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Effects of whole cottonseed supplementation on performance, semen quality, and manganese superoxide dismutase concentrations in blood and semen of beef bulls*

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ABSTRACT

Objective: This research aimed to determine the effects of whole cottonseed on performance, semen quality, and manganese superoxide dismutase (MnSOD) concentrations in the blood and semen of beef bulls.

Materials and Methods: In a completely randomized design, 46 (BW = 457 ± 12 kg; 16–18 mo old) Angus bulls were stratified by BW and randomly assigned to 1 of 3 treatments: DD (n = 14, control; 3.18 kg/d dried distillers grain), WD [n = 16; 1.59 kg/d whole cottonseed (0.56 and 0.82% free gossypol in yr 1 and 2, respectively) and 1.59 kg/d dried distillers grain], or WW (n = 16; 3.18 kg/d whole cottonseed). Bulls were weighed and blood and semen were sampled on d 0, 30, and 60. Body weights and morphological characteristics of spermatozoa were measured in both years, and MnSOD concentrations of blood and semen were measured in yr 1.

Results and Discussion: There was a treatment effect for ADG ($P = 0.002$) as bulls fed WW had a decreased ($P < 0.03$) ADG compared with bulls fed WD and DD. A time effect was observed for scrotal circumference ($P < 0.001$). Scrotal circumference measurements increased on d 60 compared with d 0 and 28 ($P \leq 0.02$). There was no time main effect for percentage of spermatozoa with other abnormalities ($P = 0.87$); however, there was a treatment effect ($P = 0.04$). Bulls in the WW treatment had fewer other abnormalities than bulls in DD ($P = 0.01$) but were not different than WD ($P = 0.27$).

Implications and Applications: Whole cottonseed did not affect reproductive parameters measured in this study or blood and semen concentrations of MnSOD. According to these results, WCS supplemented to beef bulls at 0.7% of BW for 60 d may not negatively affect semen quality or MnSOD concentrations in the blood or semen.

Key words: semen quality exam, beef cattle, manganese superoxide dismutase, spermatozoa

INTRODUCTION

Cotton production is an important part of the American agricultural industry, especially in the Southeast. In 2016 the United States ranked third in the world for total cotton production by producing over $3 billion worth of cotton. Byproducts from ginning cotton, such as whole cottonseed (**WCS**), have nutritional value as a source of protein, energy, or fiber and are often used as feedstuffs for ruminant animals (Stewart, 2010).

Cotton contains gossypol, a polyphenolic compound produced in the pigment glands of the cotton plant that acts as a natural defense agent against insects and can be toxic to animals (Wang et al., 2009; Gadelha et al., 2014). Gossypol is found in most parts of the cotton plant, including the stem, leaves, seeds, and flower buds, with the highest concentration found in the seed (Gadelha et al., 2014). Gadelha et al. (2014) reported interactions of gossypol with minerals in the diet such as Mn and Fe. The mechanism of gossypol toxicity is not completely understood (Prasad and Diczfalusy, 1982). However, the recommended level of WCS supplementation is 0.5% of BW per day for mature cows and bulls, and this is intended to keep the total fat in the diet below 5% to avoid the negative effect of fat on fiber digestion in the rumen (Stewart, 2010).

Gossypol toxicosis has been reported in monogastrics (Haschek et al., 1989) and ruminants (Morgan et al., 1988). Gossypol fed in excess to livestock has caused re-

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productive issues such as decreased scrotal circumference and increased sperm morphological defects (Chase et al., 1994; Chenoweth et al., 2000) in bulls. Oxidative stress affects fertility and physiology of spermatozoa as gametes are susceptible to reactive oxygen species (Agarwal et al., 2008; Bansal and Bilaspuri, 2010). Antioxidants such as a superoxide dismutase reduce damage caused by reactive oxygen species such as superoxide anion (O_2^-; Bansal and Bilaspuri, 2010). Manganese superoxide dismutase (**MnSOD**) is an enzyme that requires a cofactor, Mn, to be present for activation (Jeeva et al., 2015). Considering the potential interaction of gossypol and Mn, the inclusion of gossypol-containing feeds may decrease the activity of MnSOD; however, no data exist to explore this relationship.

In addition, the breeding season for fall calving beef herds often starts while cows are being supplemented with WCS in the winter in the southeastern United States, and the effect of WCS on mature bulls fed during the breeding season remains unclear. The objective of this study was to determine the effects of WCS on animal growth, semen characteristics, and MnSOD activity of the blood and semen of bulls fed differing levels of WCS.

MATERIALS AND METHODS

Treatments and Performance

All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (AUP # A2019 08-044-Y1-A0).

Across 2 yr, 46 (n = 46) 16- to 18-mo-old (BW = 457.3 ± 11.9 kg) Angus and Red Angus bulls were transported to the University of Georgia Tifton campus (Tifton, GA) from a cattle producer in Osierfield, Georgia. Bulls were weighed and subjected to a semen quality examination, and blood and semen samples were collected. Twenty-eight bulls were delivered each year, and bulls that did not pass the initial semen quality examination were removed from the study (n = 5 each year). Bulls were stratified by weight and randomly assigned to 1 of 3 treatment groups indicative of no supplementation of WCS or supplementation below the recommended level of WCS (0.33% BW) or above the recommended level of WCS (0.70% of BW). Treatments included (amounts reported on as-fed basis) **DD** (n = 14, control; 3.18 kg of dried distillers grain and 1.36 kg of soyhulls), **WD** [n = 16; 1.59 kg of WCS (9 and 13 g of free gossypol in yr 1 and 2, respectively), 1.59 kg of dried distillers grain, and 1.36 kg of soyhulls], or **WW** [n = 16; 3.18 kg of WCS (18 and 26 g of free gossypol in yr 1 and 2, respectively) and 1.36 kg of soyhulls]. Each bull received 113.4 g of Pasture Mineral (including 16% NaCl, 18.2% Ca, 6.5% Mg, 2.8% P, 1.1% K, 8,550 mg/kg Fe, 4,420 mg/kg Zn, 3,420 Mn, 2,710 mg/kg Cu, 26.5 mg/kg Se, 143,300 IU/kg vitamin A, 28,700 IU/kg vitamin D, and 230 IU/kg vitamin E; Multi-Kare Inc.) and 56.7 g of calcium carbonate (Multi-Kare Inc.) each day. Dried distillers grains were used as a protein source similar to WCS, and soybean hulls were used to increase the energy on all diets. Both ingredients are commonly used in the southeastern United States for supplementation. Bulls were fed for 60 d to mimic a controlled breeding season and to allow spermatogenesis to occur. Additionally, bulls did not have prior exposure to WCS to mimic production settings in which bulls do not have access before the breeding season. The experimental diet was intentionally limit fed at approximately 1% of BW throughout the experiment to ensure complete consumption. The WCS, dried distillers grain, soy hull pellets, mineral, and calcium were premixed each week and hand delivered each day. The bunks were inspected daily, and orts were collected on a weekly basis; however, no feed refusals were observed. In addition to the experimental diet, bulls had ad libitum access to bermudagrass (*Cynodon dactylon*) hay. Individual ingredients were sampled on d 0, 30, and 60; pooled across date; and submitted for analysis at Cumberland Valley Analytical Services (Waynesboro, PA). The chemical analysis of each feed ingredient is presented in Table 1. Each bull was housed individually in a 3.65 × 10.97 m covered pen with fresh, clean water. Bulls were weighed (Model 450 For-Most Portable Scale Frame) on d 0, 30, and 60 in the morning before feeding.

Semen Quality Examinations, Semen Evaluation, and Blood Collection

Semen quality exams were administered on d 0, 30, and 60 according to the guidelines of Koziol and Armstrong (2018). Approximately 3.5 mL of semen was collected using a 60-mm, 2-electrode straight bull probe, electroejaculator (Lane Manufacturing Inc.) from each bull. Semen was analyzed under a microscope immediately after collection and examined for gross motility and sperm morphology. To evaluate sperm morphology, a small drop of Wright's stain (Lane Manufacturing) was placed on a slide and a small drop of semen sample was also placed adjacent to the stain. The drops are mixed with a separate slide, and the stained sample spread across the slide carefully making sure the smear is thin enough to allow light to pass through the slides so cells can be seen with a microscope. Sperm cells were examined at 1,000 × magnification (oil immersion objective) for normal morphology. Abnormalities of the head, midpiece, or tail were also recorded. Approximately 1 mL of semen was flash frozen using cryotubes (Fisher Scientific) in liquid nitrogen immediately after being evaluated by a veterinarian and transported back to the laboratory for MnSOD analysis.

Twenty milliliters of blood was collected from the coccygeal vein of each bull into a vacutainer with sodium heparin, mixed, and (BD Vacutainer) immediately placed on ice until processing. Blood tubes were centrifuged at 4°C and 780 × *g* for 10 min (Jouan 3.12; Jouan Inc.). The supernatant was removed, and an equal volume of 1 × phosphate buffered solution was added before blood

Table 1. Chemical analysis of feed[1]

	Whole cottonseed		Dried distillers grain		Soy hull pellets		Hay[2]	
Item	**Yr 1**	**Yr 2**	**Yr 1**	**Yr 2**	**Yr 1**	**Yr 2**	**Yr 1**	**Yr 2**
Chemistry analysis								
DM, % DM	90.7	90.2	89.6	88.8	87.9	87.7	95.0	91.3
CP, % DM	24.6	24.6	29.8	30.7	11.8	11.7	13.5	13.6
ADF, % DM	38.2	35.1	11.4	10.6	50.1	48.2	34.8	35.0
NDF, % DM	45.8	47.8	32.7	31.6	70.9	67.3	69.8	71.0
Crude fat, % DM	16.3	14.6	8.54	5.76	2.02	1.97	—	2.11
TDN, % DM	74.9	72.4	83.3	78.4	63.0	66.0	58.8	58.0
Gossypol,[3] % DM								
Free	0.56	0.84	—	—	—	—	—	—
Total	0.91	1.15	—	—	—	—	—	—
Minerals								
Ash, %	4.84	4.18	5.12	5.28	4.76	4.92	6.68	6.43
Ca, %	0.25	0.19	0.05	0.07	0.71	0.61	0.57	0.46
P, %	0.72	0.67	0.90	0.95	0.12	0.12	0.25	0.27
Mg, %	0.44	0.43	0.32	0.37	0.29	0.30	0.32	0.39
K, %	1.49	1.38	1.30	1.35	1.39	1.51	1.49	1.91
Na, %	0.02	0.02	0.30	0.24	0.02	0.02	0.18	0.05
Fe, mg/kg	101	54	77	81	424	370	210	108
Mn, mg/kg	24	21	17	19	20	16	47	37
Zn, mg/kg	46	41	68	74	44	55	38	25
Cu, mg/kg	9	9	4	5	6	9	6	10

[1]Feed analysis was performed by Cumberland Valley Analytical Services (CVAS; Waynesboro, PA).

[2]Bermudagrass (*Cynodon dactylon*).

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

was centrifuged a second time as described previously. The process, as stated previously, was repeated for a third time, and chilled deionized water was added to lyse the red blood cells. Lysed red blood cells and semen samples were stored at −80°C until further processing.

MnSOD

The protocol described by Hill et al. (1999), Johnson and Murphy (1988), and Marklund and Marklund (1974) was followed to determine MnSOD concentration in blood, and minor modifications suggested by Nichi et al. (2006) were used for semen analysis. After thawing, lysed blood samples were diluted to 1:10 with Tris HCl – DTPA buffer (pH 8.2) + KCN to inactivate copper/zinc superoxide dismutase. Each sample was plated in triplicate on a 12-well tissue culture plate (VMR International) for analysis. Fifty microliters of pyrogallol as added, and absorbance was determined at 320 nm using a spectrophotometer (Biotek μQuant; Biotek Instruments Inc.). Plates were analyzed for 3 min, and 1 unit of MnSOD activity was defined as the amount required for 50% inhibition of pyrogallol.

To lyse semen cells, samples were thawed in a water bath at 37°C for 1 min, immediately frozen in liquid nitrogen, thawed for a second time in a 37°C water bath, and centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatant was collected, and the method described above was followed for MnSOD determination.

Protein Determination

To express MnSOD activity per unit of soluble protein, protein was quantified by following the protocol as described by Lowry et al. (1951) with minor modifications. A standard curve was created by a serial dilution of bovine serum albumin. After thawing, blood samples were diluted to 1:200 with deionized water and 150 μL of sample was placed in a 16 × 100 mm culture tube (VMR International) with 5 mL of a copper reagent. Samples were shaken and incubated for 45 min at 39°C, allowed to cool to room temperature, and incubated for 30 min at room temperature after adding 1 mL of phenol (Sigma Aldrich). Sample absorbance at 660 nm was determined using a spectrophotometer (GENESYS 30; Thermo Scientific).

Statistical Analysis

A completely randomized design was used with the bull being the experimental unit, and all data were analyzed using the PROC MIXED procedure of SAS (Version 9.4; SAS Institute Inc.). Treatment and sampling date were

used as fixed effects, and year was considered a random variable. The treatment × time interaction was analyzed for all semen parameters. No interaction was observed, so it was removed from the model. Significance was determined at $P \leq 0.05$, and a tendency was considered when $0.05 < P < 0.1$.

RESULTS AND DISCUSSION

Animal Performance

Supplementation treatment did not affect initial ($P = 0.99$) and final BW ($P = 0.58$; Table 2). There was a treatment effect for ADG ($P = 0.002$) as bulls fed DD and WD had a greater ADG than bulls fed WW ($P < 0.02$), but DD and WD were not different ($P = 0.18$). Greater ADG in the DD and WD treatment of the current study is similar to the results published by Chase et al. (1994), where bulls fed WCS and cottonseed meal had reduced ADG compared with bulls fed gossypol-free soybean meal. These results differ from Calhoun et al. (1990b), who reported live weights linearly increased and feed requirements linearly decreased when WCS was increased in the diet from 0 to 30%. Moore et al. (1986) reported that fat additions of 2 and 4% from WCS, cottonseed oil, or animal fat did not decrease digestibility of diet components, but fat additions of 6.3% or greater reduced mean ADF digestibility from 40 to 28% (Moore et al., 1986). Crude fat values in the current study (Table 1) are similar to those reported by Bertrand et al. (2005) and may be a potential explanation, in part, for lower ADG in bulls fed WCS. Additionally, the diets in the current study were balanced using the TDN value (93%) reported by the NASEM (2016). However, the actual TDN of WCS in the current research (Table 1) was lower and can partially explain the difference observed in ADG.

Scrotal Circumference

There was not a treatment effect for scrotal circumference ($P = 0.52$); however, a time effect was observed ($P = 0.001$; Figure 1). Scrotal circumference measurements on d 60 were greater compared with d 0 and 28 ($P < 0.02$), which tended to be different ($P = 0.051$) from each other. Scrotal measurements increasing from d 0 to 60 are likely explained by the natural development of the animal. These results are similar to the results published by Jimenez et al. (1989) and Risco et al. (1993), who reported no differences in scrotal circumference of bulls supplemented diets with or without gossypol. Chase et al. (1994) reported scrotal circumferences and paired testicular volume tended to be less for developing bulls fed WCS compared with soybean meal. Bulls in the study by Chase et al. (1994) were fed from weaning to puberty, whereas bulls in the current study were 16 to 18 mo old, and all recorded scrotal measurements were adequate to pass a breeding soundness exam (>30 cm) at all time points.

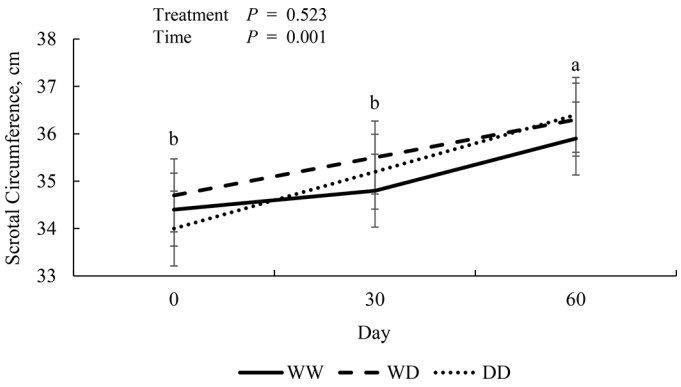

Figure 1. Scrotal circumference measurements taken on 16- to 18-mo-old beef bulls fed differing levels of whole cottonseed for 60 d. Values presented are LSM within treatment. Diets included DD (n = 14) = 3.18 kg of dried distillers grain, WD (n = 16) = 1.59 kg whole cottonseed and 1.59 kg of dried distillers grain, and WW (n = 16) = 3.18 kg of whole cottonseed. [a,b]Differing letters indicate differences across time ($P < 0.01$). Error bars represent pooled SEM (0.66).

Sperm Morphology

Although scrotal circumference was not affected by treatment, past research indicates that diets containing

Table 2. Weights and growth performance of 16- to 18-mo-old bulls fed differing levels of whole cottonseed

	Treatment[1]				
Variable	**WW**	**WD**	**DD**	**SE**	***P*-value**
Initial weight, kg	457	457	459	37.0	0.998
Final weight, kg	516	532	544	26.3	0.581
ADG, kg/d	0.96[b]	1.23[a]	1.39[a]	0.08	0.002

[a,b]Means with differing superscripts with a row are different ($P < 0.05$).

[1]DD (n = 14) = control, 3.18 kg/d dried distillers grain; WD (n = 16) = 1.59 kg/d whole cottonseed and 1.59 kg/d dried distillers grain; WW (n = 16) = 3.18 kg/d whole cottonseed.

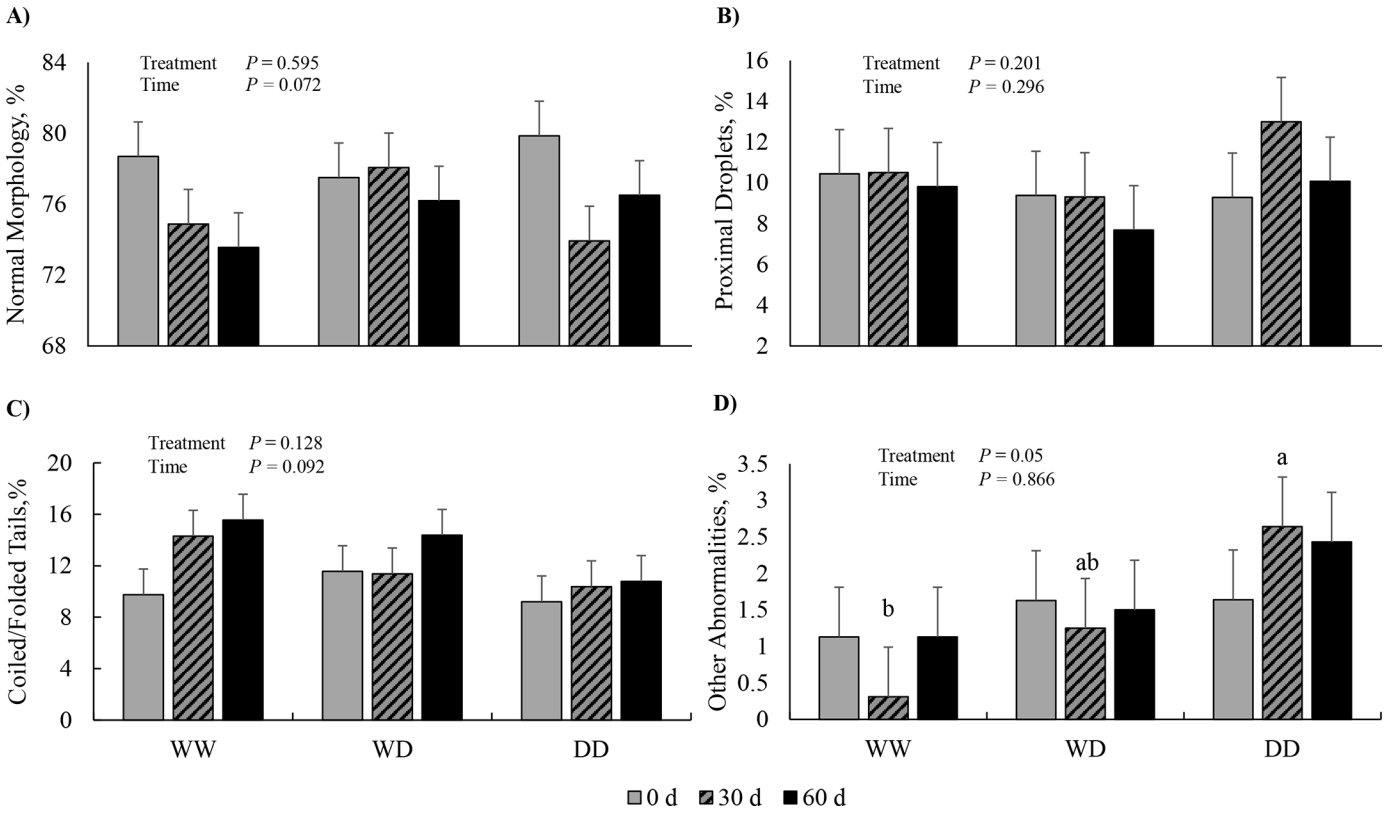

Figure 2. Spermatozoa classified as morphologically normal (A), proximal droplets (B), coiled and folded tails (C), and other abnormalities (D) in 16- to 18-mo-old beef bulls fed differing levels of whole cottonseed for 60 d. Diets included DD (n = 14) = 3.18 kg of dried distillers grain, WD (n = 16) = 1.59 kg of whole cottonseed and 1.59 kg of dried distillers grain, and WW (n = 16) = 3.18 kg of whole cottonseed. [a,b]Differing letters above treatments indicate difference ($P < 0.05$). Error bars represent pooled SEM [(A) 1.89, (B) 2.14, (C) 1.94, (D) 0.66].

8.2 g of free gossypol from cottonseed meal can result in sperm morphological defects (Chenoweth et al., 2000), and WCS included at 15% of the diet can elicit changes in the seminiferous tubules such as increased lumen diameter and decreased cell layers and wall thickness (Arshami and Ruttle, 1988). There were no treatment effects for percentages of normal spermatozoa or spermatozoa with coiled or folded tails or proximal droplets ($P \geq 0.12$; Figure 2). Percentage of normal morphology tended ($P = 0.07$) to decrease and percentage of coiled or folded tailed tended ($P = 0.09$) to increase from d 0 to 60. There was no time effect for percentage of spermatozoa with other abnormalities or proximal droplets ($P \geq 0.20$); however, there was a treatment effect ($P = 0.05$) where bulls in the WW treatment had fewer ($P = 0.01$) other abnormalities than bulls in WD and DD, which were not different ($P = 0.17$; Figure 2). The results from the present study are similar to those of Cusack and Perry (1995), who fed Hereford bulls 7.6 to 19.8 g of free gossypol per day via WCS and reported no significant difference in total spermatozoa abnormalities, secondary sperm abnormalities, or scrotal circumference. Smith et al. (1991) reported no differences in volume, color, motility, concentration, percentage of live dead spermatozoa, or percentage of spermatozoa with normal morphology when 2-yr-old Holstein bulls were fed 64 or 75 mg of free gossypol per kilogram of live mass from WCS. Chenoweth et al. (2000) reported supplementing bulls with gossypol-containing cottonseed meal negatively affected morphological characteristics; however, bulls in the current study were fed at approximately 1% of BW, which may indicate the amount of gossypol intake may have been below the toxic threshold to alter semen morphology.

MnSOD in Blood and Semen

There were no treatment effects for blood or semen MnSOD concentrations ($P = 0.255$; Table 3). Velasquez-Pereira et al. (1998) supplemented bulls with gossypol-containing cottonseed meal with or without vitamin E and reported that bulls receiving vitamin E had an increased amount of percentage motile, normal, and live spermatozoa, suggesting that negative effects of gossypol on the reproduction of bulls could be due to oxidative stress. Vince et al. (2018) reported MnSOD values of 43.1 U/g of protein in seminal plasma and 724.6 U/g of protein in spermatozoa of young (2 to 4 yr) bulls during the cold months of the year. The values presented by Vince et al. (2018) are similar to the values reported in the current study. Values may have remained unchanged between treatments

Table 3. Manganese superoxide dismutase concentrations in the blood and semen of 16- to 18-mo-old beef bulls fed differing levels of whole cottonseed for 60 d

	Treatment[1]				
Item	DD	WD	WW	SE	*P*-value
Semen, IU/mg of protein	0.74	0.78	1.02	0.14	0.333
Blood, IU/mg of protein	1.20	1.01	1.18	0.09	0.255

[1]DD (n = 7) = control, 3.18 kg/d dried distillers grain; WD (n = 8) = 1.59 kg/d whole cottonseed and 1.59 kg/d dried distillers grain; WW (n = 8) = 3.18 kg/d whole cottonseed.

because the bulls used in the current study were fed diets at approximately 1% of BW and were of similar age and health. Gossypol in WCS chelates minerals in the rumen (Gadelha et al., 2014) such as iron, zinc, and manganese, but whether this interaction affects the availability of these minerals to be used as a cofactor for superoxide dismutase is not sufficiently understood to date.

APPLICATIONS

Whole cottonseed was included as a supplemental feedstuff at a rate of up to 0.7% of BW for 60 d in 16- to 18-mo-old beef bulls without any negative effects on semen quality exams or concentrations of MnSOD in the blood and semen. However, feeding this amount of WCS resulted in a slower rate of growth compared with bulls receiving dried distillers grains. Based on these results, 16- to 18-mo-old bulls can be supplemented up to 0.7% of BW with WCS per day without a negative effect on fertility over a 60-d period.

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