed with a TMR containing ICS compared to UCS (Table 4).

Table 4. Dry matter intake (DMI), milk yield and milk composition of Holstein cows fed total mixed diets containing either uninoculated (UCS) or inoculated corn silage (ICS).

	Treatment		
Items	UCS	ICS	P value
DMI (kg/100kg BW)	3.75±0.12	3.79±0.12	ND
Milk yield (kg/day)	33.59±1.1	33.93±1.1	0.83
Milk composition (%)			
Fat	3.74±0.08	3.78±0.08	0.74
Protein	3.11±0.05	3.15±0.05	0.61
Lactose	4.66±0.06[b]	4.85±0.06[a]	0.04
Solid not fat	8.64±0.09[b]	8.90±0.09[a]	0.05
Milk urinary nitrogen	0.054±0.002	0.054±0.002	0.41

[a, b, c] Different superscripts within a row indicate a significant difference between mean values ($p < 0.05$).

Figure 3. Dry matter intake and milk production of cows fed with uninoculated (UCS) and inoculated (ICS) corn silage during the experimental period.



Discussion

Silage characteristics

Microbial inoculants have been commonly reported to improve the fermentation of silages, by decreasing pH and increasing lactic acid levels. Decreases in NH_3-N, acetic acid, and butyric acid concentrations and a reduction in DM content of silage have also previously been reported (Higginbotham *et al.*, 1998; Kung *et al.*, 2003; Aksu *et al.*, 2004; Hassanat *et al.*, 2007). With respect to maize silage, Jatkauskas and Vrotniakiene (2004) demonstrated that lactic acid bacteria (LAB) inoculants could enhance fermentation, increase WSC and lactic acid content, and decrease acetic acid, butyric acid and NH_3-N concentrations. In our study, ICS was found to contain more residual WSC than UCS ($P < 0.05$), which is in agreement with a previous study demonstrating an increase the concentration of residual WSC in silages due to homolactic inoculants (Kung *et al.*, 2003). However, Johnson *et al.* (2003) reported that inoculation had only a minor effect on corn silage fermentation characteristics. Meesk and Basson (1998) concluded that the lack of a response in the enhancement of silage fermentation could be attributed to the high numbers of lactic acid bacteria which are commonly present on the maize crop at the point of ensiling. The notable increase in lactic acid concentration and the decrease in pH value ($P < 0.05$) of ICS in the present study are in agreement with a previous review of 17 studies by Harrison *et al*. (1996), which demonstrated a numerical increase in lactic acid levels and a reduction in pH of corn silage when inoculants were used. The butyric acid and the NH_3-N levels of ICS were lower ($P < 0.05$) than those in UCS (Harrison *et al.,* 1996). This finding is in agreement with previous reports (Weinberg *et al.*, 1993; Chen *et al.*, 1994; Aksu *et al.*, 2004). McDonald *et al.* (1991) showed a reduction in pH of silage in the presence of inoculants, which inhibited protein degradation. This is the proposed reason for the lower concentration of NH_3-N in ICS compared to UCS. However, reported data on the effects of inoculation on the proteolysis of barley (Hristov and McAllister, 2002), maize (Sucu and Filya, 2006a) and millet (Hassanat *et al.*, 2007) are inconsistent. Hassanat *et al.* (2007) stated that factors other than the type of inoculants such as species, DM content and the initial pH of forage can influence the extent of proteolysis in silage. There has been considerable interest in the use of biological inoculants to overcome the problem of the aerobic instability of silage. Classical microbial inoculants have been reported to have no effect on aerobic instability, or evento accelerate the problem (Muck and Kung, 1997; Sucu and Filya 2006b). Weinberg *et al.* (1993) hypothesized that the high level residual WSC, high lactic acid and insufficient protective VFA in silage inoculated with homofermentative LAB were associated with aerobic spoilage. Their reasoning is that both the WSC and lactic acid are substrates for fungi, and VFA often inhibit these organisms. Therefore, in order to overcome the problem of aerobic deterioration of silage, the use of other types of inoculants, such as *Bacillus* species and propionic acid bacteria (PAB) has been proposed (Pahlow and Honig, 1994). It is expected that such additives would produce substances

in the silage with antimycotic properties, which would inhibit the development of yeasts and molds upon aerobic exposure. PAB ferment sugar and lactate, converting them to the short-chain aliphatic acids acetate and propionate, which inhibit yeasts and molds (Filya *et al.*, 2006). Bolsen *et al.* (1996) reported that the PAB *P. acidipropionici* improved aerobic stability of maize silage. In addition, Filya *et al.* (2004) found that the use of *P. acidipropionici* for wheat, sorghum and corn ensiling, with or without homofermentative LAB, decreased yeast and mold numbers and improved aerobic stability. However, the combination of *P. acidipropionici* and *L. plantarum* was not found to improve aerobic stability of silage. Dawson *et al.* (1998) reported that inoculation with *P. acidipropionici* improved fermentation characteristics and aerobic stability of high moisture maize silage. However, Higginbotham *et al.* (1998) showed that the addition of two levels of PAB inoculants had no appreciable effect on the aerobic stability of maize silage. The primary reasons for the ineffectiveness of these organisms may be attributed to the fact that they are strict anaerobes with slow growing and relatively acid intolerant activities (Kung, 2001). In the present study, aerobic stability was notably affected by bacterial inoculant (containing *P. acidipropionici and L. plantarum*), such that the aerobic stabilities of both forms of silage were very low. In agreement with these findings, Higginbotham *et al.* (1998) and Filya (2003) found that maize silage harvested at an early dent maturity stage was not stable aerobically when was exposed to air.

Degradability parameters

Published reports on the effects of inoculants on the degradability of silage reveal some inconsistencies. In the study of Sanderson (1993), no enhancement in fiber degradability was observed when corn silage was inoculated with *L. plantarum* and *E. faecium*. Hristov and McAllister (2002) reported that the *in situ* potentially degradable fractions of water-soluble carbohydrates (WSCs) and the rate of degradation, or effective degradability, of silage DM were not affected by inoculants. In addition, Filya (2003) showed that inoculation with *L. buchneri*, alone or in combination with *L. plantarum*, did not affect the *in situ* rumen degradability of DM, organic matter, or NDF of corn and sorghum silage. Furthermore, Zahiroddini *et al.* (2004) verified that inoculation had no effect on the kinetic parameters and the effective degradability of DM and NDF of whole crop barley silage. Sucu and Filay (2006b) also found that inoculation with the homofermentative LAB had no effect on *in situ* rumen degradability of DM and organic matter of wheat silage. However, Muck (1993) reported that DM and fiber digestibility were improved in inoculated silage in 30 - 55% of case studies. Davies *et al.* (1998) concluded that the degree of improvement in the digestibility of inoculated silages was dependent on the availability of WSC in the biomass during the silage fermentation phase. In light of this, the enhancement of degradability parameters in ICS observed in the present study can be attributed to the difference in WSC content between UCS and ICS. Chen *et al.* (1994) indicated that the treatment of haycrop silage with commercial enzyme-inoculants increased DM loss at incubation times of 0, 4, 8, 12, 18 and 24 h, and the NDF loss at times of 0, 8, 12, 18 and 24 h. The slowly-degradable DM and the NDF content were also reduced. However, the rapidly-degradable DM, the NDF constituents and the rates of degradation of slowly-degradable DM and NDF fractions appreciably increased, even though both rates of degradation increased by 35%. The authors concluded that the increased DM and NDF loss in the first 24 h of ruminal incubation and the resulting changes in the degradation parameters indicated that treatment of haycrop silage with an enzyme-inoculant mixture modified forage cell-wall structure and made the components more readily accessible to ruminal microorganisms. Salawu *et al.* (2001) found that the application of *L. plantarum* to pea-wheat silage increased the rate of nitrogen and NDF degradation in the rumen. The improvement in silage nutrient degradability was particularly surprising, since LAB inoculants do not degrade polysaccharides. Some researchers have hypothesized that the greater rate and extent of pH decline in inoculated silage enhances acid hydrolysis and may account for the higher degradability of structural carbohydrates.

Cattle performance

Milk production and composition have previously been reported to be affected by microbial inoculants by lessening the decrease in silage quality. Kung *et al.* (1987) reported that inoculated alfalfa silage stored for 120 days increased the milk production of dairy cows by 6-8%. Fresian cows fed with inoculated grass silage produced about 2 kg/day more milk than cows fed with uninoculated silage (Gordon, 1989). Chen *et al.* (1994) indicated that treating haycrop silage with a commercial enzyme-inoculant mixture increased DMI, BW ratio and milk production in cows. Meeske *et al.* (2002) demonstrated that inoculant-treated silage increased the MY/day of cows by 1 kg/day. The intake of cows fed with inoculated silage was also higher compared to the control group. Kurtoglu and Coskun (2003) found an elevation of 9.2% in milk production of cows. In a review of 11 milk production trials using a variety of silage inoculants, Mayne and Steen (1993) reported an 8.8% improvement in silage DM intake and a 2.9% increase in MY/day when inoculated silage was used. However, others (Martinson, 1991; Kung *et al.*, 1993) reported that inoculation had no effect on milk

production. Magalhaes and Rodrigues (2003) also noted that DMI and MY/day of cows were not affected by silage inoculation. Furthermore, Raeth-Knight *et al.* (2007) reported that the MY/day and DMI were not affected when dairy cows were fed *L. acidophilus* and *P. freudenreichii* added diets. These differences in the effect of inoculated silages on the milk production of dairy cows cmay be attributed to the microbial inoculants used, the conditions of trials, insufficient inoculation and the use of unsuitable inoculants for silage materials (Kurtoglu and Coskun, 2003). In agreement with Kurtoglu and Coskun (2003), in the present study, no significant differences were found between the milk fat content of cows in the two treatment groups (Figure 3). Kung *et al.* (1993) concluded that cows fed with inoculated silage produced milk with a higher fat percentage (3.62%) than those fed with uninoculated silage (3.43%). In addition, Gordon (1989) reported milk fat contents of 3.61% and 3.63% for cows in control and inoculated grass silage groups, respectively. The relative increase in milk fat may be correlated with the increase in quality of inoculated silages, leading to a positive effect of inoculation on the digestibility of organic matter and associated nutrients. In support of our results, previous studies have shown that milk protein level is not affected by the use of inoculated silages (Meeske *et al.*, 2002; Magalhaes and Rodrigues 2003; Abido *et al.*, 2007). Interestingly, Kung *et al.* (1987) found that inoculants had a considerable effect on the percentage of protein content. However, the numerical differences reported might be due to several factors, such as the animal breed, nutrition or lactation periods. The lactose and SNF content of milk was found to be higher in ICS fed cows than those in the UCS group. In contrast, Magalhaes and Rodrigues (2003) reported that silage inoculation did not influence the fat, protein, lactose, MUN and SNF percentages of milk in dairy cows. Additionally, no differences were found in the fat and protein content of milk from dairy cows in the study by Meeske *et al.* (2002). The finding in the present study that MUN was independent of the treatment group is in agreement with the findings of Magalhaes and Rodrigues (2003). It has been reported that the protein and SNF contents of milk increased with haycrop silage inoculated with a commercial enzyme-inoculant mixture (Chen *et al*., 1994). In agreement with our findings, Abido *et al.* (2007) reported that animals fed inoculated maize silage had higher contents of milk total solids, milk fat and lactose compared to those in the control group. These results can potentially be attributed to higher glucose and protein concentrations in the blood serum of cows in ICS group. In the present study, this led to an increase in milk lactose synthesis and consequently, a slight increase in milk production (Figure 3). However, Sucu and Filya (2006a) suggested that the likely success of utilizing bacterial inoculants as silage additives is dependent on several factors, such as the type and properties of the crops to be ensiled, climatic conditions, epiphytic microflora, inoculant composition, the amount and method of inoculant application, the ensiling technique and the specific properties of the inoculant. In conclusion, it is suggested that a combined treatment of *propioni bacterium acidipropionici* and *Lactobacillus plantarum* is used as a silage additive, in light of the positive effects on fermentation, the aerobic stability and degradability of corn silage observed in the present study. A moderately positive effect of the inoculant on the performance of dairy cows can also be expected with this treatment approach.

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