EFFECTS OF DIETS CONTAINING GOSSYPOL ON SPERMATOGENIC TISSUES OF YOUNG BULLS

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ABSTRACT

Eighteen yearling beef bulls were used in a study to determine the effects of diets containing gossypol on spermatogenic tissues, Sertoli cells and Leydig cells. Bulls were randomly assigned to one of three (n = 6) isonitrogenous diet groups formulated from alfalfa hay and corn (gossypol-free control), control plus whole cottonseed and cottonseed hulls, or control plus cottonseed meal and cottonseed hulls as sources of gossypol. Testicular tissues collected were examined histologically, and tissues from bulls fed gossypol-free diets were compared with those fed diets containing cottonseed products. Following a 2-mo period (P_1) when bulls were fed diets containing gossypol, one-half of the bulls were placed on a gossypol-free diet for an additional 2 mo (P_2) to determine if gossypol effects were reversible. At the end of P_1, bulls fed whole cottonseed and cottonseed meal had larger ($P<0.01$) lumens in their seminiferous tubules, decreased ($P<0.001$) wall thickness in their seminiferous tubules, and a reduced ($P<0.0001$) number of cell layers in their seminiferous tubles, when compared with bulls fed a gossypol-free diet. These histological changes indicate detrimental effects to the spermatogenic tissues and associated cells. Following the initial two months (P_1) of receiving feed containing gossypol, herd mates that were fed a gossypol-free diet for 2 mo (P_2) showed improvement in histological characteristics, indicating that gossypol-induced effects were partially reversible.

Key words: gossypol, spermatogenic tissues, Sertoli cell, Leydig cell, bovine

INTRODUCTION

Gossypol, a specific toxic substance present in the pigment glands of cottonseed, is a yellow polyphenolic compound of empirical formula $C_{30}H_{28}O_8$ (1). The exact mechanism by which gossypol is detoxified by the ruminant animal is not known. Since most differences in metabolism between ruminant and nonruminant animals may be traced to the activity of rumen microorganisms, the possibility is that these organisms might

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also act upon gossypol to metabolize it or change the rumen environment (2). Reiser and Fu (3) concluded that free gossypol was rapidly bound by soluble proteins in the rumen, and that bound gossypol was not released by proteolytic enzymes. No evidence for direct microbial involvement in detoxification was found. This chemical agent is regarded as a male contraceptive drug, which directly or indirectly disturbs development of spermatozoa. The contraceptive effect of gossypol has been attributed to an impairment of spermatogenesis, an accompanying reduction in sperm output, and the presence of morphologically abnormal, immotile spermatozoa in the duct systems of bulls, hamsters, rats and rabbits (4-6). Ultrastructure studies of testis have demonstrated that late and mid-spermatids are more sensitive to and suffer most from gossypol administration (7). A diet high in gossypol content may result in temporary sterility. A report from China (8) indicates low dosage levels of gossypol is an effective and reversible oral contraceptive for use in the human male. Because of the routine use of cottonseed products as foodstuffs for ruminants, the potential effects of gossypol on reproduction is an area that needs investigation. The objectives of this study were to examine the effects of gossypol on spermatogenic tissues of young bulls, to determine the mode of damage by histological examination of tissues, and to ascertain if damage was reversible.

MATERIALS AND METHODS

Eighteen crossbred bulls (1 yr old) were randomly assigned to one of three treatment groups for a period of 4 mo. Diets consisted of 1) alfalfa hay and ground corn (control), 2) control diet and cottonseed meal[a] plus cottonseed hulls (3% of body weight), and 3) control diet and whole cottonseed plus cottonseed hulls (3% of body weight) fed once a day for 2 mo (P_1). At the end of this period, three bulls from each group were slaughtered and one testicle was removed from each bull for a separate histological study. The remaining bulls were fed the control ration (P_2) for 2 additional months to determine if any gossypol-induced effects were reversible. At the end of P_2, the remaining bulls were slaughtered and one testicle was removed from each bull for histological study. Rations as fed are shown in Table 1.

Testicular tissue samples, approximately 10 x 10 x 5 mm, were removed immediately after slaughter from each animal, numbered and placed in 10% buffered formalin solution for 4 d. Following fixation, tissues were dehydrated, infiltrated and embedded according to Luna (9). Tissues were sectioned at 2 microns and stained with hematoxylin and eosin for histological examination.

For quantitative evaluation, 20 randomly chosen, circular,

[a] Cottonseed meal is usually fed to animals in the form of flour or pellets mixed with other ingredients. It is more digestible (higher gossypol absorption) in comparison with whole cottonseed (lower gossypol absorption), which contains high fiber and fed to animals as is.

Table 1. Composition of rations as fed to determine the effects of gossypol on crossbred bulls

	Control	Whole cottonseed Cottonseed hulls	Cottonseed meal Cottonseed hulls
		(%)	
Alfalfa hay	68	48	47
Ground corn	32	20	28
Whole cottonseed		15[a,d]	
Cottonseed meal			7[c,d]
Cottonseed hulls		17[b,d]	18
NEm-Mcal/kg	1.36	1.37	1.36
NEg-Mcal/kg	0.71	0.71	0.72
Crude protein, %	13.22	13.22	13.22
Grain, lbs/day	2.09	2.10	2.12

[a] 0.69% free gossypol.
[b] 0.08% free gossypol.
[c] 0.075% free gossypol.
[d] Free gossypol determined by Pope Testing Laboratories, Inc., Dallas, TX.

Table 2. Histological characteristics of bovine testis from young bulls fed cottonseed meal (CSM), whole cottonseed (WCS) or a gossypol-free control diet during the first period[a]

		Seminiferous tubule means				Cell means	
Treatment	No. bulls	Diameter[b] (μ)	Lumen[c] (μ)	Layers[d] (no.)	Thickness[e] (μ)	Sertoli[f] (μ)	Leydig[g] (μ)
CSM	3	35.7	25.3**	3.0**	10.2**	11.9**	11.7
WCS	3	34.9	20.8**	3.2**	14.0**	12.3**	10.6
Control	3	35.5	17.9	6.3	17.4	13.1	11.4
Pooled SEM	-	0.9	0.9	0.4	0.8	0.6	0.6

[a] Treatment period was 2-mo duration.
[b] Seminiferous tubule diameter.
[c] Lumen diameter.
[d] Number of layers in seminiferous tubule.
[e] Wall thickness of seminiferous tubule.
[f] Sertoli cell diameter.
[g] Leydig cell diameter.
** = $P < 0.01$.

seminiferous tubules were studied for each bull. The diameter of each seminiferous tubule, lumen, 20 Sertoli cell, and wall thickness was measured in microns by the use of a calibrated micrometer eyepiece. The number of layers of spermatogenic cells in each tubule was counted. In addition, 20 randomly selected Leydig cells were measured and recorded.

Data were subjected to analysis of variance (3 x 2 factorial arrangement) using the General Linear Models procedure of the Statistical Analysis System (10,11). In this study, contrast comparison was used to compare all possible interactions between treatments and two periods of treatment application.

RESULTS

There were no significant differences in the diameter of seminiferous tubules and Leydig cells when compared to the control group during P_1 and P_2. During P_1, when bulls were fed cottonseed meal and whole cottonseed (Table 2), histological examination showed an increase in lumen diameter ($P<0.01$) and a decrease in the number of cell layers ($P<0.0001$), seminiferous tubules wall thickness ($P<0.001$) and Sertoli cell size ($P<0.01$) when compared with tissues from bulls fed the control diet. Mean diameters of Leydig cells are shown in Table 2. The slight variation in size was not different ($P>0.01$). Following P_2, when all bulls had been fed the control diet (Table 3), tissues from bulls fed either cottonseed meal or whole cottonseed during P_1 still had increased lumen diameters ($P<0.01$), decreased seminiferous tubules wall thickness ($P<0.001$), decreased Sertoli cell size ($P<0.001$) and a reduced number of cell layers ($P<0.0001$) when compared to the control groups.

DISCUSSION

Gossypol-induced alterations in the histology of bulls' testis were investigated. Light microscopy was used to examine the fine structure of control animal testicular tissue (Figure 1). The morphology observed was similar to that described by Blom (12). Light micrograph (Figure 2) of a seminiferous tubule from cottonseed meal-treated bulls showed ruptured basement membranes, with presence of spermatocytes in the lumen and interstitial tissues due to the destruction of the seminiferous tubules and interruption of the spermatogenic tissue.

Light micrograph (Figure 3) of a seminiferous tubule from a whole cottonseed-treated bull showed only a single layer of cells, consisting of the Sertoli cells and spermatogonia in the tubule wall. This finding agrees with Shepu et al. (13), who also found a single layer of cells containing Sertoli cells and spermatogonia in the tubules of rats treated with gossypol. Presence of these cells in the lumen indicates degeneration of the tubule structure.

The structure of Sertoli cells observed in samples from control bulls appeared to be essentially identical to that described by Bawa (14) and Banks (15). Sertoli cells are normally triangular, with a triangle-shaped nucleus located centrally in the cell. Sertoli cells from cottonseed meal and whole cottonseed-fed bulls showed rounded

Table 3. Histological characteristics of bovine testis from young bulls fed a gossypol-free control diet for 2 mo following feeding of cottonseed meal (CSM) or whole cottonseed (WCS)[a]

Treatment	No. bulls	Seminiferous tubule means				Cell means	
		Diameter[b] (μ)	Lumen[c] (μ)	Layers[d] (no.)	Thickness[e] (μ)	Sertoli[f] (μ)	Leydig[g] (μ)
CSM	3	35.3	25.6**	2.8**	9.7**	12.2**	11.2
WCS	3	34.3	24.2**	2.7**	10.1**	12.1**	10.5
Control	3	34.6	19.5	5.4	15.1	13.4	10.8
Pooled SEM	-	0.9	0.8	0.5	0.8	0.5	0.6

[a] Treatment period was 2 mo duration.
[b] Seminiferous tubule diameter.
[c] Lumen diameter.
[d] Number of layers in seminiferous tubule.
[e] Wall thickness of seminiferous tubule.
[f] Sertoli cell diameter.
[g] Leydig cell diameter.
** = $P < 0.01$.

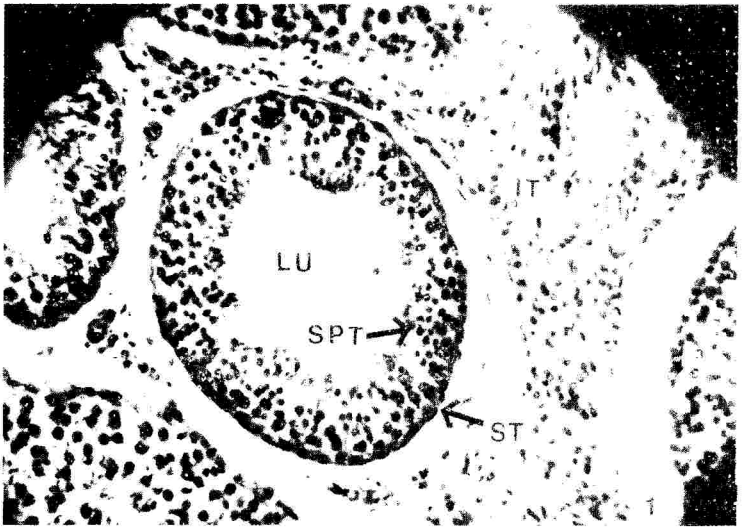

Figure 1. Light micrograph of normal seminiferous tubule (ST) from a young bull fed a control diet containing no gossypol. Spermatogenic tissues (SPT) are arranged uniformly inside the seminiferous tubules with an opening in the center (LU). Seminiferous tubule is surrounded by interstitial tissues (IT). Hematoxylin and Eosin stain. 80x.

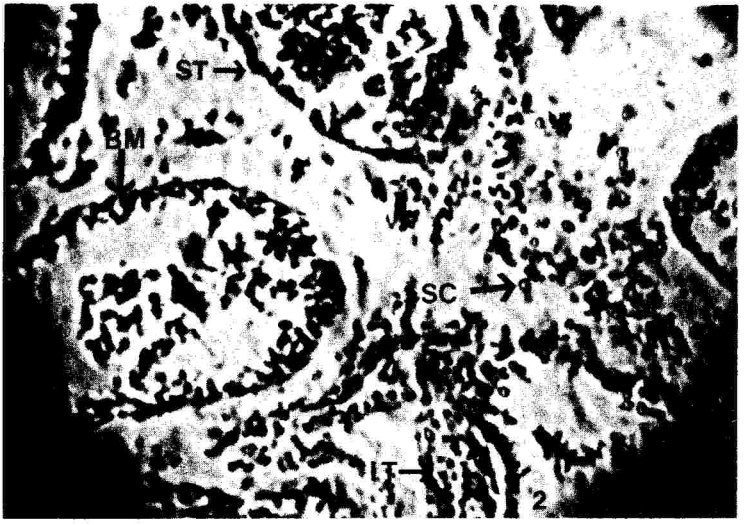

Figure 2. Light micrograph of ruptured seminiferous tubule (ST) from testis of a young bull fed a diet containing cottonseed meal (CSM) as a source of gossypol. The basement membrane (BM) is broken and spermatocytes (Sc) are present in interstitial tissues (IT). Hematoxylin and Eosin Stain. 80x.

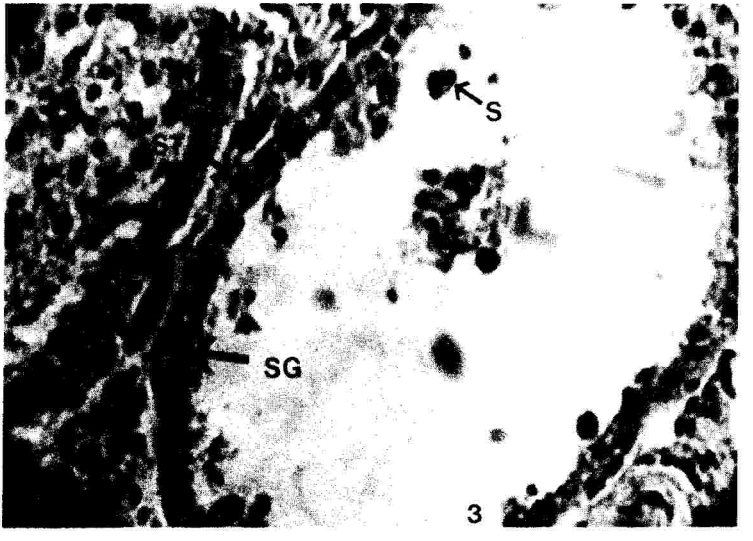

Figure 3. Light micrograph of seminiferous tubule (ST) from testis of a young bull fed a diet containing whole cottonseed (WCS) as a source of gossypol. Spermatogonia cells (SG) and a few Sertoli cells (SC) are all that remain of spermatogenic tissues. Hemotoxylin and Esosin stain. 160x.

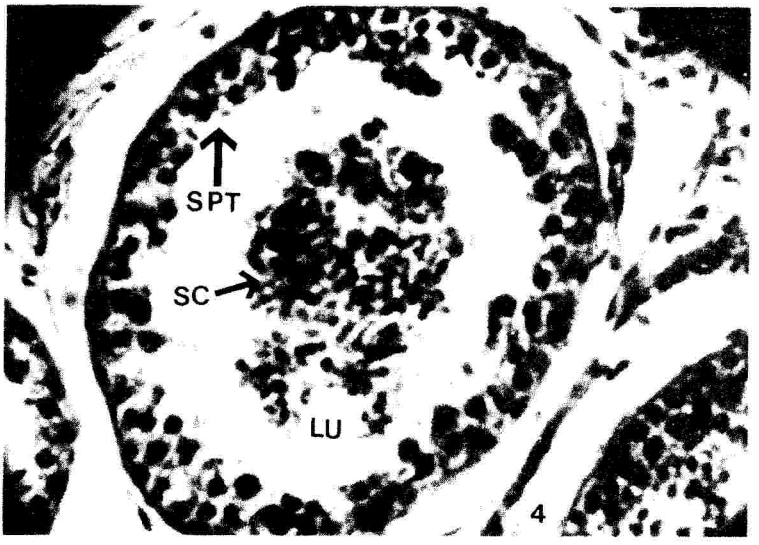

Figure 4. Light micrograph of spermatogenic tissues (SPT) from testis of a young bull fed a gossypol-free control diet after consuming a diet containing cottonseed meal (CSM) as a source of gossypol for a period of two months. Spermatocytes (SC) have detached from the tubule wall and are accumulated in the lumen (LU) area. Hemotoxylin and Eosin stain. 160x.

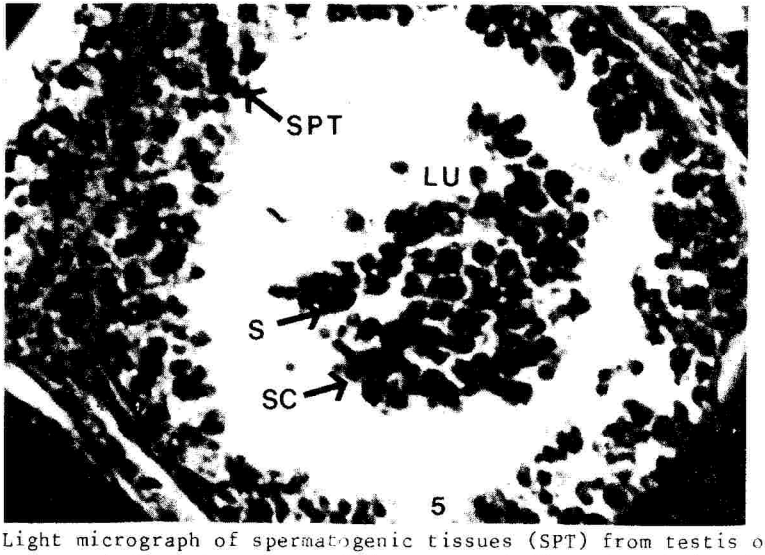

Figure 5. Light micrograph of spermatogenic tissues (SPT) from testis of a young bull fed a gossypol-free control diet after being fed a diet containing whole cottonseed (WCS) as a source of gossypol for a period of two months. Spermatocytes (SC) and a few Sertoli cells (SC) have detached and accumulated in the lumen (LU) area. Hematoxylin and Eosin stain. 160x.

Sertoli cells, with an abnormal shaped nucleus and dark spots in the cytoplasm compared with tissue from bulls fed the control diet. Size of Sertoli cells was found to be smaller ($P<0.01$) among bulls receiving gossypol, suggesting impairment of normal cellular activity. However, due to the irregular shape of the Sertoli cell and difficulty in measurment, further study may be needed to more clearly define gossypol effects. Kerr et al. (16) and Nakamura and Hall (17) reported that testis treated with heat, or during cryptorchidism, showed depolarization of the seminiferous tubules and an increase in membrane permeability. Consequently, spermatogenesis was inhibited and the ultrastructure of Sertoli cells was damaged. In addition, Yanwan et al. (7) found that disfunction of the Sertoli cells disturbs the physiological environment for developing spermatocytes.

The structure of Leydig cells in control bulls was observed to be basically similar to that described by Banks (15) and Maximow and Bloom (18). The elongated Leydig cells are located in the interstitial tissue and have large spherical nuclei. Leydig cells from cottonseed meal and whole cottonseed-treated bulls did not differ ($P>0.01$) in size when compared to Leydig cells from control bulls. Shepu et al. (13) reported no detectable damage in the Leydig cells from rats treated with gossypol, which confirms the findings of our study. Coutinho (19) and Shandilya and Clarkson (20) reported no significant decrease in plasma levels of testosterone in humans and monkeys fed gossypol, which suggests no effect on Leydig cells. In our study, the number of layers and the wall thickness of seminiferous tubules in the two groups of bulls fed cottonseed meal and whole cottonseed during the first period were higher in comparison with the same groups of bulls fed gossypol-free diet during the second period. This effect was attributed to the short length of recovery time during the second period. However, light micrographs (Figures 4 and 5) of seminiferous tubules of animals fed a gossypol-free diet in the second period showed more circular arrangement of spermatocytes in comparison with that seen in light micrographs (Figures 2 and 3) of tissues from bulls fed cottonseed meal and whole cottonseed during the first period. The improved histological characteristics of spermatogenic tissues following the second period indicates gossypol-induced effects are reversible. While this study pertained only to histological effects of gossypol-containing diets, semen parameters for these bulls was reported by Stahnke (4). He found that semen volume, concentration and percentage of motility was not ($P>0.20$) affected by gossypol in the diet. However, the percentage abnormal cells was higher ($P<0.06$) among bulls receiving diets containing cottonseed meal as a gossypol source. This finding may be related to the histological damages observed in our study.

Histological and structural examination of tissues from bulls fed diets containing gossypol revealed numerous morphological changes. The morphology of spermatogenic tissues of the seminiferous tubules was altered by feeding cottonseed meal and whole cottonseed. Partial recovery and regeneration of testicular tissue was observed at the end of the last period, when animals were fed a gossypol-free diet. Results of this study suggest that feeding gossypol-containing rations to breeding bulls may reduce their fertility and be detrimental to their

reproductive performance. Histological changes observed in tissues of bulls in this study that were fed gossypol-containing diets suggest that rumen detoxification of gossypol may be incomplete. Considering the importance of cottonseed products as a protein source in ruminant diets, the previously accepted theory that the rumen adequately detoxifies gossypol may need to be reconsidered. Results of this study suggest that alternate protein sources may be necessary for breeding males.

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