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TESTICULAR HISTOMETRY, GONADAL AND EXTRA GONADAL SPERM RESERVE OF RED SOKOTO BUCKS FED COTTON SEED CAKE

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ABSTRACT

An experiment was conducted to determine the effect of cotton seed cake inclusion on testicular histometry of red sokoto bucks. Sixteen sexually matured Red Sokoto bucks were randomly allotted to four dietary treatments wit four animals per treatment. Treatment compared were T_1 = 0% cotton seed cake (CSC), T_2 = 10% CSC, T_3 = 20% and T_4 = 30% CSC respectively. The results of chemical composition show that the crude protein (CP) content was generally high for all the diets. The ash, ether extract (EE), neutral detergent fibre (NDF) and gross energy values (GE) were all highest in T_3. The gonadal sperm reserve ($x10^6$) reveals the right and left testis to be significantly higher ($p<0.05$) in T_1 than the other treatment groups (2762.6×10^6) and (2761×10^6). The result of the extra gonadal sperm reserve showed significant difference ($p<0.05$) in all the parameters observed except for left caput epididymides and right caudal epididymides. The result of testicular morphometry showed significant effect ($p<0.05$) in all the parameters observed. The result also shows histological changes at higher level (30% CSC inclusion). Base on these findings, 10 and 20% level of CSC inclusion does not have any negative effect on testicular morphometry of Red Sokoto buck as reveal by the photomicrograph section of the testis.

Keywords: Testicular, Gonadal, Goats, Sperm, Cotton seed cake.

INTRODUCTION

Small ruminants such as sheep and goat, are numerically important domesticated animals due to their contribution of meat, milk, skin, fiber and manure and as the sole or subsidiary source of livelihood for a large number of small and marginal farmers and landless labourers; they therefore contribute significantly to subsistence and socio-economic livelihoods of a large human population in low-input, small holder production systems in developing countries [1, 2]. Of the three breeds of goats in Nigeria, the Red Sokoto goat is the predominant and the most widely used and distributed breed in the northern savannah belts of the country [3]. The breed has a uniform dark red colour, short haired and with sexes having horns and the animal has short drooping ears and short legs. The skin is very valuable among other goats.

Reproduction is one of the most important factors affecting livestock production and its success greatly depends on a mixture of factors including genetic merit, physical environment, nutrition and management [4]. It is well documented that reproductive well-being and performance of farm animals is largely dependent on their nutritional status. Evidence from the literature suggests that nutritional factors are the most crucial in terms of their direct effect on reproductive phenomenon and have the potential to moderate the effects of other factors [5-7].

Cottonseed cake is used as an alternative to soy because of its low cost and accessibility in areas, where it is grown [8]. However, cotton seed presents a substance with toxic potential in its composition. The gossypol is a phenolic yellow pigment produced by pigment glands found in cotton roots, branches, leaves, and seeds [9;10] that confers resistance of the plant of pathogens [11].

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MATERIALS AND METHODS

Experimental Location

The study was conducted in Kano which is located within the lines of longitude 9˚30 and 12˚30 north and latitude 9˚30 and 8˚42 east. The area is characterized by tropical wet and dry climate; a wet season (May to September) and dry season (October-April) with annual rain fall that ranges between 600 to 1000mm and temperature regimes between 20 and 40˚C in the months of September to February [12].

Experimental Animals and Management

The experimental animals were divided into four treatments of four animals each. Treatment 1 were given 0% cottonseed cake, treatment 2 10% cotton seed cake, treatment 3, 2% cotton seed cake and treatment four (4) 30% cotton seed cake. The animals in each treatment were given the diet in the morning at exactly 7:30 am and the left over was measured on daily basis, water and mineral salt lick were given *ad-libitum* throughout the experimental period. Hay was also given in the evening. Animals were given antibiotics prophylaxis using 20% oxytetracycline (Long acting) at the dose rate of 10 mg kg^{-1} body weight. Also, animals were dewormed using Albendazole suspension at dose rate of 7.5 mg per kg body weight. The experimental house was thoroughly clean and disinfected before the arrival experimental animals using morigard at dose rate of 2 ml per liter. House sanitation was done on daily basis throughout the experimental period. Feederss and water containers were washed and clean on daily basis to avoid contamination

Statistical Analysis

Data collected were coded and then subjected to analysis of variance (ANOVA) in a Randomize Complete Block Designed (RCBD) using SAS package [24] where significant differences are observed means were separate using LSD method of analysis.

Determination of Gonadal and Extra Gonadal Sperm/Spermatid Reserve

Gonadal and epididymal sperm/spermatid reserve were determined by the method of Igbocli and Rakha [13], Rekwot*et al* [5]. Each testis were homogenized in 50 ml of saline that contained the antibiotics (1.h/1m of streptomycin and 100 IU/ml sodium penicillin G); using high-speed blender operated at full speed for two minutes and the homogenate volume is measure after rinsing the blender container with 20 ml of saline and adding the effluent. From the homogenate, 5 ml is transferred into a conical flask and diluted further with 80 ml of saline. The homogenate was store overnight at 5^0C. The sperm/spermatid concentration was determined with a hemocytometer. The epididymis is carefully separated from each testis with scalpel blade and the lengths and weights of each caput; corpus and caudaportion was measured. The caput, corpus and caudaepididymides were separated minced in 20 ml of saline with sharp scissors and stored overnight at 5^0c. The minced caput, corpus and cauda epididymides were filtered through gauze and the volume of the filtrate measured. One milliliter of the epididymal filtrate was diluted with 2 ml of saline. The concentration of the spermatozoa was determined with hemocytometer as described for gonadal sperm [14].

RESULTS

Chemical composition of experimental diets

The chemical composition of the experimental diets is shown in Table 1. From the Table, the dry matter content of experimental diets ranged from 934.00 in T_2 (10% Cotton seed cake inclusion level) to 94.50 g kg^{-1} DM in T_3 (20% cotton seed cake level of inclusion). T_1 was observed to have the lowest ash content compared to other treatments. Significant differences ($p < 0.05$) were observed in the crude protein values for all dietary treatments, highest value was observed in T_3, 382.00. The ADF was significantly different ($p < 0.05$), with T_3 recoding the highest value 388.40 g kg^{-1} DM and lowest in T_1 341.30, 501.10, 441.00 g kg^{-1} DM respectively compared to other treatments.

Effect of CSC inclusion on gonadal sperm reserve ($x10^6$) of Red Sokoto bucks

The result of cotton seed cake on gonadal sperm reserve of Red Sokoto bucks is shown in Table 5. Significant differences ($p< 0.05$) were observed among the treatments. T_1 having the highest value of 2762.6 and T_4 recorded the lowest value of 705.7 $X10^6$ right testis.

Effects of CSC inclusion on extra gonadal sperm reserve of Red Sokoto bucks (ml)

The result of extra gonadal sperm reserve is presented in Table 6.The result shows no significant effects ($p> 0.05$) among the treatments observed for both left and right epididymides volume. With T_2 recording the highest epididymides volume 14.00 ml while the lowest value was observed in T_1 and T_4 (12.00 ml).

Effects of CSC inclusion on testicular morphometry of Red Sokoto bucks.

Significant effects ($p<0.05$) were observed among the left and right testicular weights as shown in Table 8. T_4, 36.00 g being the lowest and T_2 recording the highest value of 64.10 g. The right testicular length significantly ($p<0.05$) differed among the treatments, T_4 recorded the lowest value 36.00 cm and T_2 recorded the highest value 64.10 cm for right testis. However, the left testicular length differed significantly with T_1 recording the value of 58.40 and T_4 had the value of 35.10

The photomicrograph of a section of the testis

The photomicrograph of the testis reveals normal structure for T_1, T_2, and T_3 but T_4 showed reduced number of spermatogenic cell layers in seminiferous tubules and scanty primary spermatids.

Table 1. Composition of the experimental diets (%)

Ingredients	Treatments			
	T_1	T_2	T_3	T_4
Cottonseed Cake	0	10	20	30
Groundnut Cake	30	20	10	0
Rice Bran	19	19	19	19
Maize Offal	20	20	20	20
Sorghum husk	10	10	10	10
Wheat Offal	20	20	20	20
Bone Meal	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**
Calculated ME (MJ/Kg DM)	9.77	9.47	9.01	9.19
Calculated CP (g Kg^{-1} DM)	165.00	164.00	161.00	158.00

CP = crude protein, ME = metabolized energy

Table 2. Chemical composition of experimental diets on dry matter (DM) (g kg^{-1} DM)

	DM	Ash	CP	EE	ADF	NDF	ADIA	CELL	HCELL	ADL	GE
T_1	942.50	89.50^{c}	354.00^{d}	87.00^{d}	341.30^{d}	441.00^{d}	78.10a	99.70^{c}	216.20^{d}	125.10^{a}	5286.50^{c}
T_2	934.00	103.00^{b}	372.00^{b}	112.00^{b}	352.60^{c}	463.40^{c}	69.30c	110.80^{ab}	256.30^{c}	96.30^{b}	5469.49^{b}
T_3	945.00	112.50^{a}	366.00^{c}	145.00^{a}	388.40^{a}	501.10^{a}	58.80d	112.70^{a}	299.50^{b}	88.90^{c}	5663.28^{a}
T_4	942.10	92.80^{c}	382.00^{a}	102.90^{c}	376.40^{b}	492.80^{b}	76.30a	116.40^{a}	304.10^{a}	72.30^{d}	5665.21^{a}
SEM	**1.26**	**2.11**	**0.97**	**1.02**	**0.87**	**0.21**	**0.62**	**3.21**	**0.76**	**0.24**	**0.78**

a, b, c= means within the same rows with different super scripts are significantly different. (P< 0.05); DM=Dry matter; CP=Crude protein; EE= Ether Extract; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; Acid detergent lignin; ADIA=Acid detergent insoluble ash; Cell.=Cellulose and Hemi cellulose; SEM= Standard Error of Means; GE=Gross Energy.

Table 3. Effect of cotton seed cake supplementation on gonadal sperm reserve ($x10^6$) of Red Sokoto bucks

Treatment					
Parameter	**T1**	**T2**	**T3**	**T4**	**SEM**
Right testis	2762.6^{a}	2610.8^{a}	2184.9^{b} 1	705.7^{c}	198.98
Left testis	2761.8^{a}	2620.9^{a}	2189.7^{b}	1710.9^{c}	199.99

abcMean within the same rows with different superscript are significantly different. (P<0.05).SEM = standard error of mean

Table 4. Effects of cotton seed cake supplementation on extra gonadal sperm reserved of Red Sokoto bucks (ml)

Treatment					
Parameter	**T1**	**T2**	**T3**	**T4**	**SEM**
Left caput epididymides	12.00	14.00	13.00	12.00	1.83
Right caput epididymides	14.00^{a}	13.00^{ab}	13.00^{ab}	12.00^{b}	1.53
Left carpus epididymides	8.90^{ab}	9.50^{a}	9.00^{ab}	8.40^{b}	0.60
Right carpusepididymides	12.00^{a}	11.20^{ab}	11.00^{ab}	10.00^{b}	1.52
Left caudaepididymides	13.00^{ab}	14.60^{a}	13.00^{ab}	11.50^{b}	1.50
Right caudaepididymides	13.00	14.30	13.00	12.30	2.20

Means within the same rows are not significantly different (P<0.05). SEM = Standard error of mean

Table 5. Effects of cotton seed cake supplementation on testicular morphometry of Red Sokoto bucks

Treatment					
Parameter	**T1**	**T2**	**T3**	**T4**	**SEM**
Right testicular weight (g)	59.60^{a}	64.10^{a}	45.00^{b}	36.00^{c}	2.21
Left testicular weight (g)	58.40^{a}	58.20^{a}	46.30^{b}	35.10^{c}	0.22
Right testicular length (cm)	59.60^{b}	64.10^{a}	45.00^{c}	36.00^{d}	1.56
Left testicular length (cm)	58.40^{a}	58.20^{a}	46.30^{b}	35.10^{c}	0.15

abcd means within the same rows with different super scripts are significantly different (P < 0.05).SEM = Standard error of mean.

Fig 1. The photomicrograph of a section of the testis of a buck fed 0% cotton seed cake showed, prominent spermatogenic cells in normal layers.Intact seminiferous tubules.A large number of primary spermatids towards the lumen of primary spermatids. Intact intertubular spaces (H and E X 400).

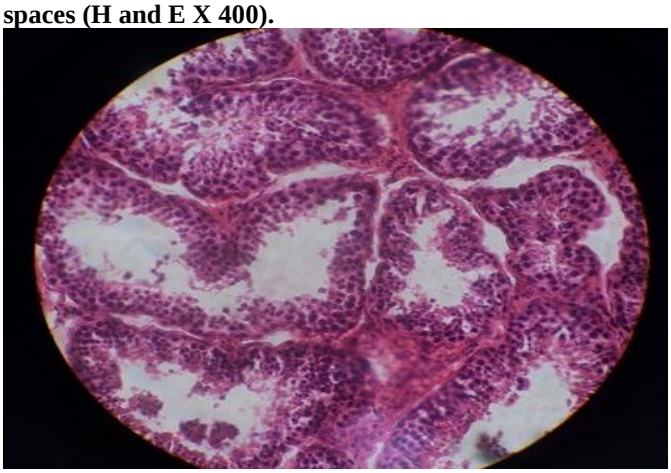


Fig 2.The photomicrograph of a section of a testis of a buck fed 10% cotton seed cake showed normal cells layer of spermatogenic cells. Moderate numbers of primary spermatids. Intact intertubular spaces and also moderate number of spermtogenic cells (H and E x400).

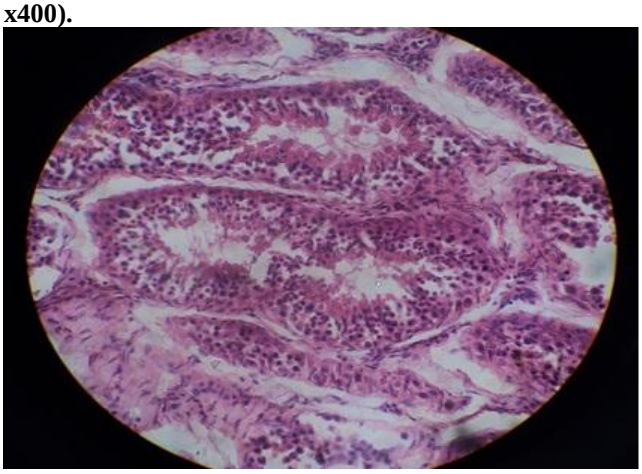
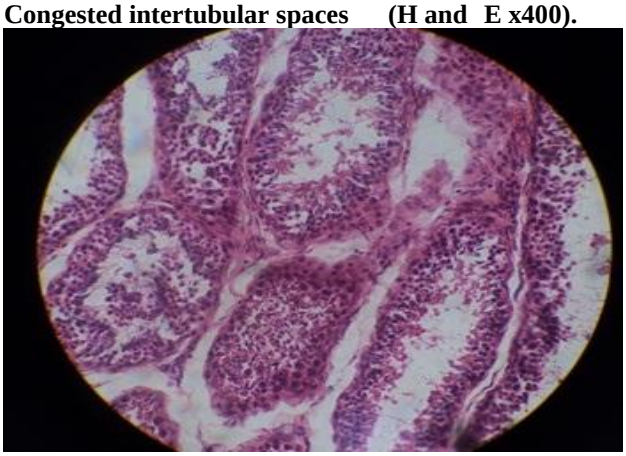


Fig 3. The photomicrograph of a section of the testis of a buck fed 20% cotton seed cake showing reduced number of spermatogenic cells layers.Moderate numbers of primary spermatids. Intact seminiferous tubules and intratubular spaces (H and E x400).

Fig 4. The photomicrograph of a section of the testis of a buck fed 30% cotton seed cake showed congested testis. Reduced number of spermatogenic cells layers in seminiferous tubules. Scanty primary spermatids. Congested intertubular spaces (H and E x400).



DISCUSSION

The chemical composition of experimental diets is shown in Table 1.The crude protein (CP) content of cotton seed cake studied was generally higher. The (CP) content ranging from 354.00 to 382.00 kg $^{-1}$ DM is above 7% CP requirement for ruminants that should provide the ammonia required by rumen micro-organism to support the optimum microbial growth. Brown *et al* [15] and Bonsi*et al* [16] justified the use of cotton seed cake in small quantities especially for the animals in the breeding stock in order to supplement poor pasture and crop residue. High CP content of cotton seed cake is well documented raging from 35 to 46%. Brown *et al* [15] gave the minimum value of 22%. Generally the CP content of cotton seed cake has been shown to be above the minimum level required (7%) for microbial activities in the rumen (Brown 2009). Cotton plants have a higher protein content compared to other species, although species in *G barbadense* family have an average of 35% more protein than species of *G hirsutum.* Bonsi*et al* [16]also noted that all family of cotton plants is able at all their phonological stage to meet the protein and energy requirements of livestock at maintenance, production and lactating level. The difference in CP can be explained by inherent characteristics of each species in relation to its ability to extract and accumulate nutrients from the soil. The EE content ranging from 87.00 to 145.00 g kg $^{-1}$ DM. Zahid [17] reported that the protein content of cotton seed cake is depend on the efficiency of oil extraction method. The crude fibre (CF) content of the experimental diets

studied is 76.00 to 86.00 g kg $^{-1}$ DM. Obrient [18] reported that a ruminant requires at least 17% CF for normal physiological function. With regard to fibre content Zahid [17] reported similar mean for NDF and ADF as fallows 441.00 to 501.10 g kg $^{-1}$ DM and 341.30 to 388.40 g kg^{-1} DM. Cellulose content in the present study ranged from 99.70 to 116.40 g kg $^{-1}$ DM. The cell wall content of hemicelluloses was observed to be fairly high with the mean value of 216.20 to 301.10 g kg $^{-1}$ DM.

The gonadal sperm reserve ($X10^6$) were significantly different among the four treatments ($P<0.05$). T_1 had the highest value of sperm count 2762.6 $X10^6$ and T_4 had the lowest value of sperm count 1705.7 $X10^6$ right testis. This was probably due to the facts that cotton seed cake supplementation significantly affect the spermatogenesis. Raji*et al* [19] reported that the anti nutritional factors in the cotton seed is positively correlated with rainfall and negatively correlated with temperature, this means that the end product of oil seed extraction would have high amount of gossypol when cotton seed from temperate environment is used and the reverse is the case. Therefore, the sperm concentrations in left testis were also found significantly different ($p<0.05$) among the four treatments. Batista *et al* [20] reported the same value with the present study 2762.6, 2761.8 right and left testis.

The result of investigation in to the extra gonadal sperm reserve of the present study shows no significant differences among the treatments ($P > 0.05$). Both left and right epididymides volumes were not significant different, the caput, corpus and caudaepididymides were also not significantly different among the treatments. Gundogan [21] reported that the epididymides homogenate and filtrate volumes were statistically different ($p<0.05$) in Red Sokoto bucks. The difference noted was the tissues content. Rogers *et al* reported the same values as in the present study. T_2 recorded the highest value of 14.30 ml and T_4 having the lowest value of 1.30 ml right caudal epididymides

The study revealed that the testicular weight was found to be significantly different ($p< 0.05$) among the treatments studied, T2 recorded the highest value of 64.10 g and T4 had the lowest value of 36.00 g, this was probably due to the fact that cotton seed cake supplementation had a significant effects on testicular morphometry as observed in this study, while the testicular length values ranged from 64.10 to 36.00 cm. T_2 having the highest testicular length while T_4 had the lowest testicular length. Raji*et al* [19] Reported high values of left testicular weight and length of 68.00 g and 65.00 cm.

Histological studies as presented in figures 1 to 4, reveals some features of the testis under the four treatments. In all the slides the plates reveals normal structures as described by Kessler [22]. These included the seminiferous tubules, prominent spermatogenic cells, primary spermatid in the lumen. This findings show that inclusion up to 10% and 20% of cotton seed cake in the diets of the bucks have no deleterious effect on the histology of the testes of the bucks, but higher levels (20%) show histological changes.

CONLUSION

Based on the result of this research, it is recommended that animals should be given heat dehulled cotton seed byproduct at 10%and 20% inclusion level.

Animals should be given the diets with fibre supplementation at least 17% to meet the ruminant fibre requirements. The study reveals that cotton seed supplementation shows effect on seminal characteristics 30% inclusion level therefore animals for meat production can be fed since the result shows positive correlation between intake and body performance. Further studies should be done to determine the effects of cotton seed cake supplementation on haemotological parameters and serum Biochemistry of Red Sokoto bucks at 40% and 50% inclusion level.

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