The Effect of Treating Alfalfa with *Lactobacillus buchneri* 40788 on Silage Fermentation, Aerobic Stability, and Nutritive Value for Lactating Dairy Cows¹

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ABSTRACT

Lactobacillus buchneri 40788 and enzymes (β -glucanase, α -amylase, xylanase, and galactomannase) were applied to chopped alfalfa (39% DM) to study their effects on the fermentation and nutritive value of the silage. Alfalfa was treated with nothing, or *L. buchneri* 40788, for a final application rate of 1×10^5 , 5×10^5 , or 1×10^6 cfu/g of fresh forage and ensiled in laboratory silos for 2, 4, 8, and 56 d. Treatment with *L. buchneri* 40788 had few effects on the end products of fermentation through 8 d of ensiling. However, after 56 d of ensiling, treated silages had a higher pH (4.55 vs. 4.38) and higher concentrations of acetic acid (6.40 vs. 4.24%), propionic acid (0.18 vs. 0.06%), and ammonia-N (0.35 vs. 0.29%) when compared to untreated silage. Lactic acid was also numerically lower in treated (3.51%) than untreated (4.12%). Silages treated with the moderate and highest dose of *L. buchneri* 40788 also resulted in greater recoveries of DM than did untreated silage. Alfalfa (43% DM) was also untreated or treated with a commercial application of *L. buchneri* 40788 (4×10^5 cfu/g, a commercial dose) in farm-scale bag silo. Holstein cows were fed a diet comprised of 32% untreated or treated alfalfa silage, 11% corn silage, 5% chopped alfalfa hay, and 52% of concentrate (DMB) for a 6-wk treatment period. Dry matter intake and milk composition were unaffected by treatment, but cows fed silage treated with *L. buchneri* 40788 produced 0.8 kg more milk than did cows fed untreated silage. Treated

silage had a higher concentration of acetic acid (5.67 vs. 3.35%) but lower lactic acid (3.50 vs. 4.39%) than untreated silage. When exposed to air, the total mixed ration containing treated alfalfa silage remained stable for 100 h, whereas the ration containing untreated silage spoiled after 68 h. Treating alfalfa silage with *L. buchneri* 40788 increased the concentration of acetic acid, and when the silage was combined into a total mixed ration and fed to lactating cows, it improved the aerobic stability of the ration and increased milk production.

(Key words: aerobic stability, *Lactobacillus buchneri*, silage)

Abbreviation key: WSC = water-soluble carbohydrates.

INTRODUCTION

Excess air trapped in the forage mass severely compromises the subsequent ensiling process by promoting the growth of aerobic microorganisms (Woolford, 1990). These microbes compete with lactobacilli for nutrients, and their excessive growth can lead to undesirable fermentations. Air is also detrimental to silage after fermentation is complete because yeasts, naturally present in many silages, can assimilate lactic acid, which results in reduced DM recovery and poor nutritive value. Packing silos quickly, densely, and sealing silos helps to minimize the exposure of forage to air. However, silages are often stored for prolonged periods of time, and air can penetrate into the silage mass. When spoiled silages are fed to cows, depressions in intake and performance have been observed (Hoffman and Ocker, 1997; Whitlock et al., 2000).

Ironically, many silage inoculants that improve the active fermentation phase of the ensiling process worsen the stability of the silage during storage and feedout because of lowered production of antifungal compounds. In addition, homolactic inoculation often results in high concentrations of residual water-soluble

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sugars. Thus, buffered propionic acid, which is highly toxic to yeasts, has been added to silages to specifically improve its aerobic stability (Kung et al., 2000). However, these products are often costly. In lieu of chemical additives, *Lactobacillus buchneri* 40788 is a biological alternative that, when added to forage at harvest, has resulted in an increase in the concentration of acetic acid, a lower number of yeasts, and improved aerobic stability in a variety of silages (Muck, 1996; Weinberg et al., 1999; Kung and Ranjit, 2001).

Although research on improving the aerobic stability of forage crops has primarily been focused on corn silage and high-moisture corn, alfalfa silage can be a major portion of the forage in diets for dairy cows, and it can also spoil when exposed to air. To date, there are no reports on the ability of *L. buchneri* 40788 to improve the aerobic stability of alfalfa silage. Thus, the objectives of this study were to 1) evaluate the effects of *L. buchneri* 40788 on the fermentation and aerobic stability of alfalfa silage ensiled in laboratory and farm silos, and 2) to determine whether feeding cows a total mixed ration (TMR) containing treated silage would have adverse effects on intake and animal performance because, in some past studies, silages with high levels of acetic acid have been associated with depressed animal intakes (Wilkins et al., 1971; Rook and Gill, 1990).

MATERIALS AND METHODS

Laboratory Silo Study

Alfalfa silage was mowed and allowed to wilt to a DM content of about 39%. Forage was chopped with a conventional forage harvester to a theoretical cut of 0.95 cm. Within 30 min of chopping, the following treatments were applied to forage: 1) water (0.5 ml/kg of fresh forage), control, 2) *Lactobacillus buchneri* 40788 (a final application rate of 1×10^5 cfu/g of fresh forage), β -glucanase (42,000 IU/tonne of fresh forage), α -amylase (21,000 IU/tonne), xylanase (22,800 IU/tonne), and galactomannase (3840 IU/tonne; Biotal, Eden Prairie, MN), 3) *L. buchneri* (5×10^5 cfu/g) and enzymes as in treatment 2, and 4) *L. buchneri* (1×10^6 cfu/g) and enzymes as in treatment 2. Forage from each treatment was packed into polyvinyl chloride laboratory silos (0.203×0.064 m) in triplicate to achieve a packing density of 250 kg of DM/m³ and sealed immediately on both ends with number 1 rubber stoppers. One of these stoppers was equipped with a Bunsen release valve for escape of gas. Weights of the empty and full silos were recorded, and silos were then stored at ambient temperature (20 to 27°C) in an enclosed barn. Representative samples of forage were obtained after application of the appropriate additive but prior to ensiling from each treatment. Samples were stored on ice (about 1 h) until

returned to the laboratory for processing. Dry matter of the samples was determined in a forced-draft oven set at 60°C for 48 h.

Twenty-five grams of fresh forage from each replicate was also homogenized for 1 min with 225 ml of sterile one-quarter-strength Ringer's solution (Oxoid BR52, Unipath, Basingstoke, UK). Yeasts and molds were enumerated by pour plating in malt extract agar (Oxoid CM59) that had been acidified by the addition of 85% lactic acid at the rate of 0.5% vol/vol. Plates were incubated aerobically at 32°C for 48 h. Lactic acid bacteria were enumerated by pour plating on de Mann Rogosa Sharpe agar (Oxoid CM361, Oxoid, Basingstoke, UK). Sterile cyclohexamide was added after autoclaving to obtain a concentration of 0.01% wt/vol. Plates were incubated aerobically at 32°C for 48 h. For yeasts and molds and lactic acid bacteria, colonies were counted from the plates of appropriate dilutions containing a minimum of 30 colonies. A portion of the water extract was filtered through Whatman 54 filter paper (Whatman, Clifton, NJ), acidified with 50% H₂SO₄ (25 μ l), and frozen before to further analysis. Ammonia-N was analyzed by the phenol-hypochlorite procedure described by Weatherburn (1967). Water-soluble carbohydrates (WSC) were determined as described by Nelson (1944).

Triplicate silos for each treatment were opened after 2, 4, 8, and 56 d of ensiling. Silage samples were processed and analyzed as described for fresh forages. Final weights of the full silos were recorded, and each silo was opened, and the silage was mixed thoroughly. Dry matter recovery was calculated by subtracting the final DM weight from the initial DM weight in each silo and then by dividing that number by the initial DM weight in each silo. In addition, the filtered and acidified water extracts of silages were analyzed for lactic acid (kit 826-UV, Sigma, St. Louis, MO) and VFA. For the analysis of *D*-lactic acid, *L*-lactic dehydrogenase was replaced with a similar amount of *D*-lactic dehydrogenase (Sigma L-9636). *L*-Lactic acid (Sigma L-2250) and *D*-lactic acid (Sigma L-1000) were used for standards for their respective assays. The sum of the *L*- and *D*-lactic acids was reported as the total lactate concentration. For analysis of VFA, one ml of each filtered and acidified water extract was combined with 200 μ l of 25% meta-phosphoric acid containing isocaproic acid as an internal standard. Samples were centrifuged for 15 min at $10,000 \times g$ and analyzed on a gas chromatograph (Hewlett-Packard 5890 GC, Hewlett-Packard, Avondale, PA) with a 530- μ m Carbowax 20 M column (Supelco, Bellefonte, PA). The chromatograph oven was programmed as follows: 70°C for 1 min, 5°C increase/min to 100°C, 45°C increase/min to 170°C, and a final holding time of 5 min.

Ethanol concentration of the water extracts was determined only on samples after 56 d of ensiling as per the alcohol procedure for markedly turbid samples using multi-assay vials (Sigma procedure no. 332-UV).

Lactation Study

Alfalfa from one field was wilted to approximately 40% DM before chopping and packing (Kelly Ryan Equipment Co., Blair, NE) into a bag silo (Klerk's Plastic Products Manufacturing, Inc., Richburg, SC), which was filled and sealed within 6 h. One-half (about 45 tonne) of the alfalfa forage was untreated, and the remaining half was treated with *Lactobacillus buchneri* 40788 (Lallemand Animal Nutrition, Milwaukee, WI) applied in water (2 L/tonne) via a mounted sprayer (Nevro Sales, Ltd., London, Ontario, Canada) in water to achieve a final application rate of 4×10^5 cfu/g of fresh forage. The inoculant also contained the following enzymes: β -glucanase (42,000 IU/tonne of fresh forage), α -amylase (21,000 IU/tonne of forage), xylanase (22,800 IU/tonne of forage), and galactomannase (3840 IU/tonne of forage).

After 10 mo of storage, both ends of the bag silo were opened simultaneously for feeding in a lactation study. Twenty-four multiparous and six primiparous Holstein cows averaging 81 ± 24 DIM and average milk production of about 39 kg/d were offered a complete diet as a TMR once daily for a 14-d pretreatment period. The first 4 d were used to accustom cows to feeding (once daily at 0700 h) via Calan gates (American Calan, Northwood, NH), and the data from the next 10 d were used for adjustment to the diet. During the pretreatment period, cows were fed a diet comprised of 16% (DM basis) untreated alfalfa silage, 16% treated alfalfa silage, 11% corn silage, 5% chopped alfalfa hay, and 52% of a pelleted concentrate (Table 1). Diets were balanced to meet National Research Council (2001) requirements (Table 2). Cows were randomly allocated into two treatments based on pretreatment milk production, parity, and DIM and fed a similar diet for a 6-wk treatment period with the exception that all the alfalfa silage was either untreated or treated. Cows were fed ad libitum, and feed refusals were measured daily. Fresh water was available at all times, and the care of animals was via accepted protocols (Anon., 1999). Throughout the study, a computer recorded milk production twice daily at 0700 and 1900 h. Once weekly, milk was sampled proportionately to milk yield from consecutive p.m. and a.m. milkings and analyzed for fat, protein, and somatic cells (Milk-O-Scan, Foss Technology, Hillerød, Denmark). Body weights were recorded at the start and end of the study. Samples of silages and TMR were collected three times per week

Table 1. Ingredient composition of the concentrate fed to lactating dairy cows.

Item	%, DM basis
Corn meal	43.53
Wheat midds	10.58
Distillers dried grains	10.45
Corn gluten feed	10.22
Roasted soybean meal	4.45
Molasses mix	4.30
Soybean meal	3.48
Soybean hulls	3.39
Fat blend	1.74
Sodium sesquicarbonate	1.74
Corn starch	1.39
Protein blend ¹	1.39
Limestone, 37%	1.17
Corn gluten meal	1.07
Salt	0.85
Selenium ²	0.09
Magnesium oxide	0.07
Vitamin pack ³	0.04
Trace mineral salts ⁴	0.04

¹Dried blood meal, hydrolyzed poultry feathers, fishmeal, calcium carbonate, and ethoxyquin.

²Contained (DM basis) 612 mg/kg selenium.

³Contained (DM basis) 13,043 KIU/kg of vitamin A, 4348 KIU/kg of vitamin D, and 81,521 IU/kg of vitamin E.

⁴Contained (DM basis) 14.4% S, 7.5% Ca, 1632 mg/kg of Co, 40,816 mg/kg of Cu, 4081 mg/kg of I, 6122 mg/kg of Fe, 153,061 mg/kg of Mn, and 163,265 mg/kg of Zn.

and composited for analyses. Silages were analyzed as previously described. The TMR were analyzed for DM in a forced-draft oven set at 60°C for 48 h. After drying, forage samples were ground through a Wiley Mill (1-mm screen, Arthur H. Thomas, Philadelphia, PA) and analyzed for DM (100°C oven for 24 h), NDF using sulfite and amylase (Van Soest et al., 1991), and ADF (Roberston and Van Soest, 1981). Crude protein was calculated by multiplying total N by 6.25 after total combustion (LECO CNS 528 Analyzer, LECO Corporation, St. Joseph, MI). Minerals and starch were ana-

Table 2. Nutrient composition of TMR fed to lactating cows.

Item	Control	<i>Lactobacillus buchneri</i> 40788
DM, %	56.6	59.6
CP, %	16.8	17.1
ADF, %	22.3	22.5
NDF, %	31.3	32.0
Starch, %	29.4	24.4
Ca, %	0.95	0.95
P, %	0.45	0.46
Mg, %	0.27	0.26
K, %	1.79	1.89
Na, %	0.58	0.61
Fe, mg/kg	489	426
Zn, mg/kg	63	62
Cu, mg/kg	41	39
Mn, mg/kg	61	56

Table 3. Chemical (DM basis) and microbial composition of fresh alfalfa after treatment but before ensiling in laboratory silos.

Item	Control	<i>Lactobacillus buchneri</i> 40788			SE
		1 × 10 ⁵ cfu/g of fresh forage	5 × 10 ⁵ cfu/g of fresh forage	1 × 10 ⁶ cfu/g of fresh forage	
DM, %	41.5 ^a	37.9 ^{bc}	36.4 ^c	39.3 ^{ab}	0.6
pH	5.96	6.00	5.99	5.98	0.01
WSC ¹ , %	4.56	3.69	4.03	3.45	0.32
Ammonia-N, %	0.033	0.032	0.038	0.029	0.003
LAB ² , log ₁₀ cfu/g	6.85	6.74	6.77	7.14	0.15

^{a,b,c}Means in rows with unlike superscripts differ $P < 0.05$.

¹Water-soluble carbohydrates.

²Lactic acid bacteria.

lyzed by Cumberland Valley Analytical Services (Hagerstown, MD).

Silages and TMR were also analyzed for aerobic stability every other week during the treatment period. Approximately 3 kg of silage or TMR was placed in a 20-L pail and exposed to air in the laboratory (23 to 25°C). The temperature of the forage mass was measured every 10 min by a data logger. Aerobic stability was defined as the number of h the forage mass remained at baseline temperature before rising 2°C (Moran et al., 1996).

Statistical Analyses

All microbial data were transformed to log₁₀. Chemical data are presented on a DM basis. Data from the laboratory silo study were analyzed as a randomized complete block design with treatments and blocks (days) as the main effects, via the general linear models procedure of SAS (SAS, 1998). Data from the lactation study were analyzed as a completely randomized design using the general linear models procedure of SAS (1998). Data from the pretreatment period were used

for covariate adjustment. Differences among means were tested using Tukey's test (Snedecor and Cochran, 1980) and an α level of $P < 0.05$.

RESULTS

Laboratory Silo Study

The chemical and microbial content of fresh alfalfa before ensiling was similar among treatments with the exception of small differences in the DM content (Table 3). Untreated alfalfa contained more lactic acid bacteria (6.85 log₁₀ cfu/g) than levels applied with the inoculants. Yeasts and molds were not detectable in any of the laboratory silages at any sampling time (data not shown). After 2 d of ensiling (Table 4), there was a large increase in lactic acid bacteria (from 6 log₁₀ to > 9 log₁₀ cfu/g), a decrease in pH and WSC, and an increase in fermentation acids. The composition of silages was similar among treatments, with the exception that ammonia-N was greater in silage treated with the highest level of *L. buchneri* 40788. After 4 d of ensiling (Table 5), treated silages had lower pH than did untreated

Table 4. The chemical (DM basis) and microbial composition of alfalfa silage after 2 days of ensiling in laboratory silos.

Item	Control	<i>Lactobacillus buchneri</i> 40788			SE
		1 × 10 ⁵ cfu/g of fresh forage	5 × 10 ⁵ cfu/g of fresh forage	1 × 10 ⁶ cfu/g of fresh forage	
DM, %	36.9	38.0	37.4	39.2	1.0
pH	4.99	4.97	4.88	4.94	0.03
WSC ¹ , %	2.43	2.29	2.05	2.19	0.09
Ammonia-N	0.127 ^b	0.122 ^b	0.130 ^b	0.169 ^a	0.004
Lactic acid, %	2.43	2.31	2.41	2.41	0.16
Acetic acid, %	2.11	2.48	2.38	2.02	0.20
Propionic acid, %	< 0.05	< 0.05	< 0.05	< 0.05	...
LAB ² , log ₁₀ cfu/g	9.23	9.42	9.36	9.47	0.07

^{a,b}Means in rows with unlike superscripts differ $P < 0.05$.

¹Water-soluble carbohydrates.

²Lactic acid bacteria.

Table 5. The chemical (DM basis) and microbial composition of alfalfa silage after 4 d of ensiling in laboratory silos.

Item	Control	<i>Lactobacillus buchneri</i> 40788			SE
		1 × 10 ⁵ cfu/g of fresh forage	5 × 10 ⁵ cfu/g of fresh forage	1 × 10 ⁶ cfu/g of fresh forage	
DM, %	38.5	38.2	36.8	38.3	0.8
pH	4.66 ^a	4.51 ^b	4.46 ^b	4.51 ^b	0.02
WSC ¹ , %	2.10	1.81	1.79	1.81	0.17
Ammonia-N, %	0.141	0.137	0.152	0.138	0.010
Lactic acid, %	3.03	3.25	3.58	3.25	0.192
Acetic acid, %	2.60	2.28	2.65	2.28	0.19
Propionic acid, %	< 0.05	< 0.05	< 0.05	< 0.05	...
LAB ² , log ₁₀ cfu/g	9.46	9.38	9.26	9.17	0.07

^{a,b}Means in rows with unlike superscripts differ $P < 0.05$.

¹Water-soluble carbohydrates.

²Lactic acid bacteria.

silage, and concentrations of lactic acid were numerically but not statistically greater for these silages. There were no other detectable differences in silage composition among treatments at this sampling time. The concentration of lactic acid was lowest in silage treated with the low level of *L. buchneri* 40788 after 8 d of fermentation, but this silage also had the numerically lowest concentration of acetic acid (Table 6). After 56 d of ensiling, DM recovery was greater in silages treated with the moderate (97.6%) and high (93.4%) levels of *L. buchneri* 40788 compared with control silage (86.8%; Table 7). Silage pH and ammonia-N content was higher in silages treated with *L. buchneri* 40788. The concentration of acetic acid was similar (average of 6.40%) but higher among silages treated with *L. buchneri* 40788 compared with control silage (4.24%). Silage treated with the low and moderate level of *L. buchneri* 40788 also had higher concentrations (average 0.21%) of propionic acid than did control silage (0.06%). The concentration of ethanol was lower in untreated silage (0.45%)

than in silage treated with the highest level of *L. buchneri* 40788 (0.62%).

Lactation Study

The ambient temperature ranged from 24 to 30°C during the study. The chemical compositions of the alfalfa silages fed during the lactation study are presented in Table 8. Silage pH was higher, and WSC concentration was lower in silage treated with *L. buchneri* 40788. The concentrations of lactic acid and acetic acid were lower and higher, respectively, in silage treated with *L. buchneri* 40788. Silages did not heat when exposed to air (data not shown); however, when mixed into a TMR, silages treated with *L. buchneri* 40788 showed an improvement in aerobic stability of the TMR (68 vs. 100 h).

Dry matter intake and milk composition were similar between treatments (Table 9), but cows fed silage treated with *L. buchneri* 40788 produced more milk per

Table 6. The chemical (DM basis) and microbial composition of alfalfa silage after 8 d of ensiling in laboratory silos.

Item	Control	<i>Lactobacillus buchneri</i> 40788			SE
		1 × 10 ⁵ cfu/g of fresh forage	5 × 10 ⁵ cfu/g of fresh forage	1 × 10 ⁶ cfu/g of fresh forage	
DM, %	34.7 ^b	37.6 ^a	37.1 ^{ab}	38.2 ^a	0.6
pH	4.56	4.52	4.61	4.55	0.03
WSC ¹ , %	1.47 ^a	1.36 ^{ab}	1.01 ^b	1.38 ^{ab}	0.10
Ammonia-N, %	0.26	0.23	0.25	0.25	0.02
Lactic acid, %	4.45 ^a	3.61 ^b	3.94 ^{ab}	4.12 ^{ab}	0.18
Acetic acid, %	3.36	2.71	3.84	3.04	0.31
Propionic acid, %	< 0.05	< 0.05	< 0.05	< 0.05	...
LAB ² , log ₁₀ cfu/g	8.96	9.20	9.28	9.27	0.07

^{a,b}Means in rows with unlike superscripts differ $P < 0.05$.

¹Water-soluble carbohydrates.

²Lactic acid bacteria.

Table 7. The DM recovery and chemical composition (DM basis) of alfalfa silage after 56 d of ensiling in laboratory silos.

Item	Control	<i>Lactobacillus buchneri</i> 40788			SE
		1×10^5 cfu/g of fresh forage	5×10^5 cfu/g of fresh forage	1×10^6 cfu/g of fresh forage	
DMR ¹ , %	86.8 ^b	92.7 ^{ab}	97.6 ^a	93.4 ^a	1.3
DM, %	36.8	36.4	35.7	37.4	0.6
pH	4.38 ^c	4.49 ^b	4.57 ^{ab}	4.58 ^a	0.02
WSC ² , %	0.76	0.68	0.71	0.65	0.10
Ammonia-N, %	0.29 ^b	0.36 ^a	0.34 ^a	0.34 ^a	< 0.01
Lactic acid, %	4.12	3.73	3.37	3.42	0.17
Acetic acid, %	4.24 ^b	6.42 ^a	6.28 ^a	6.49 ^a	0.34
Propionic acid, %	0.06 ^c	0.22 ^a	0.20 ^{ab}	0.11 ^{bc}	0.02
Ethanol, %	0.45 ^b	0.56 ^{ab}	0.56 ^{ab}	0.62 ^a	0.03

^{a,b,c}Means in rows with unlike superscripts differ $P < 0.05$.

¹Dry matter recovery.

²Water-soluble carbohydrates.

day and tended to produce more FCM/d than did cows fed untreated silage.

DISCUSSION

The results of our study showed that adding of *L. buchneri* 40788 to alfalfa at the time of ensiling increased the concentration of acetic acid in alfalfa silage stored in both laboratory and farm silos. Minor differences in response to treatment between treated alfalfa in laboratory and farm-scale silos may have been due to the fact that farm-scale treatment did not include enzymes. However, in a previous study, we found that enzymes were ineffective in altering fermentation end products (Taylor et al., 2002). In the laboratory silo study, all levels of inoculation (from 1×10^5 to 1×10^6 cfu/g) increased the concentration of acetic acid, but the effect was not dependent on dose. In a past study, Ranjit and Kung (2000) reported that inoculation with 1×10^5

cfu/g of *L. buchneri* was ineffective in increasing the acetic acid concentration in corn silage, but in another study (Ranjit et al., 2002), concentrations of acetic acid increased with increasing doses of *L. buchneri* 40788. In most silages, the concentration of lactic acid is usually greater than the concentration of acetic acid with typical ratios of approximately 3:1 (Ward, 2000). In contrast, treating silage with *L. buchneri* 40788 has resulted in a decrease in the ratio of lactic:acetic acid, and in some instances the resulting silage has had more acetic than lactic acid (Driehuis et al., 2001). Lack of effects of *L. buchneri* 40788 on the concentration of acetic acid during the early silage fermentation process in this study is similar to findings reported in corn silage (Driehuis et al., 1999a). The probable reason for this finding is that anaerobic conversion of lactic acid to acetic acid, 1,2 propanediol, and ethanol by *L. buchneri* 40788 occurs only at a low pH (Oude Elferink et al., 2001).

Table 8. Chemical composition (DM basis) of alfalfa silage and alfalfa silage treated with *Lactobacillus buchneri* stored in a bag silo and fed during the lactation study.

Item	Control	<i>L. buchneri</i> 40788 ¹	SE
DM, %	43.4	41.5	1.6
pH	4.36 ^b	4.66 ^a	0.03
WSC ² , %	1.20 ^a	0.65 ^b	0.08
Lactic acid, %	4.39 ^a	3.50 ^b	0.19
Acetic acid, %	3.35 ^b	5.67 ^a	0.40
Propionic acid, %	0.08	0.11	0.02
Butyric acid, %	0.01	0.02	< 0.01
Ammonia-N, %	0.17	0.21	0.02
Aerobic stability, ³ h	68 ^b	100 ^a	6

^{a,b}Means in rows with unlike superscripts differ $P < 0.05$.

¹Added to achieve 4×10^5 cfu/g of fresh forage.

²Water-soluble carbohydrates.

³Hours before the temperature of the total mixed ration increased more than 2°C above baseline when exposed to air at a controlled temperature of 23 to 25°C.

Table 9. Dry matter intake, milk production, and milk composition from cows fed TMR containing untreated alfalfa or alfalfa treated with *Lactobacillus buchneri* 40788.

Item	Control	<i>L. buchneri</i> 40788 ¹	SE
DMI, kg/d	25.1	25.4	0.4
Milk, kg/d	39.9 ^b	40.7 ^a	0.3
Milk fat, %	3.37	3.43	0.08
Milk fat, kg	1.34	1.38	0.03
Milk protein, %	3.07	3.27	0.14
Milk CP, kg	1.22	1.35	0.08
Lactose, %	4.82	4.84	0.01
Somatic cells, log ₁₀	2.5	2.3	0.09
3.5% FCM, kg/d	38.9 ^c	40.0 ^d	0.5
Feed efficiency, kg milk/ kg DMI	1.60	1.62	0.02

^{a,b}Means in rows with unlike superscripts differ $P < 0.05$.

^{c,d}Means in rows with unlike superscripts differ $P < 0.10$.

¹Added to achieve 4×10^5 cfu/g of fresh alfalfa forage.

Higher concentrations of propionic acid were found in alfalfa silages treated with *L. buchneri* 40788 and stored in laboratory silos but not in treated silage stored in the farm silo. We reported higher concentrations of propionic acid in barley silage (Kung and Ranjit, 2001) but not corn silage (Ranjit and Kung, 2000) treated with *L. buchneri* 40788. Inconsistent effects of *L. buchneri* 40788 on increasing concentrations of propionic acid have also been reported by Driehuis et al. (2001). Further studies should be conducted to determine the factors that control the appearance of propionic acid in silages treated with *L. buchneri* 40788 because this acid is also highly antifungal.

Yeasts were not detected in any of the alfalfa silages stored in laboratory and farm silos and, thus, alfalfa silages alone did not heat (data not shown). However, when silage treated with *L. buchneri* 40788 at the time of ensiling was mixed with other feeds, it improved the aerobic stability of the TMR. A similar finding was reported by us (Taylor et al., 2002) when barley silage treated with *L. buchneri* 40788 was mixed into a TMR and showed that protective effects of *L. buchneri* 40788 on aerobic stability can be carried over into other feeds.

In the current study, DM recovery from laboratory silos was greater in silages treated with the moderate level (97.6%) of *L. buchneri* 40788 and numerically greater in other *L. buchneri* 40788 treatments (average of 93.1%) when compared to untreated silage (86.8%). In contrast, treatment with *L. buchneri* 40788 on corn silage slightly reduced DM recovery (Driehuis et al., 1999a). In a previous study, we (Kung and Ranjit, 2001) reported varying effects of *L. buchneri* 40788 on DM recovery in barley silage.

In past studies, high concentrations of acetic acid in silages have sometimes been associated with lower DMI (Wilkins et al., 1971; Rook and Gill, 1990). Thus, our lactation study was designed to evaluate potential neg-

ative effects on animal performance from feeding silages treated with *L. buchneri* 40788 during moderate weather (24 to 30°C). The results from the current study showed that feeding alfalfa silage treated with *L. buchneri* 40788, which contained a high concentration of acetic acid, had no effect on the DMI of lactating cows but increased milk production. To date, no depressions in intake have been reported in three lactation studies (the current study, Driehuis et al., 1999b, and Taylor et al., 2002), suggesting that high concentrations of acetic acid in silage treated with *L. buchneri* 40788 do not limit intake.

CONCLUSIONS

Inoculating alfalfa with *L. buchneri* 40788 at the time of ensiling altered the resulting fermentation by causing a moderate accumulation of acetic acid. Because acetic acid is a highly effective antifungal agent, when treated silage was mixed into a TMR for dairy cows, the ration remained fresher for a longer period of time compared with a ration with untreated silage. Cows fed a TMR containing alfalfa inoculated with *L. buchneri* 40788 ate similar amounts of DM as those cows fed untreated silage, but they produced more milk. This study further supports the concept that *L. buchneri* 40788 improves the aerobic stability of silages, and although the resulting silage is higher in acetic acid, when fed to cows, there are no deleterious effects on dry matter intake or subsequent milk production.

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