Chemical composition, intake by sheep, and in situ disappearance in cannulated cows of bermudagrass hayed at two moisture concentrations and treated with a non-viable Lactobacillus-lactic acid preservative

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Abstract

Bermudagrass [Cynodon dactylon (L.) Pers.] is commonly used for grazing and haying in the southern USA, but hay curing can be challenging due to frequent rainfall events during spring and early summer. An existing stand of ‘Greenfield’ bermudagrass was divided into 12 plots using a randomized complete block design with a 2×2 factorial treatment arrangement to evaluate the influence of a non-viable Lactobacillus-lactic acid preservative and moisture concentration at baling on chemical composition, intake by sheep, and in situ disappearance in cattle. At time of mowing, half of the plots in each block were either spray-treated (T) or not treated (U) with 81 mL/t forage dry matter (DM) of the preservative solution. Hay was then baled at target moisture concentrations of either 174 g/kg DM (L) or 267 g/kg DM (H). Maximum temperature and heating degree days were greater (P<0.05) from H compared with L during the 42-d storage period. An interaction between spray and moisture treatments tended (P<0.10) to affect recovery of DM; recoveries for LT (0.992) differed (P<0.10) from HT (0.913), but LU and HU were intermediate between the spray-treated hays, and did not differ from either (P>0.10). Post-storage nutritive value was largely influenced by moisture treatments only. Intake and digestibility, and in situ DM disappearance of these same hays were determined using 16 wether lambs (43 ± 3.7 kg initial BW), or six ruminally cannulated cows (617 ± 3.5 kg initial BW), respectively. Dry matter intake by sheep was not affected by either treatment factor (P>0.05), but DM digestibility and digestible DM intake were greater (P<0.05) from U compared with T. The in situ immediately soluble DM portion was greater from (P<0.05) L compared with H, but the reverse was true for the potentially degradable DM fraction. The lag time tended (P<0.10) to be greater from H compared with L. Treating bermudagrass with a non-viable Lactobacillus acidophilus-lactic acid spray product at time of baling may not offset the negative effects on forage quality and digestibility of baling bermudagrass hay at excessive moisture concentrations.

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Keywords:
Bermuda grass
Hay preservative
Dry matter disappearance

Abbreviations: ADF, acid-detergent fiber; ADIN, acid-detergent insoluble N; CP, crude protein; DM, dry matter; HDD, heating-degree days >35 °C; L, low moisture (174 g/kg DM); lignin (Sa), lignin; H, high moisture (267 g/kg DM); NDF, neutral detergent fiber; U, not treated with non-viable Lactobacillus-lactic acid preservative; T, treated with non-viable Lactobacillus-lactic acid preservative.

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1. Introduction

Bermudagrass [Cynodon dactylon (L.) Pers.] is a major summer hay crop in the southeastern U.S., making it one of the most important forage crops throughout that region (Hoveland, 2000). In spite of its success, frequent precipitation events early in the growing season make desiccation of bermudagrass challenging and put this valuable cash crop at risk (Coblentz et al., 2000). Producers are often forced to bale bermudagrass at moisture concentrations that are greater than optimum levels. However, baling hay at moisture concentrations greater than 200 g/kg dry matter (DM) may result in spontaneous heating and diminishing nutritive value during storage when packaged in small rectangular (~45-kg) bales (Coblentz et al., 2004). To reduce the negative effects of baling at moisture concentrations greater than recommended levels, preservatives have been developed and marketed, mainly for use on legumes. For example, Johnson et al. (1983) demonstrated that alfalfa (Medicago sativa L.) treated at the time of cutting with a solution containing potassium carbonate and emulsified methyl lardowate or methyl lardate and baled at 750–800 g/kg of DM retained nutritive value after a 42-d storage period. While various studies evaluated the effects of traditional chemical preservatives, less information is available regarding the use of microbiological compounds with the objective of improving hay quality. Rotz (2003) and Deetz et al. (1989) reported that although strains of Lactobacillus have been applied to hay as forage-stabilizing compounds, no major effects on mold, color, heating, DM loss, or change in forage quality could be observed. Rotz (2003) also argued that while products containing Bacillus bacteria may be better suited to the aerobic hay environment, there has been little evidence that these products provide substantial storage improvements. In principle, the addition of bacteria that produce lactic, acetic, or propionic acid may serve as alternatives to more corrosive inorganic preservatives.

Some practitioners throughout the southeastern USA claim to have used a product successfully that contains non-viable Lactobacillus-lactic acid to preserve bermudagrass hay and retain hay quality. Therefore, our objective was to study the effects of this product on the post-storage chemical composition of bermudagrass hay baled at two moisture concentrations. Our second objective was to determine the intake by sheep and in situ disappearance of these hays in fistulated beef cows.

2. Materials and methods

2.1. Experimental site and treatments

The research presented in this paper was conducted at the University of Arkansas Division of Agriculture-Watershed Research and Education Center (WREC) located at Fayetteville, AR (94°10’W; 36°05’N; 394 m elevation). The soil at the site was classified as a Captina silt loam soil (fine-silty, siliceous, active, mesicTypicFragiudults; NRCS, 2009). The area is defined by a humid sub-tropical climate with an annual mean precipitation of approximately 117 cm and an average annual temperature of 16.7 °C (AAREC, 2007). Climatic data for 2007 are summarized in Fig. 1.

An established field of ‘Greenfield’ bermudagrass was used for obtaining the hay that was used in this study. An area of approximately 100 m x 50 m of the stand was divided into 3 field blocks, each containing 4 plots, and then fertilized with 112 kg N/ha as ammonium nitrate (34–0–0) in May of 2007. Annual weeds were controlled in March of the same year with glyphosate (Roundup®; The Scotts Company LLC, Marysville, OH, USA) at an application rate of 1.44 L/ha. On 4 July 2007, bermudagrass plots were mowed between 09:30 h and 10:30 h using a mower-conditioner (Model 1411; CNH America, LLC, Racine, WI, USA), leaving a 0.6-m buffer strip between experimental units. Bermudagrass at the time of cutting was in stem elongation stage (mean stage count, MSC = 2.66; Moore et al., 1991). Species composition at the experimental site was 70% bermudagrass, using procedures described by Evans and Love (1957). The remaining 30% was comprised of species including johnsongrass [Sorghum halepense (L.) Pers.], goosegrass [Eleusine indica (L.) Gaertn.], and crabgrass [Digitaria ciliaris (Retz.) Koeler]. The bermudagrass used represented the second cut of the season; the stand was cut previously at the beginning of June 2007.

![Figure 1](image_url)  
**Fig. 1.** Monthly average temperature and rainfall for Fayetteville, AR, USA comparing 2007 with the 30-yr mean.
At the time of cutting, half of the plots within each block were treated (T) with 81 mL/t DM of a solution containing 110 g/kg lactic acid and 25.0 × 10^7 colony forming units (cfu)/mL of a non-viable Lactobacillus acidophilus (Pro-Serve II®, Conklin Company Inc., Shakopee, MN, USA); half of plots were not treated (U). The preservative was combined with a non-ionic wetting agent (Wex®, Conklin Company Inc., Shakopee, MN, USA) and applied at a pressure of 248 kPa through spray nozzles that were spaced at 74-cm intervals underneath the front cover of the mower. Plots were mowed in consecutive order and from the same end of the study area to prevent driving over unmowed forage. This necessitated driving over both spray-treated and untreated plots after they were mowed. After mowing each spray-treated plot, a section of bermsagrass in the same field but outside of the plot area was mowed to ensure all of the spray treatment was removed from the rollers of the mowing equipment prior to mowing a non-treated plot. Also, the tractor and mower were driven around the outside of the plot area after mowing each plot to ensure the spray was removed from the tractor tires before moving to another plot.

Swaths were teddered on July 7, 2007 to facilitate drying before hay was baled with a rectangular baler (Model 320; CNH America, LLC, Racine, WI, USA) at moisture concentrations of 267 g/kg (H) during the early afternoon on July 8, 2007 and 174 g/kg (L) during late afternoon of the same day. Prior to baling, moisture concentrations were repeatedly monitored by collecting random grab samples at different locations within each plot and drying each sample to a constant weight in a microwave oven.

2.2. Hay sample collection

After baling, 6 bales from each plot were selected at random, weighed, and stacked on dry, non-heating bales in 3 layers of 2 bales each in a single row within a closed metal barn. The twelve 6-bale stacks were then surrounded on all exterior sides with dry, non-heating bales, and a 2.5-cm thick sheet of Styrofoam insulation board was placed between each 6-bale stack. Bales were insulated to decrease the effects of fluctuations in ambient temperature and to reduce heat transfer between adjacent stacks. Internal bale temperatures were monitored from 2 bales selected at random, located at the bottom and center of each stack using an Omega 450 AKT Type K thermocouple thermometer (Omega Engineering, Stamford, CT, USA). Temperature readings were taken twice daily at 08:00 and 16:00 h. Heating degree days (HDD) were computed as the summations of the daily increment by which the mean internal bale temperature was >35°C during a 42-d storage period.

Prior to storage, 3 core samples (46-cm depth; 2.54-cm diameter) were taken from one end of 3 bales per stack at different locations using a Uni-Forage Sampler (Star Quality Samplers, Edmonton, AB, Canada) to determine bale moisture concentration and pre-storage chemical composition. Core sampling holes were filled immediately after sampling with insulating foam sealant (Great Stuff™; The Dow Chemical Company, Wilmington, IL, USA) to maintain bale integrity. After the 42-d storage period the remaining 3 bales per stack were weighed and core sampled using the same procedure as described previously, and analyzed for moisture concentrations and nutritive value. Dry matter recovery was calculated from DM weights of each group of bales obtained before, and then after the 42-d storage period.

2.3. Hay sample analysis

Initial and post-storage core samples were dried under forced air at 50°C to a constant weight and ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA). Concentrations of total N were determined by rapid combustion (AOAC, 1998; method 992.23). Neutral detergent fiber, acid detergent fiber (ADF), and lignin (sa) (solubilization of cellulose with sulfuric acid) were analyzed sequentially using the batch procedures outlined by ANKOM Technology Corp. (Fairport, NY, USA; Van Soest et al., 1991; Vogel et al., 1999); no sodium sulfite or heat-stable α-amylase was included in the neutral detergent solution. Hemicellulose was calculated as the difference between ADF and NDF. Residual ash was not separately determined and is thus contained in ADF values. Acid detergent insoluble N (ADIN) was determined using combustion procedures identical to those described for total N and were calculated and reported on DM and total N basis (Licitra et al., 1996; Turner et al., 2002). The ADF residues for ADIN analyses were obtained using separate analyses from the ADIN determination. These samples were not subjected to NDF determination prior to ADIN analysis (Van Soest et al., 1991).

2.4. Intake and digestibility by sheep

Sixteen black-faced wether lambs (Ovis aris; 43 ± 3.7 kg initial BW) were purchased from private ownership on November 1, 2007, and relocated to an enclosed field (0.3 ha) for a 28-d adjustment period during which they were offered untreated bermsagrass hay ad libitum. Water was provided for ad libitum consumption. Lambs were offered a small quantity of a commercial sheep mineral1 (Land O’ Lakes Purina Feed LLC, Shoreview, MN, USA) upon arrival; the mineral amount was increased slowly until the lambs reached ad libitum access. Any lamb developing health-related disorders was treated with either 1 mL Excene® RTU (Pfizer Animal Health, New York, NY, USA) or 1.5 mL Nuflox (Intervet/Schering-Plough Animal Health, Summit, NJ, USA). This study was conducted in conformance with the approved IACUC protocol #08026.

1 Land O’Lakes sheep and goat mineral contained 147–176 g/kg Ca, 283–338 g/kg salt, and not less than 70 g/kg P, 3 g/kg K, 0.027 g/kg Se, 342,222 IU/kg vitamin A, 40,000 IU/kg vitamin D₃, and 667 IU/kg vitamin E.
The 16 wether lambs were weighed and placed randomly in individual pens (1.1 m × 1.5 m) with metal grated floors and nipple watering systems in an insulated metal research barn. Temperature was maintained at 16 °C in the barn throughout the time of study, and lighting in the barn was maintained between 07:00 and 21:00 h each day.

Four lambs each were assigned randomly to one of the four treatment combinations. Lambs were fed forage from field block 1 during the adaptation period and during the collection period from field block 2. All six of the hay bales from each of the twelve stacks were chopped with a chipper shredder (Briggs and Stratton 1450 series, Cleveland, OH, USA) to a length of approximately 6 cm and stored temporarily in plastic bags. This reduction in length was necessary to minimize hay wastage by the lambs. Each lamb was offered its respective hay for a 14-d dietary adaptation period. The assigned treatment hay was offered daily beginning at 09:00 h, and additional hay was offered throughout the day to ensure ad libitum access to forage while minimizing hay wastage. Grab samples of each forage treatment were taken daily each time the hay was weighed for each lamb. These samples were placed in paper bags and dried to a constant weight at 50 °C for DM determination. The commercial granulated mineral was provided for ad libitum consumption.

On day 12 of the adaptation period, lambs were fitted with fecal collection bags that remained open. Bags were closed on day 14 and total feces were collected twice daily at 08:30 h and 17:00 h for 7 d. At each collection, total feces were removed from the bag, weighed, put in labeled paper sacks and dried to a constant weight at 50 °C. Beginning on day 13 of the adaptation period, unconsumed hay was collected each morning before the 09:00 h feeding, weighed, placed in labeled paper sacks, and dried to a constant weight at 50 °C. During the adaptation period, lambs were removed from their pens and allowed to socialize and exercise for 2 h every 3 d in an enclosed area (12 m × 15 m) inside the barn. Lambs were again weighed after the trial.

2.5. In situ digestibility in cannulated cows

Six ruminally cannulated cows (Bos primigenius f. Taurus; 617 ± 3.5 kg initial BW) were placed in individual pens (2.7 m × 4.3 m) with concrete flooring in an enclosed facility on January 28, 2008. Cows were offered a basal diet of bermudagrass hay for ad libitum intake along with a supplemental concentrate\(^2\) at 3 g/kg BW during a 10-d adaptation followed by a 5-d in situ sampling period. Samples of the concentrate mixture and forage were collected for each day of the study. Both bermudagrass hay and supplement were offered in equal portions at 08:00 and 16:30 h. Water was provided ad libitum from automatic bowl-type waterers. Lighting was provided from 07:00 to 21:00 h daily. Pen floors were cleaned twice daily. This study was conducted in conformance with the approved IACUC protocol #09001.

Samples of bermudagrass hay were obtained from the chopped material of each plot at the time it was chopped for the lamb study. These samples were ground to pass through a 2 mm screen and placed in Dacron bags (10 cm × 20 cm, 53 ± 10-μm pore size, Ankom Technology Corp., Fairport, NY, USA) for determination of in situ ruminal disappearance kinetics. Forage samples (5 g) from each of the 12 plots of bermudagrass were placed in a total of 20 dacron bags per plot. The bags were sealed with 31-mm × 1.6-mm small rubber bands, and then placed in 36-cm × 42-cm mesh polyester laundry bags. Forage samples were grouped by field block according to the experimental field layout and each of the forage samples from each of the 4 plots within each block was assigned to two cows that represented one of the three field blocks. Although this procedure only provides two animal observations for the forage from each individual forage sample, field block integrity was maintained and a total of 6 animal observations were obtained per treatment. Samples were placed in tepid (39 °C) water for 20 min to decrease the lag time associated with wetting (Ogden et al., 2006), then inserted into the ventral rumen of each cow simultaneously and incubated for 6, 12, 18, 24, 36, 48, 72, 96, or 120 h. Immediately after sample removal from the rumen, mesh bags were immersed in cold water to prevent any further microbial degradation. A separate set of bags was pre-incubated and rinsed without ruminal incubation (0 h). Dacron bags containing in situ residues were rinsed in a top-loading washing machine for 2 min and were spun for 1 min. This process was repeated a total of 10 times for each incubation period. Bags were checked frequently for tears and evident ruptures were isolated with a rubber band. After the rinsing process was completed, bags were placed in a forced-air drying oven at 50 °C in a large, paper sack until dry. Dacron bags were allowed to equilibrate with atmospheric moisture before the final weight was recorded (Vanzant et al., 1996).

2.6. Statistical analysis

Initial and post-storage bale characteristics and nutritive values were analyzed as a randomized complete-block design with a 2 × 2 factorial arrangement of treatments with three field replications consisting of two moisture concentrations (H and L) and presence or absence of a preservative (T or U) using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC, USA, 2008) according to the following model:

\[
Y_{i(k)} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{k(ij)}
\]

\(^2\) The supplemental concentrate consisted of cracked corn at 372 g/kg, wheat midds at 200 g/kg, soybean meal at 347 g/kg, molasses at 40 g/kg, limestone at 3 g/kg, salt at 33 g/kg, vitamin A, D, E (premix) at 2 g/kg, and vitamin E (premix) at 3 g/kg.
Table 1
Concentrations of moisture, bale weight, and heating characteristics of bermudagrass hay baled at two concentrations of moisture and treated or not treated at mowing with a non-viable Lactobacillus-lactic acid preservative and stored in small stacks for 42 d.

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Pre-storage</th>
<th>Post-storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU</td>
<td>LT</td>
</tr>
<tr>
<td>Moisture concentration (g/kg)</td>
<td>171</td>
<td>177</td>
</tr>
<tr>
<td>Bale weight (as is) (kg)</td>
<td>23.9</td>
<td>24.6</td>
</tr>
<tr>
<td>Bale weight (DM basis) (kg)</td>
<td>19.8</td>
<td>19.1</td>
</tr>
<tr>
<td>Moisture concentration (g/kg)</td>
<td>106</td>
<td>102</td>
</tr>
<tr>
<td>Bale weight (as is) (kg)</td>
<td>21.0</td>
<td>21.1</td>
</tr>
<tr>
<td>Bale weight (DM basis) (kg)</td>
<td>18.7</td>
<td>18.9</td>
</tr>
<tr>
<td>DM recovery</td>
<td>0.947ab</td>
<td>0.992a</td>
</tr>
<tr>
<td>Maximum temperature (°C)</td>
<td>41.4</td>
<td>39.3</td>
</tr>
<tr>
<td>Heating degree daysd</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Days above 35°C</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Means within a row without a common superscript letter differ (P<0.1).

* Treatments, bermudagrass hay was baled at 174 g/kg (L) or 267 g/kg (H) moisture concentrations and spray-treated (T) or not treated (U) at time of mowing with a non-viable Lactobacillus-lactic acid preservative.

* SED, standard error of the difference of the means.

* M, moisture effect (P<0.05; m×t, tendency of moisture by spray treatment interaction effect (P<0.1); ns, no significant difference.

* Heating-degree days were calculated as the summations of the increment each day by which the internal bale temperature was >35 °C during the 42-d storage period.

where \( Y_{ij} \) was the observation, \( \mu \) the population mean, \( A_i \) the hay preservative effect, \( B_j \) the moisture effect, \( AB_{ij} \) the interaction thereof, and \( e_{ijk} \) the residual error. Bermudagrass plots were considered the experimental unit and block was used as the random factor. There were three observations per treatment for presented forage data. When interactions between the main effects were detected (P<0.05), mean separations were performed using an F-protected t-test (PDIIFF option). All data are reported as least squares means. Effects were considered significant at P≤0.05. Differences referred to as tendencies are those with a P-value between 0.05 and 0.10.

Data for intake and digestibility by sheep were analyzed statistically using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA, 2008) for a 2×2 factorial treatment arrangement. In total, there were four observations (sheep) per treatment used for data analysis. Effects of bale moisture, spray treatment, and their interaction were included in the model. In the event of a significant interaction (P<0.05), means were compared using an F-protected t-test.

Dry matter was divided into 3 fractions based on relative susceptibility to ruminal disappearance. The forage fractions were defined as: fraction A, being the immediately soluble portion; fraction B, being the portion that disappeared at a measurable rate; and fraction U, being the portion undegraded in the rumen. Fractions B and U, the lag time (L), and the disappearance rate (Kd) were determined directly by the non-linear model. Fraction A was calculated as [1000 – (B + U)]. The effective ruminal degradability (ED) was calculated as \( A + B \times [K_d \times ([K_d + 0.0353]/h)] \) (Orskov and McDonald, 1979), where 0.0353/h is the estimated particulate passage rate for basal diets of this type based on past work (Scabrough et al., 2006). Residual DM remaining in the in situ study was fitted to a non-linear statistical model (Mertens and Loften, 1980) using PROC NLIN of SAS (SAS Inst. Inc., Cary, NC, USA, 2008) to determine DM degradation kinetic parameter estimates. There was a total of six observations per treatment (2 cows per field block and three field blocks). The model included effects of bale moisture, spray treatment, and the bale moisture by spray treatment interaction. Block was considered a random effect.

3. Results

3.1. Bale moisture and storage characteristics

Pre- and post-storage bale characteristics are described in Table 1. Initial bale moisture concentration, bale weight (as is), and bale DM weight were greater (P<0.05) under high moisture compared with low moisture. These moisture effects were still present at the end of the storage period. Bale DM recovery tended to be affected by an interaction between moisture and spray treatments (P<0.1), under which LT resulted in greater (P=0.056) DM recovery than HT, but did not differ (P>0.1) from LU and HU. Maximum bale temperature and HDD were greater (P<0.05) from H than from L.

3.2. Pre- and post-storage chemical composition

No differences regarding chemical composition were observed between treatments pre-storage; however, differences were present after the storage period ended (Table 2). Concentrations of NDF, ADF, and lignin (sa) were greater (P<0.05) from H than from L but spray treatment effects were not observed (P>0.1). Hemicellulose concentrations were affected by moisture and additionally by an interaction between moisture and spray treatments (P<0.05 and P<0.01, respectively). Further, hemicellulose concentrations from both H treatments were greater (P<0.05) than from L treatments, but additionally,
Table 2
Pre- and post-storage chemical composition (g/kg dry matter, DM, unless otherwise noted) of bermudagrass baled at two concentrations of moisture and treated or not treated at mowing with a non-viable Lactobacillus-lactic acid preservative and stored in small stacks for 42 d. There were no differences between treatments pre-storage; thus, means were combined.

<table>
<thead>
<tr>
<th>Component (pre-storage)</th>
<th>SED</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>3.3</td>
</tr>
<tr>
<td>Neutral detergent fiber (NDF)</td>
<td>672</td>
<td>22.8</td>
</tr>
<tr>
<td>Acid detergent fiber (ADF)</td>
<td>329</td>
<td>9.6</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>343</td>
<td>2.0</td>
</tr>
<tr>
<td>Lignin (sa)</td>
<td>44</td>
<td>6.8</td>
</tr>
<tr>
<td>Acid detergent insoluble N (ADIN)</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>ADIN (g/kg N)</td>
<td>95.1</td>
<td>10.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component (post-storage)</th>
<th>Treatments</th>
<th>SED</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU</td>
<td>LT</td>
<td>HU</td>
</tr>
<tr>
<td>N</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>NDF</td>
<td>718</td>
<td>712</td>
<td>743</td>
</tr>
<tr>
<td>ADF</td>
<td>337</td>
<td>342</td>
<td>353</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>381b</td>
<td>370c</td>
<td>390a</td>
</tr>
<tr>
<td>Lignin (sa)</td>
<td>38.0</td>
<td>43.5</td>
<td>51.2</td>
</tr>
<tr>
<td>ADIN</td>
<td>2.4</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>ADIN (g/kg N)</td>
<td>99.5</td>
<td>104.4</td>
<td>116.8</td>
</tr>
</tbody>
</table>

Means within a row without a common letter differ (P<0.05).

a SED, standard error of the difference of the means.
b M, moisture effect (P<0.05); M×T, moisture by spray treatment interaction effect (P<0.05); m, tendency of moisture effect (P<0.1); ns, no significant difference.
c Treatments, bermudagrass hay was baled at 174 g/kg (L) or 267 g/kg (H) moisture concentrations and spray-treated (T) or not treated (U) at mowing with a non-viable Lactobacillus-lactic acid preservative.

centations of hemicellulose from LU were greater (P<0.05) than those from LT. Acid detergent insoluble N (g/kg DM) tended to be affected by moisture concentrations at baling (P<0.1). Further, concentrations of ADIN (expressed as g/kg total N) were (P<0.05) affected by initial bale moisture as well.

3.3. Intake and digestibility by sheep

Differences were not observed among treatment combinations (P<0.05) and no interaction of moisture level and spray application was present (P>0.1) for DM intake expressed either as g/d or as g/kg of body weight (Table 3). Dry matter digestibility was greater (P<0.05) from U compared with T and tended (P<0.1) to be greater from H compared with L. Actual digestible DM intake (g/d) was greater (P<0.05) from U vs. T, but this difference did not persist when expressed on a body-weight basis.

3.4. In situ digestibility in cows

The water–soluble fraction (A) was greater (P<0.05) from L than from H, but the slowly degradable fraction (fraction B) was greater (P<0.05) H than from L (Table 4). The undegradable fraction (U), ruminal DM disappearance rates (Kd), and effective ruminal disappearance did not differ (P>0.1) among moisture concentrations at baling. Lag times reflecting the onset of digestion of fraction B had a tendency to differ (P<0.1) among moisture concentrations at baling. Application of the hay preservative at the time of mowing did not affect (P>0.10) any of the DM disappearance measurements. Likewise,

Table 3
Dry matter intake and digestibility by sheep of bermudagrass hay baled at high or low moisture concentrations and treated or untreated with a nonviable lactic acid–Lactobacillus preservative at time of mowing.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>SED</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LU</td>
<td>LT</td>
</tr>
<tr>
<td>Dry matter (DM) intake (g/d)</td>
<td>730</td>
<td>634</td>
</tr>
<tr>
<td>DM intake (g/kg) bodyweight (BW)</td>
<td>16.9</td>
<td>14.9</td>
</tr>
<tr>
<td>DM digestibility</td>
<td>0.541</td>
<td>0.487</td>
</tr>
<tr>
<td>Digestible DM intake (g/d)</td>
<td>394</td>
<td>308</td>
</tr>
<tr>
<td>Digestible DM intake (g/kg BW)</td>
<td>9.15</td>
<td>7.23</td>
</tr>
</tbody>
</table>

a Treatments, bermudagrass hay was baled at 174 g/kg (L) or 267 g/kg (H) moisture concentrations and spray-treated (T) or not treated (U) at mowing with a non-viable Lactobacillus–lactic acid preservative.
b SED, standard error of the difference of the means.
c T, spray treatment effect (P<0.05); m, tendency of moisture effect (P<0.1); ns, no significant difference.
interactions between moisture and spray treatments (P>0.05) were not present in any of the in situ DM disappearance measurement.

4. Discussion

4.1. Moisture effects

Moisture content at baling is considered the major factor influencing DM recovery of hay (Turner et al., 2002; Collins, 1995; Rotz and Muck, 1994). The greater the moisture concentration the higher the internal bale temperature, which in turn affects DM recovery negatively (Coblentz et al., 2000). In this study, DM recovery was not directly affected by moisture. However, the fact that the moisture by spray treatment interaction tended to be present does not necessarily contradict previous work. We speculate that the inconsistency of DM recovery was simply a result of sample variation.

Our data support the findings of previous studies (Rotz, 2003; Coblentz et al., 2000) that increased bale moisture will typically result in elevated bale temperature. Results for both maximum bale temperatures and HDD clearly showed significantly higher values for H in opposite to L, but application of the spray product did not reduce bale temperature during storage.

With above-optimal tissue moisture at time of baling, oxidation of water-soluble compounds will extend further into the storage period. Spontaneous heating began immediately in all bales (Fig. 2) as a result of respiratory processes of plant enzymes and microflora adhered to the hay (Hlodverson and Kaspersson, 1986). A second peak of heating in sample bales as described by Coblentz et al. (2000) was observed in 3 out of 4 treatments. This second peak in temperature has been associated with respiratory processes of storage microorganisms (Riddell et al., 1980). In the present study, temperatures under high-moisture treatments equilibrated with low-moisture treatments after approximately 10 d in storage, and equilibration with ambient temperature of all treatments was reached after a total of 18 d.

The delay in bale-to-ambient temperature equilibration under high moisture treatments appeared to negatively affect post-storage nutritive value. Fiber contents under H were greater than L, indicating a reduced concentration of water-soluble cell compounds. While N concentrations did not differ across treatments, decreased digestibility of hay baled at high moisture could be expected because of an increase in NDF concentration of 30 g/kg from L to H.

Based on ADIN values from our study, it appears bales were slightly affected by heat damage under all treatment combinations. Acid detergent insoluble N is used as a heat damage indicator for the non-enzymatic browning reaction (Licita et al., 1996). There also is evidence that ADIN and HDD are correlated (McBeth et al., 2001). In our study, both ADIN and HDD were

![Figure 2: Temperature vs. time curves for bermudagrass hay baled at high or low moisture concentrations and treated or not treated with a nonviable Lactobacillus-lactic acid preservative at time of mowing at a rate of 80 mL/Mg DM of forage mass. Storage length was 42 d. Ambient represents ambient temperature within the hay barn. Treatments: bermudagrass hay was baled at 174 g/kg (L) or 267 g/kg (H) moisture concentrations and spray-treated (T) or not treated (U) at time of mowing with a non-viable Lactobacillus-lactic acid preservative.](image-url)
elevated under H. Yu (1976) indicated that the average ADIN (g/kg N) for undamaged forage is considered to be 70 g/kg. This value was exceeded by all samples in the present study. Between pre- and post-storage, the ADIN value increased for both T and U (from 2.3 to 2.6 g/kg DM for each; P < 0.05), and for H (from 2.35 to 2.8 g/kg DM; P < 0.05). Therefore, it appears that the spray product has no measurable effect on reducing storage losses when hay is baled at elevated moisture concentrations.

Except for a moisture effect tendency affecting DM digestibility in sheep, other intake and digestion measurements were unaffected by moisture treatments. In addition, results for the sheep intake experiment should be treated conservatively and with caution, as the four animal observations per treatment are derived from forage from a single block.

With respect to the in situ DM disappearance in canulated cows, differences in the A and B fractions based on moisture concentration at baling were expected. During storage of hay above a recommended moisture concentration of 180 g/kg, enzymatic activity is prolonged and not impeded through desiccation as in low-moisture hay (Rotz, 2003), thus reducing hay nutritive value. In the present study, the decrease in fraction A from H compared with L is comparable to the increase in NDF concentrations from H compared with L, again indicating the loss of water soluble components during the heating process.

Various studies have been conducted on different forages in the past that support our observations in general. McBeth et al. (2003) reported on decreased DM disappearance of the immediate soluble fraction with elevated moisture in bermudagrass hay. In another study on alfalfa hay, Coblentz and Hoffman (2009) showed that NDF disappearance inversely related to increasing heat damage in bales resulting from elevated moisture concentrations.

4.2. Effects of the Lactobacillus-lactic acid preservative

There were no spray treatment effects measurable on hay quality post-storage other than a tendency for an interaction with moisture treatments pertaining to DM recovery in hay bales, and a significant spray by moisture treatment interaction related to hemicellulose. In both cases, a biological explanation for these effects may only be speculative. It appears that interactions were incidental. Main spray treatment effects of importance to post-nutritive value were all inconclusive. Therefore, our results support findings by Rotz (2003) that using a Lactobacillus product had little effect on nutritive value preservation in high-moisture hay. Similarly, Duchaine et al. (1995) demonstrated that the addition of a lactic acid-producing bacterium did not alter alfalfa and timothy hay quality at moisture level larger than 200 g/kg DM.

It is not clear why the spray treatment affected the hay in such a way that it reduced digestible DM intake in sheep by almost 200 g/kg. As stated above, intake data and effects are based on a single field block of forage only, and are therefore being discussed with caution.

Emanuele et al. (1992) found that intake in lambs was not affected by either low-moisture hay or high-moisture hay inoculated with the microbial inoculant Bacillus pumilus. Hardin et al. (2008) reported both moisture and spray treatment effects on DM digestibility and digestible DM intake by lambs offered crabgrass hay treated with Lactobacillus-lactic acid, but no difference among treatments for DM intake. Digestible DM intake in the present study was slightly less than that of Coastal and Tifton bermudagrass by sheep (10.3 and 11.7 g/kg BW: Burns and Fisher, 2007).

The observed average rate of in situ DM disappearance was somewhat lower than values reported by Emanuele and Staples (1988) for various particle sizes of Tifton 78 bermudagrass, but somewhat greater than values reported by Galdámez-Cabrera et al. (2003) from common bermudagrass (0.034/h). Observed values for effective ruminal DM disappearance were numerically higher than data reported by Scarbrough et al. (2006) who investigated in situ digestibilities of stockpiled bermudagrass harvested late in fall and winter. Their values ranged between 220 and 379 g/kg, likely reflecting lower nutritive value compared with bermudagrass harvested in July as in our case. However, the observed values in this study were somewhat lower than those observed by Galdámez-Cabrera et al. (2003) who reported ranges of 498–553 g/kg from common bermudagrass fertilized with up to 168 kg/ha and harvested in late May or mid-August.

5. Conclusions

Only few studies have investigated the effects of Lactobacillus-based hay preservatives. Based on the results from the present study, treating bermudagrass at time of mowing with a non-viable Lactobacillus-lactic acid preservative may not positively influence hay nutritive value. Baling bermudagrass at moisture concentrations greater than the generally recommended 180 g/kg DM may negatively affect post-storage hay quality and induce spontaneous heating similar to other hayed forages. Additionally, utilizing this product to mitigate the negative effects of baling bermudagrass hay at moisture concentrations that exceed moisture concentrations recommended currently may not result in advantages regarding intake and digestibility by either sheep or cows.

References


Arkansas Agri. Experiment Station Research Series 563, pp. 43–45.


