

EFFECTS OF GOSSYPOL ON MICROMETRY OF TESTES OF TEDDY MALE GOATS

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ABSTRACT

The effects of gossypol on micrometry of testes of Teddy male goats were studied. Nine Teddy male goats were randomly divided into three equal groups named A, B and C. Animals in group A were fed control ration (without gossypol), animals in group B were fed ration which contained unboiled cottonseed cakes (free and bound gossypol contents), while those in group C were given ration containing boiled cottonseed cakes (bound gossypol only). These experimental rations were fed to animals of respective groups for a period of 90 days. At the termination of experimental period, the right testis of each male goat was removed surgically. Histological sections from these testes were examined and the micrometric characteristics of testes were recorded. The mean values for total diameter (μ), lumen diameter (μ), wall thickness (μ), spermatogenic cell layers (Nos.) of seminiferous tubules and Leydig cell diameter (μ) of right testicle of Teddy male goats in group A were 71.24 ± 0.52 , 63.03 ± 0.94 , 8.21 ± 1.85 , 6.63 ± 0.08 and 31.88 ± 0.58 , respectively. The corresponding figures for the male goats in group B were 74.13 ± 0.52 , 66.89 ± 0.94 , 7.24 ± 1.85 , 4.86 ± 0.08 and 31.02 ± 0.58 , while in group C these values were 72.32 ± 0.52 , 64.45 ± 0.94 , 7.87 ± 1.85 , 5.32 ± 0.08 and 31.40 ± 0.58 , respectively. Statistical analysis revealed a significant ($P < 0.05$) increase in the total diameter and lumen diameter and a significant ($P < 0.05$) decrease in wall thickness of seminiferous tubules in male goats fed diet containing unboiled cottonseed cakes compared with those in control group. Feed containing boiled cottonseed cakes did not affect these parameters significantly. The number of spermatogenic cell layers was also significantly ($P < 0.05$) lower in the testes of male goats fed unboiled cottonseed cakes compared with those of controls and non-significantly lower compared with those fed boiled cottonseed cakes indicating a disruption in spermatogenesis. The Leydig cell diameter, however, was not affected.

Key words: Micrometry; seminiferous tubules, Leydig cells, gossypol, Teddy male goats

INTRODUCTION

Gossypol is a yellow pigment found in various parts of the cotton plants, including seeds, of the genus *Gossypium* (Adams *et al.*, 1938). Gossypol has been shown to exert antifertility effects in males (Randel *et al.*, 1992). It has direct damaging effects on the epididymides, testes and the developing germ cells (Frick and Danner, 1985).

Cottonseeds (CS) and their by-products, e.g., cottonseed cakes (CSC) and cottonseed hulls (CSH), being an inexpensive and rich source of high quality protein have been used extensively for supplementing dairy animals' rations to increase fat and milk production in these animals (Ahmad, 1993). Cottonseed cakes contain 0.28 ± 0.02 per cent free and 1.44 ± 0.04 per cent total gossypol (Zahid, 2002). The indiscriminate use of cottonseeds or their by-products can adversely affect the

reproductive performance of ruminants including Teddy male goats. The present study was, therefore, carried out to determine the effects of gossypol on micrometry of testes tissue of Teddy male goats.

MATERIALS AND METHODS

In the present study, nine sexually mature and healthy (10–11 months of age) Teddy male goats with clinically normal reproductive tract were used. The animals were randomly divided into three groups named A, B and C, with three animals in each group. Male goats in group A were fed on control ration, without cottonseed cakes (without gossypol). Animals in group B were fed ration containing unboiled cottonseed cakes (which contained both free and bound gossypol contents of 620 and 1750 ppm, respectively) and those in group C were fed ration containing cottonseed cakes boiled at 100°C for

1 hour (which contained only inactivated or bound gossypol contents of 2370 ppm). In addition, male goats in all the 3 groups were offered good quality chaffed seasonal green fodder and clean water *ad libitum*. These experimental rations were fed to the male goats at the rate of 3% of their body weight for a period of 90 days.

At the termination of the treatment, the right testis of each male goat was removed surgically. Immediately after removal, each testis was bisected. Tissue samples of measuring approximately 10 x 10 x 5 mm size were taken from the proximal, middle and distal portions of each half of the testis and placed in 10 per cent buffered formal saline solution for four days for fixation. Following fixation, the tissues were dehydrated, infiltrated with melted paraffin at 58°C, embedded in paraffin blocks, sectioned at 5 micron thickness and stained with haematoxylin and eosin using the standard staining procedures (Luna, 1968; Humason, 1972). In this way, a total of 25 slides from each testis were prepared for histological studies.

For quantitative evaluation of testicular tissue, 20 randomly selected seminiferous tubules were examined, under microscope, at 100X and the total diameter, lumen diameter, wall thickness and spermatogenic cell layers of each seminiferous tubule and diameter of Leydig cells were studied. These parameters were measured by using a calibrated micrometer eye-piece. The number of spermatogenic cell layers in seminiferous tubules was also counted.

Statistical analysis was performed using analysis of variance (ANOVA) technique (Steel and Torrie, 1984) under completely randomised design. For this purpose, general linear model (GLM) procedure under SAS computer programme (SAS, 1990) was adopted. Least significant difference (LSD) test was used for comparison of means (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

The mean values (\pm SE) of total diameter (μ), lumen diameter (μ), wall thickness (μ), spermatogenic cell layers (Nos.) and Leydig cell diameter (μ) of seminiferous tubules of testes of male goats in all the groups are presented in Table-1 and illustrated by figures-1, 2 and 3.

The total diameter (μ) of seminiferous tubules of animals in groups A, B and C averaged 71.24 ± 0.52 , 74.13 ± 0.52 and 72.32 ± 0.52 , respectively. In group B it was significantly ($P < 0.05$) greater than that of animals in group A. However, there was no difference in the diameter of seminiferous tubules between the groups A and C and groups B and C.

The lumen diameter (μ) of seminiferous tubules of animals in groups A, B and C was 63.03 ± 0.94 , 66.89 ± 0.94 and 64.45 ± 0.94 , respectively, the highest value was in male goats of group B, while the lowest value was in control group. In male goats of group B it was significantly ($P < 0.05$) greater than that of animals in group A and non-significantly greater than that of animals in group C. However, the difference was non-significant in lumen diameter of seminiferous tubules between the groups A and C.

The wall thickness (μ) of seminiferous tubules of animals in groups A, B and C was 8.21 ± 1.85 , 7.24 ± 1.85 and 7.87 ± 1.85 , respectively. The highest wall thickness was recorded in animals of control group, while the lowest in male goats of group B fed diet containing unboiled CSC. The difference in this parameter was significant ($P < 0.05$) between the male goats of groups A and B. The difference between groups A and C and groups B and C was, however, non-significant.

The number of spermatogenic cell layers in seminiferous tubules of animals in groups A, B and C was 6.63 ± 0.08 , 4.86 ± 0.08 and 5.32 ± 0.08 , respectively. The highest number of spermatogenic cell layers in the seminiferous tubules was recorded in animals of control group, while the lowest number was noted in male goats of group B. The difference in this parameter was significant ($P < 0.05$) between groups A and B while non-significant ($P > 0.05$) between groups A and C and B and C.

The results on the total diameter, lumen diameter, wall thickness and spermatogenic cell layers of the seminiferous tubules indicate that unboiled CSC adversely affected these parameters in male goats used in this study.

The Leydig cell diameter (μ) of animals in groups A, B and C was 31.88 ± 0.58 , 31.02 ± 0.58 and 31.40 ± 0.58 , respectively. The highest value for Leydig cell diameter was recorded in male goats of control group, while the lowest value was observed in male goats of group B. However, the differences in Leydig cell diameter among three groups were statistically non-significant.

Information on the effects of gossypol feeding on the micrometry of testes in Teddy male goats and other species of goats is scanty. However, Smith *et al.* (1991) reported a non-significant effect of gossypol treatment on micrometry of seminiferous tubules in Holstein bulls. The results of the present study are also not in line with the findings of Akhtar (1997), who showed non-significant differences in micrometry of seminiferous tubules including lumen diameter and number of spermatogenic cell layers between control and those Nili-Ravi buffalo bulls fed cottonseed cakes and whole cottonseeds as a source of gossypol.

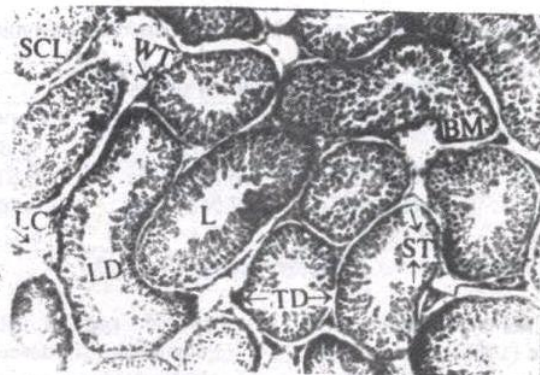


Figure 1: Light micrograph of testis of an experimental male goat fed control diet without gossypol. Spermatogenic cell layers (SCL) are arranged uniformly inside the seminiferous tubules (ST) with an opening in its lumen (L). Seminiferous tubules are surrounded by Leydig cells (LC). Total diameter (TD), lumen diameter (LD), wall thickness (WT) and basement membrane (BM) of seminiferous tubules are normal, stain; H&E; 100X.

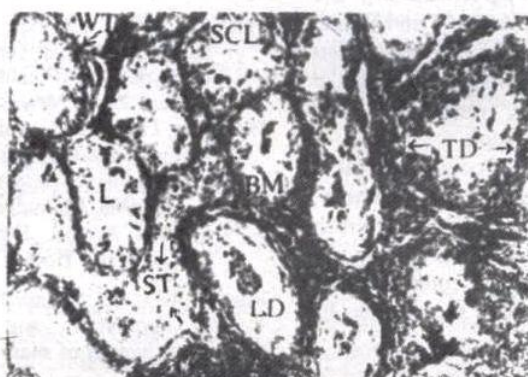


Figure 2: Light micrograph of testis of an experimental male goat fed diet containing un-boiled cottonseed cakes as a source of free and bound gossypol. Seminiferous tubules (ST) show ruptured basement membrane (BM), increased total diameter (TD) and lumen diameter (LD) and decreased wall thickness (WT) and spermatogenic cells layers (SCL). Lumen (L) is well prominent, stain; H&E; 100X



Figure 3: Light micrograph of testis of an experimental male goat fed diet containing boiled cottonseed cakes as a source of inactivated or bound gossypol only. Seminiferous tubules (ST) show intact basement membrane (BM), lumen (L) is prominent, spermatogenic cell layers (SCL) are arranged uniformly. Total diameter (TD) and lumen diameter (LD) are slightly greater when compared with control group, while wall thickness (WT) shows slight decrease. Leydig cells (LC) are prominent, stain; H&E; 100X.

Table 1: Effects of feeding gossypol on micrometry (mean±SE) of the seminiferous tubules and Leydig cell diameter of Teddy male goats.

Groups of animals	Micrometry of seminiferous tubules				Leydig cell diameter (μ)
	Total diameter (μ)	Lumen diameter (μ)	Wall thickness (μ)	Spermatogenic cell layers (Nos.)	
A	71.24 ± 0.52b	63.03 ± 0.94b	8.21 ± 1.85a	6.63 ± 0.08a	31.88 ± 0.58a
B	74.13 ± 0.52a	66.89 ± 0.94a	7.24 ± 1.85b	4.86 ± 0.08b	31.02 ± 0.58a
C	72.32 ± 0.52ab	64.45 ± 0.94ab	7.87 ± 1.85ab	5.32 ± 0.08ab	31.40 ± 0.58a

Values bearing different letters in the same column differ significantly (P<0.05).

The results of present study are in agreement with the findings of Arshami and Ruttle (1988), who reported significant (P<0.01) increase in the lumen diameter, a significant (P<0.01) decrease in the number of spermatogenic cell layers and non-significant effect on Leydig cell diameter in the testes of Holstein bulls fed whole cottonseeds rations as a source of gossypol. Similarly, these results are also in agreement with the findings of Fengyuan *et al.*, (1987); Arshami and Ruttle, (1989) and Singh and Rath (1990), who reported similar changes in the micrometric characteristics of testicular tissue of rabbit, ram and mouse, respectively, fed cottonseed rations as a source of gossypol.

The rations containing unboiled cottonseed cakes adversely affected the total diameter, lumen diameter, wall thickness and number of spermatogenic cell layers in seminiferous tubules of Teddy male goats. It may be concluded that indiscriminate use of diets containing free gossypol (unboiled cottonseed cakes) should not be encouraged in dairy animals.

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