Effect of *Lactobacillus* inoculants and forage dry matter on the fermentation and aerobic stability of ensiled mixed-crop tall fescue and meadow fescue

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ABSTRACT

This study evaluated the effects of *Lactobacillus plantarum* with or without *Lactobacillus buchneri* on the fermentation and aerobic stability of mixed tall fescue (*Festuca arundinacea* Schreb) and meadow fescue (*Festuca pratensis* Huds.) silage ensiled at different dry matter (DM) contents. The first cut was harvested at boot stage and second-cut grasses were harvested when 30- to 35-cm tall. Four DM content treatments of the first cut were 17.9, 24.9, 34.6, and 48.7%; and of the second cut were 29.1, 36.3, 44.1, and 49.2%. Chopped grasses at each DM content were treated with (1) deionized water (control), (2) *Lb. plantarum* MTD-1 (LP), or (3) a combination of *Lb. plantarum* MTD-1 and *Lb. buchneri* 40788 (LP+LB). The application amount of each inoculant to the fresh forage was 1×10^6 cfu/g. Grasses were ensiled in vacuum-sealed polyethylene bags containing 150 g of DM for 60 d, with 4 replicates for each treatment. Silages inoculated with LP+LB had greater pH compared with untreated or LP-treated silages. Lactate was greater in LP silage than control or LP+LB silages. As silage DM increased, lactate in untreated and LP-treated silages decreased, but increased in LP+LB-treated silage. Acetate concentration decreased with increased DM in all silages. The LP+LB-treated silage had the longest and control silage the shortest aerobic stability for both harvests. The greatest values in aerobic stability were observed in silages with highest DM content. In this study, aerobic stability of grass mixes ensiled between 18 and 44% DM content increased as the percentage of DM increased. The LP and LP+LB inoculants improved aerobic stability of silages harvested between 18 and 44% DM content.

Key words: grass silage, *Lactobacillus*, fermentation quality, aerobic stability

INTRODUCTION

Forage DM concentration at ensiling has substantial effects on silage fermentation, feed intake, and performance. An extensive investigation regarding effects of DM content and silage additives on the fermentation of bunker-made ryegrass silage suggested that increasing DM content from 18 to 30% without additive had beneficial influences on fermentation (Haigh et al., 1996). Feeding trials indicated that grass silages with high (44.9%) DM concentration improved silage DMI of dairy cows (Romney et al., 2000) and milk yield of dairy cows when compared with the lower-DM alfalfa silage (Campbell and Buchanan-Smith, 1991). However, fermentation of grass silages with DM content above 40% and without addition of inoculants often results in ethanol production and high DM losses after ensiling (Xiccato et al., 1994).

To improve fermentation quality and to decrease DM losses during ensiling, homofermentative lactic acid bacteria such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* spp. are often used to promote adequate production of lactic acid and decrease in pH. However, these inoculants did not improve (and in some cases worsened) the aerobic stability of cereal grain silages (Weinberg et al., 1993). Combination of *Lb. plantarum* and *Lactobacillus buchneri* has been shown to improve fermentation and aerobic stability of cereal grain silages (Filya, 2003b) and grass silages (Adesogan et al., 2004). Hu et al. (2009) showed that inoculation of *Lb. plantarum* and *Lb. buchneri* had different effects on the fermentation and aerobic stability of corn silage made at normal (33%) and moderately (41%) high DM content. Therefore, we proposed that *Lb. plantarum* and *Lb. buchneri* might provide differing benefits at different moisture levels for the fermentation and aerobic stability of ensiled grasses. Few studies have been conducted to investigate the effect of *Lb. plantarum* and *Lb. buchneri* on the fermentation and aerobic stability of grass silage when ensiled at different DM contents.

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Tall fescue (*Festuca arundinacea* Schreb) and meadow fescue (*Festuca pratensis* Huds.) are widely grown cool-season pasture and cultivated grasses in the Midwestern United States and southern Canada. Recently developed endophyte-free tall fescue and meadow fescue varieties possess several desirable agronomic traits, such as high yield, drought and disease tolerance, good compatibility when grown with alfalfa, winter hardiness, and persistency. Newer fescue cultivars also have soft texture, high nutritive value, and improved palatability compared with older varieties (Brink and Casler, 2009). Most grass silage fermentation information is on ryegrass (*Lolium perenne* L.) silages, with little research on fermentation of tall fescue and meadow fescue available (Kuoppala et al., 2008). Thus, the objective of current study was to investigate the contribution of *Lb. plantarum* alone or in combination with *Lb. buchneri* on the fermentation and aerobic stability of mixed-crop tall fescue and meadow fescue silages ensiled at different DM contents.

MATERIALS AND METHODS

Forage and Ensiling

Mix-cropped tall fescue and meadow fescue forages were harvested on June 1, 2011, at the boot stage as first-cut forage, and on August 18, 2011, at the vegetative stage (30- to 35-cm tall) as second-cut forage from the University of Wisconsin Arlington Research Station. The stand was about 60% tall fescue and 40% meadow fescue. After harvesting with a commercial mower/conditioner, the fresh forage was divided into 4 piles, and each pile placed on a wooden drying frame with a screen bottom. Forage on each frame was exposed to ambient temperature and sunlight and weighed from time to time to estimate the forage DM content. Our goal was to wilt subsamples of the forage to 4 moisture levels; fresh cut (approximately 20% DM), 30% DM, 40% DM, and 50% DM. The 4 DM content treatments obtained were 17.9 (directly harvested fresh forage), 24.9, 34.6, and 48.7% for the first-cut forage. For the second-cut forage, the DM contents at ensiling were 29.1 (directly harvested fresh forage), 36.3, 44.1, and 49.2%.

The fresh and wilted forages were chopped into 1.5-to-2 cm pieces by using a paper cutter. Chopped forages of each DM treatment were then assigned to one of the following treatments: (1) untreated (deionized water), (2) *Lb. plantarum* MTD-1 (**LP**; Vita Plus Corp., Madison, WI), or (3) a combination of *Lb. plantarum* MTD-1 with *Lb. buchneri* 40788 (**LP+LB**; Vita Plus Corp.). The application rate of each inoculant to the fresh forage was 1×10^6 cfu/g. The same application

rates were applied according to the DM content of the fresh and wilted forages. All inoculants were dissolved in 500 mL of deionized water, and mixed thoroughly with the forages after uniform spraying onto each pile of the chopped forages. The treated forages were packed into vacuum-sealing polyethylene plastic bags (dimensions 270 mm $\times$ 300 mm; Embossed Food saver bag; Taizhou Wenbwu Soft-Packing Color-Printing Co. Ltd., Zhejiang, China) with 2 layers and vacuum-sealed tightly. An attempt was made to conserve approximately 150 g of forage DM for each treatment, so for making silage from the first-cut forage, 4 replicate bags were packed with 800 g of fresh forage or 580, 430, or 300 g of wilted forages for the 24.9, 34.6, and 48.7% DM treatments, respectively; and for making second-cut forage silages, 4 replicate bags were packed with 500 g of fresh forage or 440, 340, or 300 g of wilted forages for the 36.3, 44.1, and 49.2% DM treatments, respectively; The silos were then stored in closed coolers (dimensions 649 $\times$ 357 $\times$ 35.9 mm; Igloo Island Breeze 48-quart cooler; Igloo Products Corp., Katy, TX) for 60 d at ambient temperature (20 to 22°C).

Chemical Analysis

Samples of the untreated fresh and wilted forages of each DM treatment were collected before ensiling. DM content was measured by drying the samples in a forced-air oven at 65°C for 72 h. Samples were then ground with a Wiley mill (1-mm screen; Thomas Scientific, Swedesboro, NJ). Ground samples were analyzed for Kjeldahl N (AOAC, 1990; method 954.01). Crude protein content was calculated as Kjeldahl N $\times$ 6.25. Neutral detergent fiber and acid detergent fiber concentration was determined according to the methods of Van Soest et al. (1991) using an Ankom 200 fiber analyzer (Ankom Technology Corp., Fairport, NY). Heat-stable α -amylase and sodium sulfite were added to the NDF solution during refluxing.

After 60 d of ensiling, sealed bags were opened and thoroughly mixed. Dry matter recovery of the second-cut forage silages was calculated according to weight differences between the silos and DM concentrations of the fresh and ensiled material. Dry matter recovery was not calculated for the first-cut material because fresh weights were not recorded. Subsamples of the 4 replicate silos for each treatment were immediately frozen (-20°C) in sealed plastic bags until further chemical analysis. The DM, NDF, and total N were analyzed by the methods described for the fresh and wilted forages. A 25-g sample from each silo was placed in a blender jar, diluted with distilled water to 250 g, and macerated for 30 s in a high-speed blender, and then filtered through 2 layers of cheesecloth. Silage water extract pH

was measured immediately using a portable pH meter. The filtrate was centrifuged for 15 min at $10,000 \times g$ at 4°C , filtered with a dialyzer ($0.45 \mu\text{m}$), and then analyzed for VFA and lactic acid by HPLC using a Shimadzu SCL-10 AVP controller (Shimadzu Scientific Instruments, Columbia, MD) equipped with a refractive index detector (RID-10A; Dairyland Laboratories Inc., Arcadia, WI) according to Muck and Dickerson (1988). An aliquot of 10 mL (250 g/L , wt/vol) TCA was added to 40 mL of the filtrate and the solution was allowed to stand at room temperature for 1 h or was held at 4°C overnight to precipitate the protein. The solution was then centrifuged at $18,000 \times g$ for 15 min and the supernatant was analyzed for $\text{NH}_3\text{-N}$ (Broderick and Kang, 1980). Nonfiber carbohydrate concentration in the fresh forage and silage was estimated as described by Hall (2000).

Aerobic Stability Measurement

After sampling the silages from each bag for fermentative and chemical analysis, silages from 4 replicates of each treatment were mixed thoroughly and then triplicate silage samples (350 g for each sample) were incubated in insulated mugs (1.54 L ; Bubba Brands Inc., Atlanta, GA) at room temperature ($24 \pm 1^{\circ}\text{C}$). A thermocouple wire was placed in the geometric center of the silage mass, and connected to a data logger for measuring the temperature. The mug was kept uncovered and temperature of the silage mass was recorded every 8 h each day. Aerobic stability was defined as the amount of time after silo opening for the silage temperature to increase by 2°C above ambient temperature.

Statistical Analysis

Data were analyzed by using the GLM procedure of SAS (SAS Institute, 2004) according to a randomized complete block design, and the statistical model was

$$Y_{ijk} = \mu + \alpha_i + b_j + (\alpha \times b)_{ij} + e_{ijk},$$

where Y_{ijk} is the chemical composition and fermentative characteristics of the ensiled forages, μ is the overall mean, α_i is the effect of forage DM content i ($i = 1, 2, 3$, or 4), b_j is the effect of inoculation treatments j ($j = 1, 2$, or 3); $(\alpha \times b)_{ij}$ is the effect of interaction between forage DM content and inoculation treatment, and e_{ijk} is the error term. Contrasts of means were constructed to evaluate the effects of treatments within the DM contents by the Tukey test (Snedecor and Cochran, 1980), and significance was declared at $P < 0.05$. Parameters of the fermentation profiles (pH, lactate, acetate, and $\text{NH}_3\text{-N}$) from the first-cut and second-cut forage silages

Table 1. Dry matter content and chemical composition of the first- and second-cut tall fescue and meadow fescue mixed crops at different DM contents before ensiling

Item	First-cut forage	Second-cut forage	SEM
DM ¹ (%)	—	—	—
CP (% of DM)	20.6 ^a	13.9 ^b	1.32
NDF (% of DM)	54.3 ^a	59.5 ^b	1.22
NFC (% of DM)	13.9	13.7	0.46

^{a,b}Means within a row with different superscripts differed ($P < 0.05$).

¹DM contents for the first-cut forage at ensiling were 17.9, 24.9, 34.6, and 48.7%; for the second-cut forage, DM contents were 29.1, 36.3, 44.1, and 49.2%.

were plotted when inoculation $\times$ DM interactions were significant ($P < 0.05$) to aid interpretation of results.

RESULTS

Fresh Intercrops of Tall Fescue and Meadow Fescue

The chemical composition of first-cut and second-cut grasses before ensiling is shown in Table 1. The first-cut grass had lower NDF and higher CP content than the second-cut grass ($P < 0.05$), but no difference existed in NFC concentration between the first- and second-cut grasses ($P > 0.05$).

Chemical Composition of Mix-Crop Tall Fescue and Meadow Fescue Silages Ensiled for 60 d

The main effects of inoculation and DM on the chemical composition of mix-crop tall fescue and meadow fescue silages after 60 d of fermentation are shown in Tables 2 and 3 for the first-cut grass silages and the second-cut grass silages, respectively. The first-cut grass silages treated with LP+LB had greater NDF and lower NFC than untreated silages and silages treated with LP ($P < 0.05$). For the second-cut grass silages, however, no effects of inoculation on these 2 variables were observed. Dry matter content had little effect on NDF concentration in the first-cut grass silages, and increased DM content increased the CP concentration but decreased the NFC concentration. In the second-cut grass silages, the lowest values in NDF and NFC concentrations were found in silages with the lowest DM content, and no differences were observed in these 2 variables among the other DM contents. Inoculation with LP or LP+LB decreased DM recovery of silages compared with untreated silages in the second-cut grass silages, and silages inoculated with LP+LB had the lowest DM recovery ($P < 0.05$).

Fermentation Characteristics of Mix-Crop Tall Fescue and Meadow Fescue Silages Ensiled for 60 d

Inoculation had effects on the fermentation profiles of the first-cut (Table 4) and the second-cut grass si-

Table 2. Chemical composition of the first-cut grass silages ensiled at different DM contents for 60 d and inoculated with *Lactobacillus plantarum* or with *Lb. plantarum* plus *Lactobacillus buchneri*

Item	Inoculation treatment (I) ¹				DM content at ensiling					ANOVA (<i>P</i> -value)		
	Control	LP	LP+LB	SEM	18%	25%	35%	49%	SEM	I	DM	I × DM
DM (%)	30.8	31.0	31.1	0.23	17.9 ^A	26.4 ^B	34.8 ^C	44.7 ^D	0.27	0.72	<0.001	<0.001
CP (% of DM)	19.7	19.8	19.6	0.09	18.7 ^A	19.8 ^B	20.1 ^C	20.2 ^C	0.11	0.57	<0.001	0.033
NDF (% of DM)	46.6 ^a	47.0 ^a	48.1 ^b	0.20	47.2 ^{AB}	46.6 ^A	47.7 ^B	47.4 ^B	0.24	<0.001	0.015	<0.001
NFC (% of DM)	22.5 ^b	22.0 ^b	21.1 ^a	0.17	22.8 ^B	22.4 ^B	21.0 ^A	21.2 ^A	0.20	<0.001	<0.001	<0.001

^{A-D}Means of DM content at ensiling within a row with different superscripts differ ($P < 0.05$).

^{a,b}Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).

¹LP = *Lb. plantarum* MTD-1; LP+LB = LP plus *Lb. buchneri* 40788.

lages (Table 5). For the first-cut grass silages, silage inoculated with LP had lower pH than untreated silage and silage treated with LP+LB ($P < 0.05$). The inoculated silages had greater concentrations of $\text{NH}_3\text{-N}$ compared with untreated silage ($P < 0.05$), and the highest concentration of $\text{NH}_3\text{-N}$ was found in LP+LB-treated silage ($P < 0.05$). For the second-cut silages, however, grass treated with LP had lower $\text{NH}_3\text{-N}$ concentration than untreated or LP+LB-treated silages ($P < 0.05$), and no difference existed in $\text{NH}_3\text{-N}$ concentration between untreated silage and silage treated with LP+LB. Silages made from the first-cut and second-cut grasses and inoculated with LP had greater concentrations of lactate and total acid than silage treated with LP+LB and untreated silage in the first-cut silage ($P < 0.05$). The lowest concentration of lactate and the greatest concentration of acetate occurred in LP+LB-treated silages ($P < 0.05$). The total acid concentration in LP-treated silage was greater than that in untreated silage in the first-cut grass silages ($P < 0.05$), but no difference was found in the second-cut grass silages, and the lowest total acid concentrations occurred both in the first-cut and second-cut grass silages ($P < 0.05$). Concentrations of propionate, butyrate, and ethanol in all samples were less than 0.01% DM (data not shown). Inoculation markedly increased aerobic stability of the ensiled mixed crops, and the silage inoculated with

LP+LB had the longest aerobic stability ($P < 0.05$) among the untreated silage and silages treated with LP and LP+LB.

Dry matter contents had different effects on pH values of the first-cut and second-cut grass silages. With an increase in DM content, the pH value increased in the first-cut silages (Table 4). A decrease was, however, observed in pH with increasing DM content in the second-cut grass silages (Table 5 and Figure 1). Concentrations of $\text{NH}_3\text{-N}$ and acetate in silages made from both harvests decreased as the DM increased (Tables 4 and 5; Figures 2 and 3). Acetate and $\text{NH}_3\text{-N}$ were markedly lower in silages with the highest DM content compared with the unwilted silage ($P < 0.05$). Lactate concentration in the first-cut grass silages decreased with the increase in DM content, but no difference was found in lactate concentration among DM contents of the second-cut grass silages. However, plotting figures on interactions between lactate concentrations and DM contents showed that with the increase in DM content, lactate in untreated and LP-treated silages decreased, but increased in LP+LB-treated silage (Figure 4).

Inoculants markedly improved the aerobic stability of ensiled grasses compared with the untreated silage, and the greatest value of aerobic stability was found in LP+LB-treated silage ($P < 0.05$). Interactions were observed between inoculation and forage DM contents

Table 3. Chemical composition of the second-cut grass silages ensiled at different DM contents for 60 d and inoculated with *Lactobacillus plantarum* or with *Lb. plantarum* plus *Lactobacillus buchneri*

Item	Inoculation treatment (I) ²				DM content at ensiling					ANOVA (<i>P</i> -value)		
	Control	LP	LP+LB	SEM	29%	36%	44%	49%	SEM	I	DM	I × DM
DM (%)	38.4	38.3	37.9	0.296	26.8 ^A	35.0 ^B	42.5 ^C	48.5 ^D	0.276	0.48	<0.001	0.001
DM recovery (%)	98.3 ^c	98.0 ^b	97.7 ^a	0.07	98.3 ^B	97.9 ^A	98.2 ^B	97.9 ^A	0.08	<0.001	<0.001	0.001
CP (% of DM)	14.9 ^b	14.6 ^a	14.7 ^{ab}	0.056	14.3 ^A	15.1 ^B	14.6 ^A	15.0 ^B	0.065	0.008	<0.001	0.001
NDF (% of DM)	57.9	57.8	58.2	0.25	59.2 ^B	57.4 ^A	57.8 ^A	57.4 ^A	0.29	0.51	<0.001	0.088
NFC (% of DM)	14.3	14.6	14.1	0.22	13.5 ^A	14.5 ^B	14.7 ^B	14.7 ^B	0.25	0.32	0.004	0.075

^{A-D}Means of DM content at ensiling within a row with different superscripts differ ($P < 0.05$).

^{a-c}Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).

²LP = *Lb. plantarum* MTD-1; LP+LB = LP plus *Lb. buchneri* 40788.

Table 4. Fermentation profiles of the first-cut grass silages ensiled at different DM contents for 60 d and inoculated with *Lactobacillus plantarum* or with *Lb. plantarum* plus *Lactobacillus buchneri*¹

Item	Inoculation treatment (I) ²			SEM	DM content at ensiling				SEM	ANOVA (<i>P</i> -value)		
	Control	LP	LP+LB		18%	25%	35%	49%		I	DM	I × DM
pH	5.04 ^b	4.86 ^a	5.07 ^b	0.019	4.83 ^A	4.93 ^B	5.09 ^C	5.10 ^C	0.022	<0.001	<0.001	<0.001
NH ₃ -N (% of total N)	18.4 ^a	22.6 ^b	25.8 ^c	0.52	24.2 ^B	24.7 ^B	23.1 ^B	17.1 ^A	0.60	<0.001	<0.001	<0.001
Lactate (% of DM)	3.75 ^b	5.25 ^c	2.84 ^a	0.155	5.59 ^C	4.25 ^B	2.89 ^A	3.05 ^A	0.179	<0.001	<0.001	<0.001
Acetate (% of DM)	1.89 ^a	2.35 ^b	3.32 ^c	0.085	4.59 ^D	2.54 ^C	1.73 ^B	1.23 ^A	0.098	<0.001	<0.001	<0.001
Total acid (% of DM)	5.64 ^a	7.60 ^c	6.43 ^b	0.221	10.57 ^C	6.79 ^B	4.62 ^A	4.27 ^A	0.256	<0.001	<0.001	<0.001
Lactate/total acid	66.9 ^b	69.4 ^c	49.4 ^a	0.65	51.9 ^A	61.9 ^B	62.3 ^B	71.5 ^C	0.75	<0.001	<0.001	<0.001
Aerobic stability ³ (h)	261 ^a	370 ^b	410 ^c	5.9	367 ^C	242 ^A	269 ^B	508 ^D	6.8	<0.001	<0.001	<0.001

^{a-c}Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).

^{A-D}Means of DM content at ensiling within a row with different superscripts differ ($P < 0.05$).

¹Concentrations of propionate, butyrate, and ethanol in all treatments were less than 0.01% of DM.

²LP = *Lb. plantarum* MTD-1; LP+LB = LP plus *Lb. buchneri* 40788.

³Defined as hours after opening for silage temperature to increase by 2°C above ambient temperature. Forages ensiled at 49% DM dried out before temperature of material increased 2°C; therefore, values reported for this treatment are time to increase 1°C above ambient temperature.

Table 5. Fermentation profiles of second-cut grass silages ensiled at different DM contents for 60 d and inoculated with *Lactobacillus plantarum* or with *Lb. plantarum* plus *Lactobacillus buchneri*¹

Item	Inoculation treatment (I) ²			SEM	DM content at ensiling				SEM	ANOVA (<i>P</i> -value)		
	Control	LP	LP+LB		29%	36%	44%	49%		I	DM	I × DM
pH	4.63 ^a	4.58 ^a	4.74 ^b	0.027	4.83 ^C	4.73 ^B	4.48 ^A	4.56 ^A	0.032	0.001	<0.001	0.002
NH ₃ -N (% of total N)	10.4 ^b	9.8 ^a	10.3 ^b	0.16	12.5 ^D	11.0 ^C	9.2 ^B	8.0 ^A	0.18	0.048	<0.001	<0.001
Lactate (% of DM)	5.04 ^b	5.13 ^b	3.62 ^a	0.190	4.72	4.55	4.58	4.53	0.220	<0.001	0.92	0.001
Acetate (% of DM)	1.29 ^b	0.89 ^a	1.41 ^c	0.060	1.57 ^B	1.66 ^B	0.86 ^A	0.71 ^A	0.069	<0.001	<0.001	0.001
Total acid (% of DM)	6.51 ^b	6.59 ^b	5.44 ^a	0.264	7.49 ^D	6.52 ^C	5.46 ^A	5.24 ^A	0.277	0.002	<0.001	0.26
Lactate/total acid	78.9 ^b	81.6 ^c	67.5 ^a	0.691	61.4 ^A	71.6 ^B	83.7 ^C	87.2 ^D	0.797	<0.001	<0.001	<0.001
Aerobic stability ³ (h)	417 ^a	434 ^b	587 ^c	2.2	518 ^C	480 ^B	397 ^A	523 ^C	2.5	<0.001	<0.001	<0.001

^{a-c}Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).

^{A-D}Means of DM content at ensiling within a row with different superscripts differ ($P < 0.05$).

¹Concentrations of propionate, butyrate, and ethanol in all treatments were less than 0.01% of DM.

²LP = *Lb. plantarum* MTD-1; LP+LB = LP plus *Lb. buchneri* 40788.

³Defined as hours after opening for silage temperature to increase by 2°C above ambient temperature. Forages ensiled at 49% DM dried out before temperature of material increased 2°C; therefore, values reported for this treatment are time to increase 1°C above ambient temperature.

Figure 1. Changes in pH with changes in DM content at ensiling of the first-cut (●; $n = 16$) and the second-cut (▲; $n = 16$) tall fescue and meadow fescue mixed-crop silages treated without inoculation (control; a), with *Lactobacillus plantarum* (b), or with *Lb. plantarum* and *Lactobacillus buchneri* (c). Color version available in the online PDF.

on aerobic stability of the ensiled mixed crops ($P < 0.001$). Aerobic stability of mixed grasses ensiled between 18 to 44% DM increased as the DM content increased (Tables 4 and 5). In both cuttings, the silages with the highest DM content dried out before reaching the predetermined 2°C rise above ambient temperature.

DISCUSSION

Different effects of inoculation or forage DM on concentrations of CP, NDF, and NFC occurred between

Figure 2. Changes in $\text{NH}_3\text{-N}$ concentration with changes in DM content at ensiling of first-cut (●; $n = 16$) and second-cut (▲; $n = 16$) tall fescue and meadow fescue mixed-crop silages treated without inoculation (control; a), with *Lactobacillus plantarum* (b), or with *Lb. plantarum* and *Lactobacillus buchneri* (c). Color version available in the online PDF.

the first-cut and the second-cut grass silages. Lower NFC concentration in the LP+LB-treated silage than in untreated or LP-treated first-cut silage was probably due to heterofermentative bacteria of *Lb. buchneri* consuming more nutrients for fermentation than homofermentative bacteria (Oude Elferink et al., 1999), which consequently caused a greater NDF concentration on a DM basis in the LP+LB-treated silage. Although the concentration of CP in the LP-treated silage made from second-cut grass was statistically lower than untreated silage, this difference was small and not of practical sig-

Figure 3. Changes in acetate concentration with changes in DM content at ensiling of first-cut (●; n = 16) and second-cut (▲; n = 16) tall fescue and meadow fescue mixed-crop silages treated without inoculation (control; a), with *Lactobacillus plantarum* (b), or with *Lb. plantarum* and *Lactobacillus buchneri* (c). Color version available in the online PDF.

nificance (14.9 vs. 14.6%). Effects were observed of forage DM on the CP and NDF concentrations in silages made from both harvests, but the difference were small.

The average concentration of NDF in the first-cut grass silage decreased about 7% after ensiling (54.3 vs. 47.2% DM), and the average concentration of NFC increased about 8% after ensiling (13.9 vs. 21.9% DM). However, concentrations of the 2 variables were comparable in the second-cut grass before and after ensiling (59.5 vs. 58.0% DM for NDF; 14.3 vs. 13.7%

Figure 4. Changes in lactate concentration with changes in DM content at ensiling of first-cut (●; n = 16) and second-cut (▲; n = 16) tall fescue and meadow fescue mixed-crop silages treated without inoculation (control; a), with *Lactobacillus plantarum* (b), or with *Lb. plantarum* and *Lactobacillus buchneri* (c). Color version available in the online PDF.

DM for NFC). These findings suggest that structural carbohydrates (i.e., hemicellulose) in the first-cut grass were more degradable than the second-cut grass and thereafter transformed into NFC after fermentation. Grasses grown in a cooler environment generally have lower NDF and less lignification than grasses grown under warm conditions (Chatterton et al., 1989; Xu et al., 2002). This might result in a higher concentration of water-soluble hemicellulose (Xu et al., 2007) after

ensiling for the first-cut forage. Therefore, although wilting grass before ensiling restricts the fermentation of ensiled forage (Dawson et al., 1999), higher concentration of NFC in the directly cut grass silage made from the first-cut grass might be as a result of accumulation of NFC after degradation of structural carbohydrates by extensive fermentation. Concentrations of NDF and NFC in the second-cut grasses, however, were comparable before and after ensiling, and much more fermentation in the directly cut grass silage resulted in lower NFC concentration compared with that in the wilted silages.

As expected, the application of *Lb. plantarum* to the grass silages resulted in a lower silage pH because homolactic acid bacteria often produce more lactic acid (Muck and Kung, 1997). The moderately higher pH in the grass silages treated with the combination of *Lb. plantarum* and *Lb. buchneri* was mainly due to the degradation of lactic acid into acetic acid by *Lb. buchneri* (Oude Elferink et al., 2001) and the addition of *Lb. buchneri* to silages has generally resulted in a moderately higher pH (Kleinschmit and Kung, 2006; Hu et al., 2009). Previous research showed that silage pH increased as the DM content increased (Xiccato et al., 1994). Nevertheless, our results showed that with the increase in DM content, silage pH increased in the first-cut grass silages, but decreased in the second-cut grass silages, regardless of the decreases in total organic acid concentrations in both harvest silages. Response of silage pH to the forage DM content could probably be codetermined by concentrations of $\text{NH}_3\text{-N}$ (DM basis) and lactate in the ensiled forage because the ratio of lactate to $\text{NH}_3\text{-N}$ decreased in the first-cut silages and increased in the second-cut silages with the increase in forage DM (Figure 5).

Most of the $\text{NH}_3\text{-N}$ in silage results from protein degradation. Extensive proteolysis causes less efficient use of silage N by ruminants (Van Vuuren et al., 1995). In agreement with a previous report on grass silage (Driehuis et al., 2001), addition of LP+LB increased the concentration of $\text{NH}_3\text{-N}$ in the first-cut grass silages, whereas the combination of *Lb. plantarum* and *Lb. buchneri* had no effect on silage $\text{NH}_3\text{-N}$ concentration in the second-cut silages as was reported in corn silages (Filya, 2003a; Hu et al., 2009). It was proposed that the higher concentration of $\text{NH}_3\text{-N}$ as a result of inoculation with *Lb. buchneri* might be associated with increased pH in *Lb. buchneri*-treated silage (Driehuis et al., 2001). Our results on effects of inoculants on silage pH and $\text{NH}_3\text{-N}$ concentration differed between the harvests of silages. The results might be attributed to the considerable difference in degrees of protein degradation that occurred in the first-cut and the second-cut grass silages as well as the difference in buffer capacity

of the 2 harvest silages. It has been generally accepted that proteolysis in the ensiled forage is decreased at lower pH (McKersie, 1985; Jones et al., 1995). For unknown reasons, in spite of the lower pH in the LP-treated silage made from the first-cut grass, the $\text{NH}_3\text{-N}$ concentration was greater compared with the untreated silage. However, $\text{NH}_3\text{-N}$ concentration was lower in the LP-treated silage than the untreated silage made from second-cut grass.

Forage DM of silage adversely affects the degree of protein degradation (Muck, 1987; Jones et al., 1992). Nevertheless, silages with high CP concentration might restrict the decreasing degree of protein degradation as forage DM increased. Unlike the second-cut grass silage, no difference in $\text{NH}_3\text{-N}$ concentrations was observed when silage DM increased from 18 to 35% in the first-cut silage with high CP concentration (20% DM).

Silages made from both harvests and treated with LP+LB had lower concentrations of lactate and higher concentrations of acetate than untreated silage or LP-treated silage. These findings are in agreement with those of Driehuis et al. (2001), Filya (2003a,b), and Hu et al. (2009) with corn, sorghum, and grass silages. Because of the restricted fermentation in wilted silages (Dawson et al., 1999), lactate concentrations in untreated and LP-treated silages and acetate concentrations in untreated and both of the inoculant-treated silages decreased with the increase in forage DM content. However, our results showed increases in lactate in the LP+LB-treated silage as the forage DM increased (Figure 3). The heterofermentative bacteria *Lb. buchneri* is known to convert lactate to acetate and 1,2-propanediol under anaerobic conditions (Driehuis et al., 1999; Oude Elferink et al., 2001). Therefore, the increment in lactate concentration with the increase in

Figure 5. Responses of the ratios of lactate concentration to $\text{NH}_3\text{-N}$ concentration (DM basis) to the DM content in silages made from the first-cut (●; n = 48) and second-cut (▲; n = 48) tall fescue and meadow fescue mixed crops. Color version available in the online PDF.

forage DM in the LP+LB-treated silage indicates that conversion of lactate by *Lb. buchneri* was restricted as forage DM increased.

Aerobic stability is of great importance because it is not only a potential cause of nutrient and DM losses, it also leads to health risks to animals and humans due to mycotoxins produced by undesirable microorganisms (Schmidt and Kung, 2010). Inoculation with *Lb. buchneri* has been reported to improve aerobic stability of ensiled forages (Muck, 1996). Although homolactic acid bacteria have sometimes led to poorer aerobic stability of silage (Weinberg et al., 1993; Filya 2003b), the results in the current study indicate that inoculation with *Lb. plantarum* alone or in combination with *Lb. buchneri* improved the aerobic stability of ensiled mixed-crop tall fescue and meadow fescue. An interaction between inoculation and forage DM on aerobic stability was detected in the current study. Based on the responses of aerobic stability to forage DM contents in the first-cut and second-cut grass silages, within the forage DM range of 18 to 44%, aerobic stability of the ensiled grasses decreased as the forage DM increased. Therefore, wilted grass silages were not as aerobically stable as directly cut grass silages.

CONCLUSIONS

Inoculation with LP decreased silage pH and increased lactate concentration in the ensiled mixed-crop tall fescue and meadow fescue. Differences in fermentation profiles due to the addition of LP between the primary growth and the regrowth silages were small. The combination of LP with LB increased silage pH and the concentration of acetate in the ensiled grasses. Both inoculants increased the aerobic stability of the ensiled grasses, and the longest aerobic stability was detected on silages treated with the inoculant of LP+LB. Interactions existed between inoculation and forage DM on the fermentation profiles and aerobic stability of the ensiled forages. Wilting grass at different DM content restricted silage fermentation and subsequently decreased fermentation products compared with the directly cut grass silages. However, with the increase in forage DM, lactate concentration in the LP+LB-treated silage increased. Aerobic stability of grass mixes ensiled between 18 and 44% DM increased as DM percentage increased, which suggests that directly cut grass silages were more aerobically stable than wilted grass silage, but LP and LP+LB inoculants improved aerobic stability of silages harvested between 18 and 44% DM.

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