

Sperm Quality of Sheep Fed Cottonseed Cake

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ABSTRACT

Background: Cottonseed cake is used as source of proteins for animal feeding. However, cotton seeds present a substance with toxic potential in their composition, the gossypol. Gossypol is a compound highly reactive that binds rapidly to different substances, including minerals and amino acids. In males, gossypol promotes reduction of motility and spermatozoid concentration. The present work evaluated the effects on spermatogenesis of male sheep fed cottonseed cake.

Materials, Methods & Results: Twelve male adult Santa Inês sheep with high genetic standard were used. They 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. Cottonseed cake or corn meal was offered moiled. At the end of the experimental period, samples of semen were collected for laboratorial determination of quality, including density and spermatic pathologies. Sample collection was done by use of 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: 1: little or no individual spermatozoa motion with no wave; 2: slow motion with no swirl; 3: rapid motion with slow swirl and eddies; and 4: vigorous progressive motion with rapid swirl and eddies. From each ejaculate, 10 µL 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. Data were compared using unpaired *t* test, with the level of significance set at $P < 0.05$. In the present study none of the experimental animals from both groups presented any clinic alteration, during the evaluated period. No statically significant differences were found at semen volume, spermatozoids concentration, total spermatozoids, motility and percentage of abnormal spermatozoids between the two groups.

Discussion: The obtained results revealed that administration of cottonseed cake to sheep did not affect the quality of produced semen. During the process of oil extraction, it occurs binding of gossypol to proteins from the seeds, probably in the radical epsilon-amine from lysine. 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. In this study, one possible explanation for the absence of deleterious effects in sheep is that the free gossypol concentration in cottonseed cake is low because of the thermic treatment performed during the oil extraction process. Further studies are necessary to determine the residual amounts of gossypol, both free and bound forms, at cotton residue. Therefore, we conclude that, based on our experimental conditions, the cottonseed cake can be administered, at the concentration and time evaluated in this study, to male adult sheep without compromising their spermatogenesis.

Keywords: gossypol, cottonseed cake, sheep, sperm pathology, spermatogenesis.

INTRODUCTION

Cottonseed is used as an alternative to soy because its low cost and accessibility in areas which it is grown. However, cotton seeds present a substance with toxic potential in their composition, the gossypol, a phenolic yellow pigment produced by pigment glands found in cotton roots, branches, leaves, and seeds [6,19].

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 [6,12,15,19].

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 [6,8,12,15,19].

In males, gossypol promotes reduction of motility and spermatozoid concentration. Besides this effect, testosterone level and testicular morphology remain unaltered [16]. 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 [17].

The present work evaluated the effects on spermatogenesis of male sheep fed cottonseed cake.

MATERIALS AND METHODS

Twelve male adult Santa Inês sheep with high genetic standard were used. They 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. Cottonseed cake (Torta de Algodão Tangará, Tangará, RN, Brazil) or corn meal was offered moiled.

At the end of the experimental period, samples of semen were collected for laboratorial determination of quality, including density and spermatoc pathologies. Sample collection was done by use of 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: 1: little or no individual spermatozoa motion with no wave; 2: slow motion with no swirl; 3: rapid motion with slow swirl and eddies; and 4: 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.

Data are reported as mean \pm standard deviation and were compared using unpaired *t* test (GraphPad Prism v.4 for Mac). The level of significance was set at $P < 0.05$.

RESULTS

In the present study none of the experimental animals from both 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 spermatoc production and the spermatoc analysis.

The results of the analysis of semen samples are shown in Table 1. Therefore, no statically significant differences were found at semen volume, spermatozoids concentration, total spermatozoids, motility and percentage of abnormal spermatozoids between the two groups.

DISCUSSION

The gossypol is a non specific enzymatic inhibitor [10] and forms chemical complexes with cations and iron [1]. It was verified that the administration of gossypol to rats can induce diarrhea [2,18], which was attributed to the possible inhibition of pepsinogen and/or other digestive enzymes [2]. 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

Table 1. Sperm analysis from sheep 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.

| Parameters | Control | Treated |
|---|-----------|-----------|
| Semen volume (mL) | 1.62±0.19 | 1.64±0.18 |
| Spermatozooids concentration (x10 ⁹ /mL) | 4.11±0.53 | 3.98±0.59 |
| Total spermatozooids (x 10 ⁹) | 6.66±0.62 | 6.53±0.74 |
| Motility | 4.0±0.0 | 4.0±0.0 |
| Abnormal spermatozooids (%) | 4.72±0.87 | 5.18±0.96 |

Data are shown as mean ± S.D; n=6.

that the animals were exposed and/or to differences in the susceptibility between species.

Gossypol has proven deleterious action on spermatogenic mobility [7,11], blocking the production, release and use of ATP in these cells [20]. Besides, abnormal spermatozooids are formed in animals exposed to gossypol for the reason that ultrastructural abnormalities, mainly in the mitochondrial membranes [9]. In this study no morphological or mobility abnormalities was found in sheep fed with cottonseed. These data is similar to an earlier study [14] conducted in goats.

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 [5]. 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 [5]. In this study, one possible explanation

for the absence of deleterious effects in sheep 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 [3,13]. Therefore, further studies are necessary to determine the residual amounts of gossypol, both free and bound forms, at cotton residue.

CONCLUSIONS

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

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