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Grazing cotton crop residue to reduce winter supplementation cost in late-gestation beef cows and assessment of the negative effects of gossypol on fermentation of mixed ruminal microorganisms*

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ABSTRACT

Objective: This research aimed to evaluate the use of standing cotton crop residue as a winter supplementation source for gestating beef cows and the effects of gossypol, a naturally occurring pigment with potential anti-quality characteristics, on in vitro mixed ruminal microorganism fermentations.

Materials and Methods: Experiment 1 used a 3-yr completely randomized study with 108 Angus cross cows (BW 559 ± 7.9 kg) enrolled in a 30-d grazing trial. Cows were weighed, stratified by weight, and randomly assigned to 1 of 2 treatments: dormant bermudagrass (*Cynodon dactylon*) pasture + bermudagrass hay (GR) or cotton (*Gossypium*) crop residue + bermudagrass hay (CT). Experiment 2 used a completely randomized in vitro mixed ruminal microorganism fermentation with 3 × 5 factorial arrangements of treatments. This included 3 substrates: corn (*Zea mays*; CC), average relative forage quality (RFQ 100) coastal bermudagrass (ABG), and high relative forage quality (RFQ 120) coastal bermudagrass (HBG), along with 5 concentrations of gossypol: 0, 0.0615, 0.123, 0.1845, or 0.246 mg of gossypol/mL.

Results and Discussion: Total hay offered and hay per head per day were greater ($P \leq 0.02$) in the GR group compared with the CT group. Economic analysis indicated that total hay cost, cost per head per day, and cost of gain were all significantly decreased for cows in the CT treatment compared with cows in the GR treatment ($P < 0.002$). In vitro DM digestibility and total VFA production were lower ($P < 0.002$) as gossypol concentration reached the highest concentration.

Implications and Applications: Results suggest that grazing cotton crop residue is a partial replacement for hay supplementation during the winter months. Increased levels of gossypol have negative effects on DM disappearance and VFA production. The gossypol amount found in crop residue is approximately the same as the lowest concentration evaluated in the in vitro experiment. These results indicate that grazing cotton crop residue should not influence ruminal fermentation.

Key words: cotton crop residue, bermudagrass, gossypol, cost of gain

INTRODUCTION

By-products of cotton production (e.g., whole cottonseed, cottonseed meal, cottonseed hulls, and cotton gin by-product, also referred to as gin trash) are often used in the beef cattle industry as supplementary or low-cost feedstuffs. Whole cottonseed and cottonseed meal are sources of protein and energy for ruminants, and cottonseed hulls and gin by-product can be used as a source of roughage (Rogers et al., 2002). Gin by-product is similar to cotton left in the field after harvest, as it consists of stems, leaves, burrs, and immature seeds (Hill et al., 2013). Cotton gin by-product can be fed to beef cows with additional supplementation such as whole cottonseed (Sagebiel and Cisse, 1984).

Unfortunately, there are limits to how much cotton by-products can be fed to cattle because of the relatively high fat content of whole cottonseed and the gossypol content (Rogers et al., 2002). Although ruminants are less sensitive to gossypol than monogastrics, due to ruminal microbial degradative activity, problems still arise when feeding cattle large amounts of cotton by-products, such as anemia, anorexia, and decreased growth rate (Chase et al., 1994; Calhoun et al., 2004).

Beef production and profitability in the southeastern United States is largely dependent on weather, rainfall, and forage production. Most herds in the southeast calve in the spring, which means that, during the winter months, nonlactating, pregnant cows have relatively low

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nutritional requirements. However, in years of inclement weather and poor forage yield, cattle are often in need of additional feed supplementation to maintain their body condition during the winter. Using cotton by-product feeds or grazing crop residues, or a combination thereof, can be economical and convenient solutions to maintaining cow body condition scores and meeting the cow's nutrient requirements. Cotton crop residue is similar to gin by-product, and grazing this residue can potentially meet the maintenance requirements of nonlactating, pregnant cows (Stewart and Rossi, 2022).

The objectives of this study were to (1) determine growth performance and economic benefits of grazing cotton crop residue as a partial replacement for hay and (2) determine the effect of gossypol on a mixed ruminal microorganism fermentation of feedstuffs commonly supplemented in forage-based cattle production.

MATERIALS AND METHODS

Experiment 1: Cattle Consumption of Cotton Crop Residue

Animal Care and Use. All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee prior to the start of the study (AUP no. A2018 11-007-Y2-A1).

Animals and Treatments. A 30-d experiment was conducted in 3 consecutive years using 108 Angus (*Bos taurus*) crossbred cows. Each year, 36 late-gestation cows at the University of Georgia's J. Phil Campbell Sr. Research Center (Watkinsville, GA) were weighed (BW 559 ± 7.9 kg), stratified by weight, and randomly assigned to 1 of 2 treatments: dormant bermudagrass (*Cynodon dactylon*) pasture + bermudagrass hay (**GR**) or cotton (*Gossypium*) crop residue + bermudagrass hay (**CT**). Bermudagrass pastures used in the GR treatment were grazed before the experiment to remove stockpiled forage in an effort to limit intake to only the hay offered. Pasture was used as the experimental unit, with 2 replicates per treatment each year. Eighteen animals per treatment were used each year. Four fields (two 2.43-ha and two 4.86 ha) were used during the study, with one field of each size in cotton residue and the other in bermudagrass. Each treatment was represented by a 2.43- and 4.86-ha field. Stocking rate was held constant across experimental units at 0.40 animals per hectare, resulting in 6 cows per 2.43-ha field and 12 cows per 4.86-ha field.

Sample Collection. Each year, after cotton was harvested from each field, standing cotton crop residue was quantified before grazing. In each field, 4 locations were randomly selected, and a 1.11-m^2 (1.22-m length × 0.91-m row spacing) section of a cotton row was harvested to a 7.5-cm stubble height. Total plant mass from each location was weighed to estimate total residue. Samples were separated into stalk, lint and seed, and boll and leaf, and each was weighed to determine individual component amounts and edible residue. Weights were recorded, and samples were transported to an oven to be dried at 60°C for 48 h. After drying, the samples were removed from the oven and weighed again to determine DM percentage. Each of the 4 samples was used to determine a pasture average of standing residue per 1.11 m^2. Dry weights for lint, seed, leaf, and boll were used in the following equation to determine standing edible residue per hectare:

$$\text{Residue per hectare} = \frac{10{,}000\,\text{m}^2 \text{ per hectare}}{1.11\,\text{m}^2 \text{ sample}} \times \text{DM weight for lint, seed, leaf, and boll.}$$

Additionally, cotton residual samples were analyzed for nutrient content. A representative sample was collected by starting in each corner of the field and walking to the adjacent corner, in the shape of an X, collecting material every 40 steps. At each location, a hand-grab sample was collected, to mimic the selection of edible material, similar to what the cows would select. This included lint, seed, and leaf. Samples were weighed, dried at 60°C for 24 h, and submitted to the Agricultural and Environmental Services Laboratory at the University of Georgia (Athens, GA) to be analyzed via wet chemistry. Each year, a cotton residue sample was submitted to the Georgia Department of Agriculture (Atlanta, GA) for pesticide screening. No compounds were detected in any year above stated tolerance levels (Code of Federal Regulations, 2022).

Animal and Hay Management. The grazing experiment was initiated during the first week of December each year. In year 1, cows were vaccinated with Pyramid 10 and drenched with Synanthic (Boehringer Ingelheim) on d 0. In years 2 and 3, cows received Synanthic, Clean Up 2 (Bayer Animal Health), and Express 5vl5 (Boehringer Ingelheim) on d 0. Weights were recorded for all cows on d 0 and 30 to calculate changes in body weight and average daily gain. Before feeding, all hay bales were identified and weighed, and a representative sample was collected and submitted for chemical analysis at the Agricultural and Environmental Services Laboratory (Athens, GA). Throughout the feeding trial, bale weights were recorded as they were delivered to each pasture. Hay was offered ad libitum to the cows in the GR treatment starting on d 0 and continuing until d 30. Hay was not offered to cows in the CT treatment until d 19 of each year, to allow the cattle to adapt to grazing the cotton residue. All pastures had ad libitum access to fresh, clean water.

Statistical Analysis. A completely randomized design was used, and pasture was the experimental unit. Treatment, pasture, and treatment × year interaction were used as fixed effects, and year was considered a random variable. All data were analyzed using PROC MIXED in SAS (Version 9.4; SAS Institute Inc.). Significance was declared when $P < 0.05$.

Experiment 2: In Vitro Fermentations

Animal Care and Use. All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (AUP no. A2018 10-023-Y2-A0).

Treatments and Stock Solution. An in vitro batch culture technique was used to evaluate multiple dietary treatments, using the Ankom Gas Production Measurement System (2019, Ankom Technology). In this, a 3 × 5 factorial design was used evaluate 3 substrates: corn (*Zea mays*; **CC**; 9.5% CP and 85.6% TDN), average relative forage quality (**RFQ** = 100, 12.0% CP, and 52.2% TDN) Tifton 85 bermudagrass (*Cynodon dactylon*; **ABG**), and high relative forage quality (RFQ = 120, 19.5% CP and 59.8% TDN) Tifton 85 bermudagrass (**HBG**), with 5 concentrations of gossypol (Sigma-Aldrich): **0**× (no gossypol), **1**× (0.0615 mg of gossypol/mL of ruminal fluid), **2**× (0.123 mg/mL), **3**× (0.1845 mg/mL), and **4**× (0.246 mg/mL). A gossypol stock solution was created by dissolving 1 g of gossypol in 10 mL of ethanol to reach a final concentration of 0.1 mg of gossypol/μL of ethanol.

Sample Preparation. Samples of the 3 feedstuff substrates were ground (Wiley Mill, Thomas Scientific) to pass through a 1-mm screen. Approximately 0.5 g of substrate was weighed and placed in an Ankom F57 filter bag (Ankom Technology) that was previously weighed. Bags were sealed using an impulse sealer (American International Electric) and randomly assigned to a 250-mL bottle (Ankom Technology). Bottles were randomly assigned to 1 of the 5 gossypol dosages, plus 1 blank bottle. Each bottle (n = 16) contained 3 bags of the same substrate, for a total of 48 bags.

Ruminal Fluid Collection and Bottle Preparation. Ruminal fluid was collected from 3 ruminally cannulated steers grazing bermudagrass pastures at the University of Georgia's J. Phil Campbell Sr. Research Station. After the cannula was removed, whole ruminal contents were agitated by hand, and samples were obtained from multiple locations in the rumen to obtain a representative sample. Ruminal contents were strained through paint strainers (Reaves and Company), to remove large particles, into a 1-L insulated bottle that had been previously heated with warm water (39°C). One liter of ruminal fluid was collected from each steer and transported back to the laboratory. Pooled ruminal fluid was added (33% vol/vol) to anoxic medium, as described by Cotta and Russell (1982) and Callaway and Martin (1996). Medium composition was as follows: 292 mg/L of K_2HPO_2, 240 mg/L of KH_2PO_4, 480 mg/L of $(NH_4)\ _2SO_4$, 480 mg/L of NaCl, 100 mg/L of $MgSO_4 \cdot 7H_2O$, 64 mg/L of $CaCl_2 \cdot 2H_2O$, 4,000 mg/L of Na_2CO_3, and 600 mg/L of cysteine·HCl. The mixed ruminal microorganisms were allowed to equilibrate while being sparged continuously with O_2-free CO_2 for 10 min before being aliquoted anaerobically (200 mL) to O_2-free, CO_2 flushed bottles. After being filled with mixed ruminal microorganisms, the bottles were sealed and incubated in an oscillating incubator (Sheldon Manufacturing Inc.). Mixed ruminal microorganism fermentations were incubated for 24 h at 39°C and 127 rpm.

IVDMD and pH. Bottles were removed after 24 h of fermentation, pH was immediately recorded (pH 11 Series, Cole-Parmer Scientific), and 10 mL from each fermentation was collected and immediately frozen at −20°C until further VFA and ammonia nitrogen analysis. Bags were removed from each fermentation bottle and rinsed with cold water to cease fermentation until rinse water appeared clear and then placed in an oven to dry at 60°C for 24 h. Once dry, bags were weighed, and IVDMD was calculated. Blank bags were used as a correction factor.

VFA and Ammonia Nitrogen. Frozen samples were thawed before VFA determination. Once thawed, samples were centrifuged at 10,000 × *g* for 10 min at 20°C, and 1 mL of the resulting supernatant was combined in a 5:1 ratio with 0.2 mL of metaphosphoric acid (25% wt/vol), mixed, and frozen overnight. The following day, samples were thawed and centrifuged at 10,000 × *g* for 10 min at 20°C before 0.8 mL of the supernatant was mixed with 1.6 mL of ethyl acetate (1:2 ratio), vortexed for 15 s, and allowed to sit and separate for 5 min. Next, 0.8 mL of the top portion was transferred to screw-thread vials for analysis of short-chain fatty acids in a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation) equipped with a flame ionization detector and a capillary column (Zebron ZB-FFAP; 30 m × 0.32 mm × 0.25 μm; Phenomenex Inc.). Sample injection volume was 1.0 μL, and helium was used as the carrier gas. Column temperature was initially set at 110°C and gradually increased to 200°C, increasing 14°C/min for 6.5 min. Injector and detector temperatures were set at 250 and 350°C, respectively.

Ammonia nitrogen was measured in duplicate by adding 50 μL of sample into disposable 16 × 100 culture tubes with 1 mL of phenol reagent, 1 mL of hypochlorite reagent, and 10 mL of distilled water (Chaney and Marbach, 1962). Samples were incubated at room temperature for 30 min before absorbance was measured. Ammonia concentrations were determined using absorbance (630 nm), which was determined using a model Genesys 30 spectrophotometer (Thermo Scientific).

Statistical Analysis. A completely randomized design with 3 × 5 factorial arrangements of treatments was used. The bottle was the experimental unit for pH, ammonia, acetate, butyrate, propionate, and total VFA, with substrate and level of gossypol as main effects. The bag was the experimental unit for DM disappearance, and substrate, level of gossypol, and their interaction were used as main effects. All data were analyzed using the PROC GLM procedure of SAS (Version 9.4; SAS Institute Inc.). Differences were determined by comparing least squares means. Significance was determined at $P < 0.05$ and tendency at $0.05 < P < 0.10$.

Table 1. Amount of cotton crop residue left in the field after harvest[1] on a DM basis

Component,[2] kg/ha	Year 1	Year 2	Year 3	SEM	*P*-value
Seed	248.8[b]	130.0[c]	317.3[a]	22.7	<0.001
Lint	252.5[b]	129.1[c]	328.0[a]	21.6	<0.001
Boll/leaf	571.6[a]	413.7[b]	575.2[a]	47.9	0.049
Stalks	2,392.2[a]	701.9[c]	1,259.1[b]	124.7	<0.001
Edible residue[3]	1,073.0[a]	670.8[b]	1,220.5[a]	80.4	<0.001

[a–c]Means with differing superscripts within a row are different ($P < 0.05$).

[1]Cotton was harvested with a spindle picker.

[2]Means presented are averages of eight 1.22-m samples taken each year.

[3]Edible residue per hectare excludes stalks.

RESULTS AND DISCUSSION

Experiment 1

Cotton Crop Residue. There were greater amounts ($P < 0.05$) of edible cotton crop residue and boll or leaf per hectare in yr 1 and 3 compared with yr 2, but yr 1 and 3 did not differ ($P > 0.21$). The amount of seed and lint per hectare was greatest in year 3 and was greater in yr 1 than yr 2 ($P < 0.01$). There was a greater amount of stalks per hectare in yr 1 than 2 and 3 ($P < 0.001$) and more stalks in yr 3 compared with yr 2 ($P < 0.007$). A different cotton harvester was used in yr 2, due to mechanical issues unrelated to this study. The use of different harvesting equipment each year could explain, in part, the large differences in the amounts of cotton crop residue left in the field. Environmental factors, such as rainfall, may also explain the differences in cotton crop residue from yr 1 to 2. The J. Phil Campbell Sr. Research Station received 60.5, 42.1, and 54.6 cm of rain from May 1 to September 30 in 2018, 2019, and 2020, respectively (University of Georgia Weather Network, 2020).

Nutrient Analysis and Gossypol. Nutrient analyses for cotton and hay across 3 yr are presented in Table 1. Whole cottonseed is a great source of protein, energy, and fiber (Rogers et al., 2002). Although cotton crop residue consists of all parts of the cotton plant, the amount of seeds present (20–24%; Table 2) in residue is likely the reason for protein and energy values greater than those reported in NASEM (2016) for cotton gin by-product (48.5% TDN, 12.3% CP), yet lower than whole cottonseed (22.8% CP, 93% TDN). Lower CP and TDN values in yr 2 could be explained by the reduced amount of seeds present due to differences in harvesters (Table 1) and decreased rainfall. Despite the variation in CP and TDN values across years, all cotton and hay exceeded the nutrient requirements of late-gestation beef cows, which is 7.9% CP and 54% TDN (NASEM, 2000).

Gossypol content is presented in Table 1. There are 2 forms of gossypol: free and bound (Gadelha et al., 2014). The free form of gossypol is biologically active and toxic, whereas the binding process renders gossypol biologically inactive. The bound form is created when gossypol binds with another compound (i.e., minerals, AA; Gadelha et al., 2014), often during processing due to high pressure and temperature. Additionally, gossypol can be bound in the rumen or intestine and excreted in the feces or absorbed by the animal and removed by bile (Barraza et al., 1991). Total gossypol is a summation of the free and bound forms. Total gossypol production is affected by several factors, including temperature, rainfall, and species. Gossypol production has shown to be positively correlated with the amount of rainfall and negatively correlated with temperature (Pons et al., 1953). Gossypol is present in all parts of the cotton plant, but it is most highly concentrated in the seeds. Reported free gossypol amounts in the current study are similar to amounts reported by Santos et al. (2002), which were 0.71 to 0.73% free gossypol. Bertrand et al. (2005) reported total gossypol (0.62–0.64%) of traditional and 3 genetically modified cottonseed; these amounts were lower compared with the current research. However, Jacobs et al. (2022) reported similar total ($1.2 \pm 0.2\%$) and free ($1.0 \pm 0.2\%$) gossypol ranges

Cow Performance. Initial and final body weights for cows in the GR and CT treatments were similar ($P > 0.70$; Table 3). There was no treatment effect, year effect, or treatment × year interaction for average daily gain ($P > 0.10$). Cranston et al. (2006) and Huerta-Leidenz et al. (1991) reported that cattle supplemented with cottonseed had similar ADG to cattle supplemented with other common feedstuffs in finishing rations. Also, Hill et al. (2000) reported that cotton gin by-product could serve as a replacement for hay for winter supplementation of nonlactating beef cows. Although grazed residue has different nutrient composition compared with cotton gin by-product and whole cottonseed, this research demonstrates that it will serve as a replacement for hay.

Hay and Economic Analysis. Total amount of hay offered, hay per day, and hay per head per day were greater in the GR group compared with the CT group ($P < 0.05$; Table 4). There was a year effect ($P \leq 0.02$) and

Table 2. Nutrient analysis of cotton crop residue and bermudagrass hay on a DM basis[1]

	Year 1			Year 2			Year 3		
	Cotton[2]			Cotton			Cotton		
Chemistry analysis	**WB**	**P1**	**Hay**	**WB**	**P1**	**Hay**	**WB**	**P1**	**Hay**
Crude protein, %	24.1	22.9	12.3	14.0	14.3	11.2	10.7	11.2	9.8
Crude fiber, %	38.4	37.8	28.5	50.2	47.9	30.5	49.8	56.4	29.1
Acid detergent fiber, %	56.4	58.1	37.5	64.0	60.9	39.0	63.3	60.7	37.1
Neutral detergent fiber, %	66.6	65.4	62.0	65.7	66.3	66.9	70.4	70.0	63.4
Total fat, %	5.69	8.35	—	5.75	5.95	—	5.77	7.69	—
Total digestible nutrients,[3] %	62.7	62.9	59.0	59.7	59.6	56.5	56.5	56.8	57.5
NE_m, Mcal/kg	1.40	1.40	1.27	1.30	1.29	1.19	1.19	1.20	1.22
NE_g, Mcal/kg	0.81	0.81	0.69	0.72	0.71	0.28	0.62	0.63	0.65
Gossypol,[4] %									
Free	0.73	0.82	—	0.66	0.73	—	1.13	1.05	—
Total	1.12	1.17	—	0.79	0.87	—	1.35	1.19	—

[1]Chemical analysis was performed by the Feed and Environmental Services Laboratory (Athens, GA).

[2]WB = Wellbrook, 2.43-ha cotton field; P1 = 4.86-ha cotton field.

[3]Hay TDN = 86.96 + 0.43 × (0.827 × CP − 3.52) − 1.080 × (3.72 + 0.4 × NDF); cotton TDN = 90.93 + 0.41 × (0.827 × CP − 3.52) − 1.150 × (3.72 + 0.4 × NDF).

[4]Gossypol analysis was performed by ATC Scientific (Little Rock, AR).

treatment × year interaction ($P \leq 0.05$) for hay per head per day and hay cost per head per day. Hay per head per day and hay cost per head per day were decreased in yr 3 ($P \leq 0.001$) compared with yr 1 and 2, which were not different ($P \geq 0.83$). There was not a year effect or treatment × year interaction for total hay, hay per day, total hay cost, or cost of gain ($P \geq 0.054$). Economic analysis results indicated that total hay cost, cost per head per day, and cost of gain were all significantly lower for cows in the CT treatment compared with cows in the GR treatment ($P < 0.05$). A hay value of \$110/metric ton was used for economic calculations. The stocking rate was maintained at 0.40 cows/ha throughout the study, to identify how many days cows could graze cotton crop residue at this rate. Cows in the CT treatment consumed less hay due to the cotton residue providing enough nutrients for the first 19 d of each grazing periods and partially for the following 11 d. Similarly, Stewart and Rossi (2022) reported that grazing cotton stalks reduced the amount of hay fed (0.6 kg/d) compared with hay only (12.2 kg/d) over a 30-d period to nonlactating, pregnant beef cows. However, the amount fed while grazing cotton residue was lower compared with the current study and is likely due to differences in harvest technique.

Table 3. Body weight measurements of late-gestation beef cows (n = 106)

Weight, kg	GR[1]	CT[2]	SEM	*P*-value
Initial[3]	557	560	11.7	0.858
End[4]	584	578	11.8	0.701
ADG	0.87	0.59	1.10	0.104

[1]GR = bermudagrass pasture and bermudagrass hay.

[2]CT = cotton crop residue and bermudagrass hay.

[3]Initial weights were recorded on d 0.

[4]End weights were recorded on d 30.

Experiment 2

IVDMD. Gossypol concentration and the type of substrate used both influenced IVDMD ($P < 0.001$), and there was a tendency ($P = 0.057$) for an interaction between substrate and gossypol concentration in the in vitro mixed ruminal microorganism fermentations (Figure 1). Dry matter disappearance was greatest for 0× (57.7%), decreased at other concentrations ($P < 0.002$) except that 3× (48.6%) was similar to 1× (51.1%) and 2× (47.0%), and was lowest at 4× (41.4%). Dry matter disappearance was greatest ($P < 0.001$) for CC (71.4%), and both CC and HBG (40.2%) were greater than ABG (35.8%; $P < 0.001$). Gossypol binds to protein (Willard et al., 1995) and minerals (Gadelha et al., 2014) in the rumen, which may explain decreased IVDMD. Binding of gossypol to nutrients could make them unavailable to both the ruminal microorganisms and the animal, producing a change in the ruminal and gastrointestinal microbial population. In vitro DM digestibility values in the current study for CC are similar to those reported via in situ by Peter et al. (2000), and the lower IVDMD values for ABG and HBG are likely related to the maturity and quality of the forage.

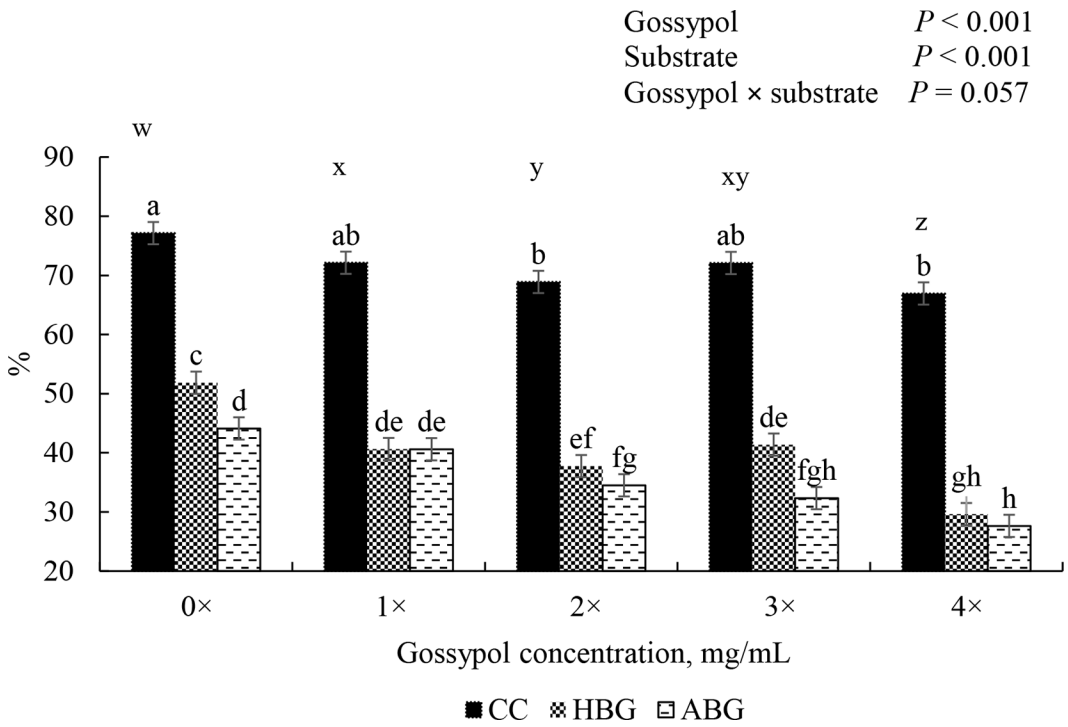

Figure 1. Dry matter disappearance of 0.5 g of corn (CC), bermudagrass with average relative forage quality (RFQ 100; ABG), and bermudagrass with high RFQ (RFQ 120; HBG) in in vitro mixed ruminal microorganism fermentations at 39°C for 24 h treated with gossypol at 0, 0.0615, 0.123, 0.1845, or 0.246 mg of gossypol/mL of ruminal fluid. a–h: Columns with differing lowercase letters are different ($P < 0.05$). w–z: Differing lowercase letters indicate differences between gossypol concentrations ($P < 0.05$).

In the current study, the maximum free gossypol intake as a percentage of the total diet is equivalent to the free gossypol content of the cotton residue presented in Table 2. Considering that 1.5 g of substrate were present in each incubation bottle, the amount of free gossypol present in the 1× treatment is 0.82%. Therefore, cattle in yr 1 and 2 consumed an amount of gossypol equal to or less than the equivalent amount in the1× treatment. Considering there was no difference in IVDMD for 2 of the 3 substrates, this indicates that grazing cotton should have minimal effect on nutrient utilization.

Fermentation pH. After 24 h of in vitro fermentation, pH tended ($P = 0.082$) to be higher in the 4× treatment compared with 0× and 1× (Table 5); however, substrate had a greater ($P < 0.001$; Table 5) effect on final pH. Fermentations of CC (6.34) had lower ($P < 0.001$) final pH compared with forage treatments, as expected, but ABG (6.63) and HBG (6.63) were not different ($P >$

Table 4. Hay and economic analysis[1]

Variable	GR[2]	CT[3]	SEM[4]	*P*-value
Total hay,[5] kg	4,185[a]	1,391[b]	562	0.013
Hay per day, kg	136.11[a]	46.37[b]	19.58	0.018
Hay/head per day, kg	16.22[a]	5.69[b]	0.59	<0.001
Total hay cost,[6] $	461.33[a]	153.32[b]	62.01	0.013
Cost/head per day, $	1.79[a]	0.63[b]	0.06	<0.001
Cost of gain, $/kg	2.37[a]	1.09[b]	0.17	0.002

[a,b]Differing superscripts within a row indicate difference between means ($P < 0.05$).

[1]Means presented are least squares means.

[2]GR = bermudagrass pasture and bermudagrass hay.

[3]CT = cotton crop residue and bermudagrass hay.

[4]SEM is standard error of the least squares means.

[5]Total hay is total amount of hay offered over 2 yr.

[6]A price of $110/metric ton was used for economic calculations.

Table 5. Mean pH and ammonia and VFA concentrations (in m*M*) from 3 substrates (corn, bermudagrass with average relative forage quality, and bermudagrass with high relative forage quality)

	Gossypol content[1]						
Item	0×	1×	2×	3×	4×	SEM	*P*-value
pH	6.48^{z}	6.49^{z}	6.53^{yz}	6.55^{yz}	6.60^{y}	0.02	0.082
Ammonia nitrogen	26.2	24.6	23.5	25.1	25.0	1.5	0.786
Acetate	42.0^{a}	38.6^{ab}	33.3^{bc}	29.1^{cd}	24.7^{d}	1.9	0.001
Butyrate	4.2^{y}	3.2^{yz}	2.4^{z}	2.6^{z}	2.1^{z}	0.5	0.086
Propionate	9.6^{a}	8.9^{a}	8.9^{a}	7.3^{a}	3.5^{b}	0.9	0.012
Total VFA[2]	57.0^{a}	51.6^{ab}	45.3^{bc}	39.8^{c}	31.2^{d}	2.3	<0.001

$^{a–d}$Rows without common superscript are different ($P < 0.05$).

^{y,z}Rows without a common superscript indicate a tendency ($0.05 < P < 0.10$).

[1]Gossypol concentrations were as follows: 0× = no gossypol; 1× = 0.0615 mg of gossypol/mL of ruminal fluid; 2× = 0.123 mg/mL; 3× = 0.1845 mg/mL; and 4× = 0.246 mg/mL.

[2]Total VFA includes acetate, propionate butyrate, isobutyrate, valerate, and isovalerate.

0.90). Increased pH values in response to increased gossypol could be due to inhibitory effects of gossypol on the mixed ruminal microorganism fermentation. The present results are similar to those of Bhatta et al. (2009), in which polyphenolic compounds (e.g., tannins) resulted in higher pH. However, the higher pH related to gossypol concentrations in the present study may be due to reduced VFA production levels (Table 5). Final pH in the current study were similar to those reported by Russell (1998), and it should be noted that the higher pH in the present forage-containing fermentations can be attributed to the fermentation of cellulose versus starch. In the presence of increased amounts of starch, starch degrading bacteria (e.g., *Lactobacillus* spp., *Streptococcus bovis*) produce copious amounts of lactic acid, which is a strong acid and decreases ruminal pH (Russell and Chow, 1993).

Ammonia Nitrogen. Ammonia is released from the degradation of dietary protein, NPN, and other nitrogenous compounds. Substrate ($P = 0.003$) affected NH_3-N concentrations after 24 h of fermentation, but level of gossypol was not different ($P = 0.79$; Table 5). Ammonia nitrogen produced from CC (9.2% CP, 85.6% TDN) fermentations was lowest ($P > 0.004$), and NH_3-N levels for ABG (12% CP, 52.2% TDN) and HBG (19.5% CP, 59.8% TDN) were not different ($P = 0.58$) from each other. Differences in NH_3-N concentrations between CC and forage treatments could potentially be explained by the increased IVDMD of CC relative to the forages and decreased CP content of CC. Satter and Slyter (1974) suggested that gossypol inhibits ruminal fermentation, resulting in lower ATP availability for MCP synthesis. Similarly, in the present study, ammonia concentrations were greater in the presence of increased gossypol concentrations, likely due to reduced fermentation activity. Bhatta et al. (2009) previously reported that polyphenolic compounds, such as tannins, decreased NH_3-N production.

VFA Production. Gossypol affected ($P \leq 0.012$) total VFA, acetate, and propionate production (Table 5) in the in vitro mixed ruminal microorganism fermentations. Butyrate production tended ($P = 0.086$) to decrease as gossypol concentration increased. Substrate affected ($P \leq 0.026$) total VFA, propionate, and butyrate production. Total VFA and acetate production decreased linearly ($P < 0.001$) with increasing gossypol concentrations. Propionate production was greater in fermentations containing gossypol up to 3× but was reduced ($P < 0.03$) in the presence of 4× mg/mL. The CC treatment had greater total VFA production compared with other substrates ($P \leq 0.012$). The CC substrate fermentations produced more propionate and butyrate compared with all other substrates ($P \leq 0.02$), which was expected due to the greater availability of starch for fermentation to propionate. We hypothesize that gossypol addition at increasing concentrations altered the rumen microbial population, resulting in reduced ruminal fermentation of feedstuffs.

APPLICATIONS

The present results provide an in-depth assessment of the benefits of grazing cotton crop residue and how gossypol influences ruminal fermentation of common feedstuffs. The cotton residue grazing trial demonstrated that producers can extend the grazing period and decrease feed cost for late-gestation beef cows in the winter months. These results indicate that cotton crop residue is a viable replacement for hay supplementation in the winter months. The in vitro fermentations demonstrated that gossypol did have negative effects on ruminal fermentation of feedstuffs and altered end product concentrations. Results demonstrated that DM digestibility and VFA production were inhibited by gossypol above 0.0615 mg/mL. Additional research is needed to evaluate the effects of

gossypol on the rumen microbial population and fermentation. Collectively, our data indicate both the usefulness and the potential limitations associated with increasing the use of cotton by-products in cattle production to supplement low forage availability.

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