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Reproductive Characteristics of Growing Beetal Goat Fed on Fermented Cotton Seed Cake

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Abstract

Background: Cotton seed cake is the byproduct of cotton which is produced worldwide

Aims and Objectives: This study was aimed to investigate the effect of Fermented Cotton Seed Cake (FCSC) on reproductive characteristics of growing beetal goats.

Methodology: Thirty goats (n=6 per treatment) were randomly assigned to five dietary treatments arranged in a 2x2 factorial with a control within a Completely Randomized Design (CRD). For Solid state fermentation (SSF) purposes Aspergillus terreus, was employed. Diet was offered by adding FCSC at 0 (control), 10%, and 20%, 30% and 40% levels for 12 weeks. At the end three animals per treatment were slaughtered and dissection of reproductive organs was done by measuring and processing for sperm evaluation. The reproductive parameters evaluated include testicular and epididymal morphometrics, Daily Sperm Production (DSP), Daily Sperm Production per gram testis (DSP/g), Gonadal Sperm Reserve (GSR), sperm count and motility.

Results: Fermented Cotton Seed Cake (FCSC) level resulted in significant ($p<0.05$) reduction in testicular weights, lengths and volume and significantly ($p<0.05$) lowering of epididymal weight as compared to the control group. 10% FCSC in diet did not compromise with DSP, DSP/g, GSR, sperm count and motility, while T3, T4 and T5 FCSC diet level significantly reduces these parameters.

Conclusion: It was concluded that feeding FCSC - based diets to goats adversely affected the testicular and epididymal morphometrics.

Keywords: Beetal goats; Cottonseed cake; Fermentation; Testis and Sperm characteristics

Introduction

Cotton seed cake (CSC) is a by-product of the cotton processing industry (Apata, 2010 [1]). This is protein rich meal (42%) but presence of toxic material gossypol results in abnormalities in ruminants (Dereje et al. 2015[2]).

In monogastrics, gossypol interfered with protein digestion, depressed appetite, and caused reproductive impairment and intestinal and other internal organ abnormalities (Rathore et al. 2020 [3]). Earlier, Sivilai and Preston, (2019) [4] had reported that feeding sows and gilts diets containing free gossypol either reduced conception rates or caused conception failure. Gossypol has been shown to exert anti-fertility effects in males (Itodo et al. 2020 [5]). It has direct damaging effects on the epididymides, testes and the developing germ cells (Taylor et al. 2019 [6]). Different methods have been used either to completely or partially remove the gossypol from CSC for animal feeding. These include ferrous sulphate treatments and microbial fermentation (Hill et al. 2017 [7]). Microbial fermentation has been proved to be more effective in the detoxification of CSC (Soares Neto et al. 2021 [8]). This study was aimed to investigate the effect of fermented CSC-based diets on the reproductive characteristics of growing beetal goats.

Materials and Methods

All the experimental procedures were performed at The Islamia University Bahawalpur, Pakistan. Thirty (30) growing beetal goats, aged between 9-10 weeks, were randomly divided into five (5) groups (n = 6 animals per treatment) arranged in a 2x2 factorial with a control within a completely randomized design (CRD). Each animal served as a replicate. Solid State Fermentation (SSF) of CSC was done by using *Aspergillus terreus*. For isolation and purification purpose Potato Dextrose Agar (PDA); supplemented with 20% sucrose at p^h 5.5, temperature of 30^0C and preserved at 4^0C was used. A loopful of the mycelium of *Aspergillus terreus* was inoculated into Kirks' basal inoculum medium, thus, an aqueous suspension of *Aspergillus terreus* was prepared. The suspension was collected and stored in a refrigerator until it was used. Moisture content of dry CSC was raised to 60%. The CSC was then sterilized in a locally fabricated 24 kg-capacity fermenter. Sterilization was done by autoclaving at 121^0C for 3 hours and the CSC was allowed to cool. After inoculation of CSC, plates were kept for 7 days at 30 ± 1^0C for fermentation and the plates were covered with muslin cloth and aluminum foil. The goats were offered with soybean meal-based diet and clean water "containing anti-stress (stress strok, at 5g/10 litres) and antibiotics (Anicilin)" for one week. Animals were fed the experimental diets for 12 weeks. The diets were prepared to contain 18% crude protein such that soybean meal (SBM) for the control diet (T_1). In diet 2 (T_2) and diet 3 (T_3), 10% and 20% of raw cotton seed cake (RCSC), respectively, were included to replace SBM; while 10% and 20% fermented cotton seed cake (FCSC) were included in diet 4 (T_4) and diet 5 (T_5), respectively. The gross composition of the experimental diets is presented in Table 1.

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Table 1: Experimental diets composition for growing beetal goats.

Ingredients	T_1(Control)	T_2(10% FCSC)	T_3 (20% FCSC)	T_4 (30% FCSC)	T_5 (40% FCSC)
Maize	52.21	46.99	42.43	40.46	39.61
Soybean Meal	22.54	17.76	12.32	11.29	10.14
Cotton Seed Cake	-	10.00	20.00	30.00	40.00
Fish Meal	2.00	2.00	2.00	2.00	2.00
Wheat Offal	15.00	15.00	15.00	15.00	15.00
Bone Meal	3.00	3.00	3.00	3.00	3.00
Blood Meal	3.00	3.00	3.00	3.00	3.00
Oyster Shell	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Premix*	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Crude Protein (%)	21.00	21.00	21.00	21.00	21.00
Metabolizable Energy (Kcal/kg)	2796.98	2703.27	2614.40	2606.76	2515.72
Crude Fibre (%)	3.91	5.93	7.99	8.91	9.98

*Premix composition: Each feed contained Vit A, 1500IU: Vit D3, 2500IU, Vit E, 11IU, Vit B2, 10mg: Vit B3, 40mg: Vit B6, 20mg; cholinc chloride, 400mg; Mn, 120mg; Fe, 70mg; cu, 10mg: 12., 2.2mg; Se, 0.2mg; Zn, 45mg; Co., 0.02mg.

For investigation purposes 3 animals were slaughtered at the end of trial and their reproductive organs were dissected and measured carefully. After separating testes and epididymides of each animal and data were obtained for testicular and epididymal morphometrics, sperm characteristics, Gonadal Sperm Production (GSP), Gonadal Sperm Reserve (GSR) and Daily Sperm Production (DSP). Vernier Caliper was used to measure the length and width of the testes, while the volume of testis was determined by Archimede's principles of water displacement, using a measuring cylinder. Right and left testis weight; paired testis weight; paired testis volume; right and left testis length; right and left testis width were measured; right and left epididymal weight and length were measured. The GSP and GSR were determined haemocytometrically by homogenate technique as described by Vandu et al. (2021 [9]). Daily sperm production (DSP) was determined from the GSR. The DSP was obtained using the formula proposed by Osayande et al. (2017 [10]), Sperm morphology was determined by Gatimel et al. (2017 [11]) method.

$$DSP = \frac{\text{Testis sperm count (GSR)}}{\text{Time divisor}}$$

The value of time divisor for goats = 3.56.

DSP/g parenchyma = Gonadal Sperm Reserve 1

Gross testis wt – Tunica albuginea wt 3.56

Statistical analysis

Data generated were subjected to analysis of variance (ANOVA) appropriate for a 2x2 factorial and one-way using SAS (2002). Duncan's option was used for means separation.

Results

It was observed that testicular parameters of growing beetal goats fed on FCSC shows significant ($p<0.05$) effect by reducing testis weight, length, and volume, as compared to control group, but FCSC diet induces no change in testis width (Table 2). It was observed that all the testicular characteristics were non-significant ($p>0.05$) when measured at different FCSC diet levels, but FCSC treatment significantly ($p<0.05$) affected the left, right and paired epididymal weights; left and right epididymal lengths Table 3. The epididymal weights (left, right, paired and mean) were significantly ($p<0.05$) lower for the goats that were fed on FCSC diets compared to the control group. The epididymal lengths (left, right and mean) of goats offered FCSC were shorter than those fed the control. All sperm parameters (sperm count, live and normal sperm, sperm motility, gonadal sperm reserve and daily sperm production) were significantly ($p<0.05$) lower at T3, T4 and T5 FCSC diets. While 10% FCSC level did not affected these sperm parameters Table 4.

Discussion

The significant reduction observed in most of the testicular parameters (testis weights, lengths and volume) of goats fed on FCSC-based diets suggests that gossypol compromised the growth of the testes and fermentation was not able to completely ameliorate the adverse effect of gossypol contained in the FCSC-based diets. Thomas, (2021[12]) reported that testis size reflects both the present and future sperm production capacity, as well as the breeding quality of a male animal. Hence, reduced testicular size as indicated by reduction in the weight, length and volume of the testis of goats fed FCSC-based diets suggests that the testes of these goats could have a reduced sperm production capacity and the goats may not perform maximally for breeding purpose. Although most testicular parameters were significantly reduced, few exceptions were recorded for the width of the testis. The width of the testis was not significantly affected by the treatment. This observation therefore makes the results of this study to be in partial agreement with the report of Vandu et al. (2021 [9]) observed that when rabbits fed on cottonseed cake-based diets showed no adverse effect on testicular morphometrics, most especially when the period of dietary treatment is short. The significant reduction in the epididymal weights and lengths of goats fed on FCSC-based diets suggested a low sperm storage capacity. Observations on the epididymal weights in this study are contrary to that reported by Amao and Showumi (2016 [13]) which stated that the epididymal weight of rabbit bucks fed on raw CSC-based diets was heavier than the control.

Table 2: Testicular effects of fermented cottonseed cake on different treatments.

Parameter	T1 (Control)	T2 (10% FCSC)	T3 (20% FCSC)	T4 (30% FCSC)	T5 (40% FCSC)
Left Testis Weight (g)	31.23^{a}	16.53^{b}	15.60^{b}	15.13^{b}	10.50^{c}
Right Testis Weight (g)	29.83^{a}	16.86^{b}	16.06^{b}	15.90^{b}	11.40^{c}
Paired Testis Weight (g)	61.06^{a}	33.40^{b}	33.25^{b}	32.93^{b}	20.90^{c}
Left Testis length (cm)	5.66^{a}	4.03^{b}	3.96^{b}	3.80^{b}	3.37^{b}
Right Testis Length (cm)	5.80^{a}	4.30^{b}	4.16^{b}	4.13^{b}	4.00^{b}
Mean Testis Length (cm)	5.73^{a}	4.16^{b}	4.11^{b}	3.98^{b}	3.85^{b}
Left Testis Width (cm)	3.03	2.50	2.53	2.54	2.56
Right Testis Width (cm)	3.46	3.16	3.26	3.19	3.33
Mean Testis Width (cm)	3.25	3.13	2.90	3.06	3.06
Left Testis Volume (cm)	33.33^{a}	17.00^{bc}	20.00^{bc}	38.66^{bc}	43.33^{c}
Right Testis Volume (cm)	32.00^{a}	17.66^{b}	19.50^{bc}	28.43^{b}	42.33^{c}
Paired Testis Volume (cm)	65.33^{a}	34.66^{bc}	39.50^{bc}	47.10^{bc}	55.66^{c}
a, b, c: *Means within the same row with different superscripts differ significantly ($p<0.05$)					

Table 3: FCSC effects on epididymal characteristics.

Parameter	T1 (Control)	T2 (10% FCSC)	T3 (20% FCSC)	T4 (10% FCSC)	T5 (20% FCSC)
Left Epididymal weight (g)	15.10^{a}	8.96^{b}	6.50^{b}	5.83^{b}	3.96^{c}
Right Epididymal Weight (g)	13.70^{a}	9.03^{b}	6.66^{bc}	5.33^{bc}	3.50^{c}
Paired Epididymal Weight (g)	28.80^{a}	18.00^{b}	13.16^{bc}	12.96^{bc}	9.46^{c}
Mean Epididymal Weight (g)	14.40^{a}	9.00^{b}	6.58^{bc}	6.08^{bc}	4.73^{c}
Left Epididymal Length (cm)	10.90^{a}	9.40^{ab}	8.46^{ab}	8.06^{ab}	7.03^{b}
Right Epididymal Length (cm)	10.36^{a}	8.40^{ab}	7.90^{ab}	7.50^{ab}	6.93^{b}
Mean Epididymal Length (cm)	21.27^{a}	17.80^{ab}	16.37^{ab}	16.07^{ab}	14.93^{b}
a, b, c: *Means within the same row with different superscripts differ significantly ($p<0.05$)					

Table 4: FCSC levels effects on sperm characteristics of growing goats.

Parameter	T1(Control)	T2(10% FCSC)	T3(20% FCSC)	T4(30% FCSC)	T5(40% FCSC)
Sperm count (x10^{6}/g)	48.50^{a}	36.30^{b}	30.97^{b}	19.20^{c}	15.77^{c}
Sperm motility (%)	68.01^{a}	67.95^{a}	61.45^{b}	56.05^{b}	57.10^{b}
Normal sperm (%)	71.34^{a}	70.90^{a}	61.87^{b}	52.76^{c}	54.61^{c}
Live sperm (%)	74.76^{ab}	77.29^{a}	67.98^{bc}	61.54^{c}	64.41^{c}
GSR (x10^{6})	1928.31^{a}	1921.56^{a}	1199.18^{ab}	748.50^{b}	610.81^{b}
DSP (x10^{6})	335.26^{a}	332.16^{a}	274.41^{b}	171.28^{c}	139.77^{c}
DSP/g (x10^{6})	17.54^{a}	17.44^{a}	20.21^{b}	16.02^{b}	13.53^{b}
a,b,c: *Means within the same row with different superscripts differ significantly ($p<0.05$)					

Significant reduction in the epididymal weight in this study is however justifiable as gossypol contained in the raw CSC could have adversely affected the growth of the epididymides. The observation that there was significant reduction in sperm count and motility; normal and live sperm; GSR, DSP and DSP/g of goats fed on FCSC-based diets is an indication of the anti-spermatogenic effect of gossypol contained in CSC-based diets and the inability of fermentation to completely get rid of gossypol toxicity. Anti-spermatogenic effect of gossypol had been linked with the tendency of gossypol to damage the germinal epithelium of seminiferous tubules (Babashani et al. 2014 [14]). This was like the report of Shandilya et al. (1982 [15]) which stated that gossypol inhibits sperm motility and decreased sperm concentration in male cynomolgus monkeys (*Macaca fascicularis*). The inability of fermentation to ameliorate the deleterious effect of gossypol contained in CSC observed in this study contradicts the report of Amao and Showumi (2016 [13]) which stated that anti-spermatogenic effect of gossypol was neutralized by fermentation when rabbits are used. This could probably be due to the differences in the species of animal used for the experiments. Furthermore, possibly fermentation by *Aspergillus terreus* degraded the nutrients in the cottonseed cake such that the protein quality of the FCSC could not support the testicular and epididymal growth in the goats.

Conclusion

It was concluded that feeding FCSC - based diets to growing beetal goats adversely affected the testicular and epididymal morphometrics; however, 10% FCSC in diet did not compromise with gonadal sperm characteristics. Further studies to investigate the use of other micro-organisms apart from *Aspergillus terreus* to ferment CSC are recommended.

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Conflict of Interest

The author should declare no conflict of interest.

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