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INTERNAL ORGANS MORPHOMETRIC, SPERM AND CARCASS CHARACTERISTICS OF RABBIT BUCKS AS INFLUENCED BY BLACK SEED (Nigella sativa)

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ABSTRACT

This study assessed internal organs morphometric, sperm indices and carcass characteristics of rabbit bucks as influenced by dietary supplementation of unprocessed black seed. A total of 40 weaned male rabbits (Chinchilla X New Zealand) were weigh-balanced into four dietary treatments of 10 replicates each. The rabbits were fed 5% of their body weight with diet containing 16% crude protein, 14% crude fibre and 2300 Kcal/kg metabolizable energy. The four experimental treatments consisted of unprocessed black seed at levels of 0, 0.5, 1.0 and 1.5% of feed in T1, T2, T3 and T4 respectively. The feeding trial lasted for 84 days. At the end of the trial, 5 rabbits from each treatment were humanely sacrificed and the internal organs, and reproductive organs were carefully dissected for morphometric and semen evaluation respectively. Carcass indices were also assessed. The data collected were subjected to one way analysis of variance (ANOVA). The result revealed a significant ($p<0.05$) increase in carcass indices as the supplementation level increased up to 1%. A significantly ($p<0.05$) similar trend was observed in all the internal organs morphometric. Significant ($p<0.05$) increase was also observed in all the sperm variables (sperm count, sperm motility, sperm morphology and sperm viability) as black seed supplementation increased up to 1%. Supplementation of unprocessed black seed up to 1.5% in male rabbit's diet has beneficial effects on internal organs morphometric, sperm and carcass characteristics. Dietary supplementation up to 1% however demonstrated the best response in terms of the aforementioned indices.

INTRODUCTION

The efficiency of rabbits in terms of meat production is high compared to other farm animals. They are excellent source of animal protein for human consumption and may play important role in solving meat shortage problem in developing countries of the world (Abdel-Azeem *et al.,* 2007). Various additives and supplements have been reportedly added to rabbit feed or water as a way to enhance productive and reproductive performance of rabbits (Verga *et al.,* 2007). Black seed (*Nigella sativa*) is commonly used for many purposes, such as natural feed additive, supplement and medical purposes (Khodary *et al.,* 1996).

Black seed is also considered a good source of major minerals such as Ca, P, K, Mg and Na (Abdel-Aal and Attia, 1993). *Black* seed contains carbonyl fractions, thymoquinone, dithymoquinone, carvacrol, *p*-cymene, *t*-anethole and thymohydroquinon (Woo *et al.,* 2011; Ahmad *et al.,* 2013) which make it to have several therapeutic effects such as anti-inflammatory (Amin and Hosseinzadeh, 2016), antioxidant, anticancer and immune system stimulation (Gholamnezhad *et al.,* 2015), as well as antitumor (Gupta *et al.,* 2012).

There is a dearth of information on the appropriate dietary supplementation level of unprocessed black seed (*Nigella sativa*) in order to enhance productive and reproductive performances in rabbit bucks**.** Hence, the present experiment aimed to study the influence of black seed supplementation at various levels of dietary inclusion on internal organs morphometric, sperm indices and carcass characteristics of rabbit bucks.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Rabbit Production and Research Unit, Teaching and Research Farm of the Ladoke Akintola University of Technology, Ogbomoso. Ogbomoso is situated in a derived savannah zone of southwest of Nigeria and lies on latitude 8° 8' 31.7940" N and longitude 4° 14' 42.6696" E. The altitude is between 300m and 600m above sea level while the mean temperature and annual rainfalls are 27^0C and 1247mm respectively (Google Earth Map, 2021).

Source of Experimental Materials

The experimental rabbits were obtained from Rabbit Production Unit of the Department of Animal Nutrition and Biotechnology, Ladoke Akintola University of Technology Ogbomoso, Oyo State, Nigeria and the Black seed was procured from Sabo market, Ogbomoso, Oyo state, Nigeria.

Chemical Analysis of Black Seed (*Nigella sativa*)

The chemical analysis was done at Animal Production and Health Laboratory, Ladoke Akintola University of Technology Ogbomoso. The crude protein, ether extract, ash and crude fiber content were determined through the procedure of AOAC (2005). Total nitrogen was determined by micro-Kjeldahl method using Marham's distillation apparatus while the CP content was calculated by multiplying %N by factor of 6.25.

Experimental Design and Management of Rabbits

A total of forty (40) male rabbit (Chinchilla X New Zealand) weaners (6 weeks of age) were used for this study. They were individually housed in wooden hutches for a period of two weeks for physiological adjustment. They were dewormed using Ivermectin injection (administered subcutaneously at 0.15 ml per kilogram body weight) against potential ecto-parasites and endo-parasites.

Experimental Procedure

The animals were fed 5% of their body weight (Wafar *et al*., 2019), with diets containing 16% crude protein, 2300 Kcal/kg metabolizable energy and crude fibre of 14%. They were also offered forage comprising *Tridax procumbens* in the evening as basal diet. Freshwater was provided to the animals during the study period. Other routine and periodic management practices necessary for rabbit production was carried out.

Experimental treatments

Forty (40) weaned male rabbits were weight-balanced into 4 treatment groups of ten (10) rabbits each in a completely randomized design. The four (4) treatment groups were designated as T_1, T_2, T_3 and T_4. All the groups were fed pelletized diet containing 16% crude protein and 2300 ME Kcal/kg. *Nigella sativa* was supplemented in their diets at different levels as thus;

T1 (**Contro**l**):** No *Nigella sativa* supplementation, **T2:** 5g of *Nigella sativa* per kg of diet, **T3:** 10g of *Nigella sativa* per kg of diet, **T4:** 15g of *Nigella sativa* per kg of diet.

Table 1: Gross Composition of experimental diet

Feed Ingredients (%)	**T1 Control 0g/kg *N.sativa***	**T2 0.5% *N.sativa***	**T3 1% *N.sativa***	**T4 1.5% *N.sativa***
Maize	32.61	32.61	32.61	32.61
SBM	16.39	15.89	15.39	14.90
N.Sativa	0.00	0.50	1.00	1.50
BDG	15.00	15.00	15.00	15.00
Rice husk	30.00	30.00	30.00	30.00
Fish meal (72%)	3.00	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25	0.25
Vitamin premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15
Methionine	0.10	0.10	0.10	0.10
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
Phosphorous	0.27	0.27	0.27	0.26
Calcium	1.04	1.04	1.03	1.03
EE	5.61	5.76	5.91	6.06
Lysine	0.81	0.80	0.78	0.77
Methionine	0.67	0.67	0.66	0.66
Determined Nutrients				
CP (%)	16.17	16.14	16.11	16.15
CF (%)	13.73	13.74	13.74	13.73
EE (%)	5.62	5.65	5.62	5.64

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.

Semen evaluation

Five rabbits were randomly selected and humanely sacrificed from each treatment group at day 84 post commencement of the experiment. Testes were carefully dissected from the sacrificed animals. The epididymides were separated from the testes and other adhering tissues. The cauda epididymides were then individually placed in the petri dish containing 5ml physiological saline solution and several longitudinal incisions were made on them so that the spermatozoa would swim out. Spermatozoa were harvested and evaluated for sperm concentration, motility, percentage live spermatozoa and morphology of spermatozoa by methods described by (Zemjanis, 1977).

Internal Organs Morphometrics

The following internal organs (heart, lungs, liver and kidney) were immediately removed

from the sacrificed rabbits and weighed using a sensitive digital electronic scale.

Statistical analysis

Data collected from the experiment was subjected to one way analysis of variance (ANOVA), using the procedure of statistical analysis (SAS, 2001) and means were separated using Duncan's multiple range test (DMRT) of the same statistical package.

RESULTS AND DISCUSSION

Carcass characteristics of rabbit bucks fed varying levels of black seed (*Nigella sativa*) supplemented diets

Table 2 reveals the carcass characteristics of rabbit bucks as influenced by graded supplementation of black seed (*Nigella sativa*) in their diets. The treatments had significant ($p<0.05$) effects on all the variables. Rabbits fed T3 had a significantly ($p<0.05$) higher values in terms of final live weight, percentage bled weight, percentage eviscerated weight, percentage decapitated weight and percentage dressed weight. T1, T2 and T4 however had significantly ($p<0.05$) similar mean values in those variables with the exception of final live weight where T1 recorded the least weight. T2 and T3 recorded significantly ($p<0.05$) low values in terms of percentage dressed weight and head weight respectively when compared to the rest of treatment groups.

Table 2: Effect of black seed (*Nigella sativa*) supplemented diets on the Carcass characteristics of rabbit bucks

Parameter	T1	T2	T3	T4	±SEM	P Value
Final live weight (g)	1808.00^{c}	2115.40^{b}	2446.50^{a}	2169.33^{b}	53.45	0.00
Percentage bled weight	87.37^{b}	86.57^{b}	96.69^{a}	88.95^{b}	0.97	0.00
Percentage eviscerated weight	71.87^{b}	68.33^{b}	80.27^{a}	69.02^{b}	1.30	0.00
Percentage decapitated weight	61.15^{b}	59.10^{b}	72.25^{a}	60.04^{b}	1.42	0.00
Percentage dressed weight	58.93^{b}	53.01^{c}	68.31^{a}	54.21^{bc}	1.50	0.00
Percentage head weight	10.71^{a}	9.22^{b}	8.03^{c}	8.98^{b}	0.23	0.00

abc: Means on same row with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Mean

The rabbits fed diets supplemented with different levels of black seed (*Nigella sativa*) showed an improvement in percentage bled weight, percentage eviscerated weight, percentage decapitated weight and percentage dressed weight compared to the control group. The higher percentage of the carcass characteristics variables in T3 might be attributed to the presence of biological antioxidant activities and antimicrobial activities (Packiyasothy and Kyle, 2002) of black seed. The present findings are in agreement with the finding of Yasser *et al*. (2016), who reported that inclusion of black seed meal in rabbits' diet significantly increased the dressing percentage compared to the control diet. The present study however contradicts the report of El-Ghammry *et al*. (2002). In their findings, they reported that feeding broiler chickens, rations containing 0.2% or 0.4% crushed Nigella sativa did not improve carcass characteristics compared to the control treatment.

On the other hand, T4 (treatment with the highest *Nigella sativa* supplementation) recorded lower percentage carcass variables when compared to T3. These observations are below expectation because T3 with lower *Nigella sativa* supplementation recorded significantly higher percentages in the evaluated variables. These maybe attributed to some secondary metabolites such as tannin, saponin and alkaloids present in black seed, which tend to increase as the supplementation level increases (Mamun and Absar, 2018). These metabolites can reduce the nutritional value of the diet as tannin binds to feed protein thereby making protein unavailable to ceacal microorganisms (Salem *et al*., 2007). The efficient performance and lack of toxicity at low supplementation of *Nigella sativa* in rabbit bucks' feed may suggest the existence of threshold limit.

Internal organs morphometric of rabbit bucks fed varying black seed (*Nigella sativa*) supplemented diets

The results of internal organ morphometrics as influenced by varying supplementation levels of *Nigella sativa* is presented in Table 3. The result shows significant differences ($p<0.05$) among the dietary treatments for all the parameters weighed. A significantly increasing ($p<0.05$) trend was observed in all the weighed indices as the supplementation level increased up to 1% (T3). Rabbits fed T3 had significantly higher ($p<0.05$) heart weight, liver weight, paired lungs weight, left and right kidney weight than those fed T1, T2 and T4. T1 recorded significantly ($p<0.05$) lowest value for all the listed parameters compared to the rest of the treatment groups while T2 and T4 reveals a significantly ($p<0.05$) similar values in all weighed variables.

Table 3: Effect of black seed (*Nigella sativa*) supplementation on the internal organs morphometric of rabbit bucks

Organ weight (g)	T1	T2	T3	T4	SEM	P value
Heart weight	3.48^{c}	3.90^{b}	4.74^{a}	4.12^{b}	0.11	0.00
Liver weight	48.75^{c}	52.27^{bc}	66.93^{a}	55.03^{b}	3.01	0.00
Left kidney weight	4.33^{c}	5.06^{b}	6.13^{a}	4.80^{bc}	0.17	0.00
Right kidney weight	4.38^{c}	5.06^{b}	6.15^{a}	4.80^{bc}	0.17	0.00
Paired lungs weight	10.10^{c}	11.89^{b}	16.05^{a}	12.19^{b}	0.50	0.00

abc: Means on same row with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Mean

Supplementation of black seed (*Nigella sativa*) into the diets of rabbit bucks affected the internal organs morphometrics: heart weight, liver weight, left kidney weight, right kidney weight and paired lungs weight (Table 3). The observation follows the same trend as the carcass characteristics. The observation in the present study agrees with the report of Hassen *et al*. (2012), who reported a positive phenotypic correlation of body weight with other internal organs weight. Ayyat *et al*. (1995) and Oke *et al*. (2011) also reported a similar positive phenotypic correlation among body weights and internal morphometric variables at 90 days in Newzealand white rabbits.

Orheruata *et al*. (2006) and Okoro *et al*. (2010), further opined that internal organs morphometrics can be used to predict live body weight of Newzealand white, red Baladi and black Baladi rabbits at 12 weeks. The values obtained for all the internal organs morphometric variables falls within the average

numerical range reported for male rabbits of various ages and breeds (Brown *et al*., 1925). Thus, there's no fear of hypertrophy or atrophy in these organs that might be as a result of the diets offered. The favourable effects of Nigella sativa on the internal organs morphometrics and overall performance are thought to be due to high nutritive value as well as pharmacologically active substances present in the seeds (Mahmoud and Bendary, 2014).

Sperm characteristics of rabbit bucks fed black seed (*Nigella sativa*) supplemented diets

The sperm characteristics of rabbit bucks fed varying dietary supplementation of *Nigella sativa* is presented on Table 4. The dietary treatments imposed significant variation ($p<0.05$) on all the evaluated sperm indices. A significantly ($p<0.05$) increasing trend was observed as the supplementation level increases up to 1% *Nigella sativa* (T3) in the following parameters: sperm count, percentage motile sperm, percentage normal sperm, percentage live sperm, round spermatid and elongated spermatids. T3 recorded significantly ($p<0.05$) highest values in the aforementioned variables while T1 recorded significantly ($p<0.05$) lowest values when compared to the rest of the treatment groups. T3 however recorded the lowest ($p<0.05$) values in percentage non-motile sperm, percentage abnormal sperm and percentage dead sperm when compared to the rest of the dietary groups. T2 and T4 recorded a significantly similar ($p<0.05$) trend for all variables with the exception of percentage normal sperm that recorded a significantly similar ($p<0.05$) value with T3.

Table 4: Effect of black seed (*Nigella sativa*) supplemented diets on the Sperm characteristics of rabbit bucks

Parameter	T1	T2	T3	T4	±SEM	P value
Sperm count ($\times10^6$)	51.84^{c}	66.80^{b}	84.00^{a}	74.00^{b}	2.94	0.00
Percentage motile sperm	79.43^{c}	84.06^{b}	89.45^{a}	84.01^{b}	0.99	0.00
Percentage non-motile sperm	20.56^{a}	15.94^{b}	10.55^{c}	16.03^{b}	0.99	0.00
Percentage normal sperm	82.50^{b}	87.99^{a}	89.51^{a}	85.72^{b}	0.85	0.01
Percentage abnormal sperm	17.51^{a}	12.00^{b}	10.50^{b}	14.10^{ab}	0.84	0.01
Percentage live sperm	89.83^{ab}	88.62^{ab}	91.91^{a}	86.82^{b}	0.66	0.04
Percentage dead sperm	10.15^{ab}	10.82^{ab}	8.09^{b}	13.08^{a}	0.63	0.03
Round spermatid ($\times10^6$)	64.40^{c}	87.40^{b}	104.25^{a}	85.00^{b}	4.66	0.00
Elongated spermatid ($\times10^6$)	49.60^{c}	73.00^{b}	90.50^{a}	74.83^{b}	3.58	0.00

$^{abc:}$ Means on same row with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Mean

Increasing trends was observed in sperm characteristics variables of rabbit bucks as supplementation levels increases up to T3. This observation is consistent with similar work in rabbits (El-Tohamy *et al*., 2010), Albino rats (Bashaday, 2007; Mohammad *et al*.,2009; Al-Sa'aidi *et al.,* 2009, Parakah, 2010; Paradin *et al*., 2012; Al-Zarahani *et al*., 2012; Zohara *et al*., 2012; Sultan *et al*., 2014) and male chickens (Abdulkarim and Sardary, 2009). These authors suggested that effect of *Nigella sativa* on pituitary gland triggered the rise in spermatogenesis hormones. Furthermore, due to the presence of unsaturated fatty acids including

linoleic acid (about 60%) and oleic acid (about 20%), *Nigella sativa* seeds are able to boost sperm parameters like sperm count and motility as well as remove the abnormal, non-motile and dead sperm. The authors further suggested that the spermatogenetic effects can also be attributed to thymoquinone presence in *Nigella sativa* seed. The protective effect of thymoquinone on testicular parameters, further stimulates the production of testosterone by the leydig cells, which in turn act on the sertoli cells and peritubular cells of the seminiferous tubules, thus indirectly stimulates sperm production.

CONCLUSION

Based on the current findings, supplementation of unprocessed black seed up to 1.5% in male rabbit's diet has beneficial effects on the internal organs morphometric, sperm and carcass characteristics. Dietary supplementation up to 1% however demonstrated the best performance in terms of the aforementioned indices.

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