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The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents

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ABSTRACT

Whole-plant corn was harvested at 33 (normal) and 41% (moderately high) dry matter (DM) and ensiled in quadruplicate 20-L laboratory silos to investigate the effects of *Lactobacillus buchneri* 40788 (LB) or *L. plantarum* MTD-1 (LP) alone, or in combination, on the fermentation and aerobic stability of the resulting silage. Aerobic stability was defined as the amount of time after exposure to air for the silage temperature to reach 2°C above ambient temperature. The chopped forage was used in a 2 × 2 × 2 factorial arrangement of treatments: normal and moderately high DM contents, LB at 0 (untreated) or 4×10^5 cfu/g of fresh forage, and LP at 0 or 1×10^5 cfu/g. After 240 d of ensiling, corn silage harvested at the moderately high DM had higher pH, higher concentrations of ethanol, and more yeasts compared with the silage ensiled at the normal DM content. Inoculation with LB did not affect the concentration of lactic acid in silages with a moderately high DM, but decreased the concentration of lactic acid in the silage with normal DM. Higher concentrations of acetic acid were found in the silage treated with LB compared with those not treated with this organism. Inoculation with LP increased the concentration of lactic acid only in the silage with the normal DM content. The concentration of acetic acid was lower in silage treated with LP with a moderately high DM content, but greater in the silage treated with LP with the normal DM content when compared with silages without this inoculant. Appreciable amounts of 1,2-propanediol (average 1.65%, DM basis) were found in all silages treated with LB regardless of the DM content. The addition of *L. buchneri* increased the concentration of NH_3-N in silages but the addition of *L. plantarum* decreased it. Aerobic stability was improved in all silages treated with LB, with greater aerobic stability occurring in the silage with moderately high DM compared with silage with normal DM content. Inoculation with LP had no effect on aerobic stability. There were no interactions between *L. buchneri* and *L. plantarum* for most fermentation products or aerobic stability of the silages. This study showed that inoculating whole-plant corn with *L. buchneri* 40788 or *L. plantarum* MTD-1 has different beneficial effects on the resulting silage. There appear to be no major interactions between these organisms when added together to forage. Thus, there is potential to add both organisms simultaneously to improve the fermentation and aerobic stability of corn silage.
Key words: corn silage, inoculant, aerobic stability

INTRODUCTION

Lactic acid bacteria (**LAB**) such as *Lactobacillus plantarum*, *Enterococcus faecium*, and various *Pediococcus* species are often added to forage crops at the time of ensiling to improve the ensuing fermentation. These added organisms rapidly produce lactic acid, which lowers the pH of the silage and helps to preserve the forage mass. Recently, bacterial inoculants have been developed that improve the aerobic stability of silages after the primary fermentation process has ended. For example, *Lactobacillus buchneri*, a heterofermentative LAB, has been shown to convert lactic to acetic acid under anaerobic conditions (Oude Elferink et al., 2001). Acetic acid has good antifungal properties and thus improves the stability of silages when they are exposed to air (Kleinschmit and Kung, 2006a). Combining *L. buchneri* with other LAB to gain the positive attributes when silages are exposed to air and active fermentation, respectively, has been studied in cereal grain silages (Weinberg et al., 1999; Filya, 2003a) and in grass silages (Driehuis et al., 2001; Adesogan et al., 2004). Kleinschmit and Kung (2006b) reported that corn silage treated with *L. buchneri* 40788 and *Pediococcus pentosaceus* R1094 had normal fermentation characteristics, but the aerobic stability of silage was not consistently improved with this combination of organisms. In contrast, Weinberg et al. (2002) and Filya (2003a) found that the combination of *L. buchneri* and *L. plan-*

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tarum improved the aerobic stability of corn silages. In those studies, the specific strain(s) of *L. plantarum* used was (were) not documented in their publications. However, to our best knowledge, no research has been conducted to test the efficacy of a combination of *L. buchneri* 40788 with *L. plantarum* MTD-1.

The DM at which forage crops are ensiled also has profound effects on silage fermentation because a lack of moisture in dry forages restricts the overall fermentation process. Nishino and Touno (2005) reported that inoculation of *L. buchneri* decreased lactic acid and increased acetic acid and 1,2-propanediol, with the effects being greater in direct-cut than in wilted grass silages. Little is known about the effect of the combination of *L. buchneri* and *L. plantarum* on corn silage with moderately high forage DM content. We hypothesized that inoculants may elicit different degrees of response from the ensiling process depending on the DM content of the forage and that there might be an interaction between inoculants when they are added in combination. Thus, the objective of this study was to evaluate the effects of *L. buchneri* 40788 and *L. plantarum* MTD-1, alone and in combination, on the fermentation and aerobic stability of corn silage harvested at normal and moderately high DM contents.

MATERIALS AND METHODS

Forage and Ensiling Conditions

Whole-plant corn (Pioneer 33B51, Pioneer Hi-Bred International, Des Moines, IA) was harvested at 2 DM contents: 33.1 and 40.6% DM. Forages were chopped to a theoretical length of 0.95 cm using New Holland FP230 pull-type harvester equipped with a mechanical processor (New Holland, PA). Within 1 h of chopping, the chopped forages were divided into four 75-kg piles at each DM. Each pile was assigned to 1 of the following treatments: 1) untreated (deionized water only); 2) *L. buchneri* 40788 (**LB**; Lallemand Animal Nutrition, Milwaukee, WI) applied at 4×10^5 cfu/g of fresh corn forage; 3) *L. plantarum* MTD-1 (**LP**; Ecosyl Products Ltd., Stokesley, UK) applied at 1×10^5 cfu/g; and 4) a combination of *L. buchneri* 40788 with *L. plantarum* MTD-1 applied at 4×10^5 and 1×10^5 cfu/g, respectively. All inoculants were first dissolved in 200 mL of deionized water and then sprayed onto each pile of the corn forages uniformly with thorough mixing. The microbial inoculants were plated on de Man, Rogosa, and Sharpe agar (Oxoid CM0361, Unipath, Basingstoke, UK) before the start of the study. The *L. buchneri* inoculant contained 2.0×10^{10} cfu of viable LAB/g. The *L. plantarum* inoculant contained 6.5×10^{10} cfu of viable LAB/g. An appropriate amount of each inoculant was applied to forage to achieve the desired rate of inoculation.

The treated forages were packed into quadruplicate 20-L experimental silos (30 cm in diameter × 36 cm in height) to achieve a final packing density of 184 ± 9 kg of DM/m^3. The silos were covered with 2 layers of polyethylene plastic, sealed tightly, and stored to allow ensiling for 240 d at ambient temperature (18 to 25°C) in a closed barn.

Sample Collection and Analyses

Triplicate samples of the untreated fresh forage were collected before ensiling. Dry matter content was determined by drying samples in a 60°C forced-air oven for 48 h. Dried samples were ground with a Udy Cyclone sample mill (1-mm screen, Udy Corp., Fort Collins, CO). Samples were analyzed for NDF and ADF by the methods of Goering and Van Soest (1970) using an Ankom 200 fiber analyzer (Ankom Technology, Fairport, NY). Amylase and sodium sulfite were used for the NDF procedure. Crude protein was determined by multiplying total N by 6.25 after total combustion of the sample (Leco CNS 2000 Analyzer, Leco, St. Joseph, MI).

Twenty-five grams of the fresh forages were homogenized with 225 mL of sterile 25% strength Ringer's solution (Oxoid BR52) for 1 min. The pH of the water extract was measured. A portion of the water extract was filtered through Whatman 54 filter paper (Whatman Inc., Clifton, NJ), acidified with 50% H_2SO_4 (pH <2.0), and frozen for further analysis. The filtered and acidified water extracts were analyzed for water-soluble carbohydrates (**WSC**; Nelson, 1944) and NH_3-N (Weatherburn, 1967). Another portion of the water extract was filtered through a double layer of cheesecloth into a sterile tube for microbial analyses. For the enumeration of LAB, serial dilutions of the water extract were made in sterile Ringer's solution. Samples were plated in de Man, Rogosa, and Sharpe agar using pour plates and incubated at 32°C for 48 to 72 h before counting. Yeasts were enumerated by pour-plating samples in malt extract agar (Oxoid CM59, Unipath) that had been acidified by the addition of 85% lactic acid at the rate of 0.5% (vol/vol) after autoclaving, and incubated as described for LAB. Colonies were counted from plates of appropriate dilutions that contained a minimum of 30 colonies.

After 240 d of ensiling, each silo was opened and mixed thoroughly. Recovery of forage DM was calculated based on the weights of empty silo, the initial forage, and final silage, and the DM concentration of forage and its respective silage. Silage samples were analyzed for DM, pH, ADF, NDF, CP, WSC, NH_3-N, LAB, and

yeast, following the procedures described for the fresh forages. The filtered and acidified silage water extracts were analyzed by HPLC (Shimadzu, Columbia, MD) for concentrations of VFA, lactic acid, 1,2-propanediol, and ethanol (Muck and Dickerson, 1987).

The aerobic stability of each silo was determined by adding 3 kg of a sample of silage back to its respective silo and exposing it to air at 22°C. A thermocouple wire was placed in the geometric center of the silage mass. The wire was attached to a data logger (CR10X Measurement and Control System, Campbell Scientific Inc., Logan, UT) that recorded the temperature every 10 min and averaged these values every 2 h. Each silo was covered with a double layer of the sterile cheesecloth to avoid contamination and drying of the silage, but allowing air to infiltrate the silage mass. Aerobic stability was defined as the amount of time (number of h) after silo opening for the silage temperature to increase by 2°C above ambient temperature.

The 24-h digestibility of NDF was determined on the silage samples after 240 d of ensiling by using the in vitro rumen fluid NDF procedure (using amylase and sodium sulfite) described by Goering and Van Soest (1970). Samples were incubated in 50-mL polycarbonate tubes sealed with a rubber stopper fitted with a glass tube and a rubber policeman (14-105A, Fisher Scientific) with a 5-mm slit to allow for venting of gas pressure. Tubes were placed in a heated (39°C) orbital shaker at a speed of 120 rpm for 24 h.

Statistical Analysis

All microbial data were transformed to $\log_{10}$ and presented on a wet weight basis, whereas chemical data were presented on a DM basis. Data were analyzed with the GLM procedure of SAS (SAS Institute, 2004) according to the model for a 2 × 2 × 2 factorial treatment design:

$$Y_{ijk} = \mu + D_i + P_j + T_k + (D \times P)_{ij} + (D \times T)_{ik} + (P \times T)_{jk} + (D \times P \times T)_{ijk} + e_{ijk},$$

where μ = overall mean; D_i = effect of forage DM content i (i = 1, 2); P_j = effect of inoculation of *L. buchneri* 40788 j (j = 1, 2); T_k = effect of inoculation of *L. plantarum* MTD-1 k (k = 1, 2); $(D \times P)_{ij}$ = effect of interaction between forage DM content i and inoculation of *L. buchneri* 40788 j; $(D \times T)_{ik}$ = effect of interaction between forage DM content i and inoculation of *L. plantarum* MTD-1 k; $(P \times T)_{jk}$ = effect of interaction between inoculation of *L. buchneri* 40788 j and inoculation of *L. plantarum* MTD-1 k; $(D \times P \times T)_{ijk}$ = effect of interaction among forage DM content i, inoculation of *L. buchneri* 40788 j, and inoculation of *L. plantarum* MTD-1 k; and e_{ijk} = error term.

Least squares means were compared using the PDIFF option of SAS. Significance was defined as $P \leq 0.05$.

Table 1. Dry matter, chemical (DM basis), and microbial (wet basis) composition of freshly chopped whole-plant corn at normal and moderately high DM contents before ensiling

Item	Normal DM	Moderately high DM
DM, %	33.1	40.6
pH	5.46	5.54
NDF, %	37.9	42.9
ADF, %	23.1	25.9
CP, %	6.64	5.59
NH_3-N, %	0.038	0.027
Water-soluble carbohydrates, %	7.43	6.37
Lactic acid bacteria, $\log_{10}$ cfu/g	6.80	6.80
Yeasts, $\log_{10}$ cfu/g	5.30	5.33
Molds, $\log_{10}$ cfu/g	4.99	4.66

RESULTS AND DISCUSSION

Fresh Corn Forage

The chemical and microbial composition of the freshly chopped corn is shown in Table 1. The DM concentrations of the fresh forage at 2 maturities were 33.1 and 40.6%. Harvesting at a moderately higher DM (later maturity) resulted in predictable changes in plant composition. For example, the concentrations of NDF and ADF increased, whereas the concentration of WSC decreased with advancing maturity. Microbial populations on the fresh forages were similar between DM.

Chemical Composition of Corn Silage Ensiled for 240 d

The DM, DM recovery, and nutrient composition of silages are shown in Table 2. There were effects of LB and LP on DM concentrations, but the differences were small and most likely a random effect of sampling because there is no known effect of inoculation on DM content. The recovery of DM was extremely high (>98%) for all silages and there was no effect of adding LP or LB on DM recovery. Although there was an LB × DM interaction for DM recovery, the effect was extremely small and of no practical significance.

Higher concentrations of WSC were found in silages with a normal versus moderately high concentration of DM (1.22 vs. 0.89%; $P < 0.01$). The concentration of WSC in the silages treated with LB was lower than in silages not treated with this inoculant but this difference was extremely small and not of practical significance (1.01 vs. 1.10%; $P < 0.01$). Although there were

Table 2. Chemical composition of corn silages ensiled at normal and moderately high forage DM for 240 d (DM basis)[1]

			No LB		LB			Effect (*P*-value)						
Variable	Forage DM[2]		No LP	LP	No LP	LP	SE	DM	LB	LP	DM × LB	DM × LP	LB × LP	DM × LB × LP
DM, %	Normal	32.7	33.4	32.0	33.1	32.1								
	Moderately high	39.1	39.8	39.2	38.6	39.0								
	Average		36.6	35.6	35.9	35.5	0.5	<0.01	0.04	<0.01	NS[3]	<0.01	0.08	NS
DM recovery, %	Normal	99.0	99.1	99.0	99.0	98.8								
	Moderately high	98.6	98.7	98.5	98.7	98.7								
	Average		98.9	98.8	98.8	98.7	0.1	<0.01	0.10	0.07	<0.01	NS	NS	NS
WSC,[4] %	Normal	1.22	1.25	1.34	1.13	1.17								
	Moderately high	0.89	0.91	0.90	0.93	0.82								
	Average		1.08	1.12	1.03	1.00	0.07	<0.01	<0.01	NS	0.03	0.02	NS	NS
NH_3-N, %	Normal	0.12	0.12	0.09	0.13	0.13								
	Moderately high	0.15	0.16	0.09	0.26	0.10								
	Average		0.14	0.09	0.20	0.11	0.03	0.01	<0.01	<0.01	NS	<0.01	NS	0.02
CP, %	Normal	7.48	7.43	7.34	7.48	7.65								
	Moderately high	6.42	6.45	6.38	6.58	6.28								
	Average		6.94	6.86	7.03	6.96	0.17	<0.01	NS	NS	NS	0.08	NS	0.03
ADF, %	Normal	24.5	23.7	25.2	23.8	25.1								
	Moderately high	25.5	23.3	26.5	24.9	27.4								
	Average		23.5	25.8	24.4	26.3	1.0	0.01	0.09	<0.01	0.10	0.05	NS	NS
NDF, %	Normal	39.6	39.9	39.0	39.9	39.7								
	Moderately high	42.3	40.3	42.4	42.1	44.5								
	Average		40.1	40.7	41.0	42.1	1.7	<0.01	0.12	NS	NS	0.06	NS	NS
NDF-D,[5] %	Normal	43.7	44.3	41.0	45.7	43.8								
	Moderately high	42.8	42.1	43.0	43.2	43.0								
	Average		43.2	42.0	44.5	43.4	2.4	NS	NS	NS	NS	NS	NS	NS

[1]LB = *L. buchneri* 40788 (Lallemand Animal Nutrition, Milwaukee, WI), applied at 4×10^5 cfu/g of fresh forage; LP = *L. plantarum* MTD-1 (Ecosyl Products, Ltd., Stokesley, UK), applied at 1×10^5 cfu/g.

[2]Normal DM content of harvested forage = 33.1%; moderately high DM content of harvested forage = 40.6%.

[3]NS = $P > 0.15$.

[4]WSC = water-soluble carbohydrates.

[5]NDF-D = 24-h in vitro NDF digestibility.

interactions between DM × LB and DM × LP, these differences were also very small.

Silages treated with LB had higher concentrations of NH_3-N compared with those that were not treated with LB (0.16 vs. 0.12%; $P < 0.01$). In contrast, treatment with *L. buchneri* 40788 did not affect the ammonia concentration of corn silages in past studies (Ranjit and Kung, 2000; Kleinschmit et al., 2005). However, addition of LB increased the concentration of NH_3-N in grass and low DM corn and sorghum silages (Driehuis et al., 2001; Filya, 2003b). The higher concentration of NH_3-N as a result of inoculation of LB might be associated with increased pH in silage treated with LB (Driehuis et al., 2001). Low concentrations of NH_3-N can be an indicator of a more homolactic fermentation in silage. For unknown reasons, in the present experiment, inoculation of LP only resulted in a lower concentration of NH_3-N in the silage with a moderately high DM content (0.09 vs. 0.21%; $P < 0.01$).

As expected, the concentration of CP ($P < 0.01$) was lower but the concentrations of ADF ($P = 0.01$) and NDF ($P < 0.01$) were higher in silages with a moderately high versus normal concentration of DM. The addition of LP increased the concentration of ADF in silages with both moderately high (26.9 vs. 24.1%; $P < 0.01$) and normal (25.1 vs. 23.8%; $P = 0.01$) concentrations of DM and we have no explanation for this finding.

Regardless of DM content, inoculation with *L. buchneri* alone, *L. plantarum* alone, or a combination of both did not affect the in vitro rumen digestibility of NDF of corn silages. This result is similar to that reported by Filya (2003a). In contrast, Weinberg et al. (2007) reported that corn silage treated with *L. plantarum* MTD-1 or *L. buchneri* 40788 improved NDF digestibility after 24 h of in vitro incubation in their study. Inconsistent effects of these inoculants on improving NDF digestibility could be related to many factors (e.g., factors affect plant composition, hybrid specifics) and the base diets that the fistulated animals were fed.

Fermentation Characteristics of Corn Silage Ensiled for 240 d

Silage pH was <3.8 (Table 3) for all treatments, which is typical for corn silage in previous studies from our lab (Kleinschmit et al., 2005; Kleinschmit and Kung, 2006b). The pH was lower in silage with a normal versus a moderately high DM content ($P < 0.01$), and there were DM × LB and DM × LP interactions for pH, but the absolute differences were very small.

A moderately higher pH in silage treated with *L. buchneri* is a common finding (Kleinschmit and Kung, 2006a) because of the degradation of lactic acid to acetic acid by LB (Oude Elferink et al., 2001). The addition of homolactic acid bacteria to silages has generally resulted in lower silage pH because there is often greater production of lactic acid (Muck and Kung, 1997). In a past study, treating corn silage with *L. plantarum* MTD-1 (Kung et al., 1993) did affect the pH of silage. Homolactic inoculants probably have a lesser effect on silage pH of corn silage versus alfalfa silage because the buffering capacity is relatively low in corn (Muck and Kung, 1997).

There was a DM × LB interaction ($P < 0.01$) on concentrations of lactic acids. Treatment with LB resulted in a lower concentration of lactic acid in silage with a normal DM but not in silage with a moderately high DM. There was also a DM × LP interaction for lactic acid. Treatment with LP resulted in more lactic acid in silage with a normal DM content but not in silage with a moderately high DM. The former was as expected, but the reasons for a lack of response at the higher DM are unknown. There were DM × LB and DM × LP interactions for acetic acid. As expected, inoculation with LB resulted in silages with more acetic acid, but the effect was greater in silage with a moderately high versus normal DM. Although addition of LP decreased the concentration of acetic acid in silage with a moderately high DM, it unexpectedly resulted in an increase in acetic acid in silage with the normal DM content.

Lactobacillus buchneri also produces 1,2-propanediol during the anaerobic degradation of lactic acid to acetic acid (Oude Elferink et al., 2001). As expected, appreciable amounts of 1,2-propanediol were found in all silages treated with LB regardless of DM content at ensiling (average 1.65%). In contrast, the addition of LP decreased the concentration of 1,2-propanediol in silage treated with LB with a moderately high DM content ($P < 0.01$), but it increased concentration of 1,2-propanediol in the silage treated with LB with normal DM content ($P < 0.01$). We are unable to explain this finding at this time, but organisms such as *Lactobacillus diolivorans* (Krooneman et al., 2002) have the capacity to convert 1,2-propanediol to 1-proponal or propionic acid in silages (Driehuis et al., 2001).

Propionic, butyric, and isobutyric acids were not detected in any silages except that a small amount of butyric acid (0.14%) was found in the silage with a moderately high forage DM content with the sole inoculation of LB. Corn silages typically do not contain butyric acid because their low pH is not conducive for the growth of clostridia and thus we have no explanation for this anomaly.

The concentration of ethanol was higher ($P < 0.01$) in the silage ensiled at the moderately high versus normal DM content (Table 3). The addition of LB did not affect the concentration of ethanol in the silage with a

Table 3. Fermentation characteristics, microbial composition, and aerobic stability of corn silages ensiled at normal and moderately high forage DM for 240 d[1]

			No LB		LB			Effect (P-value)						
Variable	Forage DM[2]		No LP	LP	No LP	LP	SE	DM	LB	LP	DM × LB	DM × LP	LB × LP	DM × LB × LP
pH	Normal	3.60	3.54	3.56	3.65	3.66								
	Moderately high	3.69	3.60	3.65	3.73	3.78								
	Average		3.57	3.60	3.69	3.72	0.02	<0.01	<0.01	<0.01	0.05	0.02	NS[3]	NS
Lactic acid,[4] %	Normal	3.02	3.39	4.12	1.97	2.60								
	Moderately high	3.21	2.97	3.13	3.83	2.91								
	Average		3.18	3.63	2.90	2.75	0.50	NS	<0.01	NS	<0.01	0.01	NS	NS
Acetic acid,[4] %	Normal	1.36	0.84	1.02	1.33	2.23								
	Moderately high	1.36	0.55	0.60	2.58	1.69								
	Average		0.70	0.81	1.96	1.96	0.23	NS	<0.01	NS	<0.01	<0.01	NS	<0.01
1,2-Propanediol,[4] %	Normal	0.78	0.00	0.06	1.24	1.83								
	Moderately high	0.88	0.00	0.00	2.23	1.31								
	Average		0.00	0.03	1.73	1.57	0.13	0.04	<0.01	NS	<0.01	<0.01	0.05	<0.01
Ethanol,[4] %	Normal	0.40	0.54	0.55	0.23	0.26								
	Moderately high	0.91	0.89	0.88	0.87	0.99								
	Average		0.72	0.72	0.55	0.63	0.12	<0.01	0.02	NS	<0.01	NS	NS	NS
LAB,[5] $\log_{10}$ cfu/g[6]	Normal	7.81	5.40	7.40	9.27	9.18								
	Moderately high	8.43	7.28	7.83	9.06	9.53								
	Average		6.34	7.61	9.17	9.36	0.66	0.02	<0.01	<0.01	0.03	NS	0.03	0.04
Yeast, $\log_{10}$ cfu/g[6]	Normal	3.90	3.62	4.93	4.03	3.04								
	Moderately high	5.45	6.26	5.65	5.39	4.50								
	Average		4.94	5.29	4.71	3.77	1.12	<0.01	0.04	NS	NS	NS	NS	NS
Aerobic stability, h	Normal	79	53	47	112	106								
	Moderately high	159	49	53	236	300								
	Average		51	50	174	203	41	<0.01	<0.01	NS	<0.01	NS	NS	NS

[1]LB = *L. buchneri* 40788 (Lallemand Animal Nutrition, Milwaukee, WI), applied at 4×10^5 cfu/g of fresh forage; LP = *L. plantarum* MTD-1 (Ecosyl Products, Ltd., Stokesley, UK), applied at 1×10^5 cfu/g.

[2]Normal DM content of harvested forage = 33.1%; moderately high DM content of harvested forage = 40.6%.

[3]NS = $P > 0.15$.

[4]DM basis.

[5]Lactic acid bacteria.

[6]Wet basis.

moderately high concentration of DM, but decreased the concentration of ethanol in the silage with normal forage DM content (0.24 vs. 0.55%; $P < 0.01$). Nishino et al. (2003) reported a lower concentration of ethanol in corn silage ensiled at 27.3% of DM as a result of the inoculation of LB. Others have observed increased ethanol concentrations in barley silages treated with LB (Kung and Ranjit, 2001; Taylor et al., 2002). Ethanol could be produced by LB during the anaerobic degradation of lactic acid to acetic acid and 1,2-propanediol (Oude Elferink et al., 2001) or by fermentation of yeasts or other organisms. In the present experiment, the higher concentration of ethanol in silage with a moderately high DM appeared to be because of the increased numbers of yeasts (Table 3) when compared with the silage with a normal DM content. Moreover, the lower concentration of ethanol in silages treated with LB ensiled with a normal DM content might be related to inhibited activity of yeasts resulting from the inoculation with LB. The addition of LP did not affect the production of ethanol in silage.

Microbial Composition of Corn Silage Ensiled for 240 d

In uninoculated silages, higher numbers of LAB were found in silage with a moderately high versus normal concentration of DM ($P < 0.01$). As expected, inoculation with either LB or LP increased the numbers of LAB in silage. More than 100-fold higher numbers of LAB were found in the silages treated with than in silage without LB (9.26 vs. 6.98 $\log_{10}$ cfu/g of fresh silage; $P < 0.01$), with a greater increase occurring in the silage treated with LB with a normal DM content. A greater number of LAB were detected in silage inoculated with LP versus those without this inoculant (8.48 vs. 7.75 $\log_{10}$ cfu/g of fresh silage; $P < 0.01$). There was an interaction effect of LB × LP on numbers of LAB ($P = 0.03$). Lactic acid bacteria increased with the inoculation of LP ($P < 0.01$) in the silages without LB, but was unaffected in the silage treated with LB. This finding suggested that LB dominated the fermentation after 240 d of ensiling. Schmidt et al. (2008) and Mari et al. (2008) also reported greater numbers of LAB in corn silages treated with *L. buchneri* than in untreated corn silage and further confirmed that treated silages had markedly greater numbers of *L. buchneri* (as determined by quantitative real-time PCR). The silage with a moderately high forage DM had more yeasts when compared with the normal DM silage ($P < 0.01$), and the numbers of yeasts that were detected were lower in silage treated with LB versus silage without this inoculant (4.24 vs. 5.11 $\log_{10}$ cfu/g of fresh silage; $P = 0.04$). Greater numbers of yeasts in corn silages with moderately high DM contents are often a result of lower packing densities or lower concentrations of fermentation acids. However, these factors were not present in the current study and thus we are unable to explain this finding. The addition of LP did not affect the numbers of yeasts in silage.

Aerobic Stability of Corn Silages Ensiled for 240 d

The aerobic stability of corn silage was improved in all silages treated with LB compared with those not treated with this organism (treated, 189 h vs. untreated, 50 h; $P < 0.01$), and the effect of LB was greater in moderately high DM versus normal DM silage although the reason for this finding was not consistent with the fact that moderately high DM silage with LB had more yeasts than lower DM silage with LB. However, the improvement in aerobic stability from inoculation with LB is consistent with the previous research showing that inoculation with LB enhances the aerobic stability of ensiled forage crops most likely because of its production of acetic acid (Kleinschmit and Kung, 2006a). Inoculation with homolactic acid bacteria has sometimes led to the worsening of aerobic stability of silage (Weinberg et al., 1993; Filya, 2003a). For example, Filya (2003a) found that wheat, sorghum, and corn silages were aerobically unstable when inoculated solely with LP. However, there was neither a LP effect nor an interaction between LB and LP on the aerobic stability in the present experiment.

CONCLUSIONS

Corn silage treated with *L. buchneri* 40788 had higher concentrations of acetic acid, fewer yeasts, and improved aerobic stability compared with untreated silage. These effects were consistent regardless of silage DM. However, the magnitude of the effects for acetic acid and aerobic stability were greater in moderately high than in normal DM silage. Addition of LP in the current study increased lactic acid only in silage with a normal DM content, and decreased acetic acid only in silage with a moderately high DM content. The concentration of NH_3-N decreased most in silage with a moderately high DM content. The addition of LP had no effect on yeasts or aerobic stability. With the exception of concentration of 1,2 propanediol and numbers of LAB, there were no other interactions between LP and LB. This study shows that the LB and LP inoculants used in our study had different beneficial effects on the ensiling process and that these beneficial effects were generally maintained when the inoculants were added together. The data also suggest that their effects on some variables were more pronounced in silage with

a moderately high DM content. This is important because silages with a moderately high DM content are a greater challenge to manage in farm silos because they are more difficult to pack; this finding supports the recommendations that microbial inoculants used in this study may especially be helpful under this condition.

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REFERENCES

Adesogan, A. T., N. Krueger, M. B. Salawu, D. B. Dean, and C. R. Staples. 2004. The influence of treatment with dual-purpose bacterial inoculants or soluble carbohydrates on the fermentation and aerobic stability of bermudagrass. J. Dairy Sci. 87:3407–3416.

Driehuis, F., S. J. W. H. Oude Elferink, and P. G. Van Wikselaar. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass Forage Sci. 56:330–343.

Filya, I. 2003a. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. J. Appl. Microbiol. 95:1080–1086.

Filya, I. 2003b. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low DM corn and sorghum silages. J. Dairy Sci. 86:3575–3581.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agriculture Handbook. No. 379. ARS-USDA, Washington, DC.

Kleinschmit, D. H., and L. Kung Jr. 2006a. A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89:4005–4013.

Kleinschmit, D. H., and L. Kung Jr. 2006b. The effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn silage. J. Dairy Sci. 89:3999–4004.

Kleinschmit, D. H., R. J. Schmidt, and L. Kung Jr. 2005. The effects of various antifungal additives on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 88:2130–2139.

Krooneman, J., F. Faber, A. C. Alderkamp, S. J. H. W. Oude Elferink, F. Driehuis, I. Cleenwerck, J. Swings, and J. C. Gottschal. 2002. *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. Int. J. Syst. Evol. Microbiol. 52:639–646.

Kung, L. Jr., J. H. Chen, E. M. Kreck, and K. Knutsen. 1993. Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows. J. Dairy Sci. 76:3763–3770.

Kung, L. Jr., and N. K. Ranjit. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. J. Dairy Sci. 84:1149–1155.

Mari, L. J., R. J. Schmidt, L. G. Nussio, C. M. Hallada, and L. Kung Jr. 2008. An evaluation of the effectiveness of Lactobacillus buchneri 40788 to improve the aerobic stability of corn silage in farm silos. J. Dairy Sci. 91(E Suppl. 1):31. (Abstr.)

Muck, R. E., and J. T. Dickerson. 1987. Storage temperature effects on proteolysis in alfalfa silage. Trans. ASAE 31:1005–1009.

Muck, R. E., and L. Kung Jr. 1997. Effect of silage additives on ensiling. Proc. Silage: Field to feedbunk. North Am. Conf. Hershey, PA. NRAES-99.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375–380.

Nishino, N., and E. Touno. 2005. Ensiling characteristics and aerobic stability of direct-cut and wilted grass silages inoculated with *Lactobacillus casei* or *Lactobacillus buchneri.* J. Sci. Food Agric. 85:1882–1888.

Nishino, N., M. Yoshida, H. Shiota, and E. Sakaguchi. 2003. Accumulation of 1,2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with *Lactobacillus buchneri.* J. Appl. Microbiol. 94:800–807.

Oude Elferink, S. J. W. H., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Faber, and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri.* Appl. Environ. Microbiol. 67:125–132.

Ranjit, N. K., and L. Kung Jr. 2000. The effect of *Lactobacillus buchneri, Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. J. Dairy Sci. 83:526–535.

SAS Institute. 2004. SAS system software: Release 9.1 (TS1M3). SAS Institute Inc., Cary, NC.

Schmidt, R. J., M. G. Emara, and L. Kung Jr. 2008. The use of a quantitative real-time polymerase chain reaction assay for identification and enumeration of *Lactobacillus buchneri* in silage. J. Appl. Microbiol. 105:920–929.

Taylor, C. C., N. J. Ranjit, J. A. Mills, J. M. Neylon, and L. Kung Jr. 2002. The effect of treating whole-plant barley with *Lactobacillus buchneri* 40788 on silage fermentation, aerobic stability, and nutritive value for dairy cows. J. Dairy Sci. 85:1793–1800.

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. 39:971–974.

Weinberg, Z. G., G. Ashbell, Y. Hen, and A. Azrieli. 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. J. Appl. Bacteriol. 75:512–518.

Weinberg, Z. G., G. Ashbell, Y. Hen, A. Azrieli, G. Szakacs, and I. Filya. 2002. Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri.* J. Ind. Microbiol. Biotechnol. 28:7–11.

Weinberg, Z. G., O. Shatz, Y. Chen, E. Yosef, M. Nikbahat, D. Ben-Ghedalia, and J. Miron. 2007. Effect of lactic acid bacteria inoculants on in vitro digestibility of wheat and corn silages. J. Dairy Sci. 90:4754–4762.

Weinberg, Z. G., G. Szakacs, G. Ashbell, and Y. Hen. 1999. The effect of *Lactobacillus buchneri* and *L. plantarum*, applied at ensiling, on the ensiling fermentation and aerobic stability of wheat and sorghum silages. J. Ind. Microbiol. Biotechnol. 23:218–222.