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Ensiling corn silage with different levels of a multi-species lactic acid bacteria inoculant

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Abstract. A multi-species lactic acid bacterial inoculant (Lactisil maize, LM) was applied to whole-crop corn at different maturities in laboratory silos, to evaluate its effects on biochemical characteristics and aerobic stability. The corn crop was harvested at hard dough (HD, 253.1 g/DM kg), one-third milkline (ML, 293.7 g/DM kg) and one-third milkline with a killing frost (MLF, 297.6 g/DM kg). Crops were chopped to a 2.5-cm theoretical cut length, subsampled and treated with two levels of inoculant (LB1 = 1.5×10^5 cfu/g forage, LB2 = 3×10^5 cfu/g forage) or untreated (WO). The chemical composition of MLF crops was very similar to that of ML crops. However, lower ($P < 0.01$) numbers of lactic acid bacteria and higher numbers of yeast were enumerated in MLF than in ML crops. Higher percentages of DM and neutral detergent fibre and higher pH, but lower ($P < 0.01$) concentrations of water soluble carbohydrate and crude protein were measured in ML and MLF crops than in HD crops. Application of the inoculant increased ($P < 0.01$) concentrations of volatile fatty acids, neutral detergent fibre and acid detergent fibre in silages. Lactic acid concentration increased ($P < 0.01$) in HD treatments with an increasing level of inoculant. In contrast, the highest ($P < 0.01$) lactic acid concentration was measured in LB1 treatment compared with WO and LB2 in ML and MLF silages. Silages prepared from ML and MLF crops had higher ($P < 0.01$) lactic and acetic acid concentrations but lower ($P < 0.01$) butyric acid concentrations than did those prepared from HD. The pH in LB1 and LB2 silages was higher ($P < 0.01$) than that measured in WO silages. Aerobic stability was not influenced by inoculant treatment but low-DM silages were more ($P < 0.01$) resistant to spoilage. Frost-killed corn crops had a good potential to produce well fermented silage. Using LM resulted in silages with slightly higher fermentation products but it failed to improve aerobic stability of silage after 120 days of ensiling. These results indicated that inoculation of corn crops with LM for a short-duration ensilage period cannot enhance aerobic stability of silages due to insufficient acetic acid production from lactic acid conversion.

Additional keywords: aerobic stability, frost-killed corn, heterofermentative fermentation, homofermentative lactic acid bacteria, *Lactobacillus buchneri*, maturity stage.

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Introduction

Ensiling is a common preservative method for forage crops. Anaerobic conditions in silo and fermentation of water soluble carbohydrates (WSC) into lactic acid with lactic acid bacteria (LAB) preserve the nutrients in crops (McDonald *et al.* 1991). In Iran, corn (*Zea mays*) fodder is one of the major forages for ensiling and makes up approximately half of the dietary forage on many commercial dairies. However, in Iran, corn silage is usually planted in July as a second crop and is ensiled with low DM content (20–25% DM) due to insufficient time to reach an ideal maturity for ensiling (Khorvash *et al.* 2006). Moreover, late planting date and early falling in ambient temperature may result in situations where corn is killed by frost. Frosting reduces the level of LAB on the standing plant and elevates numbers of spoilage organisms (Mohammadzadeh *et al.* 2012) and then may produce silages with restricted fermentation and low aerobic stability.

To improve the nutritive value of silage, various types of additives, including bacterial inoculants, have been developed. Most bacterial inoculants consist of homofermentative LAB (e.g. *Lactobacillus plantarum*) rather than heterofermentative LAB (e.g. *Lactobacillus buchneri*). Homofermentative LAB inoculants enhance the rate of acidification and reduce the final pH or protein breakdown in silages (Sheperd *et al.* 1995; Weinberg and Muck 1996; Driehuis *et al.* 1997; Aksu *et al.* 2004). However, homofermentative LAB could induce aerobic deterioration of whole-crop cereal silages due to insufficient production of volatile fatty acids (VFA) to inhibit fungi (Weinberg *et al.* 1993; Filya *et al.* 2000). *L. buchneri* is the main heterofermentative LAB strain that produces higher

concentrations of acetic acid than does *L. plantarum* in silage (Kleinschmit and Kung 2006). Improvement of aerobic stability using *L. buchneri* has been demonstrated in laboratory (Driehuis *et al.* 1999; Filya 2001, 2003*b*; Kung and Ranjit 2001; Weinberg *et al.* 2002) and field (Mari *et al.* 2009; Kristensen *et al.* 2010) studies.

The aim of the present study was to investigate the effects of a new multi-species LAB additive, Lactisil maize (Medipharm, Kågeröd, Sweden), on chemical composition, fermentation characteristics and aerobic stability of corn silages prepared from crops at different maturities as well as on frost-killed corn crops. This inoculant consists of homofermentative (*Enterococcus faecium M74*, *Lactobacillus plantarum LS1*, *L. casei* and *Pediococcus pentosaceus*) and heterofermentative (*Lactobacillus buchneri*) LAB. *E. faecium* and *P. pentosaceus* have a high optimum pH and start to produce lactic acid at a high pH of forage crops. This leads to faster falling in pH and provides optimal environmental conditions for *L. plantarum* and *L. casei* (low optimum pH). At latter phases of ensilage period, the inhibitory effect of low pH on homofermentative LAB provides a suitable situation for *L. buchneri* to proliferate and compete with homofermentative LAB (Nishino *et al.* 2003). As a result, Lactisil maize (LM) may simultaneously enhance both fermentation rate and aerobic stability of corn silage, especially in matured and frost-killed corn crops.

Materials and methods

Experimental silages

The whole-crop corn (hybrid 700; Plant Breeding, Karaj, Iran) was sown on 17 June and was harvested on 28 September at a hard-dough maturity stage (HD, 253.1 g DM/kg), on 13 October at a one-third milkline maturity stage (ML, 293.7 g DM/kg) and on 26 October at a one-third milkline maturity stage after a killing frost (MLF, 297.6 g DM/kg). When the ambient temperature decreased below 0°C (−4°C) for one night, forages in the field were harvested as frost-killed forages. In frost-killed corn crops, leaves were of brown or blackish-green colour. Crops at each maturity stage were cut by a chopper (Model 965, Claas, Omaha, NE, USA) to a average cut length of 2.5 cm and subsampled to be inoculated and ensiled. For inoculant-treated silages, 4 or 8 g of LM powder was suspended in 2 L of distilled water according to manufacturer's recommendations and the suspension was then sprayed over the 400-kg subsamples of crop using a pressure sprayer, to get the final inoculation rates of 1.5×10^5 colony forming units (cfu)/g (LB1 treatment) and 3×10^5 cfu/g (LB2 treatment). The subsamples were each mixed thoroughly to obtain an even and homogeneous distribution of inoculums over the crops. The same amount of distilled water was applied to the control crops (silages without inoculants, WO). Laboratory PVC silos, 70 cm in height, 10 cm in diameter and equipped with a sink at the bottom to allow seepage outflow, were used for ensiling the crops. Before filling, each laboratory silo was weighed and then filled with ~2.5 kg of fresh forage. On filling, the silos were packed with a manual steel packer and were capped tightly. There were five silo replicates for each treatment. The silos were stored in the dark at room temperature (20–23°C), until opened for sampling after 120 days of ensiling.

Analytical procedures

Five replicated samples of corn crops and silages were analysed. DM of the samples was determined after drying in a forced-air oven for 72 h at 60°C, and corrected for loss of volatiles using the equation of Porter and Murray (2001). The dried samples were ground to pass through a 1-mm screen by using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA, USA). Organic matter was determined by burning at 550°C for 12 h. The ground samples were analysed for total N using the Kjeldahl method (Kjeltec 1030 Auto Analyzer, Tecator, Höganäs, Sweden). Neutral detergent fibre (NDF) was determined according to Van Soest *et al*. (1991) using α amylase and reported ash free. Concentrations of acid detergent fibre (ADF) were measured as described by AOAC (1990).

Subsamples of 20 g of corn crop or fresh silage were mixed with 180 mL of distilled water for 30 s in a blender to obtain extracts. Immediately after extracting, the pH was measured using a portable pH meter (HI8314, Hanna Instruments, ClujNapoca, Romania). The WSC concentration of samples was determined using the phenol–sulfuric acid method of DuBois *et al*. (1956). The silage extracts were filtered through filter paper (Whatman No. 54) for determination of concentration of fermentation products and ammonia-N (NH_3-N). VFA were determined using gas chromatography (0.25 × 0.32, id of 0.3 µ WCOT Fused Silica Capillary, CHROMPACK CP 9002, Model CP-9002, Delft, The Netherlands) according to Mohammadzadeh *et al*. (2012). Lactic acid concentration was determined spectrophotometrically according to Barker and Summerson (1941), as modified by Pennington and Sutherland (1956). Ammonia-N concentration was measured by distillation in a Kjeltec Auto Analyzer (Tecator), without previous digestion step, as described by Filya (2003*a*).

Aerobic-stability measurement and microbiological analysis after exposure to air

Aerobic stability in air-exposed silage was defined as the number of hours the silage remained stable before temperature rising >2°C above the ambient temperature (Moran *et al.* 1996). When the silos were opened, ~1200 g of silage in each silo was placed in a plastic open-top container. The silage was loosely packed to fill approximately one-half of the volume of the container. A thermometer was placed in the geometric centre of the silage mass. The containers were covered with two layers of sterile cheesecloth to minimise drying. The containers were kept at room temperature and the temperatures were measured at 2-h intervals (Mohammadzadeh *et al.* 2012). Water extracts were obtained from silages just before exposing to air, for enumerating microbial population according to Adesogan and Salawu (2004). Yeast and mould populations were enumerated in triplicate by using potato dextrose agar (Merck, Darmstadt, Germany) with 0.15% tartaric acid and incubation at 25°C for 4 days under aerobic conditions (Higginbotham *et al.* 1998). The LAB population was enumerated in triplicate by using MRS agar (Difco Laboratories, Detroit, MI, USA) and incubation at 35°C for 2 days. Colonies were counted from the plates of appropriate dilutions containing a minimum of 30 colonies. All microbial data were $\log_{10}$ transformed and are presented on a wet-weight basis.

Statistical analyses

This research was conducted using a factorial experiment based on completely randomised design. Main effects of stage of maturity (HD, ML and MLF), inoculation (WO, LB1 and LB2) and their interactions were included in the model. General linear model of SAS (2003) was used for analyses. Data for the composition of crops were analysed using one-way ANOVA. Significant differences among means were identified by Tukey's studentised range test and $P < 0.05$ was designated as significant.

Results

Table 1 reports the chemical and microbial composition of whole corn crop at different maturities before ensiling. Proportions of DM, OM and NDF and pH were higher ($P < 0.01$) in ML and MLF crops than in the HD crop. In contrast, lower ($P < 0.01$) WSC and crude protein (CP) concentrations were measured in ML and MLF crops than in the HD crop. The ML and MLF crops had larger numbers of epiphytic fungi ($P < 0.01$) and lower numbers of epiphytic LAB ($P < 0.01$) than did the HD crop. Chemical composition of MLF crops was similar to that of the ML crop. However, lower ($P < 0.01$) numbers of LAB and higher ($P < 0.01$) numbers of yeasts were found in the MLF than in ML crop.

Chemical composition of corn silages is given in Table 2. After 120 days of storage, there was no effect of inoculant application on the percentage of DM in the silages (Table 2). However, inoculation of MLF silage with a higher dosage of LM resulted in a silage with a higher ($P < 0.01$) percentage of DM. Inoculated silages had a higher ($P < 0.01$) concentration of NDF than did the control silage. The increase in the concentration of NDF in response to inoculation was more evident in MLF silages. A higher application level of the inoculant further increased ($P < 0.01$) NDF and ADF fractions in the silages. Although the LB2 treatment increased ($P < 0.01$) the concentration of CP in the HD and ML silages, a reduction was observed in the MLF silage.

Fermentative characteristics of corn silages are given in Table 3. Concentration of lactic acid increased ($P < 0.01$) in the HD crop with an increasing application level of the inoculant. A higher ($P < 0.01$) lactic acid concentration was measured in the LB1 treatment than in the WO or LB2 treatments of ML and MLF silages. Higher ($P < 0.01$) concentrations of acetic, propionic and butyric acids were measured in treated silages than in the respective control. Moreover, the higher application level of the inoculant increased ($P < 0.01$) the concentration of VFA in the silages. ML and MLF silages had a higher ($P < 0.01$) acetic acid concentration than did the HD silage. In contrast, HD silage had a higher ($P < 0.01$) butyric acid concentration than did the ML and MLF silages. A higher ratio of lactic to acetic acid was measured in LB1 than in LB2 treatments. The pH of the inoculated silages was higher ($P < 0.01$) than that of untreated silages. Inoculation resulted in an increase ($P < 0.01$) in NH_3-N concentration in the MLF silage, while a decrease ($P < 0.01$) was observed in the ML and HD silages.

Parameters of aerobic stability of inoculated and untreated corn silages are given in Table 4. Low-DM silages were more ($P < 0.01$) resistant to spoilage than were silages with a higher DM percentage. Greater ($P < 0.01$) numbers of yeasts and moulds were found in ML and MLF silages than in the HD silage. Silages prepared from crops at the HD maturity stage had greater

Table 1. Chemical and microbial composition of the fresh corn forages before ensiling

HD = hard-dough stage; ML = one-third milkline stage; MLF = one-third milkline with a killing frost; LAB = lactic acid bacteria; WSC = water soluble carbohydrates; cfu = colony forming units; OM = organic matter; CP = crude protein; NDF= neutral detergent fibre; and ADF = acid detergent fibre. Within a column, means followed by the same letter are not significantly different ($P = 0.01$); s.e. = standard error

Maturity	DM (g/kg)	pH	Chemical component (g/kg DM)					Microbial component (log_{10}, cfu/g)		
			OM	CP	NDF	ADF	WSC	Yeast	Mould	LAB
HD	253.1b	5.83b	921.5b	95.1a	468.5b	258.1b	118a	4.15c	4.2c	4.48a
ML	293.7a	6.27a	933.8a	75.8b	489.1a	246.8c	101b	4.4b	5.01a	4.17b
MLF	297.6a	6.38a	933.6a	81b	493.3a	280.6a	97b	5.19a	4.71b	3.43c
s.e.	4.7	0.08	3.4	3.5	5.3	4.1	4	0.08	0.11	0.12

Table 2. The effects of different inoculation levels of Lactisil maize (Medipharm, Kågeröd, Sweden) on the chemical composition of corn silages prepared from crops at different maturity stages

HD = hard-dough stage; ML = one-third milkline stage; MLF = one-third milkline with a killing frost M = main effect of maturity; T = main effect of treatment; M × T = interaction of maturity and treatment; WO = silages without inoculant; LB1 = silages with Lactisil maize at 1.5×10^5 colony forming units (cfu)/g; LB2 = silages with Lactisil maize at 3×10^5 cfu/g· **, $P < 0.01$; n.s. = non-significant

Component	HD			ML			MLF			s.e.	Significance		
	WO	LB1	LB2	WO	LB1	LB2	WO	LB1	LB2		M	T	M × T
DM (%)	22.99	22.82	22.63	26.34	26.26	25.87	27.01	27.16	27.64	0.465	**	n.s.	**
OM (%DM)	90.58	90.04	89.76	93.06	92.81	92.29	91.94	91.61	91.43	0.845	**	n.s.	n.s.
NDF (%DM)	53.18	56.31	58.19	56.40	58.91	60.88	53.93	57.81	61.98	0.614	**	**	**
ADF (%DM)	28.65	29.10	29.58	28.91	29.39	29.68	28.64	29.21	29.73	0.407	n.s.	**	n.s.
CP (%DM)	8.35	8.49	8.88	6.88	6.92	7.25	7.02	6.78	6.21	0.188	**	n.s.	**

Table 3. The effects of different inoculation levels of Lactisil maize (Medipharm, Kågeröd, Sweden) on fermentation parameters of corn silages prepared from crops at different maturity stages

HD = hard-dough stage; ML = one-third milkline stage; MLF = one-third milkline with a killing frost M = main effect of maturity; T = main effect of treatment; M × T = interaction of maturity and treatment; WO = silages without inoculant; LB1 = silages with Lactisil maize at 1.5×10^5 colony forming units (cfu)/g; LB2 = silages with Lactisil maize at 3×10^5 cfu/g. *, $P < 0.05$; **, $P < 0.01$; n.s. = non-significant

Parameter	HD			ML			MLF			s.e.	Significance		
	WO	LB1	LB2	WO	LB1	LB2	WO	LB1	LB2		M	T	M × T
Lactic acid (%DM)	10.7	14.0	14.6	6.6	8.7	7.5	5.3	6.2	5.6	0.312	**	**	**
Acetic acid (%DM)	1.26	1.48	1.64	1.39	1.69	1.83	1.36	1.87	1.95	0.119	**	**	n.s.
Lactate:acetate ratio	8.52	9.50	8.94	4.79	5.15	4.10	3.90	3.32	2.87	0.443	**	*	*
Propionic acid (%DM)	0.12	0.15	0.19	0.12	0.16	0.18	0.11	0.15	0.19	0.021	**	**	n.s.
Butyric acid (%DM)	0.098	0.129	0.151	0.092	0.118	0.144	0.084	0.112	0.121	0.008	**	**	n.s.
pH	3.73	3.79	3.86	3.78	3.83	3.85	3.72	3.78	3.97	0.032	n.s.	**	**
WSC (%DM)	5.21	5.13	5.02	4.99	4.96	4.92	5.02	5.36	5.45	0.324	n.s.	n.s.	n.s.
NH_3-N (%total N)	6.06	5.47	5.03	5.99	5.36	4.80	4.39	5.34	5.89	0.273	n.s.	n.s.	**

Table 4. The effect of different inoculation levels of Lactisil maize (Medipharm, Kågeröd, Sweden) on aerobic stability and microbial numbers of corn silages prepared from crop at different maturity stages

HD = hard-dough stage; ML = one-third milkline stage; MLF = one-third milkline with a killing frost; M = main effect of maturity; T = main effect of treatment; M × T = interaction of maturity and treatment; WO = silages without inoculant; LB1 = silages with Lactisil maize at 1.5×10^5 colony forming units (cfu)/g; LB2 = silages with Lactisil maize at 3×10^5 cfu/g. **, $P < 0.01$; n.s. = non-significant

Parameter	HD			ML			MLF			s.e.	Significance		
	WO	LB1	LB2	WO	LB1	LB2	WO	LB1	LB2		M	T	M × T
Aerobic stability (h)	172	167	169	80	76	80	85	90	87	3.79	**	n.s.	n.s.
Lactic acid bacteria (log_{10}, cfu/g)	5.22	5.46	5.37	4.63	4.88	4.58	4.84	4.99	4.85	0.108	**	**	n.s.
Yeasts (log_{10}, cfu/g)	2.05	2.18	2.15	4.22	4.65	4.19	3.76	3.17	3.71	0.035	**	n.s.	**
Mould (log_{10}, cfu/g)	<2	<2	<2	2.46	2.64	2.41	2.75	2.24	2.69	0.033	**	**	**

($P < 0.01$) numbers of LAB than did ML and MLF silages. Silages with a greater resistance to spoilage had a lower ($P < 0.01$) DM percentage and fewer ($P < 0.01$) fungi.

Discussion

Percentages of DM and NDF were higher in corn crops at a later maturity stage. These crops also had lower concentrations of WSC and CP and a higher pH. Our findings are in agreement with those of Johnson *et al*. (2003) who reported a lower concentration of WSC and a higher pH for high-DM corn crops. Frosting did not affect the chemical composition of corn crops except ADF. The increase in the proportion of ADF in response to frosting may have been due to binding of soluble cell contents to the cell wall, thus increasing the concentration of ADF. However, an increase in the numbers of yeasts and a decrease in the numbers of moulds and LAB in corn crops occurred due to frosting. These findings implied that fermentation rate can be limited in frosted or matured crops due to a higher proportion of DM, a lower concentration of WSC and fewer LAB. Furthermore, the greater number of fungi on matured and frosted crops may enhance spoilage of produced silage (Mohammadzadeh *et al.* 2012).

A higher concentration of lactic acid was found in silages at an earlier maturity stage. It has been demonstrated that a lower percentage of DM, low pH and a higher concentration of WSC in immature crops increase the production of organic acids in produced silage (Goodrich *et al.* 1975; Baron *et al.* 1986; Bal *et al.* 1997). Lactic and acetic acid concentrations were higher in inoculated silages than in the respective control silage. This finding agrees with those of Kleinschmit and Kung (2006) and Mohammadzadeh *et al*. (2011) who reported higher concentrations of lactic acid and acetic acid in response to inoculation of corn crops with a mixture of homo- and heterofermentative LAB. A higher application level of the inoculant resulted in elevated concentrations of lactic and acetic acid in the HD silage. In ML and MLF silages, the LB1 treatment resulted in a higher concentration of lactic acid and a lower concentration of acetic acid than did the LB2 treatment. The latter may have been due to higher numbers of *L. buchneri* in LB2 treatments and the inability of homofermentative LAB to compete with the heterofermentative LAB. The high DM percentage, low concentration of WSC and low numbers of LAB in ML and MLF crops had an inhibitory effect on the higher concentration of homofermentative LAB in the LB2 treatments. However, the higher concentration of heterofermentative LAB in LB2 treatments and the inhibitory effects on these bacteria resulted in a higher heterolactic activity than in the LB1 treatment, and in an increase in the concentration of acetic acid and a decrease in the concentration of lactic acid. Nishino *et al*. (2003) suggested that the high acetic acid concentration in silages inoculated with *L. buchneri* could be attributed mainly to lactic acid degradation and not to heterolactic fermentation. In general, these findings imply that LM causes an increase in numbers and activity of *L. buchneri* rather than homofermentative

LAB in high-DM silages which then results in an increase in acetic acid and a decrease in lactic acid concentrations. However, a greater increase in lactic acid concentration in HD silage, in response to applying the inoculant at a high concentration, than in silages prepared from crops at ML and MLF stages suggested the dominance of homofermentative LAB in silage at an earlier maturity stage. The probable reason for this finding is the greater suspension in activity of homofermentative LAB in more matured crops due to relatively less available WSC, lower water activity and low numbers of epiphytic LAB in respective crops (Bernardes *et al.* 2005). Suspension in the activity of WSC-fermentative LAB provides an opportunity to heterolactic LAB (e.g. *L. buchneri*) to grow and develop by lactic acid to acetic acid conversion. Then, acetic acid accumulates faster and earlier in silages prepared from more matured corn forages. Nishino *et al*. (2003) showed that an increase in acetic acid concentration was more distinct at 120 days than at 60 days of ensiling.

Higher concentrations of propionic and butyric acids were measured in inoculant-treated silages due to heterolactic fermentation of *L. buchneri*. Krooneman *et al*. (2002) speculated that certain members of the epiphytic microfloora (*L. diolivorans*) are involved in the conversion of 1,2-propanediol (produced by *L. buchneri*) to propionic acid. The pH was higher in inoculant-treated silages due to a higher concentration of acetic acid and a lower ratio of lactic to acetic acid. Silages inoculated with *L. buchneri* usually have a higher pH than does the control or silages treated with homofermentative LAB (Mari *et al.* 2009; Kristensen *et al.* 2010; Mohammadzadeh *et al.* 2011). LAB use mainly WSC as a substrate during fermentation and produce lactic acid and VFA to gain energy for growth and proliferation (McDonald *et al.* 1991). Concentrations of residual WSC were substantially reduced in silages compared with those found in fresh corn forages. However, concentrations of WSC in silages were above 3.5% DM, a value considered to be the minimum required for a good fermentation to occur (Wilkinson 1990). The concentration of ammonia-N was lower in treated HD and ML silages. Filya (2003*b*) and Driehuis *et al*. (1999) reported that the concentration of ammonia-N was lower in the *L. plantarum*- and *L. buchneri* + *L. plantarum*-inoculated silages than in untreated silages or silages inoculated with *L. buchneri*. Previously, Zahiroddini *et al*. (2004) and McDonald *et al*. (1991) demonstrated the inhibitory effect of a rapid fall in silage pH on proteolytic activity of aerobic microorganisms and plant enzymes. However, in MLF silage, concentrations of CP and NH_3-N decreased and increased, respectively, in response to inoculation. This may be due to a delay in pH drop in frost-killed corn silages because of lower concentrations of WSC and epiphytic LAB and a higher pH in parent crops, which limits LAB activity (McDonald *et al.* 1991). A higher pH in the MLF silage at a higher application level of the inoculant is in agreement with this finding. However, concentrations ammonia-N in all silages were very low (due to low pH in silages), indicating that all silages had a good fermentation.

In the current study, the inoculant had no significant effect on the aerobic stability of silages, in spite of the higher lactic acid concentration. This is due to the fact that LM increases the concentration of acetic acid in silage simultaneously with an increasing lactic acid concentration. Thus, the ratio of lactic acid to acetic acid can be a better tool to predict the aerobic stability of silage within each maturity stage, instead of the sole lactic acid concentration. Driehuis *et al*. (1999) and Nishino *et al*. (2003) demonstrated the inhibitory effects of acetic acid on fungi. The results achieved by Ranjit and Kung (2000), Driehuis *et al*. (2001) and Filya (2003*a*) showed that inoculation with *L. buchneri* alone or in combination with homofermentative LAB impairs the growth of moulds on the surface of silage. Acetic acid acts as a growth inhibitor of spoilage organisms by decreasing the maximum growth rate and, therefore, acetic acid increases the aerobic stability (Holzer *et al.* 2003). Moreover, *L. buchneri* produces other metabolites, as yet unidentified, with antifungal activity (Mann and Spoelstra 2001). Oude Elferink *et al*. (2001) showed that 1,2-propanediol, coupled with acetic acid, is produced during anaerobic degradation of lactic acid by *L. buchneri* and this enhances aerobic stability. The parameters of aerobic stability implied that the 120 days of ensiling was not long enough for *L. buchneri* (and consequently for LM) to produce enough acetic acid from lactic acid conversion or to produce high concentrations of propionic acid by heterolactic fermentation to improve the aerobic stability of silages.

Also, a great difference was found in the aerobic stability between high- and low-DM silages and HD silages showed greater aerobic stability. In a previous study, Weinberg *et al*. (2010) reported that the porosity of the silage and its susceptibility to air ingress depend on the degree of compaction in the silo, which is affected by the DM content of the crop. It was also reported that wet-pack density in the silo tended to decline as the corn plant matured (Harrison *et al.* 1998). Porosity determines the rate that air can infiltrate the silo and, subsequently, affects the amount of spoilage at the time of feed out (Muck and Holmes 1999).

A major cause of spoilage in silage is undesirable activity of some microorganisms, such as yeasts and moulds (Woolford 1990). The lowest numbers of yeasts and moulds in silages contributed to the highest aerobic stability (Stryszewska and Pys 2006). Silage yeast, but not airborne ones, initiates deterioration of silages on exposure to air (Lindgren *et al.* 1985; McDonald *et al.* 1991; Oude Elferink *et al.* 2001). In our study, within each maturity stage, silages with a low ratio of lactic to acetic acid had low numbers of yeasts and moulds on the day of opening. This finding was confirmed with a higher measured aerobic stability in silages with a higher acetic to lactic acid ratio. The results of Driehuis *et al*. (2001) showed that inoculation with a combination *of L. buchneri* and homofermentative LAB impairs the growth of moulds on the surface of silage. Filya (2003*a*) reported that after 90 days of ensiling, yeasts and moulds were not found in silages inoculated with *L. buchneri* + *L. plantarum*, whereas appreciable numbers of yeasts and moulds were detected in the control silages. In a study by Ranjit and Kung (2000), the numbers of yeasts were reduced dramatically in the silage inoculated with *L. buchneri* compared with the untreated silage.

Conclusions

The maturity stage of corn forage significantly affected anaerobic fermentation characteristics in produced silages. Lower concentrations of fermentation end products were measured in silages prepared from more matured forages due to a higher

DM percentage, a higher pH and lower concentrations of WSC and LAB. Homofermentative fermentation was dominant over heterofermentative fermentation in high-moisture crops, but a reverse was found in more matured crops. Frosting of one-third milkline corn crop resulted in silages with good fermentation characteristics without any considerable effects on chemical composition. Frost-killed matured corn crops have a good potential to be ensiled and can be considered as a good feed source for ruminants when frosting occurs before harvesting the crops. During the short ensiling period, using LM as a LAB inoculant (especially in higher doses) accelerated homofermentative fermentation in unmatured corn silages and heterolactic fermentation in silages prepared from more matured crops. However, LM failed to improve aerobic stability of silage after 120 days of ensiling, maybe due to insufficient time to produce a higher concentration of acetic acid by lactic acid conversion. Lactisil maize may enhance aerobic stability and fermentation rate of matured corn crops when ensiled for a long time period.

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