SEMEN CHARACTERISTICS OF RABBIT BUCKS FED COTTONSEED CAKE DIETS SUPPLEMENTED WITH SUSSAGE FRUITS (*KIGELIA AFRICANA*)

***Akanbi, O. J. Ajayi, B. A. and Osunkeye, O. J.**
Department of Animal Science, Faculty of Agricultural Production and Management, College of Agriculture, Ejigbo campus, Osun State University, Osogbo, Nigeria
*Corresponding author's email: mayowakanbi@gmail.com

ABSTRACT
A study was conducted to investigate the impact of feeding sausage fruits (Kigelia africana) on semen characteristics of rabbit bucks fed cottonseed cake (CSC)-based diets. Sixty rabbit bucks were randomly assigned to five dietary treatments: T1 (0% CSC), T2 (100% CSC), T3 (CSC + 100g/100kg Kigelia africana powder), T4 (CSC + 100mg/bodyweight Kigelia africana extract), and T5 (CSC + powder and extract). After 14 weeks of feeding, semen evaluation was conducted. T2 bucks showed significantly lower sperm count and volume but higher abnormalities. Supplemented groups exhibited improvements in semen parameters. Conclusion: CSC negatively affected semen characteristics, mitigated by Kigelia africana supplementation. Rabbits intended for breeding should avoid CSC unless supplemented with Kigelia africana fruits..

Keywords: Rabbits, Cottonseed cake, Kigelia *africana* fruits, sperm characteristics, supplementation.

INTRODUCTION

Cottonseed cake (CSC) is one of such conventional feed ingredients that have not been used fully exploit on non- ruminants despite its good source of protein and fiber in animal diets however its utilization is widely known to feed various class ruminants (Amao *et al.,* 2014). Its use is limited to ruminant feeding due to the presence of gossypol, a polyphenolic compound of significant physiological implication (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, damage the basement membrane of spermatogenic tubules (Carruters *et al*., 2007). Several attempts have been made to solve this problem, researchers studied for many years to find way of either reducing of inactivating free gossypol or 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 to block absorption of free gossypol and increased the cost of input. Also breeding of plant types with lower level of gossypol have also been investigated (Sunikumar *et al*., 2006). This study aimed at evaluating 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 Kigelia africana fruits

Cottonseed cake (CSC) w utilized as substrate and obtained from a cotton milling industry in Kano, Nigeria. The material was sundried, milled and the initial moisture content was determined by drying until constant weight at 110^0C in hot-air oven. The matured ripe fruits of *Kigelia africana* were collected based on ethno-pharmacological information from the forest in Oko-Ara town area, Osun State. The matured *Kigelia africana* fruits were harvested, washed, peeled, cut into small pieces; sun-dried into a constant weight then ground into fine powder particles pa using a grinding machine. Aqueous extraction was performed according to the method of Gwatido *et al.* (2018) with minor modification. Four concentrate diets were compounded with T1- Control (SBM-based diet), T2-

Cotton seed cake without *K. africana*, T3- Cotton seed cake with *Kigelia africana* powder, T4- Cotton seed cake with *Kigelia africana* extract, T5- CSC with *Kigelia africana* fruits powder and extract.

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
Brewer's dried grains	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
Common 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 Kigelia africana powder, T4- Cotton seed cake with Kigelia africana extract, T5- CSC with Kigelia africana fruits powder and extract.

Animal and management

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

Reproductive characteristics

At the end of feeding trial, 3 animals per treatment were randomly selected and slaughtered and reproductive organs (testis and epididymis) were carefully dissected out 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 cheese cloth and the filtrate diluted to ratio 1:20 with de-ionized water. Some drops of the homogenate were introduced into an improved Neubauer haemocytometer counting chamber. All the elongated spermatids and nature sperm cells in the four diagonal and the center 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.

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

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

Daily Sperm Production (DSP)

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

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

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

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

$$DSP/g\ \text{parenchyma} = \frac{\text{Gonadal Sperm Reserve}}{\text{Gross testis weight} - \text{Tunica albuginea weight}} \times 3.43$$

Extra – Gonadal Sperm Reserve (EGSR)

Samples of cauda epididymis were homogenized separately in normal saline solution at 100mg/ml of the saline solution and then filtered through a double layer cheese cloth. The resulting homogenate was diluted with de-ionized water at 25 x dilution factor. The sperm count was determined using the improved Neubauer hemocytometer (Adejumo, 2007).

Sperm morphology and Analysis

Sperm morphology was determined according to the method Zemjanis of (1970) modified by Raji and Hart (2012). The epididymis was carefully separated from the left testis and the cauda 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-nigrosine dye that had been thoroughly mixed was 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 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 *Kigelia africana* supplementation is presented in the 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 CSC diet without supplementation of *Kigelia africana* fruits were significantly ($P<0.05$) lower in non-supplemented treatments but significantly ($P<0.05$) higher in supplemented groups. Increased values observed in T5 was as a result of combined effect of *Kigelia 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

Antioxidants like those found in Kigelia africana scavenge reactive oxygen species (ROS), countering the deleterious effects of free radicals, such as those generated by CSC-gossypol toxicity (Alice Nabatanzi *et al*., 2020). The presence of bioflavonoids, saponins, and tannins in Kigelia africana contributes to its antioxidant properties. Saponins may elevate testosterone levels by increasing luteinizing hormone (Oyetayo *et a*l., 2007). Supplementation with Kigelia africana powder tended to increase sperm count more than extract supplementation, possibly due to synergistic action promoting unhindered spermatogenesis. Similar effects were observed in mice (Kone *et al.*, 2021). Bucks fed CSC without supplementation showed reduced motile sperm, likely due to unmitigated gossypol effects, as reported in bulls. Lower sperm abnormalities in supplemented bucks suggest Kigelia africana's protective role against gossypol-induced damage to seminiferous tubules, possibly by inhibiting ROS damage and preserving germinal epithelial integrity. This underscores its antioxidant

potential in enhancing spermatogenesis by neutralizing oxygen free radicals generated by gossypol (Arshami and Ruttle, 1998; Fisher, 1994).

Table 2: Semen quality assessment of rabbits fed cotton seed cake-based diet supplemented with or without *Kigelia 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. *africana*, T3- Cotton seed cake with *Kigelia africana* powder, T4- Cotton seed cake with *Kigelia africana* extract, T5- Cotton seed cake with *Kigelia africana* powder and extract extract GSR- Gonadal Sperm Reserve, EGSR- Extra Gonadal Sperm, DSP- Daily Sperm Production.

CONCLUSION

It was concluded that, CSC diet supplemented with *Kigelia africana* fruits powder could enhance sperm count and CSC diet supplemented with *Kigelia africana* fruits extract could improve sperm motility and morphology of rabbit bucks without adverse effect. Rabbits bucks intended for breeding purpose should not be fed CSC unless supplemented with *Kigelia Africana*.

REFERENCES

Adejumo, D.O, (2006). The effect of supplementing cocoa husks on testicular characteristics of pigs. *Nigerian Journal of Animal Production, 33(1): 151-156*

Alice Nabatanzi, Sanah M. Nkadimeng, N. L, John. D., K. and, Lyndy J. M, (2020). Antioxidant and anti-inflammatory activities of *Kigelia africana* (Lam.) benth.. Evidence based complementary and alternative medicine. Ecam, 2020:4352084.

Amann,R.P, (1970). In D.O., Jounson, W.R..G and N.L van Demark (Eds). The sperm production rates. Vol.1.

Amao, O. A., Adejumo, D. O., Togun, V.A. and Oseni, B. S. A. (2012). Physiological response of rabbit bucks to prolonged feeding of cotton seed cake-based diets supplemented with vitamin E. *African Journal of Biotechnology*, 11(22): 6197-6206.

Amao, O. A., Adejumo, D. O., Togun, V.A. and Oseni, B. S. A. (2012). Physiological response of rabbit bucks to prolonged feeding of cotton seed cake-based diets supplemented with vitamin E. *African Journal of Biotechnology,* 11(22): 6197-6206

Arshasmi, J, and Ruttle J.L, (1988). The effect if diets containing gossypol on spermatogenic tissues of young bulls..Theriogenology, 30(3), 507-516.

Gwatido, L, Dzomba P, and Mangena M, (2018). TLC separation and antioxidant activities of flavonoid from Carissa, bisopinasa, Ficus syscomorus and gwewia bicolor fruits. *Nutrire* volume 43 article number 3.

Koné, M.C., Kpan, B.W., Kandé, I., Kouakou, R.K., Koman, R.S., Konan, Y. and Kouakou, K. (2021) Effect of Different Parts of Kigelia africana Fruit Aqueous Extracts on Sperm Parameters and Testis. *Advances in Reproductive Sciences*, 9, 171-188.

Nagalakshmi, D. Sastry, V.R.B and Pawde, A. (2003). Rumen fermentation pattern and nutrient digestion in lambs fed cottonseed meal supplemental diets *Animal Feed Science and Technology*; 103;1-4.

Oyetayo, F.L., Oyetayo, V. O, Ajewole, V. (2007). Phytochemical profile and antibacterial properties of the seed and leaf of the Luffa plant (Luffa cylindrical). *Journal of Pharmacological Toxicology,* 2:586-589.

Raji, Yand Hart, V.O, (2012). Influence of prolounging exposure. *Nigerian Journal Physiological Science* 27: 055-063.

SAS Institute, (2000). SAS user guide: statistics, version 9 edition SAS institute, Inc., cary, NC. USA.

Sunilkumar, G Campbell, L.M, Puckhaber, L. Stipanovic, R.D and Rathore, K.S, (2006). Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *Proceeding Nat. Acad Sci.* 103, 18054-18059.

Tabatabai, F. Goilan, A and Salarmoeini, M (2002). Determination and detoxification methods of

Zemjanis, R. (1970). Collection and evaluation of semen. In: Diagnostic and therapeutic techniques in animal production 2nd edition, willams and Wilkins Co. Baltimore. M.D pp. 139-156.