Ruminal in situ degradability of forage components and in vitro organic matter digestibility of warm-season grasses treated with calcium oxide

Francine M. Ciriaco,[†,‡,©] Darren D. Henry,[‡,©] Romina Beierbach,[ǁ] Tessa M. Schulmeister,[†,©] Martin Ruiz-Moreno,[†] Mariana E. Garcia-Ascolani,[ǁ,$] Federico Podversich,[†] Jose C. B. Dubeux Jr.,[†] and Nicolas DiLorenzo[†,1,©]

[†]Department of Animal Sciences, North Florida Research and Education Center, University of Florida, Marianna, FL 32446-7906, USA [‡]Department of Animal and Dairy Science, University of Georgia, Tifton, GA 31793-5766, USA [ǁ]Universidad Nacional del Sur, Departamento de Agronomia, Sand Andrés 800 Bahía Blanca, 8000 Buenos Aires, Argentina [$]Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo 111421, Paraguay

ABSTRACT: An experiment was designed to evaluate the effects of CaO on ruminal in situ degradability (RISD) of forage components and in vitro organic matter digestibility (IVOMD) of warm-season forages. Bahiagrass (*Paspalum notatum*; BH) or Tifton 85 bermudagrass (*Cynodon* spp.; BM) hay were stored in 20-L buckets in two consecutive years (n = 4/treatment) as follows: 1) untreated dry BH or BM (DH); 2) hydrated BH or BM stored for 7 d (W7); 3) hydrated BH or BM stored for 14 d (W14); 4) hydrated BH or BM + 5% [dry matter (DM) basis] CaO stored for 7 d (CO5-7); 5) hydrated BH or BM + 5% (DM basis) CaO stored for 14 d (CO5-14); and 6) hydrated BH or BM + 10% (DM basis) CaO stored for 14 d (CO10). With exception of the dry treatment (DH), tap water was added to forages under the remaining treatments to reach a DM concentration of 50%. Ruminal in situ degradability of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) of BH and BM was determined for 24, 48, and 72 h in two ruminally cannulated steers consuming BH. Data were analyzed as a randomized block design using bucket as the experimental unit. The model included the fixed effect of treatment and the random effect of year. Concentration of NDF was reduced (P < 0.001) when BH and BM were treated with 10% and 5% CaO and compared with DH. However, only CO10 promoted a reduction (P = 0.007) in ADF concentration of BH, whereas CO10 and CO5, regardless of storage length, reduced (P ≤ 0.006) ADF concentration of BM, when compared with DH. At all ruminal incubation time points, a treatment effect (P < 0.001) was observed on RISD of DM, OM, CP, NDF, and ADF of BH and BM, where all treatments containing CaO promoted greater degradability when compared with DH, W7, and W14, which did not differ (P > 0.05). Ruminal degradability of forage components was greatest (P < 0.05) for CO10, followed by CO5-7 and CO5-14, which did not differ (P > 0.05). In vitro organic matter digestibility was increased (P < 0.001) in both BH and BM when CaO was applied and compared to DH. Treatment of BH and BM with CaO seems to be an effective method of promoting increased digestibility of forage components, including fiber fractions, when applied at 5% of the forage DM with potential additional benefits to BH when applied at 10%.

Key words: alkali treatment, degradability, digestibility, fiber fractions, warm-season forages

INTRODUCTION

Ruminants have a competitive advantage over other domesticated animals: the ability to digest plant cell wall components through a symbiotic relationship with ruminal microorganisms (Van Soest, 1994). The southeastern United States has ideal conditions to grow large quantities of forages. Consequently, most of the beef cattle operations located in this region rely on forage-based diets (Ciriaco et al., 2015). However, the predominant forages can be of limited nutritive value for several reasons, such as high-fiber content when compared with more digestible components. Although ruminants have evolved to consume and utilize fibrous feedstuffs, physical barriers such as lignin attachment to the cell wall polysaccharides make the task more difficult to accomplish (Jung and Deetz, 1993).

With the goal of increasing forage digestibility, forage breeders have attempted to reduce lignin and cellulose in forages; however, the main function of these compounds in nature is to provide structure and support to plants, making it difficult to improve digestibility without reducing forage yield (Pedersen et al., 2005). Hydrolytic chemicals, the majority being alkaline compounds, can improve digestibility by action of hydroxide groups that are capable of disruption of the cell wall structure and possible swelling that can result in increased microbial attachment (Fahey et al., 1993). Therefore, chemical treatment of poor-quality roughages is a promising method of improving fiber digestibility (Klopfenstein et al., 1972; Shreck et al., 2015; Watson et al., 2015), allowing ruminants to more efficiently convert poor-quality fibrous feedstuffs into meat, milk, and fiber.

Sodium hydroxide (NaOH) has been extensively used in the past and up to 30% improvements in total tract digestibility of dry matter (DM) has been observed (Fahey et al., 1993). Compared with NaOH, calcium oxide (CaO), when applied at 5% of the forage DM with the addition of water to reach a final concentration of 50% DM in the treated residue, has been observed to provide similar results and, besides providing a source of Ca in the diet, is considered safer, easier to handle, and less caustic (Shreck et al., 2015). Therefore, it was hypothesized that treating warm-season forages with the alkali CaO would be an effective method of improving the digestibility of nutrients, with major impacts on the fiber fraction. An experiment was designed to test this hypothesis with the following objectives: evaluate the effects of two concentrations of CaO on ruminal in situ degradability of forage components and IVOMD of bahiagrass (*Paspalum notatum*) hay (BH) and Tifton 85 bermudagrass (*Cynodon* spp.) hay (BM) under storage for 7 or 14 d.

MATERIALS AND METHODS

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee (Protocol # 201508733).

Experimental Design and Treatments

Hay (BH or BM) was stored in 20-liter sealed buckets in quadruplicate (2 consecutive years; 1 bucket in year 1 and 3 buckets in year 2; $n = 4$/treatment) as follows: 1) untreated dry BH or BM as is (DH); 2) hydrated BH or BM stored for 7 d (W7); 3) hydrated BH or BM stored for 14 d (W14); 4) hydrated BH or BM + 5% CaO (DM basis) stored for 7 d (CO5-7); 5) hydrated BH or BM + 5% CaO (DM basis) stored for 14 d (CO5-14); and 6) hydrated BH or BM + 10% CaO (DM basis) stored for 14 d (CO10). With exception of the dry treatment (DH), tap water was added to forages under the remaining treatments to reach a DM concentration of 50%. After 7 or 14 d, buckets were opened, and contents were dried for 72 h at 55 C. In a Wiley mill, a set of samples was ground to pass a 4-mm screen for further ruminal in situ degradability analysis and another set was ground to pass a 2-mm screen for further forage composition and IVOMD analysis. Samples were stored at 4 C.

Treatment of Forages

Hay (BH or BM) from round bales was chopped, using a tub grinder (model H-1000; Haybuster, Jamestown, ND) with a 12.7-cm screen to reduce particle size. Forages were placed in a large plastic container with enough surface area to provide proper mixing and homogenization. Subsequently, half of the volume of water needed for each treatment was poured over the forage followed by the previously weighed amount of CaO (97+% available CaO, Acros Organics, Fair Lawn, NJ), which was sprinkled evenly across the top. Using gloves appropriate for handling caustic material, the forage was mixed by hand until there were no visual clumps of CaO, when the remainder of the water was added, and everything was mixed until all water was absorbed by the forages. The treated forage was compacted into the 20-L buckets until completely full, when a lid was placed, sealing the buckets. The amount of forage treated at one time [approximately 3 kg (as is) of hay] was enough to fill one bucket completely, ensuring everything was compacted in the bucket before another batch was treated for the next bucket. Although temperature increase was not measured during forage treatment and storage periods, it is anticipated that elevated temperatures that occur from the exothermic reaction or during extended storage periods were not maintained, since buckets are small enough and heat dissipated faster than it would in large amounts of treated forages, such as in a true ensiling process scenario.

The choices of 5% CaO, water addition to reach 50% DM, and 7 d of storage were based on what has been most commonly reported in the literature when treating crop residues (Shreck et al., 2011; Shreck et al., 2015). It has been reported that at least 7 d of storage is needed for proper reaction to occur (Watson et al., 2015); therefore, 14 d of storage was included to evaluate potential benefits of increased storage length. The treatment containing 10% CaO was chosen in an attempt to observe potential additional effects of CaO.

Ruminal In Situ Degradability

Ruminal in situ degradability of forage components of BH and BM was determined in triplicate in two ruminally cannulated steers (323 ± 42 kg of body weight [BW]; average BW $\pm$ SD) that were consuming BH for at least 14 d. Dried and ground (4 mm) samples of BH and BM after removal from bucket were weighed (5 g) into 10×20 cm Ankom in situ bags (R1020, Ankom Technology Corp., Macedon, NY). The bag pore size was 50 µm and the sample size to free bag surface area ratio was 12.5 mg/cm². Bags were heat-sealed and placed in zippered mesh bags attached to a rope and carabiner, weighted, immersed in warm (39 C) water for 15 min, and incubated in the ventral sac of the rumen for 24, 48, and 72 h. All bags were placed at the same time in the rumen and removal occurred at each predetermined time point. After removal, all bags were placed in a cooler with ice water to halt fermentation, subsequently rinsed with cold running tap water to remove adherent particles and bacteria, frozen overnight, and then rinsed in a domestic washing machine using a cool-wash regular cycle without soap. The same rinsing procedure was applied for bags not incubated in the rumen. Rinsed bags were dried for 48 h at 55 C and weighed. After weighing, residues from the ruminal incubations (3 bags/time point/steer/treatment) were composited by incubation time within steer. Composited samples and original whole samples were analyzed for determination of DM, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) to calculate ruminal degradability of each component.

Laboratory Analyses

Chemical composition of forages. To determine sample DM and OM of treated BH and BM before and after ruminal in situ digestion, approximately 0.5 g of samples were weighed, in duplicate, into ceramic crucibles, dried in a forced-air oven at 105 C for 24 h, and subsequently ashed at 650 C for 6 h. Approximately 0.5 g of samples were weighed, in duplicate, into F57 bags (Ankom Technology Corp.) and analyzed for NDF, using heat-stable α-amylase and sodium sulfite, and subsequent ADF was performed as described by Van Soest et al. (1991) in an Ankom 200 Fiber Analyzer (Ankom Technology Corp.). For total N concentration, and further CP determination, samples were ball-milled and analyzed using a C, H, N, and S analyzer by the Dumas dry combustion method (Vario Micro Cube; Elementar, Hanau, Germany). Crude protein was calculated by multiplying the N concentration of the dry sample by 6.25.

In Vitro Organic Matter Digestibility

A modified Tilley and Terry (1963) procedure was used to determine IVOMD of BH and BM. Briefly, after removal from bucket, 0.7 g of dried

and ground (2 mm) samples were incubated with 50 mL of a 4:1 McDougall's buffer:ruminal fluid inoculum in 100-mL plastic centrifuge tubes for 48 h under constant agitation (60 rpm) at 39 C. Two ruminally cannulated steers consuming BH for at least 14 d were used as ruminal fluid donors. Two tubes per treatment per roughage and two blank (without substrate) tubes were incubated in each of the three separate replicate days. After the initial 48 h, 6 mL of 20% HCl was added to the tubes followed by 2 mL of a 5% pepsin solution. Tubes were then incubated for an additional 48 h under constant agitation (60 rpm) at 39 C. Samples were then filtered through P8 filters (Fisherbrand; Thermo Fisher Scientific Inc.). Filters with wet samples were then dried at 105 C in a forced air oven for 24 h to determine in vitro dry matter digestibility (IVDMD; data not shown). Dry filters with residual samples were ashed at 650 C for 6 h. The ash was then placed in a 105 C oven for 24 h prior to recording weight.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For ruminal in situ degradability of forage components and IVOMD of BH and BM, data were analyzed as a randomized block design. Bucket was considered the experimental unit and the model included the fixed effect of treatment and the random effect of year (block). Significance was declared at $P \leq 0.05$ and Tukey–Kramer adjustments were utilized.

RESULTS AND DISCUSSION

Composition of BH before and after treatment with CaO and storage for either 7 or 14 d is presented in Table 1. Besides CP concentration, which was not affected by treatment ($P = 0.073$), OM, NDF, and ADF concentrations were all reduced ($P < 0.001$) when CaO was applied at a rate of 10% of the total DM (CO10). The lack of reduction in CP with the reduction in OM is of great interest, possibly indicating that the fiber fractions of the forage are being affected the most. There was a treatment effect ($P < 0.001$) on NDF concentration of BH, where a reduction of 23% was observed when the forage was treated with CO10 and compared with DH. Although to a lesser extent, when CaO was applied at a 5% rate, there was a reduction ($P \leq 0.029$) of 7% on NDF concentration of BH when compared with the non-treated forage (DH), regardless of number of storage days. Nevertheless, treating BH with 5% CaO was not effective at reducing ADF concentration when compared with DH. The proposed mode of action of alkali treatment has been suggested to include some solubilization of hemicellulose and consequently increased extent and rate of cellulose digestion (Klopfenstein, 1978). This could explain the reduction in NDF concentration when CaO was applied at either 5% or 10% of the total DM, which could have solubilized the hemicellulose, when compared with no treatment of the forage. Moreover, it has been reported that the core lignin contents are generally not reduced by treatment (Klopfenstein et al., 1972), potentially explaining the lack of reduction on ADF concentration with 5% CaO. However, ADF concentration was reduced when CaO was applied at 10% (CO10), indicating that perhaps lignin may be affected by treatment when using greater amounts of the chemical.

Ruminal in situ degradability of forage components of BH treated or not with CaO and stored for either 7 or 14 d are presented in Table 2. At all

Table 1. Composition of bahiagrass (*Paspalum notatum*) hay (BH) after treatment with water and calcium oxide (CaO) and storage for either 7 or 14 d

Item*, % DM	Treatment[†]						SEM[‡]	*P*-value
	DH	W7	W14	CO5-7	CO5-14	CO10		
DM	89.2	51.8	52.8	53.8	54.4	56.3	–	–
OM	95.1[c]	91.9[b]	94.6[c]	90.9[b]	90.6[b]	87.0[a]	0.57	< 0.001
CP	8.7	9.2	9.1	8.4	8.4	8.2	0.26	0.073
NDF	74.3[c]	74.6[c]	75.8[c]	70.3[b]	67.8[b]	57.5[a]	0.83	<0.001
ADF	37.0[b]	39.4[d]	38.9[cd]	38.3[bcd]	37.4[bc]	34.8[a]	0.40	<0.001

*DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

[†]DH, untreated dry BH; W7, hydrated BH stored for 7 d; W14, hydrated BH stored for 14 d; CO5-7, hydrated BH + 5% CaO stored for 7 d; CO5-14, hydrated BH water + 5% CaO stored for 14 d; CO10, hydrated BH + 10% CaO stored for 14 d. Water was added to reach a final DM concentration of 50%.

[‡]Standard error of treatment means; *n* = 4 buckets/treatment.

[a–d]Within a row, means with different superscripts differ, $P \leq 0.05$.

Table 2. Ruminal in situ degradability of forage components of bahiagrass (*Paspalum notatum*) hay (BH) after treatment with water and calcium oxide (CaO) and storage for either 7 or 14 d

Item*	DH	W7	W14	CO5-7	CO5-14	CO10	SEM[‡]	*P*-value
DMD, %								
24 h	43.4[ab]	35.6[a]	40.3[a]	57.0[cd]	51.6[bc]	61.2[d]	2.07	<0.001
48 h	54.7[a]	47.6[a]	53.4[a]	72.0[b]	72.7[b]	83.0[c]	2.00	<0.001
72 h	58.5[a]	51.9[a]	58.0[a]	77.6[b]	77.7[b]	90.1[c]	1.87	<0.001
OMD, %								
24 h	40.1[ab]	33.1[a]	38.2[a]	54.2[cd]	49.3[bc]	63.3[d]	2.37	<0.001
48 h	54.2[a]	48.0[a]	51.5[a]	70.6[b]	71.5[b]	82.0[c]	2.15	<0.001
72 h	58.4[a]	52.6[a]	57.6[a]	76.0[b]	76.9[b]	89.7[c]	1.78	<0.001
CPD, %								
24 h	40.3[a]	36.1[a]	39.1[a]	41.5[a]	42.4[a]	53.6[b]	2.06	<0.001
48 h	56.1[ab]	51.6[a]	53.5[ab]	61.7[ab]	68.1[bc]	78.5[c]	2.36	<0.001
72 h	59.1[a]	54.5[a]	59.3[a]	72.7[b]	74.2[b]	83.2[b]	2.13	<0.001
NDFD, %								
24 h	36.9[ab]	30.0[a]	35.1[ab]	48.6[cd]	41.7[bc]	57.3[d]	2.14	<0.001
48 h	50.0[a]	42.2[a]	50.4[a]	67.4[b]	70.4[b]	79.0[c]	2.08	<0.001
72 h	55.6[a]	48.1[a]	55.2[a]	74.2[b]	73.6[b]	88.0[c]	2.09	<0.001
ADFD, %								
24 h	38.6[ab]	31.4[a]	36.9[a]	49.2[bc]	42.7[ab]	55.7[c]	2.65	<0.001
48 h	52.6[b]	44.0[a]	54.0[b]	70.0[c]	71.9[c]	82.5[d]	1.94	<0.001
72 h	59.2[b]	50.9[a]	59.0[b]	76.3[c]	75.9[c]	88.5[d]	1.88	<0.001

*Degradability of dry matter (DMD), organic matter (OMD), crude protein (CPD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD).

[†]DH, untreated dry BH; W7, hydrated BH stored for 7 d; W14, hydrated BH stored for 14 d; CO5-7, hydrated BH + 5% CaO stored for 7 d; CO5-14, hydrated BH water + 5% CaO stored for 14 d; CO10, hydrated BH + 10% CaO stored for 14 d. Water was added to reach a final DM concentration of 50%.

[‡]Standard error of treatment means; *n* = 4 buckets/treatment.

[a–d] Within a row, means with different superscripts differ, *P* ≤ 0.05.

ruminal incubation time points, a treatment effect was observed (*P* < 0.001) for degradability of DM, OM, CP, NDF, and ADF, where CaO treatment promoted greater in situ degradability of BH when compared with the non-treated forage. The treatment difference is more prominent after 48, and up to 72 h of ruminal incubation, where CO10 promoted the greatest increase (*P* < 0.05) in degradability of DM, OM, NDF and ADF, followed by CO5-7 and CO5-14, which were not different (*P* > 0.05) from each other. Crude protein degradability was increased (*P* ≤ 0.011) after 24 h incubation in the rumen only when CO10 treatment was applied, whereas, after 72 h of incubation in the rumen, CP degradability was increased (*P* ≤ 0.028) by treating the forage with either 5 or 10% CaO. At 24 h of incubation in the rumen, it was observed that OM degradability was not different between CO10 and CO5-7 (*P* = 0.110); however, CO10 was greater than CO5-14 (*P* = 0.006). Although mold counts were not performed in the current experiment, the visual presence of mold after 14 d of bucket storage and lack of it at 7 d of bucket storage could explain this difference. This negative effect that could have
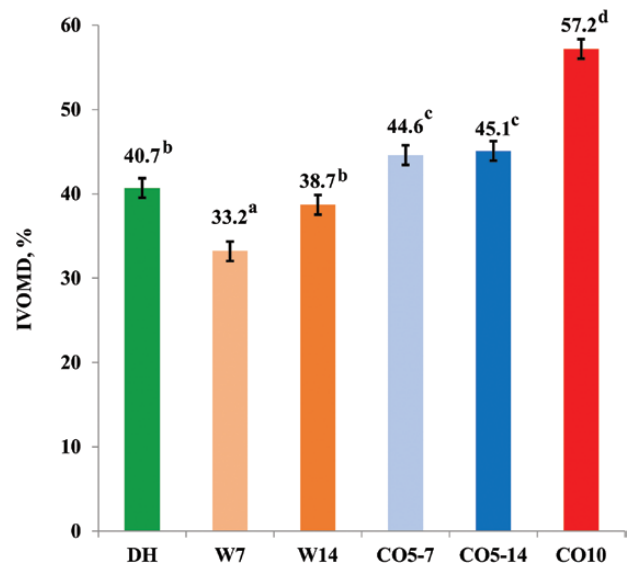

Figure 1. In vitro organic matter digestibility (IVOMD) of bahiagrass (*Paspalum notatum*) hay (BH) after treatment with water and calcium oxide (CaO) and storage for either 7 or 14 d. DH, untreated dry BH; W7, hydrated BH stored for 7 d; W14, hydrated BH stored for 14 d; CO5-7, hydrated BH + 5% CaO stored for 7 d; CO5-14, hydrated BH water + 5% CaO stored for 14 d; CO10, hydrated BH + 10% CaO stored for 14 d. Water was added to reach a final DM concentration of 50%. [a,b]Means with different superscripts differ; *P* < 0.001; error bars represent SEM; *n* = 4/treatment.

been caused by mold was probably diluted by the longer incubation in the rumen for 48 or 72 h, as indicated by the greater degradability of OM when CaO was applied at 10% when compared to 5%, regardless of bucket storage for 7 or 14 d.

In vitro OM digestibility of BH was increased (Figure 1; $P < 0.001$) with CaO treatment and was greatest when CO10 was applied (57.2% IVOMD), promoting a 41% increase when compared with DH (40.7% IVOMD) and a 28% increase when compared with CaO applied at 5% (average of 44.85% IVOMD between CO5-7 and CO5-14), regardless of length of storage. Although to a smaller extent, applying CaO at 5%, regardless of length of storage, promoted an increase of 10% in IVOMD of BH when compared with the dry forage (44.85 vs. 40.7% IVOMD).

Composition of BM before and after treatment with CaO and storage for either 7 or 14 d is presented in Table 3. Similar to BH, besides CP concentration, which was not affected ($P = 0.096$) by treatment, OM, NDF, and ADF concentration were reduced ($P < 0.001$) when CaO was applied compared with DH. The greatest reduction (36%) in NDF concentration was observed when CO10 was applied, followed by 5% CaO (18%), regardless of days of storage.

Ruminal in situ degradability of forage components of BM treated or not with CaO and stored for either 7 or 14 d are presented in Table 4. At all ruminal incubation time points, a treatment effect was observed ($P < 0.001$) for degradability of DM, OM, CP, NDF, and ADF. At 24 h, CO10 promoted greater ($P < 0.05$) degradability of DM, OM, and CP, when compared with 5% CaO application rate; however, NDF and ADF degradability did not differ ($P > 0.05$) among CO10, CO5-7, and CO5-14. At 48 and 72 h, no differences ($P > 0.05$) were

observed on degradability of DM, OM, CP, NDF, and ADF between CO10 and CO5-7 or CO5-14.

Similar to BH, IVOMD of BM was increased (Figure 2; $P < 0.001$) by 31% when CaO was applied to the forage and compared with DH; however, no differences ($P > 0.05$) were observed between CO10 and CO5, regardless of length of storage. These results indicate that the use of a greater amount of CaO is beneficial to improve degradability of nutrients and IVOMD of BH to a greater extent; however, these benefits of additional CaO do not apply to BM to the same extent and 5% CaO should be sufficient to generate near maximal benefit for BM. Chemical treatment is, in general, most beneficial when treating more lignified substrates (Fahey et al., 1993), meaning that forages with greater concentrations of lignin have a larger number of bonds between polysaccharides and lignin. Therefore, it could be hypothesized that bahiagrass hay potentially has more ester bonds between polysaccharides and lignin that can be impacted by treatment.

The results observed in the current experiment with BH and BM are in accordance with previous research where crop residues were treated in the same manner and reduction in NDF concentration, improved NDF digestibility, and improved in vitro DM digestibility were observed (Dahlke and Euken, 2013). Furthermore, when treating corn cobs, wheat straw, and corn stalks with CaO at 5% of the residue DM and adding water to reach 50% DM concentration, increases in IVOMD by 26%, 49%, and 41% were observed, respectively (Shreck, 2013).

The presence of water is necessary in order for the CaO to be converted into calcium hydroxide ($Ca(OH)_2$); thus, the addition of treatments containing just water was necessary to exclude its possible confounding effects. No effects of water

Table 3. Composition of Tifton 85 bermudagrass (*Cynodon* spp.) hay (BM) after treatment with water and calcium oxide (CaO) and storage for either 7 or 14 d

Item, % DM*	Treatment[†]						SEM[‡]	*P*-value
	DH	W7	W14	CO5-7	CO5-14	CO10		
DM	89.3	51.3	50.0	52.3	53.3	55.3	–	–
OM	93.7[c]	93.3[c]	93.0[c]	86.7[ab]	88.2[b]	84.1[a]	0.57	<0.001
CP	11.1	14.1	14.0	14.0	14.4	13.0	0.26	0.096
NDF	77.1[d]	74.7[cd]	72.0[c]	63.6[b]	63.4[b]	49.2[a]	0.83	<0.001
ADF	42.0[c]	39.4[bc]	37.8[b]	36.9[ab]	36.8[ab]	33.2[a]	0.40	<0.001

*DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

[†]DH, untreated dry BM; W7, hydrated BM stored for 7 d; W14, hydrated BM stored for 14 d; CO5-7, hydrated BM + 5% CaO stored for 7 d; CO5-14, hydrated BM water + 5% CaO stored for 14 d; CO10, hydrated BM + 10% CaO stored for 14 d. Water was added to reach a final DM concentration of 50%.

[‡]Standard error of treatment means; $n = 4$ buckets/treatment.

[a–d] Within a row, means with different superscripts differ, $P ≤ 0.05$.

Table 4. Ruminal in situ degradability of forage components of Tifton 85 bermudagrass (*Cynodon* spp.) hay (BM) after treatment with water and calcium oxide (CaO) and storage for either 7 or 14 d

Item*	DH	W7	W14	CO5-7	CO5-14	CO10	SEM[‡]	P-value
DMD, %								
24 h	46.3[a]	49.3[a]	49.5[a]	62.7[b]	67.1[b]	73.0[c]	1.26	<0.001
48 h	54.2[a]	61.5[ab]	63.0[b]	79.9[c]	77.9[c]	86.1[c]	1.77	<0.001
72 h	58.0[a]	64.3[a]	65.4[a]	78.9[b]	81.3[b]	88.1[b]	1.92	<00001
OMD, %								
24 h	44.1[a]	48.3[a]	48.1[a]	60.7[b]	66.2[c]	71.9[d]	1.24	<0.001
48 h	53.2[a]	60.6[b]	62.1[b]	78.9[c]	77.4[c]	85.6[c]	1.87	<0.001
72 h	54.6[a]	64.0[b]	64.3[b]	78.4[c]	81.0[c]	90.4[c]	1.85	<0.001
CPD, %								
24 h	65.1[a]	62.4[a]	61.2[a]	74.4[b]	79.2[b]	85.4[c]	2.02	<0.001
48 h	70.0[ab]	75.9[ab]	78.5[b]	89.2[c]	88.2[c]	94.0[c]	1.93	<0.001
72 h	75.1[a]	81.0[a]	80.1[a]	91.1[b]	90.7[b]	93.8[b]	1.55	<0.001
NDFD, %								
24 h	38.6[a]	43.0[a]	39.2[a]	56.1[b]	62.0[b]	60.3[b]	2.02	<0.001
48 h	46.8[a]	54.5[a]	55.7[a]	71.5[b]	75.3[b]	81.8[b]	2.33	<0.001
72 h	51.5[a]	59.9[a]	59.9[a]	78.3[b]	75.3[b]	81.1[b]	2.25	<0.001
ADFD, %								
24 h	34.9[a]	40.6[ab]	36.5[a]	50.0[bc]	55.5[c]	54.9[c]	2.25	<0.001
48 h	45.6[a]	53.8[a]	53.8[a]	69.6[b]	72.9[b]	78.9[b]	2.23	<0.001
72 h	48.5[a]	57.5[ab]	59.5[b]	76.4[c]	73.5[c]	78.9[c]	2.12	<0.001

*Degradability of dry matter (DMD), organic matter (OMD), crude protein (CPD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD).

[†]DH, untreated dry BM; W7, BM treated with water for 7 d; W14, BM treated with water for 14 d; CO5-7, BM treated with water + 5% CaO for 7 d. CO5-14, BM treated with water + 5% CaO for 14 d; CO10, BM treated with water + 10% CaO for 14 d. Water was added to reach a final DM concentration of 50%.

[‡]Standard error of treatment means; n = 4 buckets/treatment.

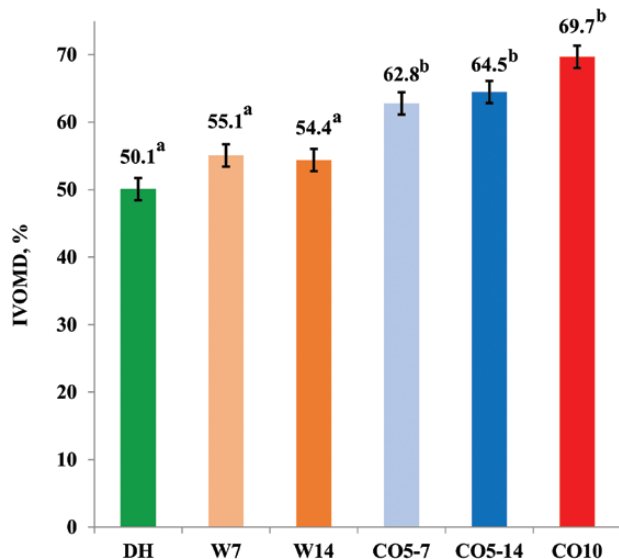[a-c]Within a row, means with different superscripts differ, P ≤ 0.05.

Figure 2. In vitro organic matter digestibility (IVOMD) of bermudagrass (*Cynodon* spp.) hay (BM) after treatment with water and calcium oxide (CaO) and storage for either 7 or 14 d. DH, untreated dry BM; W7, BM treated with water for 7 d; W14, BM treated with water for 14 d; CO5-7, BM treated with water + 5% CaO for 7 d. CO5-14, BM treated with water + 5% CaO for 14 d; CO10, BM treated with water + 10% CaO for 14 d. Water was added to reach a final DM concentration of 50%. [a,b]Means with different superscripts differ; P < 0.001; error bars represent SEM; n = 4/treatment.

addition compared to dry treatment were expected, and a few exceptions that were observed between W treatments and DH are speculated to be attributed to microbial degradation that might have occurred without the presence of a strong base such as CaO. The length of storage needed to allow the reaction to occur between forage and chemical treatment has been debated, and 7 or 14 d were chosen in the current experiment based on the most common reported values when treating crop residues (Shreck et al., 2011; Shreck et al., 2012). Research comparing IVDMD of corn stalks treated with 5% CaO at 3, 7, or 14 d after treatment showed that no changes were observed after 3 d of treatment and an increase in digestibility did not occur until 7 d of storage in most cases (Euken et al., 2013). Moreover, a small increase above 7 d was observed when IVDMD was performed after 14 d of storage (Euken et al., 2013). These results indicate that in order for treatment to be effective, a minimum of 7 d of storage is necessary and, perhaps, longer periods can promote greater effects; however, results were inconsistent since there was considerable variation in the IVDMD of treated

samples (Euken et al., 2013). Conversely, when different crop residues were treated with 5% CaO and stored for either 7, 14, or 28 d, no differences were observed between 7 and 14 d for corn cobs; however, storing the residue for 28 d reduced IVDMD by 6% (Shreck, 2013). In contrast, when evaluating wheat straw, storage for 14 d increased IVDMD by 11%, when compared to storage for 7 d, while it was not different from 28 d of storage (Shreck, 2013). These results indicate that the length of storage can be variable depending upon the type and composition of roughage.

CONCLUSION

Chemical treatment of warm-season forages common to the southeastern United States, such as bahiagrass and bermudagrass hay, with calcium oxide was shown to be an effective method of promoting greater digestibility of nutrients when applied at 5% of the forage DM and, in some cases, even greater when applied at a 10% rate. Moreover, treating the forages for longer than 7 d seemed to be unnecessary, since no differences between 7 and 14 d of storage were observed. Therefore, increase in forage digestibility through chemical treatment using CaO might potentially result in improved animal performance when forage-based diets are provided and translate into greater efficiency in beef production systems. Results obtained in the current experiments warrant further in vivo studies to be performed in order to evaluate the effects of treating warm-season forages with CaO on beef cattle ruminal fermentation parameters, total tract digestibility of nutrients, performance, and methane emissions.

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LITERATURE CITED

Ciriaco, F. M., D. D. Henry, V. R. Mercadante, T. Schulmeister, M. Ruiz-Moreno, G. C. Lamb, and N. DiLorenzo. 2015. Effects of different levels of supplementation of a 50:50 mixture of molasses:crude glycerol on performance, Bermuda grass hay intake, and nutrient digestibility of beef cattle. J. Anim. Sci. 93:2428–2438. doi:10.2527/jas.2015-8888.

Dahlke, G. R., and R. M. Euken. 2013. Calcium oxide and ammoniated whey treatment of cornstalks, oat hulls, wheat straw and drought stressed corn plants. In: Iowa State animal industry report. AS 659:ASL R2773. Ames: Iowa State University. doi:10.31274/ans_air-180814-169

Euken, R. M., N. Willamsen, M. J. Cecava, A. Grusby, and A. D. Midland. 2013. Demonstrating calcium oxide treatment of forages. In: Iowa State animal industry report. ASL R2776. Ames: Iowa State University. doi: 10.31274/ans_air-180814-602

Fahey, G. C., L. D. Bourquin, E. C. Titgemeyer, and D. G. Atwell. 1993. Postharvest Treatment of Fibrous Feedstuffs to improve their nutritive value. In: H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph, editors. Forage cell wall structure and digestibility. Madison, WI: ASA, CSA, SSSA; p. 715–766.

Jung, H. G., and D. A. Deetz. 1993. Cell wall lignification and degradability. In: H. G. Jung, D. R. Buxton, R. D. Hatfield, and J. Ralph, editors. Forage cell wall structure and digestibility. Madison, WI: ASA, CSA, SSSA; p. 315–346.

Klopfenstein, T. 1978. Chemical treatment of crop residues. J. Anim. Sci. 46:841–848. doi:10.2527/jas1978.463841x

Klopfenstein, T. J., V. E. Krause, M. J. Jones, and W. Woods. 1972. Chemical treatment of low quality roughages. J. Anim. Sci. 35:418–422. doi:10.2527/jas1972.352418x

Pedersen, J. F., K. P. Vogel, and D. L. Funnell. 2005. Impact of reduced lignin on plant fitness. Crop Sci. 45:812–819. doi:10.2135/cropsci2004.0155

Shreck, A. L. 2013. Use of alkaline treated crop residues as partial grain replacements for finishing cattle. PhD Diss. Univ. of Nebraska, Lincoln.

Shreck, A. L., C. D. Buckner, G. E. Erickson, T. J. Klopfenstein, and M. J. Cecava. 2011. Digestibility of crop residues after chemical treatment and anaerobic storage. In: 2011 Nebraska beef cattle report. Rep. No. 633. Lincoln: University of Nebraska; p. 35–36.

Shreck, A. L., B. L. Nuttelman, W. A. Griffin, G. E. Erickson, T. J. Klopfenstein, and M. J. Cecava. 2012. Chemical treatment of low-quality forages to replace corn in cattle finishing diets. In: 2012 Nebraska beef cattle report. Rep. No. 690. Lincoln: University of Nebraska; p. 106–107.

Shreck, A. L., B. L. Nuttelman, J. L. Harding, and W. A. Griffin. 2015. Digestibility and performance of steers fed low-quality crop residues treated with calcium oxide to partially replace corn in distillers grains finishing diets. J. Anim. Sci. 93:661–671. doi:10.2527/jas2013-7194

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18:104–111. doi:10.1111/j.1365-2494.1963.tb00335.x

Van Soest, P. J. 1994. Nutritional concepts. In: Nutritional ecology of the ruminant. 2nd ed. Ithaca, NY: Cornell University Press; p. 7–21.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Watson, A. K., J. C. MacDonald, G. E. Erickson, P. J. Kononoff, and T. J. Klopfenstein. 2015. Forages and pastures symposium: optimizing the use of fibrous residues in beef and dairy diets. J. Anim. Sci. 93:2616–2625. doi:10.2527/jas.2014-8780.