Effect of Whole Cottonseed Amount and Delivery Frequency on the Performance of Gestating and Lactating Beef Cows

by

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Abstract

Whole cottonseed (WCS) is a common byproduct feedstuff in the southeastern United States due to its affordability, high energy, and high protein content. Since developing feeding recommendations in the 1980's, seed quality characteristics have continued to change. Additionally, beef producers in Alabama utilize WCS in rations at inclusion rates contrary to recommendations and may not offer supplemental feed daily due to labor and management constraints. Therefore, the objective of this thesis was to determine appropriate WCS supplementation level in beef cattle diets and to investigate the effect of delivery frequency on beef cow performance. We found that lactating beef cows fed WCS at or above established recommendations consumed greater nutritive value with no negative measured physiological effects. Furthermore, we found that greater feeding intervals did not have a negative effect of gestating beef cows. Overall, WCS can be fed at infrequent and high inclusion rates to match management strategies.

Digital Accessibility Use Disclosure Statement

In the preparation of this thesis, the following digital accessibility tools were used to ensure this document complies with federal requirements: Adobe. The author acknowledges full responsibility for the intellectual content of this work and has made a good faith effort to comply with digital accessibility requirements in publishing, wherein the nature of the content does not significantly change in order to do so. Furthermore, all content has been reviewed and revised to meet these requirements prior to final publication.

Artificial Intelligence (AI) Use Disclosure Statement

In the preparation of this thesis, the following artificial intelligence (AI) tools were used: Chat-GPT. These tools were used primarily to proofread and format text. The author acknowledges full responsibility for the intellectual content of this work and has ensured that all AI-assisted sections have been reviewed and revised for accuracy and appropriate academic style. All AI-generated content was reviewed and validated for relevance, appropriateness, and accuracy before incorporation into the final document to maintain scholarly integrity of this research.

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List of Abbreviations

2X	Whole cottonseed offered 2 d per week
3X	Whole cottonseed offered 3 d per week
7X	Whole cottonseed offered 7 d per week
ADF	Acid detergent fiber
ADG	Average daily gain
AAN	Apparently absorbed nitrogen
BMG	Bermudagrass hay
BR	Signal grass
BUN	Blood urea nitrogen
BW	Body weight
CON	No whole cottonseed inclusion
CP	Crude protein
d-	deci – (1×10^{-1} ; prefix for physical units)
DE	Digestible energy
DM	Dry matter
DMD	Dry matter disappearance
DMI	Dry matter intake
DMY	Dry matter yield
EE	Ether extract
FAT4	Whole cottonseed inclusion at 4% crude fat allowance
IACUC	Institutional Animal Care and Use Committee
GE	Gross energy
m-	milli - (1×10^{-3} ; prefix for physical units)
M-	mega – (1×10^{6} ; prefix for physical units)
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
NRC	Nutrient requirements of beef cattle
LIB	*ad libitum* whole cottonseed inclusion
OM	Organic matter
SG	Signal-grass
SS	Sorghum-sudangrass
TMR	Total mixed ration
WCS	Whole Cottonseed
WCS15	15% dry matter inclusion of whole cottonseed
µ-	micro- (1 × 10-6 ; prefix for physical units)

Chapter I

Introduction

Background of the Study

Cotton (*Gossypium hirsutum*) is a common row crop in the southeastern United States which is used in the textile industry, but processing of cotton lint leaves whole cottonseed (WCS) as a byproduct. Whole cottonseed has gained popularity as a cost effective byproduct protein feed for beef producers in the southeastern US, and feeding recommendations, developed in the 1980s, advised that whole cottonseed should be limited to no more than 15% of the dry matter intake (DMI) of dairy cattle (Palmquist and Jenkins, 1980). Additionally, Moore et al. (1986) found that WCS inclusion should be restricted to no more than 0.5% of body weight (BW), which relates to approximately 4% fat in the total diet.

Whole cottonseed is categorized as a high protein feed with 23% crude protein (CP), 19% ether extract (EE), 49% neutral detergent fiber (NDF), and 43% acid detergent fiber (ADF) (Nutrient Requirements of Beef Cattle, 2016). This relatively high fat concentration, when compared to other similar protein feeds, can lead to inhibited microbial digestion in the rumen through physical covering of fiber particles and antimicrobial properties of fatty acids (Moore et al., 1986; Hall and Eastridge, 2014; Jacobs et al., 2022).

Additionally, cotton varieties present in the Southeast have continued to undergo genetic improvement in regard to lint and seed quality since the original feeding recommendations were developed. These genetic improvements have led to reduced fat and greater crude protein content as well as greater digestibility of WCS (Jacobs et al., 2022). Although these changes have been demonstrated, updated feeding recommendations have not been developed. Beef producers utilizing WCS therefore continue to feed at or above established recommendations, often ad libitum, and frequently adopt feeding intervals of up to once weekly to accommodate management and labor constraints. As J. R. R. Tolkien observes, “such is oft the course of deeds that move the wheels of the world: small hands do them because they must, while the eyes of the great are elsewhere,” a concept that reflects how incremental and often overlooked changes in feed composition may meaningfully influence animal performance, particularly when recommendations fail to evolve alongside underlying inputs (Tolkein, 1954).

Statement of the Problem

While WCS has remained a popular byproduct protein feed in the southeastern US, changes in seed nutritive quality, particularly fat and crude protein content, have been documented, and little progress has been made on updating original feeding recommendations to align with modern seed nutritive quality. Additionally, with demonstrated changes in seed fat concentration, there is a gap in addressing how these changes impact the digestibility of whole cottonseed in beef cattle. Finally, while utilizing updating feeding recommendations, understanding how different feeding frequency, utilized in typical production practices, impact beef cattle performance was necessary.

Research Objectives

The objectives of this study were as follows:

1. To determine the effect of whole cottonseed supplementation, at and above established feeding recommendations, on the performance of lactating beef cows.
2. To determine the effect of whole cottonseed supplementation, at an above established feeding recommendations, on the digestibility of whole cottonseed fed to lactating beef cows.
3. To determine the effect of delivery frequency of whole cottonseed, at established feeding recommendations, on the performance of gestating beef cows.

Style and Form

This thesis was prepared according to the "Instructions to Authors" of the Journal of Animal Science (American Society of Animal Science). All efforts were made to adhere to this style, except in cases where divergence was necessary to comply with the formatting requirements of the Auburn University Graduate School Electronic Thesis and Dissertation Guide or to enhance clarity in the document.

Chapter II

Effects of Whole Cottonseed Supplementation on Beef Cow Performance: A Systematic Review

Introduction

Cotton production is a major component of agricultural systems throughout the southern United States, generating several byproducts that can be utilized as feed resources for beef cattle. Among these byproducts, whole cottonseed (WCS) has become a common supplemental feed ingredient in cow-calf production systems due to its widespread availability, favorable nutrient composition, and economic value. Whole cottonseed is unique in that it provides substantial amounts of energy, protein, and effective fiber within a single feedstuff. According to the Nutrient Requirements of Beef Cattle (2016), WCS contains approximately 52% neutral detergent fiber (NDF), 24% crude protein (CP), and 18% crude fat on a dry matter basis. This nutrient profile allows WCS to simultaneously address deficiencies in energy and protein that commonly occur in forage-based beef cattle diets, particularly during periods of reduced forage quality or availability.

Historically, WCS has been incorporated into beef cattle diets as a supplemental feed to support animal performance while reducing reliance on more expensive concentrate feeds (Mullenix et al., 2022). The high energy density associated with its lipid content, combined with its protein and fiber fractions, makes WCS an attractive option for cow-calf producers seeking to improve nutrient intake and production efficiency (Hall and Eastridge, 2014; Mullenix et al., 2022). However, concerns regarding dietary fat inclusion, variability in nutrient composition among cotton cultivars, and differences in supplementation strategies have led to inconsistent recommendations regarding its use in beef cattle systems (Jacobs et al., 2022; Mullenix et al., 2022).

Given the continued importance of WCS as a feed resource in the southern United States, a comprehensive evaluation of the available literature is warranted. Therefore, the objective of this systematic review was to synthesize existing research evaluating WCS supplementation in cow-calf production systems and to assess its effects on animal performance. Particular emphasis was placed on identifying management practices and inclusion rates that optimize the utilization of WCS while maintaining or improving productive performance.

Materials and Methods

Publication Selection Criteria and Relevance Screening

This systematic review followed PRISMA guidelines (Page et al., 2021). Strategy for conducting the search was structured by the PICO framework, which defines the terms population, intervention, comparison, and outcome (Pati and Lorusso, 2018). For this study, comparison was not applicable. Mature female beef cattle and beef calves were the population explored in this study. Different inclusion rates of WCS in the diet was the intervention investigated. Overall animal performance parameters such as body weight, average daily gain, lactation, and blood metabolites were the outcomes of interest.

In order to be included in this systematic review, publications had to meet three criteria. First, the paper had to include WCS as a supplemental feed ingredient. Second, the diet must have been fed to mature female cattle. Third, animal performance parameters had to be collected. Date of publication was not a factor in publication inclusion. The systematic literature search was conducted on 20 March 2026, in the electronic databases Agricola (Table II-I), CAB Abstracts (Table **II-II**), and Web of Science (Table II-III).

Publications were screened using Covidence (Veritas Health Innovation, Melbourne, Australia), a web-based collaboration platform that streamlines the production of systematic and other literature reviews. Screening of papers occurred in three steps in Covidence (2024) to track the status of each paper. First, duplicates were removed that were recognized across the three databases. Second, the titles, keywords, and abstracts were screened to ensure they aligned with five questions (Table II-IV). Finally, full texts were reviewed and screened by answering three questions (Table II-V). Throughout the screening process, each reviewer considered the questions assigned to each step (Table II-IV and Table II-V) and answered “yes” or “no”. A unanimous “yes” meant the paper could move to the next step in the screening process. A “no” meant the paper was excluded from the review.

The reviewer rules were set in Covidence (2024) to require two reviewers for each step of the screening process in order to eliminate bias. A third reviewer resolved any conflicts. The proceeding step could not take place until the reviewer rules for each paper were met.

Methodological Assessment and Data Collection Process

The primary author conducted data extraction for all studies included in the review. Relevance of each study was confirmed through full-text evaluation, and only publications written in English were considered. Information from each study was extracted and organized according to study characteristics, including authors, publication year, journal, experimental methods, study population, treatments, and reported outcomes. Data extraction procedures were adapted from the data extraction template available in Covidence (2024).

Following data extraction, each study underwent a quality assessment to evaluate methodological rigor and determine eligibility for inclusion in the review. Quality assessment criteria were adapted from the quality assessment template provided by Covidence (2024).

Outcome data relevant to the objectives of the review were extracted and compiled from each study. Extracted information included livestock species, number of animals enrolled, and reported means for all response variables of interest. Animal performance variables included initial and final body weight (BW), average daily gain (ADG), and milk yield (MY).

Results and Discussion

Publication Selection

After the database search, 2,411 citations were identified (241 Web of Science; 1,607 Agricola; 563 CAB; Figure II-I). Initial screening for duplicates eliminated 910. Of the identified duplicates, Covidence (2024) identified 782, and the reviewer manually removed 128 during the screening process (Figure II-I).

From the identified publications, 1511 were included in the first step of the screening process, where titles, keywords, and abstracts were reviewed to ensure the study met the criteria (Table II-IV). Once the reviewer rules were met for the title and abstract screening, 80 studies proceeded to the third step of the screening process (Figure II-I).

Full-text retrieval was attempted for all 80 publications identified during the screening process. Prior to full-text review, 34 studies were excluded because the complete publication could not be obtained. Open-access availability was not an inclusion criterion for this review. Consequently, 46 studies underwent full-text evaluation. Following assessment of the complete manuscripts, an additional 30 studies were excluded from the systematic review for various reasons (Figure II-I). During the full-text review, each excluded study was assigned a reason for exclusion from a predetermined set of categories, including wrong patient population, particularly small ruminants, wrong language, wrong outcomes, wrong intervention, not primary research, and wrong study design. Of the 30 studies excluded following full-text evaluation, nine were removed due to an inappropriate patient population, seven because they were published in a language outside the scope of this review, and two because they were not primary research articles. An additional seven studies were excluded because they did not report outcomes relevant to the objectives of the review, including measures of nutritive value or animal performance. One study was excluded because of an inappropriate study design, as it did not contain at least one treatment evaluating WCS supplementation. Finally, four studies were excluded due to an intervention that did not align with the objectives of the review.

After the final screening, 16 papers were eligible for data extraction (Figure II-I). During the title, keyword, and abstract screening stages, 176 conflicts occurred between the two independent reviewers. All conflicts were resolved through evaluation by a third reviewer. During the full-text review stage, an additional 31 conflicts arose and were similarly resolved by a third reviewer. Studies ultimately included in the systematic review are presented in Appendix 1.1, which provides the title, author(s), publication year, treatment structure, and animal species evaluated in each study.

Data Evaluation

Whole Cottonseed Characteristics

Across the studies included in this systematic review, WCS contained an average crude protein (CP) concentration of 21.6% and an average ether extract (EE) concentration of 21.1% on a dry matter basis. This is comparable to published nutritive value of WCS of 24% CP, and 18% EE on a dry matter basis. These values demonstrate the dual role of WCS as both a protein and energy supplement for beef cattle, contributing substantial concentrations of degradable and undegradable nutrients while also providing a highly concentrated source of dietary lipid. The relatively high concentrations of CP and EE observed across studies are consistent with the widespread use of WCS in cow-calf production systems, particularly in regions where cotton production provides a readily available source of byproduct feedstuffs.

Whole cottonseed was supplemented among studies at a rate 1.36 kg/d or 0.42% of body weight (BW). This average inclusion rate closely aligns with traditional feeding recommendations that suggest limiting WCS supplementation to approximately 0.5% of BW to avoid excessive dietary lipid concentrations and potential negative effects on ruminal fermentation. Although substantial variation existed among studies in both nutrient composition and supplementation rate, the average inclusion level indicates that most experiments evaluated WCS within the range recommended by previous research; however, this recommendation may not always be followed by producers.

Variation in CP, EE, and supplementation rate among studies likely reflect differences in cotton cultivar, environmental growing conditions, processing methods, animal class, and production objectives. These factors should be considered when interpreting animal performance responses, as the nutritional value of WCS can differ considerably among sources. Nevertheless, the overall nutrient composition observed in this review supports the classification of WCS as a nutrient-dense supplement capable of providing both protein and energy to grazing and confined beef cattle.

Animal Performance

Across the studies included in this review, supplementation with WCS was generally associated with improved cow and calf performance. Average dry matter intake (DMI) was less for cattle receiving WCS supplementation (10.0 kg) compared with control treatments (11.1 kg), suggesting that the additional energy supplied by dietary lipid may have partially substituted for forage intake (Table II-VI). Despite the reduction in DMI, cows supplemented with WCS (0.5 kg) exhibited greater average daily gain (ADG) than control animals (0.2 kg; Table II-VI). Similarly, final cow body weight was greater for WCS supplemented cattle (451.6 kg) than for cattle receiving control diets (444.0 kg), despite comparable initial body weights between treatments (Table II-VI).

Milk production, measured among lactating dairy cattle, also tended to be greater in cows receiving WCS supplementation, averaging 18.2 kg compared with 17.3 kg for control cows (Table II-VI). Increased milk yield may partially explain the improvements observed in calf growth performance. Calves nursing WCS-supplemented dams exhibited greater ADG than calves from control treatments and achieved heavier final body weights, 456.3 kg and 338.9 kg, respectively (Table II-VI). These findings suggest that the additional energy and protein supplied by WCS may support both maternal performance and calf growth.

Blood urea nitrogen (BUN) concentrations were greater in WCS-supplemented cattle (9.8 mg/dL) than in control animals (7.8 mg/dL; Table II-VI). Elevated BUN concentrations may indicate increased nitrogen intake and metabolism associated with the protein contribution of WCS. Overall, the animal performance data included in this review suggest that WCS supplementation can improve cow weight gain, milk production, and calf growth while maintaining DMI at levels similar to or slightly lower than non-supplemented control diets.

Conclusion

Whole cottonseed remains a valuable supplemental feed ingredient for cow-calf production systems due to its ability to provide substantial concentrations of energy and protein within a single feedstuff (Mullenix et al., 2022). Results from this systematic review demonstrated that WCS contained an average crude protein concentration of 21.6% and an average EE concentration of 21.1%, supporting its classification as a nutrient dense byproduct feed. According to the studies evaluated, WCS was typically supplemented at 1.36 kg/d or 0.42% of body weight, a level consistent with traditional feeding recommendations intended to limit excessive dietary fat intake.

Overall, WCS supplementation was associated with improved animal performance compared with non-supplemented control diets. Despite a slight reduction in dry matter intake, cows receiving WCS exhibited greater average daily gain, heavier final body weights, and increased milk production. Improvements in maternal performance were accompanied by increased calf growth, as evidenced by greater calf average daily gain and heavier final body weights. Additionally, elevated blood urea nitrogen concentrations in supplemented cattle suggested increased nitrogen intake and utilization. Collectively, these findings indicate that WCS can be effectively incorporated into cow-calf production systems to enhance productive performance while maintaining acceptable levels of feed intake. Future research should continue to evaluate the effects of modern cottonseed cultivars and alternative supplementation strategies to further refine recommendations for WCS utilization in beef cattle diets.

Table II-I Results of an online database (Agricola) search to identify animal performance with and without whole cottonseed inclusion as part of a systematic review.

Database: Agricola

Search Date: 03/20/2026

Query	Boolean Phrase	Results (n)
1	(cattle* OR cow* OR heifer* OR calf* OR calves*).ti OR (cattle* OR cow* OR heifer* OR calf* OR calves*).ab	211898
2	(supplement* AND cotton*).ti OR (supplement* AND cotton*).ab	1182
3	1 AND 2	241

Table II-II Results of an online database (CAB Abstracts) search to identify animal performance with and without whole cottonseed inclusion as part of a systematic review

Database: CAB Abstracts (1910 – present, CABI)

Search Date: 03/20/2026

Query	Boolean Phrase	Results (n)
1	(cattle* OR cow* OR heifer* OR calf* OR calves*).ti OR (cattle* OR cow* OR heifer* OR calf* OR calves*).ab	829004
2	(supplement* AND cotton*).ti OR (supplement* AND cotton*).ab	5774
3	1 AND 2	1607

Table II-III Results of an online database (Web of Science) search to identify animal performance with and without whole cottonseed inclusion as part of a systematic review.

Database: Web of Science

Search Date: 03/20/2026

Query	Boolean Phrase	Results (n)
1	TI= (cattle* OR cow* OR heifer* OR calf* OR calves*) OR AB= (cattle* OR cow* OR heifer* OR calf* OR calves*) OR AK= (cattle* OR cow* OR heifer* OR calf* OR calves*)	445495
2	TI= (supplement* AND cotton*) OR AB= (supplement* AND cotton*) OR AK= (supplement* AND cotton*)	2520
3	1 AND 2	563

Table II-IV Questions used to identify citations of interest in screening manuscript titles, abstracts, and keywords to identify animal performance with and without whole cottonseed inclusion as part of a systematic review.

Question	Yes	No
1. Is this paper published in English?		
2. Does this abstract investigate primary research?		
3. Does this abstract investigate WCS inclusion in diets?		
4. Does this abstract investigate the species and sex of interest?		
5. Does this abstract investigate the effect of WCS inclusion on animal performance?		

Table II-V Questions used to identify citations of interest in screening manuscripts to identify animal performance with and without whole cottonseed inclusion as part of a systematic review.

Question	Yes	No
1. Does this study have at least one treatment that is WCS?		
2. Does this study investigate the effect of WCS on animal performance?		
3. Are sufficient raw or unadjusted data provided for animal performance?		

Table II-VI Average difference between whole cottonseed and control treatments of papers included in systematic review of the effect of whole cottonseed supplementation of cattle performance.

	Treatment[2]	
Item[1]	**CON**	**WCS**
CP, %	-	21.6
EE, %	-	21.1
WCS Inclusion, kg	-	1.36
WCS Inclusion, % BW	-	0.42
DMI, kg	11.1	10.0
Initial Cow BW, kg	427.5	439.1
Final Cow BW, kg	444.0	451.6
Cow ADG, kg	0.2	0.5
Milk Production, kg	17.3	18.2
Initial Calf BW, kg	202.1	262.78
Final Calf BW, kg	338.9	456.3
Calf ADG, kg	0.57	0.63
BUN, mg/dL	7.8	9.8

[1]CP = crude protein; EE = ether extract; DMI = dry matter intake; BUN = blood urea nitrogen

[2]CON = Control; WCS = Whole cottonseed

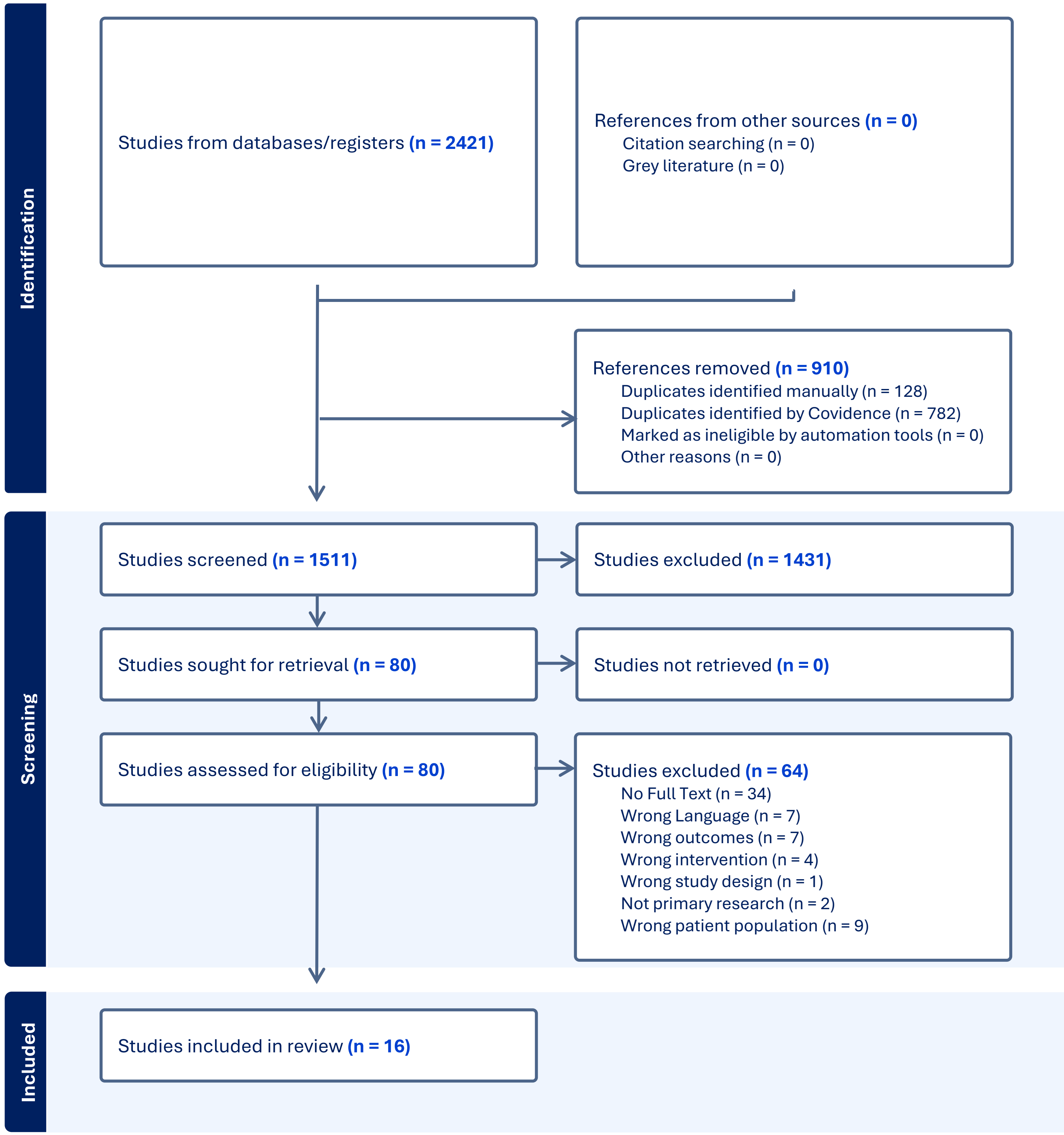

Figure II-I PRISMA flow diagram indicating the number of citations and publications included and excluded in each level to identify animal performance and forage nutritive value of forages in tall fescue systems with and without legume inclusion as part of a systematic review.

Chapter III

Performance, Digestion, and Passage of Whole Cottonseed Offered Either *Ad Libitum* or Under Restriction to Beef Cows Nursing Calves

Synopsis

Whole cottonseed (WCS) is a common byproduct feedstuff in the southeastern United States due to its affordability, high energy, and high protein content. Feeding recommendations for WCS were primarily developed in the 1980s using commercially available varieties of cotton at the time. Since this time, seed quality characteristics, such as crude fat and crude protein, have continued to change over time. Additionally, beef producers in Alabama utilize WCS in rations at inclusion rates above established recommendations. Therefore, the objective of this study was to determine appropriate WCS supplementation level in beef cattle diets using currently available seed supplies. In a randomized complete block design, lactating cows (n = 16) were randomly allocated to one of four WCS inclusion rates across two pens (blocks): no WCS inclusion (CON); WCS at 15% of DM intake (WCS15); WCS inclusion to supply fat at 4% DM intake (FAT4); WCS offered *ad libitum* intake (LIB). Cows were fed for 75 d with two digestion collection periods at 28-35 d and 68-75 d. Intake of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) did not differ among treatments ($P \geq 0.11$). Similarly, digestibility of DM, OM, NDF, ADF, ether extract (EE), and apparently absorbed and retained nitrogen (N) did not differ among treatments ($P \geq 0.10$). There was no effect of treatment on milk production, cow average daily gain (ADG), calf ADG, digestible energy

(DE), blood urea nitrogen (BUN), or non-esterified fatty acid (NEFA; $P \geq 0.07$). Cows assigned to LIB had the greatest WCS intake as a percent of total dry matter intake (DMI) (41.2%), followed by FAT (26.2%), DMI (17.0%), then CON (0.0%; $P < 0.01$). Consequently, EE ($P < 0.01$) and CP intake ($P < 0.01$) were also greatest from LIB (6.9% and 15.7%. respectively) followed by FAT (5.5% and 15.2% respectively), DMI (4.3% and 14.1% respectively), then CON (2.0% and 12.2% respectively). Additionally, LIB had the greatest gross energy (GE) in the diet (4.6 Mcal/kg) followed by FAT (4.1 Mcal/kg), CON (3.8 Mcal/kg), then DMI (4.0 Mcal/kg ; $P < 0.01$). Results from this study suggest that increased inclusion rates of WCS have minimal negative effects on lactating cow performance and digestibility.

Introduction

Feeding recommendations for WCS in beef cattle diets were primarily developed in the 1980s and tested through the early 2000s (Moore et al., 1986; Rogers et al., 2002; Mullenix et al., 2022). Palmquist and Jenkins (1980) recommended that WCS should be limited to no more than 15% of the DMI of dairy cattle. Additionally, Rogers et al. (2002) and Moore et al. (1986) stated that WCS inclusion should be limited to not more than 0.5% of body weight (BW), which relates to approximately 4% fat in the total diet.

In the southeastern United States, WCS is a common byproduct feedstuff as it is a secondary product of cotton (*Gossypium hirsutum* [L.]) row crop production. Cotton varieties produced in the Southeast are continually being improved through plant breeding efforts for lint and seed quality. These genetic improvements have downstream implications of nutritive value and digestibility of WCS (Jacobs et al., 2021). While changes in seed quality have been demonstrated, and studies have been conducted to investigate free-choice WCS feeding in short term feeding trials, seed degradation, passage rate, and fat digestibility have not been conducted (Jacobs et al., 2022; Mullenix et al., 2022). Therefore, the objective of this study was to determine appropriate WCS supplementation levels, WCS digestibility, and WCS passage rate in beef cattle diets.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2024-5432.

Experimental Design

This experiment was conducted as a generalized complete block design at the Beef Cattle Evaluation Center at the Stanley P. Wilson Beef Teaching Center, Auburn, AL. The single blocking factor was pen (n = 2; approx. 0.4 ha). Sixteen cow-calf pairs were fed for a 75-d experiment. Across the 75 d, there were two 7-d collection periods at 28 to 35 d and 68 to 75 d. Cow-calf pairs were randomly assigned to treatments such that each treatment in each blocking factor contained two cow-calf pairs. Each cow was assigned an individual bunk in a Calan Broadbent Feed System (Calan Precision Feeding Systems, New Hampshire).

Animals

Sixteen Angus-cross cow-calf pairs were used in this experiment. Cows varied in age from approximately 5 to 9 years old with an average initial body weight (BW) of 616 ± 32 kg. Each calf was at least 30 days of age before the experiment began with an average BW of 97 ± 6 kg.

Whole Cottonseed Treatments

Dietary treatments for this experiment included four inclusion rates of WCS in addition to a basal diet of Coastal bermudagrass hay (*Cynodon dactylon* [L.] Pers.; BMG): a control group receiving only BMG (CON), BMG supplemented with WCS at 15% of targeted dry matter intake (WCS15), BMG supplemented with WCS to reach a crude fat intake of 4% DM (FAT4), and BMG supplemented with WCS on an ad libitum basis (LIB). Upland Whole cottonseed was obtained from Milstead Farm Group in Shorter, Alabama in the 2024 growing season. The seed was gin-run and stored in an aerated storage facility until sourced.

Experimental Procedures

Experimental Timeline

Fourteen days before the initiation of the experiment, cows were fitted with sensor key collars which were calibrated to specific American Calan gates. Cow-calf pairs were then randomly placed in their assigned pens and provided free-choice bermudagrass hay and up to 1% BW/d WCS to encourage daily consumption from the bunk and use of the Calan gate. The training and adaptation phase continued until the initiation of the experiment on d 1. Following the initiation of the experiment, cows and their calves were weighed every 7 d. On d 28 to 35 and 68 to 75, cows were sampled for a 7 d fecal collection phase.

Feeding and Sampling

Respective dietary treatments were offered daily at 0800 h. On the first day of feeding, dietary treatments were offered at 2.5% BW on an as-fed basis. Each day thereafter, feed refusals, known as orts, were collected immediately prior to feeding to determine the subsequent offering. Bermudagrass hay was offered for *ad libitum* consumption, defined as at least 10% refusal. On each day of the experiment, each cow received 10 g of titanium dioxide (TiO_2) homogenized with feed as an external marker of digestibility. This marker was used to estimate total fecal output and apparent digestibility.

From d 28 to 35 and 68 to 75, samples of each dietary treatment were collected and WCS and BMG were separated to determine nutritive value. From d 29 to 36 and 69 to 76, samples of orts from each dietary treatment were collected to determine nutritive value of refused feed. These values were used to calculate nutrient intake per animal (Table III-I).

Fecal Collections

Fecal collections were conducted on d 28 to 35 and 68 to 75. Fecal grab samples (approx. 700 g) were collected from each cow each day at 0800 h and frozen at -20°C for further processing.

Blood Collections

On d 1, 35, and 75, while in the squeeze chute for BW measurement, blood was collected via jugular venipuncture into two 10 mL evacuated tubes, one containing K_2-EDTA and the other a clot activator. After collection, blood tubes were inverted, placed on ice, and, within two hours of collection, centrifuged at 2500 x g for 15 min. Following centrifuging, 2 subsamples of serum and plasma were collected and frozen at -20°C for further analysis.

Whole Cottonseed Passage

On d 68 and 73, each cow in WCS15, FAT4, and LIB were fed 100 g of whole cottonseed which was mordanted with chromium(III) oxide (Cr_2O_3) using the fiber mordanting method specified in Udén et al. (1980). Fecal grab samples collected on d 69 and 75 were rinsed and sieved through a 4.25-mm screen to recover undigested WCS. Recovered WCS was dried at 55°C and weighed. The recovered WCS and unfed Cr-mordanted WCS were then assayed for minerals at the University of Georgia Soil, Plant & Water Laboratory via the USEPA Method 3052 (1995) and Creed et al. (1994).

Milk Production

On d 1, 35, and 75, cow milk production was estimated using the weigh-suckle-weigh technique described by Rutledge et al. (1971). Calves were removed from their dam at 0800 h and fasted for 8 h. At 1600 h, calves were weighed, allowed to suckle for approximately 1 h, then weighed again to determine lactation rate.

Analytical Procedures

Nutritive Value

Bermudagrass hay, orts, and fecal samples were dried to 55°C for DM determination. Dried samples were then ground to pass through a 2-mm screen. A subsample was then ground to pass through a 1-mm screen using a Eberbach E3500

Series Mill (Eberbach Corporation, Charter Township, MI, USA). Ground subsamples were combined in equal potions to create a composite sample of animal and period.

Dry matter and organic matter (OM) were determined according to the AOAC (2000) protocols. Detergent fiber [neutral detergent fiber (NDF) and acid detergent fiber (ADF)] was determined according to the procedure of Vogel et al. (1999). Hemicellulose was determined as the difference in NDF and ADF. Acid detergent lignin (ADL) was determined using the sulfuric acid method (AOAC, 2000) with an automated fiber analyzer (ANKOM^{DELTA}, ANKOM Technology, Macedon, NY). Cellulose was determined as the difference in ADF and ADL.

Composited BMG, WCS, and ort samples were assayed for crude protein by combustion in a commercial laboratory (Crude Protein in Animal Feed [AOAC Method 990.03]; Cumberland Valley Analytical Services, Waynesboro, PA, USA). Composite samples were assayed for ether extract by high temperature solvent extraction in a commercial laboratory (AOCS Standard Procedure Am 5-04; Cumberland Valley Analytical Services, Waynesboro, PA, USA).

Gross energy (GE; Mcal/kg) of composite BMG, WCS, ort, and fecal samples was determined via bomb calorimetry using a LECO AC600 Automatic Calorimeter (Method Isoperibol; AOAC, 2000; Leco 31 828 Dry Combustion Analyzer, Leco Corporation, St. Joseph, MO). The vessel was calibrated using benzoic acid pellets. Pure oxygen (O_2) (95% purity; 450 psi), compressed air (12 psi), and distilled water for the chamber (15°C) was required for the method. Compared to the standard recommendation (1.0 g), the method was adapted to utilize a smaller sample weight (0.20 g) due to sample burning through ignition wires. Samples were ignited using cotton string fuses. Three benzoic standards were run at the start of each day of analysis. An additional standard was assayed after every 10 samples to ensure calibration quality and avoid drift.

Using the recorded weights of collected BMG, WCS, orts, and estimated total fecal matter throughout the collection periods, GE of samples was determined (Mcal/kg). Digestible energy (DE) was calculated as the difference in intake GE and output GE, as a proportion of intake GE.

Total Fecal Estimation

Fecal samples collected on d 28 to 35 and 68 to 75 were assayed for TiO_2 using the method outlined by Myers et al. (2004) to estimate total fecal excretion per cow per day. In duplicate, 50 mg of ashed feces were added to a tube containing Na_2SO_4 and H_2SO_4. Tubes were vortexed and heated at 120°C for 24 h. After heating, samples were diluted to 100 mL and contents were allowed to settle for 12 h. An aliquot was then taken from each sample and measured using a chromatograph at 410 nm.

Gossypol

Gossypol concentration was determined using ground WCS kernels that were initially processed to pass through a #12 screen and subsequently ground with a Braun® hand blender until the material passed through a #20 sieve. The ground kernel samples were analyzed via high-performance liquid chromatography (HPLC; Agilent 1100, Palo Alto, CA) with an Inertsil ODS column (SGE, Austin, TX), a diode array detector, and a mobile phase flow rate of 1m/min using the AOCS Ba 8a-99 method. Absorbance at 254 nm was recorded and used a detection wavelength. Detectable limit of gossypol was 0.46 μg, therefore a normal distribution was created of values between 0 and 0.46 and measurements that fell below the measurement threshold were assigned a value randomly along the normal distribution.

Gossypol exists as two naturally occurring optical isomers, (+)-gossypol and (−)-gossypol, which differ in their three-dimensional configuration despite having the same chemical composition (Zhang et al., 2007). The (−)-isomer exhibits greater biological activity and toxicity because it binds more readily to proteins and other cellular components, whereas the (+)-isomer is less biologically active (Gadelha et al., 2014). Gossypol concentration can vary between cotton species and variety as well as being influenced by environmental conditions (Nunes et al., 2010).

Blood Metabolites

Blood urea nitrogen (BUN) was determined from serum samples via a colorimetric ELISA blood metabolite assay kit (EIABUN, ThermoFisher Scientific, Life Technologies Corporation, Frederick, MD, USA). Non-esterified fatty acid (NEFA) was determined from serum samples via colorimetric blood metabolite assay kit (ACS-ACOD Method; RayBiotech Inc., Peachtree Corners, GA, USA). Plasma gossypol was determined from plasma samples via high-performance liquid chromatography (HPLC; Agilent 1100, Palo Alto, CA) with an Inertsil ODS column (SGE, Austin, TX), a diode array detector, and a mobile phase flow rate of 1 nm/min using the AOCS Ba 8a-99 method. Absorbance at 254 nm was recorded and used a detection wavelength.

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the feeding experiment was a generalized complete block design with four dietary treatments.

Bermudagrass and WCS nutritive value and energy concentrations were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX). The fixed effect was dietary treatment, and denominator degrees of freedom were adjusted using the 2^{nd}-order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were pen and Calan gate.

Blood metabolites, BW, and milk production were analyzed as repeated measurements using PROC GLIMMIX. The model fixed effects included dietary treatment and day. Denominator degrees of freedom were adjusted using the 2^{nd}-order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were pen and Calan gate.

A canonical discriminate analysis was conducted in order to determine the overall effect of treatment across the multivariate response space (Hair et al., 2019). This technique is useful for guiding statistical discovery when the number of response variables exceeds the number of observations. A dataset was generated for response variables using mean values for each animal × block × treatment combination. Mean responses were analyzed for correlation (PROC CORR) and a stepwise procedure (PROC STEPDISC) was conducted to determine the ideal combination of response variables in order to generate linear combinations. A canonical discriminant analysis (PROC CANDISC) was then performed using the selected variables to visualize treatment clustering.

Results and Discussion

As there were numerous responsible variables measured, stepwise discriminant analysis was conducted for this dataset. The stepwise procedure revealed that WCS intake and BUN were the influential variables of interest. The first discriminant function was positively associated with WCS intake (standardized coefficient = 0.99) and the second discriminant function was positively associated with BUN (0.80; Figure III-I). This analysis successfully separated the WCS treatments. Treatments WCS15 and FAT clustered together at the origin of the first discriminant factor with CON separating along the negative side and LIB separating along the positive side. This primarily indicates a clustering of treatment by WCS intake. Observations from FAT separated from WCS15 along the second discriminate function primarily indicating separation with an association of BUN but also separating from other treatments by an increased physiological response. This distribution indicates that treatments CON, WCS15 and FAT4, and LIB were separated from one another primarily based on their WCS intake and CON, WCS15, and LIB treatments were separated from FAT4 primarily by BUN. Furthermore, this separation indicates that FAT4 had an increased biological response with similar WCS intake to WCS15. Subsequent univariate analysis supported the observed grouping patterns from the multivariate analysis.

Intake

There was no effect of treatment on overall dietary DMI (10.6 ± 0.69 kg/d; $P = 0.16$; Table III-II). Studies have demonstrated that the inclusion of WCS can suppress intake

(Coppock et al., 1985; Rogers et al., 2002); however, others found that WCS has been fed at nearly double the recommended 0.5% BW limit observed no negative effects (Mullenix et al., 2022). Additionally, research conducted by Hill et al. (2009) found that feeding WCS at the recommended rate stimulated hay intake.

By design, and as revealed in the discriminant analysis, there was an effect of treatment on WCS intake ($P < 0.01$; Table III-II). Cows assigned LIB (41.2%) had the greatest ($P \leq 0.05$) WCS intake followed by FAT4 (6.2%) then WCS15 (17%). As a result, there was also an effect of treatment on EE ($P < 0.01$) and CP intake ($P < 0.01$; Table III-II). Cows assigned to LIB (6.9%) had the greatest EE intake followed by FAT4 (5.5%), then WCS15 (4.3%), and CON (2.0%). Whole cottonseed utilized during this study had an EE content of 16.4%. The NRC (2016) lists the average fat content of whole cottonseed as 19.5%. Therefore, treatments with higher inclusion rates and intake of WCS had a greater intake of EE. Cows from LIB (15.7%) had the greatest CP intake, followed by WCS15 (14.1%), and CON (12.2%) had the least CP intake with FAT4 (15.2%) as an intermediate. Whole cottonseed utilized during this study had a CP content of 26.2%, additionally the NRC (2016) lists the fat content of whole cottonseed as 22.9%, therefore, treatments with higher inclusion rates and intake of WCS had a greater intake of CP.

Digestibility

There was no effect of treatment on NDF digestibility (NDFD) (72.63 ± 9.26%; P = 0.27; Table III-III) or ADF digestibility (ADFD) (65.9 ± 12.29%; P = 0.49; Table III-III). For apparently absorbed nitrogen (AAN), there was no effect of treatment (73.4 ± 5.1%; P = 0.49; Table III-III). There was no effect of treatment (Table III-III) on DMD (67.43 ± 11.80%; P

= 0.17) or EED (81.2 ± 3.74%; P = 0.47). While it has been hypothesized in the past that high levels of WCS in cattle diets could lead to undigested WCS lost in fecal, there was no effect of treatment on WCS disappearance (99.9%; P = 0.48; Table III-III).

According to Nutrient Requirements of Beef Cattle (NRC) (2016), high fat inclusion in the diets of cattle can disrupt ruminal fermentation and the digestion of structural carbohydrates. Jenkins (1993) found that ruminal digestion of carbohydrates can be reduced by 50% or more by an inclusion of 10% fat in the diet of cattle. However, with the inclusion of WCS at 4% crude fat allowance, as well as *ad libitum*, digestibility of WCS and BMG was not inhibited. It was noted that the digestion of non-structural carbohydrates, such as starch, were less affected by the inclusion of fats.

While Jenkins (1993) noted that dietary lipids can interfere with the ruminal digestion of proteins, other studies have found that lipid have no effect on protein degradability (Broderick et al., 1991). Additionally, Coppeck et al. (1985) found that dairy cattle supplemented with 30% WCS inclusion rate had increased CP digestibility.

Past studies have found evidence that the inclusion of WCS, due to its high fat content, can negatively impact DMD. However, modern WCS, including WCS used on this study, is lower in EE concentration than WCS utilized in previous decades (Palmquist and Jenkins, 1980; Coppock et al., 1985; Jenkins, 1993; Jacobs et al., 2021). It is probable that the fat content of modern WCS is at a low enough concentration where DMD is not adversely affected as severely as in previous studies, such as in Coppock et al. (1985) where WCS had a EE of 17.3%. Additionally, the hard seed coat of WCS can slow the release of unsaturated fats in the rumen and reduce its effect on rumen fermentation (Adeniji et al., 2025).

We found that nearly all WCS consumed was unrecoverable. In contrast, a similar experiment was conducted with dairy cattle fed a total mixed ration (TMR) diet with a 15% inclusion rate of WCS. Approximately 2.5% of the fed WCS was recorded, intact and undigested, in the fecal material of the animals (Adeniji et al., 2025). It should be noted that the dairy cows in the WCS treatment had a DMI of 26.7 kg/d while lactating cows in this study had DMI values of less than 16 kg/d. The low recovery of intact seeds in the feces likely reflects the prolonged retention of WCS within the rumen, providing greater opportunity for rumination and microbial degradation before passage from the gastrointestinal tract (Adeniji et al., 2025). Furthermore, the high-forage diet likely increased ruminal retention time by promoting rumen mat formation and reducing particulate passage rate, thereby allowing a greater proportion of WCS to be mechanically disrupted and digested prior to excretion (Colucci et al., 1982).

Blood Metabolites

As expected, there was an effect of treatment on plasma gossypol concentration ($P = 0.03$; Table III-IV). Treatment FAT4 (2.6 μg/mL) had the greatest plasma gossypol followed by LIB (2.2 μg/mL) and WCS15 (1.9 μg/mL), while CON had the least plasma gossypol (0.8 μg/mL). Based on the blood metabolite levels, no evidence of gossypol toxicity was present in any of the treatments. According to Tang et al., (2018), established safe levels of plasma concentrations of gossypol are <5 μg/mL for (±)-gossypol and <3.5 μg/mL for (-)-gossypol. Safe levels of both isomers of gossypol were not exceeded in any treatment.

Though BUN was an influential variable in the multivariate analysis, there was no effect of treatment (Table III-IV) on NEFA (604.3 ± 173.7 μM ; P = 0.55) or BUN (27.6 ± 3.6 mg/dL; P = 0.07). Non-esterified fatty acid concentrations greater than approximately 600 μM indicate substantial mobilization of body fat reserves, and concentrations exceeding 800 μM are associated with severe negative energy balance during early to peak lactation (Oetzel, 2004). Additionally, blood urea nitrogen (BUN) concentrations in beef cattle are commonly used as an indicator of protein and energy balance, with normal concentrations typically ranging from approximately 7 to 15 mg/dL. Concentrations below this range may indicate inadequate dietary protein, while concentrations above 20 mg/dL often indicate excess rumen degradable protein relative to available energy, resulting in inefficient nitrogen utilization (Herath et al., 2018). Overall, these levels suggest that the cows in each treatment were in a negative energy balance, possibly due to the energy demands of lactation.

Growth and Lactation

Contrary to hypothesized projections, there was no effect of treatment on milk production (2.8 ± 0.40 kg; P = 0.34; Table III-V). This is consistent with previous studies that found no response in milk yield when WCS was supplemented to dairy cow diets (Arieli, 1998). In other studies, milk production was found to increase (Sklan et al., 1992; Harrison et al., 1995) or decrease (Smith et al., 1981) in dairy cow with rations supplemented with WCS. Additionally, there was no effect of treatment on calf average daily gain (0.7 ± 0.05 kg; P = 0.39; Table III-V). This is likely due to the lack of response of lactation to WCS supplementation.

The absence of a lactational response suggests that energy intake was not limiting under the conditions of the present study. Because cows grazed relatively high-quality bermudagrass forage, the additional energy supplied by WCS may have been insufficient to elicit a measurable increase in milk synthesis. Furthermore, previous research has demonstrated that responses to supplemental fat are often greatest when basal diets are energy deficient or when cows are in a more severe state of negative energy balance (Huston et al., 1999). Under conditions where nutrient requirements are largely met by forage alone, supplemental fat may instead contribute to maintenance of body condition rather than increased milk production. Consequently, milk production and calf growth were likely constrained more by the cows' physiological capacity for lactation than by dietary energy supply, explaining the absence of treatment effects on calf average daily gain.

Energy Fractionation

There was an effect of treatment on gross energy (GE) of intake ($P < 0.01$; Table III-VI). Diet of LIB had the greatest GE of intake (4.4 Mcal/kg) with an intermediate FAT4 (4.3 Mcal/kg), followed by WCS15 (4.2 Mcal/kg) and CON (4.0 Mcal/kg) with the least GE of intake. According to Nutrient Requirements of Beef Cattle (2016), the estimated energy value of fate is 9.3 Mcal/kg (Hall and Eastridge, 2014). Therefore, diets with greater fat inclusion will have greater GE of intake. There was no effect of treatment on DE (2.8 ± 0.12 Mcal/kg; $P = 0.18$; Table III-VI), estimated ME (2.3 ± 0.01 Mcal/kg; $P = 0.18$; Table III-VI), estimated net energy of maintenance (NEM) (1.4 ± 0.09 Mcal/kg; $P = 0.16$; Table III-VI), or estimated net energy of gain (NEG) (0.8 ± 0.08 Mcal/kg; $P = 0.15$; Table III-VI).

Although gross energy increased with increasing dietary inclusion of WCS, this additional energy was not reflected in the digestible or metabolizable energy fractions. The lack of treatment differences in DE, ME, NEM, and NEG suggests that the additional gross energy supplied by WCS was largely offset by similar digestive efficiency among treatments. These findings are consistent with the lack of treatment effects observed for apparent total tract digestibility, indicating that WCS supplementation increased the energy density of the diet without altering the proportion of energy that was ultimately available for metabolism. Collectively, these results suggest that the level of WCS supplementation used in the present study did not appreciably improve the efficiency of energy utilization despite increasing the total energy content of the diet.

Conclusion

When evaluating the performance of lactating beef cows supplemented with WCS, FAT and LIB had greater WCS, CP, and EE intake, with an increase in nutritive value. Additionally, there was no effect on digestibility or nitrogen absorption between treatments, in contrast to the expected inhibition of fiber from a high fat inclusion. While no change in milk production was observed in any treatment, previous research has been inconclusive on the effect of WCS supplementation on lactation. Subsequently, there was no effect of treatment on calf and cow ADG. This supports the utilization of WCS in lactating beef cow diets at or above recommended levels.

Additionally, while FAT and LIB diets showed greater nutritive value, free-choice WCS supplementation may not meet production goals. It was noted that cows assigned to FAT performed at a similar level to those assigned to LIB, while consuming less total WCS.

Therefore, while it does not have a negative physiological effect, *ad libitum* supplementation of WCS may be financially sub-optimal to beef producers. Additionally, future research should be conducted to address varying feeding intervals commonly practiced in production systems such as weekly or bi-weekly feeding.

Table III-I Nutritive value of whole cottonseed and bermudagrass hay used in the evaluation of whole cottonseed on digestibility and performance in lactating beef cows.

	Feed[2]	
Item[1]	**WCS**	**BMG**
DM, %	90.7	93.0
OM, % DM	96.4	91.3
NDF, % DM	49.1	69.0
ADF, % DM	19.9	35.5
ADL, % DM	14.4	4.5
Hemicellulose, % DM	29.2	33.5
Cellulose, % DM	5.5	31.0
Ash, % DM	3.7	8.8
CP, % DM	26.2	12.1
EE, % DM	16.4	2.1
Gossypol, % DM	0.6	0.0
GE, Mcal, kg	4.6	4.0

[1]DM = dry matter; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; CP = crude protein; EE = ether extract; GE = gross energy

[2]WCS = Whole cottonseed; BMG = ‘Coastal’ bermudagrass hay

Table III-II Daily intake of dry matter, nutrients, and gossypol in lactating beef cows fed bermudagrass hay across treatments differing in whole cottonseed inclusion rates.

	Treatment[2]					
Item[1]	**CON**	**WCS15**	**FAT4**	**LIB**	**SEM[3]**	***P*-value**
DMI, kg	9.2	10.3	11.6	11.4	0.69	0.16
Gossypol, g	0[d]	19[c]	34[b]	75[a]	3.49	< 0.01
WCS Intake, %	0.0[d]	17.0[c]	26.2[b]	41.2[a]	0.82	< 0.01
OM Intake, %	87.3	87.7	87.9	87.5	0.40	0.25
NDF Intake, %	79.2[a]	76.1[a]	75.6[a]	73.7[b]	0.16	0.11
ADF Intake, %	40.8	41.9	43.2	44.6	0.96	0.54
CP Intake, %	12.2[c]	14.1[b]	15.2[ab]	15.7[a]	0.36	< 0.01
EE Intake, %	2.0[d]	4.3[c]	5.5[b]	6.9[a]	0.22	< 0.01

[1]DMI = Dry matter intake

[2]CON = No whole cottonseed inclusion; WCS15 = Whole cottonseed inclusion at 15% dry matter intake; FAT4 = Whole cottonseed inclusion to supply fat at 4% dry matter intake; LIB = Whole cottonseed offered for *ad libitum* intake

[3]SEM = standard error of the mean. When SEM differed for various levels of treatment, the greatest value was reported.

[a,b,c,d] Means within a row with different superscripts are different ($P \leq 0.05$)

Table III-III Apparent digestibility of nutrients in lactating beef cows fed bermudagrass hay across treatments differing in whole cottonseed inclusion rates.

	Treatment[2]					
Item[1]	**CON**	**WCS15**	**FAT4**	**LIB**	**SEM[3]**	***P*-value**
DMD, %	70.3	66.0	71.6	61.8	11.80	0.17
NDFD, %	72.8	68.2	73.4	64.2	10.55	0.23
ADFD, %	74.5	72.3	76.1	67.6	9.26	0.10
AAN, %	65.8	64.3	70.3	63.2	12.29	0.49
EED, %	73.1	72.9	77.2	70.5	5.10	0.47
WCS Disappearance, %	99.9	99.9	99.9	99.9	< 0.01	0.48

[1]DMD = dry matter digestibility; NDFD = NDF digestibility; ADFD = ADF digestibility; AAN = apparently absorbed nitrogen; EED = ether extract digestibility

[2]CON = No whole cottonseed inclusion; WCS15 = Whole cottonseed inclusion at 15% dry matter intake; FAT4 = Whole cottonseed inclusion to supply fat at 4% dry matter intake; LIB = Whole cottonseed offered for *ad libitum* intake

[3]SEM = standard error of the mean. When SEM differed for various levels of treatment, the greatest value was reported.

[a,b,c,d] Means within a row with different superscripts are different ($P \leq 0.05$)

Table III-IV Blood metabolite concentrations in lactating beef cows fed bermudagrass hay across treatments differing in whole cottonseed inclusion rates.

	Treatment[2]					
Item[1]	**CON**	**WCS15**	**FAT4**	**LIB**	**SEM[3]**	***P*-value**
NEFA, μM	573	565	700	577	173.7	0.55
BUN, mg/dL	19.3	25.6	38.1	27.3	3.59	0.07
Gossypol, μg/mL	0.8[c]	1.9[b]	2.6[a]	2.2[b]	0.22	< 0.01

[1]NEFA = Non-esterified fatty acid; BUN = Blood urea nitrogen

[2]CON = No whole cottonseed inclusion; WCS15 = Whole cottonseed inclusion at 15% dry matter intake; FAT4 = Whole cottonseed inclusion to supply fat at 4% dry matter intake; LIB = Whole cottonseed offered for *ad libitum* intake

[3]SEM = standard error of the mean. When SEM differed for various levels of treatment, the greatest value was reported.

[a,b,c,d] Means within a row with different superscripts are different ($P \leq 0.05$)

Table III-V Cow and calf performance in lactating beef cows fed bermudagrass hay across treatments differing in whole cottonseed inclusion rates.

	Treatment[1]					
Item	**CON**	**WCS15**	**FAT**	**LIB**	**SEM[2]**	***P*-value**
Initial Cow BW, kg	632.0	622.0	608.0	604.0	31.89	0.92
Final Cow BW, kg	530.0	573.0	556.0	574.0	30.16	0.71
Cow ADG, kg	-1.5	-0.7	-0.8	-0.5	0.42	0.39
Milk Production, kg	2.1	2.7	3.7	2.5	0.61	0.34
Initial Calf BW, kg	96.0	83.0	89.0	88.0	6.39	0.57
Final Calf BW, kg	148.0	138.0	136.0	142.0	8.29	0.73
Calf ADG, kg	0.7	0.8	0.6	0.7	0.05	0.39

[1]CON = No whole cottonseed inclusion; WCS15 = Whole cottonseed inclusion at 15% dry matter intake; FAT4 = Whole cottonseed inclusion to supply fat at 4% dry matter intake; LIB = Whole cottonseed offered for *ad libitum* intake

[2]SEM = standard error of the mean. When SEM differed for various levels of treatment, the greatest value was reported.

[a,b,c, d] Means within a row with different superscripts are different ($P \leq 0.05$)

Table III-VI Energy fractionation in lactating beef cows fed bermudagrass hay across treatments differing in whole cottonseed inclusion rates.

	Treatment[2]					
Item[1]	**CON**	**WCS15**	**FAT4**	**LIB**	**SEM[3]**	***P*-value**
GE, Mcal/kg	3.8[c]	4.0[c]	4.1[b]	4.6[a]	0.21	< 0.01
DE, Mcal/kg	2.7	2.6	2.9	3.0	0.64	0.14
ME, Mcal/kg	2.2	2.1	2.4	2.5	0.53	0.14
NEM, Mcal/kg	1.3	1.3	1.5	1.6	0.49	0.14
NEG, Mcal/kg	0.7	0.7	0.9	1.0	0.44	0.15

[1]GE = Gross energy; DE = Digestible energy; ME = Metabolizable energy; NEM = Net energy of maintenance; NEG = Net energy of growth

[2]CON = No whole cottonseed inclusion; WCS15 = Whole cottonseed inclusion at 15% dry matter intake; FAT4 = Whole cottonseed inclusion to supply fat at 4% dry matter intake; LIB = Whole cottonseed offered for *ad libitum* intake

[3]SEM = standard error of the mean. When SEM differed for various levels of treatment, the greatest value was reported.

[a,b,c,d] Means within a row with different superscripts are different ($P \leq 0.05$)

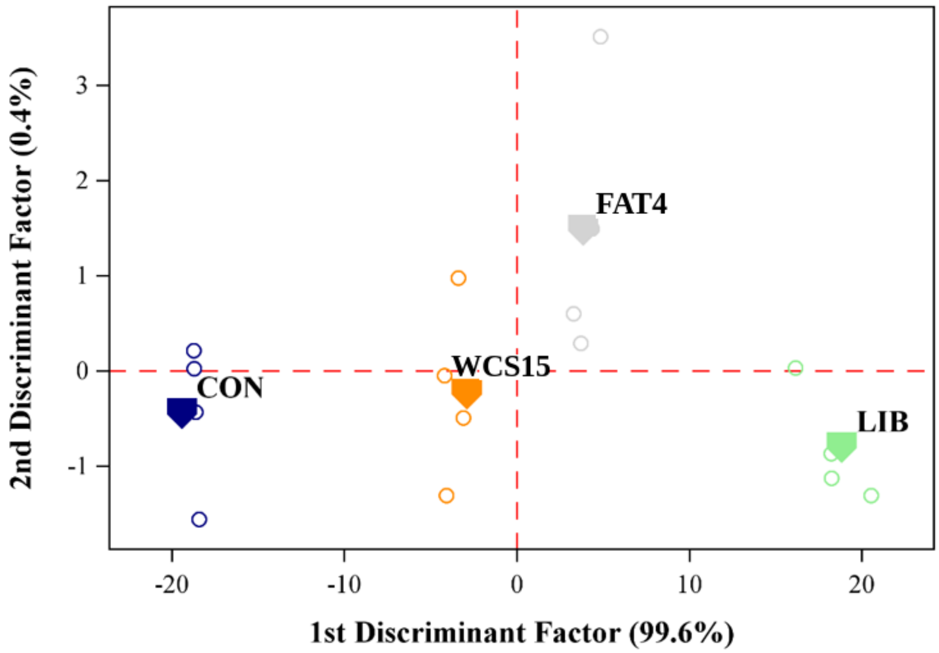

Figure III-I Canonical discriminant analysis illustrating the multivariate separation of whole cottonseed (WCS) supplementation treatments based on the response variables selected by stepwise discriminant analysis. The first canonical discriminant function was primarily associated with WCS intake, whereas the second discriminant function was primarily associated with blood urea nitrogen (BUN). Large, filled symbols represent treatment centroids (means of the canonical discriminant functions), and open circles represent individual animal × block × treatment observations. (CON = No whole cottonseed inclusion; WCS15 = Whole cottonseed inclusion at 15% dry matter intake; FAT4 = Whole cottonseed inclusion to supply 4% crude fat dry matter intake, LIB = Whole cottonseed offered for *ad libitum* intake)

Chapter IV Effect of Whole Cottonseed Delivery Frequency on Beef Cattle Performance in Warm-Season Forage Systems

Synopsis

Whole cottonseed (WCS) is a common byproduct feedstuff in the southeastern United States due to its affordability, high energy, and high protein content. Feeding recommendations for WCS were developed in the 1980s using seeds available at the time. Since this initial development, seed quality characteristics have continued to change over time. Additionally, beef producers in Alabama utilize WCS in rations at inclusion rates contrary to established feeding rate recommendations. Recent research has demonstrated that WCS can be fed at or above established feeding recommendations with minimal negative effects on digestibility and performance. However, these studies relied on daily feeding of WCS at a fixed target rate or *ad libitum*, but do not reproduce common reduced feeding frequencies utilized by beef producers. Therefore, the objective of this study was to determine the effect of feeding frequency of WCS on the performance of gestating cows. In a completely randomized experiment, gestating cows (n = 32) were randomly allocated to one of four WCS delivery frequencies: no supplemental WCS (CON); supplemental WCS offered 2 d per week (2X); supplemental WCS offered 3 d per week (3X); supplemental WCS offered daily (7X). Whole cottonseed was fed to achieve a crude fat intake of 4% dry matter. Cows were fed for 84 d on paddocks established with signalgrass (*Urochloa platyphylla* [Munro ex C.Wright]) and sorghum-sudangrass (*Sorghum bicolor* [L.] Moench ssp. *drummondii*). Forage sampling occurred every 7 d and blood

collection with body weight measurement occurred every 14 d. There was no effect of delivery frequency on body weight, blood urea nitrogen (BUN), non-esterified fatty acid (NEFA), or plasma gossypol concentration ($P \geq 0.49$). Likewise, there was no effect of delivery frequency on forage mass, forage allowance, or the botanical composition of signal grass, sorghum-sudangrass, weeds and dead material ($P \geq 0.47$). While not significant, there was a visual trend of decreasing forage allowance as feeding interval increased which could indicate a possible increase in forage intake among animals with reduced feeding frequency. Results from this study suggest that feeding interval has no measured effect on beef cow performance.

Introduction

Cotton (*Gossypium hirstum* [L.]) cultivars produced in the Southeast are being improved by plant breeding efforts to improve lint and seed quality. These genetic improvements have caused an effect on the nutritional value of whole cottonseed (Jacobs et al., 2021; Jacobs et al., 2022). To address these changes, short-term feeding trials have been conducted to investigate free-choice WCS feeding (Jacobs et al., 2022; Mullenix et al., 2022).

In southeastern United States, whole cottonseed (WCS) is a common supplemental byproduct feedstuff for cow-calf producers as it is a secondary product of cotton. Whole cottonseed feeding recommendations were developed in the 1980s based on seed characteristics of the time (Moore et al., 1986; Rogers et al., 2002; Mullenix et al., 2022). At that time Palmquist and Jenkins recommended that WCS should be limited to no more than 15% dry matter intake (DMI) of dairy cattle (1980). Additionally, Roger et al. (2002)

stated that WCS inclusion should be limited to not more than 0.5% of body weight (BW), which relates to approximately 4% fat in the total diet.

Recent research has demonstrated that WCS can be fed at, or above, feeding recommendations with no measured negative effect on animal performance in cow-calf systems. However, this research was conducted in a feeding scenario in which animals were fed rations every 24 hours, which does not represent the range of feeding frequencies used by beef operations using WCS. Therefore, the objective of this study was to determine the effect of feeding frequency of WCS on the performance of gestating cows when supplemented at a rate above feeding recommendations.

Materials and Methods

All procedures for this experiment were approved by the Animal Care and Use Committee (IACUC) of Auburn University under the Animal Use Protocol 2025-5616.

Experimental Design

This experiment was conducted as a completely randomized design at the E.V. Smith Research Center Beef Unit in Shorter, Alabama. Sixteen paddocks (n = 16; approx. 0.8 ha). Thirty-two gestating cows were fed on pasture for a 84 d experiment. Each paddock had a base forage of perennial signalgrass (*Urochloa platyphylla* [Munro ex C.Wright]; SG) and annual sorghum-sudangrass (*Sorghum bicolor* [L.] Moench ssp. *drummondii*; SS). Across the 84 d, available forage was sampled every 14 d. Animal sampling occurred every 28 d. Gestating cows of each paddock were randomly assigned to treatments such that each treatment was applied to four paddocks and 8 cows.

Animals

Thirty-two Angus-cross gestating cows were used in this experiment. Cows varied in age from approximately five to eight years old with an average initial body weight (BW) of 697 ± 48 kg. Cows were approximately 160 d gestation at the initiation of the experiment.

Whole Cottonseed Treatments

For this experiment gestating cows were allowed to graze a base forage of SG and SS while assigned to one of four delivery rates of supplemental WCS to achieve a crude fat intake of 4% dry matter (0.625% BW on DM basis) in addition to a basal diet of SG and SS: a control group receiving no supplemental WCS (CON), a group receiving supplemental WCS 2 d per week (2x), a group receiving supplemental WCS 3 d per week (3x), and a group receiving supplemental WCS every d (7x). Whole cottonseed was obtained from Milstead Farm Group in Shorter, Alabama in the 2025 growing season.

Experimental Procedures

Experimental Timeline

Gestating cows were placed in their assigned paddocks and provided supplemental WCS, according to their weight, at their assigned frequency. Every 14 d, existing forage was sampled to determine forage biomass and nutritive value. Every 28 d, animals were weighed and blood was collected to determine animal performance. The study continued for 84 d and was concluded two weeks prior to expected parturition. Following each 14 d

DMY determination, each paddock was resized using temporary fencing to maintain a target forage allowance of 1 kg DM / 1 kg BW ratio.

Feeding and Sampling

Respective dietary treatments were offered at their assigned frequency at 0800 h. On each day of feeding, dietary treatments were offered at 0.625% BW. On 1 d, samples of each dietary treatment were collected to determine nutritive value (Table IV-1).

Every 28 d from the initiation, while in the squeeze chute for body weight (BW) collection, blood was collected via jugular venipuncture into three 10 mL evacuated tubes, two containing K_2-EDTA and the other a clot activator. After collection, blood tubes were inverted and centrifuged at 2500 x g for 15 minutes. Following centrifuging, 2 subsamples of serum and 4 subsamples of plasma were collected from the red top and purple top tubes, respectively, and frozen at -20°C for further analysis.

Forage samples (n = 6 per paddock) were collected every 14 d with a 0.16m^2 quadrat that was made of polyvinyl chloride, harvested to ground level from six random, representative locations throughout each paddock to determine forage biomass and nutritive value. Two out of six of the forage samples per paddock were separated and used to determine botanical composition of each paddock.

Analytical Procedures

Nutritive Value

Forage samples were dried to 55°C for DM determination. Dried samples were then ground to pass through a 2-mm screen. A subsample was then of each sample was ground to pass through a 1-mm screen using a Eberbach E3500 Series Mill (Eberbach Corporation, Charter Township, MI, USA). Ground subsamples were then combined to create a composite sample of paddock and date.

Dry matter and organic matter (OM) were determined according to the procedure of AOAC (2000) protocols. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined according to the procedure of Vogel et al. (1999). Hemicellulose was determined as the difference in NDF and ADF. Acid detergent lignin (ADL) was determined using the sulfuric acid method (AOAC, 2000) using the ANKOM^{DELTA}. Cellulose was determined as the difference in ADF and ADL.

Composite samples were assayed for crude protein by combustion analyzer in a commercial laboratory [Protein (Crude) in Animal Feed (990.03)]; Cumberland Valley Analytical Services, Waynesboro, PA, USA). Whole cottonseed composite samples were assayed for ether extract by high temperature solvent extraction in a commercial laboratory (AOCS Am 5-04; Cumberland Valley Analytical Services, Waynesboro, PA, USA).

Gossypol

Gossypol concentration was determined using ground WCS kernels that were initially processed to pass through a #12 screen and subsequently ground with a Braun® hand blender until the material passed through a #20 sieve. The ground kernel samples were analyzed via high-performance liquid chromatography (HPLC; Agilent 1100, Palo Alto, CA) with an Inertsil ODS column (SGE, Austin, TX), a diode array detector, and a mobile phase flow rate of 1m/min using the AOCS Ba 8a-99 method. Absorbance at 254 nm was recorded and used a detection wavelength. Detectable limit of gossypol was 0.46 μg, therefore a normal distribution was created of values between 0 and 0.46 and measurements that fell below the measurement threshold were assigned a value randomly along the normal distribution.

Blood Metabolites

Blood urea nitrogen (BUN) was determined with via colorimetric ELISA blood metabolite assay kit (EIABUN, ThermoFisher Scientific, Life Technologies Corporation, Frederick, MD, USA). Non-esterified fatty acid (NEFA) was determined via colorimetric blood metabolite assay kit (ACS-ACOD Method; RayBiotech Inc., Peachtree Corners, GA, USA). Total plasma gossypol was determined via high-performance liquid chromatography (HPLC; Agilent 1100, Palo Alto, CA) with an Inertsil ODS column (SGE, Austin, TX), a diode array detector, and a mobile phase flow rate of 1m/min using the AOCS Ba 8a-99 method. Absorbance at 254 nm was recorded and used a detection wavelength.

Gossypol exists as two naturally occurring optical isomers, (+)-gossypol and (−)-gossypol, which differ in their three-dimensional configuration despite having the same chemical composition (Zhang et al., 2007). The (−)-isomer exhibits greater biological activity and toxicity because it binds more readily to proteins and other cellular components, whereas the (+)-isomer is less biologically active (Gadelha et al., 2014).

Statistical Analysis

Data were analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). The design of the feeding experiment was a completely randomized design with four dietary treatments.

Forage and WCS nutritive value concentrations were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX). The fixed effect was dietary treatment, and denominator degrees of freedom were adjusted using the 2nd-order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were paddock. Botanical compositions, blood metabolites, and body weight were analyzed as repeated measurements using PROC GLIMMIX. The model fixed effects included dietary treatment. Denominator degrees of freedom were adjusted using the 2nd-order Kenward-Roger approximation (Kenward and Roger, 2009). Random effects were paddock. Response variables were analyzed using mixed model regression to evaluate the effects of WCS delivery frequency using PROC MIXED. Response variables were treated as continuous variables and a regression was performed across delivery frequencies.

Results and Discussion

Forage Parameters

Mixed model analysis indicated that, as expected, there was no effect of delivery frequency on botanical composition of SS (P = 0.47), SG (P = 0.50), weed (P = 0.80), or dead material (P = 0.84; Figure IV-I). There was an effect of time on botanical composition of SS (P < 0.01) and SG (P < 0.01), but there was no effect on weed (P = 0.22) or dead material (P = 0.22; Figure IV-I. Subsequently, there was no effect of delivery frequency on OM (P = 0.57), NDF (P = 0.69), ADF (P = 0.76), ADL (P = 0.80), or CP (P = 0.88) while there was an effect of d on OM (P < 0.01), CP (P < 0.01), and NDF (P < 0.01; Figure IV-I).

There was no effect of delivery frequency on forage mass (P = 0.96) or forage allowance (P = 0.57). In contrast, time affected both forage mass (*P* < 0.01; and forage allowance (*P* = 0.02; Figure IV-III). While there was no effect of delivery frequency on forage allowance, there was a visual pattern of decreasing forage allowance among infrequent feeding frequencies as seen in Figure IV-III. This is possibly due to a stimulatory effect of the WCS on total DMI. This is supported by research conducted by Huston et al. (1993) which found that adult cows supplemented on rangeland had increased total DMI. Additional research found that the inclusion of WCS can suppress intake due to its high fat content (Coppock et al., 1985). While this visual pattern of decreased forage allowance was not observed in animals high feeding frequencies of WCS every, it is possible that the inconsistent supplementation of WCS had a stimulatory effect on DMI that was not seen in those animals with daily feeding. While not using WCS, a similar effect was found by Huston et al. (1999) with beef cows fed supplemental cottonseed meal. Animals fed once weekly had less variable DMI than those fed daily. Additionally, Huston et al. (1993) found that low level feeding (0.15% BW) of high protein supplements can stimulate forage intake.

Animal Performance

There was no effect of delivery frequency on cow BW (*P* = 0.13); however, there was an effect of time (*P* < 0.01; Figure IV-IV). While ADG between delivery frequencies did not differ, all cows had a greater ending BW than starting BW, likely due to their proximity to parturition. While cows fed WCS had greater nutritive performance, it is likely that an 84 d feeding period was not long enough to determine BW changes between delivery frequency.

Additionally, cattle had access to the same nutritive value forage throughout the feeding and therefore the same basal diet.

Blood Metabolites

There was no effect of treatment on BUN (15.5 ± 2.43 mg/dL; *P* = 0.49; Figure IV-V) or NEFA (597.0 ± 95.81 µM; *P* = 0.54; Figure IV-VI). Additionally, BUN concentrations in beef cattle are used as an indicator of protein and energy balance. Normal BUN concentrations typically range from approximately 7 to 15 mg/dL (Herath et al., 2018). Concentrations below this range may indicate inadequate dietary protein in lactating cow diets, while concentrations above 20 mg/dL often indicate excess rumen degradable protein relative to available energy, resulting in inefficient nitrogen utilization (Herath et al., 2018). Non-esterified fatty acid concentrations greater than approximately 600 µM indicate substantial mobilization of body fat reserves. Concentrations exceeding 800 µM are associated with severe negative energy balance during early to peak lactation of dairy cows (Oetzel, 2004). Overall, these levels suggest that the cows in this study, regardless of delivery frequency or inclusion of WCS, were in a positive energy balance with adequate dietary protein.

There was no effect of treatment on plasma gossypol concentration among treatments supplemented with WCS (1.1 ± 0.22 µg/mL; *P* = 0.89; Figure IV-VII). Established safe levels of plasma concentrations, established by Tand et al. (2018), are < 5 µg/mL for (±)-gossypol and <3.5 µg/mL for (-)-gossypol. Safe levels of both isomers of gossypol were not exceeded in any delivery frequency. Based on the blood metabolite levels, no evidence of gossypol toxicity was present in any of the treatments.

Conclusion

When evaluating the performance of gestating beef cows in a warm-season grazing system supplemented with WCS at different feeding intervals, there was no change in BW, BUN, NEFA, or plasma gossypol. Therefore, feeding supplemental WCS at longer intervals that conform to normal practice among beef cattle producers had no measured effect on the performance of gestating beef cows. Additionally, there was a visual trend of decreasing forage allowance as delivery interval increases. This visual trend may suggest that cows supplemented with WCS weekly had stimulated forage DMI. This possible effect of increased DMI by supplemental feeding has been documented in past research when high protein feed was fed at a low level. Overall, this supports the feeding of WCS at longer weekly intervals to accommodate labor and management limitations of producers.

Future research should investigate the effect of feeding interval on forage and total DMI of beef cows supplemented with WCS in order to investigate the trend of decreasing forage allowance among cows with increasing feeding intervals. This may give insight into how infrequent feeding of high fat feeds such as WCS can alleviate the reduced DMI measured in past research.

Table IV-I Nutritive value of whole cottonseed used in the evaluation of performance of gestating cows offered supplemental whole cottonseed in differing delivery frequencies.

Item, %[1]	**Whole Cottonseed**
DM	90.4
OM	96.6
NDF	43.3
ADF	37.2
ADL	10.8
Hemicellulose	6.1
Cellulose	31.1
Ash	4.4
CP	24.1
EE	14.1
Gossypol	0.96

[1]DM = Dry matter; OM = Organic matter; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; CP = Crude protein; EE = Ether extract

Table IV-II Nutritive value of whole cottonseed and forage used in the evaluation of performance of gestating cows offered supplemental whole cottonseed in differing delivery frequencies.

Item[1]	**Intercept** [2]	**Frequency** [3]	**Day** [4]	**Frequency × Day**	***P*-value (Freq.)**	***P*-value (Day)**	***P*-value (F × D)**
OM, %	65.0 [62.2, 67.8]	(0.1) [(0.9), 0.6]	0.1 [0.0, 0.1]	(0.0) [(0.0), 0.0]	0.69	< 0.01	0.88
NDF, %	89.1 [88.6, 89.6]	0.0 [(0.1), 0.2]	0.0 [0.0, 0.0]	(0.0) [(0.0), 0.0]	0.57	< 0.01	0.53
ADF, %	36.2 [28.6, 43.9]	(0.2) [(2.1), 1.7]	0.0 [(0.0), 0.1]	0.0 [(0.0), 0.0]	0.76	0.17	0.89
ADL, %	5.9 [(1.5), 13.2]	(0.2) [(2.1), 1.6]	0.0 [(0.1), 0.1]	0.0 [(0.0), 0.0]	0.80	0.83	0.87
CP, %	11.4 [11.0, 11.9]	(0.0) [(0.1), 0.1]	(0.0) [(0.1), (0.0)]	(0.0) [(0.0), 0.0]	0.88	< 0.01	0.80

[1]OM = Organic matter; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; ADL = Acid detergent lignin; CP = Crude protein

[a,b] Means within a row with different superscripts are different ($P \leq 0.05$)

[2]Intercept represents the mean forage nutritive value at Day 0 with 95% confidence interval listed in brackets (intercept of the regression model)

[3]Estimated linear change in forage nutritive value with increasing feeding frequency with 95% confidence interval listed in brackets

[4]Estimated linear change in forage nutritive value over the experimental period with 95% confidence interval listed in brackets

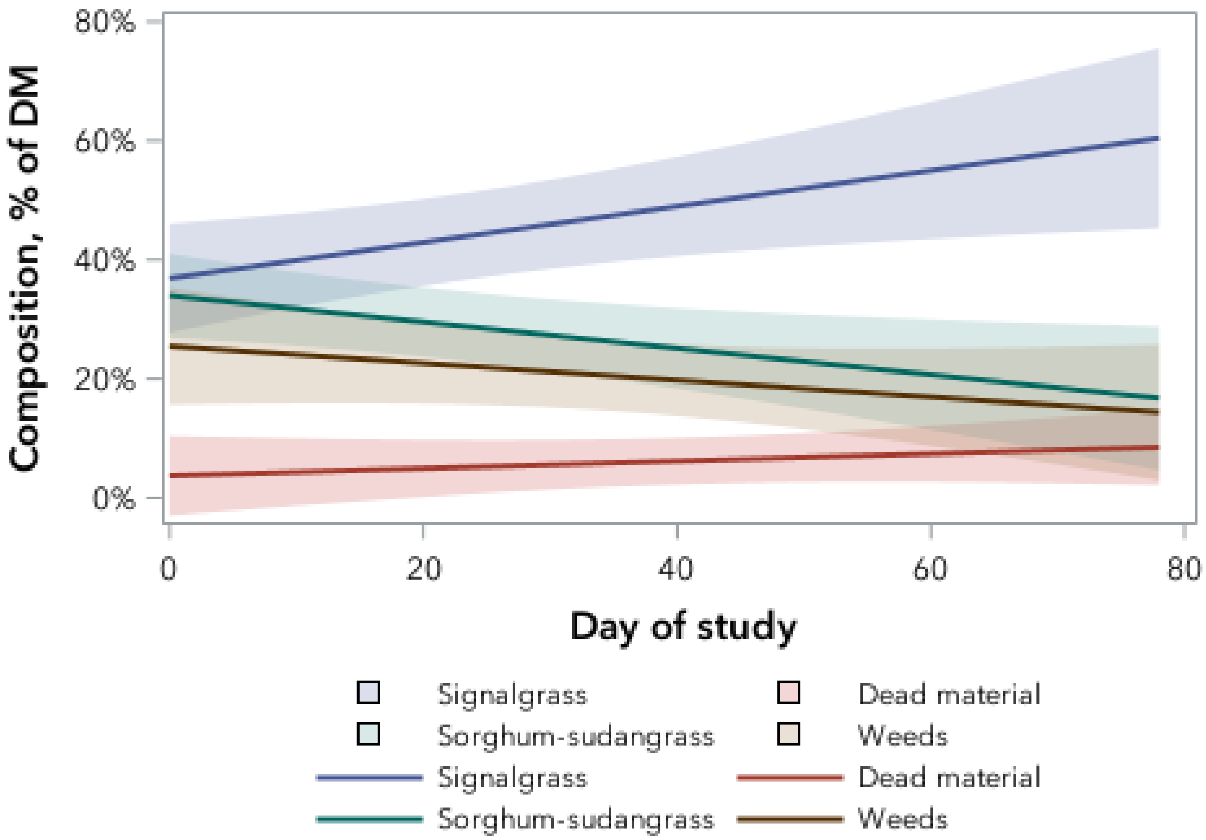

Figure IV-I Effect of time on botanical composition of signalgrass, sorghum-sudangrass, weeds, and dead material in paddocks with gestating cows receiving whole cottonseed supplementation at different intervals.

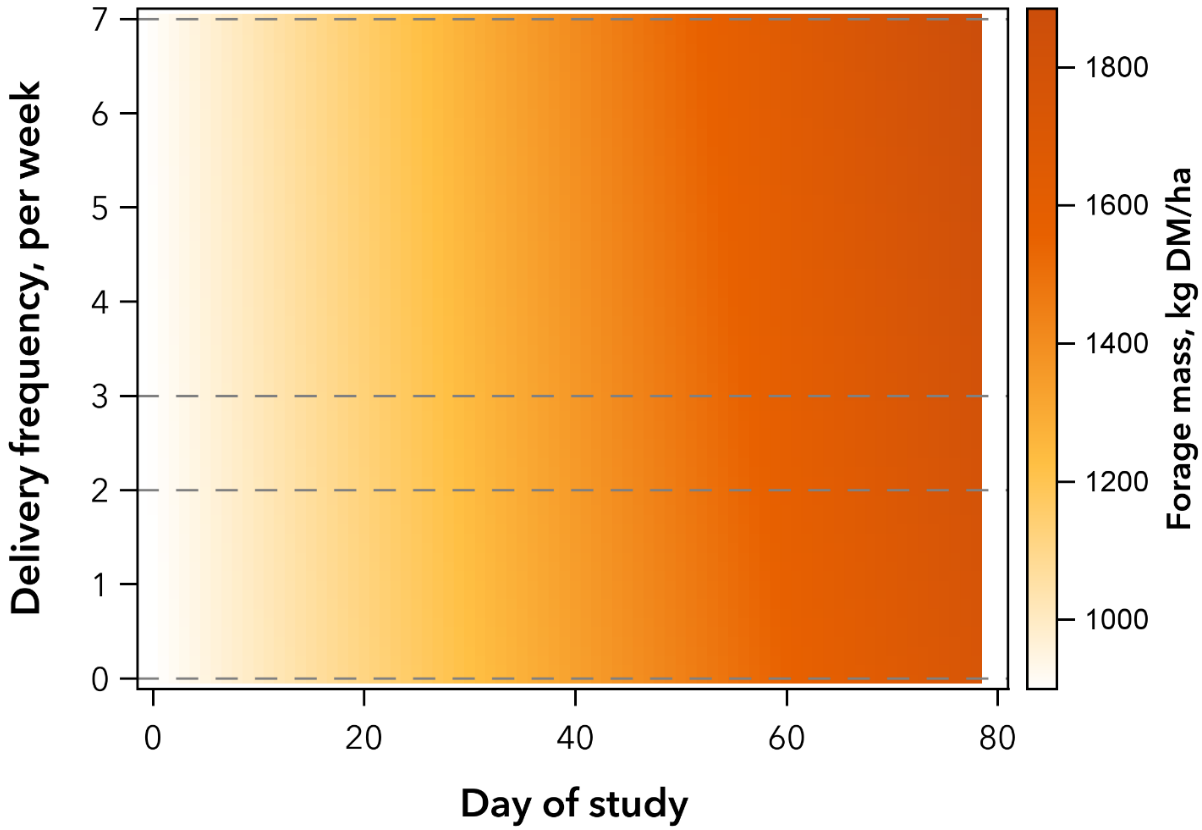

Figure IV-II Predicted forage mass (kg DM) as influenced by supplementation delivery frequency and day of study for gestating beef cows receiving whole cottonseed (WCS). Color intensity represents model-predicted forage mass, with lighter colors indicating lower values and darker colors indicating greater values. Dashed horizontal lines indicate the supplementation frequencies evaluated experimentally (0, 2, 3, and 7 deliveries per week), whereas the continuous gradient between these lines represents predictions from the fitted regression model..

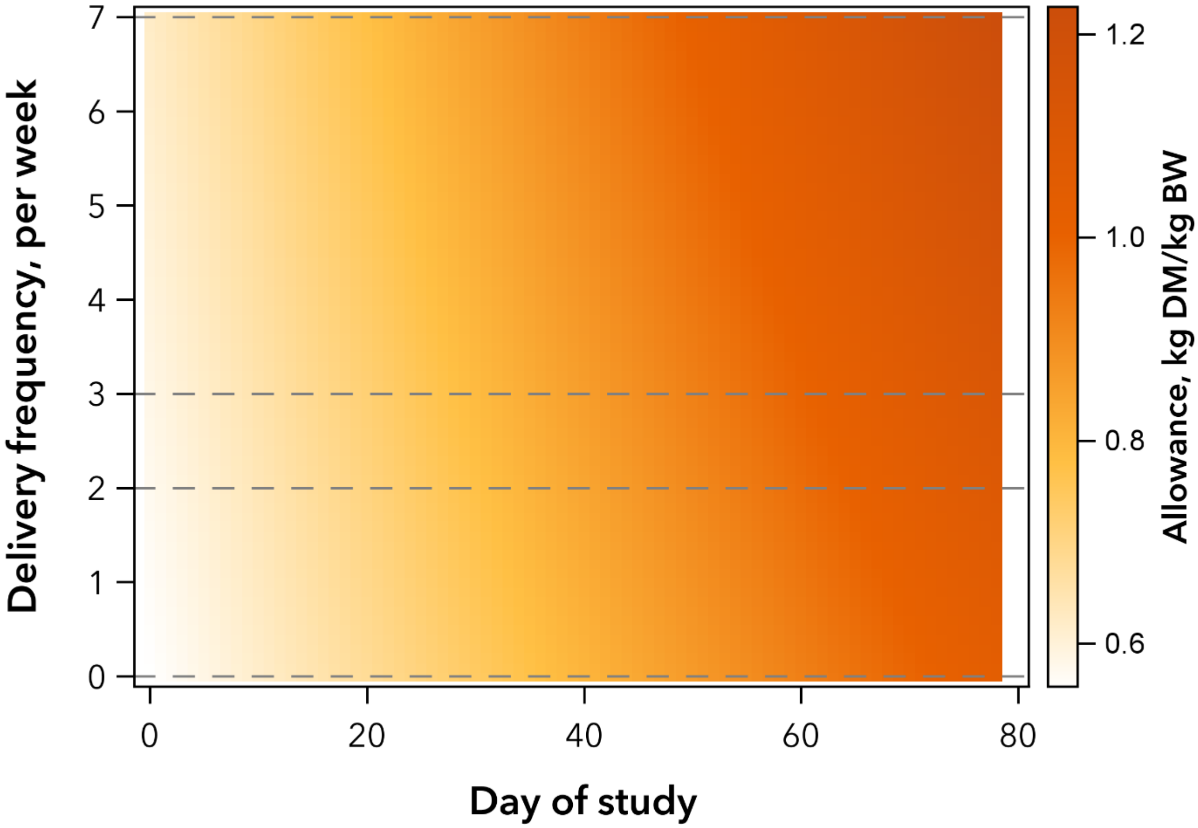

Figure IV-III Predicted forage allowance (kg DM/kg BW) as influenced by supplementation delivery frequency and day of study for gestating beef cows receiving whole cottonseed (WCS). Color intensity represents model-predicted forage allowance, with lighter colors indicating lower values and darker colors indicating greater values. Dashed horizontal lines indicate the supplementation frequencies evaluated experimentally (0, 2, 3, and 7 deliveries per week), whereas the continuous gradient between these lines represents predictions from the fitted regression model.

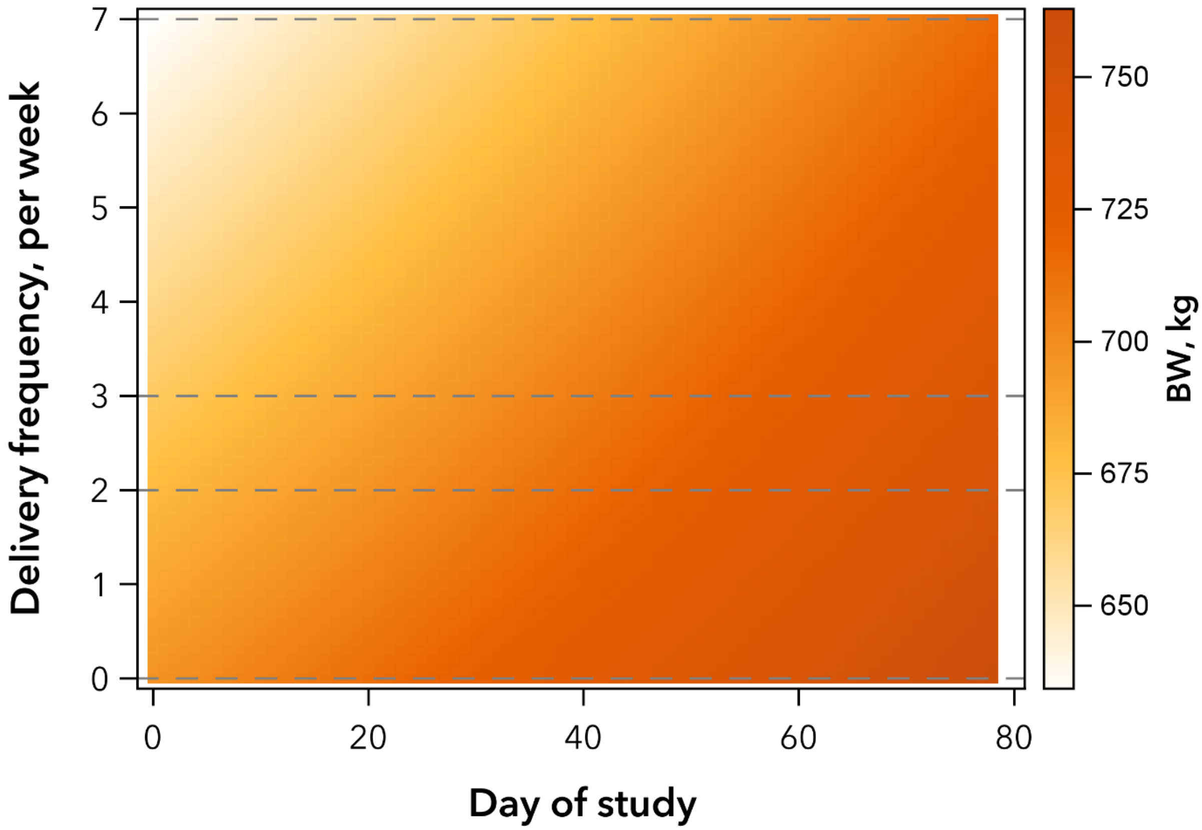

Figure IV-IV Predicted body weight (BW; kg) as influenced by supplementation delivery frequency and day of study for gestating beef cows receiving whole cottonseed (WCS). Color intensity represents model-predicted BW, with lighter colors indicating lower values and darker colors indicating greater values. Dashed horizontal lines indicate the supplementation frequencies evaluated experimentally (0, 2, 3, and 7 deliveries per week), whereas the continuous gradient between these lines represents predictions from the fitted regression model.

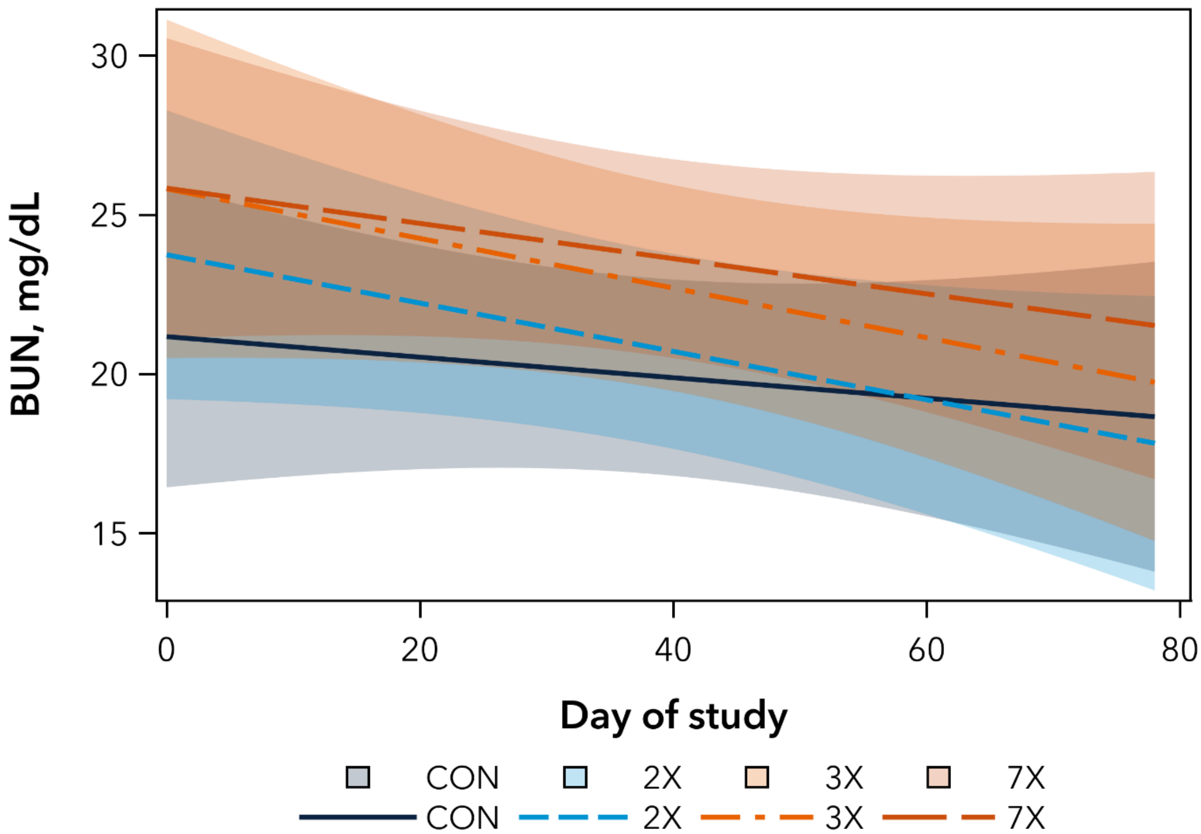

Figure IV-V Effect of delivery frequency and time on blood urea nitrogen serum concentration of gestating cows receiving whole cottonseed supplementation at different intervals.

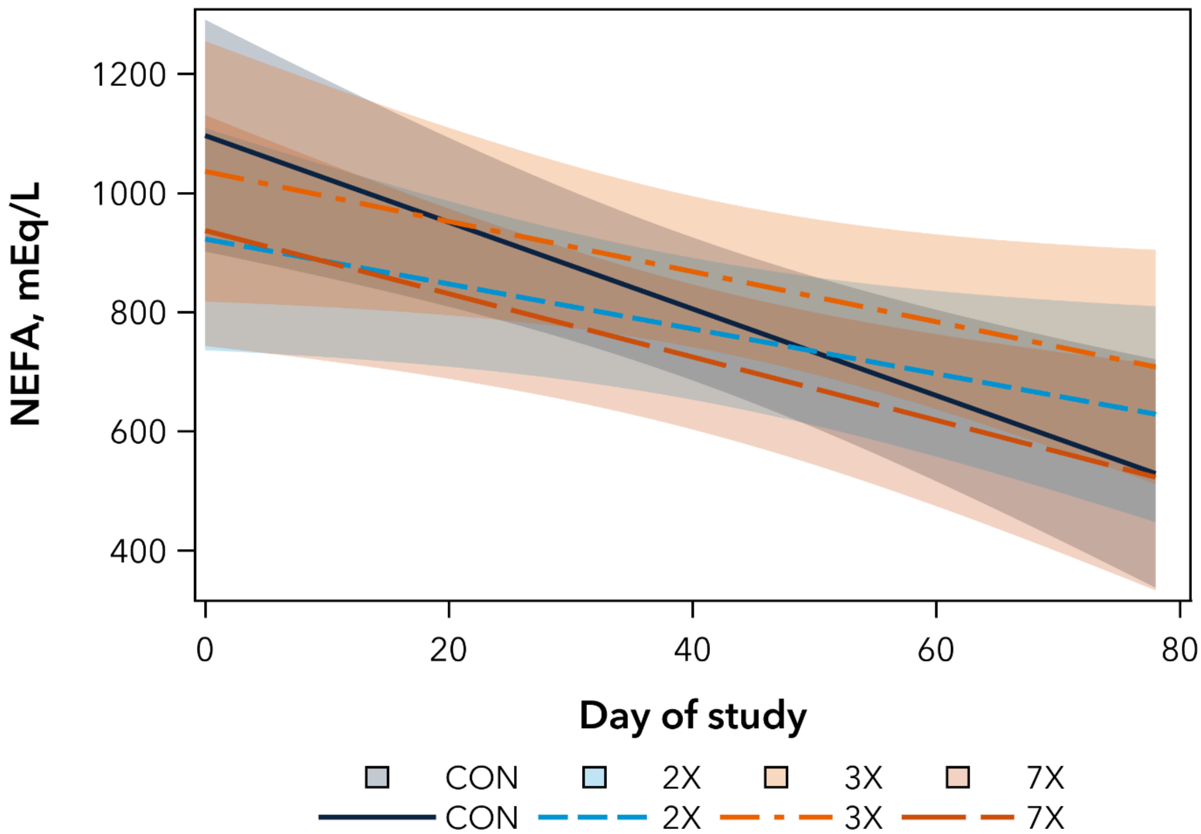

Figure IV-VI Effect of delivery frequency and time on non-esterified fatty acid serum concentration of gestating cows receiving whole cottonseed supplementation at different intervals.

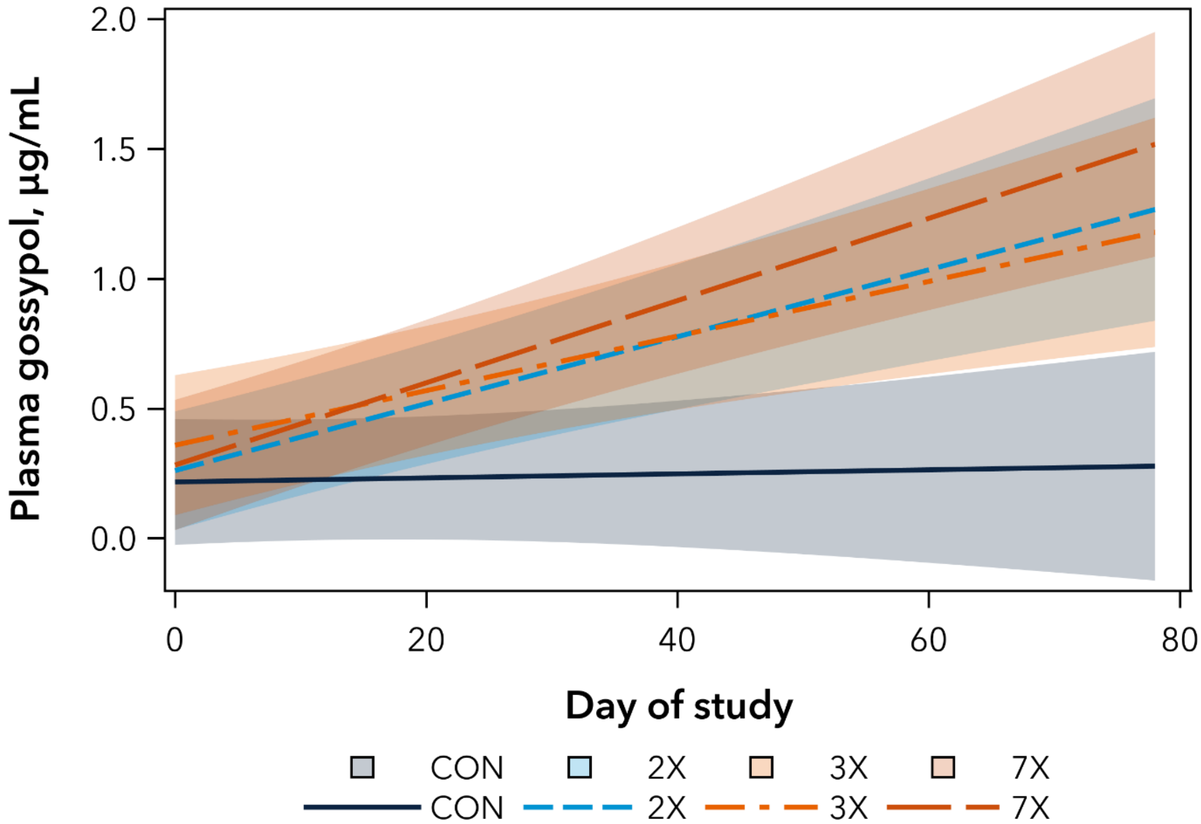

Figure IV-VII Effect of delivery frequency and time on plasma gossypol concentration of gestating cows receiving whole cottonseed supplementation at different intervals.

Chapter V

Conclusions and Implications

Summary of Findings

When evaluating the performance of lactating beef cows supplemented with WCS, FAT and LIB had greater WCS, CP, and EE intake, showing an increase in nutritive value. While no change in milk production was observed in any treatment, previous research has been inconclusive on the effect of WCS supplementation on lactation. Subsequently, there was no effect of treatment on calf and cow ADG. Additionally, there was no effect on digestibility or nitrogen absorption between treatments, in contrast to the expected inhibition of fiber from a high fat inclusion. When evaluating the performance of gestating beef cows in a warm-season grazing system supplemented with WCS at different feeding intervals, there was no change in BW, BUN, NEFA, or plasma gossypol. Additionally, there was a visual trend of decreasing forage allowance as delivery interval increased.

Implications

Overall, the results of these studies demonstrate that whole cottonseed (WCS) can be utilized effectively in beef cow diets at or above traditionally recommended inclusion rates without negatively affecting nutrient digestibility, physiological responses, or animal performance. Increased WCS intake improved dietary nutritive value; however, greater consumption did not consistently translate into additional performance benefits, suggesting that excessive supplementation may reduce economic efficiency. Furthermore, extending supplementation intervals to frequencies commonly used by beef producers did not adversely affect the performance of gestating beef cows. Although forage allowance tended to decrease as feeding interval increased, this response may indicate increased forage dry matter intake and warrants further investigation. Collectively, these findings suggest that WCS is a flexible supplementation strategy that can be incorporated into beef production systems at elevated inclusion rates and extended delivery intervals, allowing producers to accommodate labor and management constraints while maintaining animal performance.

Limitations

Although every effort was made to ensure the integrity of these experiments, several limitations should be considered when interpreting the results. As with many animal nutrition studies, occasional equipment and management challenges occurred during data collection. Minor issues related to feed delivery and animal access to individual feeding stations, such as aged and faulty equipment, were identified and corrected promptly to minimize their potential influence on treatment responses. Additionally, the incorporation of marker compounds into supplemental feed may have introduced a small degree of analytical variation in estimates of fecal output. However, all identified issues were addressed according to established protocols, and no evidence suggested that these limitations systematically biased treatment comparisons. Therefore, while a modest increase in experimental variation may have occurred, the authors are confident that the overall findings and conclusions of these studies remain valid, and representative of the biological responses observed.

Additionally, although appropriate statistical procedures were employed to account for the relatively small sample size, increasing the number of animals included in future studies would improve statistical power and increase the ability to detect more subtle biological responses. A larger sample size would also reduce the influence of individual animal variation, allowing treatment effects that may have been biologically meaningful but statistically indistinguishable in the present study to be detected with greater confidence.

Future Research

Future research should evaluate the effects of whole cottonseed (WCS) supplementation frequency on forage intake and total dry matter intake in beef cattle to better understand the observed trends in forage utilization under extended feeding intervals. A greater understanding of intake responses may provide insight into how infrequent delivery of high-fat supplements influences grazing behavior, nutrient consumption, and overall animal performance. Additionally, these responses should be evaluated across different stages of production, such as lactation, gestation, and post-weaning, as nutrient requirements and metabolic demands vary considerably throughout the production cycle. Research conducted under different seasonal grazing conditions and forage qualities would also help determine whether the effects of supplementation frequency are consistent across production environments or are influenced by changes in forage availability and nutritive value.

Additionally, despite the high fat concentration of WCS, the current studies observed no detrimental effects on nutrient digestibility or animal performance at elevated levels of supplementation. While these findings suggest that WCS can be incorporated into beef cattle diets at greater inclusion rates than traditionally recommended, the biological mechanisms responsible for this response remain poorly understood. Future research should focus on characterizing the ruminal digestion, passage kinetics, and metabolic fate of WCS within the ruminant digestive tract to better understand how its nutrients are utilized. Such information would help explain the apparent tolerance of beef cattle to greater dietary fat concentrations from WCS and provide a physiological basis for revising current feeding recommendations for modern cottonseed cultivars.

Beyond digestion and metabolism, additional research is warranted to evaluate the practical application of WCS supplementation under grazing conditions. One area of particular interest is the effect of supplementation frequency on forage intake, grazing behavior, and animal performance. Whole cottonseed is commonly delivered at intervals ranging from daily to once weekly to reduce labor and fuel costs, yet limited research has evaluated how feeding frequency influences substitution of forage with supplement. A controlled feeding trial comparing multiple supplementation frequencies while maintaining equal weekly nutrient delivery would provide valuable information on changes in daily dry matter intake, forage consumption, ruminal fermentation patterns, body weight gain, and circulating metabolites such as blood urea nitrogen and non-esterified fatty acids. Incorporating behavioral measurements, including grazing time, bunk attendance, and feeding activity through electronic monitoring systems, would further improve understanding of how cattle adapt to less frequent supplementation.

Quantifying these responses would allow researchers to determine whether infrequent supplementation alters forage utilization efficiency compared with daily feeding. Such information would have direct implications for producers seeking to minimize labor requirements without sacrificing animal performance or pasture utilization. Ultimately, integrating digestion studies with applied grazing experiments would provide a more complete understanding of WCS supplementation strategies and support evidence-based recommendations that maximize both biological efficiency and economic return in beef production systems.

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Appendices

Appendix 1.1

First author	Year	Title	Animal	Outcomes
Adams	1995	Effects of varying forage types on milk-production responses to whole cottonseed, tallow, and yeast	Dairy cattle	DMI, Cow ADG, Milk yield
Beck	2018	Whole cottonseed supplementation improves performance and reduced methane emission intensity of grazing beef steers	Beef cattle	Calf ADG
Beck	2019	Fat supplements differing in physical form improve performance but divergently influence methane emissions of grazing beef cattle	Beef cattle	DMI, Calf BW, Calf ADG
Brosch	1989	Cottonseed for protein and energy supplementation of high-roughage diets for beef cattle.	Beef cattle	DMI, Calf BW, Calf ADG
Ehrlich	1993	Use of whole cotton seed as a dietary supplement for grazing dairy cows.	Dairy cattle	Milk yield, BW change
Grainger	2008	Supplementation with whole cottonseed reduces methane emissions and can profitably increase milk production of dairy cows offered a forage and cereal grain diet	Dairy cattle	Milk yield, BW change
Grainger	2008	Effect of whole cottonseed supplementation on energy and nitrogen partitioning and rumen function in dairy cattle on a forage and cereal grain diet	Dairy cattle	DMI, Milk yield
Gunter	2021	Evaluation of nitrogen-delivery methods for stocker cattle grazing annual ryegrass	Beef cattle	Calf ADG
Hernandez	2025	Whole Cottonseed as an Effective Strategy to Mitigate Enteric Methane Emissions in Cattle Fed Low-Quality Forages	Beef cattle	DMI, Cow BW, Cow ADG
Holland	2018	Stockpiled "Tifton 85" bermudagrass for cow-calf production as influenced by nitrogen fertilization	Beef cattle	Cow BW, Calf BW
Holm	1971	The effects of cottonseed supplementation on the productivity of an East Kimberley Shorthorn cattle herd.	Beef cattle	Cow ADG, Calf ADG
Little	1991	Comparison of sesame cake and cottonseed as supplementary sources of protein to weaned Ndama bull calves in the gambia	Beef cattle	Calf ADG
O'kelly	1968	The effect of drought feeding with whole cottonseed, and vitamin therapy, on the serum lipids and liveweight changes of beef cattle in north-western Australia.	Beef cattle	Calf BW
Poore	2008	Effect of whole cottonseed supplementation on energy and nitrogen partitioning and rumen function in dairy cattle on a forage and cereal grain diet	Dairy cattle	Calf BW, Calf ADG, BUN
Rekwot	2004	Effects of nutritional supplementation and exposure to bulls on resumption of post-partum ovarian activity in Bunaji (Bos indicus) cattle.	Beef cattle	Cow ADG
Thomas	2009	Effects of prepartum whole fuzzy cottonseed or whole raw soybean supplementation on response to timed artificial insemination by suckled mature beef cows following ovulation synchronization.	Beef cattle	Cow BW, Calf BW