Indian J. Anim. Nutr. 2021. 38 (3): 272-277
doi: 10.5958/2231-6744.2021.00040.2

Indian Journal of Animal Nutrition

Effect of Long time Feeding of Cotton Seed Cake (*Gossypium sp.*) on Blood Profile, Testicular Biometry and Semen Attributes in Growing Barbari Goats

Ravindra Kumar*, Chetna Gangwar and Kamendra Swaroop
Division of Animal Nutrition Management and PT,
ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura -281 122, India

ABSTRACT

A long term (319 days) feeding trial was conducted on eighteen male growing Barbari goats (age approx. 5 m and mean BW 10.37±0.19 kg). Animals were distributed into three groups (Gr I, Gr II and Gr III) of six each as per completely randomized design and were fed with concentrate pellet and Bengal gram (*Cicer arietinum*) straw to meet their nutrient requirement. Gr I was fed with concentrate pellet (having linseed (*Linum usitatissimum)* cake as protein source), while animals in Gr II and Gr III were fed with T_1 (having equal proportion of linseed and cotton seed (*Gossypum* sp.) and T_2 (having cotton seed (*Gossypum* sp.) cake as protein source, respectively. Testicular biometry was measured at 9 and 12 months of age. Blood was collected at the end of experimental feeding and semen was collected at around 13 months of age. No statistically (P>0.05) significant difference was reported in testicular breadth and circumference between different groups of goats. Hematology and different blood metabolites were similar in all three groups of goats. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and super oxide dismutase (SOD) activity was also statistically similar (P>0.05) in all three groups of goats. Testosterone concentration (ng/ml) was 11.97 for Gr I, 12.17 for Gr II and 11.96 for Gr III. No statistically significant (P<0.05) difference was reported in the serum testosterone level in different group of goats. The colour of semen from Gr I was creamy yellowish while in other groups (Gr II and Gr III) it was creamy. Gossypol feeding had no detrimental effect on consistency, mass motility, progressive motility and viability of sperm in Gr II and Gr III. Malondialdehyde (MDA) in seminal plasma was significantly (P<0.05) increased by feeding of cotton seed cake. The study inferred that long time feeding of cottonseed cake (*Gossypium* sp.) had no deleterious effect on blood and semen attributes in goats.

Key words: Cotton seed cake, Goats, Gossypol, Semen, Testosterone

INTRODUCTION

Protein supplements, those conventionally used in goat feeding includes oil meals of ground nut, soybean meal, linseed and til *etc.*, which are costly and they are mostly utilized in the pig and poultry ration. Cottonseed cake (CSC) is consumed as an alternative ingredient to soy bean meal because of its low cost and accessibility in areas, where it is grown. India is one of the largest producers of cotton seed cake in the world with annual production of around 75 to 90 Lakh MT. All the cotton seed cake produced in the country is consumed domestically. Market value of this commodity is approximately 73 billion dollars. However, cotton seed contains a potential toxic compound, gossypol. This gossypol is a phenolic compound produced by pigment glands found in cotton roots, branches, leaves, and seeds (Cheeke, 1998). A number of toxic effects of gossypol limit the use of cottonseed meal as a feed ingredient. However, in farm animals, acute toxicity of gossypol is little; there are substantial chronic effects, especially in monogastric animals like pig and poultry. Ruminants can endure higher levels of gossypol as free gossypol has been reported to bind to proteins in the rumen (Randel *et al.,* 1992; Schneider *et al.,* 2002).This complexed gossypol is non-toxic in nature. The effects of gossypol are accumulative and may appear suddenly after a variable period of ingestion in non-ruminants (Patton *et al.,* 1985) as well as ruminants (Cheeke, 1998; Kerr, 1989). Keeping these facts in background, this study was conducted to observe the effect of long duration feeding of different level of cotton seed cake (*Gossypol*) on testicular biometry, blood and semen attributes in male Barbari goats.

*Corresponding authors E-mail: ravindra.srivastava@gmail.com

MATERIALS AND METHODS

Feeding trial was conducted at animal experimental unit of Animal Nutrition Management and Product Technology Division of ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura, India. Geographically, the institute is located at 27° N latitude, and 78° N E longitude on 176 m above the sea level. Eighteen male Barbari goats (age approx. 5 months and average body weight 10.37 kg ± 0.19) were distributed into three groups (Gr I, Gr II and Gr III) of six each as per completely randomized design. Three types of concentrate pellet was formulated and prepared using different oil cakes using feed pelleting machine. Type I pellet contained 55% crushed barley (*Hordeum vulgare)*, 25% linseed (*Linum usitatissimum)* cake, 17% wheat (*Triticum aestivum*) bran, 2% mineral mixture and 1 % salt. In type II and type III pellets, 50 and 100% of linseed cake was replaced with cotton seed (*Gossypum* sp.) cake. All these pellets were made iso-nitrogenous. Animals of Gr I was fed with type I concentrate pellet while Gr II and Gr III was fed with type II and type III pellet, respectively. Each animal was treated for external and internal parasite before start of the experimental feeding and was regularly dewormed during the experimentation. Water was provided *ad lib* during experimental feeding.

The duration of the experimental feeding was 319 days (approx. 10.5 months) from 5 months to 15.5 months age. Animals were fed with Bengal gram (*Cicer arietinum)* straw and concentrate pellet at 60:40 ratios to fulfill their nutrient requirements. Animals of Gr I was fed with type I concentrate pellet while those of Gr II and Gr III was fed with type II and type III pellet, respectively. The animals were fed with concentrate pellet at 8 AM and after consumption of pellet they were fed gram straw *ad libitum*. Green fodder was provided two days in a week.

At the end of experimental feeding (at 319 days of experimental feeding) about 10 ml blood was collected from each goat. The site of collection was jugular vein and time was 0 h post feeding. Out of 10 ml, 8 ml blood was used for serum separation by taking blood into a clean and dry glass test tube and keeping it in slanting position for 45 minutes to separate serum. Fresh blood (2 ml) was kept in Eppendorf tube containing anticoagulant (EDTA) for hematological analysis. For serum separation, blood samples in test tube were brought to the laboratory and centrifuged at 3000 rpm for 15 min to separate serum. Serum obtained were stored in small plastic Eppendorf tubes (2 ml) at -20^0 C till further metabolites analysis.The whole blood was analyzed for hematological parameters using hematology analyzer (Melet Schloesing Laboratories, France) standardized for goats as per manufacturer protocol.

Serum was tested for different metabolites using commercial diagnostic kits (Autospan, Span diagnostic LTD.). Concentration of glucose, total protein, total albumin, triglycerides, urea, HDL cholesterol were quantified using end-point assay using double beam spectrophotometer (UV-Vis spectrophotometer, Optizen, 3220UV, Mecasys.co. Ltd, Korea). The company written procedure on kits leaflet was strictly followed for analysis.

Superoxide dismutase (SOD) activity in serum samples was estimated by using SOD determination kit by SIGMA-ALDRICH Co. LLC. Free radical scavenging activity was measured using 2, 2-diphenyl2-picryl-hydrazyl (DPPH) (Blois,1958). All the tests were performed in triplicates and the results were averaged. Testosterone was estimated in the serum using BG TESTO ELISA kit using competitive enzyme immuno-assay technique utilizing goat specific anti-testo antibody and TESTO-HRP conjugate. Testicular biometry was measured at 9 and 12 months of age of goats. Length and circumference was recorded using measuring tape while breadth was measured using vernier caliper. All the units are in centimeter.

After attainment of 13 months of age, animals were trained for mounting and after 4 weeks of training each animal was subjected to semen ejaculation. Semen ejaculates from each buck were collected twice at weekly intervals with the help of artificial vagina during morning time. A dummy non-oestrous doe was utilized for buck mounting, and semen was collected into the graduated cups. Semen was tested immediately

after collection for colour, consistency, mass motility and concentration. Just after collection, semen was maintained in hot water bath at 37°C and subjected to evaluation. Each ejaculated volume was measured using graduated collection cup. Mass motility was measured at low power magnification (10×) using a compound microscope with neat semen on thermo stage maintained at 37°C. Semen samples with mass motility greater than 3 were diluted and after dilution evaluated for progressive motility, live/dead count and abnormalities. Diluted semen (10 µl) was placed on a grease free clean pre-warmed slide (37°C) with cover slip and observed under 40×magnification of phase contrast microscope for evaluating the progressive motility. The average values of two experts were recorded for determining the progressive motility. Live and dead sperm count was measured as per standard staining procedure (Hancock, 1951). A drop of diluted semen mixed with 2-3 drops of stain (Eosin (0.67 g/100 ml) and Nigrosin (5 g/100 ml)) was incubated at 30°C for 1 min. Then smears made on pre-warmed slides were allowed to dry at 30°C. Then smear was examined under high power lens of the phase contrast microscope. Same staining technique was used for measuring the sperm abnormalities and about 200 sperm were counted to calculate the per cent abnormal sperm in the semen. Malondialdehyde concentration, as an index for lipid peroxidation (LPO) in the semen samples, was estimated using the thiobarbituric-acid reaction (Esterbauer and Cheeseman, 1990).

Statistical analysis

The data collected in the study were analyzed by ANOVA as per Snedecor and Cochran (1989) according to a completely randomized design using statistical software package (SPSS). Individual animals were considered as experimental units. The difference between means was considered significant at 95% significance level ($P<0.05$).

RESULTS AND DISCUSSION

The aim of present study was to evaluate the effect of long duration feeding of CSC having gossypol as a toxic constituent on hematology, overall health and semen attributes in male goats. Acute toxicity of gossypol is less, while chronic effects are reported especially in mono gastric animals like pig and poultry. Ruminants in which rumen is not fully functional are also susceptible to gossypol toxicity (Randel *et al.,* 1992). They can tolerate higher levels of gossypol as free gossypol because free gossypol bound to proteins to form complex in the rumen (Coppock *et al.,* 1987, Randel *et al.,* 1992, Schneider *et al.,* 2002), thereby lowering the toxicity of gossypol. There may also be other incompletely defined mechanisms for rumen detoxification of gossypol involved. Free gossypol content in CSC (*Gossypum sp.*) used as ingredient in concentrate pellet preparation was estimated and found to have 0.125 mg/g. The average dose of consumed free gossypol in Gr I was nil, in Gr II 0.25 mg/kg BW/day and in Gr III 0.50 mg/kg BW/day. None of the goats used in experimentation exhibited any sign of toxicity during the period of study. Animal growth rate in different group of goats were also studied (data not shown), but no significant difference in the body weight gain was found in control and treatment group of goats. Body weights and fortnightly body weight changes were not significantly ($P>0.05$) different among groups.

There was no significant ($P>0.05$) difference in hematological parameters like Blood cell counts, hemoglobin, PCV, HCT and differential WBC counts between different groups of goats. Different leukocytes were in the normal range. Blood hemoglobin (g/dl) was 7.64 for Gr I, 7.51 for Gr II and 7.84 for Gr III. No adverse effect on total RBC, WBC, thrombocytes count and differential WBC count was reported. (Table1).

Free gossypol has been reported to bind dietary Fe in the small intestine and thus reduce its absorption and retention (Lindsey *et al.,* 1980).In our study all the hematological variables were within the normal reference range for goats. Hematocrit, packed cell volume and hemoglobin, indicators of dietary Fe adequacy, were in normal range. Even though the experimental rations used in this study contained a certain amount of free gossypol, there was no adverse effect on hematological parameters by feeding of gossypol in growing goats. However, increased

erythrocyte fragility was observed in beef heifers fed a diet with cottonseed meal (Gray *et al.,* 1993, Velasquez-Pereira *et al.,* 1998) and in goat (Luginbuhl *et al.,* 2000). However, the doses were quite higher in earlier cases. No significant difference was reported in serum glucose, total protein, serum urea, cholesterol, triglycerides and HDL in different groups of goats (Table 1). Serum calcium (mg /dl) was 8.54 for Gr I, 8.80 for Gr II and 9.09 for Gr III. Serum antioxidant activity (DPPH) and antioxidant enzyme SOD was also measured. There was no significant difference between different groups of goat. DPPH (% inhibition) was 67.34 for Gr I, 63.11 for Gr II and 62.57 for Gr III while SOD (% inhibition) was 93.03 for Gr I, 93.33 for Gr II and 94.31 for Gr III. There was no significant (P>0.05) difference in serum biochemical parameters *viz.*, glucose, total protein, urea, triglycerides, cholesterol, HDL and calcium among different groups suggesting that the cotton seed cake containing gossypol had no adverse effect on physiological functions of body. However, serum albumin was significantly higher (P<0.05) in groups of goats fed with cotton seed cake. This might be due to more bypass protein fraction in cotton seed cake, making more protein available at intestinal level.

There was no significant difference in DPPH (% inhibition) and SOD activity among the different groups suggesting no adverse effect of gossypol feeding on antioxidant status of the animals. Testosterone concentration (ng/ml) was 11.97 for Gr I, 12.17 for Gr

Table 1. Effect of gossypol feeding on hematology, serum metabolites, antioxidant activity and testosterone of Barbari goats

Parameters	Gr I	Gr II	Gr III	Mean	Sig
WBC (m/mm^3)	8.52±0.92	6.98±0.90	10.35±1.08	8.61±0.61	0.076
RBC (M/mm^3)	12.36±0.53	12.26±0.31	12.35±0.39	12.32±0.23	0.951
HCT (%)	18.41±0.76	19.08±0.68	18.28±1.26	18.53±0.51	0.813
MCV (fl)	14.97±0.35	15.61±0.42	15.34±0.7	15.30±0.30	0.709
Hb (g/dl)	7.64±0.25	7.51±0.18	7.84±0.17	7.66±0.12	0.576
THR(m/mm^3)	325.85±22.77	418.28±49.52	361.57±61.00	368.57±27.23	0.397
MCHC (g/dl)	30.57±0.41	28.88±0.41	30.44±0.45	29.96±0.29	0.21
Lymphocytes (%)	64.77±2.69	64.01±2.17	58.60±2.05	62.46±1.41	0.153
Monocytes (%)	10.07±1.09	9.87±1.14	11.04±1.19	10.32±0.63	0.746
Granulocytes (%)	25.20±1.90	26.11±1.38	30.35±1.43	27.22±1.00	0.075
Serum metabolites					
Glucose (mg/dl)	69.67±3.63	74.01±3.39	72.47±3.19	72.05±1.91	0.666
Total protein (g/dl)	6.37±0.23	6.34±0.38	6.81±0.06	6.51±10.10	0.105
Albumin (g/dl)*	3.58±0.09^b	4.37±0.24^a	4.78^a±0.31	4.24±0.17	0.007
Serum urea (mg/dl)	27.17±2.33	28.80±2.70	28.10±1.72	28.02±1.26	0.882
Cholesterol (mg/dl)	79.46±1.31	75.70±1.67	78.06±2.72	77.74±1.14	0.422
Triglycerides (mg/dl)	92.28±2.12	106.28±7.00	124.00±6.23	107.52±4.20	0.003
HDL (mg/dl)	55.52±3.31	56.55±2.20	55.96±3.03	56.01±1.58	0.969
Calcium (mg/dl)	8.54±0.05	8.80±0.38	9.09±0.59	8.74±0.27	0.34
Antioxidant activity					
DPPH (% inhibition)	67.34±2.01	63.11±1.62	62.57±2.64	64.34±1.26	0.251
SOD (% inhibition)	93.03±2.93	93.33±2.78	94.31±3.15	93.52±1.62	0.953
Testosterone (ng/ml)	11.97±1.44	12.17±4.40	11.96±2.84	12.37±2.03	0.51

*Mean with different superscript in a row differ significantly (P<0.05)

Table 2. Effect of gossypol feeding on testicular biometry and fresh semen quality of Barbari goats

Parameters	Gr I	Gr II	Gr III	Mean	Sig
Body weight (kg)	28.0±0.64	27.18±0.96	26.54±1.51	27.24±0.62	0.254
Testis length (cm)	11.64[a]±0.32	11.50[a]±0.36	12.71[b]±0.28	11.95±0.21	0.032
Testis breadth(cm)	7.49±0.14	7.43±0.13	7.87±0.20	7.60±0.10	0.170
Testis Circumference(cm)	21.47±0.40	20.98±0.42	22.20±0.52	21.55±0.27	0.192
Semen Colour	Creamy Yellowish	Creamy	Creamy		
Mass motility	3.30±0.13	3.69±0.08	3.60±0.15	3.51±0.07	0.056
Consistency	1.66±0.08	1.78±0.08	1.75±0.09	1.73±0.05	0.579
Progressive motility (%)	73.12[b]±3.03	79.64[a]±1.69	73.50[ab]±3.0	75.38±1.56	0.018
Viability (%)	79.69±2.18	85.90±2.79	84.20±2.12	83.06±1.05	0.24
Abnormality (%)	15.62±0.85	14.14±0.74	15.18±1.06	14.96±0.78	0.35
Seminal plasma MDA (nmol/ml)	9.83[b]±0.83	12.07[ab]±1.67	15.03[a]±1.98	11.97±0.92	0.034

[*]Mean with different superscript in a row differ significantly (P<0.05)

II and 11.96. Male sex hormone testosterone concentration was also similar among all the groups showing no detrimental effect of gossypol on testo sterone production. Though effects of gossypol are accumulative and may appear suddenly after a variable period of ingestion affecting male reproductive parameters in non-ruminants (Randel *et al.,* 1992) in our study no effect on male sex hormone was reported as reported by Qian and Wang (1984) in cross bred bull. This may be due to binding of free gossypol in the rumen of ruminants.

Morphometric analysis on the testes of any species or breed is helpful in anticipation of sperm production as well as storage potentials and fertilizing ability of the breeder male. Testicular biometry was studied at 9 and 12th months of age to assess the effect of gossypol feeding. No adverse effect on testicular biometry was reported in goats. At 12 months of age, testicular length was significantly higher (P<0.05) in Gr III (12.71 cm) as compared to Gr I (11.64 cm) and Gr II (11.50 cm). No statistically significant difference was reported in testicular breadth and circumference between different groups of goats (Table 2). Qian and Wang (1984) also reported that testicular morphology remained unaltered in cross bred bull, however, Nassir *et al.* (2014) reported that higher levels (30%) of cottonseed cake-based diets showed some negative effect on testicular morphometry in red Sokoto bucks. But in the present study the level of cotton seed cake was much lower (5 and 10% of diet).

The colour of semen from Gr I was creamy yellowish while in the other groups (Gr II and Gr III) it was creamy. The mass motility was 3.30 for Gr I, 3.69 for Gr II and 3.60 for Gr III. The consistency was also similar in all three groups of Barbari goats being 1.66 for Gr I, 1.78 for Gr II and 1.75 for Gr III. No detrimental effect on sperm progressive motility and viability was reported after incorporation of CSC (*Gossypium sp* in the ration of growing goats after long time feeding. Malondialdehyde concentrations (nmol/ml) was significantly increased (P<0.05) in seminal plasma by feeding of cotton seed cake in goats. This showed that the lipid peroxidation was increased due to gossypol ingestion however no adverse effect on serum antioxidant enzyme SOD was reported (Table 1). Gossypol has proven deleterious action on spermatic mobility (Chongthammakun *et al.,* 1986; Hong *et al.,* 1989), blocking the production, release and use of ATP in these cells (Ueno *et al.,* 1988). Besides, abnormal spermatozoids are formed in animals exposed to gossypol for the reason that ultra-structural abnormalities, mainly in the mitochondrial membranes (Haffer, 1983). Gossypol seems to stop spermatogonial cells entry into meiotic prophase. In this study, no morphological or mobility abnormalities was found in goats fed with cottonseed cake. The possible

explanation for the absence of deleterious effects in goats, due to low intake of gossypol from cotton seed cake and non-absorbance of protein bound gossypol by the gastrointestinal tract of the ruminants. Ruminal micro biota of adult goats is capable to detoxify the gossypol by binding it to proteins (Calhoun *et al.,* 1995).

CONCLUSION

Present study concluded that long time feeding of low level of cotton seed cake in goats had no adverse effect on hematology, serum antioxidant status and metabolites. There was also no negative effect on semen attributes and testosterone hormone.

ACKNOWLEDGEMENTS

The research facilities and funds provided by Director, ICAR-Central Institute for Research on Goats, Makhdoom for this work are greatly acknowledged.

REFERENCES

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. *Nature.* 181: 1199.

Calhoun, M.C., Kuhlmann, W. and Baldwin, B.C. 1995. Assessing the gossypol status of cattle fed cottonseed products. In: Proceedings of the Pacific Northwest Animal Nutrition Conference, Oct. 11. Portland, USA, pp: 147A-157A.

Cheeke, P.R. 1998. *Natural Toxicants in Feeds, Forages, and Poisonous Plants*. 2nd edn. Danville: Interstate Publishers, 479p.

Chongthammakun, S. Ekavipat, C. Sanitwongse, B. and Pavasuthipaisit, K. 1986. Effects of gossypol on human and monkey sperm motility *in vitro*. *Contraception.* 34: 323-331.

Coppock, C.E., Lanham, J.K. and Horner, J.I. 1987. A review of the nutritive value and utilization of whole cottonseed, cottonseed meal, and associated by-products by dairy cattle. *Anim. Feed Sci. Technol.* 18: 89-129.

Esterbauer, H. and Cheeseman, K.H. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. In: Lester, G. Packer, A.N. (Eds). *Methods in Enzymology*, Academic Press pp.407-421.

Gray, M.L., Greene, L.W. and Williams, G.L. 1993. Effects of dietary gossypol consumption on metabolic homeostasis and reproductive endocrine function in beef heifers and cows. *J. Anim. Sci.* 71: 3052-3059.

Haffer, A.P. 1983. Effects of gossypol on the seminiferous epithelium inthe rat: A light and electron microscope study. *Biol. Reprod.* 28: 1000-1003.

Hancock, J.L. 1951. A staining technique for the study of temperature shock in semen. *Nature.* 167: 323.

Hong, C.Y., Huang, J.J. and Wu P. 1989. The inhibitory effect of gossypol on human sperm motility: Relationship with time, temperature and concentration. *Human Toxicol.* 8: 49-51.

Kerr, L.A. 1989. Gossypol toxicosis in cattle. *Compendium on Continuing Education for the Practicing Veterinarian* 11: 1139-1146.

Lindsey, T.O., Hawkins, G.E. and Guthrie, L.D. 1980. Physiological responses of lactating cows to gossypol from cottonseed meal rations. *J. Dairy Sci.* 63: 562-573.

Luginbuhl, J.M., Poore, M.H. and Conrad, A.P. 2000. Effect of level of whole cottonseed on intake, digestibility, and performance of growing male goats fed hay-based diets. *J. Anim. Sci.* 78: 1677-1683.

Nasir, M., Njidda, A.A., Duwa, H. and Chibuogwu, C.I. 2014. Testicular morphometry of red sokoto bucks fed cotton seed cake. *Scholars J. Agric. Vet. Sci.* 1:242-248.

Patton, C.S., Legendre, A.M., Gompf, R.E. and Walker, M.A. 1985. Heart failure caused by gossypol poisoning in two dogs. *J. Am. Vet. Med. Assoc.* 187: 625-627.

Qian, S.Z. and Wang, Z.G. 1984. Gossypol: a potential antifertility agent for males. *Ann. Rev. in Pharmacol. Toxicol.* 24: 329-360.

Randel, R.D., Chase, C.C. and Wyse, S.J. 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *J. Anim. Sci.* 70: 1628-1638.

Schneider, I.C., Ames, M.L., Rasmussen, M.A. and Reilly, P.J. 2002. Fermentation of cottonseed and other feedstuffs in cattle rumen fluid. *J. Agric. Food Chem.* 50: 2267-2273.

Snedecor, G.W. and Cochran, W.G. 1989. *Statistical Methods*. 8th ed. The Iowa State University Press, Ames, Iowa, USA.

Ueno, H., Sahni, M.K., Segal, J. and Koide, S.S. 1988. Interaction of gossypol with sperm macromolecules and enzymes. *Contracetion.* 37: 333-341.

Velasquez-Pereira, J., McDowell, L.R., Risco, C.A., Prichard, D., Martin, F.G., Calhoun, M.C., Williams, S.N., Wilkinson, N. S. and Ogebe, P. 1998. Effects on performance, tissue integrity and metabolism of vitamin E supplementation for beef heifers fed a diet that contains gossypol. *J. Anim. Sci.* 76: 2871-2884.

Received on 23.09.2021 and accepted on 17.11.2021