

Inoculant Effects on Alfalfa Silage: Fermentation Products and Nutritive Value

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ABSTRACT

The effect of 14 microbial inoculants on the fermentation and nutritive value of alfalfa silages was studied under laboratory conditions. The first cut (477 g of dry matter/kg) and second cut (393 g of dry matter/kg) of a second-year alfalfa stand were ensiled in 2 trials. In both trials alfalfa was harvested with standard field equipment. All inoculants were applied at 1.0×10^6 cfu/g of crop. Uninoculated silages served as controls. After inoculants were added, the chopped forages were ensiled in 1.0- and 0.5-L anaerobic glass jars, respectively, at a density of 500 g/L. Each trial had 15 treatments (uninoculated control and 14 inoculants), with 4 silos per treatment. Silos were stored for a minimum of 30 d at room temperature ($\sim 22^\circ\text{C}$). In first-cut silage, all inoculants but one reduced pH relative to the uninoculated control, and all but 2 of the homofermentative strains shifted fermentation toward lactic acid. In second-cut silage, the epiphytic lactic acid bacterial population was 2.7×10^7 cfu/g, and only commercial inoculants produced significant shifts in fermentation. Overall, microbial inoculants generally had a positive effect on alfalfa silage characteristics in terms of lower pH and shifting fermentation toward lactic acid with homofermentative lactic acid bacteria or toward acetic acid with heterofermentative lactic acid bacteria, *Lactobacillus buchneri*. These effects were stronger in the commercial products tested. In spite of the positive effects on silage fermentation, 48-h in vitro true DM digestibility was not improved by inoculation with lactic acid bacteria.

Key words: alfalfa, lactic acid bacteria, nutritive value, silage

INTRODUCTION

Inoculants are the most common biological additives used in silage preservation in the United States and

Europe. These products have selected strains of homofermentative lactic acid bacteria (**LAB**), such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. When used, such inoculants often result in a faster decrease in pH, lower final pH values, higher lactate:acetate ratios, lower ethanol and ammonia nitrogen, and a 1 to 2% improvement in DM recovery (Weinberg and Muck, 1996). Recently, a heterofermentative LAB inoculant species, *Lactobacillus buchneri*, has become available commercially and produces high concentrations of acetic acid in silage that inhibit fungi and thus preserve silages susceptible to spoilage upon exposure to air (Weinberg et al., 2002; Filya, 2003a,b). Although the 2 types of inoculants take different approaches to directing fermentation in the silo, the principal goal of both is to preserve as much of the nutritive value of the crop at harvest as possible for the livestock consuming the resulting silage.

Although inoculants have been used for several decades, there are still unanswered questions about the interaction of inoculant LAB with other microorganisms and how this interaction drives fermentation and affects utilization of the silage by animals (Weinberg and Muck, 1996). Inoculants do not consistently improve silage fermentation or animal performance characteristics such as intake, feed efficiency, rate of gain, or milk production (Weinberg and Muck, 1996; Kung et al., 2003). In part, this may be due to characteristics of the crop at harvest: epiphytic LAB population, sugar availability, and plant DM concentration. For example, when the epiphytic LAB population is sufficiently greater than the level of LAB applied to the crop, the inoculant LAB can be overwhelmed and not significantly affect fermentation (Muck, 1989). However, variation in the results of inoculant studies may be due to the efficacy of the inoculant strains. This has been most clearly illustrated in occasional animal trials in which inoculants have had no significant effect on silage pH or fermentation products but the inoculated silages increased milk production or gain (Weinberg and Muck, 1996). Because the crop and its epiphytic microbial populations affect the results of inoculant trials, the relative efficacy of various inocu-

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Table 1. Inoculants used in the trials

Number ¹	Inoculant	Source
1	<i>Lactobacillus buchneri</i> (Pioneer 11A44)	Pioneer Hi-Bred International Inc., Des Moines, IA
2	<i>L. buchneri</i> (Biotal)	Biotal Canada Limited, Calgary, Alberta, Canada
3	<i>Lactobacillus plantarum</i> and <i>Enterococcus faecium</i> (Pioneer 1174)	Pioneer Hi-Bred International Inc., Des Moines, IA
4	<i>L. plantarum</i> and <i>Pediococcus cerevisiae</i> (Biomate LP/PC)	Chr. Hansen Biosystems, Milwaukee, WI
5	<i>L. plantarum</i> (Biomax5)	Chr. Hansen Biosystems, Milwaukee, WI
6	<i>Pediococcus pentosaceus</i> and <i>Propionibacterium jensenii</i> (Biotal Plus)	Biotal Canada Limited, Calgary, Alberta, Canada
7	<i>E. faecium</i> , <i>L. plantarum</i> , and <i>Pediococcus</i> spp. (H/M Plus)	Medipharm USA, Des Moines, IA
8	<i>L. plantarum</i> MTD1 (Ecosyl)	Ecosyl, Yorkshire, UK
9	<i>E. faecium</i> C (Agri-King)	Agri-King, Fulton, IL
10	<i>E. faecium</i> Q (Agri-King)	Agri-King, Fulton, IL
11	<i>Lactobacillus pentosus</i> (Agri-King)	Agri-King, Fulton, IL
12	<i>L. plantarum</i> (Agri-King)	Agri-King, Fulton, IL
13	<i>P. pentosaceus</i> (Agri-King)	Agri-King, Fulton, IL
14	<i>P. pentosaceus</i> (Ecosyl)	Ecosyl, Yorkshire, UK

¹Inoculants 1 to 8 are commercially marketed.

lants can be measured accurately only by head-to-head comparisons on the same forage. A wide head-to-head comparison may also help reveal whether there are substantial or consistent inoculant effects on the silage characteristics that are typically measured to develop live-stock rations.

The objective of this study was to test a wide variety of inoculant LAB on the ensiling of alfalfa and to determine the effects on silage fermentation, nutritive value, and rumen in vitro fermentation. This paper reports solely on the effects on silage fermentation and standard measures of nutritive value. Two subsequent papers address the effects on rumen in vitro fermentation [Muck et al., 2007; unpublished manuscript of the authors and D. R. Mertens, and P. J. Weimer (both at USDA, ARS, US Dairy Forage Research Center, Madison, WI)].

MATERIALS AND METHODS

Mini Silo Experiments

In 2003, alfalfa was ensiled in 2 trials (first cut, 477 g of DM/kg; second cut, 393 g of DM/kg) on June 9 and July 2, respectively. In both trials, alfalfa was harvested with standard field equipment (mower-conditioner, forage harvester, 10-mm theoretical length of cut) without inoculation. The chopped alfalfa was ensiled in 1.0- and 0.5-L anaerobic glass jars (Weck, Weyerhaeuser, Germany), respectively, at a density of 500 g/L. Each trial had 15 treatments (uninoculated control and 14 inoculants), with 4 silos per treatment. Eight inoculants were commercial products (inoculants 1 to 8; Table 1); the others were single strains provided by 2 companies. All inoculants were applied at a rate of 1.0×10^6 cfu/g of crop (not label rates) to help ensure domination of fermentation. All inoculants were diluted with distilled water so that they were applied at the same rate (10 g of solution/kg of crop). The control received 10 g of water/

kg of crop. The amount of chopped alfalfa for a given silo was weighed, sprayed with the appropriate inoculant solution with a plant sprayer (one sprayer for each treatment), mixed by hand, and then placed into the silo by hand with periodic tamping. Equipment coming into contact with treated alfalfa was washed and wiped with ethanol between treatments to prevent cross-contamination. Over the course of ensiling for all treatments, 4 samples of untreated chopped alfalfa were taken for analysis of initial characteristics, and all inoculant solutions were analyzed for LAB counts. Silos were stored for 35 and 47 d, respectively, at room temperature (~22°C).

Analyses

The untreated chopped alfalfa at ensiling and the silages at silo opening were analyzed for the same constituents, with the exception that fermentation products were determined only on silages. Duplicate samples (50 g) were taken for moisture determination by freeze-drying. After moisture determination, the duplicate freeze-dried samples were ground together to 1-mm particle size and analyzed for total nitrogen by a Leco FP-2000A nitrogen analyzer (Leco Corp., St. Joseph, MI) and for NDF, ADF, and acid detergent lignin (**ADL**) through the procedure of Robertson and Van Soest (1977) as modified by Hintz et al. (1995) to include sodium sulfite during refluxing. Hemicellulose (**HC**) concentration was estimated by the difference of NDF minus ADF, and cellulose concentration was estimated by the difference of ADF minus ADL. In vitro true DM digestibility [**IVTDM**, i.e., (initial DM fermented – undigested NDF residue at 48 h)/initial DM fermented] was determined by the in vitro procedure of Goering and Van Soest (1970), and water-soluble carbohydrates (**WSC**) were determined by the phenol sulfuric acid method (Dubois et al., 1956). Another portion of original sample (20 g) was

Table 2. Characteristics (g/kg of DM except as noted) of the fresh alfalfa at ensiling

Cut	pH	DM, g/kg	Water-soluble carbohydrates	Total nitrogen	NDF	ADF	Acid detergent lignin	Cellulose	Hemicellulose	In vitro true DM digestibility
First	6.19	477	37	37.1	391	314	61	253	78	802
Second	6.08	393	41	37.7	282	239	53	185	43	845

diluted 10:1 with autoclaved distilled water and blended in a 0.5-L Waring commercial laboratory high-speed blender for 30 s. The diluted sample was enumerated immediately for acid-tolerant LAB by using Rogosa SL agar (Difco 0480, Becton Dickinson, Sparks, MD). After the diluted sample was subsampled for LAB, the remainder was filtered through 4 layers of cheesecloth, and pH was immediately measured on the filtrate. A 20-mL aliquot of the filtrate was placed into a 50-mL polypropylene tube and centrifuged at $25,000 \times g$ for 25 min. Liquid decanted from the centrifuge tube was frozen at -20°C and used for measuring fermentation products. Fermentation products (lactate, acetate, propionate, butyrate, and ethanol) were determined by HPLC with a refractive index detector (Muck and Dickerson, 1988).

Because of differences in initial quality characteristics between harvests, statistical analysis was performed for each cut separately with the generalized linear model procedure of SAS (SAS Inst. Inc., Cary, NC). Differences among means were tested by Fisher's protected least significant difference, and significance was declared at $P < 0.05$. Correlations among silage characteristics were determined using PROC CORR in SAS, with significance declared at $P < 0.05$.

RESULTS

Original Forage

The first and second cuts of alfalfa were different in initial characteristics. First-cut alfalfa had higher DM, NDF, ADF, and ADL, and lower WSC and IVTDMD than second-cut alfalfa (Table 2). The IVTDMD values for both cuts were high compared with what might be expected in more common measurements of IVTDMD, such as that by Tilley and Terry (1963). The primary difference between the techniques is that our procedure is more effective in removing bacterial residues from in vitro fermentation (Van Soest et al., 1966), thus providing a more accurate estimate of the degree of digestion of the original silage. In first-cut alfalfa, the epiphytic LAB population at ensiling was 1.5×10^5 cfu/g and would have been expected to be overwhelmed by the inoculant LAB applied at 1.0×10^6 cfu/g. In second-cut alfalfa, the epiphytic population (2.7×10^7 cfu/g) at ensiling was more than 10 times higher than the inoculant applica-

tion rates, providing a stiffer challenge for the added LAB.

Fermentation Products

The pH, WSC, and organic acid concentrations of first- and second-cut alfalfa silages were different among treatments (Tables 3 and 4). In first-cut silage, all inoculants except *E. faecium* C reduced pH relative to that of the control (Table 3). The commercial homofermentative inoculants produced the largest reductions in pH, whereas the 2 commercial heterofermentative (*L. buchneri*) inoculants produced the smallest reductions, as might be expected. In second-cut silage, the only treatments lowering pH values more than the control were 5 of the 6 commercial homofermentative inoculants. The *L. buchneri* inoculants had the highest pH values.

Fermentation products in both cuts were limited to 3 principal products: lactate, acetate, and ethanol (Tables 3 and 4). Propionate and butyrate concentrations were below detectable concentrations (0.1 g/kg of DM). In first-cut alfalfa silage, the inoculated treatments produced from 2.5 to 106% greater lactate concentrations than the uninoculated treatment (Table 3). In addition, alfalfa inoculated with *Lactobacillus pentosus*, the 2 strains of *L. buchneri*, *E. faecium* C, *E. faecium* Q, the combination of *L. plantarum* and *E. faecium*, and *L. plantarum* MTD1 had 15 to 259% greater acetate concentrations than the uninoculated control. Even with these increases in acetate, the only treated silages in which the lactate:acetate ratios were lower than the uninoculated control (2.88) were *L. pentosus* (2.08), *E. faecium* C (2.39), and the 2 *L. buchneri* (2.21, 2.40) strains. The lactate:acetate ratios ranged from 4.46 to 11.71 in the other treatments. Alfalfa silages inoculated with both *L. buchneri* strains and *E. faecium* Q had higher ethanol concentrations than the control and other LAB-inoculated alfalfa silages.

In second-cut alfalfa silage, the uninoculated silage had a greater lactate concentration than all but 2 inoculated alfalfa silages ($P < 0.05$; Table 4). Alfalfa silages inoculated with both *L. buchneri* strains and *L. pentosus* had higher acetate and ethanol concentrations than the control and other LAB-inoculated alfalfa silages ($P < 0.05$). These 3 inoculants were the only ones to produce lower lactate:acetate ratios (1.73 to 2.74) compared with

Table 3. Fermentation characteristics (g/kg of DM except as noted) of the first-cut alfalfa silages

Treatment	DM, g/kg	pH	Water-soluble carbohydrates	Lactate	Acetate	Ethanol	Lactate: acetate
Control	480	5.08	18	40.5	14.2	3.2	2.88
<i>Lactobacillus buchneri</i> (Pioneer 11A44)	463	4.82	8	49.5	20.6	8.4	2.40
<i>L. buchneri</i> (Biotal)	465	4.90	9	45.9	20.8	7.0	2.21
<i>Lactobacillus plantarum</i> and <i>Enterococcus faecium</i> (Pioneer 1174)	476	4.50	10	83.5	17.3	1.3	4.94
<i>L. plantarum</i> and <i>Pediococcus cerevisiae</i> (Biomate LP/PC)	471	4.43	9	82.8	13.5	1.4	6.31
<i>L. plantarum</i> (Biomax5)	477	4.33	12	81.9	8.4	1.3	9.98
<i>Pediococcus pentosaceus</i> and <i>Propionibacterium jensenii</i> (Biotal Plus)	465	4.51	10	71.0	9.1	1.7	7.96
<i>E. faecium</i> , <i>L. plantarum</i> , and <i>Pediococcus</i> spp. (H/M Plus)	470	4.38	10	79.5	8.3	1.3	9.11
<i>L. plantarum</i> MTD1 (Ecosyl)	463	4.51	8	72.7	16.3	2.0	4.46
<i>E. faecium</i> C (Agri-King)	463	5.14	13	41.6	17.5	4.1	2.39
<i>E. faecium</i> Q (Agri-King)	466	4.58	7	73.4	16.2	4.7	4.53
<i>Lactobacillus pentosus</i> (Agri-King)	463	4.66	7	76.2	36.8	3.6	2.08
<i>L. plantarum</i> (Agri-King)	467	4.46	10	68.0	10.1	2.7	6.75
<i>P. pentosaceus</i> (Agri-King)	466	4.57	13	62.8	6.0	2.2	10.73
<i>P. pentosaceus</i> (Ecosyl)	470	4.58	15	64.2	5.5	3.0	11.71
LSD ($P < 0.05$)	9.0	0.017	0.8	2.67	1.16	1.05	1.069

that of the control (2.98). This ratio ranged from 2.99 to 6.12 for the other inoculated silages.

The WSC remaining after fermentation in first-cut silage were significantly ($P < 0.05$) higher in the uninoculated control silage than in all other treatments (Table 3). The lowest WSC concentrations were in silages treated with *L. pentosus*, *E. faecium* Q, and the 2 *L. buchneri* strains. In second-cut silage, the WSC concentration in the control was in the middle of the range for all treatments (Table 4). The lowest concentrations occurred in the *L. buchneri* treatments, and the highest were in 2 of the commercial inoculant treatments and the 2 *E. faecium* treatments.

Nutritive Characteristics

On average, NDF, ADF, and ADL concentrations of the silages in both cuts (Tables 5 and 6) were higher than their respective values at ensiling (Table 2). Losses of DM from fermentation and respiration preferentially come from utilization of sugars by microorganisms (Pahlow et al., 2003), so such increases in the concentrations of cell wall fractions would be expected. However, the concentrations of polysaccharides in the cell wall varied by category. Cellulose concentrations in silages were, on average, higher than those in the unensiled alfalfa, whereas HC concentrations in the silages were numeri-

Table 4. Fermentation characteristics (g/kg of DM except as noted) of the second-cut alfalfa silages

Treatment	DM, g/kg	pH	Water-soluble carbohydrates	Lactate	Acetate	Ethanol	Lactate: acetate
Control	368	4.42	6.8	86.5	29.0	4.5	2.98
<i>Lactobacillus buchneri</i> (Pioneer 11A44)	381	4.64	4.2	61.5	35.5	8.7	1.73
<i>L. buchneri</i> (Biotal)	365	4.65	4.0	70.1	37.4	9.2	1.87
<i>Lactobacillus plantarum</i> and <i>Enterococcus faecium</i> (Pioneer 1174)	377	4.34	5.5	81.3	18.4	3.4	4.44
<i>L. plantarum</i> and <i>Pediococcus cerevisiae</i> (Biomate LP/PC)	378	4.40	5.5	78.3	20.6	3.7	3.81
<i>L. plantarum</i> (Biomax5)	366	4.29	5.4	81.2	16.6	3.2	4.90
<i>Pediococcus pentosaceus</i> and <i>Propionibacterium jensenii</i> (Biotal Plus)	372	4.42	8.8	79.0	21.0	4.6	3.78
<i>E. faecium</i> , <i>L. plantarum</i> , and <i>Pediococcus</i> spp. (H/M Plus)	379	4.32	10.8	80.4	13.1	4.4	6.12
<i>L. plantarum</i> MTD1 (Ecosyl)	363	4.40	5.7	80.5	19.0	4.1	4.28
<i>E. faecium</i> C (Agri-King)	364	4.47	8.1	84.6	28.7	4.6	2.99
<i>E. faecium</i> Q (Agri-King)	385	4.44	8.0	78.2	22.6	4.6	3.46
<i>Lactobacillus pentosus</i> (Agri-King)	365	4.46	6.7	86.0	31.7	5.5	2.74
<i>L. plantarum</i> (Agri-King)	367	4.42	7.7	80.0	20.9	6.1	3.84
<i>P. pentosaceus</i> (Agri-King)	371	4.46	7.6	78.8	23.0	4.7	3.43
<i>P. pentosaceus</i> (Ecosyl)	368	4.46	6.6	82.6	24.5	4.3	3.38
LSD ($P < 0.05$)	13.0	0.018	1.15	2.19	1.31	0.45	0.346

Table 5. Cell wall components, total nitrogen (TN), and in vitro true DM digestibility (IVTDMD) (g/kg of DM) of first-cut alfalfa silages

Treatment	NDF	ADF	Acid detergent lignin	Hemicellulose	Cellulose	TN	IVTDMD
Control	419	342	78	78	264	34.5	766
<i>Lactobacillus buchneri</i> (Pioneer 11A44)	430	354	78	77	276	35.1	762
<i>L. buchneri</i> (Biotal)	442	354	77	88	276	34.0	760
<i>Lactobacillus plantarum</i> and <i>Enterococcus faecium</i> (Pioneer 1174)	436	356	79	80	277	35.1	767
<i>L. plantarum</i> and <i>Pediococcus cerevisiae</i> (Biomate LP/PC)	420	352	76	67	277	35.1	769
<i>L. plantarum</i> (Biomax5)	441	364	81	77	283	33.9	760
<i>Pediococcus pentosaceus</i> and <i>Propionibacterium jensenii</i> (Biotal Plus)	419	354	81	65	273	35.3	769
<i>E. faecium</i> , <i>L. plantarum</i> , and <i>Pediococcus</i> spp. (H/M Plus)	421	350	75	70	276	34.8	773
<i>L. plantarum</i> MTD1 (Ecosyl)	434	361	82	73	279	34.3	764
<i>E. faecium</i> C (Agri-King)	391	330	71	61	259	35.9	778
<i>E. faecium</i> Q (Agri-King)	424	354	81	71	273	35.0	762
<i>Lactobacillus pentosus</i> (Agri-King)	421	346	78	75	267	37.0	766
<i>L. plantarum</i> (Agri-King)	404	335	73	70	261	35.0	773
<i>P. pentosaceus</i> (Agri-King)	418	337	75	80	262	35.1	772
<i>P. pentosaceus</i> (Ecosyl)	423	342	75	81	268	34.5	772
Average	423	349	77	74	271	35.0	767
LSD ($P < 0.05$)	24.5	16.3	5.7	NS	13.6	1.53	NS

cally lower, on average, than those prior to ensiling. A reduction in HC during ensiling has been reported in various forages, including alfalfa (Rooke and Hatfield, 2003), and in alfalfa the reduction in HC appears to be the result of acid hydrolysis of arabinosyl side branches from the main HC backbone (Jones et al., 1992).

In both cuts, some significant differences in cell wall constituents by treatment were observed. In first-cut silage, significant differences were observed in NDF, ADF, ADL, and cellulose (Table 5). In second-cut silage, NDF, ADF, and HC differed by treatment (Table 6). In first-cut silage, the concentrations of NDF, ADF, ADL,

HC, and cellulose of the control were in the middle of the ranges for each constituent. As a result, although there were inoculant treatments that were significantly different from one another, there were few instances in which an inoculated treatment was different from the uninoculated control. The exceptions were the low NDF and ADL concentrations for *E. faecium* C and the high ADF and cellulose values for Biomax5 and *L. plantarum* MTD1. In second-cut silage, the control had the highest ADL and HC concentrations, a high NDF, and moderate levels of ADF and cellulose. Similar to first-cut silage, the *E. faecium* C treatment had significantly lower NDF

Table 6. Cell wall components, total nitrogen (TN) and in vitro true DM digestibility (IVTDMD) (g/kg of DM) of second-cut alfalfa silages

Treatment	NDF	ADF	Acid detergent lignin	Hemicellulose	Cellulose	TN	IVTDMD
Control	307	258	62	49	196	38.1	873
<i>Lactobacillus buchneri</i> (Pioneer 11A44)	303	258	58	45	200	39.7	855
<i>L. buchneri</i> (Biotal)	314	260	56	54	204	39.2	835
<i>Lactobacillus plantarum</i> and <i>Enterococcus faecium</i> (Pioneer 1174)	293	253	58	41	195	38.4	875
<i>L. plantarum</i> and <i>Pediococcus cerevisiae</i> (Biomate LP/PC)	293	258	57	35	202	38.4	849
<i>L. plantarum</i> (Biomax5)	307	261	62	47	199	38.1	866
<i>Pediococcus pentosaceus</i> and <i>Propionibacterium jensenii</i> (Biotal Plus)	291	259	54	32	205	39.1	851
<i>E. faecium</i> , <i>L. plantarum</i> , and <i>Pediococcus</i> spp. (H/M Plus)	290	255	55	34	200	38.9	856
<i>L. plantarum</i> MTD1 (Ecosyl)	292	255	53	37	202	39.5	849
<i>E. faecium</i> C (Agri-King)	284	256	57	29	199	39.0	854
<i>E. faecium</i> Q (Agri-King)	292	261	57	31	204	38.8	863
<i>Lactobacillus pentosus</i> (Agri-King)	309	263	59	46	204	38.3	840
<i>L. plantarum</i> (Agri-King)	293	255	57	38	199	39.1	835
<i>P. pentosaceus</i> (Agri-King)	306	265	58	41	207	38.6	844
<i>P. pentosaceus</i> (Ecosyl)	300	259	56	42	202	38.7	849
Average	298	258	57	40	201	38.8	853
LSD ($P < 0.05$)	17.0	NS	4.2	14.4	NS	NS	22.2

and ADL concentrations than the control, as well as the lowest HC concentration. *Enterococcus faecium* Q had concentrations of those constituents similar to *E. faecium* C. Eight other inoculants had lower ADL values than the control. In addition to the *E. faecium* treatments, Biotal Plus and H/M Plus had lower HC values than the control.

Total nitrogen was affected by treatment in first-cut but not in second-cut silage (Tables 5 and 6). Few instances of significant differences in first-cut silage were found. Only the silage treated with *L. pentosus* had a total nitrogen value statistically different from the control.

In vitro true DM digestibility was significantly affected by treatment in second-cut but not in first-cut silage (Tables 5 and 6). In second-cut silage, IVTDMD was highest in Pioneer 1174, and the control had a value similar to that of Pioneer 1174. Seven inoculant treatments had IVTDMD values significantly lower than the uninoculated control: Biotal *L. buchneri*, Biomate LP/PC, *L. plantarum* MTD1, *L. pentosus*, Agri-King *L. plantarum*, and the 2 *Pediococcus* strains.

DISCUSSION

In first-cut silage, conditions for an inoculant to improve silage fermentation were nearly ideal: a somewhat high DM content (477 g of DM/kg) that would restrict normal fermentation (Muck et al., 2003), a limited WSC content, and an epiphytic LAB population lower than the applied rates of inoculant LAB. All inoculant treatments, except for *E. faecium* C, reduced pH relative to the control. The greatest reduction in pH relative to the control was 0.75, a substantial difference. Most inoculant LAB treatments produced silages with higher lactate:acetate ratios than the uninoculated control. The exceptions were the heterofermentative *L. buchneri* treatments and the 2 single homofermentative strains. Such shifts (lower pH and higher lactate:acetate ratios) in silage fermentation with homofermentative LAB are consistent with summaries of previous studies when such inoculants were successful (e.g., Weinberg and Muck, 1996; Muck and Kung, 1997; Kung et al., 2003). Among the 12 homofermentative inoculants, there were significant differences in pH and fermentation products. The 3 silages with the lowest pH values were produced by commercial inoculants, whereas the 2 highest lactate:acetate ratios were produced by the *Pediococcus pentosaceus* strains, not the commercial inoculants.

The second-cut trial provided a greater challenge for the inoculant LAB. The epiphytic LAB population was more than 10 times the application rates of the inoculant LAB. In addition, the uninoculated silage achieved a low pH (4.42) with a high lactic acid content (86.5 g/kg of

DM). Even with these challenges, all 8 of the commercial inoculant products produced significant shifts in pH or fermentation products, which indicated that they had affected the final outcome of silage fermentation. The *L. buchneri* treatments increased pH and shifted fermentation to acetic acid relative to the control, as expected with a heterolactic fermentation (Moon, 1983). Five of the 6 homofermentative commercial inoculants lowered pH relative to the control, and all 6 produced a more homofermentative fermentation (i.e., higher lactate:acetate ratio) even though producing less lactic acid than the control treatment. The 6 homofermentative single strains showed less evidence of affecting fermentation. The ability of the commercial inoculants to affect fermentation even when present at lower numbers than the epiphytic population has been observed previously. For example, Muck (1989) conducted 4 trials and found that the inoculant used (similar to the H/M Plus inoculant here) consistently improved silage fermentation when applied at 10% or more of the epiphytic population but had no significant effect when applied at less than 1% of the epiphytic population.

One might have expected, based on the fermentation results presented in Tables 3 and 4, that the inoculant treatments, particularly in first-cut silage, would have affected rumen in vitro fermentation. However, no effect on a 48-h IVTDMD measurement was observed in first-cut silage (Table 5). In second-cut silage, the control silage had the second highest IVTDMD and 7 inoculated treatments had IVTDMD values significantly lower than that of the control. The lack of a positive effect of inoculants on IVTDMD or other measures of potential digestibility has occurred in some studies. Muck and Kung (1997) found that fermentation was improved (i.e., reduced pH, increased the lactate:acetate ratio, or both) by inoculants in more than 60% of trials published between 1990 and 1995, whereas DM digestibility (in vitro or in vivo) was increased in only 30% of the trials. Weinberg and Muck (1996), in their review, also reported instances in which fermentation was affected by an inoculant, whereas digestibility was not.

Within a trial, IVTDMD values of individual silos were generally negatively correlated with various fiber constituents. In first-cut silage, the highest correlation was with ADF ($r = -0.630$). In second-cut silage, the highest correlation was with cellulose ($r = -0.376$). Negative correlations with fiber components would be expected because the cell wall component of the silage is the least digestible fraction. Based on the reduction of NDF, *E. faecium* C should have had the greatest effect on IVTDMD. It had the highest IVTDMD numerically, although not significantly, in first-cut silage but had an IVTDMD similar to the mean in second-cut silage. The consistent reduction of NDF by *E. faecium* C was unex-

pected because LAB are not known to have enzymes that break down structural carbohydrates (Rooke and Hatfield, 2003).

Most fermentation products and pH were not highly correlated with IVTDMD. However, there was a significant correlation ($P < 0.03$) between IVTDMD and ethanol ($r = -0.292$ and -0.373 for first- and second-cut silage, respectively). Such a correlation might be expected. Ethanol production, whether by yeast or LAB, leads to carbon dioxide production and loss of digestible DM (McDonald et al., 1991).

Although positive effects of silage inoculants on IVTDMD were not observed in this study, it is still possible that these inoculated silages could affect animal performance via the rate of digestion or some other factor (Weinberg and Muck, 1996). In subsequent papers (Muck et al., 2007; unpublished paper of authors, D. R. Mertens, and P. J. Weimer) look at the rates and products of in vitro fermentation across the silages in these 2 trials.

CONCLUSIONS

Microbial inoculants generally had a positive effect on alfalfa silage characteristics in terms of lower pH and shifting fermentation toward lactic acid with homofermentative LAB or toward acetic acid with *L. buchneri*. These effects were stronger in the commercial products tested. However in both trials, 48-h IVTDMD was not increased by treatment of the crop with LAB at ensiling.

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