



## Effect of microbial inoculants on the quality and aerobic stability of bermudagrass round-bale haylage

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### ABSTRACT

The objective of this study was to compare the efficacy of using 4 commercially available microbial inoculants to improve the fermentation and aerobic stability of bermudagrass haylage. We hypothesized that the microbial inoculants would increase the fermentation and aerobic stability of the haylages. Bermudagrass (4-wk regrowth) was harvested and treated with (1) deionized water (control); (2) Buchneri 500 (B500; Lallemand Animal Nutrition, Milwaukee, WI) containing  $1 \times 10^5$  of *Pediococcus pentosaceus* and  $4 \times 10^5$  of *Lactobacillus buchneri* 40788; (3) Biotol Plus II (BP2; Lallemand Animal Nutrition) containing  $1.2 \times 10^5$  of *P. pentosaceus* and *Propionibacteria freudenreichii*; (4) Silage Inoculant II (SI; AgriKing Inc., Fulton, IL) containing  $1 \times 10^5$  of *Lactobacillus plantarum* and *P. pentosaceus*; and (5) Silo King (SK; AgriKing Inc.), containing  $1 \times 10^5$  of *L. plantarum*, *Enterococcus faecium*, and *P. pentosaceus*, respectively. Forty round bales (8 per treatment;  $441 \pm 26$  kg;  $1.2 \times 1.2$  m diameter) were made and each was wrapped with 7 layers of plastic. Twenty bales were stored for 112 d and the remaining 20 were stored for 30 d and sampled by coring after intermediary storage periods of 0, 3, 7, and 30 d. The pH of control and inoculated haylages sampled on d 3 did not differ. However, B500 and BP2 had lower pH ( $5.77 \pm 0.04$  vs.  $6.16 \pm 0.04$ ;  $5.06 \pm 0.13$  vs.  $5.52 \pm 0.13$ ) than other treatments by d 7 and 30, respectively. At final bale opening on d 112, all treatments had lower pH than the control haylage ( $4.77 \pm 0.07$  vs.  $5.37 \pm 0.07$ ). The B500, BP2, and SI haylages had greater lactic acid and lactic-to-acetic acid ratios than SK and control haylages. No differences were detected in neutral detergent fiber digestibility, dry matter losses, dry matter, lactic and acetic acid concentrations, and yeast and coliform counts. The SK haylage had lower clostridia counts compared with the control ( $1.19 \pm 0.23$  vs.  $1.99 \pm 0.23$  cfu/g). Treatments B500, BP2, SI, and SK tended to reduce mold counts and they

improved aerobic stability by 236, 197, 188, and 95%, respectively, compared with the control ( $276 \pm 22$  vs.  $99 \pm 22$  h).

**Key words:** Bermudagrass haylage, silage inoculant

### INTRODUCTION

Ensiling is an alternative forage storage method to making hay that requires relatively less drying of the forage for successful preservation. Tifton 85 bermudagrass (*Cynodon dactylon*) is an improved tropical grass cultivar that is widely used in southern US dairy systems because it has greater NDF digestibility than other grasses adapted to the region (Hill et al., 1993). However, ensiling bermudagrass is challenging due to its low concentration of readily fermentable carbohydrates (Kunkle et al., 1988). Inoculants are applied to forage to improve their fermentation by rapidly reducing the pH, thereby reducing losses of DM or to improve aerobic stability by inhibiting the growth of spoilage-causing yeasts. Due to their ability to ferment glucose to antifungal VFA, such as acetate and propionate (Moon, 1983), propionic acid bacteria have been applied to silage to improve aerobic stability (Dawson et al., 1998; Filya et al., 2004). However, such attempts have been unsuccessful in several studies (Higginbotham et al., 1998; Filya et al., 2006; Arriola et al., 2011 a,b) because the acidic conditions in the silo inhibited their growth and ability to compete with lactic acid bacteria (Weinberg et al., 1995). Forages ensiled with obligate heterofermentative *Lactobacillus buchneri* have typically improved the aerobic stability of forages (Adesogan et al., 2003; Kleinschmit et al., 2005; Huisden et al., 2009; Arriola et al., 2011b). However, application of *L. buchneri* alone can increase DM losses slightly (Ranjit and Kung, 2000). Therefore, *L. buchneri* is often combined with homofermentative or facultative heterofermentative bacteria in inoculants to constrain DM losses and improve aerobic stability (Driehuis et al., 2001; Adesogan et al., 2004; Arriola et al., 2011 a,b).

Only a few studies have examined effects of inoculant application to bermudagrass silage on the quality and aerobic stability of the silage. Adesogan et al. (2004)

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showed that microbial inoculants or molasses could improve the fermentation and quality of bermudagrass silage. Vendramini et al. (2012) reported that aerobic stability of bermudagrass silage was greater when it was treated with microbial inoculants instead of molasses or nothing. Both of these studies were conducted on bermudagrass that had been ensiled in mini-silos. No published studies were found that examined effects of inoculation of the bermudagrass round-bale haylage used in commercial practice on many dairies in the southern United States. The objective of our study was to compare the efficacy of using 4 commercially available microbial inoculants to improve the fermentation and aerobic stability of bermudagrass haylage. We hypothesized that the microbial inoculants would increase the fermentation and aerobic stability of the haylages.

## MATERIALS AND METHODS

### Forages and Treatments

An existing stand of Tifton 85 bermudagrass on a 23-ha field at the University of Florida Santa Fe Beef Unit (Alachua, FL) was fertilized with 526 kg/ha of 17-0-10 (N-P-K) on May 3, 2010, and 3.4 tons/ha of lime on May 21, 2010. Approximately 280 kg/ha of 34-0-0 (N-P-K) were applied after the first cutting of the 4-wk regrowth on June 17, 2010. The second 4-wk regrowth was harvested at a stubble height of 8 cm with a 3-m wide mower (Disco 3000 TC model, Claas, Harsewinkel, Germany) fitted with a conditioner on July 7, 2010. The grass was wilted for 2.5 h and then treated with or without inoculants in the windrow before baling. Mowing started at 1000 h to avoid the morning dew and ended at about 1600 h. Baling and wrapping started immediately after the 2.5-h wilt such that the first and last bales were made at approximately 1300 and 1830 h, respectively. A John Deere 468 (John Deere, Moline, IL) round baler and a McHale wrapper (991BC, McHale, Ballinrobe, County Mayo, Ireland) were used.

Inoculants were reconstituted in 12 L of deionized water and sprayed onto the forage in the windrow using the tractor-mounted, 57-L, continuous-flow sprayer (FIMCO, North Sioux City, SD) described by Krueger et al. (2008). The sprayer was fitted with a 3-nozzle boom and mounted on the tractor in front of the baler to ensure the forage was sprayed with the inoculant just before the grass entered the baler chamber. The sprayer was switched off and about half a windrow of untreated forage was run through the baler after each treatment to clean out the baler and avoid cross contamination. The field was divided into 5 blocks and each treatment was applied to windrows in each block to ensure

that each treatment was applied to comparable forage. The following treatments were applied in the stated sequence: (1) water (control); (2) Buchneri 500 inoculant (**B500**; Lallemand Animal Nutrition, Milwaukee, WI), applied at 9.9 mg/kg of fresh forage to supply  $1 \times 10^5$  cfu/g of a mixture of *Pediococcus pentosaceus* and  $4 \times 10^5$  *L. buchneri* 40788 as well as sufficient *Trichoderma reesei*  $\beta$ -glucanase, xylanase, and galactomannase to release 441, 1,258, and 20 mg of glucose/min per gram, respectively; (3) Biotal Plus II inoculant (**BPII**; Lallemand Animal Nutrition), applied at 4.0 mg/kg of fresh forage to supply  $1 \times 10^5$  cfu/g of a mixture of *P. pentosaceus* and *Propionibacteria freudenreichii*, as well as sufficient  $\beta$ -glucanase, xylanase, and galactomannanase to release 1,215 and 3,456, and 54 mg of glucose/min per gram, respectively; (4) Silage Inoculant II (**SI**; AgriKing Inc., Fulton, IL) applied at 4.0 mg/kg of fresh forage to supply  $1.2 \times 10^5$  cfu/g of a mixture of *Lactobacillus plantarum* and *P. pentosaceus* as well as sufficient  $\beta$ -glucanase (*T. reesei*),  $\alpha$ -amylase (*Aspergillus oryzae*), xylanase (*T. reesei*), and galactomannanase (*T. reesei*) to release 1,260, 630, 684, and 115 mg of glucose/min per gram, respectively; and (5) Silo King inoculant (**SK**; AgriKing Inc.), applied at 7.9 mg/kg of fresh forage to supply  $1 \times 10^5$  of a mixture of *L. plantarum*, *Enterococcus faecium*, and *P. pentosaceus*, as well as sufficient *Aspergillus oryzae* amylase, *Bacillus subtilis* amylase, and *Trichoderma longibrachiatum* cellulase for 5,000,000, 2,100,000, and 520,000 dinitrosalicylic acid units, respectively. The bacteria composition and enzyme activities of the inoculants were stipulated by the manufacturers. Their lactic acid bacteria populations were counted by pour plating on de Man, Rogosa, Sharpe agar (Oxoid, Basingstoke, UK) and incubating for 48 h at 32°C before they were applied.

Forty round bales (8 per treatment;  $441 \pm 26$  kg;  $1.2 \times 1.2$  m diameter) were made and each was wrapped with 7 layers of white, 6 mil (0.152 mm) stretch plastic (Sunfilm silage wrap, AEP Industries Inc., Montvale, NJ) and stored. Twenty bales (4 per treatment) were opened after 112 d of storage. Bale weights were recorded immediately after they were wrapped on d 0 and just before they were opened on d 112. These weights and the corresponding DM concentrations were used to calculate DM losses. The remaining 20 bales were stored for 30 d and 3 core samples were taken from different sites on each one with a silage corer (Star Quality Samplers, Edmonton, Canada) and frozen ( $-4^\circ\text{C}$ ) after 0 (approximately 30 min after wrapping), 3, 7, and 30 d of storage. Silage tape was used to seal the coring site after each sampling. Four representative samples of the forage taken from several windrows at different times after mowing but before wilting on d 0 were frozen ( $-4^\circ\text{C}$ ) for subsequent analysis.

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