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Reproductive Characteristics of Rabbit Bucks Fed Diet Containing Raw or Fermented Cottonseed Cake

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Authors' contributions

This work was conducted in collaboration between both authors. Author OAA designed the experiment, coordinated and monitored the data collection, performed the statistical analysis, extracted the tables and interpreted the data. Author KAS did data collection, managed the literature search and wrote the first draft of the manuscript. Both authors read, edited and approved the final manuscript.

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ABSTRACT

This study was conducted to investigate the effect of diets containing raw or fermented cottonseed cake on reproductive characteristics of rabbit bucks. Eighteen (18) weaned crossbred (New Zealand White X Chinchilla) rabbit bucks, 6-7 weeks old, were randomly allotted to three treatments with six animals per treatment in a Completely Randomized Designed experiment. The treatment diets were T1, (Control) containing soyabean meal (SBM) as the main protein source; T2, Raw Cottonseed Cake (RCSC) - based diet and T3, Fermented Cottonseed Cake (FCSC) - based diet. The fermentation was done by inoculation with *Aspergillus niger*. Animals were acclimatized for one week. The feeding trial lasted for 9 weeks. At the end of this trial, 3 bucks per treatment were slaughtered and the reproductive organs dissected out for testicular and epididymal morphometrics as well as gonadal sperm assessment. Testicular morphometrics were not significantly (P>0.05) affected by the treatment. Treatment had significant (P<0.05) effect on the

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epididymal weight and length of rabbit buck. Testicular sperm count for the RCSC group ($124.67x10^6$) was significantly ($P<0.05$) lower than that of the control ($182.22x10^6$). Bucks on FCSC diet had comparable sperm count ($181.50x10^6$) with the control group. The proportion of motile sperm was significantly ($P<0.05$) increased from 48% in the control to 66.33% in the FCSC group. It was concluded that raw CSC had adverse effect on the sperm characteristics of rabbit bucks. However fermentation, using *Aspergillus niger* mitigated the adverse effect of CSC on the sperm characteristics. It is recommended that CSC to be included in diet of rabbit bucks meant for breeding purpose should be fermented by *Aspergillus niger.*

Keywords: Reproductive characteristics; Rabbit buck; cottonseed cake; fermentation.

1. INTRODUCTION

Rabbits have been shown to possess potentials to bridge the gap in the supply of animal protein to humans, especially in the developing and underdeveloped world. They are efficient converter of feed to meat and can utilize up to 30% crude fibre as against 10% by most poultry species [1]. Although rabbit can be raised on forage-based diet, the poor and unbalanced quality of grasses, as well as seasonal availability of the forage are major constraints limiting the successful production of rabbit for consumption. Cheeke and Raharjo [2] concluded in a review of rabbit production on tropical feed sources that tropical grasses were unsuitable as the sole feed for rabbits due to the low digestibility (less than 10%) of the forage. There is a serious shortage in the supply of convectional feed ingredients such as soybean meal, maize and groundnut cake for concentrate diet for rabbit. In addition, production of grains in developing countries is mostly for human consumption, leading to very high prices of the ingredients [3]. The utilization of unconventional feed resources holds great relevance to developing countries, where the main constraint to livestock production is the scarcity and fluctuation of the quality and quantity of all-year-round animal feed supply. The shortage of forages during the dry season, coupled with man's competition with animals for conventional feed ingredients has greatly reduced the availability of animal protein for human consumption. Hence, it becomes necessary for animal producers and nutritionists to source for means of replacing or supplementing the conventional ingredients with the unconventional ones.

Over the years, groundnut cake (GNC) and soybean (SBM) meal have remained the major protein sources in the diets of non-ruminant animals. These ingredients are also highly consumed by man, and as such, there is stiff competition between man and livestock for their consumption. Hence their prices are becoming more and more prohibitive. A possible alternative that is not popular for feeding non-ruminants is cottonseed cake (CSC). Cottonseed cake is a by-product from the extraction of oil from whole cottonseed. The CSC is an excellent source of protein, energy and fibre for a variety of livestock species [4].

The CSC is one of the industrial by-products not consumed directly by human beings and as such can reduce livestock feed cost [5]. The nutritional potential of CSC has been documented. It is rich in crude protein (35 to 46%) and contains over 1% phosphorus and 70 to 80% TDN [4,6]. However, it has not been fully exploited for monogastric feeding. This is because it contains gossypol, a polyphenolic compound that is very toxic to the monogastric animals [7].

Gossypol is a natural toxin present in the cotton plant to protect it from insects. Non-ruminant animals such as pigs, poultry and rabbits cannot tolerate much gossypol before toxicity signs develop [8]. Deleterious effects of gossypol-containing diets on various physiological processes in animal's body have been reported. Cottonseed cake has been reported to impact negatively on reproduction in various animal species such as cattle [9] and rabbit [7,10] Reproductive toxicity is seen particularly in males, where gossypol affects sperm motility, inhibits spermatogenesis and depresses sperm counts, causes sertoli cell toxicity and may also affect leydig cells [11]. The implication of this is overall poor quality semen with attendant poor reproductive efficiency.

Zhang et al. [12] reported that microbial fermentation could greatly reduce free gossypol level in cottonseed cake and detoxification efficiency deferred between species of micro organism used. *Aspergillus niger* was reported to be highly efficient for detoxification of gossypol

[12]. The precise mechanism of action of gossypol remains to be elucidated. The rabbits are considered as an excellent model for reproductive toxicological studies but only few studies on such effect of gossypol have been conducted on male rabbits [10]. This study was therefore conducted to investigate the effect of raw or fermented cottonseed cake on the reproductive characteristics of rabbit buck.

2. MATERIALS AND METHODS

2.1 Location of Experiment

The experiment was conducted at the Rabbit Research and Production Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo state Nigeria. Ogbomoso`s geographical attributes have been described by [13]. Ogbomoso is situated on Latitude 8° 15′ N and Longitude 4°15′ E. The mean annual rainfall is 1,247mm and the relative humidity is between 75% and 95%. The altitude is between 300 m and 600 m above the sea level. The town is located within the derived savanna zone of Nigeria.

2.2 Animals and Management

Eighteen (18) weaned crossbred (New Zealand White X Chinchilla) rabbit bucks, aged 6 - 7 weeks were used for this experiment. The rabbits were balanced for weight and allocated to three dietary treatments in a completely randomized design. Before the commencement of the experiment, animals were allowed to acclimatize for one week and were treated against endo- and ecto-parasites. Feed was supplied generously with an allowance of 120 g/animal/day. Clean, cool drinking water was available all the time. During the acclimatization period, animals were maintained on the control diet containing 16% crude protein and about 2500 kcal/kg (ME). Hutches were cleaned daily and refusals from the previous feeding were measured daily to determine feed intake. Rabbits were housed individually in wooden metabolic cages, each unit measuring 44 × 44 × 34 cm, with screened floors raised to a height 45 cm from the concrete floor. The screened floor permitted faeces and urine to fall out of the reach of the rabbits and were retained in the collecting tray at the base of the cage. This allowed for easy emptying of the tray. Feeding was done twice a day, 08:00h (8:00 am) and 16:00h (4:00 pm). Six rabbit bucks were allocated to each treatment, each rabbit constituting a replicate. They were fed test diets which were compounded to contain raw cottonseed cake (RCSC) and fermented cottonseed cake (FCSC) at 100% replacement for soya bean meal (SBM).

2.3 Isolation of Organisms and Preparation of Inoculum

Isolation of organism and preparation of inoculum followed the method of [14]. *Aspergilus niger* was isolated from CSC using potato dextrose agar (PDA) supplemented with 20% sucrose. Stock culture was maintained on media after serial dilution. The strain was bred and obtained from the research laboratory of Pure and Applied Biology, LAUTECH, Ogbomoso. Inoculum medium was sterilized and incubated at 30±1℃ for 48 hrs. Inoculum was developed by transferring a loopful of mycelium into inoculum medium (1% sucrose, 0.2% yeast extract, pH 5.50). The inoculated bottles were incubated at 30±1℃ on a rotary shaker at 100 rev/min for 24hrs. The harvested suspension was stored in the refrigerator until used.

2.4 Preparation of Solid Substrate

The initial moisture content of dried CSC was determined by drying to constant weight at 110℃ in a hot-air oven. Sterilization was carried out in a “fermenter” with 24 kg carrying capacity of the substrate. Moisture content was raised to 60%. The CSC with fermenter were sterilized by autoclaving at 121℃ for 3 hr and allowed to cool, inoculated with the inoculum and left to ferment for 7 days at 30±1℃. Prior to autoclaving, each tray containing the CSC was covered with muslin cloth and aluminum foil. The substrate was supplemented with sucrose and yeast extract to enhance microbial growth.

2.5 Preparation of Experimental Diets

Three treatment diets were compounded such that soybean meal (SBM) was the main protein source for the control diet denoted as Control. Diet 2 had the SBM completely replaced by Raw Cottonseed Cake (RCSC) while diet 3 had the SBM completely replaced with Fermented Cottonseed Cake (FCSC).

All diets were isonitrogenous and isocaloric, containing 16% crude protein (CP) and about 2500 kcal/kg ME. The gross composition of the experimental diets is shown in Table 1, while the proximate composition and gossypol level of test ingredients are presented in Table 2.

At the end of 9 weeks of feeding trial, three animals per treatment were randomly selected and slaughtered for reproductive organs evaluation.

2.6 Testicular and Epididymal Morphometry

After slaughtering, the epididymis was trimmed off the testis; the right and left testis were weighed using a digital scale. The length and the width of testis were also measured using a pair of vernier caliper. The volume of the testis was determined using water displacement of Archimede's principle using a measuring cylinder.

2.7 Sperm Analysis

Sperm morphology was determined according to the method of [15]. A smear of the semen was made by cutting the left testis along the equatorial region and rubbing the cut surface on a clean glass slide. 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 slide. The normal cells were then expressed as the percentage of number of cells counted on each field of the slide. Only the mature sperm cells were observed for normality and abnormality. Dead cells were also identified and recorded.

Sperm count was determined haemocytometrically by homogenization technique as described by [16] and [7]. The tunica albuginea was carefully removed from the testis and the testicular parenchyma was weighed. A portion of the parenchyma tissue was taken and homogenized by maceration with a pair of sharp scissors for 5 minutes in a beaker containing 10 ml of physiological saline solution. The homogenate was filtered through a double layer 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 mature sperm cells in the four diagonal and the centre squares of the haemocytometer were counted in each diluted homogenate. Motility was determined by a modification of the method of Ewuola and Egbunike (2010). A drop of the homogenate was placed on a sterile slide, covered with a cover slip and observed under the microscope at X 400 Magnification and scored between 0 and 100%.

Table 1. Gross composition and calculated nutrients of experiment diets

Ingredients (%)	T1 (SBM)	T2 (RCSC)	T3 (FCSC)
Maize	46.32	43.46	44.85
Soybean meal	17.68	-	-
Cottonseed cake	-	20.54	19.15
Rice husk	20.00	20.00	20.00
Wheat offal	10.00	10.00	10.00
Fishmeal (72%)	2.00	2.00	2.00
Oyster shell	2.00	2.00	2.00
Bone meal	1.25	1.25	1.25
Premix*	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Lysine	0.15	0.15	0.15
Methionine	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated nutrients			
Crude protein (%)	16.00	16.00	16.00
Crude Fiber (%)	8.95	12.88	12.91
ME (Kcal/kg)	2606.33	2457.20	2475.12

**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; iodine, 155mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg. SBM: soybean meal CSC: cottonseed based diets*

2.8 Statistical Analysis

All data generated were analyzed by one-way analysis of variance (ANOVA) using the General Linear Model (GLM) according to SAS [17]. Means were separated by Duncan's multiple range test of the same statistical software.

3. RESULTS

The results of this experiment are presented in Tables 3 to 5. Testicular characteristics of rabbit buck fed raw or fermented cottonseed cake are presented in Table 3. Testicular morphometrics were not significantly ($P>0.05$) different among treatments. However, rabbit buck fed FCSC-based diets tended to have higher values ($P>0.05$) than other treatment. Table 4 shows the epididymal characteristics of rabbit bucks fed diet containing raw or fermented cottonseed cake. Treatment had significant effect on the epididymal morphometrics. Bucks that fed on RCSC-based diet had significantly ($P<0.05$) higher values for left, right and mean epididymal weights than for bucks on other treatments.

Rabbit bucks fed with control diet had longest (P<0.05) epididymides (left, right and mean) compared to those on other treatments.

The results of testicular sperm characteristics are presented in Table 5. Rabbit bucks fed RCSC-based diet had significantly (P<0.05) lower sperm count than the control and FCSC. Morphologically normal sperm cells (50.67%) was also significantly (P<0.05) lower than that of the FCSC group but similar to that of the control. The proportion of motile sperm cells was significantly (P<0.05) higher than that of the other treatments. However, non-motile sperm, abnormal sperm cells, round and elongated spermatids were not significantly (P>0.05) affected by experimental diets.

4. DISCUSSION

The observation that the testicular characteristics were not significantly affected by the treatment is consistent with the report of [7] who reported that cottonseed cake with or without vitamin E supplementation had no significant effect on testicular and epididymal variables of rabbit buck. Similar observation has been reported by earlier workers for bulls [9,18]. This is an indication that adverse effect of cottonseed cake diet on the reproductive organs of farm animals may not be detectable by morphometric evaluation. Probably the effect of gossypol in CSC did not cause hyperplasia and or hypertrophy in the testis of bucks in this study. According to Amao et al. [7] hypertrophy and hyperplasia of body organs lead to increased size of the organs and invariably the weight of the organs. Probably the duration of the study was not long enough to elicit any discernible changes in the testicular variables. Randel et al. [11] reported that the effects of gossypol on reproductive organs of male animals were both dose and time dependent.

It was observed that rabbit bucks fed RCSC-based diet had significantly higher values for left, right and mean epididymal weight compared to control and FCSC suggesting a larger capacity for storage of sperm. This observation disagrees with the report of Amao et al. [7] No explanation could be offered for the observation as it could not be reconciled with the lower sperm count recorded for the same treatment in this study.

Table 2. Proximate composition and gossypol content of test ingredients (raw and fermented cotton seed cake)

Parameters(%)	**Raw CSC**	**Fermented CSC**	**SEM**
Crude Protein (%)	37.45[b]	39.55[a]	0.58
Crude Fibre (%)	11.60[a]	9.85[b]	0.51
Ether Extract (%)	8.15[a]	2.45[b]	1.64
Ash (%)	5.54[b]	7.15[a]	0.18
Moisture (%)	5.37[b]	7.25[a]	0.54
NFE (%)	30.81[b]	33.67[a]	0.83
Total Gossypol (%)	0.57	0.14	

[a,b:] Mean along the same row with different superscripts differ significantly (P<0.05)

Table 3. Testicular characteristics of rabbit bucks fed diet containing raw or fermented cottonseed cake

Parameters	**T1 (SBM)**	**T2 (RCSC)**	**T3 (FCSC)**	**SEM**
Paired testis weight (g**)**	3.33	3.45	3.67	0.32
Left testis weight (g)	1.67	1.80	1.90	0.16
Right testis weight (g)	1.67	1.65	1.77	0.16
Mean testis length (cm)	2.97	2.95	2.95	0.10
Left testis length (cm)	2.90	3.00	3.00	0.10
Right testis length (cm)	3.03	2.90	2.90	0.15
Mean testis width (cm)	1.12	1.11	1.18	0.04
Left testis width (cm)	1.10	1.15	1.20	0.05
Right testis width (cm)	1.13	1.07	1.17	0.04
Paired testis volume (ml)	6.67	7.25	7.50	0.67
Left testis volume (ml)	3.50	3.50	3.83	0.38
Right testis volume (ml)	3.17	3.75	3.67	0.14

Table 4. Epididymal characteristics of rabbit bucks fed diet containing raw or fermented cottonseed cake

Parameters	T1 (SBM)	T2 (RCSC)	T3 (FCSC)	SEM
Mean Epid. Weight (g)	2.27^{b}	3.50^{a}	2.50^{b}	0.30
Left Epid. Weight (g)	2.27^{b}	3.40^{a}	2.47^{b}	0.31
Right Epid. Weight (g)	2.27^{ab}	3.60^{a}	2.03^{b}	0.32
Mean Epid. Length (cm)	16.33^{a}	14.75^{ab}	12.87^{b}	0.65
Left Epid. Length (cm)	17.30^{a}	15.35^{ab}	14.30^{b}	0.58
Right Epid. Length (cm)	15.37^{a}	14.10^{a}	11.43^{b}	0.80

$^{a,b:}$ *Mean along the same row with different superscripts differ significantly (P<0.05)*

Table 5. Gonadal sperm characteristics of rabbit bucks fed diet containing raw or fermented cottonseed cake

Parameters	T1 (SBM)	T2 (RCSC)	T3 (FCSC)	SEM
Sperm count (x10^{6})	182.00^{a}	124.67^{b}	181.50^{a}	14.48
Motile sperm %	48.00^{b}	54.00^{b}	66.33^{a}	3.20
Non motile sperm %	9.67	11.33	12.00	0.73
Normal sperm %	50.00^{b}	50.67^{b}	63.50^{a}	2.81
Abnormal sperm %	10.33	9.67	10.50	0.86
Round spermatids (x10^{6})	34.33	22.00	26.00	2.68
Elongated spermatids (x10^{6})	11.33	11.33	14.00	1.22
Total spermatids (x10^{6})	45.67	33.33	40.00	3.15

$^{a,b:}$ *Mean along the same row with different superscripts differ significantly (P<0.05*

The significantly lower sperm count, higher proportion of motile and normal sperm cells observed for RCSC group confirm the spermatoxic effect of cottonseed cake-based diets recently reported [7] This observation is consistent with similar work in bulls [9] and rabbit [10]. This antispermatogenic effect has been largely attributed to the presence of gossypol in the CSC which has been known to cause damages to the germinal epithelium of the seminiferous tubules [11,19].

The significantly higher sperm count, motile and morphologically normal cells for the FCSC group indicate that the antispermatogenic effect of CSC was neutralized by fermentation. This could be due to reduction in gossypol level occasioned by microbial activities during fermentation. This suggests that fermentation could be a useful method of detoxifying cottonseed cake.

5. CONCLUSION

It was concluded from this study that feeding rabbit buck with cottonseed cake-based diet had deleterious effect on testicular sperm characteristics. This adverse effect was however corrected by fermenting the cottonseed cake using *Aspergillus niger*. It was recommended that cottonseed cake should not be included in diets of breeding rabbit buck unless fermented by *Aspergillus niger*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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