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Full Length Research

Semen characteristics of rabbit bucks fed cottonseed cake diets supplemented with sausage fruits (*Kigelia africana*)

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ABSTRACT: This study was conducted to investigate the effect of sausage fruits (*Kigelia africana)* on semen characteristics of rabbit bucks fed cottonseed cake (CSC) based diets. Sixty (60) rabbit bucks, 7-8 weeks old with an average weight of 868.52 g of heterogeneous population were randomly assigned to five dietary treatments with twelve animals per treatment in a Completely Randomized Design (CRD) experiment. The CSC replaced soybean meal (SBM) at 0% (T1) and 100% in the other four treatments. Treatment two (T2) was CSC without *Kigelia africana* supplementation. Treatment three (T3) was supplemented with powder of *Kigelia africana* fruits at 100 g/100kg feed. Treatment four (T4) received an aqueous extract of *Kigelia africana* fruits supplementation at 100 mg/bodyweight). Treatment five (T5) was a combined blend mixture of powder and extract of K*igelia africana* fruits at 100 mg/body weight. Animals were fed for 14 weeks after which semen was collected through artificial virginal techniques for evaluation. Parameters evaluated were sperm count, semen volume, semen colour, live sperm, dead sperm, abnormal sperm, sperm morphology, gonadal sperm reserve (GSR), extra-gonadal sperm reserve (EGSR), and daily sperm production (DSP). Results showed that rabbit bucks fed T2 had significantly (p≤0.05) lower sperm count and sperm volume whereas bucks fed T3 revealed a significantly (p≤0.05) higher sperm count and sperm volume. Detached head, mid-piece and curve tail were significantly (p≤0.05) higher in T2 while lower (p≤0.05) in supplemented treatment groups. It was concluded that powder of *kigelia africana* fruits supplementation to CSC diets ameliorated the adverse effect of CSC-gossypol in rabbits buck than extract and blend mixture of *Kigelia africana* fruits. Rabbit bucks intended for breeding purposes should not be fed CSC unless supplemented with *Kigelia africana* fruits.

Keywords: Cottonseed cake, Gossypol, Kigelia *africana* fruits, rabbit bucks, sperm characteristics.

INTRODUCTION

Cottonseed cake (CSC) is one of such conventional feed ingredients that have not been fully exploited on non-ruminants despite its good source of protein and fibre in animal diets; however, its utilization is widely known to feed various classes of ruminants (Amao *et al.,* 2014). Its use is limited to ruminant feeding due to the presence of gossypol, a polyphenolic compound with significant physiological implications (Amao and Akanbi, 2017). Gossypol is a phenolic compound produced by the cotton plant and it is highly reactive, binding rapidly to different substances, including minerals and amino acids. Gossypol appears to exert unique and selective effects upon the male reproductive system causing infertility by inhibiting spermatogenesis, damaging the basement membrane of spermatogenic tubules (Carruthers *et al*., 2007). Several attempts have been made to solve this problem, researchers studied for many years to find ways of either reducing or inactivating free gossypol and propose a few methods such as solvent extraction by liquid acetone (Gadner *et al.,* 1976), addition of iron salts to cottonseed meal-based diets to bind gossypol (Tabatabai *et al.,* 2002), use of calcium hydroxide (Nagalakshmi *et al.,* 2003).

Unfortunately, some of these methods affected the protein quality and were inefficient in blocking the absorption of free gossypol and increased the cost of input. Also, the breeding of plant types with lower levels of gossypol has been investigated (Sunilkumar *et al*., 2006). Therefore, this study aimed to evaluate the ameliorative effect of *Kigelia africana* fruits on semen characteristics of rabbit bucks fed cottonseed cake-based diets.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the rabbit research and production unit of the teaching and research farm, Osun State University, College of Agricultural Sciences and Management Ejigbo, Campus, Ejigbo.

Preparation of CSC and *K. africana* fruits

Cottonseed cake (CSC) was utilized as substrate and obtained from a cotton milling industry in Kano, northern Nigeria. The material was sundried and milled. The initial moisture content was determined by drying until constant weight at 110°C in the hot-air oven. The matured ripe fruits of *K. africana* were collected based on ethno-pharmacological information from the forest in Oko-Ara town area, Osun State, southwestern Nigeria. The matured *K. africana* fruits were harvested, washed, peeled, cut into small pieces; sun-dried (≥90%DM), then ground into fine powder particles (≤2mm) using a grinding machine. Aqueous extraction was performed according to the method of Gwatido *et al.* (2018) with minor modifications. Table 1 showed gross composition of experimental diets and calculated nutrients. Five concentrate diets were compounded with T1- Control (SBM-based diet), T2- CSC without *K. africana*, T3- CSC with *K. africana* powder, T4- CSC with *K. africana* extract, T5- CSC with K. *africana* fruits powder and extract.

Animals and management

Sixty (60) weaned rabbit bucks of a heterogeneous population aged between 6-8 weeks old were obtained from a reputable breeding farm. The animals were randomly assigned to four (5) dietary treatments of 12 replicates, each buck serving as replicates in a completely randomized design. The rabbits were acclimatized for three weeks during which a soybean meal-based diet, water-based anti-stress and antibiotics were given. Feed and clean drinking water were supplied *ad-libitum.*

Reproductive characteristics

At the end of the feeding trial, 3 animals in each treatment were randomly selected and slaughtered and reproductive organs (testis and epididymis) were carefully dissected for evaluation. Data were obtained for the following parameters: testicular sperm count, semen volume, semen colour, semen motility, dead sperm, abnormal sperm, sperm morphology, Gonadal Sperm Reserve (GSR), Extra-Gonadal Sperm Reserve (EGSR), Gonadal Sperm Production (GSP), Gonadal Sperm Production (GSP) and Gonadal Sperm Reserve (GSR)

Gonadal sperm reserve and gonadal sperm production were determined haemocytometrically by homogenate technique using a modification of the method of Amao *et al.* (2012). The tunica albuginea was removed from the testis and the testicular parenchyma weighed. A portion of the parenchyma tissue was taken and homogenized by maceration with a pair of sharp scissors for about 5 minutes in a beaker containing 10 ml physiological saline solution. The homogenate was filtered through a double layer of cheesecloth and the filtrate was diluted to a ratio of 1:20 with deionized water. Some drops of the homogenate were introduced into an improved Neubauer haemocytometer counting chamber. All the elongated spermatids and mature sperm cells in the four diagonal and centre squares of the haemocytometer were counted in each diluted homogenate. The concentration of the sperm cells per gram of testis parenchyma was calculated as follows.

$$Conc. per\ gram = \frac{No\ of\ sperm\ cells\ x\ volume\ used}{Weight\ of\ the\ sample}$$

Gonadal sperm reserve was calculated as; Concentration/gram testis X total weight of right testis.

Daily Sperm Production (DSP) was determined from gonadal sperm reserve (GSR) using the formula proposed by Amann (1970).

$$DSP = \frac{Testis\ Sperm\ Count\ (GSR)}{Time\ divisor}$$

The value of the time divisor for rabbits = 3.43 (Amann, 1970)

Daily sperm production per gram parenchyma (testis) per animal was estimated by the formula:

$$DSP/g\ parenchyma = \frac{Gonadal\ Sperm\ Reserve}{GTW\ -\ TAW\ \ X\ 3.43}$$

Where: GTW = Gross testis weight and TAW= Tunica albuginea weight.

Extra-Gonadal Sperm Reserve (EGSR)

Samples of cauda epididymis were homogenized separately in normal saline solution at 100 mg/ml of the

Table 1. Gross composition of experimental diets and calculated nutrients.

Ingredients (%)	T1	T2	T3	T4	T5
Maize	43.57	43.24	43.23	43.24	43.24
Soybean meal	20. 43	-	-	-	-
Cottonseed cake	-	20.76	20.76	20.76	20.76
Rice husk	21.00	21.00	21.00	21.00	21.00
BDG	10.00	10.00	10.00	10.00	10.00
Fish meal 72%)	2.00	2.00	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100
Calculated Nutrient					
Crude protein (%)	16.34	15.72	15.72	15.72	15.72
Crude Fibre (%)	10.22	11.57	11.57	11.57	11.57
ME (Kcal/kg)	2582	2436	2436	2436	2436
Lysine	0.86	0.64	0.64	0.64	0.64
Methione	0.27	0.25	0.25	0.25	0.25

*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D3,2500 IU; vitamin E, 50.00k3, 2.50mg; vitamin B1, 3.00mg; vitamin B2, 6.00mg; vitamin B6. 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotion, 0.08mg; vitamin B12,0.26mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg; zinc, 45mg; copper, 2.00mg; meal. BDG- Brewer's dried grain, ME- Metabolizable Energy, T1- Control (SBM-based diet), T2- Cotton seed cake without *K. africana*, T3- Cotton seed cake with *K. africana* powder, T4- Cotton seed cake with *K. africana* extract, T5- CSC with K. *africana* fruits powder and extract.

saline solution and then filtered through a double-layer cheesecloth. The resulting homogenate was diluted with deionized water at 25 x dilution factor. The sperm count was determined using the improved Neubauer hemocytometer (Adejumo, 2006).

Sperm morphology and analysis

Sperm morphology was determined according to the method of Zemjanis (1970) modified by Raji and Hart (2012). The epididymis was carefully separated from the left testis and the cauda was severed from its remaining part. A smear of the semen samples was obtained by laceration and rubbing the cut surface on a clear glass slide. Sperm motility was determined immediately. Two drops of eosin-nigrosin dye that had been thoroughly mixed were added. A smear was made on another slide and viewed under a light microscope to identify normal and abnormal cells from several fields on the slides. The normal cells were then expressed as the percentage of the number of cells counted on each field of the slide. Dead cells were also identified and recorded. Sperm count was determined under a microscope with the aid of the improved Neubauer hemocytometer.

Statistical analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using SAS (2000). Means were separated by Duncan's Multiple Range option of the same statistical package.

RESULTS

Sperm characteristics of the rabbit buck fed CSC with or without K. *africana* supplementation are presented in Table 2. Sperm count, sperm volume, sperm colour, sperm motility, non-motile sperm, detached head, mid-piece, curve tail, and EGSR were significantly ($p<0.05$) affected by dietary treatment. Abnormal sperm, bent tail, dead sperm, GSR and DSP were not significantly ($p>0.05$) affected by dietary treatments. Sperm count, semen volume, motile sperm, detached head, mid-piece, curve tail and EGSR of the bucks fed T2 were significantly ($p<0.05$) lower meanwhile significantly ($p<0.05$) higher for bucks in T3 and T5 and the increased value observed for T5 was statistical similar with T2. The observed semen colour in bucks fed T2 was watery but changed to creamy in T3 and T5. The increased value observed in T5 was

Table 2. Semen quality assessment of rabbits fed cotton seed cake-based diet supplemented with or without *K. africana* fruits.

Parameters	T1	T2	T3	T4	T5	SEM
Sperm count ($x10^6$/ml)	22.00^b	5.00^c	25.33^a	22.00^b	23.05^{ab}	1.77
Sperm volume (ml)	0.98^c	0.99^c	2.20^a	1.40^b	1.70^{ab}	0.11
Semen colour	Milky	Watery	Creamy	Milky	Creamy	-
Motile sperm (%)	78.17^{ab}	71.73^c	73.20^b	79.97^a	80.12^a	1.77
Non-motile sperm (%)	21.83^b	28.27^a	26.80^a	20.03^b	19.88^{ab}	1.77
Abnormal sperm (%)	15.83	18.54	14.12	15.33	15.50	1.47
Detached head (%)	3.20^{bc}	4.24^a	3.02^c	3.50^b	3.25^{bc}	0.20
Mid-piece (%)	2.45^d	5.18^a	3.00^{cd}	4.45^b	3.05^c	0.53
Curve tail (%)	6.10^a	5.10^a	4.02^b	3.88^c	6.08^{ab}	1.04
Bent tail (%)	4.08	4.02	4.08	4.13	3.12	0.17
Dead sperm (%)	6.10	10.00	4.02	4.87	4.38	0.52
GSR ($x10^6$)	52.99	48.17	48.73	66.44	63.79	3.83
EGSR ($x10^6$)	63.25^c	14.05^d	91.18^b	109.38^a	91.68^{ab}	7.47
DSP ($x10^6$)	10.07	10.01	13.95	12.51	12.84	1.12

^{abcd} Means with different superscript along the same row are significantly different (P<0.05). SEM-Standard Error of Mean, T1- Control (SBM-based diet), T2- Cotton seed cake without *K. africana*, T3- Cotton seed cake with *K. africana* powder, T4- Cotton seed cake with *K. africana* extract, T5- Cotton seed cake with *K.* africana powder and extract extract GSR- Gonadal Sperm Reserve, EGSR- Extra Gonadal Sperm, DSP-Daily Sperm Production.

because of the combined effect of *K. africana* fruits (powder and extract) which had similar statistical values (p>0.05) as the T3. The observed semen colour in bucks fed CSC without supplementation was watery but changed to creamy and milky in supplemented groups.

DISCUSSION

The observed CSC diet supplemented with powder K. *africana* fruits tended to increase sperm count more than extract *K. africana* supplementation. The observed increase for bucks fed combined blend-mixture of CSC with powder and extract *K. africana* fruits is an indication of synergistic action possibly to pave the way for unhindered spermatogenesis that resulted in higher sperm count. Feeding CSC diets without supplementation has been reported to cause infertility in non-ruminants (Carruthers *et al*., 2007). This suggests that *K. africana* fruits might have played an important role in the metabolism of CSC-gossypol. *K. africana* fruits possess a number of antioxidants such as flavonoids, phenolic compounds, terpenoids etc. They are very scavenging in nature. The antioxidant activities of *K. africana* fruits were to act by scavenging free radicals produced by CSC-gossypol thereby reducing reactive oxygen species (ROS) which damage testicular cells and tissues (Nabatanzi*et al*., 2020). The mechanism action of gossypol toxicity was the generation of free radicals which are the deleterious by-product of metabolism. Possibly, the scavenging activities of *K. africana* worked against the activities of gossypol on the seminiferous tubules of the bucks. *K. africana* is rich in bioflavonoids, saponins and tannins. Phytochemical studies on *K. africana* fruit extract secondary metabolites revealed the presence of saponins which might be contributory to increasing the endogenous testosterone levels (Oyetayo *et al.,* 2007) by raising the level of luteinizing hormone. This observation is consistent with the report of Koné *et al,* (2021) that *K. africana* fruits increased sperm number in mice. Significant reduction observed for motile sperm in bucks fed CSC diets without supplementation could be associated with a strong effect of gossypol in the diets on sperm cells which could not be neutralized. A similar observation was reported by Arshasmi and Ruttle (1998) who reported a decrease in the number and motility of spermatozoa for bull-fed CSC without supplementation. The significantly lower detached head, mid-piece and curve tail observed in bucks fed CSC without *K. africana* fruit supplementation could be associated with the effect of gossypol in the CSC diets which might have caused some damage to the germinal epithelium of the seminiferous tubules leading to lower concentration of cytoplasmic defensive mechanism enzymes against lipid peroxidation in the late spermiogenesis (Fisher, 1994). However, bucks fed CSC with *K. africana* fruit supplementation possibly counteracted the reduction by inhibiting the damage that might have been caused by reactive oxygen species (ROS). Moreover, *K. africana* fruits might have inhibited possible destruction by gossypol to the germinal epithelial layer of seminiferous tubules, demonstrating its antioxidant potential by capturing the oxygen-free radicals that have been generated by gossypol, thereby enhancing spermatogenesis.

Conclusion

Based on this study, it was concluded that the CSC diet supplemented with *Kigelia africana* fruit powder could enhance sperm count and the CSC diet supplemented with K. *africana* fruit extract could improve sperm motility and morphology of rabbit bucks without adverse effects. Rabbit bucks intended for breeding purposes should not be fed CSC unless supplemented with *Kigelia africana* fruits.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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