

EFFECTS OF DIETS CONTAINING COTTONSEED MEAL ON SEMEN QUALITY
AND TESTICULAR TISSUE IN FINE-WOOL RAMS¹

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Summary

Twelve fine-wool rams (18 mo old) were used to determine the effects of cottonseed meal on spermatogenic tissues and associated cells and semen quality. Animals were randomly assigned to two diets formulated using a) alfalfa hay, ground corn and soybean meal (gossypol-free, control) and b) alfalfa hay, ground corn, soybean meal and 12% cottonseed meal (gossypol source) for 26 wk. Diets were fed at 3% of body weight. Body weight was measured monthly and semen collected biweekly and evaluated. Right testicles from each ram were removed surgically at the end of the trial for histological examination. Cottonseed meal had no effect ($P > .10$) on semen characteristics or weight gain. However, overall percentage abnormal sperm was higher among gossypol-treated rams (14% vs 17%) than for control rams. Histological examination revealed significant effects on testicular tissues. These tissues from rams fed cottonseed meal had larger ($P < .01$) lumen diameters, fewer ($P < .01$) number of layers, and reduced ($P < .01$) wall thicknesses in seminiferous tubules, and reduced ($P < .01$) size of Sertoli and Leydig cells as compared to tissue from control rams. Results of this study suggest diets containing gossypol may alter testicular tissues and semen quality in rams.

Introduction

Gossypol, a promising male contraceptive isolated from cottonseeds, inhibited spermatozoal motility in laboratory mammals (Wangy et al., 1979), and produced abnormal spermatozoa in semen of crossbred bulls (Stahnke, 1986). In vitro studies with mature boar spermatozoa showed that gossypol abolished spermatozoal forward motility (Tso and Lee, 1981). Ultrastructure studies of testis have demonstrated that late and mid-spermatids are more sensitive to, and suffer most from, gossypol administration (Yanwan et al., 1985). Histological changes in testicular tissues were observed in bulls fed cotton products (Arshami and Ruttle, 1988). This suggests rumen microbes may not detoxify gossypol completely.

Reiser and Fu (1962) reported free gossypol was rapidly bound by soluble proteins in the rumen, and bound gossypol was not released by proteolytic enzymes. The potential effects of gossypol on reproduction among farm animals that may consume cotton products is an area that needs investigation. The objectives of this study were to examine the

effects of cottonseed meal (CSM) on semen characteristics, weight gain and spermatogenic tissues of fine-wool rams, and determine the mode of possible damages.

Materials and Methods

Twelve fine-wool rams (18 mo old) were randomly selected and divided into two groups. One group ($n = 6$) received a control diet (gossypol free), and the other group received 12% CSM in their diet. Diets were formulated to be isonitrogenous and isocaloric on an organic matter basis, and offered daily to rams as a mixed ration at a rate of 3% of their body weight (BW) for 26 wk. Rams were individually penned and fully consumed their feed every day.

Semen samples were collected from each ram by electroejaculation every 2 wk and evaluated for volume (vol), percent motility (% mol), concentration (con), percent abnormal cells (% abn) and rate of forward movement (RFM). In addition, scrotal circumference and BW were measured throughout the study, using method described by Ruttle et al. (1983). At the end of the treatment period, the right testicle was removed surgically from each ram for histological examination. Testicular tissue samples approximately 5 x 5 x 3 mm were removed immediately from each animal, numbered, washed in 0.2 M phosphate buffer (pH = 7.2) and fixed in 2.5% glutaraldehyde for 6 h, then postfixed in 2% buffered osmium tetroxide solution overnight. Following fixation, tissues were dehydrated, infiltrated and embedded in epoxy resin³. Tissues were sectioned at 2 microns and stained with 1% Borax Toluidine Blue for histological examination.

For quantitative evaluation, 20 randomly chosen, circular, seminiferous tubules from each ram were examined. Diameter of each seminiferous tubuli (ST), Sertoli cell (SC), Leydig cell (LC) and wall thickness (WT) were measured in microns using a calibrated micrometer eyepiece. The number of layers (NL) of spermatogenic cells in each tubule was counted. Data were analyzed as a completely randomized design for histological analysis using the General Linear Models procedure of the Statistical Analysis System (SAS, 1982). Semen characteristics and BW were subjected to a split-plot analysis of variance for repeated measurements over time (Gill and Hafs, 1971).

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Results

The results of analysis revealed a significant ($P < .01$) reduction in the diameter of lumen, WT, size of Sertoli and Leydig cells, and NL in the seminiferous tubules, compared to tissues from rams fed the control diet (Table 1). Semen quality and weight gain data were similar ($P > .10$) among treatments. However, overall percentage of abnormal sperm was lower (14% vs 17%) for rams fed control diets (Table 2).

Discussion

Gossypol-induced alterations in the semen quality and testicular tissues of rams' testis were investigated. Light microscopic examination showed the fine structure of control animal testicular tissue similar to that described by Arshami and Ruttle (1988). Light microscopic examination of ST from CSM-treated rams revealed no significant differences in size when compared to control animals. Furthermore, the basement membrane of ST was undisturbed, where in previous studies with bulls fed a shorter time and at a lower level of gossypol, destruction of seminiferous tubules was observed. This suggests rams may detoxify gossypol more effectively than bulls. Number of layers in ST of the CSM-treated group were reduced ($P < .01$), and presence of spermatocytes in the lumen of the ST were observed and attributed to the interruption of spermatogenic tissue.

The structure of SC observed in the ST of control rams appeared to be essentially identical to that described by Banks (1974). Size of Sertoli cells from CSM-fed rams were reduced ($P < .01$) in the ST as a result of gossypol effect, suggesting impairment of normal cellular activity of spermatogenic cells. This finding agrees with Yanwan et al. (1985), who reported that dysfunction of the SC disturbs the physiological environment for developing spermatocytes. The structure of Leydig cells in control rams was observed to be similar to that described by Banks (1974). Leydig cells from rams fed CSM were significantly reduced in size when compared to the control animals. This finding is in disagreement with Shepu et al. (1980), who reported no detectable damage in the LC from rats treated with gossypol.

Numbers of layers in the ST of rams fed CSM were significantly ($P < .01$) reduced in comparison to control animals, suggesting disappearance of secondary spermatozoa in the seminiferous tubuli and reduction of WT of the seminiferous tubules. Similarly, Shepu et al. (1980) found only a single layer of cells in the ST of rats treated with gossypol.

There were no differences ($P > .10$) among the two groups of rams for weight gain, scrotal circumference and semen characteristics. However, overall means for percentage of abnormal cells was higher for rams fed cottonseed meal. This finding suggests gossypol involvement in the ultrastructural compartment of spermatozoa during and after maturation. Similarly, Bozek et al. (1981) reported spermatozoa from gossypol-treated rats inhibited, wrinkled and disorganized all membranes in the head and tail regions. The cell membranes were missing from segments of the tail midpiece and other regions. Retention of cytoplasmic droplets, looped tails and malformed heads were also observed.

Conclusion

Light microscopic examination of testicular tissues of rams fed 12% CSM in their diets for 26 wk significantly reduced NL, WT, and size of Sertoli cells and Leydig cells in comparison to the rams from control group. No significant differences were detected for BW, SC and semen quality. The results of this study suggest mature fine-wool rams may tolerate a higher percentage of CSM in their diet for a longer period than bulls (Arshami and Ruttle, 1988). Overall, histological damage in rams was not as obvious as in bulls. Furthermore, CSM-treated rams gained weight similar to the control rams by the end of the treatment period, which suggests feeding CSM may not be a desirable supplement for replacement rams.

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TABLE 1. HISTOLOGICAL CHARACTERISTICS OF OVINE TESTIS FROM FINE-WOOL RAMS FED COTTONSEED MEAL (CSM) OR A GOSSYPOL-FREE CONTROL DIET.

Variable (μ)	N	Diet		SE ^a	P ^b
		Control	CSM		
Seminiferous tubule	120	180.5	175.2	3.77	.33
Lumen	120	68.7	92.3	2.58	.0001
Wall thickness	120	108.0	83.0	2.46	.0001
Number of layers	120	6.1	4.4	.13	.0001
Sertoli cell	120	7.5	6.4	.18	.0001
Leydig cell	120	8.8	6.4	.20	.0001

^aStandard error of mean.

^bProbability value.

TABLE 2. SEMEN CHARACTERISTICS OF OVINE TESTIS FROM FINE-WOOL RAMS FED COTTONSEED MEAL (CSM) OR A GOSSYPOL-FREE CONTROL DIET^a

Variable	N	Diet		SE ^b	P ^c
		Control	CSM		
Body weight (kg)	42	82.4	82.5	2.40	.9827
Scrotal circumference (cm)	78	36.0	35.0	.42	.1164
Semen volume (ml)	78	1.27	1.34	.15	.7342
Motility, %	78	86.1	86.7	1.80	.8242
Concentration x 10 ⁷	78	309.0	301.9	33.0	.8813
Abnormal cell, %	78	14.4	17.0	2.73	.5167
Rate forward movement	78	3	3.05	.03	.3035

^aSplit-plot analysis of variance showed no diet by time interaction (P > .10), therefore means pooled across time.

^bStandard error of mean.

^cProbability value.