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Medical Science**  
Journal homepage: [www.Sjournals.com](http://www.Sjournals.com)**Original article****Effects of dietary addition of toasted soybean seed meal on libido, semen quality and testis histology of rabbit bucks****I.P. Ogbuewu<sup>a,\*</sup>, C. Enumaibe<sup>a</sup>, O.E. Kadurumba<sup>a</sup>, C.T. Iwuji<sup>a</sup>, U.E. Ogundu<sup>a</sup>, I.F. Etuk<sup>a</sup>, M.N. Opara<sup>a</sup>, I.C. Okoli<sup>a</sup>, M.U. Iloeje<sup>a</sup>**<sup>a</sup>*Department of Animal Science and Technology, Federal University of Technology, Owerri, P.M.B.1526, Owerri, Nigeria*<sup>\*</sup>Corresponding author; Department of Animal Science and Technology, Federal University of Technology, Owerri, P.M.B.1526, Owerri, Nigeria

## ARTICLE INFO

## ABSTRACT

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The study was aimed to evaluate the effect of toasted soybean seed meal on sexual libido, semen quality and testis histology of rabbit bucks. The experimental rabbits were divided into two treatment groups (BT<sub>1</sub> and BT<sub>2</sub>) of 12 bucks each. Bucks in BT<sub>1</sub> group were fed control diet while those in BT<sub>2</sub> group were fed control diet + 10% toasted soybean seed meal. The rabbit bucks were trained for semen collection into an artificial vagina using matured cyclic rabbit does. Semen was collected twice a week for four consecutive weeks. Results obtained revealed that sperm motility and sperm concentration were significantly ( $p < 0.05$ ) enhanced by toasted soybean seed meal. The result also revealed that testis weight and sexual libido was higher in BT<sub>2</sub> group than those in BT<sub>1</sub> group. The photomicrograph of the BT<sub>2</sub> bucks revealed an increase in the number of active spermatogenic cells at different stages of development when compared with those bucks in BT<sub>1</sub> group. In conclusion, dietary addition of toasted soybean seed meal to mature rabbit diet improved semen quality and histoarchitecture of the testis.

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**1. 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 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 *et al.*, 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 $\beta$ -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 and rabbits have demonstrated retardation of atherogenesis during dietary isoflavone phytoestrogen administration (Yamakoshi *et al.*, 2000; Adams *et al.*, 2005).

In recent years, it has been suggested that isoflavones, may be involved in the regulating the renewal of spermatogonial stem cells<sup>7</sup>, and male reproductive tissue with estrogen receptors (Amin *et al.*, 1969; Miura *et al.*, 2003). 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 dietary addition of toasted soybean seed meal on sexual libido, testicular histology and semen quality of rabbit bucks.

## 2. Materials and methods

### 2.1. 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.

### 2.2. 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 90°C and 100°C. 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.

### 2.3. Experimental design and diet

Twenty four clinically healthy crossbred rabbit bucks were used for 10 weeks to evaluate the effect of toasted soybean seed meal on sexual libido, semen quality and testicular histology. The animals were allowed to acclimatize for a period of 14 days before the commencement of treatments. Nine matured rabbit bucks (group BT<sub>1</sub>) were fed normal rabbit diet for ten consecutive weeks while, another group of nine bucks (group BT<sub>2</sub>) 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 of the experimental diets are presented in Table 1.

### 2.4. 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 Zemjanis (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.

<b>Nutrient</b>	<b>Percent</b>
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
<b>Calculated nutrient composition</b>	
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.

### 2.5. 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).

### 2.6. Statistical analysis

Data on sexual libido and semen quality were subjected to the student's t – test. Where significant differences were detected between the treatment means, mean separation was done using General Linear Model procedure of SAS (1999).

## 3. Results and discussion

The data on sexual libido, testis weight, semen quality and testis weight of bucks fed toasted soybean seed meal based diet are presented in Table 2 and Figure 1 respectively. Maturation of mammalian testes is dependent

on the normal proliferation and differentiation of germinal epithelium and sertoli cells 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 BT<sub>2</sub> treatment group in this study is a pointer that toasted soybean seed meal promotes testicular growth. Concerning the reaction time, the rabbit bucks in BT<sub>1</sub> and BT<sub>2</sub> groups had similar (p>0.05) sexual libido which fell within the range reported by Ogbuewu *et al.* (2009) in mature rabbit bucks.

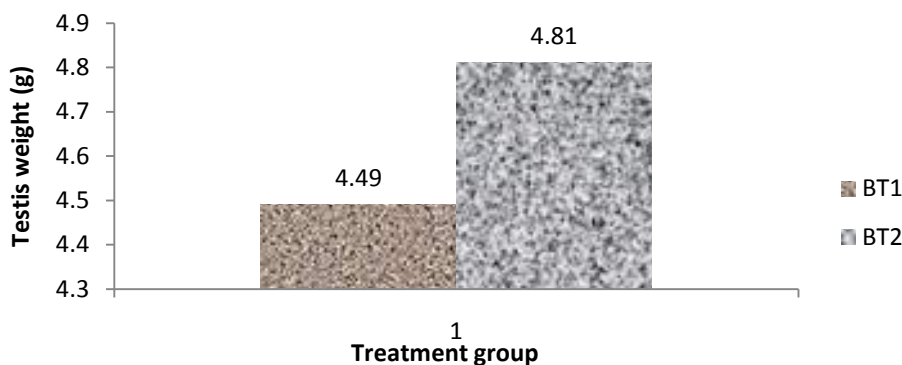


Fig. 1. Effect of dietary addition of toasted soybean seed meal on testis weight of rabbit bucks.

Table 2

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

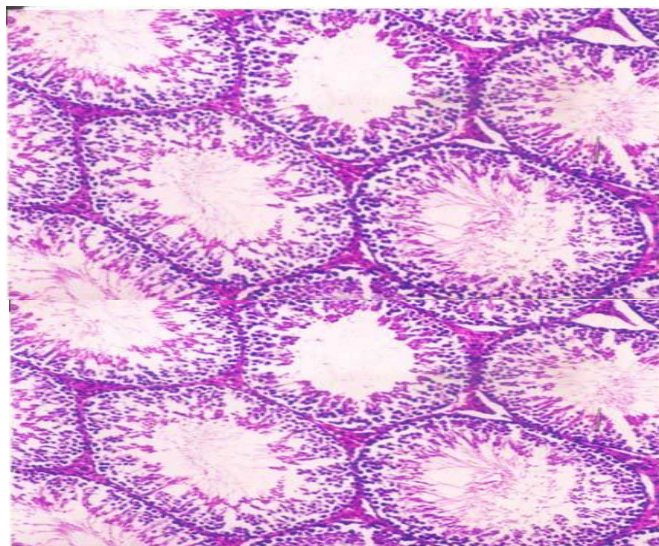
Parameters	BT <sub>1</sub>	BT <sub>2</sub>	p<0.05
Live spermatozoa (%)	80.90 ± 1.75	82.02 ± 1.98	ns
Semen volume (ml)	0.50 ± 0.02	0.60 ± 0.04	ns
Sperm motility (%)	69.5 ± 1.05	72.50 ± 1.42	*
Sperm conc. (× 10 <sup>9</sup> / ml)	1.39 ± 0.82	1.86 ± 0.29	*
Semen colour	Creamy white	Creamy white	-
Sexual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

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

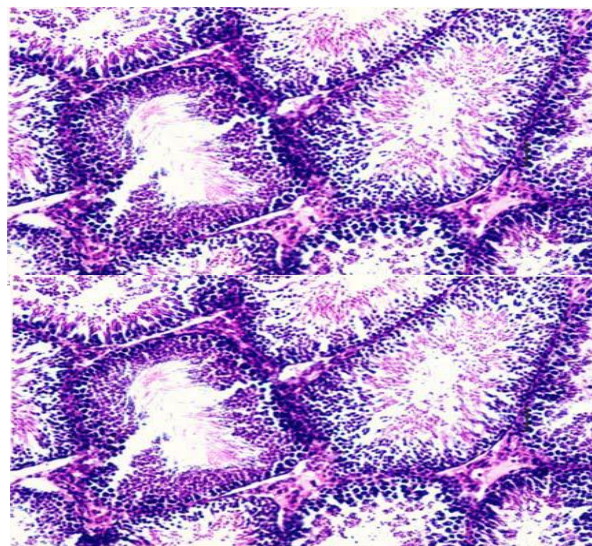
Semen evaluation is an important tool for clarifying the effect of external and internal agents affecting male reproduction. Data on semen quality parameter in the present study revealed that rabbit bucks in BT<sub>2</sub> group had the best semen picture in most of the semen quality parameters studied. Sperm motility and sperm concentration value of BT<sub>1</sub> rabbits were significantly (p<0.05) lower than those in BT<sub>2</sub> treatment group. The motility of sperm cells is important index for fertilization since only sperm cells with progressive motility are involved in fertilization processes. The significant increases in the sperm motility in this study could be attributed to the actions of pharmacological active ingredients in soybean which were well documented to improve spermatogenic activities (Mitchell *et al.*, 1001; Ogbuewu *et al.*, 2010). Also the observed increase in all the semen quality parameters of bucks fed diet supplemented with 10% 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. Their seminiferous tubule epithelia are structurally intact and show normal germ cells. Results showed that bucks in BT<sub>2</sub> group had complete spermatogenesis than those in BT<sub>1</sub> group. This observation was consistent with the increase in semen quality

parameters of bucks in BT<sub>2</sub> 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 revealed that toasted soybean seed meal positively affect gonad developments (increased proliferation of many types of testis cells involved in spermatogenesis) in rabbit buck and similar results were reported in studies with rabbits by (Oyeyemi and Okediran, 2007). This indicates that toasted soybean seed meal supplementation enhanced primary reproductive organ of male rabbits.



**Plate 1.** Micrograph of seminiferous tubule of rabbit bucks in BT<sub>1</sub> group showing active in spermatogenic cells, X240.



**Plate 2.** Micrograph of seminiferous tubule of rabbit bucks in BT<sub>2</sub> group showing active spermatogenic cells, X240.

#### 4. Conclusion

In this study dietary toasted soybean meal enhanced testis development 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.

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