
Sexual libido, testicular histology and sperm physiology of rabbit bucks fed diets supplemented with toasted soybean seed meal

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Abstract The effect of toasted soybean seed meal on sexual libido, testicular histology and sperm physiology of New Zealand white rabbit bucks were investigated. The rabbits were divided into two groups (BT₁ and BT₂), each group made up of 12 bucks. Bucks in group BT₂ were fed diet supplemented with 10% toasted soybean seed meal while bucks in group BT₁ served as control. The rabbit bucks were trained for semen collection into an artificial vagina using matured cyclic does. Semen was collected twice a week for four consecutive weeks. The weight of the testis was determined, and after tissue processing, their histology were studied by light microscope. Results obtained revealed that sperm motility and concentration were significantly ($p < 0.05$) enhanced by the toasted soybean seed meal treatment. The result also revealed that testis weight and sexual libido scores were higher in BT₂ treatment group than in BT₁ treatment group. Histological examination of the testis of rabbits in BT₂ group revealed an increased seminiferous tubule diameter with active spermatogenic cells at different stages of development when compared with those animals in BT₁ treatment group. In conclusion, toasted soybean seed meal supplementation improved semen quality and histoarchitecture of rabbit testis. This suggests that toasted soybean seed meal could be used to enhance rabbit fertility.

Key words: Rabbit, soybean, testis, semen, histology

Introduction

The need to abridge the animal protein intake is a major problem especially in developing countries where population practically erodes increases in food production leaving no hope of ever increasing surplus grain sources to compound livestock feeds. Hence efforts are being made to source for alternative methods of processing conventional feed ingredients that can

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lead to a reduction in the cost of feed and hence total cost of production. Locally processed soybean seed meal has such potentials (Oyenuga, 1987; Amaefule and Onwudike, 2000).

Soybean seeds contain approximately 36% protein and 18 % fat. After oil extraction the meal may have as much as 45% protein and 1-5% fat. The main phytochemicals in soybean are saponins, phytic acid and isoflavones. Isoflavones, the principle soy phytochemical are similar to 17 β -oestradiol and its examples are genistein, daidzein, formononetin, biochanin A and equol (Ogbuewu *et al.*, 2010) and then the lignans, example: enterolactone and enterodiol derived from precursors in the diet by the gut microflora (Martini *et al.*, 1993). Studies in non-human primates (Adams *et al.*, 2005) and rabbits (Yamakoshi *et al.*, 2000) have demonstrated retardation of atherogenesis during dietary isoflavone phytoestrogen administration.

In recent years, it has been suggested that isoflavones, may be involved in the regulating the renewal of spermatogonial stem cells (Miura *et al.*, 2003), and male reproductive tissue with estrogen receptors (Amin *et al.*, 1969). Investigations have revealed that soybean seed contain estrogenic materials as a gonad stimulating compounds that improve male fertility (Mitchell *et al.*, 2001; Ogbuewu *et al.*, 2010). Soy meal has been shown to improve sperm motility and fertility of rabbit bucks (Oyeyemi and Okediran, 2007).

The cellular elements of the semen are sperm cells suspended in the seminal plasma (Ogbuewu *et al.*, 2009). These myriads of metabolites in the seminal plasma, provides a valuable medium for clinical investigation and nutritional status of animal hence its recommended use of the semen profile in the medical and nutritional assessment. In view of this, the present study was conducted to determine the effects of toasted soybean seed meal on sexual libido, testicular function and sperm physiology of rabbit bucks.

Materials and methods

Animal model

Eighteen mature 11-12 months male New Zealand white rabbits used for this study weighed between 1.8 and 2.0 kg. The experiment was conducted at the Rabbit Unit of the Teaching and Research Farm, Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria. Procedures involving animals and their care were conducted in conformity with international laws and policies.

Processing of toasted soybean seed meal

Soybean seeds were acquired from the National Root Crop Research Institutes (NRCRI), Umudike, Abia State, Nigeria. These seeds were cleaned of dirt and subjected to toasting; a process which involved spreading of the seeds thinly in preheated sand inside a frying pan for 30 minutes at a temperature fluctuating between 90⁰C and 100⁰C. After toasting, the seeds were removed by sieving the hot sand, cooled and later ground to the desired particle size to produce the meal used.

Experimental design and diet

Twenty four clinically healthy crossbred rabbit bucks were used for 10 weeks to study the influence of toasted soybean seed meal on sexual libido, testicular parameter and sperm physiology. The animals were allowed to acclimatize for a period of 14 days before the commencement of treatments. Nine matured rabbit bucks (group BT₁) were fed normal rabbit diet for ten consecutive weeks while, another group of nine bucks (group BT₂) were fed normal buck diet supplemented with 10% toasted soybean seed meal for the same period in a completely randomized design experiment. Feed and water were given *ad libitum*. Chemical compositions (AOAC, 1990) of the experimental diets are presented in Table 1.

Semen quality

The artificial vagina (AV) for semen collection was built based on a model described by Herbert and Adejumo (1995). Its mucosa was filled with glycerin, and it was used when the inner temperature was between 45° and 50°C. A collector tube was attached onto one of the edges, and the free edge was positioned to penis intromission. Before semen collection, bucks were allowed one false mount and, at the subsequent mounting, the AV was adequately positioned on the dorsum of the stimulus female allowing penis intromission.

Sexual activity (libido) was estimated as time between introduction of the teaser female into the male's hutch and ejaculation. Sperm concentration was assessed by Neubauer haemocytometer (Improved Neubauer chamber, Germany) after dilution (1:100 v/v). Semen colour and motility was estimated according to Zemjams (1970) and percentages of live spermatozoa were determined for each sample using the procedure of Oyeyemi *et al.* (2008). Ejaculate volume was read using graduated collection tube and recorded in milliliters.

Table 1. Chemical compositions of the experimental diet

Nutrient	Percent
Maize	35.00
Fish meal	3.00
GNC	3.00
Spent grain	55.00
Bone meal	2.00
Oyster shell	1.00
Premix*	0.50
Common salt	0.50
Total	100.00
Calculated nutrient composition	
Crude protein	18.87
Crude fibre	10.1
Ether extract	5.97
Calcium	1.41
Phosphorus	0.66
ME (MJ/kg)	10.42

Vitamin and mineral premix contributed the following to each kilogram of diet: vit. A 500 IU; Vit. D2 1500 IU; Vit. E 3 IU; Vit. K 2 mg; riboflavin 3 mg; panthothenic acid 6 mg; niacin 15 mg; Vit B12 0.8 mg; choline, 3 mg; folic acid 4 mg; Mn 8 mg; Zn 0.5 mg; iodine 1.0 mg; Co 1.2 mg.

Testicular histology: At the end of the study 3 bucks each whose weights were closer to the mean were selected at random from each treatment group. The bucks were sacrificed and the testes was carefully milked out from the scrotal sac, trimmed of all fat and blotted dry to remove any blood. The testicular weight was recorded and testis sample was stored in a bottle containing 10% formalin solution. The samples were immediately taken to the laboratory for analysis within 3 hours of collection. Samples were cut and fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated by different grades of alcohol (70, 85, 96 and 99%). Thereafter, samples were cleared by xylene and embedded in paraffin wax and sectioned 5 to 6 microns using microtome. The sections were stained by Hematoxyline and Eosin (H & E) and then examined by light microscope for histopathological changes according to Pearse (1968).

Data analysis: Data obtained on sexual libido, testicular and semen quality characteristics were subjected to the Student's t – test. The statistical analyses were performed using General Linear Model procedure of SAS (SAS, 1999).

Results and discussions

The data on sexual libido, testis weights and semen quality characteristics of rabbit bucks fed toasted soybean seed meal based diets are presented in table 2. Maturation of mammalian testes is dependent on the normal proliferation and differentiation of germinal epithelium and sertoli cells and the number of sertoli cell which established during the pre puberal period which determines the final testicular size and the number of sperm produced in sexually mature animals (Johnson, 1994). Testicular weights have been reported to have a high correlation with sperm reserve in the testis or epididymis and therefore a reflection of sperm production (Ogbuewu *et al.*, 2009). The increase in testicular weights of rabbit bucks in BT₂ treatment group is a pointer that toasted soybean seed meal promotes testicular growth. Ejaculate colour of the BT₁ and BT₂ animals were creamy white. Concerning the reaction time, the rabbit bucks in BT₁ and BT₂ groups had similar ($p>0.05$) sexual libido which fell within the range reported by Ogbuewu *et al.* (2009) in mature rabbit bucks.

Table 2. Effect of toasted soybean seed meal on semen quality characteristics of rabbit bucks

Parameters	BT ₁	BT ₂	p<0.05
Live spermatozoa (%)	80.90 ± 1.75	82.02 ± 1.98	ns
Ejaculate volume (ml)	0.50 ± 0.02	0.60 ± 0.04	ns
Sperm motility (%)	69.5 ± 1.05	72.50 ± 1.42	*
Sperm conc. (× 10 ⁹ / ml)	1.39 ± 0.82	1.86 ± 0.29	*
Ejaculate colour	Creamy white	Creamy white	-
Sexual libido (sec)	22.60	23.10.00	ns
Testicular weight (g)	4.49 ± 0.41	4.81 ± 0.32	ns

* Significantly different at $p<0.05$; ns - Not significantly different at $p<0.05$

Semen evaluation is an important tool for clarifying the effect of external and internal agents affecting male reproduction. Data on semen quality parameter in the present study revealed that rabbit bucks in BT₂ treatment group had the best results in most of the semen quality parameters studied. The sperm motility and concentration of BT₁ rabbits was significantly ($p<0.05$) lower than those in BT₂ treatment group. The motility of sperm cells is important for fertilization since only sperm cells with progressive motility are involved in fertilization processes. The significant increases in the sperm motility could also be attributed to the actions of active ingredients in soybean which were well documented to improve spermatogenic activities (Mitchell *et al.*, 2001; Ogbuewu *et al.*, 2010). The observed increase in semen quality parameters of rabbit bucks fed toasted soybean seed could be attributed in part

by the antioxidant activity of soy isoflavones (Ogbuewu *et al.*, 2010). Isoflavone rich toasted soybean seed meal for instance, could also increase sperm motility and viability by increasing glucose metabolism leading to the production of pyruvate and energy. Pyruvate is known to be the preferred substrate essential for the activity and survival of sperm cells (Egbunike *et al.*, 1986). In addition, arginine the predominant amino acid in soybean is a biochemical precursor in the synthesis of putrescine, spermidine and spermine which are essential for sperm motility (Steven, 2000).

Micrographs of histological sections of the testis are shown in plate 1 and 2. The histological structure of testis differed among the treatment at slaughtering. Results showed that bucks in BT₂ treatment group had slightly elevated seminiferous tubule lumen diameter with complete spermatogenesis than in BT₁ treatment group. Their epithelia are structurally intact and show normal germ cells. In addition, the slight increase in the number of spermatogonia, spermatocyte and spermatids in BT₂ treatment group showed a progress in spermatogenesis. This observation was consistent with the increase in semen quality parameters of bucks in BT₂ treatment group. However, it is ideal that dietary supplementation of toasted soybean meal may increase activity and development of testicular tissue in rabbits. The result showed that toasted soybean seed meal positively affected gonad developments (increased proliferation of many types of testis cells involved in spermatogenesis) of the rabbit and similar results were reported in studies with rabbits by Oyeyemi and Okediran (2007). This indicates that toasted soybean seed meal supplementation did not elicit adverse effects on the primary reproductive organ of the male rabbits.

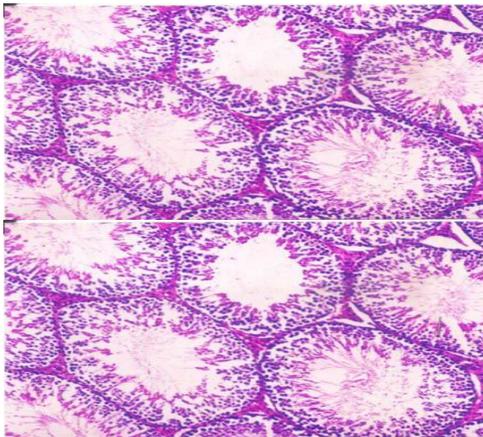


Fig. 1. Micrograph of seminiferous tubule of rabbit bucks in BT₁ group showing active spermatogenic cells, X240

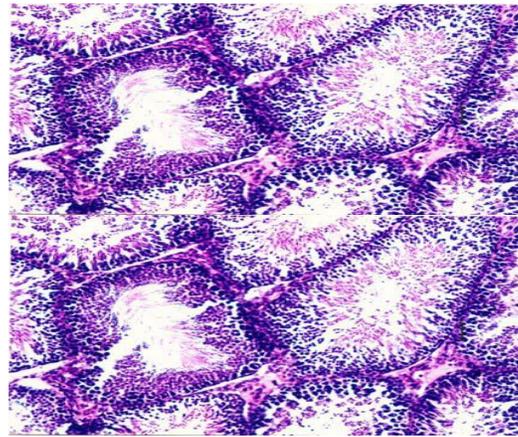


Fig. 2. Micrograph of seminiferous tubule of rabbit bucks in BT₂ group showing active spermatogenic cells, X240

Conclusion

In this study dietary toasted soybean meal enhance testes developments and spermatogenesis in rabbits. The association of toasted soybean seed meal with improved spermatozoa production and semen output is a welcome development. Therefore, toasted soybean seed meal is recommended in rabbit breeding programme where fertility of the animal is fundamental to productivity.

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