

Effects of Gossypol Present in Cottonseed Cake on the Spermatogenesis of Goats

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Abstract: The present research evaluated the effects on spermatogenesis of feeding male goats with cottonseed cake. It was used thirty adult male goats, which were separated into two groups: one fed with 0.5 kg/animal/day of cottonseed cake and other fed with 0.5 kg/animal/day of corn, for 120 days. At the end of the experimental period, blood samples were collected for determination of serum levels of total proteins, glucose, cholesterol, urea, creatinine and triglycerides. Samples of semen were collected for laboratorial determination of quality, including density and spermatic pathologies. The obtained results revealed that administration of cottonseed cake to goats did not affect the quality of produced semen.

Key words: Cotton residue, gossypol, semen, reproductive toxicology, goats

INTRODUCTION

Cottonseed cake is used as an alternative to soy because its low cost and accessibility in areas, which it is grown (Hagens *et al.*, 2009). However, cotton seeds present a substance with toxic potential in their composition, the gossypol. The gossypol is a phenolic yellow pigment produced by pigment glands found in cotton roots, branches, leaves and seeds (Cheeke, 1998; Rogers *et al.*, 2002) that confers resistance of the plant of pathogens (Abdurakhimov *et al.*, 2009).

Multiple factors may influence the presence of gossypol in the plant. Among them, weather conditions play a significant role and the gossypol concentration is positively correlated with rainfall and negatively with temperature. Variation between cotton species is another important factor, *G. barbadense* presents higher concentration than *G. hirsutum*. On the other hand, the storage of cotton has low influence on gossypol content (Cheeke, 1998).

Gossypol is a compound highly reactive that binds rapidly to different substances, including minerals and amino acids. Iron is the most important mineral capable to bind to gossypol, originating the complex gossypol-iron. Iron bound to gossypol becomes inaccessible and iron deficiency may occur affecting the hematopoiesis. In addition, the presence of this complex in the yolk of eggs determines the formation of a green color (Kerr, 1989; Cheeke, 1998; Rogers *et al.*, 2002; Hassanabadi *et al.*, 2009).

Since, the levels of this substance in the cotton are not high enough to cause acute intoxication, the natural intoxication by gossypol occurs through prolonged ingestion of the plant. The effects of gossypol are cumulative and may appear suddenly after a variable period of ingestion (Kerr, 1989; Cheeke, 1998; Rogers *et al.*, 2002).

In males, gossypol promotes reduction of motility and spermatozoid concentration. Besides this effect, testosterone level and testicular morphology remain unaltered (Qian and Wang, 1984; Yang *et al.*, 2004). In non ruminant females, the exposure to gossypol has been associated to the interruption of estrous cycle and pregnancy and early embryo development. On the other hand, females from non ruminant species are less sensitive (Randel *et al.*, 1992).

Therefore, the present study evaluated the effects of gossypol on male goats fed with cottonseed cake, especially on the reproductive system.

MATERIALS AND METHODS

Thirty male adult crossbreed goats were separated into two groups, the first was fed with 0.5 kg/animal/day cottonseed cake (treated group) and the second was fed with 0.5 kg/animal/day corn meal (control group), both for 120 consecutive days. Complete clinic evaluation was done weekly and animals were weighed individually once a month.

At the end of the experimental period, blood samples were collected for determination of serum levels of total proteins, glucose, cholesterol, urea, creatinine and triglycerides. Samples of semen were collected for laboratorial determination of quality, including density and spermatic pathologies. Semen samples were collected from all goats using an artificial vagina. Semen ejaculates were collected directly into a graduated tube, the volume recorded and samples were immediately examined. Motility was estimated by examining a fresh drop of semen on slide without cover slip with a light microscope at 100x magnification. Motility was scored as: little or no individual spermatozoa motion with no wave; slow motion with no swirl; rapid motion with slow swirl and eddies and vigorous progressive motion with rapid swirl and eddies. From each ejaculate, 10 μ L of semen were suspended in 2 mL of 10% buffered formol saline solution and spermatozoids were counted in a Neubauer chamber using a light microscope. Total spermatozoids counts were obtained by multiplication of spermatozoids concentration by semen volume. The percentage of abnormal sperm was calculated for a total of 200 sperm from each sample with a microscope under 1000x magnification.

The results are represented as mean \pm standard deviation. Parametric t-test was applied with level of significance $p < 0.05$ and Pearson test of correlation was done. The statistic analysis was performed with SAS software for Windows v.8.

RESULTS

None of the experimental animals from both groups presented any clinic alteration. In addition, there was no significant differences in the gain of weight between groups (7.8 kg in the group treated with cottonseed and 8.6 kg in the control group; Table 1). The determination of serum levels of total proteins, glucose, cholesterol, urea, creatinine and triglycerides did not show significant differences between groups (Table 1).

The results of the analysis of semen samples are shown in Fig. 1. Therefore, no statically significant differences were found between the two groups. The only relevant result obtained of spermatic pathology was the

Table 1: Body weight gain, biochemical panel and erythrom from goats treated with 0.5 kg/animal/day corn meal (control group) or 0.5 kg/animal/day cottonseed cake (treated group) for 120 consecutive days. Data are shown as mean \pm SD (n = 15)

Parameters	Control group	Treated group
Body weight gain	2.60 \pm 0.70	5.00 \pm 4.60
Total proteins (mg dL ⁻¹)	69.1 \pm 8.60	78.7 \pm 11.8*
Urea (mg dL ⁻¹)	34.1 \pm 10.9	52.9 \pm 14.9*
Creatinine (mg dL ⁻¹)	1.08 \pm 0.37	1.04 \pm 0.31
Cholesterol (mg dL ⁻¹)	102.4 \pm 27.6	104.8 \pm 22.6
Triglycerides (mg dL ⁻¹)	54.5 \pm 19.8	60.5 \pm 22.9
Glucose (mg dL ⁻¹)	53.6 \pm 11.4	53.8 \pm 15.7
Hemoglobin (g%)	7.70 \pm 1.00	7.40 \pm 0.80
Packed cell volume (%)	23.7 \pm 3.40	23.7 \pm 3.10
Mean corpuscular hemoglobin concentration (%)	32.6 \pm 3.40	31.6 \pm 3.10

* $p < 0.05$, Student t-test

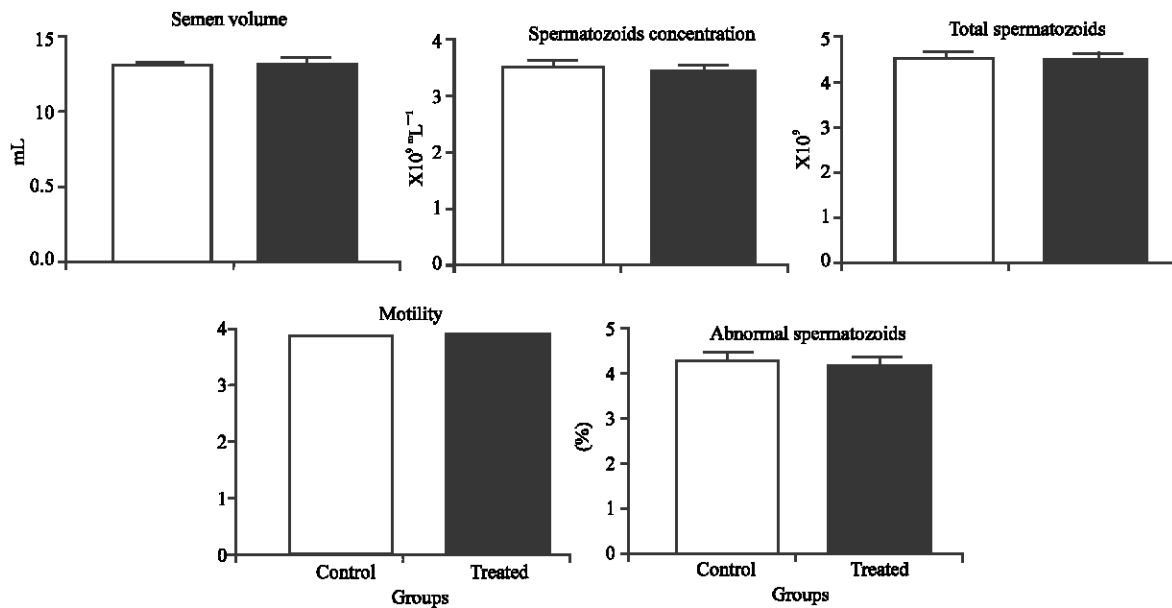


Fig. 1: Sperm analysis from goats treated with 0.5 kg/animal/day corn meal (control group) or 0.5 kg/animal/day cottonseed cake (treated group) for 120 consecutive days. Data are shown as mean \pm SD (n = 15)

presence of curved tails in 86.7% of goats treated with cottonseed and 73.3% in goats from control group and one of the goats treated with cottonseed presented isolated heads.

DISCUSSION

In the present study showed that none of the animals from the two experimental groups presented any clinic alteration, during the evaluated period. This fact is relevant because it indicates that both groups presented similar nutritional metabolic state, specially related to the similar gain of weight. Therefore, no clinic alteration was observed indicative of diseases that could affect the spermatid production and the spermatid analysis.

The gossypol is a non specific enzymatic inhibitor (Herve *et al.*, 1996) and forms chemical complexes with cations and iron (Abou-Donia, 1976). It was verified that the administration of gossypol to rats can induce diarrhea (Bender *et al.*, 1988; Silva *et al.*, 2002), which was attributed to the possible inhibition of pepsinogen and/or other digestive enzymes. In the present study no diarrhea or any other disturbance of the digestive tract was found. This can be attributed to differences in the gossypol concentration that the animals were exposed and/or to differences in the susceptibility between species.

Gossypol has proven deleterious action on spermatid mobility (Chongthammakun *et al.*, 1986; Hong *et al.*, 1989; Ojha *et al.*, 2008), blocking the production, release and use of ATP in these cells (Ueno *et al.*, 1988). Besides, abnormal spermatozooids are formed in animals exposed to gossypol for the reason that ultrastructural abnormalities, mainly in the mitochondrial membranes (Haffer, 1983). Gossypol seems to stop spermatogonial cells entry into meiotic prophase or by production of altered tetraploid primary spermatocytes incapable of further proliferation (Ojha *et al.*, 2008). In this study, no morphological or mobility abnormalities was found in goats fed with cottonseed cake.

In the nature, gossypol can be found in the free form or bound to proteins. The intact cotton seeds present gossypol mainly in its free form. During the process of oil extraction, it occurs binding of gossypol to proteins from the seeds, probably in the radical epsilon-amine from lysine (Calhoun *et al.*, 1995).

Gossypol bound to proteins is not absorbed by the gastrointestinal tract of the ruminants and this form is considered non toxic. Moreover, ruminal microbia of developed animals is capable to detoxify the gossypol by binding it to proteins (Calhoun *et al.*, 1995). In this study, one possible explanation for the absence of deleterious effects in goats is that the free gossypol

concentration in cottonseed cake is low because of the thermic treatment performed during the oil extraction process.

Furthermore, the ruminal microbiota action could also have contributed to the reduction of the amount of free form of toxin. Yet, the binding of gossypol to proteins can be broken during digestion, releasing the toxin (Blackwelder *et al.*, 1998; Noftsker *et al.*, 2000). Therefore, further studies are necessary to determine the residual amounts of gossypol, both free and bound forms, at cottonseed cake.

CONCLUSION

Therefore, conclusion based on the experimental conditions, the cottonseed cake can be administered at the concentration and time evaluated in this study, to male adult goats without compromising their spermatogenesis.

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