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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 × 300 mm; Embossed Food saver bag; Taizou Wenbwu Soft-Packing Color-Printing Co. Ltd., Zhejiang, China) with 2 layers and vacuum-sealed tightly. An attempt was made to conserve approximately150 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 × 357 × 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 × 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