HISTOLOGICAL CHANGES IN TESTIS OF RABBIT BUCKS FED WITH DIET CONTAINING RAW OR FERMENTED COTTONSEED CAKE SUPPLEMENTED WITH GINGER

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ABSTRACT
The aim of this study was to evaluate the histological changes in testis of rabbit bucks (New Zealand White X Chinchilla) fed with raw or fermented cottonseed cake (CSC) - based diets supplemented with ginger. Solid state fermentation (SSF) was employed to biodegrade CSC using Aspergilus niger. Thirty (30) weaner rabbit bucks aged between 5 - 6 weeks were acclimatized for two weeks and allocated to five dietary treatments with equal number of animals (n=6) per treatment in a 2x2 factorial arrangement within a completely randomized design (CRD). Treatment 1, (control) had soybean meal (SBM) as protein source; other four treatments were divided into two groups. The first group comprised raw or fermented CSC without ginger supplementation while the second group comprised raw or fermented CSC with ginger supplementation. The SBM was completely replaced with CSC weight for weight in the four treatments. Ginger was supplemented at a dosage of 30mg/kg diet. Feeding trial lasted for 12 weeks. Testis samples were processed for histological evaluation. The photomicrographs of the testis revealed that feeding raw CSC diets without ginger supplementation resulted in severe testicular damage. Either the fermentation of CSC by A. niger or ginger supplementation, or their combination exhibited the potential to greatly ameliorate the adverse effects. It was therefore, concluded that feeding CSC to rabbit buck intended for breeding purpose should either be fermented with A. niger or supplemented with ginger or their combination.
Keywords: Testis, Fermentation, Cottonseed cake, Ginger, Rabbit bucks.

INTRODUCTION

Cottonseed cake (CSC) is a cheap and excellent source of animal protein, energy and fiber for variety of animal species (NCPA, 2002). It is one of the feedstuffs that have not been fully exploited for livestock feeding, most especially non-ruminant animals, despite its rich nutritional potential. Its crude protein content ranges from 35 to 46% and fibre 11-13% as well as 70-80% TDN (Coppock *et al.,* 1987; Jibrin *et al*., 2020). Non ruminant animals are not usually fed with CSC because of the presence of gossypol, a toxic factor which has been reported to have anti-fertility effects on reproductive organs at both macroscopic and microscopic levels (Chase *et al*., 1994; Amao and Showunmi, 2016; Amao and Akanbi, 2017). Extensive research has demonstrated a variety of toxicological effects of gossypol on male reproductive characteristics of farm animals. Exposure to toxic factors during fetal development and puberty inhibits the multiplication of Sertoli and Leydig cells in the testis (Taha *et al.*, 2006). One of the mechanisms of action of gossypol in animal's body is generation of free radicals. Sperm are extremely susceptible to damage from free radicals. The sperm cell membrane is primarily composed of polyunsaturated fatty acids, particularly omega-3 essential fatty acids which are especially vulnerable to free radical damage.

Several attempts have been made to tackle these problems either by reducing or inactivating free gossypol through a number of methods, such as, addition of iron salts to cottonseed meal based diets to bind gossypol (Tabatabai *et al.*, 2002) and use of calcium hydroxide (Nagalakshmi *et al.*, 2003). Multiple strategies have also been attempted to detoxify cottonseed (Liu *et al*., 2022). Unfortunately, some of these methods affected the protein quality, were inefficient to block absorption of free gossypol and increased cost of input. Microbial fermentation by *Aspergillus niger* has been investigated (Zhang *et al.,* 2006) and was found to reduce gossypol levels in CSC.

It has been reported that antioxidants are important in the metabolism of gossypol on sperm cells (Amao *et al.,* 2012). Sperm lack their own defensive enzymes so they are completely dependent upon antioxidants for protection. In view of this, feeding CSC diets to non ruminant animals requires

adequate supplementation with antioxidants so as to neutralize its negative effect on sperm producing cells. Ginger (*Zingiber officinale*) is a natural source of antioxidant that could possibly neutralize the negative effect of gossypol in CSC. This study therefore investigated the possibility of using ginger and microbial fermentation by *A. niger* to mitigate the adverse effect of CSC on the histological architecture of testis of rabbit bucks.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at the rabbit research and production unit of the teaching and research farm, LAUTECH, Ogbomoso, Oyo State, Nigeria. The climatic condition of Ogbomoso has been described by Ayinla *et al*. (2015).

Animals and management

Thirty (30) weaned crossbreed (New Zealand White X Chinchilla) rabbit bucks, aged 5 - 6 weeks with an average weight of 768.54g were weight-balanced and assigned to five dietary treatments. The experiment was laid out in a 2 x 2 factorial arrangement with a control in a completely randomized design having six animals per treatment. The bucks were housed individually in wooden hutches with screened floor made of quarter size rods and wire mesh with dimensions of 45 x 45 x 35cm. The rabbits were acclimatized for a period of two weeks; thereafter they were fed the treatment diets for a period of 12 weeks. Clean drinking water was supplied *ad libitum*. Feeding was twice per day at 8.00am and 4.00pm, with an allowance of 120 g per animal per day.

Fermentative Organism

The isolated and purified culture of the fungus *A. niger* was obtained from Department of Pure and Applied Biology Laboratory, LAUTECH Ogbomoso. The fungal culture was grown on potato dextrose agar (PDA) supplemented with 20% sucrose at pH 5.5 and temperature of 30^0C and was preserved at 4^0C in a refrigerator. Cultures were reactivated periodically as needed at their respective optimal growth temperature (Lateef *et al.,* 2008).

Solid state fermentation (SSF)and Preparation of experimental diet

The solid state fermentation was accomplished by the method of Amao and Akanbi, (2017). After the fermentation, the substrate was sun-dried to constant weight and tagged “fermented CSC” while the unfermented was labeled as “raw CSC”. The raw CSC and fermented CSC were used in preparing the experimental diets. Raw ginger was peeled, cut into chips and then sun-dried to constant weight. The chips were ground into powder form and used to supplement two of the diets at 30 mg/kg according to the recommended dose (Ibrahim *et al*., 2011). Five experimental diets were formulated such that the first diet (Control) had soybean meal (SBM) as the main protein source. Four other diets were formulated by replacing SBM with either raw or fermented CSC weight for weight with or without ginger supplementation. The treatment diets were: T1 – Control (SBM); T2 – Raw CSC without Ginger supplementation; T3 - Raw CSC with Ginger supplementation; T4 – Fermented CSC without Ginger Supplementation; T5 – Fermented CSC with Ginger supplementation. Table 1 shows the gross composition of experimental diets.

Histological slide preparation and evaluation

Histological slide preparation followed a standard procedure of fixing, clearing, dehydration, infiltration, embedding, sectioning and staining. Slides were viewed under the light microscope and captured for evaluation.

RESULTS AND DISCUSSION

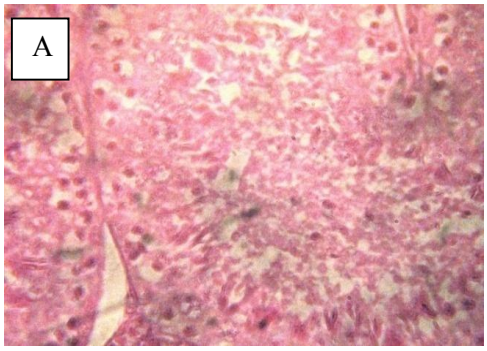
The Results of this experiment are presented in Plates 1A to 1E. No histological changes or aberrations were observed in the testis of the bucks fed the control diets (Plate 1A). The seminiferous tubules were packed with mature spermatozoa as well as spermatids in various stages of development. Plate 1B shows severe erosion and degeneration of germinal epithelial layer of the seminiferous tubules with consequent few sperm cells in the tubules. The lumen of seminiferous tubule was wide and appeared scanty and disorganized. In Plate 1C, mature spermatozoa and some spermatids in their various stages of development were observed. Histological structure of each seminiferous tubule was normal but mildly affected by CSC diets. It was observed in Plate 1D that ginger supplementation showed the potential to ameliorate the adverse effect of CSC on the testis of rabbit bucks. All histological anomalies observed in Plate 1B were corrected in Plate 1D. The seminiferous tubules

appeared more organized. In Plate 1E, the observation was similar to that of the control. The lumen was filled with mature spermatozoa and developing spermatids. It was observed that a combination of fermentation and ginger supplementation tended to increase the population of sperm cells in the seminiferous tubules.

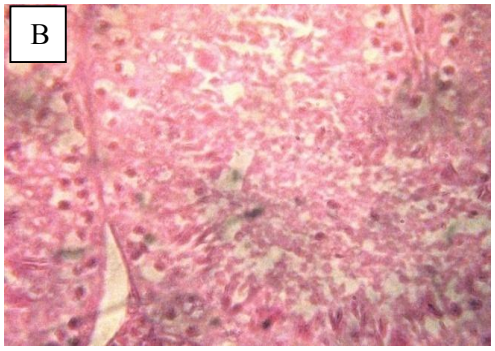
Table 1: Gross composition of experimental diets and calculated nutrients

Ingredients (%)	Control	- Ginger		+ Ginger	
	SBM	Raw CSC	Fermented CSC	Raw CSC	Fermented CSC
Maize	43.57	43.24	43.57	43.24	43.57
Soybean meal	20.43	-	-	-	-
Cottonseed cake	-	20.43	20.43	20.43	20.43
Brewers dry grain	10.00	10.33	10.00	10.33	10.00
Fixed ingredients	26.00	26.00	26.00	26.00	26.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Ginger (mg/kg)		-	-	30	30
Calculated nutrients					
Crude protein (%)	16.32	15.72	16.13	15.72	16.13
Crude Fibre (%)	10.22	11.63	11.57	11.63	11.57
ME (Kcal/kg)	2582.00	2469.00	2436.00	2469.00	2436.00

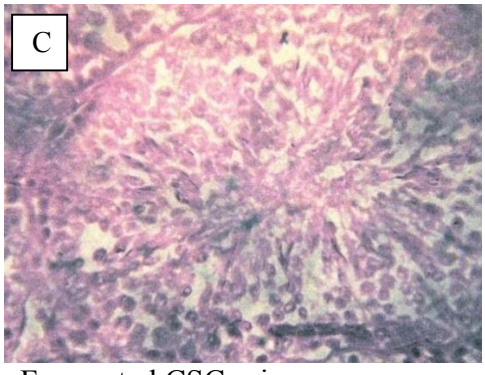
*Premix composition (per kg of diet): vitamin A, 12,500 IU; vitamin D_3, 2500 IU; vitamin E, 50.00K3, 2.50mg; vitamin B_1, 3.00mg; vitamin B_2, 6.00mg; vitamin B_6, 6.00mg; niacin, 40mg; calcium pantothenate, 10mg; biotion,0.08mg; vitamin B_{12},0.26mg; folic acid, 1.00mg; chlorine chloride, 300mg; manganese, 100mg; iron, 50mg;zinc,45mg; copper, 2.00mg; iodine, 155mg; cobalt, 0.25mg; selenium, 0.10mg; antioxidant, 200mg. Ginger was added to the diets at 30mg/kg SBM: soybean meal, CSC: cottonseed based diets



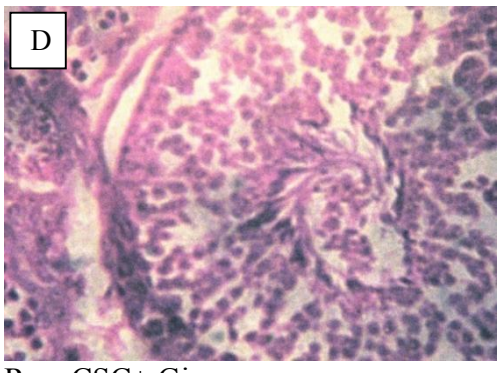
SBM- Control



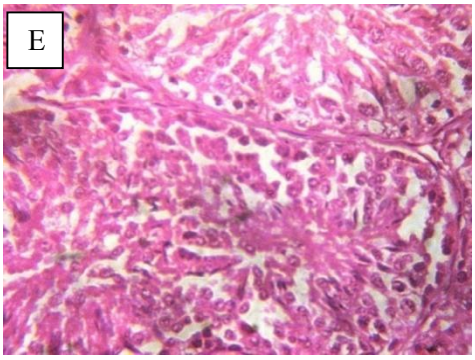
Raw CSC - Ginger



Fermented CSC- ginger



Raw CSC+ Ginger



Fermented CSC+ Ginger

Plate 1: The photomicrograph of the seminiferous tubules of testis of rabbit buck fed raw or fermented CSC supplemented with ginger (Mag: X400)

The observed degenerative changes in which there were no spermatozoa in the seminiferous tubules recorded for the groups of bucks fed raw or fermented CSC without ginger supplementation suggests the adverse effect of the residual gossypol that might be present in the CSC. Probably fermentation alone was not potent enough to overwhelm the residual gossypol in the fermented CSC used. The fact that ginger supplementation showed the potential to ameliorate the adverse effect of gossypol indicates that the mechanism of antioxidant activities of ginger could be deployed for the enhancement of utilization of CSC in rabbit bucks' diet (Amao and Akanbi, 2017). The observation that a combination of fermentation and ginger supplementation proved to be more potent in neutralizing the adverse effect of gossypol on testicular tissues suggests that a synergistic effect of fermentation by *A. niger* and ginger supplementation may be required to handle a fairly high level of CSC in diet of rabbit buck intended for breeding.

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