

## EFFECTS OF GOSSYPOL ON SEMEN CHARACTERISTICS IN TEDDY MALE GOATS

I.A. Zahid, L.A. Lodhi<sup>1</sup>, N. Ahmad<sup>1</sup>, Z.I. Qureshi<sup>1</sup>, N.U. Rehman<sup>1</sup> and M.S. Akhtar<sup>2</sup>

*Research Institute for Physiology of Animal Reproduction, Bhunikey (Pottoki), Distt. Kasur.*

<sup>1</sup>*Department of Animal Reproduction, University of Agriculture, Faisalabad.*

<sup>2</sup>*Department of Veterinary Physiology and Pharmacology, University of Agriculture, Faisalabad*

### ABSTRACT

In the present study, the effects of gossypol on semen characteristics in Teddy male goats were studied. Nine Teddy male goats were randomly divided into three equal groups named A, B and C. Animals in all groups were fed concentrated ration without cottonseed cakes (CSC) at the rate of 3% of their liveweight for a period of 30 days and it was named as pre-treatment period. Just after the completion of this period, animals in group A were fed control ration (without gossypol), those in group B were fed ration which contained unboiled CSC as a source of free and bound gossypol, while animals in group C were given ration containing CSC boiled at 100°C for 1 hour as a source of bound gossypol. These experimental rations were fed to animals of respective groups at the rate of 3% of their liveweight for a period of 90 days and it was named as treatment period. Feeding of ration containing gossypol to Teddy male goats did not affect the colour, volume, mass activity, sperm concentration, percentage of dead spermatozoa, liveability and absolute index of liveability of spermatozoa at 37°C. However, it affected significantly ( $P < 0.05$ ) the pH, per cent motility of spermatozoa and percentage of morphologically abnormal spermatozoa. The Teddy male goats fed rations containing a combination of free and bound gossypol showed a significant ( $P < 0.05$ ) increase in the pH and a decrease in motility of spermatozoa which was statistically lower than those fed control diet or diet containing bound gossypol. It was concluded that rations containing a combination of free and bound gossypol (unboiled CSC) or bound gossypol only (boiled CSC) adversely affected the semen quality of Teddy male goats in terms of sperm motility and morphologically abnormal spermatozoa in ejaculates.

**Key Words:** Gossypol, cottonseed cakes, Teddy male goats, semen characteristics.

### INTRODUCTION

Goat breeding, a very basic form of livestock production, is practiced worldwide and makes an important contribution to overall global agricultural production. Goat breeding plays a vital role in the improvement of economy of many people, particularly those in the more primitive areas of sparse vegetation where rearing of other livestock is difficult, the goat is an important provider of milk, meat and fibers such as mohair, cashmere and cashgora. Goat farming appears to be an attractive proposition for a small farm, since the milk yield of a good dairy goat is at least 50 per cent greater than that of a good dairy cow, calculated on a weight for weight comparison (Mowlem, 1985).

Gossypol is a yellow pigment, found in various parts of the cotton plants, including seeds, of the genus *Gossypium* (Adams *et al.*, 1960). Chemically, it is 1, 1', 6, 6', 7, 7'-hexahydroxy-5, 5'-diisopropyl-3, 3'-dimethyl (2, 2'-binaphthalene)-8,8'-dicarboxaldehyde

with the empirical/molecular formula of  $C_{30}H_{20}O_8$  (Abou-Donia, 1976) and a molecular weight of 518.54 (Adams *et al.*, 1960). Gossypol is known to cause toxicity in monogastric (Haschek *et al.*, 1989) as well as ruminant animals (Holmberg and Kutches, 1991). This compound has been shown to exert antifertility effects in males (Randel *et al.*, 1992) and females (Gu *et al.*, 1990). It has direct damaging effects on the testes and the developing germ cells (Zahid *et al.*, 2002). Cottonseed cakes contain  $0.28 \pm 0.02$  per cent free and  $1.44 \pm 0.04$  per cent total gossypol (Zahid, 2002).

Cottonseeds and their by-products, e.g., cottonseed cakes (CSC) and cottonseed meals (CM), have been used extensively as protein supplements in dairy animals' rations to increase fat and milk production in these animals (Ahmad, 1993). As a cheaper but rich source of high quality protein for supplementing animal diets, the use of cottonseed products has been continued over the last many decades. This indiscriminate use of

cottonseeds or their by-products can adversely affect the reproductive performance of dairy animals.

However, there is little information in the literature on the effects of gossypol on the semen quality of Teddy male goats. Therefore, the present study was carried out to determine the effects of gossypol on semen characteristics in Teddy male goats.

## MATERIALS AND METHODS

In the present study, nine adult healthy (10–11 months of age) Teddy male goats were used. These were randomly divided into three groups A, B and C, with three animals in each group. Male goats in all groups were kept under the same managerial and environmental conditions. In order to let the animals adjust to the new environment and adopt themselves to concentrate feeding, all the experimental animals were fed concentrated ration other than cottonseed cakes at the rate of 3% of their liveweight for a period of 30 days, in addition to good quality chaffed seasonal green fodder and clean water *ad libitum*. This period was named as pre-treatment period. Just after the completion of this period, animals in group A were fed control ration without cottonseed cakes (without gossypol), those in group B were fed ration consisting of unboiled cottonseed cakes which contained both free and bound gossypol (620 and 1750 ppm, respectively), while animals in group C were given ration containing cottonseed cakes boiled at 100°C for 1 hour which contained bound gossypol only (2370 ppm). These rations were fed to animals of respective groups at the rate of 3% of their liveweight for a period of 90 days, in addition to good quality chaffed seasonal green fodder and clean water *ad libitum*. This period was named as treatment period. During this period, semen samples were collected on weekly intervals by using artificial vagina. On each collection day two consecutive ejaculates were taken from each animal in all experimental groups. Immediately after collection, the semen collecting tubes were taken to the laboratory and placed in a water bath at 37°C. First and second ejaculates were pooled and each pooled sample was evaluated for its physical characteristics, i.e. colour, volume, pH, mass activity, per cent motility of spermatozoa, sperm concentration, dead spermatozoa, per cent morphologically abnormal spermatozoa, liveability of spermatozoa at 37°C and absolute index of liveability of spermatozoa, using the standard procedures for evaluation of semen. To measure the volume of semen, two consecutive ejaculates were averaged.

Statistical analysis was performed using analysis of variance technique (Steel and Torrie, 1984) under

completely randomised design. For this purpose, general linear model procedure under SAS computer programme (SAS, 1990) was adopted. Least significant difference test was used for comparison of means (Steel and Torrie, 1984).

## RESULTS AND DISCUSSION

At the completion of treatment period, the mean ( $\pm$  SE) values for characteristics of semen in male goats of groups A, B and C are presented in Table-1. The highest values for semen volume ( $0.79 \pm 0.04$  ml) were noted in male goats of control group, while the lowest ( $0.68 \pm 0.04$  ml) in animals of group B fed diet containing unboiled cottonseed cakes. Statistical analysis of the data revealed that the difference was non-significant ( $P > 0.05$ ) among all the groups. Similar trend was observed for mass activity (scores), motility of spermatozoa (%), liveability of spermatozoa (hours) and absolute index of liveability of spermatozoa at 37°C. The pH of ejaculates in animals of group B was greater ( $6.73 \pm 0.01$ ) than animals of groups A and C for which these values were  $6.69 \pm 0.01$  and  $6.70 \pm 0.01$ , respectively. Statistical analysis of the data revealed that the difference was significant ( $P < 0.05$ ) between groups A and B, and B and C while it was non-significant ( $P > 0.05$ ) between groups A and C.

The values for sperm concentration were higher in group A ( $3.09 \pm 0.01 \times 10^9$ /ml) while lower values ( $3.08 \pm 0.01 \times 10^9$ /ml) were observed in groups B and C. However, the difference was non-significant ( $P > 0.05$ ) among all the groups. The values for dead spermatozoa were highest in group B ( $17.80 \pm 1.09\%$ ) while lowest ( $15.90 \pm 1.09\%$ ) in group A. However, statistically all the groups differed non-significantly ( $P > 0.05$ ). The values for morphologically abnormal spermatozoa were highest in group B ( $7.62 \pm 0.22\%$ ) while lowest ( $3.69 \pm 0.22\%$ ) in group A. Statistical analysis of the data revealed that the difference was significant ( $P < 0.05$ ) among all the groups (Table 1).

Information on the effects of gossypol feeding on semen characteristics in Teddy male goats and other breeds of goats is scanty. However, Jainudeen *et al.* (1982) and Akhtar (1997) observed no change in the colour or mass activity of ejaculates of buffalo bulls fed rations containing gossypol. The results of the present study are in line with the observations of these workers. The results of the present study regarding the ejaculatory volume are in agreement with the findings of Smith *et al.* (1991) and Akhtar (1997), who reported no change in volume of semen of Holstein and Nili-Ravi buffalo bulls, respectively, fed on rations containing gossypol. Similarly, the percentage of dead

**Table 1: Effects of gossypol on semen characteristics in Teddy male goats**

Semen characteristics	Group A	Group B	Group C
Ejaculatory volume (ml)	0.79 ± 0.04	0.68 ± 0.04	0.76 ± 0.04
The pH of ejaculates	6.69 ± 0.01 <sup>b</sup>	6.73 ± 0.01 <sup>a</sup>	6.70 ± 0.01 <sup>b</sup>
Mass activity (Scores)	4.01 ± 0.04	3.95 ± 0.04	3.99 ± 0.04
Motility of spermatozoa (%)	79.66 ± 0.40 <sup>a</sup>	77.36 ± 0.40 <sup>b</sup>	79.70 ± 0.40 <sup>a</sup>
Sperm concentration (x 10 <sup>9</sup> /ml)	3.09 ± 0.01	3.08 ± 0.01	3.08 ± 0.01
Dead spermatozoa (%)	15.90 ± 1.09	17.80 ± 1.09	15.93 ± 1.09
Morphologically abnormal spermatozoa (%)	3.69 ± 0.22 <sup>c</sup>	7.62 ± 0.22 <sup>a</sup>	5.88 ± 0.22 <sup>b</sup>
Liveability of spermatozoa at 37°C (hours)	7.67 ± 0.75	6.11 ± 0.75	6.78 ± 0.75
Absolute index of liveability at 37°C	149.67 ± 13.21	115.33 ± 13.21	126.67 ± 13.21

Group A = Animals were fed ration without gossypol

Group B = Animals were fed ration containing unboiled cottonseed cakes (combination of free and bound gossypol)

Group C = Animals were fed ration containing boiled cottonseed cakes at 100°C for 1 hour (bound gossypol only)

Values bearing different superscripts in the same row differ significantly (P<0.05).

spermatozoa, liveability and absolute index of liveability of spermatozoa at 37°C and sperm concentration were not affected by feeding rations containing gossypol. Akhtar (1997) also reported non-significant (P>0.05) differences in these parameters of semen collected from Nili-Ravi buffalo bulls fed rations with or without gossypol.

The feeding of ration containing gossypol affected the pH, motility of spermatozoa and percentage of morphologically abnormal spermatozoa. Teddy male goats fed rations containing a combination of free and bound gossypol showed an increase in the pH. However, the pH of semen collected from all these groups remained within the range reported by Mann (1981). The percentage of motility of spermatozoa in animals fed a combination of free and bound gossypol was decreased and statistically it was lower than those fed control diet or ration containing bound gossypol. These results are supported by the findings on semen of man (Hong *et al.* 1989), rats (Swan *et al.*, 1990) and Brahman bulls (Chenoweth *et al.*, 1994), where a deleterious effect of free gossypol feeding on sperm motility was reported.

In the present study, feeding of rations containing a combination of free and bound gossypol or bound gossypol only, adversely affected the morphologically abnormal spermatozoa. Similarly, Arshami and Ruttle (1989) reported higher percentage of abnormal spermatozoa in the ejaculates from rams fed rations containing 14 to 17 per cent cottonseeds (gossypol) than those fed control ration.

Morphology of spermatozoa plays an important role in their fertilizing ability. An increased number of morphologically abnormal spermatozoa in an ejaculate is associated with a decrease in fertility (Lagerlof, 1934). Infertility is a reflection of lesions in the testes and/or of the excurrent duct system. Hikum and Hoffer (1987) reported reduced spermatogenesis accompanied by increased abnormal sperms in rats after feeding gossypol. An increased number of morphologically abnormal spermatozoa in ejaculates has been reported following feeding ration containing gossypol to rats (Swan *et al.*, 1990) and cocks (Mohn *et al.*, 1989). The results of the present study are supported by the findings of these workers as the feeding of ration containing gossypol showed a significant increase in the pH and a decrease in motility of spermatozoa which was statistically lower than those fed control diet or diet containing bound gossypol.

Based on the findings of the present study, it can be concluded that rations containing a combination of free and bound gossypol (unboiled CSC) or bound gossypol only (boiled CSC) adversely affected the semen quality of Teddy male goats in terms of pH, sperm motility and morphologically abnormal spermatozoa in ejaculates. Thus, gossypol seems to be a potential antifertility agent, as increased number of abnormal spermatozoa, increase in pH and decrease in sperm motility in an ejaculate are associated with a decrease in fertility.

## REFERENCES

- Abou-Donia, M.B., 1976. Physiological effects and metabolism of gossypol. *Residue Rev.*, 61: 125-160.
- Adams, R., T.A. Geissman and J.D. Edwards, 1960. Gossypol, a pigment of cottonseed. *Chem. Rev.*, 60: 555-574.
- Ahmad, M.K., 1993. Few cheeper and balanced rations for animals. Livestock Production Research Institute, Bahadarnagar, Okara, Pakistan.
- Akhtar, N., 1997. Effect of cottonseed (gossypol) on the reproductive performance of male and female buffaloes. Ph.D. Thesis, Univ. Agri., Faisalabad, Pakistan.
- Arshami, J. and J.L. Ruttle, 1989. Effects of diets containing cottonseed meal on semen quality and testicular tissue in fine wool rams. *Proc. West. Sec. Amer. Soc. Anim. Sci.*, 40: 277-282.
- Chenoweth, P.J., C.A. Risco, R.E. Larsen, J. Velez, T. Tran and C.C. Chase Jr., 1994. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. *Theriogenology*, 42: 1-13.
- Gu, Y., C.J.G. Chang, Y. Rikihisa and Y.C. Lin, 1990. Inhibitory effect of gossypol acetic acid on human chorionic gonadotrophic hormone (hCG)-induced progesterone secretion in cultured bovine luteal cells. *Life Sci.*, 47: 407-414.
- Haschek, W.M., V.R. Beasley, W.B. Buck and J.H. Finnell, 1989. Cottonseed meal (gossypol) toxicosis in swine herd. *J. Amer. Vet. Med. Assoc.*, 195: 613-615.
- Hikum, A.P.S. and A.P. Hoffer, 1987. Quantitative analysis of germ cells and Leydig cells in rats made infertile with gossypol. *Contraception*, 35: 395.
- Holmberg, C.A. and A. Kutches Jr., 1991. Cottonseed meal feeding trials in young calves. *Proc. Ruminant Nutrition-Pharmacology and Toxicology. Special Session on Effects of Gossypol on Domestic Animals. Annual Mtg. Amer. Soc. Anim. Sci. Laramie.*
- Hong, C.Y., J.J. Huang and P. Wu, 1989. The inhibitory effect of gossypol on human sperm motility: relationship with time, temperature and concentration. *Hum. Toxicol.*, 8: 49-51.
- Jainudeen, M.R., T.A. Bongso and S. Dass, 1982. Semen characteristics of swamp buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.*, 4: 213-217.
- Lagerlof, N., 1934. Morphological studies on the changes in the sperm structure and in the testes of bulls with decreased or abolished fertility. *Acta Path. Microbiol. Scand.*, 19: 254.
- Mann, T., 1981. *Biochemistry of the Semen and of the Male Reproductive Tracts*. Methuen, London.
- Mohan, J., J.N. Panda, U.S. Singh and R.P. Moudgal, 1989. Studies oil antifertility effects of gossypol acetic acid in domestic cocks. *J. Reprod. Fert.*, 85: 73-78.
- Mowlem, A., 1985. Milk and meat production from goats. *Goat Vet. Soc. J.*, 6: 32-37.
- Randel, R.D., C.C. Chase Jr. and S.J. Wyse, 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *J. Anim. Sci.*, 70: 1628-1638.
- SAS, 1990. *SAS/STAT User's Guide (Release 6.04)*. SAS Inst., Inc., Carry, NC.
- Smith, W.A., L.P. Vosloo, F.P. Theron and C.H. Van Niekerk, 1991. Effect of free gossypol in whole cottonseed on the semen quality of Holstein bulls. *J. Amer. Sci.*, 21: 16-20.
- Steel, R.G.D. and J.H. Torrie 1984. *Principles and Procedures of Statistics*, 2<sup>nd</sup> edition. Mc Graw Hill Co. Inc. New York, USA. pp:107-109.
- Swan, M.A., R. Vishwanath, I.G. White and P.D. Brown-woodman, 1990. Electron microscopic observations on the effect of gossypol on rat cauda epididymis. *Z Mikrost-Ant-Forsch.*, 104: 273-286.
- Zahid, I. A., L.A. Lodhi, N. Rehman and M. S. Akhtar, 2002. Effects of gossypol on micrometry of testes of teddy male goats. *Pakistan Vet. J.* 22: 101-104
- Zahid, I. A., 2002. Studies on the effects of diets containing gossypol on testes of adult teddy buck. Ph.D. Thesis, Dept. Anim. Reprod., Univ. Agri., Faisalabad.