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DRAFT

EFFECTS OF GOSSYPOL ON SEMEN CHARACTERISTICS AND TESTOSTERONE PROFILES IN FINE-WOOL RAMS[1]

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ABS[illegible] Twelve fine wool rams [illegible] were used in a study [illegible] effects of a diet containing gossypol on body weight (BW), scrotal circumference (SC), semen characteristics and testosterone profiles. Rams were randomly assigned to one of two groups (n = 6) and fed [illegible] isocaloric diets (2 % BW) formulated from alfalfa hay, ground corn, [illegible] cottonseed meal (gossypol-free, control), or control plus [illegible] (CSM, free gossypol, .76 %) as a source of gossypol. [illegible] control diets during the first period (P1 = 6 mo) and [illegible] the second period (P2 = 3 mo) of the trial to determine [illegible]-induced effects were reversible. Semen samples were collected every 2 wk and evaluated for volume, percentage motility, concentration, percentage abnormal cells (% Abn), and rate of forward movement. In addition, SC and BW were measured every 2 wk and monthly, respectively. Blood samples for serum testosterone (T) evaluation were obtained 1) before treatment, 2) after 3 mo treatment, 3) after 6 mo treatment, and 4) at the end of P2. Semen characteristics, BW, and SC were not affected by changes (P > .10) between treatment groups within a [illegible]. However, all % Abn sperm was lower (12.3 % vs 18 %) for rams [illegible] the control diet during P2. Serum T of treated and control rams did not differ (P > .15). Results of this study showed the only semen [illegible] affected was sperm morphology, which was evident in P2. Serum T was not influenced by gossypol in the diet. These results suggest that rams may be less sensitive to gossypol effects than are bulls.

Key Words: Gossypol, semen, testosterone, sheep

Introduction

[illegible] forms of cottonseed meal (CSM) were produced in 19[illegible] [illegible] were distributed by the U.S. Navy (USDA, 1985). Cottonseed is utilized as a livestock feed throughout the world. Cottonseed poisoning was mentioned in early work after isolation of gossypol by Marchlewski and Carruth (1915). Gossypol is toxic to non-ruminants including humans (Abou-Donia, 1976; Clawson and Shull, 1985). Ruminants, however, are able to detoxify this substance partially by binding to soluble proteins in the rumen environment, and that which is bound is not released by proteolytic enzymes (Reiser and Fu, 1962).

The ability of gossypol to cause male infertility was first brought to the attention of the world by Chinese scientists (National Coordination Group, 1977). They indicated that gossypol, at low dosages, is an effective and reversible oral contraceptive for use in human males. Gossypol, a promising male contraceptive, inhibited sperm motility in laboratory mammals (Wang et al., 1982), abolished forward motility of boar spermatozoa (Tso and Lee, 1982) and produced abnormal sperm ($P < .06$) in semen of young bulls (Stahnke, 1986). However, Hahn et al. (1981) reported that the mouse is relatively resistant to the antifertility effects of gossypol. Dosages as high as 40 mg·kg^{-1}·d^{-1} for 8 wk produced no antifertility effects but resulted in a high ($P < .01$) mortality rate.

Studies regarding the effect of gossypol on testosterone (T), LH and FSH are somewhat conflicting. Lin et al. (1981) reported a decrease in T and LH concentrations without a change in serum FSH in gossypol-treated rats, whereas, Bardin et al. (1980) found no change in FSH, LH and T in gossypol-treated rats. Recently, Chase et al. [illegible] no change in serum T in Brahman bulls fed diets containing [illegible]. The objectives of this study were to investigate effects of gossypol [illegible] quality and testosterone profiles to determine the mode of action and to ascertain if damages were reversible in fine-wool rams.

Materials and Methods

Twelve half - brother fine-wool rams (18-mo-old) were randomly selected and divided into two groups (n = 6) received a control (gossypol free), and the other group received 12% cottonseed meal (gossypol source, .76 % free gossypol, CSM) in their diet. Diets were formulated to be isonitrogenous and isocaloric on an organic matter basis (Table 1), and were offered daily to rams as a mixed ration at a rate of 2 % of BW.

Rams were individually penned and fully consumed their feed [illegible]. The study was conducted in two periods: 1) in the first period (P1), rams were fed treatment diets for 6 mo and 2) in the second period (P2) rams were fed the control diet for 4 mo to determine if any gossypol - induced effects were reversible. On average, treated rams consumed a total of 40.8 grams of free gossypol during the 6-mo treatment period. One testicle was removed surgically at the end of P1 for histological study and enzyme assay; therefore, scrotal circumference (SC) was not measured during P2. Semen parameters and scrotal circumference (SC) were measured every 2 wk and BW was recorded monthly for each ram throughout the study. Semen samples were collected from each ram by electroejaculation (Rutle et al., 1983) and evaluated for volume, percentage motility, concentration, percentage abnormal cells, and rate of forward movement.

Blood samples for serum testosterone evaluation were obtained four times throughout the study 1) before treatment, 2) after 3 mo treatment, 3) after 6 mo treatment, and 4) at the end of the study. Rams were bled intensively every hour for 7 h via jugular venipuncture into serum separation tubes (15 ml) and allowed to clot at room temperature for 30 min before centrifugation (1,100 xg, 15 min at 4°C). Serum was separated and stored at -20°C and assayed for testosterone using a method described by Tierney and Hallford (1985). Intensive bleeding was begun at 0700 and immediately after the first bleeding each ram received 50 μg GnRH (i.m.).

Analysis of variance for completely randomized design was used to evaluate the effects of a diet containing gossypol (CSM) on animal weight, SC, and semen parameters in fine-wool rams (Steel and Torrie, 1980). Serum testosterone profiles were subjected to a split-plot analysis of variance procedure for repeated measurements of rams fed different diets during two periods of treatments (Gill and Hafs, 1971). Data were analyzed for the first and second period of study using GLM of SAS (1985).

Results and Discussion

Body weights ($P > .90$) and SC ($P > .11$) were similar between control and treatment groups in P1 (Table 2). Weights of rams

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in the P2 (Table 3) revealed similar results ($P > .65$) in both groups with greater gain for rams consuming CSM in their diet during P1 (93.3 kg vs 85.2 kg, respectively). Analysis was conducted on semen characteristics (P1) and no differences were observed ($P > .30$) between treatments. Sperm concentration was slightly greater for control rams compared with rams fed CSM (309.0 vs 301.9 x 10^9); however this difference was not significant ($P > .90$). Percentage of abnormal cells during P1 also did not differ ($P > .50$). Rate of forward movement and volume were also essentially the same. Overall BW was slight greater for rams receiving CSM in the diet (Table 3). Percentage motility was greater for control rams compared with treated rams (85.1 vs 79.8%), respectively. Sperm concentration and RFM in the two treatment groups showed no differences during P2. Percentage abnormal cells was greater for rams fed CSM (18%) during P1 compared with control rams (12.3%) by the end of P2.

Split plot analysis of variance revealed no treatment differences ($P > .18$) or treatment x period interactions ($P > .16$) for serum T within each period. Testosterone concentrations within time (Table 4) revealed no diet by sampling time interactions ($P > .20$).

In a previous study (Stanake, 1986), the effect of diets containing gossypol (CSM or WCS) on semen characteristics revealed more abnormal spermatozoa ($P < .06$) in young bulls. Results from this study showed no change in semen quality. However, the overall percentage of abnormal sperm was greater for rams fed CSM than for those fed control diets (18 vs 12.3%, respectively). Sperm motility was greater in P2 for control rams compared with the group fed CSM in P1. Similarly, Pons et al. (1983) reported low sperm motility in human males consuming gossypol in their diet. Sperm concentration increased throughout the trial in both treatment groups and was considered to be a reflection of increasing age. Greater concentration was observed for control rams compared with CSM-treated rams during P1. Several studies have shown a reduction in number of sperm cells in rat and cynomolgus monkey receiving gossypol in their diet (Shandilya et al., 1982; Sotelo et al., 1982), respectively. Body weights and SC were similar between control and CSM-treated rams during P1 and P2. Similar effects of BW and SC were reported in young bulls and ram lambs consuming CSM in their ration (Stanake, 1986; Kramer et al., 1989).

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Table 1. Composition of control and treatment rations fed to rams.

Item	Control (%)	Treatment (%)
Alfalfa	64.75	58.00
Cottonseed meal[a]	—	12.00
Soybean meal	10.00	1.00
Corn	25.00	28.75
NH_4Cl	0.25	0.25

[a]Free-gossypol, .076%. Analysis by Pope Laboratories, Dallas, Texas.

Table 2. Semen characteristics, scrotal circumference and body weight of fine-wool rams fed a diet containing cottonseed meal (CSM) or a control diet (gossypol-free) for 6 months[a,b] (1st period).

Item	Treatment			
	Control	CSM	SE[c]	OSL[d]
Body weight, kg	82.4	82.5	.91	.98
Scrotal circumference, cm	36.0	35.0	.12	.11
Semen volume, ml	1.3	1.3	.04	.73
Motility, %	86.1	86.7	.50	.82
Concentration, no x 10^9	309.0	301.9	9.16	.88
Abnormal cells, %	14.4	14.0	.76	.51
Rate of forward movement	3.1	3.0	.01	.30

[a]Semen collected biweekly.
[b]Body weights were taken monthly.
[c]Standard error of the mean (n = 6).
[d]Observed significance level.

Table 2 [illegible] body weight of five-week-old rats fed a [illegible] (goodyear-diet) for 3 weeks[a] [illegible] (a mod).

Item	Treatment		SEM[b]	OSL[c]
	Control	CSM		
Body weight, g	95.3	95.3	1.8	.65
Volume, ml	1.4	1.3	.05	.66
Modified [illegible]	85.1	79.9	1.51	.52
[illegible]	315.1	314.1	17.6	.99
[illegible]	12.3	18.0	1.7	.34
Ratio of [illegible]	3.6	2.7	.04	.12

[illegible] collected [illegible]
[b]Pooled weighted [illegible] collected [illegible]
[c]Standard error of the mean [illegible]
[d]Observed significance level

Table 4 [illegible] GnRH challenge in rats fed control or cottonseed meal (CSM) diets[a].

Hour [illegible] or after [illegible]	Treatment			Time before or after initiating feeding, hr					
				3			6		
	CSM	SE	Control	CSM	SE	Control	CSM	SE	Control	CSM
[illegible]	[illegible]	[illegible]	7.9	7.2	2.0	5.2	4.6	1.6	3.9	3.0
[illegible]	[illegible]	.8	11.8	9.9	1.6	10.9	12.4	.4	6.3	7.3
[illegible]	[illegible]	1.5	12.8	12.4	1.1	13.7	13.4	1.0	9.3	8.3
[illegible]	[illegible]	[illegible]	13.6	10.6	.7	14.2	12.2	1.3	6.4	7.5
[illegible]	[illegible]	[illegible]	12.8	10.7	1.2	12.0	11.05	.8	6.3	6.8
[illegible]	[illegible]	[illegible]	8.6	7.7	1.0	11.5	8.4	.8	4.1	4.0
[illegible]	[illegible]	[illegible]	6.9	6.1	.9	8.1	7.3	.9	[illegible]	2.7
Overall [illegible]	[illegible]	[illegible]	10.1	9.2	.7	10.9	9.9	.6	[illegible]	5.1

[illegible] [illegible] during the [illegible] period when only five controls were available as a result of the death of one rat.
[b]Observed [illegible] [illegible] 17th [illegible] four-quarter.
[c]The GnRH [illegible] was administered as a single i.m. injection immediately after the hour 0 blood sample was collected.
Split plot analysis of variance with time revealed no diet by sampling hour interaction ($P > .20$); effects of diet on serum testosterone were examined [illegible] sampling hours. Such values and [illegible] do not differ ($P > .10$)