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Effect of diets containing whole cottonseed on the quality of sheep semen

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ABSTRACT. This study evaluated the semen quality of Santa Ines sheep fed diets containing different levels of whole cottonseed (WCS). Twenty sheep were distributed into four groups that were given diets with 0; 20; 30; 40% of WCS. For this study the semen was collected by electroejaculation, and a monthly andrological examination was performed during nine months. The analyzed variables were: scrotal circumference (SC), appearance and color of the ejaculate, progressive motility (PM), vigor, mass movement (MM), total sperm concentration ($spzX10^9$), sperm morphology (SM) and blood hematocrit. The SC had no difference among diets, with mean 31.83 cm. The animals fed diets with WCS, presented mean intake of 9.16 mg gossypol kg BW, and were prone to present greenish semen, and appearance ranging from thin creamy to watery. The PM decreased linearly ($p < 0.05$) with increasing level of WCS, while the vigor had a quadratic trend. The total defects increased linearly in relation to reference values described in literature. The WSC had no influence on hematocrit levels, but did affect the PM and vigor of spermatozoa, thus not recommended to diets of animals for breeding.

Keywords: andrological, gossypol, ram, reproduction, sperm motility.

Efeito de dietas contendo caroço de algodão sobre a qualidade do sêmen de ovinos

RESUMO. Este estudo objetivou avaliar a qualidade do sêmen de carneiros da raça Santa Inês, alimentados com dietas contendo diferentes níveis de caroço de algodão integral (CAI). Foram utilizados 20 carneiros distribuídos em quatro dietas e alimentados com 0, 20, 30, 40% do caroço de algodão integral. Para este estudo realizou-se a coleta de sêmen dos animais pelo método da eletroejaculação e durante nove meses procedeu-se o exame andrológico mensal. As variáveis analisadas foram: circunferência escrotal (CE); volume, aspecto e cor do ejaculado, motilidade progressiva (MP), vigor, movimento de massa (MM), concentração espermática total ($spzX10^9$), morfologia espermática (ME) e hematócritos sanguíneos. A CE não variou entre as dietas com média de 31,83 cm. Os animais que receberam (CAI) tiveram ingestão média de 9,16 mg de gossipol kg^{-1} PV, e tenderam a apresentar o sêmen de coloração esverdeada, com aspecto variando de cremoso fino a aquoso. Nas características microscópicas a MP decresceu linearmente com aumento do nível de CAI, enquanto o vigor mostrou comportamento quadrático. Os defeitos totais tiveram efeito linear crescente em relação aos valores de referência descritos na literatura. O CAI não influenciou os níveis de hematócritos, porém afetou a motilidade progressiva e o vigor dos espermatozoides, não devendo ser usado em dietas de animais destinados à reprodução.

Palavras-chave: andrológico, gossipol, carneiro, reprodução, motilidade espermática.

Introduction

The use of cotton derivatives in animal feeding is widespread for the good quality of its protein and other components, such as calcium, phosphorus, iron, and vitamins, besides the energy value. However one of the main problems when using the whole cottonseed or some of its derivatives is the presence of gossypol, an antinutritional factor. The free form of gossypol is predominant in the whole cottonseed (CALHOUN et al., 1995) but during processing to obtain the meal, the gossypol binds to proteins. In the bound form, it is considered less toxic, because its absorption by the digestive tract is impaired (MENA et al., 2001), although there are evidences that the bound form can become free during the fermentation in the rumen (BLACKWELDER et al., 1998).

Kerr (1989) observed that all gossypol in the whole cottonseed is found in free form and is readily absorbed after intake and acts on the metabolism of amino acids, binding to proteins with free amino acids. It is important to know the gossypol levels in the diets as increases the amount of dry matter ingested by animals, due to increased production.

Randel (1992) mentions that the decrease in sperm motility and sperm count are among negative effects of gossypol. Also Chase Jr. et al. (1994) verified that these reproductive problems are due to degeneration of testicular tissue, resulting in decreased the number of sperm that reach maturity and increased percentage of morphological defects.

The fertility and productivity of a herd are directly associated with the male's reproductive capacity. Thus, the libido, the sperm production and its fertilization power are attributes that define the reproductive efficiency of the animal, which is also subjected to environmental factors, such as temperature, air relative humidity, age, nutrition, and management system (SALGUEIRO; NUNES, 1999).

Given the above, this study evaluated the influence of whole cottonseed on the macroscopic, microscopic and morphology of semen and blood hematocrit of Santa Ines sheep, with breeding age.

Material and methods

The study was conducted at the Experimental Station Pendência, of the State Enterprise for Agricultural Research of Paraíba (EMEPA), in the microregion of 'Cariri paraibano', in the municipality of Soledade, Paraíba State. The trial as performed between October 2004 and June 2005, with average temperature of 30ºC and relative humidity of 60%.

Twenty sheep of Santa Ines breed, aged five months, and initial mean weight of 30.0 ± 2.6 kg. The animals were vaccinated against clostridiosis, wormed and kept in intensive management system in collective pens, with five animals per pen. After the adaptation to installations and management (10 days), the animals were submitted to clinical and andrological examination, aiming to standardize the experimental groups.

Diets have been formulated with increasing levels of whole cottonseed (0, 10, 20 and 30%) in the dry matter, and calculated to meet the protein and energy requirements for gains of 150 g day^{-1} (NRC, 1985). The compositions of the diets are listed in Table 1. The mean intake of dry matter was determined by the daily control of supplied food and leftovers. The diet was given at 8 am and 4 pm, and the leftovers were weighed and registered on the following morning. Samples of the diets and of the leftovers were gathered fortnightly to analyze: dry matter (DM), crude protein (CP), mineral matter (MM), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), according to Silva and Queiroz (2002). The total gossypol of the cottonseed was analyzed through high pressure liquid chromatography (HPLC) according to described technique (ABOU-DONIA et al., 1981).

In order to estimate the gossypol intake, the WCS was weighed daily and mixed with other ingredients when the diet was supplied, in the subsequent day, we quantified the WCS in the leftovers, determining thus its consumption.

Each month the animals were submitted to clinical and andrological assessment, when the semen was collected through electroejaculation, using an automatic electroejaculator (Autoject, Neovet) with 12 V and 5 A, the ejaculates were obtained by the automatic method with animals standing in a sheep chute, followed by a sequence of 1-35 stimuli, adding up a total of 30 seconds to 5 minutes. The volume of the ejaculate was measured by means of a collection tube, which was usually 1.2 mL per ejaculate. After this, it was kept in a water bath at 37ºC, and we evaluated the macroscopic (volume, appearance and color) and microscopic characteristics (mass movement, progressive motility, vigor, sperm morphology and concentration). Blood samples were obtained by puncturing the external jugular vein in heparinized tubes at the beginning, middle and end of the trial, in order to determine the hematocrit levels in the animals' blood.

Table 1. Percentage and bromatological composition of experimental diets with different levels of whole cottonseed (% DM).

Ingredients (%)	Levels of whole cottonseed (%)			
	00	20	30	40
Tifton 85 hay	33.10	30.50	36.60	31.20
Ground corn grain	32.60	21.60	6.40	-
Soybean meal	7.80	-	-	-
Whole cottonseed	00	20.00	30.00	40.00
Forage palm	24.40	25.00	25.20	25.80
Livestock urea	1.10	1.00	0.80	0.50
Limestone	0.50	1.40	0.50	2.00
Mineral mix[1]	0.50	0.50	0.50	0.50
	Bromatological composition			
Dry matter	31.40	30.90	30.80	30.30
Crude protein	15.00	15.00	15.90	16.70
Ether extract	2.90	6.00	7.40	9.00
Neutral detergent fiber[1]	36.40	41.60	49.60	49.60
Acid detergent fiber	19.80	24.90	30.10	31.70
Total carbohydrate	75.20	71.60	70.10	66.20
Non fiber carbohydrate	41.00	32.30	23.20	19.40
Mineral matter[2]	6.90	7.40	6.60	8.10
Total digestible nutrients	66.10	66.72	65.50	66.43

[1]Neutral detergent fiber corrected for protein – NDFp; [2]Assurance levels (nutrient kg^{-1}): calcium 130 g; phosphorus 70 g; magnesium 1,320 mg; iron 2,200 mg; cobalt 140 mg; manganese 3,690 mg; zinc 4,700 mg; iodine 61 mg; selenium 45 mg; sulfur 12 g; sodium 170mg; chlorine 276 g; maximum fluorine 700 mg; minimum solubility of P2O5 in citric acid at 2% = 90%.

The measures of the testis were made during semen collection, the scrotal circumference was assessed using a measuring tape surrounding the central surface of the testicles and expressed in centimeters. The length of the testis was made using a caliper open between the caudal end of the head and the cranial end of the tail of the epididymis and expressed in centimeters.

Other assessments were made in the testis, like symmetry (symmetrical standard), shape (ovoid standard), position (vertical standard), consistence (turgid elastic standard), sensitivity (absent standard) and motility (present standard). The epididymis was evaluated by palpation of the presence and integrity of its parts, head, body and tail; and the scrotum, by visualization of lack of continuity solutions or diverse injuries.

Considering the semen, it was evaluated its volume soon after obtaining the sample, through direct reading of millimeter graduation of the collection vial, and the result was expressed in milliliter (mL). The sample was then kept in water bath at 37°C, and the evaluations were made in sequence, according to CBRA (1998).

The appearance of the semen was determined by visualization of consistence of ejaculates and classified as: creamy marble, creamy, thick milky, milky and watered. To evaluate the mass movement, a 10 μL-aliquot was placed on preheated slides (37°C) and observed under optical microscope (100 x magnification). The result was expressed in an increasing scale of 0-5 points.

The progressive motility and vigor were determined by placing 10 μL of semen into 1 mL of Tris dilution buffer (Hydroxymethyl-aminomethane) (3.0 g), sodium citrate (2.0 g) and fructose (1.0 g). Then a 10 μL-aliquot of the diluted semen sample was placed between preheated slide and coverslip (37°C) and evaluated under optical microscope (100 x magnification). The progressive motility was expressed in percentage and the vigor in an increasing scale of 0-5 points.

Sperm concentration was determined by direct counting of spermatozoids in a Neubauer chamber, by diluting a sample of 20 μL into 2 mL of distilled water, obtaining a final dilution of 1:200, being the result presented in mm^3.

Sperm morphology was performed from 95 smears stained with Giemsa at 7.5% (Doles Laboratory), diluted in distilled water and immersing the slides in this solution for two hours. Afterwards the slides were kept upright until completely dry. It was counted 100 sperm cells of each smear, and the result was expressed in percentage.

A completely randomized design was used, with four diets and five replicates. The data of microscopic characteristics of the semen was submitted to an analysis of variance and regression analysis, as a function of the whole cottonseed levels. Statistical analysis were made using the software SAS (Statistical Analysis System) described by (SAS, 1999). The subjective variables, color and appearance, are presented only for description, they were not included in the statistical models.

Results and discussion

Table 2 presents the data about the performance of the animals and their mean intake, in kg day^{-1} of dry matter, of whole cottonseed and gossypol in mg kg^{-1} LW. The mean concentration of gossypol in the whole cottonseed was 1,547 mg kg^{-1} in the dry matter. Considering that the sheep had a mean intake of 0.249, 0.320 and 0.399 kg day^{-1} of whole cottonseed, the estimated intake of gossypol corresponded to 6.8, 9.2 or 11.5 mg kg^{-1} LW in diets with 20, 30 or 40% of whole cottonseed.

The daily weight gain, expressed in g day^{-1}, had no influence from the levels of whole cottonseed, with a mean value of 164.0 g day^{-1}, indicating that the animals presented a good development of weight throughout the study period.

Table 2. Mean value, regression equation, coefficient of variation (CV) of the performance and intake of food of Santa Ines sheep fed whole cottonseed.

Variables	Levels of whole cottonseed (%)				Regression	CV (%)
	0	20	30	40		
Initial live weight (kg)	30.46	30.80	29.10	30.10	$\hat{Y}$= 30.1	9.31
Final live weight (kg)	75.00	76.90	73.10	70.30	$\hat{Y}$=73.8	12.70
Daily weight gain (g day^{-1})	170.0	170.0	165.0	151.0	$\hat{Y}$=164.0	16.80
Mean intake DM(kg)	1.953	2.003	1.878	1.841	$\hat{Y}$ =1.919	-
Mean intake, %LW	3.71	3.80	3.70	3.71	$\hat{Y}$ =3.72	10.83
Mean intake of WCS (g day^{-1})	0.0	249.0	320.0	399.0	$\hat{Y}$ =323.0	8.55
Mean intake of gossypol (mg)	0.0	6.80	9.20	11.50	$\hat{Y}$ =9.16	19.67

The mean daily intake of dry matter was similar for the four levels of whole cottonseed, with mean values of 1.919 kg and 3.72% of live weight, respectively. For young ruminants, Tool Vera (1997) mentions that the maximum amount of gossypol should be 10-20 mg kg^{-1}. In this study, the animals that had consumed 9.20 or 11.50 mg gossypol kg^{-1} LW day^{-1}, presented the motility and vigor affected (Table 3) in relation to the control diet.

Scrotal circumference (SC) has high correlation with sperm production, service capacity and sexual development (YARNEY et al., 1990). In this study, the SC and the length of the testis were not influenced by the levels of whole cottonseed, whose mean values were 31.84 and 1.92 cm, respectively

(Table 3) and the animals presented symmetrical testes, with turgid elastic consistence, no anomalies and mobile within the scrotum.

These results diverge from those found by Andreazzi et al. (1995), working with animals since weaning with 20-30 days until 18 months of age, which observed smaller scrotal circumference in goats receiving 30% of whole cottonseed in relation to the control diet. The results with sheep may have been influenced by age, once the animals were in process of growth during the experimental period. Therefore, mean values of 34 and 36 cm are expected for SC of adult sheep (NUNES et al., 1998; BITTENCOURT et al., 2004).

Similar results were obtained by Kramer et al. (1991), with pubescent rams receiving gossypol in the diet. In the same way, Jimenez et al. (1989), working with bulls, observed that gossypol intake had no influence on the SC, compared to the control animals.

Macroscopic characteristics of the semen, like color and appearance, are described just for information. On average, the semen had consistency from thin creamy to watery. As for the color, the animals that ingested higher levels of whole cottonseed had presented greenish semen, from the fourth month of supply the diets, pointing out the effect of gossypol on these characteristics. Under normal conditions, the semen of sheep is creamy and white.

Microscopic characteristics (Table 3) referring to volume of ejaculate, mass movement, and sperm concentration, were not influenced by the diet, whose mean values were 1.45 mL; 2.65 (0-5) and 2.16 (10^9spz mL^{-1}), respectively. The values for the volume were higher than reported by Tutida et al. (1999), in sheep of different genetic groups, and similar to those found by Salgueiro and Nunes (1999), in Santa Ines sheep, being within the range observed in literature for the species, 0.5 to 1.5 mL (CBRA, 1998). Furthermore, Ferreira et al. (1995) obtained values of 0.89, 0.86 and 1.03 mL for the levels of 0, 30 and 60% of cottonseed meal in the diet of goats. However, as this is a subjective assessment, the values found in literature are very variable and can only be observed for species with highly concentrated semen.

In relation to sperm concentration, Boundy (1993) affirmed that a good quality semen must contain 3.5-6.0 million sperm per milliliter of ejaculate. Our results were below these values, but were similar to those reported by Moreira et al. (2001) for Santa Ines sheep.

Freitas and Nunes (1992) studying sheep in the Northeastern region, observed values of 3.98 x 10^6 mm^{-3} during dry season, and 3.41 x 10^6 mm^{-3} during rainy season.

The progressive motility and vigor of sperm cells had been influenced by the whole cottonseed ($p < 0.01$), with a downward linear trend as increased the WCS level. The results corroborated the results of Risco et al. (1993) that fed bulls with a diet without gossypol and another diet with 8.2 g day^{-1} of free gossypol, and verified that the sperm motility was affected (52.0 ± 9.8 vs. 82.0 ± 6.2%) in relation to the control group, in the ninth week, whereas the percentage of normal sperm decreased from the fifth week (49.0 ± 9.8 vs. 82.0 ± 3.2%), and still the authors observed erythrocyte fragility. Moreover, Santos et al. (2008) registered a reduction in sperm motility (from 73.7 to 52.5%) and an increase of total defects of sperm cells (26.8 x 20.0%), respectively for bulls fed diet with no gossypol and diet with 2.2 kg of whole cottonseed. The sperm vigor presented quadratic trend, whose maximum was 3.3 points for the control diet. This indicates that this characteristic was affected by the WCS, since these values were below the average of 3 points, cited by CBRA (1998) as a desirable standard to select animals for breeding. Maugh (1981) evidenced that the gossypol alters the spermatogenesis, causing oligospermia and aspermia, by inhibiting the lactate dehydrogenase X, an enzyme that plays a key role in spermatozoa metabolism.

Table 3. Mean value, regression equation, coefficient of variation (CV) and of determination of reproductive parameters of Santa Ines sheep fed whole cottonseed.

Physical characteristics	Levels of whole cottonseed (%)					
	0	20	30	40	Regression	CV(%)
Scrotal circumference (cm)	31.52	32.17	31.57	32.07	$\hat{Y} = 31.84$	3.9
Lenght of the testis (cm)	10.68	11.17	10.80	11.02	$\hat{Y} = 10.92$	6.5
Ejaculate volume (mL)	1.61	1.37	1.44	1.37	$\hat{Y} = 1.45$	27.3
Progressive motility (%)	68.22	44.50	48.44	43.10	$\hat{Y} = 64.659 - 0.0604^{**}X$	18.4
Sperm concentration (10^9spz mL^{-1})	2.62	2.14	2.10	1.79	$\hat{Y} = 2.16$	72.3
Mass movement (0-5)	3.32	2.08	2.38	2.82	$\hat{Y} = 2.65$	25.5
Vigor (0-5)	3.14	2.20	2.42	2.36	$\hat{Y} = 3.121 - 0,061X + 0.001^{**}X^2$	13.6

**Significant at 1% by F test.

Chase Jr. et al. (1994) fed growing cattle diets with 0, 6 or 60 mg kg^{-1} LW, for 196 days and verified delayed puberty in the animals treated with whole cottonseed, in relation to the control group; however the quality and quantity of semen was not different between the treatments. Nevertheless, the authors mentioned an increase in the lumen diameter, with lower density of germinal epithelium, and lower amount of Leydig cells in the animals that received gossypol, leading to the conclusion that the gossypol did not affect the quality of the semen, but caused damages to testicular morphology.

The results permitted to infer a probable effect of gossypol, since the literature cites that its concentration in the plant has negative correlation with environmental temperature, and positive with rainfall (KERR, 1989). However, in this study it was not possible to determine the action of gossypol for affected variables; since testosterone and plasma gossypol have not been dosed.

Zahid et al. (2003) fed goats with gossypol and observed that it did not affect the semen quality, but rather had affected significantly ($p < 0.05$) the pH, motility, and percentage of morphologically abnormal spermatozoa.

By observing the sperm morphology (Table 4), the percentage of total defects had increased linearly. The regression analysis allowed verifying that, for each 1% increase of whole cottonseed in the diet, there was an increase of 0.2% in total defects. Meantime, these values were below the limit of 20% for sperm abnormality cited by (CBRA, 1998). The morphological analyses using Giemsa staining stood out a greater occurrence of major defects, such as swelling of the acrosome, folded tail, and strongly folded tail. In accordance with Bittencourt et al. (2004), one of the most important factors when evaluating the morphology is the acrosome integrity, which is indispensable for a high fertilization. The results were lower than found by Santos et al. 2008) that achieved a mean value of 12.2% for major defects, and 20.2% for total defects, in Nelore bulls fed diets with whole cottonseed.

Chenoweth et al. (1994) working with Young cattle, a group without gossypol, and another receiving 8.2% of free gossypol a day, for 11 weeks, have concluded that the supplementation with cottonseed meal had adverse effect on the sperm morphology and spermatogenesis, with the first alterations detected between the 3rd and 4th weeks of the established diet. Additionally, Arshami and Ruttle, (1989), feeding sheep with 0 or 12% of cottonseed meal in the diet, for 26 weeks, have verified abnormalities, such as the wider diameter of the lumen, low number of Leydig cells, smaller size of Sertoli cells, and thinner walls of seminiferous tubules.

Table 4. Sperm morphology in Santa Ines sheeps fed diets with whole cottonseed.

Sperm morphology	Levels of whole cottonseed (%)				Regression	CV (%)
	00	20	30	40		
Defeitos maiores	8,2	9,2	11,8	14,4	$\hat{Y} = 10,90$	49,0
Defeitos menores	1,2	2,4	2,8	3,6	$\hat{Y} = 2,50$	84,8
Total de defeitos	9,4	11,6	14,6	18,0	$\hat{Y} = 8,669 + 0,210^{**}X$	45,3

**Significant at 1% by F test.

Through the analysis of the Table 5, we can infer that the whole cottonseed had no influence on the hematocrit levels, which remained within the reference values for sheep (24-50%). However it was observed a drop in the values in the final phase, corresponding to the last assessment. The mechanism whereby the gossypol interfere on erythrocyte fragility is not yet fully understood, but it is speculated that it may interact directly with the membrane of red blood cells (HARVEY, 1989), and changes their fluidity (VELÁSQUEZ-PEREIRA et al., 1999).

Table 5. Mean values of blood hematocrit in Santa Ines sheep fed diets containing whole cottonseed.

Blood parameters [1]	Levels of whole cottonseed (%)				Regression	CV(%)
	00	20	30	40		
1st assessment	34.00	35.00	34.20	34.0	$\hat{Y} = 34.3$	6.65
2nd assessment	31.00	30.00	31.00	29.20	$\hat{Y} = 30.3$	6.11
3rd assessment	32.00	29.00	29.60	29.40	$\hat{Y} = 30.0$	6.14

[1]Reference value for sheep (24-50%).

Beaudoin (1985), analyzing ruminants receiving diets with cottonseed meal or diets with whole cottonseed cited a reduction in the blood hemoglobin rate. On the other hand, Risco et al. (1993) stated that the changes observed in hematological and serum composition of cows fed with 0 or 800 mg of free gossypol (FG kg^{-1}) have not been enough to be used as a diagnosis of toxicity. Likewise, Andreazzi et al. (1995) did not detect significant difference between the treatments for the blood metabolites in goats fed with 0 or 13.45 mg of FG kg^{-1} LW. Besides that, Calhoun et al. (1995) proposed that the maximum safe concentration of gossypol in the plasma should be 5 $\mu g\ mL^{-1}$.

In ruminants, Mena et al. (2001) argued that the detoxification in the rumen is reduced when increased the ruminal passage rate of foods with gossypol.

However Guedes and Soto–Blanco (2010) said 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.

Conclusion

The use of whole cottonseed in diets for sheep influences the quality of the semen, especially the progressive motility and vigor, leading to an increase in the percentage of total defects in the sheep semen; therefore it should not be supplied to animals for breeding.

References

ABOU-DONIA, S. A.; LASKER, J. M.; ABOU-DONIA, M. B. High–performance liquid chromatographic analysis of gossypol. **Journal of Chomatographic**, v. 206, n. 2, p. 606-610, 1981.

ARSHAMI, J.; RUTTLE, J. L. Effects of diets containing cottonseed meal on semen quality a n d testicular tissue i n fine-wool rams. **American Society of Animal Science**, v. 40, n. 3, p. 277-279, 1989.

ANDREAZZI, M. A.; MORAES, G. V.; SANTOS, G. T.; MARTINS, E. N.; MACEDO, F. A. F.; REIS, A. C. F. Efeito do caroço de algodão sobre o desenvolvimento e desempenho reprodutivo de caprinos machos. **Arquivos de Biologia e Tecnologia**, v. 38, n. 3, p. 799-813, 1995.

BEAUDOIN, A. R. The embriotoxicity of gossypol. **Teratology**, v. 32, n. 2, p. 251-257, 1985.

BOUNDY, T. Collection and interpretation of ram semen under general practice conditions. **In Practice**, v. 15, n. 5, p. 219-223, 1993.

BITTENCOURT, R. F.; CHALHOUB, M.; ALVES, S. G. G.; ALVES, S. G. G.; PORTELA, A. P. M.; ALMEIDA, A. K.; BISCARDE, C. E. A.; FREITAS, D. S.; FERREIRA, A. B. C.; RIBEIRO FILHO, A. L. Circunferência escrotal em carneiros da raça Santa Inês em dez categorias de idade e sua relação com peso corporal, idade e medidas corporais. **Acta Scientiae Veterinariae**, v. 32, n. 3, p. 180, 2004.

BLACKWELDER, J. P.; HOPKINS, B. A.; DIAZ, D. E.; WHITLOW, L. W.; BROWNIE, C. Milk production and plasma gossypol of cows fed cottonseed and oielseed meals with or without rumen-undergradable protein. **Journal of Dairy Science**, v. 81, n. 11, p. 2934-2941, 1998.

CALHOUN, M. C.; KERTON, K. S.; KUHLMANN, S. W.; McDONALD, E.; KIM, H. L. Total (+) and (–)-gossypol in; serum of Holstein cows fed cottonseed. **Journal of Animal Science**, v. 73, Suppl. 1, p. 33, 1995.

CBRA-Colegio Brasileiro de Reproduçâo Animal. **Manual de exame e avaliação de sêmen animal**. 2. ed. Belo Horizonte: CBRA, 1998.

CHASE JR., C. C.; BASTIDAS, P.; RUTTLE, J. L.; LONG, C. R.; RANDEL, R. D. Growth and reproductive development in Brahman bulls fed diets containing gossypol. **Journal of Animal Science**, v. 72, n. 2, p. 445-452, 1994.

CHENOWETH, P. J.; RISCO, C. A.; LARSEN, R. E. VELEZ, J. N.; SHAW, N.; CHASE, C. C.; TRAN, T. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. **Theriogenology**, v. 42, n. 1, p. 1-13, 1994.

FERREIRA, R. N.; OLIVEIRA FILHO, B. D.; DIAS, M. J.; ORSINE, G. F. Efeitos de dietas contendo gossipol na qualidade de sêmen de caprinos, **Revista Brasileira de Reprodução Animal**, v. 19, n. 3-4, p. 212-218, 1995.

FREITAS, V. J. F.; NUNES, J. F. Parâmetros andrológicos e seminais de carneiros deslanados criados na região litorânea do Nordeste Brasileiro em estação seca e chuvosa. **Revista Brasileira de Reprodução Animal**, v. 16, n. 3-4, p. 95-104, 1992.

GUEDES, F. C. B.; SOTO-BLANCO, B. Sperm quality of sheep fed cottonseed cake. **Acta Scientiae Veterinariae**, v. 38, n. 4, p. 415- 418, 2010.

JIMENEZ, D. A.; CHANDLER, J. E.; ADKINSON, R. W.; NIPPER, W. A.; BAHAM, A.; SAXTON, A. M. Effect of feeding gossypol in cottonseed meal on growth, semen, quality, and spermatogenesis of yearling Holstein bulls. **Journal of Dairy Science**, v. 72, n. 7, p. 1866-1875, 1989.

HARVEY, J. M. Erythrocyte metabolism. In: KANEKO, J. J. (Ed.) **Clinical biochemistry of domestical animals**. 4th ed. New York: Academic Press, 1989. p. 173-185.

KERR, L. A. Gossypol toxicosis in catle. **Compendium on Continuing Education for Thepraticing Veterianrian**, v. 15, n. 9, p. 1139-1146, 1989.

KRAMER, R. Y.; GARNER, D. L.; ERICSSON, S. A.; REDELMAN, D.; DOWNING, T. W. The effect of dietary cottonseed on testicular development in pubertal rams. **Journal of Animal Science**, v. 67, supl. 2, 1989.

MAUGH, T. H. Male "pills" block enzyme. **Science**, v. 212, n. 4, p. 314, 1981.

MENA, H.; SANTOS, J. E. P.; HUBER, J. T. M.; TARAZON, M.; CALHOUN, M. C. The effects of feeding varing amounts of gossypol from whole cottonseed meal in lactation dairy cows. **Journal of Dairy Science**, v. 84, n. 10, p. 2231-2239, 2001.

MOREIRA, E. P.; MOURA, A. A. A.; ARAUJO, A. A. Efeito da insulação escrotal sobre a biometria testicular e parâmetros seminais em carneiros da raça santa Inês criados no estado do Ceará. **Revista Brasileira de Zootecnia**, v. 30, n. 6, p. 1704-1711, 2001.

NRC-National Research Concil. **Nutrient requeriment of goats**: angora, dairy and meat in temperate and tropical countries. Washington, D.C.: National Academic Press, 1985.

NUNES, J. F.; CIRIACO, A. L. T.; SUASSUNA, U. **Produção e reprodução de caprinos e ovinos**. 3. ed. Fortaleza: LCR, 1998.

RANDEL, R. D.; CHASE, C. C.; WYSE, S. J. Effects of gossypol and cottonseed products on reproduction of mammals. **Journal of Animal Science**, v. 70, n. 5, p. 1628-1638, 1992.

RISCO, C. A.; CHENOWETH, P. J.; LARSEN, R. E.; VELEZ, J.; SHAW, N.; TRAN, T.; CHASE JR., C. C. The effect of gossypol in cottonseed meal on performance and on hematological and semen traits in postpubertal brahman bulls. **Theriogenology**, v. 40, n. 3 p. 629-642, 1993.

SANTOS, M. D.; PORTILHO, F. K. B. C.; RUAS, J. R. M.; FREITAS, D. H. S.; COSTA, S. D.; SIMÕES, J. M. Morfologia testicular e qualidade espermática de touros da raça Nelore, submetidos à dieta contendo gossipol. **Revista Brasileira de Ciências Veterinarias**, v. 15, n. 3, p. 134-139, 2008.

SAS-Statistical Analisys System. **User's guide to statistics**. Versão 8. Cary: Statistical Analisys System Institute, 1999.

SALGUEIRO, C. C. M.; NUNES, J. F. Estudo das características testiculares e espermáticas de caprinos e ovinos. **Revista Brasileira de Reprodução Animal**, v. 23, n. 3, p. 231-232, 1999.

SILVA, D. J.; QUEIROZ, A. C. **Análises de alimentos** (métodos químicos e biológicos). 3. ed. Viçosa: Imprensa Universitária, 2002.

TUTIDA, L.; BARBOSA, O. R.; MARTINS, E. N.; MACEDO, F. A. F.; ROMAN, M. J. R.; SIMONELLI, S. M. Influência das estações do ano nas características seminais de carneiros. **Revista Brasileira de Zootecnia**, v. 28, n. 5, p. 1141-1147, 1999.

TOOL VERA, J. R. La semilla de algodón en la alimentacón de ruminantes. **Avance Agroindustrial**, v. 17, n. 68, p. 33-35, 1997.

VELASQUEZ-PEREIRA, J.; RISCO, C. A.; McDOWELL, L. R.; STAPLES, C. R.; PRICHARD, D.; CHENOWETH, P. J.; MARTIN, F. G.; WILLIAMS, S. N.; ROJAS, L. X.; CALHOUN, M. C.; WILKINSON, N. S. Long-term effects of feeding gossypol and vitamin E to dairy calves. **Journal of Dairy Science**, v. 82, n. 6, p. 1240-1251. 1999.

YARNEY, T. A.; SANFORD, L. M.; PALMER, W. M. Pubertal development of ramambs: body weight and testicular size measurements as indices of post-pubertal reproductive function. **Canadian Journal of Animal Science**, v. 70, n. 1, p. 139-147, 1990.

ZAHID, I. A.; LODHI, L. A.; QURESHI, Z. I.; REHMAN, N. U.; AKHTAR, M. S. Effects of gossypol on semen characteristics of Teddy male goats. **Pakistan Veterinary Journal**, v. 23, n. 4, p. 173-176, 2003.

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