REPRODUCTIVE DEVELOPMENT AND FUNCTION OF BRAHMAN BULLS FED DIETS CONTAINING GOSSYPOL

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SUMMARY

Reproductive development and function was assessed in thirty Brahman bulls weaned at 180 days of age and fed diets containing soybean meal (SBM), solvent extracted cottonseed meal (CSM) or whole cottonseed (WCS). Estimates of free gossypol intake ranged from .004 to .005 lb/hd/day for CSM and from .026 to .036 lb/hd/day for WCS. Bulls fed WCS attained puberty at an older ($P < .05$) age (613 days) than SBM (550 days) or CSM (528 days), but body weights at puberty were similar ($P > .10$; 1034, 998 and 1002 lb in SBM, CSM and WCS, respectively). Average daily body weight gains through puberty were 1.67, 1.71 and 1.44 lb/d for SBM, CSM and WCS, respectively. Diet did not influence hip-heights or scrotal measurements at puberty. Following first sperm, but prior to puberty, numbers of and quality of sperm in ejaculates were depressed for bulls fed WCS, and quality of sperm also was depressed for CSM, when compared to SBM. Following puberty, when bulls were repeatedly ejaculated three times on a single day, CSM bulls were unable to maintain sperm motility values comparable to SBM and WCS bulls. Bulls were castrated on day 70 postpuberty. Gross measurements of the testes and epididymi revealed no dietary ($P > .10$) effects. Histological examination of the testes, however, indicated extensive damage to the cell layers of seminiferous tubules in CSM and WCS fed bulls. These germ cell layers are required for normal spermatogenesis. Cattlemen raising bulls or maintaining bulls for breeding purposes should be cognizant of the intake of free gossypol by these bulls and keep levels below those reported in this experiment.

INTRODUCTION

Gossypol is a toxic antiquality factor present in the pigment glands of the roots, leaves, stems and seeds of the cotton plant. The popular feedstuffs, cottonseed meal and whole cottonseed contain significant quantities of "free gossypol". Gossypol intoxication has traditionally been recognized as a problem for monogastric animals and immature ruminants. The mechanism by which gossypol is detoxified in mature ruminants appears to be by binding to soluble proteins within the rumen and not direct microbial involvement. It is quite probable,

however, that when large amounts of gossypol containing feedstuffs are consumed by ruminants, some free gossypol may escape the detoxification process of the rumen and be absorbed at the small intestine.

Controlled consumption of the gossypol by the human male appears to be the most promising form of male contraception currently being researched. In support of the human literature, spermatogenesis has been shown to be impaired in the rat, hamster and monkey. Researchers at New Mexico State University evaluated semen and testes from 18 month old crossbred beef bulls fed gossypol containing feedstuffs for 60 days. The results indicated only minimal problems with the semen, however, histological examination of testis tissue under a light microscope revealed extensive damage to the seminiferous tubules of bulls fed gossypol containing feedstuffs, compared to soybean meal fed control bulls. The seminiferous tubules located in the testes are responsible for spermatogenesis (sperm production). The results of the above experiment suggested that gossypol induced fertility problems in beef and dairy bulls could be a potential problem considering traditional supplementation strategies. The objectives of this study were: To characterize the effects of feeding gossypol containing feedstuffs (whole cottonseed or solvent extracted cottonseed meal) compared to a non-gossypol containing feedstuff (soybean meal) to Brahman bulls from weaning through puberty on growth traits, age and weight at first sperm and puberty, sperm quantity and quality and histology of the testes.

PROCEDURES

Thirty Brahman bulls were weaned at approximately 180 days of age, during the fall/winter of 1987. Bulls were stratified by age and were randomly allotted to treatments (diets) that contained either soybean meal (SBM: < .002% free gossypol), solvent extracted cottonseed meal (CSM: 1.06% free gossypol) or whole cottonseed (WCS: .54% free gossypol) as the major dietary source of protein. Diets were formulated to provide similar amounts of NEg (net energy for gain) for medium frame bulls to gain 2.0 lb/day (Table 1). Each of the protein sources contributed the same amounts of crude protein to each diet and the quantity of bermudagrass hay fed was equalized across diets. Total amounts of dietary crude protein were not equal across diets, but were above NRC requirements for all diets. The bermudagrass hay averaged 10.6% crude protein and the supplements (hay excluded) averaged 21.3, 17.5 and 18.7% crude protein for SBM, CSM and WCS, respectively. The ingredient composition (%'s from Table 1) of each diet did not

change during the conduct of the experiment, however, the total amount of each diet fed was increased as the average weight of the bulls increased. Rations were adjusted when body weights increased 100 lb. The diets fed to 750 lb bulls (for 800 lb bulls to gain 2.0 lb/day) were continued through the remainder of the experiment. The total amount of each diet fed (as-fed basis) ranged from 14.4 to 20.2, 14.7 to 20.8 and 13.6 to 18.8 lb/hd/day for SBM, CSM and WCS diets, respectively. Bulls were group fed, five bulls to a pen with two replicate pens for each treatment. A 21-day period for adaptation to the diets was allowed before bulls were considered on the experiment. Supplement refusals for each pen were weighed back daily.

At 28-day intervals, body weight, hip height, scrotal circumference and the length of each testis were measured. Paired testes volume was calculated = .0396125 x (average testis length x scrotal circumference^2). Beginning at approximately 10 month of age, bulls were electroejaculated at 14-day intervals. Puberty was defined as that time when an ejaculate contained 40 x 10^6 sperm cells with at least 10% motility. Evaluation of sperm cells at each collection included the following criteria: volume, color, gross motility rating, progressive motility rating, % motility, % live/dead, concentration (x 10^6 cells/ml) and % abnormalities. Between approximately 60 and 70 day postpuberty, semen was collected and evaluated from each bull on two consecutive days. On approximately day 70 postpuberty, bulls were castrated. Weights of each testis and epididymus and measurements of each testis were obtained. Samples of testis tissue were collected and processed for histological examination. At approximately, 110 and 200 days on experiment (310 and 400 days of age, respectively) and at 60 days postpuberty, bulls were fitted with an indwelling jugular cannulae and blood samples obtained at 20 min intervals for 6 hours. Blood samples were processed to yield sera, sera stored and later concentrations of testosterone were determined using radioimmunoassay procedures.

RESULTS

Free gossypol intake ranged from approximately .004 to .005 lb/hd/day for CSM and from .026 and .036 lb/hd/day for WCS fed bulls. At the start of the experiment (day 1) the mean ages, body weights, hip heights and testes sizes of bulls were similar between diets (Table 2). Bulls fed CSM tended ($P < .01$) to have first sperm detected in their ejaculate at an earlier age than WCS (357 vs 386 days), but at similar body weights (685 vs 682 lb). Age at first sperm in SBM bulls (380 days) did not differ ($P > .10$) when compared to CSM and WCS fed bulls.

There was a trend (P < .10) however, for SBM bulls to be heavier (734 lb) at first sperm than either CSM or WCS bulls. Hip heights and scrotal sizes were similar between diets at first sperm. Puberty was attained at an earlier (P < .05) age for SBM (550 days) and CSM (528 days) compared to WCS (613 days) fed bulls, but body weights, hip heights and scrotal measurements were similar between diets at puberty. These results indicate no distinct growth advantages associated with any diet through first sperm, however, WCS fed bulls were at a disadvantage at puberty. Age and weight are two of the most important factors influencing puberty within a particular breed of animal. In this instance therefore, puberty in WCS bulls was limited by body weight and thus these bulls attained puberty at an older age than SBM or CSM bulls.

From 56 through 98 days after first sperm, but prior to puberty, total numbers of sperm/ejaculate and the concentration of sperm/ml were depressed for WCS compared to both SBM and CSM fed bulls; while values for SBM and CSM were quite similar (Figure 1a, b). In contrast, the % motile and live sperm were consistently greater for SBM compared to both gossypol containing diets (Figure 1c, d). Furthermore, CSM fed bulls generally had higher %'s than WCS. It should be emphasized, however, that diet differences in values for the above semen traits and others (not presented) persisted until approximately 98 days after first sperm. These data support the above growth data, in that sperm numbers and quality were depressed for a period of time in WCS fed bulls, and this was reflected in age at puberty. Whereas for CSM bulls, only quality estimates were depressed, and then not as severely as with WCS.

At approximately 65 days postpuberty, bulls were electroejaculated and semen collected and evaluated three times during the day. Sperm numbers and motility estimates in both ejaculate #1 and #2 were similar or greater for gossypol fed bulls compared to SBM (Table 3). Motility estimates (gross motility, motility rating and % motile) for sperm collected in ejaculate #3 were consistently lower (P < .10) for CSM compared to SBM or WCS. Repeated electroejaculation was performed at this time to try and simulate conditions where bulls would be expected to breed cows coming in heat on a daily basis. This data indicates that the motility of sperm (an indicator of quality) may be compromised by feeding CSM to bulls.

Diet did not influence (P > .10) any gross measurements of the testes or epididymi collected on day 70 postpuberty when the bulls were castrated (Table 4). Histological evaluation of testis tissue, using a light microscope, revealed that the

diameters of the seminiferous tubules were similar between diets (Table 5). The diameter of the lumen of the tubules, however, was greatest ($P < .05$) for CSM, lowest ($P < .05$) for SBM and intermediate ($P < .05$) for WCS. Furthermore, the thickness of the wall of the tubules and the number of germ cell layers were greatest ($P < .05$) for SBM, least ($P < .05$) for CSM and intermediate ($P < .05$) for WCS. This data agrees with previous results from researchers at New Mexico State University and indicates extensive damage occurred to cell layers contained within the seminiferous tubules in bulls fed both gossypol containing diets. These tubules are required for normal sperm maturation and damage may result in abnormal spermatogenesis. Diet did not influence ($P > .10$) Sertoli or Leydig cell size (Table 5). Additionally, concentration of serum testosterone were similar ($P > .10$) between diets (Table 6). Leydig cells contained within the testes produce the male hormone testosterone. Since Leydig cell size was not altered by the diets, serum testosterone concentrations would not be expected to be affected. Furthermore, changes in serum concentrations of testosterone appeared to occur normally in all bulls as they matured (Table 7).

TABLE 1. INGREDIENT COMPOSITION OF DIETS (AS-FED BASIS)

	Diets		
Ingredient %	SBM	CSM	WCS
Soybean meal	18.9		
Cottonseed meal		19.8	
Whole cottonseed			41.4
Bermudagrass hay	38.6	37.6	41.8
Corn	35.5	35.1	9.4
Molasses	2.8	2.7	3.0
Salt	3.0	3.0	2.8
Ground limestone	.6	1.2	1.0
Vigortone-62	.6	.6	.7

TABLE 2. EFFECT OF DIET ON MEASUREMENTS OF BODY AND TESTES GROWTH AT VARIOUS CRITICAL STAGES OF DEVELOPMENT

	Time of measurement*											
	Day 1 of experiment				First sperm				Puberty			
	SBM	CSM	WCS	SE	SBM	CSM	WCS	SE	SBM	CSM	WCS	SE
Age, days	211	209	212	2.8	380^{xy}	357^{x}	386^{y}	11.4	550^{a}	528^{a}	613^{b}	18.6
Weight, lb	468	451	426	29.6	734^{y}	685^{x}	682^{x}	19.4	1034	998	1002	29.2
Hip height, in	45.8	46.1	46.4	.45	50.5	50.4	50.3	.46	54.4	54.4	54.9	.55
Scrotal circumference, cm	18.8	19.0	18.5	.27	25.5	25.3	24.6	.53	32.3	33.5	33.6	.65
Paired testes volume, cc	87	91	85	3.8	220	212	205	13.1	468^{a}	508^{ab}	561^{b}	29.8

*Puberty defined as the time an ejaculate contained 40 x 10^6 sperm cells with at least 10% motility.
Least squares means by time within a row ^{a,b}differ $P < .05$; ^{x,y}differ $P < .10$.

TABLE 3. EFFECT OF DIET ON SPERM NUMBERS AND MOTILITY IN THREE EJACULATES COLLECTED ON THE SAME DAY FOLLOWING PUBERTY

Measurement	Ejaculate #	Diet SBM	Diet CSM	Diet WCS		SE
Total/ejaculate x 10^3	1	23224^{b}	49075^{a}	23700^{b}	±	6286.6
	2	20632	18912	23630	±	6286.6
	3	10008	8412	7654	±	6286.6
Concentration/ml	1	2932^{b}	5425^{a}	2920^{b}	±	665.9
	2	2980	2425	2310	±	665.9
	3	1350	1415	1374	±	665.9
Gross motility	1	3.0^{b}	3.9^{ab}	4.1^{a}	±	.40
	2	4.0	3.9	3.7	±	.40
	3	3.3^{a}	2.1^{b}	3.3^{a}	±	.40
Motility rating	1	3.6	4.0	4.3	±	.38
	2	4.7	4.5	4.3	±	.38
	3	4.2^{x}	3.2^{y}	3.7^{xy}	±	.38
Motility (%)	1	63	71	71	±	7.3
	2	80	71	74	±	7.3
	3	77^{a}	51^{b}	62^{ab}	±	7.3

Least squares means within a row ^{a,b}differ $P < .05$; ^{xy}differ $P < .10$.

TABLE 4. EFFECT OF DIET ON TESTICULAR MEASUREMENTS AT CASTRATION (70 DAYS POSTPARTUM)

Organ	Diet			
	SBM	CSM	WCS	SE
Paired teste				
weight, g	474	531	533	37.1
volume, cc	456	525	501	37.7
Mean teste circumference, cm	17.9	18.5	18.3	.47
Paired epididymus weight, g	60.3	64.7	61.7	2.60

TABLE 5. EFFECT OF DIET ON HISTOLOGICAL CHARACTERISTICS OF TESTES OBTAINED AT CASTRATION (70 DAYS POSTPUBERTY)

Variable	Diet			
	SBM	CSM	WCS	SE
Seminiferous tubule				
Diameter, μ	183.0	181.5	179.5	2.0
Lumen, μ	74.2[a]	119.2[c]	107.8[b]	2.3
Wall-thickness, μ	108.8[c]	62.9[a]	71.8[b]	2.0
No. of layers	5.6[c]	3.5[a]	3.9[b]	.1
Sertoli cell, μ	8.7	8.6	8.7	.06
Leydig cell, μ	8.3	8.3	8.4	.07

Means within a row[a,b,c] differ $P < .05$.

TABLE 6. EFFECT OF DIET ON SERUM TESTOSTERONE CONCENTRATIONS

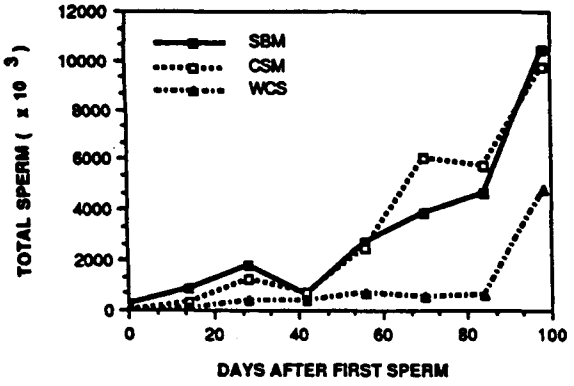
Concentrations of Testosterone[a]	Diet			
	SBM	CSM	WCS	SE
Basal ng/ml	.56	.62	.55	.032
Mean ng/ml	1.0	1.2	1.3	.11
Maximal, ng/ml	3.2	3.5	4.0	.43
No. of pulses	1.1	1.1	1.3	.11
Total area	354	436	441	38

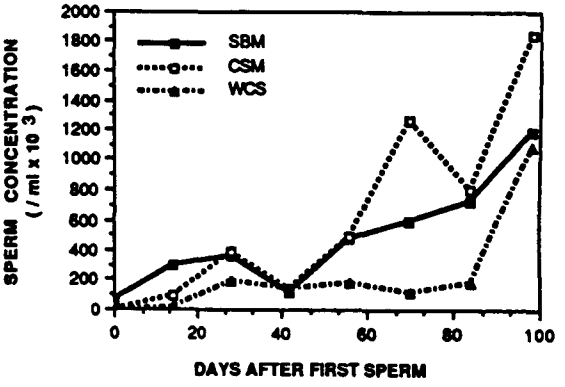
[a]During 6 hours.

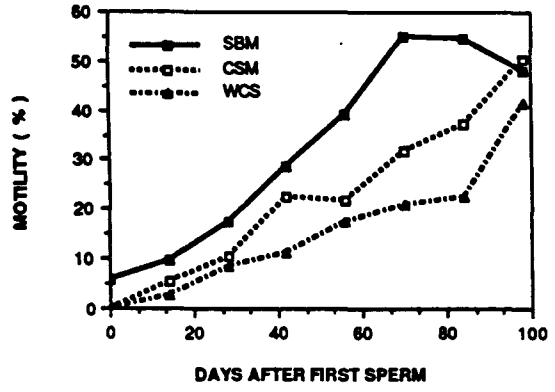
TABLE 7. EFFECT OF DAY OF SAMPLING ON SERUM TESTOSTERONE CONCENTRATIONS

Concentrations of Testosterone*	Day of Sampling			
	Day 110	Day 200	Postpuberty	SE
Basal, ng/ml	.36[a]	.66[b]	.71[b]	.033
Mean, ng/ml	.8[b]	1.4[b]	1.4[b]	.11
Maximal, ng/ml	2.6[a]	4.3[b]	3.8[b]	.44
No. of pulses	1.1	1.3	1.1	.12
Total area	272[a]	476[b]	482[b]	39

*During 6 hours.
Least squares means within a row[a,b]differ $P < .05$.







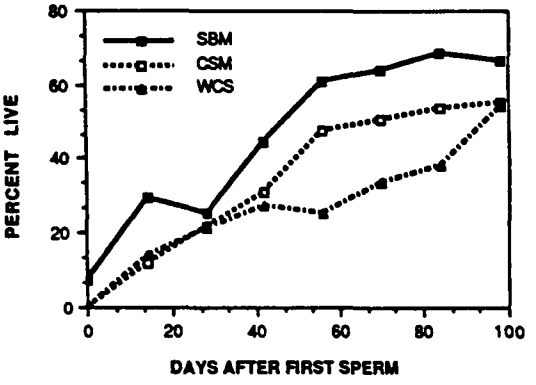


Figure 1. Effect of diet on sperm numbers (a), concentration (b), % motile (c), and % live (d) in ejaculates collected from 0 through 98 days following first sperm.