Effects of Dietary Gossypol in Cottonseed Meal on Aspects of Semen Quality and Sperm Production in Young Brahman Bulls

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Introduction

Gossypol, produced in the pigment glands of the cotton plant, can be toxic under certain conditions.[1] Nonruminants appear to be more prone to overt gossypol toxicity than ruminants which apparently can detoxify gossypol in the rumen.[2] However, this system can fail when gossypol intake or availability is high leading to toxicosis in adult ruminants.[3,4] Both male and female reproductive impairment are suggested.[5] Gossypol causes reduced spermatogenesis and sperm motility in humans and rodents[5] in association with morphological disruptions of the sperm midpiece.[6,7] In male ruminants, although dietary gossypol causes damage to the spermatogenic epithelium,[5] its deleterious effects upon either sperm motility or sperm morphology are unproven.

The objective of this study was to characterize changes in semen quality, sperm morphology and sperm production in young pubertal Brahman bulls subjected to a relatively high intake of free gossypol over a period of 11 weeks.

Materials and Methods

Eight Brahman bulls (averaging 20 months and 500 kg at commencement) from USDA/ARS Brooksville, Florida, were used. All were reproductively normal at two assessments prior to commencement. Bulls were assigned to either treatment (n=4; 2.75 kg corn and 2.75 kg cottonseed meal daily) or control (n=4; 2.75 kg corn and 2.75 kg soybean meal daily). Free gossypol content of the cottonseed meal was 3000 ppm, giving 1500 ppm in mixed concentrate.[8] Treated bulls ingested an average 8.2 g free gossypol per day from week 1 of the trial[8] and all had access to hay, water and NRC levels of vitamins and minerals. The trial ran for 11 weeks with the bulls being sacrificed the following week.

Weekly assessments included semen collection by electro-ejaculation with semen being assessed for individual sperm motility and sperm morphology (200 sperm counted) using both brightfield (nigrosin-eosin stain) and differential interference phase-contrast (DIC) microscopy.[8] At sacrifice, sperm production estimates were made from elongated spermatid counts in homogenized testicular tissue.[9] Data were analyzed using the GLM procedures of SAS with least squares means of treatment and control groups being compared either within each assessment period or at completion of the trial.

Results

Sperm motility differed at week 9 ($P<0.05$) and remained lower in the treated group over the last 2 weeks (both $P=0.06$). Normal sperm were depressed in treated bulls at week 5 ($P<0.05$), with this difference increasing at week 6 ($P<0.01$) and again from week 7 until trial end (all $P<0.001$). Sperm midpiece abnormalities were elevated in treated bulls at week 3 ($P<0.05$), increasing at week 5 ($P<0.01$) and again at week 7 ($P<0.001$). Midpiece abnormalities appeared to stabilize in the treated group at between approximately 50 to 60% of total sperm between week 5 and 11 of the trial.

Paired testicular weights did not differ between the treated and control group (551.9 and 603.0±51.2g respectively). However, daily sperm production did differ between treated and control groups (6.3 and 13.1±0.7 sperm x 10^6/g testicular parenchyma respectively; $P<0.001$) as did total daily sperm production (4.1 and 7.9±0.6 sperm x 10^9 respectively; $P<0.01$).

Paper presented at the XVIII World Buiatrics Congress, Bologna, Italy August 29 - September 2, 1994

Table 1. Least squares means (± SEM) for sperm motility, sperm midpiece abnormalities and normal spermatozoa in treated[1] (T) and control[2] (C) Bulls.

Week	Sperm Motility (%)		Abnormal Midpieces (%)		Normal Sperm (%)	
	T	C	T	C	T	C
1	68.8±10.7	65.0±9.8	8.5±2.5	7.5±2.5	79.5±6.6	74.3±5.9
2	65.0±10.6	82.5±7.5	14.0±3.1	5.3±3.1	75.8±4.5	76.3±9.0
3	63.8±16.3	73.8±4.3	32.0±5.8^{a}	8.3±5.8^{b}	70.8±9.8	79.0±4.0
4	70.0±8.7	81.3±4.3	34.5±5.4^{a}	7.8±5.4^{b}	67.3±9.2	85.3±2.3
5	68.8±15.3	76.3±3.8	52.0±5.4^{c}	10.8±5.4^{d}	49.8±9.8^{a}	83.5±3.3^{b}
6	46.3±11.3	65.0±9.4	56.5±6.5^{c}	6.0±6.5^{d}	31.8±8.0^{c}	80.3±1.7^{d}
7	48.8±11.3	71.3±5.5	60.8±5.3^{c}	6.5±5.3^{d}	26.3±9.0^{e}	80.3±2.7^{f}
8	48.8±13.6	66.3±5.5	58.5±5.7^{e}	5.5±5.7^{f}	36.8±2.7^{e}	85.5±3.1^{f}
9	52.5±9.7^{a}	82.5±6.3^{b}	46.5±4.4^{e}	6.5±4.4^{f}	37.0±4.6^{e}	81.3±2.4^{f}
10	50.0±5.4^{x}	73.8±8.5^{y}	62.5±6.0^{e}	12.0±6.0^{f}	37.0±5.6^{e}	84.5±2.7^{f}
11	66.3±8.3^{x}	85.0±2.0^{y}	59.0±5.8^{c}	15.5±5.8^{d}	36.8±5.3^{e}	81.3±6.8^{f}

Rows differ: $^{ab}P\leq0.05$, $^{cd}P\leq0.01$, $^{ef}P\leq0.001$, $^{xy}P=0.06$
[1]Treated bulls fed cottonseed meal (8.2 g free gossypol per day).
[2]Control bulls fed balanced soybean meal ration.

Discussion and Conclusions

The feeding of cottonseed meal to give an estimated intake of 8.2g of free gossypol per day to young Brahman bulls over a period of 11 weeks did not cause either overt clinical signs of gossypol toxicity or decreased growth.[8] However, treated bulls had increased sperm midpiece abnormalities from week 3, less morphologically normal sperm from week 5, and lower sperm motility from week 9 with evidence of depressed sperm production at trial completion.

The majority of abnormal sperm encountered in treated bulls had abnormal sperm midpieces. The types of midpiece abnormalities observed were similar to those reported previously[7] in gossypol-treated rats, particularly those characterized by missing segments of the mitochondrial helix and by disruptions or fractures of the axial fiber bundle. Most of these lesions have not been previously associated with the feeding of gossypol in ruminants.[5] Depressed spermatogenesis in association with the feeding of gossypol is consistent with other studies in various species including ruminants.[5]

This study shows that damage to both sperm morphology and spermatogenesis can be induced in bovine males by the feeding of cottonseed meal containing relatively high levels (3000 ppm) of gossypol. Further studies are indicated to identify those factors which might exacerbate or mollify the spermatoxic effects of gossypol in male ruminants.

Summary

Young reproductively normal Brahman bulls were fed either a cottonseed meal supplement giving 8.2g free gossypol/day (n=4) or a soybean meal supplement (n=4) for 11 weeks to study effects upon semen characteristics and sperm production. Semen was collected weekly by electro-ejaculation and assessed for sperm motility and morphology (employing both brightfield and DIC microscopy). At trial completion, bulls were sacrificed and sperm production rates were estimated. Sperm motility differed between groups over the last 3 weeks of the trial. Normal sperm were less in treated bulls from week 5, with midpiece abnormalities differing from week 3. Treated bulls had lower sperm production rates at trial completion.

Acknowledgements

The Yazoo Valley Oil Mill, Inc., Greenwood, MS; the USDA Seed Grant Research Program; USDA/STARS Brooksville and Professor D.L. Wakeman and Mr. J.W. Wasdin, Department of Animal Science.

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