Effects of a Mixture of Lactic Acid Bacteria Applied as a Freeze-Dried or Fresh Culture on the Fermentation of Alfalfa Silage

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ABSTRACT

Alfalfa (approximately 31% DM) was untreated or treated with a silage inoculant containing the lactic acid bacteria *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1. The inoculant was added at a normal and a high dose as a freeze-dried powder that had been mixed with water just prior to application, or it was grown with nutrients the day before and added as a fresh culture. The actual application rate of lactic acid bacteria was 1.19×10^5 for the normal dose, 4.30×10^5 for the high dose, and 5.10×10^5 for the fresh culture. All inoculated silages showed a faster increase in the rate of lactic acid production and a decrease in the drop in pH over the first 24 h of ensiling compared with untreated silage. The effect was greatest for silage treated with the fresh culture and was supported by the fact that this treatment had numbers of lactic acid bacteria that increased faster than in other treatments. Inoculation also generally resulted in a fermentation profile that was more homolactic (more lactic acid and less acetic acid, ethanol, and $\text{NH}_3\text{-N}$) than for untreated silage, but the effect was greatest for the fresh culture. Inoculation did not affect in vitro neutral detergent fiber digestion or the concentrations of neutral detergent fiber or total N in silages. The recovery of dry matter was greater in silage that was treated with a high level of the freeze-dried culture or with the fresh culture when compared with the untreated control. This study showed that application of a silage inoculant as a freeze-dried culture or as a fresh culture resulted in alfalfa silage with a more homolactic fermentation profile. The effect was greatest from addition of the fresh culture.

Key words: silage inoculant, fructan, alfalfa silage, *Lactobacillus plantarum*

INTRODUCTION

Inoculating silages with lactic acid bacteria (LAB) has improved silage fermentation (Ely et al., 1981; Stokes, 1992). Inoculation with these microbes has increased the rate and extent of lactic acid production in silages, decreased proteolysis, and decreased the production of volatile organic acids (Muck and Kung, 1997). Added bacteria must be revived from a freeze-dried state for them to be effective, and mixing bacterial inoculants with water prior to application to the forage crop also helps to activate the organisms. Dry and liquid applied silage inoculants can be effective, but liquid applications have some advantages over dry applications, especially in crops with low moisture. For example, Whiter and Kung (2001) reported that application of a dry and a liquid inoculant equally stimulated a drop in pH during early ensiling in alfalfa with 30% DM, but when applied to alfalfa with a DM of 54%, the effect was greater when the inoculant was applied in water.

Some microbial inoculants can be grown in water with enriched media to stimulate their growth before being applied to chopped forages. The use of such “freshly cultured” inoculants may be advantageous, because the bacteria would have a shorter lag time for being revived from a freeze-dried state. Merry et al. (1995a) reported a faster rate of silage fermentation in ryegrass silage when they applied a fresh culture of LAB, compared with untreated silage or silage treated with a freeze-dried preparation of the same inoculant. The use of a freshly cultured silage inoculant added to grass silage has also resulted in improvements in DMI and live weight gain that were equal to those of animals fed silage treated with formic acid, and results were better than for animals fed untreated silage (Winters et al., 2001).

The objective of this study was to evaluate the effect of an inoculant applied in various forms on the fermentation of alfalfa silage. The inoculant was added as a freeze-dried formulation diluted in water and applied directly to forage at the time of ensiling, or it was grown as a fresh culture with enrichment media and then diluted in water and applied to forage at ensiling.

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Figure 1. Colony-forming units (log) of lactic acid bacteria in freshly cultured growth media. Bacteria were grown in media at 32°C for 19 h and then removed and stored at a room temperature of $22 \pm 1.5^\circ\text{C}$. Each line represents the results from a separate batch. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

MATERIALS AND METHODS

A commercial inoculant (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI) containing *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 was used in this study. Prior to the silage experiment, the inoculant was grown with media (supplied with the inoculant by the manufacturer) to produce fresh cultures on 3 separate occasions. Growth medium (supplied by the manufacturer) was added to warm tap water (32°C) in a plastic drum (supplied by the manufacturer). Freeze-dried bacteria were added to the drum and the contents were gently mixed. The drum was placed on a heating pad (supplied by the manufacturer) and insulated to maintain a constant temperature between 32 and 33°C. After 19 h, the drum was removed from the heating pad and kept at room temperature ($22 \pm 1.5^\circ\text{C}$) for several days. Samples of the culture were taken 19, 24, 48, 72, 96, and 120 h after the start of fermentation and analyzed for numbers of LAB. The numbers of LAB were determined by pour plating serial 10-fold dilutions (in sterile one-quarter-strength Ringers solution made from Oxoid BR0052 tablets, Oxoid, Basingstoke, UK) on de Mann, Rogosa, Sharpe agar (Oxoid CM361). Plates were incubated anaerobically at 32°C for 48 to 72 h. There were more than 10^9 cfu of LAB/mL of culture after 19 h of culturing with heat (Figure 1). The numbers of LAB remained relatively stable in the cultures for this time frame. This finding is important because it would allow a user approximately a 4- to 5-d period when the inoculant would have full viability and would not require special storage (e.g., refrigeration).

Alfalfa was mowed during the second cutting at the early bloom stage of maturity. After wilting to a DM content of approximately 31%, forage was chopped by a conventional forage harvester to a theoretical length of 0.95 cm. Treatments applied to chopped alfalfa forage were deionized water (250 mL/25 kg of fresh matter; control), a normal level of freeze-dried formulation of the inoculant with a target of 1×10^5 cfu/g of fresh forage (FDL), a high level of the mixture just described with a target of 5×10^5 cfu/g of fresh forage (FDH), or a freshly cultured inoculant (as previously described) grown with nutrients the day before (FC, target final application rate of 5×10^5 cfu/g of fresh forage). The FDL, FDH, and FC treatments were mixed in water for 5 to 10 min prior to application and applied at a rate of 250 mL of deionized water/25 kg of fresh matter. Inoculants were applied with a hand sprayer while manually mixing the forage samples. Within 10 min of treatment, forages (400 g) were packed in nylon-polyethylene standard barrier microlayered pouches (3.5 mil thickness, 15.2×30.5 cm, Doug Care Equipment Inc., Springville, CA) and vacuum sealed with a Best Vac vacuum machine (distributed by Doug Care Equipment Inc.). The pouches had a layer of polyethylene mesh sealed into the entire length of the pouch, which assisted in the removal of air during the vacuum process. Twelve pouches (silos) were prepared for each treatment. Silos were stored between 23 and 25°C, and 3 bags were opened for each treatment at 6 h, 12 h, 24 h, and 60 d of ensiling. The DM content of forages and silages was determined by drying duplicate samples from each silo in a 60°C forced-air oven for 48 h. Dried samples of fresh forage and silages were ground with

a Udy Cyclone Sample Mill (Udy Corp., Fort Collins, CO) through a 1-mm screen. Samples were analyzed for NDF by using sulfite and heat-stable amylase (Van Soest et al., 1991) and for ADF (Robertson and Van Soest, 1981) by using an Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Fairport, NY). Total N was determined by combustion of the sample (Leco CNS 2000 Analyzer, Leco Corporation, St. Joseph, MI).

Samples of fresh forages and silages (25 g) were homogenized in 225 mL of sterile one-quarter-strength Ringer's solution for 1 min. The pH of this mixture was recorded. Liquid from the blended samples were serially diluted (10-fold) and analyzed for LAB, enterobacteria, yeasts, and molds. The numbers of LAB were determined by pour plating in de Man, Rogosa, Sharpe agar as previously described. Plates were incubated anaerobically at 32°C for 48 to 72 h. The numbers of enterobacteria were enumerated by pour plating in violet-red bile glucose agar (Oxoid CM485) with a single overlay. Plates were incubated at 36°C for 18 h prior to counting. Yeasts and molds were determined by pour plating in malt extract agar (Oxoid CM59) that had been acidified, after autoclaving, by the addition of 85% lactic acid (AC12506-5000, Fisher Scientific, Pittsburgh, PA) at a concentration of 0.5 vol/vol. Plates were incubated aerobically at 32°C for 48 to 72 h.

A water extract was collected by filtering the homogenized silage mixture through Whatman 54 filter paper (Florham Park, NJ). A portion of the filtered water extract (10 mL) was acidified with 50 μ L of 50% (wt/vol) H₂SO₄ to reduce the pH of the extract to <2.0 before freezing (-20°C). Water extracts were analyzed for lactic acid, VFA, and ethanol by HPLC (Dairyland Laboratories, Arcadia, WI) as described by Muck and Dickerson (1998). Water extracts were also analyzed for water-soluble carbohydrates (WSC; Nelson, 1944) and NH₃-N (Weatherburn, 1967). The digestibility of NDF was determined on samples after 60 d of ensiling by using the *in vitro* procedure described by Goering and Van Soest (1970) with some modifications. Those modifications included 1) incubation of samples in 100-mL polypropylene tubes, each 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; 2) gentle manual swirling of the tubes at 3, 6, 9, 20, and 26 h; and 3) incubation for 30 h. Recovery of forage DM was calculated by knowing the weight of empty silos, the initial and final weights of silos with forage, and the DM concentrations of the fresh and ensiled material.

All microbial data were transferred to log₁₀ and are presented on a wet weight basis, whereas chemical data are presented on a DM basis. Data were analyzed separately for each time period by using PROC GLM of

SAS (SAS Institute, 2001) for a completely randomized design. Differences among means were tested by using Tukey's test (Snedecor and Cochran, 1980). An alpha level of $P < 0.05$ was deemed significant.

RESULTS AND DISCUSSION

On the day of ensiling, the inoculant mixes were sampled and plated for numbers of LAB. Based on these numbers, the actual application rate of LAB was 1.19×10^5 for FDL, 4.30×10^5 for FDH, and 5.10×10^5 for FC (data not shown). The microbial populations and chemical composition of alfalfa immediately after treatment but before ensiling are shown in Table 1. Untreated silage (control) contained 5.3 log cfu/g of epiphytic LAB, which was higher than that reported by Lin et al. (1992) in alfalfa (3.76 log cfu/g). In inoculated forages, LAB ranged from 5 log cfu/g for FDL to a high of 5.44 log cfu/g for FDH. Yeasts and molds were very low and did not differ among treatments. The DM contents of forages were consistent, ranging from 31.23 to 32.02%, and there was no difference among the treatments. No differences were detected among treatments for WSC, total N, NH₃-N, and ADF. Some differences were noted among treatments in NDF but were most likely a consequence of sampling variation.

Fermentation occurred relatively rapidly for all silages (a result of the high moisture content), as shown by the decline in silage pH over the first 24 h of ensiling (Figure 2). Silage pH was lowest for FC, intermediate for FDL and FDH, and highest for the control at 6, 12, and 24 h. Applying freshly cultured microbial inoculants to forage at the time of ensiling has been shown to improve the rate of pH decline during fermentation of grass silage when compared with addition of the inoculant as a freeze-dried formulation (Merry et al., 1995a). Merry et al. (1995a) suggested that the reason for this finding was probably because freeze-dried cultures require a longer period of activation compared with a fresh culture.

Numbers of enterobacteria during the first 24 h of ensiling are shown in Figure 3. The relatively high numbers of these organisms on the plant agreed with previous findings reported by Lin et al. (1992). Treatment with FC decreased their numbers relative to other treatments at 12 h, and treatment with FC and FDH decreased their numbers at 24 h. Previous studies have also shown that populations of enterobacteria declined faster in silages treated with inoculants because these microbes are sensitive to a low pH (Kung et al., 1991; Winters et al., 1998).

The microbial populations and chemical composition of alfalfa after 6 h of ensiling are shown in Table 2. The numbers of LAB increased rapidly for all treatments

Table 1. Microbial populations (fresh matter basis), DM, and chemical composition (DM basis) of chopped alfalfa samples taken immediately after treatment but before ensiling¹

Item	Control	FDL	FDH	FC	SEM
Lactic acid bacteria, log cfu/g	5.30	5.00	5.44	5.37	0.12
Yeasts, log cfu/g	<2.00	<2.00	<2.00	<2.00	0.38
Molds, log cfu/g	2.91	<2.00	3.28	<2.00	0.66
DM, %	31.23	32.02	31.90	31.59	0.38
Water-soluble carbohydrates, %	4.83	3.68	4.33	4.77	0.39
Total N, %	2.99	2.99	2.94	2.85	0.06
NH ₃ -N, %	0.03	0.03	0.03	0.03	0.00
ADF, %	43.75	43.96	44.09	42.63	0.57
NDF, %	45.95 ^b	51.41 ^a	52.37 ^a	47.78 ^b	0.76

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

¹Control = untreated alfalfa; FDL = alfalfa treated with a low concentration of a freeze-dried inoculant diluted in water prior to application; FDH = alfalfa treated with a high concentration of a freeze-dried inoculant diluted in water prior to application; FC = alfalfa treated with an inoculant that had been grown in an overnight culture and diluted in water prior to application. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

(>8.5 log cfu/g) but were greatest for FC. Yeasts and molds were not detected at the lowest dilution that we plated. The concentrations of lactic acid were inversely related to silage pH, being lowest for the control, intermediate for FDL and FDH, and greatest for FC. Concentrations of acetic acid, ethanol, WSC, and NH₃-N were not different among treatments at this time.

The numbers of LAB increased further (>9 log cfu/g) after 12 h of ensiling and were higher in FC than in the control (Table 3). Yeasts and molds were not detected at the lowest dilution that we plated. The concentration of lactic acid was lowest and similar for the control and FDL, intermediate for FDH, and greatest for FC. Acetic

acid did not differ among the control, FDL, and FDH treatments (average 0.70%) but was lower in FC. Ethanol was greatest in the control than in the other treatments. The concentrations of residual WSC were lowest for FC, intermediate for FDH, and highest in the control and FDL treatments. Concentrations of NH₃-N were similar among treatments.

After 24 h of ensiling, the numbers of LAB were similar for the control, FDL, and FDH treatments (Table 4). However, the numbers of LAB were greater in FC than in the control and FDL treatments. Yeasts and molds were not detected at the lowest dilution plated. Treatment with FDH resulted in a higher concentration of lactic acid than in the control. When compared with

Figure 2. Effect of microbial inoculation on the pH of alfalfa during the first 24 h of ensiling. (●) Untreated silage; (□) silage treated with a normal level of a freeze-dried inoculant mixed in water; (■) silage treated with a high level of a freeze-dried inoculant mixed in water; (▲) silage treated with a freshly cultured inoculant. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

Figure 3. Effect of microbial inoculation on the numbers of enterobacteria in alfalfa during the first 24 h of ensiling. (●) untreated silage; (□) silage treated with a normal level of a freeze-dried inoculant mixed in water; (■) silage treated with a high level of a freeze-dried inoculant mixed in water; (▲) silage treated with a freshly cultured inoculant. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

Table 2. Microbial populations (fresh matter basis), DM, and chemical composition (DM basis) of chopped alfalfa silages after 6 h of ensiling¹

Item	Control	FDL	FDH	FC	SEM
Lactic acid bacteria, log cfu/g	8.62 ^b	8.67 ^b	8.65 ^b	8.91 ^a	0.03
Yeasts, log cfu/g	<2.00	<2.00	<2.00	<2.00	—
Molds, log cfu/g	<2.00	<2.00	<2.00	<2.00	—
DM, %	32.07 ^b	33.10 ^a	32.43 ^b	31.66 ^b	0.16
Lactic acid, %	0.32 ^c	0.51 ^b	0.67 ^b	1.41 ^a	0.07
Acetic acid, %	0.38	0.41	0.43	0.37	0.02
Ethanol, %	0.19	0.15	0.00	0.10	0.08
Water-soluble carbohydrates, %	5.38	5.28	4.59	4.39	0.50
NH ₃ -N, %	0.06	0.06	0.07	0.06	0.00

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

¹Control = untreated alfalfa; FDL = alfalfa treated with a normal concentration of a freeze-dried inoculant diluted in water prior to application; FDH = alfalfa treated with a high concentration of a freeze-dried inoculant diluted in water prior to application; FC = alfalfa treated with an inoculant that had been grown in an overnight culture and diluted in water prior to application. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

the control, inoculation with FC and FDL did not affect the concentration of lactic acid. Acetic acid was greater in the control than in FC. Ethanol was highest in the control and was lower in inoculated silages (average of 0.09%). Water-soluble carbohydrates ranged from 0.89% for FC to a high of 1.85% in the control. Silage treated with FC had a lower concentration of NH₃-N than silage with the other treatments.

The microbial populations and chemical composition of alfalfa after 60 d of ensiling are shown in Table 5. After 60 d of ensiling, the number of LAB had declined markedly compared with the early sampling times. In contrast to earlier sampling points, FC had the lowest numbers of LAB (5.80 log cfu/g) compared with the other treatments. Yeasts were present and were greater in inoculated silages (average of 2.56 log cfu/g) compared with the control (<2.0 log cfu/g). Molds were not different among treatments. The final silage pH was

lowest for FC, intermediate for FDL and FDH, and highest for the control. Lactic acid was not different among treatments, but silages treated with FDL, FDH, and FC had lower concentrations of acetic acid than the control. The concentration of ethanol was highest in the control, lower in FDL and FDH, and undetectable in FC. In contrast to early time points, the concentration of WSC was lowest for the control and greatest for FC. The concentration of NH₃-N was highest for the control, intermediate for FDL and FDH, and lowest for FC. Lower levels of NH₃-N have been reported in other silages treated with homolactic inoculants and may be attributed to the fact that pH declines faster and reaches a lower level, resulting in less plant proteolysis and inhibition of proteolytic bacteria (e.g., enterobacteria; Whiter and Kung, 2001). Addition of FC specifically resulted in a 61% reduction in NH₃-N compared with the control, which is quite substantial. The concen-

Table 3. Microbial populations (fresh matter basis), DM, and chemical composition (DM basis) of chopped alfalfa silages after 12 h of ensiling¹

Item	Control	FDL	FDH	FC	SEM
Lactic acid bacteria, log cfu/g	9.05 ^b	9.21 ^b	9.27 ^{ab}	9.50 ^a	0.08
Yeasts, log cfu/g	<2.00	<2.00	<2.00	<2.00	—
Molds, log cfu/g	<2.00	<2.00	<2.00	<2.00	—
DM, %	31.65 ^b	32.66 ^a	32.33 ^a	31.62 ^b	0.16
Lactic acid, %	0.92 ^c	1.19 ^c	1.75 ^b	3.16 ^a	0.10
Acetic acid, %	0.73 ^a	0.65 ^a	0.71 ^a	0.51 ^b	0.04
Ethanol, %	0.42 ^a	0.18 ^{ab}	0.00 ^b	0.11 ^b	0.08
Water-soluble carbohydrates, %	3.96 ^a	3.30 ^a	2.53 ^b	1.51 ^c	0.23
NH ₃ -N, %	0.08	0.08	0.08	0.07	0.00

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

¹Control = untreated alfalfa; FDL = alfalfa treated with a low concentration of a freeze-dried inoculant diluted in water prior to application; FDH = alfalfa treated with a high concentration of a freeze-dried inoculant diluted in water prior to application; FC = alfalfa treated with an inoculant that had been grown in an overnight culture and diluted in water prior to application. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

Table 4. Microbial populations (fresh matter basis), DM, and chemical composition (DM basis) of chopped alfalfa silages after 24 h of ensiling¹

Item	Control	FDL	FDH	FC	SEM
Lactic acid bacteria, log cfu/g	9.02 ^b	9.16 ^b	9.22 ^{ab}	9.42 ^a	0.07
Yeasts, log cfu/g	<2.00	<2.00	<2.00	<2.00	—
Molds, log cfu/g	<2.00	<2.00	<2.00	<2.00	—
DM, %	31.86 ^b	32.96 ^a	31.97 ^b	31.82 ^b	0.20
Lactic acid, %	1.68 ^b	2.58 ^{ab}	3.42 ^a	3.35 ^{ab}	0.32
Acetic acid, %	1.05 ^a	0.80 ^{ab}	0.78 ^{ab}	0.53 ^b	0.10
Ethanol, %	0.61 ^a	0.13 ^b	0.00 ^b	0.13 ^b	0.10
Water-soluble carbohydrates, %	1.85 ^a	1.46 ^{ab}	1.69 ^a	0.89 ^b	0.22
NH ₃ -N, %	0.11 ^a	0.10 ^{ab}	0.10 ^a	0.08 ^b	0.01

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

¹Control = untreated alfalfa; FDL = alfalfa treated with a low concentration of a freeze-dried inoculant diluted in water prior to application; FDH = alfalfa treated with a high concentration of a freeze-dried inoculant diluted in water prior to application; FC = alfalfa treated with an inoculant that had been grown in an overnight culture and diluted in water prior to application. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

trations of ADF were different among some of the treatments, but these differences were small and may also have been due to sampling variation. The concentrations of total N and NDF and the digestibility of NDF were not different among treatments. Dry matter recovery was greater for FC and FDH than for the control and FDL, but the overall recovery was very high, most likely because the silage was made under excellent conditions.

Of the variables that can affect the efficacy of an inoculant in silage, reactivation of the microbes can be an important factor. For example, Whiter and Kung

(2001) reported that when added to alfalfa with a high moisture content (30%), a dry and a liquid form of silage inoculant stimulated silage fermentation to an equal extent. However, for alfalfa with a higher DM content (54%), the inoculant dispersed in water resulted in a faster drop in silage pH compared with the same inoculant applied in a dry form. Similarly, Pahlow and Weissbach (1999) reported that the addition of an inoculant in water was more effective than a dry application of the same inoculant in lowering the pH of wilted (45% DM) grass silage. Low levels of metabolic water in dry forages probably slowed the reactivation of added bacte-

Table 5. Microbial populations (fresh matter basis), DM, and chemical composition (DM basis) of chopped alfalfa silages after 60 d of ensiling¹

Item	Control	FDL	FDH	FC	SEM
Lactic acid bacteria, log cfu/g	6.67 ^a	6.39 ^{ab}	6.25 ^b	5.80 ^c	0.11
Yeasts, log cfu/g	<2.00 ^b	2.66 ^a	2.66 ^a	2.35 ^a	0.34
Molds, log cfu/g	3.28	<2.00	<2.00	<2.00	0.67
DM, %	30.87	31.86	32.14	31.45	0.36
pH	4.96 ^a	4.47 ^b	4.31 ^c	4.18 ^d	0.03
Lactic acid, %	4.80	5.03	5.09	4.95	0.13
Acetic acid, %	1.92 ^a	1.39 ^b	1.04 ^{bc}	0.72 ^c	0.13
Ethanol, %	0.93 ^a	0.33 ^b	0.07 ^{bc}	0.00 ^c	0.08
Water-soluble carbohydrates, %	0.67 ^c	0.98 ^{bc}	1.23 ^{ab}	1.45 ^a	0.13
Total N, %	3.10	3.11	3.10	3.13	0.02
NH ₃ -N, %	0.28 ^a	0.19 ^b	0.16 ^b	0.11 ^c	0.01
ADF, %	44.86 ^a	43.96 ^{bc}	43.20 ^c	44.22 ^{ab}	0.28
NDF, %	50.94	50.26	51.22	52.56	1.05
NDF digestibility, ² %	47.94	47.05	48.99	50.17	1.15
DM recovery, %	98.56 ^c	98.86 ^b	98.97 ^a	99.06 ^a	0.04

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

¹Control = untreated alfalfa; FDL = alfalfa treated with a low concentration of a freeze-dried inoculant diluted in water prior to application; FDH = alfalfa treated with a high concentration of a freeze-dried inoculant diluted in water prior to application; FC = alfalfa treated with an inoculant that had been grown in an overnight culture and diluted in water prior to application. The inoculant contained a mixture of *Lactobacillus lactis*, *Lactobacillus plantarum* L-54, and *L. plantarum* Aber F1 (Powerstart, Genus plc, Cheshire, UK, distributed by ABS Global, DeForest, WI).

²In vitro digestion for 30 h.

ria. In the current study, the forage used was relatively high in moisture (approximately 31% DM) and all inoculants were added in liquid form. Thus, moisture should not have been a limiting factor affecting the inoculants. However, we observed that addition of the freshly cultured inoculant resulted in a faster drop in pH and a greater extent of fermentation when compared with addition of the freeze-dried culture mixed in water. In addition, the final fermentation profiles of inoculated silages were typical of a more homolactic acid fermentation (higher lactic acid and lower acetic acid, ethanol, and $\text{NH}_3\text{-N}$), similar to that reported in other studies in which alfalfa was treated with homolactic acid bacteria (Kung et al., 1991; Whiter and Kung, 2001), but the effect was greatest when using the freshly cultured inoculant. Similar results were reported by Merry et al. (1995a), in which an application of a fresh culture of a silage inoculant resulted in a faster drop in pH in ryegrass compared with the same inoculant that was a freeze-dried preparation. Thus, the metabolic state of the microbial inoculants that are applied to forages can also affect their efficacy.

The selected microbes and the specific crop to which they are added can also affect the outcome of silage fermentation. The inoculant we used had a specific strain of bacteria (*L. plantarum* Aber F1) that was selected for its ability to utilize fructans, which are found in relatively high concentrations in grasses (Merry et al., 1995b; Winters et al. 1998) but are low or absent in legumes. Johnson et al. (2005) reported a stimulation of silage fermentation when these bacteria (although not designed specifically for use with legumes) were applied to red clover forage (a legume), and the inoculant has been used in other experiments with red clover (Merry et al., 2006). In contrast, Speijers et al. (2002) reported that addition of a freeze-dried culture of the inoculant used in our study did not improve the fermentation of red clover or alfalfa silages. However, the results from the current study showed that the inoculant was effective in alfalfa, even when the initial load of epiphytic LAB was relatively high (5.3 log cfu/g for the control at d 0).

CONCLUSIONS

Alfalfa is a commonly grown forage that is ensiled and fed to dairy cows. However, it is a difficult crop to ensile because of its high buffering capacity. This study showed that a commercial inoculant designed primarily to improve the fermentation of grasses could also be used to improve the fermentation of alfalfa. Use of the inoculant at a normal and high rate of application in a freeze-dried form or use of the freshly cultured inoculant at a high rate of application improved silage fer-

mentation. The high rate of inoculant application in the freeze-dried form was better than the normal application rate, but inoculant application as a fresh culture was most efficacious, probably because the bacteria were in a more active state when applied to the silage. Although some concern about preparing a fresh culture and its stability (if not used immediately) has often been expressed in the field, our experience was that the culture was easy to grow and that it was stable for several days at room temperature.

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