HISTOLOGICAL CHANGES IN TESTICULAR STRUCTURE OF RABBIT BUCK FED DIETS SUPPLEMENTED WITH BLACK SEED (*NIGELLA SATIVA*)

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ABSTRACT
This study investigated the histological changes in testis of rabbit buck fed diets supplemented with Black seed (*Nigella sativa*). Twenty Chinchilla X New Zealand White, 5-6 weeks old, were balanced for weight and assigned to four treatments T1, T2, T3 and T4 of five rabbits each in a completely randomized design. The four treatment groups were supplemented with black seed (*Nigella sativa*) at 0, 0.5, 1.0 and 1.5% levels, respectively. After twelve weeks of feeding, the bucks were sacrificed and the testes were extracted for histological evaluation. The results showed that the seminiferous tubules in testes of rabbit bucks fed varying levels of Black seed had normal and larger, well arranged germ cell population than the control. As *N. sativa* supplementation increased up to 1.5%, the testicular histo-architecture remained normal. This study demonstrated that *N. sativa* seeds, at 1.5% supplementation level in diet, possess the potential to improve and preserve the histo-architecture of rabbit testis.
Keywords: Histology, Black-seed, Rabbit, Testis

INTRODUCTION
Rabbits are widely used in agricultural and biomedical research due to their physiological and anatomical similarities to other mammals (Lossi *et al.,* 2016). Assessing the histological changes in vital organs including testis of rabbit bucks fed supplemented diets can provide valuable information on the safety, efficacy and potential health benefits or otherwise of the dietary intervention on the organ (Ezea *et al.,* 2024). Testes are particularly sensitive to oxidative stress and nutritional status, directly impacting male reproductive health.
Nigella sativa, commonly referred to as black seed or black cumin, is a flowering plant of the *Ranunculaceae* family. Its seeds have been traditionally used in various cultures for their wide-range health benefits, including anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects (Manoharan *et al.,* 2021). The active compounds in black seed, particularly thymoquinone, are reported to exert protective effects against tissue damage caused by oxidative stress and other pathological conditions. These attributes make black seed a promising candidate for improving animal health and productivity (Sadeghi *et al*., 2023). In animal studies, histological evaluation of organs, such as testis, is vital for understanding the effects of dietary and environmental interventions on their structure and functions (Meng *et al*., 2023). Among various natural supplements studied for their therapeutic and protective effects, black seed (*N. sativa*), known for its medicinal properties, has gained significant attention (Manoharan *et al.,* 2021). However, there is dearth of technical information on its use as supplement and effects on the histo-architecture of testis of rabbit buck. This study therefore, investigated the effects of black seed supplementation on the histological structure of testis of buck rabbits.

MATERIALS AND METHODS
Experimental site
The experiment was conducted at the Rabbit Research and Production unit, Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The geographical and climatic characteristics of Ogbomoso have been described by Ayinla and Odetoye, (2015).

Animals and Management
Twenty growing rabbit bucks (Chinchilla X New Zealand White, 5-6 weeks old) were used for the experiment. The bucks were individually housed in wooden hutches and subjected to two weeks acclimatization period. They were treated against potential endo- and ecto-parasites and fed diet containing 16% crude protein and average of 2336.12 kcal/kg metabolizable energy. They were balanced for weight and assigned to four treatments T1, T2, T3 and T4 of five rabbits each in a completely randomized design. The rabbit bucks were weighed at the commencement of the experiment and subsequently once per week. They were offered feed *ad libitum*. Cool, clean water was made available throughout the feeding trial which lasted 12 weeks. *N. sativa* was supplemented in the diets at different levels as follows:
T1 (**Contro**l**):** No *N. sativa* supplementation; **T2:** 5g of *N. sativa* per kg of diet;
T3: 10g of *N. sativa* per kg of diet; **T4:** 15g of *N. sativa* per kg of diet.
Table 1 shows the gross composition of experimental diets.

The proximate composition of black seed was determined using the conventional procedure of AOAC (2005).

Histological slide preparation and evaluation

At the end of 12 weeks feeding, three bucks per treatment were sacrificed and their testes were carefully removed and their samples fixed for histological slide preparation. Samples of the testis were fixed in Bouin's fixative. The slide preparation was done according to standard procedure. The fixed tissues were dehydrated by dipping in concentrations of alcohol (70%, 80%, 90% and 100%). They were cleared with xylene, infiltrated with molten paraffin wax at 50ºC to 60ºC, embedded in molten paraffin wax and labeled appropriately. Thin section of 5µ was cut from the embedded tissues using a microtome (Rotary Microtome RMT-20A). The sectioned tissues were mounted on grease free, clean glass slides, dried at room temperature and stained with Haematoxylin and Eosin stain. The tissue section on slides was viewed using light microscope at 400 x magnification. Photomicrograph of the slides was taken using a digital camera, transferred to a computer and labeled appropriately.

Table 1: Gross composition of experimental diets

Feed Ingredients (%)	**T1 Control 0% *N. sativa***	**T2 0.5% *N. sativa***	**T3 1.0% *N. sativa***	**T4 1.5% *N. sativa***
Maize	32.61	32.61	32.61	32.61
Soybean meal	16.39	15.89	15.39	14.90
N. sativa	0.00	0.50	1.00	1.50
Fixed ingredients	51.00	51.00	51.00	51.00
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
CP (%)	16.20	16.13	16.06	16.00
Metabolizable Energy** (Kcal/kg)	2335.76	2336.00	2336.24	2336.49
CF (%)	13.75	13.75	13.74	13.74

*Vitamin Premix: Supply per kg diet: 2 000 000 iu vit. A; 400 000 iu D_3; 8.0 g vit. E; 4 g vit. b_1; 1.0 g vit. B_2; 0.6 g vit.; 0.4 mg vit. B_{12}; 24.0 g Niacin; 0.2 g Folic acid; 8.0 g Biotin; 48.0 g Choline; 320.0 g BHT; 16.0 g Manganese; 8.0 g iron; 7.2 g] Zinc; 0.32 copper; 0.25 iodine; 36. 0 mg cobalt; 16.0 mg selenium.** Metabolizable Energy calculated using Pauzenga, (1985).

RESULTS

The histological structures of the testes of rabbits fed diets supplemented with *N. sativa* are shown in Plate 1.

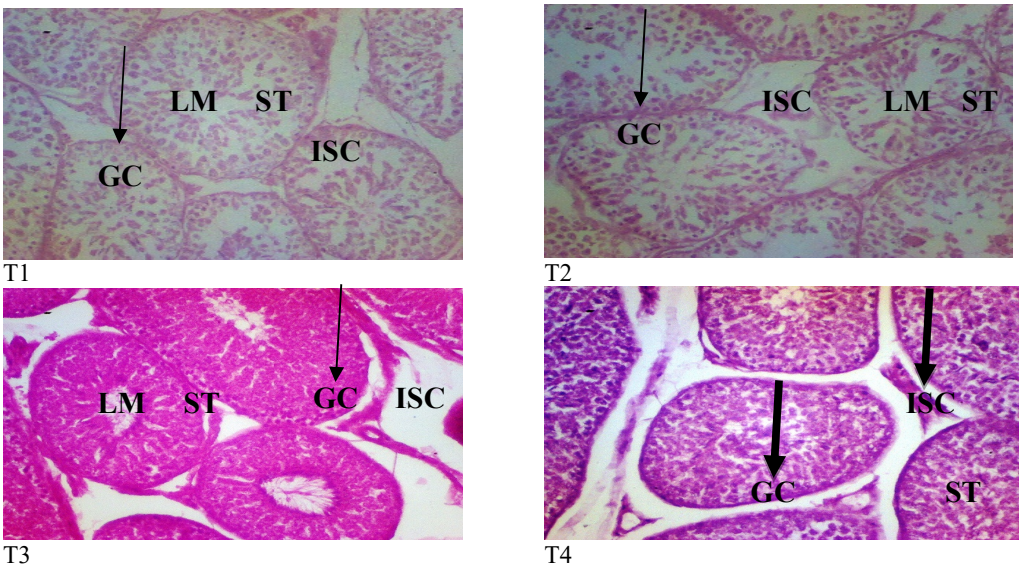

Plate1: Histological structures of testes of rabbit bucks fed diets supplemented with *N. sativa*. GC: Germ cell; LM: Lumen; ISC: Interstitial cells; ST: Seminiferous tubule; T1: Control, 0% *N. sativa*; T2: 0.5% *N. sativa*; T3: 1.0% *N. sativa;* T4: 1.5% *N. sativa*.

The histological structures of testes of rabbits fed diets supplemented with *N. sativa* are shown in Plate 1. In the control rabbits (T1), germ cell population and arrangement within the seminiferous tubules appeared normal but fewer compared to the rest of the treatments. Seminiferous tubules with numerous and closely packed (compact) spermatogenic cells were however observed in testes of bucks in T3 and T4. The germ cells were orderly arranged within the seminiferous tubules. The lumina were distinct and contained maturing spermatids. The interstitium contained interstitial cells and blood vessels. The testicular histo-architecture remained normal as *N. sativa* supplementation level increased to 1.5%.

DISCUSSION

Testis mainly consists of the seminiferous tubules in which spermatogenesis takes place, and the space between the tubules, the interstitium which contains the Leydig cells and has, among others, an endocrine function (Renkawitz-Pohl *et al*., 2005). In the present study, improved histological changes were observed in the testes of rabbits as *N. sativa* supplementation increased up to 1.5%. The improvement in testicular structure and non-deviation from the normal architecture of the control group (T1) confirmed the efficacy of *N. sativa* in enhancing male reproduction. The numerous and closely packed germ cells observed at 1.0 and 1.5% supplementation level suggest the spermatogenic potential of black seed. This observation might be influenced by the fatty acid and thymoquinone contents of the seeds as reported by Zanouny *et al*. (2013). The findings support the report of Sultan *et al*. (2014) who opined that thymoquinone has protective effect on testicular parameters. Also, Mahdavi *et al*. (2015) in a systematic review on the effects of black seed and thymoquinone on male infertility concluded that black seed could exert positive effects on testicular parameters and reproductive organs.

CONCLUSION

In conclusion, findings from this study indicate that *N. sativa* seeds possess nutritive and cyto-protective potentials at supplemental level up to 1.5% in rabbit buck diets which improved and preserved the histological architecture of the testis. Thus, supplementing diets with *N. sativa* up to 1.5% could serve as a dietary strategy for improvement and preservation of the structural integrity of testis of rabbit buck.

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