

SULFUR FERTILIZATION OF BERMUDAGRASS AND EFFECT ON DIGESTION OF NITROGEN, SULFUR, AND FIBER BY NONLACTATING COWS

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ABSTRACT

Sulfur fertilization effects on the N, S, and fiber digestibility of tropical forages have received little attention. To examine the effects of S fertilization on forage chemical composition, intake by nonlactating Holstein cows (Bos taurus), and subsequent N, S, and fiber digestion, a study was conducted using bermudagrass [Cynodon dactylon (L.) Pers. common] growing on a low S soil that had been fertilized with 0 (control) or 105 kg S ha⁻¹ as gypsum. The forage was harvested for hay after 31 d of regrowth from a 5-cm stubble height. Sulfur fertilization increased forage S concentration from 1.1 to 1.8 g kg⁻¹ dry matter (DM). The increase in forage S resulted from an increase in nonprotein S. Cows fed the S-fertilized bermudagrass hay demonstrated improved apparent digestibility of N (two percentage units), S (30.4 percentage units), and lignin (10.6 percentage units) and tended to consume more hay DM. Apparent digestibility of organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, and cellulose was similar between cows fed the different hays. High NDF concentration and low apparent S digestibility may have contributed to low DM intake (mean = 16.4 g kg⁻¹ body weight [BW]) of both hays. The present study indicates that S bioavailability may be low in bermudagrass and that further work should be done on the potential value of S supplementation or fertilization to increase the concentration of "available" S in diets based on Cynodon spp.

KEYWORDS: Cynodon dactylon, Forage Quality, Digestibility, Sulfur Fertilizers, Cattle

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INTRODUCTION

Mineralization of S in soil organic matter and deposition in rainfall may not provide adequate S for optimal forage growth in many areas of the tropics and subtropics (Pasricha and Fox, 1993). With increasing use of fertilizers having low S concentrations, tropical grasses often have unfavorably high N to S ratios (> 10 to 12) and are frequently deficient in S, preventing optimal growth of cell wall-degrading rumen microbes (Akin and Hogan, 1983; McDowell, 1985). Efficiency of N use by ruminants and plants also can be reduced at high N to S ratios (McDowell, 1985; Pasricha and Fox, 1993). The optimal N to S ratio from the perspective of the animal's requirements depends on the bioavailability of N and S in the forage. Thus N to S ratio may have little use in some situations (i.e., grasses with low S bioavailability or heat damaged forages or both; Goodrich and Garrett, 1986).

The data base for S bioavailability in tropical grasses is very limited (Goodrich and Garrett, 1986) and this may explain partially why the S requirement for ruminants consuming tropical grasses is not well defined. Estimated requirements range from about 1.5 to 3.5 g kg⁻¹ DM (McDowell, 1985). Sulfur-deficient forages characteristically have longer retention times in the reticulo-rumen and lower intake than S-adequate forages (Rees et al., 1974; Rees and Minson, 1978). In addition, animal responses to S-fertilized forages often have been greater than those obtained with S supplementation (Rees et al., 1974; 1982). These differences may be due in part to changes in cell wall composition, particularly lignin (Spears et al., 1985; Chestnut et al., 1986).

The objective of this study was to determine the influence of S fertilization of bermudagrass (one of the most important forage species in the tropics and subtropics) on forage chemical composition, intake by nonlactating Holstein cows, and subsequent N, S, and fiber digestion.

MATERIALS AND METHODS

This experiment was conducted at the University of Florida Dairy Research Unit, located 18 km north of Gainesville, FL (29° 60' N lat.). The area used was a 'Tifton 78' bermudagrass hay field that had been invaded by common bermudagrass (90% of the DM; small-leaved, strongly stoloniferous, local ecotype). The soil was a Chipley sand (thermic, coated Aquic Quartzipsammments) that was low in S. In April 1991, soil samples were collected from the Ap1 horizon (0- to 15-cm depth). Soil pH (1:2 soil:deionized H₂O ratio) was 5.8 and P, K, Ca, and Mg extractable by the Mehlich-1 "double acid" (0.05 M HCl + 0.0125 M H₂SO₄) procedure (Hanlon and DeVore, 1989) were 44, 12, 628, and 32 mg kg⁻¹, respectively. Sulfur extracted by the 0.25 M KCl 40°C procedure of Blair et al. (1991) was 5 mg kg⁻¹.

On 15 May 1991, one-half of the experimental field was fertilized with agricultural grade gypsum (157 g S kg⁻¹ and 206 g Ca kg⁻¹) at a rate of 105 kg S ha⁻¹, while the other half received no S. During the following three months the entire 1.6-ha site was harvested (5-cm stubble height) for green chop or hay three times (near daily rainfall prevented the making of good hay)

and received of NH_4NO_3 and KCl applications equivalent to a total of 130 kg N ha^{-1} and 100 kg K ha^{-1} . On 14 August (3 d after the third harvest) the entire site was fertilized with 65 kg N ha^{-1} as NH_4NO_3 . A fourth harvest was made on 11 September and clipped herbage (5-cm stubble height) from the separate halves of the field (no S [control hay] vs. S-fertilized hay) was allowed to dry outdoors for 2 d prior to storage as round bales in a barn. No rainfall occurred during the drying process. Bales of the control and S-fertilized hays were chopped to a length of 8 to 10 cm a few days prior to initiation of an intake and digestion trial on 10 Mar. 1992. Eight nonlactating Holstein cows (600 kg average body weight) were utilized in a two-period crossover design. Each period consisted of a 10-d adjustment period followed by a 5-d collection period. Daily hay offered was 105% of the previous days consumption. Voluntary intake and apparent digestibility of fiber, N, and S were measured simultaneously. Cows had free access to water ($12 \text{ mg SO}_4\text{-S L}^{-1}$) and common salt (NaCl). Chromic oxide was used as a marker to estimate daily fecal output (Williams et al., 1962). After the first 5 d of the adjustment period, animals were given 10.0 g of Cr_2O_3 in a gelatin capsule at 0700 and 1900 h for 10 consecutive days using a balling gun. During the collection period, fecal grab samples were taken immediately prior to administering the Cr_2O_3 capsules to the animals and placed in a freezer.

Chromium in the feces was determined by atomic absorption spectrophotometry following sample preparation by the procedure of Williams et al. (1962). Hay and fecal samples (corrected for DM percentage) were analyzed for total N (aluminum block digestion and colorimetric analysis; Jones and Case, 1990), total S (nitric acid digestion and inductively coupled plasma emission spectroscopy [ICPES] analysis; Jones and Case, 1990), ash-free NDF (Golding et al., 1985), ADF, and permanganate lignin (non-sequential; Goering and Van Soest, 1970). Hemicellulose was considered to be the difference between NDF and ADF, whereas cellulose was considered to be the difference between ADF and permanganate lignin (Goering and Van Soest, 1970). All samples were analyzed for DM by drying for 24 h at 105°C and OM by ashing for 15 h at 550°C . Nonprotein N and S in the hay samples were extracted under vacuum (to ensure complete wetting of the ground plant material) by the 0.015 M NaCl (30 v/w) procedure of Coto et al. (1990). Extracts were filtered through a Whatman no. 42 filter paper, deproteinized by placement in boiling water for 2 min to coagulate and precipitate protein, and refiltered through a $0.45\text{-}\mu\text{m}$ membrane filter. Nonprotein N and S were determined using a modified Kjeldahl technique (includes $\text{NO}_3\text{-N}$) and ICPES, respectively (Jones and Case, 1990). Protein N and protein S were considered to be the difference between the respective total and nonprotein fractions of N and S. In addition, hay samples were analyzed for total Ca, P, and K (nitric acid digestion and ICPES analysis; Jones and Case, 1990) and in vitro digestible OM (IVDOM; $\text{g DOM kg}^{-1} \text{ OM}$) concentration (modified two-stage procedure; Moore and Mott, 1974). Rumen fluid used in the IVDOM analysis came from a donor cow that was fed a diet of bermudagrass hay supplemented with soybean [*Glycine max* (L.) Merr.] meal.

Data were analyzed using PROC GLM of the Statistical Analysis System (SAS Institute, 1985).

RESULTS AND DISCUSSION

Chemical Composition. Chemical composition of the control and S-fertilized bermudagrass hays used in the feeding trial are presented in Table 1. Sulfur fertilization increased the total S concentration by 64% ($0.7 \text{ g kg}^{-1} \text{ DM}$). This increase was due largely to an increase in the nonprotein S fraction. Total N and nonprotein N were not affected greatly by S fertilization, although total N concentration was increased one percentage unit. Total N to S ratio was reduced as a result of S fertilization. The protein N to S ratio of approximately 16 for both hays was similar to that observed in other studies with forage grasses (Blair, 1979). It generally is accepted that the protein fraction of a particular plant species has a rather constant N to S ratio regardless of soil N and S status (Gaines and Phatak, 1982). Fiber concentrations were similar in both hays. Ash concentration was reduced in S-fertilized hay and this may be attributed to mineral dilution in approximately 18% greater DM yield (Mathews, unpublished data).

Forage Intake. Cows fed S-fertilized bermudagrass hay tended ($P = 0.14$) to consume more DM than cows fed control hay (Table 2). The DM intake in the present study was at the low end of the expected range of 15 to 25 $\text{g kg}^{-1} \text{ BW d}^{-1}$ (McDowell, 1985). This may be partially attributed to the high forage NDF concentrations (Table 1; Moore and Mott, 1973) and as will be discussed later, low availability of S. In a recent Florida study with sheep (*Ovis aries*), intake of S-fertilized bahiagrass (*Paspalum notatum* Flugge) hay grown during mid-August to early September (like the hays in the present study) was 22% ($4.6 \text{ g kg}^{-1} \text{ BW}$) lower than that observed with hays grown during mid-May to early June (Flores et al., 1993). This phenomenon has not been explained by standard forage fiber analyses for quality related characteristics. Flores et al. (1993) also observed similar trends with S-fertilized dwarf elephantgrass (*Pennisetum purpureum* Schum.), but the decrease in intake due to season was about half the magnitude of that observed with bahiagrass.

Digestibility. Apparent digestibility of OM was similar between treatment groups, averaging 53% (Table 2). This is lower than the 58% digestion coefficients reported by Bernal (1993) for nonlactating Holstein cows fed 4-wk old 'Tifton 81' bermudagrass silage. Cows consumed similar amounts of digestible OM regardless of bermudagrass hay source (Table 2).

Apparent digestibility of NDF, ADF, hemicellulose, and cellulose was not different between hays (Table 2). Apparent lignin digestibility was doubled ($P = 0.003$) as a result of S-fertilization (Table 2). Similarly, Spears et al. (1985) reported that S fertilization increased apparent digestibility of permanganate lignin by 1.7 fold in a study using steers fed orchardgrass (*Dactylis glomerata* L.) hays that had greater S concentrations than either hay in the present study. It is possible that S fertilization altered type or ratio of phenolic monomers in bermudagrass lignin, which consequently could alter lignin resistance to digestion (Chestnut et al., 1986). Alternatively, S fertilization may have enhanced the lignin digesting capability of the rumen microbial population by increasing S availability (Akin and Hogan, 1983). Apparent S digestibility was low for both hays, although apparent S digestibility with the S-fertilized hay

was 30.4 percentage units greater ($P = 0.0001$) than that observed with the control (Table 2). Apparent N digestibility was greater ($P = 0.02$) with the S-fertilized hay but the difference was small (two percentage units; Table 2).

Rees and Minson (1978) evaluated control and S-fertilized 'Pangola' digitgrass (*Digitaria decumbens* Stent.) hays in Australia with N and S concentrations and ratios similar to the hays used in the present study. They found that intake of DM by sheep was increased by 10.5% with S-fertilized hay. There was no difference in apparent OM digestibility and the difference in intake was eliminated by S supplementation to the diet. Other studies with sheep have demonstrated both intake and apparent DM digestibility responses to S-fertilized Pangola digitgrass hays (Rees et al., 1974) and intake responses to S-fertilized 'Taiwan' digitgrass (*Digitaria pentzii* Stent.) hays (Rees et al., 1982). In both studies (Rees et al., 1974; 1982) intake responses to S-fertilized hay were greater than those obtained with S supplementation of control hays. Reasons for these differences are not known (Akin and Hogan, 1983). In the study with Taiwan digitgrass (N concentration of both hays was 12.0 g kg^{-1} and S concentrations of the control and S-fertilized hays were 1.1 g kg^{-1} [N to S ratio = 10.9] and 1.7 g kg^{-1} [N to S ratio = 7.1], respectively), Rees et al. (1982) found that while S fertilization increased hay intake by 36%, apparent OM digestibility decreased by 4.9 percentage units, presumably due to reduced retention time of digesta in the reticulo-rumen (Rees and Minson, 1978).

Based on herbage N concentration and the prediction equation of Milford and Minson (1965), the apparent N digestibility values in the present study are in close agreement with expected values for tropical/subtropical pasture species. Apparent S digestibility of both hays is considered low because it generally is thought to be only slightly lower than that of apparent N digestibility (Kennedy, 1974; Spears et al. 1985). However, Kennedy (1974) and Goodrich and Garrett (1986) indicated that some forage grasses may have low S bioavailability. When S is deficient due to low bioavailability and/or concentration, intake may be reduced. If little S is provided by the diet, digestion rate and retention time of feed particles in the reticulo-rumen may be directly dependent on the rate and extent of S recycling.

As demonstrated by Spears et al. (1985), the present study indicates that the bioavailability of S in forages may be increased by fertilization to increase the concentration of nonprotein S. Further work should be conducted on S availability in relation to N in tropical grasses and the potential value of S supplementation. In a study with stargrass (*Cynodon nlemfuensis* Vanderyst) hays containing 2.0 g S kg^{-1} (N to S ratio = 7.0), Guardiola et al. (1983) found that DM intake and apparent DM digestibility were increased by 23% ($4.4 \text{ g kg}^{-1} \text{ BW}$) and $72 \text{ g kg}^{-1} \text{ DM}$, respectively, when sheep were supplemented with $1.5 \text{ g S kg}^{-1} \text{ DM}$ on offer. The form of S supplement used, Na_2SO_4 vs. D,L-methionine, had similar effects. Apparently, herbage N to S ratio or herbage S concentration is of little practical value in predicting response to S supplementation in situations where S bioavailability is low (Goodrich and Garrett, 1986).

CONCLUSION

Sulfur-fertilized bermudagrass hay improved apparent N digestibility slightly, markedly increased apparent digestibility of S (30.4 percentage units) and lignin (10.6 percentage units), tended to increase intake, but had no effect on apparent digestibility of OM, NDF, ADF, hemicellulose, or cellulose in comparison to the control hay. High NDF concentration and low apparent S digestibility may have contributed to low DM intake of both hays. Further work should be done on S bioavailability in tropical grasses (including seasonal differences) and the potential value of S supplementation or fertilization to increase the concentration of "available" S in diets based on these forages. The present study and the study by Guardiola et al. (1983) indicate that S bioavailability may be low in *Cynodon* spp. The influence of S-fertilization on lignin composition and digestibility also should be investigated further.

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Table 1. Chemical composition and IVDOM of bermudagrass hay grown with and without (control) sulfur fertilizer.

Item [†]	Control	S-fertilized
Dry matter (g kg ⁻¹)	892.0	898.0
Ash (g kg ⁻¹ DM)	48.8	43.3
NDF (g kg ⁻¹ DM)	796.0	810.0
ADF (g kg ⁻¹ DM)	368.0	369.0
Hemicellulose (g kg ⁻¹ DM)	428.0	441.0
Cellulose (g kg ⁻¹ DM)	309.0	303.0
Lignin (g kg ⁻¹ DM)	59.0	66.0
Total N (g kg ⁻¹ DM)	16.6	17.7
Protein N (g kg ⁻¹ DM)	11.5	12.4
Nonprotein N (g kg ⁻¹ DM)	5.1	5.3
Total S (g kg ⁻¹ DM)	1.1	1.8
Protein S (g kg ⁻¹ DM)	0.7	0.8
Nonprotein S (g kg ⁻¹ DM)	0.4	1.0
Total N/Total S	15.1	9.8
Protein N/protein S	16.4	15.5
Ca (g kg ⁻¹ DM)	4.6	4.2
P (g kg ⁻¹ DM)	2.7	2.4
K (g kg ⁻¹ DM)	15.3	14.3
IVDOM (g kg ⁻¹ OM)	484.0	489.0

[†] DM = dry matter, OM = organic matter, NDF = ash-free neutral detergent fiber, ADF = ash-free acid detergent fiber, hemicellulose = NDF - ADF, cellulose = ADF - lignin, lignin = permanganate lignin, IVDOM = in vitro digestible organic matter.

Table 2. Intake and apparent digestibility of bermudagrass hay grown with and without (control) sulfur fertilizer.

Item [†]	Control	S-fertilized	SE [‡]	F-test [§]
DM intake (g kg ⁻¹ BW d ⁻¹)	16.1	16.7	0.2	0.14
OM digestibility (%)	52.8	53.6	0.6	0.40
DOM intake (g kg ⁻¹ BW d ⁻¹)	8.1	8.6	0.2	0.18
NDF digestibility (%)	54.6	56.0	0.6	0.18
ADF digestibility (%)	48.3	49.8	0.7	0.18
Hemicellulose digestibility (%)	60.0	61.1	0.6	0.29
Cellulose digestibility (%)	55.9	56.5	0.8	0.59
Lignin digestibility (%)	8.7	19.3	1.6	0.003
N digestibility (%)	57.1	59.1	0.4	0.02
S digestibility (%)	6.4	36.8	1.9	0.0001

[†] DM = dry matter, BW = body weight, OM = organic matter, DOM = digestible organic matter, NDF = ash-free neutral detergent fiber, ADF = ash-free acid detergent fiber.

[‡] Standard error of a treatment mean.

[§] Probability levels.