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BLACK SEED (*Nigella sativa*) DEMONSTATES PROPHYLACTIC AND THERAPEUTIC EFFECTS ON THE REPRODUCTIVE HORMONES, SPERM CHARACTERISTICS AND TESTICULAR MORPHOMETRIC OF HEAT-STRESSED RABBIT BUCKS

By

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Abstract

The reproductive hormones, sperm characteristics and testicular morphometric was evaluated in heat-stressed rabbit bucks fed Nigella sativa (NS) supplemented diet. Thirty growing rabbit bucks were assigned to five treatments of six rabbits each in a Completely Randomized Design. The five treatments were designated as T1-Positive control (PC), T2-Heat stress (HS), T3-rabbits fed NS before (HSbNS), T4-during (HSdNS) and T5- after (HSaNS) HS inducement. At the 14th week of the experiment, Luteinizing hormone (LH) and Testosterone was evaluated. Rabbits were humanely sacrificed, the testes, epididymis were weighed and the semen was analyzed for sperm count. Luteinizing Hormone (LH), testosterone were significantly (p<0.05) elevated at NS supplemented treatments (T3, T4 and T5) when compared to positive control (PC) and negative control (HS/-Control). The rabbit bucks on HS (-Control) had significantly low (p<0.05) concentration for these hormones compared to every other treatments. Nigella sativa supplemented treatments had significantly (p<0.05) higher testicular and epidiymal weights than T1 and T2. Similarly, Significant (p<0.05) increase was observed for at the NS supplemented treatments when compared to the controls (T1 and T2). Nigella sativa supplementation in heat-stressed rabbit's bucks' diet enhanced reproductive hormone (LH, testosterone) concentration, testicular and epididymal weight. Sperm count and in heat-stressed rabbit bucks were improved by diet supplemented with Nigella sativa. Nigella sativa demonstrates a prophylactic and therapeutic effect on reproductive hormones, testicular morphometric and sperm parameters in heat-stressed rabbit bucks.

Keywords: *Heat-stress, sperm, testosterone, testes, epididymis, rabbits.*

INTRODUCTION

Climate change is one of the major challenges of our time and adds considerable stress to the environment. The impacts of climate change are global in scope and unprecedented in scale (Adedeji *et al*., 2014). Recent evidence suggests even more rapid change, which will greatly, and in some cases irreversibly, affect not just people, but also animal species and ecosystems (Fuller *et al*., 2020). One of the compelling evidence of climate change is high environmental temperature thus heat-stress (DeCourten and Brander, 2017). Heat-stress affects animals in different ways, such as reducing the feed intake (Sohail *et al.*, 2010), increasing disease susceptibility (Pollaman, 2010), affecting productive and reproductive efficiency (Hansen, 2009). In tropical and subtropical parts of the world, heat-stress appears to be the major constraint to livestock production (Najar *et al.*, 2010) and it has the

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capability to adversely affect reproductive performance of livestock and in extreme instances can result in animal death.

Fertility and reproduction can be managed to a great extent, dietetically by antioxidants, because of their roles in free radical removal, prevention of tissue and cellular lesion. Plants are sources of important phytochemical compounds that exhibit antioxidant properties (Bhadoriya *et al.*, 2011). One of such plants is Black seed (*Nigella sativa*). Black seed is an important plant in medicine and it belongs to the family of *Ranunculaceae* (Mahdavi *et al.*, 2015). Most of the pharmacological activities of *Nigella sativa* are attributed to the presence of thymoquinone as an active component (Gilani *et al.*, 2004). Thymoquinone possesses antioxidant effects by enhancing the oxidant scavenger system as well as its potent anti-inflammatory mediators, prostaglandins and leukotriene (Salem *et al*, 2007).

Rabbits are very sensitive to high temperatures since they have few functional sweat glands limiting their ability to eliminate excess body heat (Verga *et al*., 2007). High ambient temperature can impair the reproductive performance of rabbits, and 30 °C is considered as the threshold point beyond which may result to infertility (Gacek, 2002). Often times, it is not possible to achieve the thermo-neutral zone and, consequently, rabbits are subjected to heat stress (Okab *et al*., 2008) and perspiration (evacuation of water via the skin) is low due to the presence of fur. Rabbit's sensitivity to heat stress is considered as the most important factor impacting negatively on their fertility, reproductive and physiological traits (Askar and Ismail, 2012)

Many additives have been reportedly added to rabbit feed or water as a way to alleviate adverse heat-stress effects to enhance reproductive performance of rabbits. Black seed (*Nigella sativa*) is commonly used as feed additive. It contains numerous minerals, nutrients and phytochemicals which makes it to have many therapeutic effects such as antioxidant and immune system stimulation.

However, there is dearth of information on the appropriate dietary supplementation level of *Nigella sativa* to enhance physiological, and most especially reproductive performances in growing rabbit bucks under normal environment. There is also paucity of information on the use of *Nigella sativa* to mitigate the deleterious effect of heat-stress on the physiological wellbeing and reproductive efficiency of growing rabbit bucks.

This research leveraged on the numerous beneficial phytochemicals embedded in *Nigella sativa* via appropriate feed supplementation, this would avail the possibility to mitigate the enormous deleterious effects of heat stress on the physiological wellbeing and reproductive efficiency 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 lat. 8° 8' 31.7940" N and long. 4° 14' 42.6696" E. The altitude is between 300m and 600m above the sea level while the mean temperature and annual rainfalls are 270C and 1247mm respectively (Ayinla and Odetoye, 2015).

Experimental animals and Management

Thirty weaned rabbit bucks (Chinchilla X New Zealand white; average body weight of 696.72g) obtained from a reputable breeding farm in Ogbomoso, Oyo State, Nigeria 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 about 2300 kcal/kg metabolizable energy. They were balanced for weight and assigned to five treatment groups of six rabbits each in a Completely Randomized Design (CRD). The five treatment groups were designated as T1, T2, T3, T4 and T5. The rabbit bucks were weighed at the commencement of the experiment and subsequently once per week. They were offered measured quantity of pelletized feed but *ad libitum and* cool, clean water was made available throughout the experimental period. All routine management practices were well observed.

Experimental treatments was partitioned as follow:

T1 (+Control): Heat stress was not induced and rabbits were not fed diet supplemented with *Nigella sativa*.

T2 (HS/-Control): Heat stress was induced and rabbits were not fed diet supplemented with *Nigella sativa*.

T3 (HSbNS): Heat stress was induced before rabbits were fed diet supplemented with *Nigella sativa* for 2 weeks.

T4 (HSdNS): Heat stress was induced while the rabbits were being fed diet supplemented with *Nigella sativa* for 2 weeks.

T5 (HSaNS): Heat stress was induced after rabbits were fed diet supplemented with *Nigella sativa* for 2 weeks.

Table 1: Gross composition of the experimental diets and calculated nutrients

Feed Ingredients (%)	Basal diet	Diet supplemented with *N.sativa*
Maize	32.61	32.61
Soybean meal	16.39	15.39
N.Sativa	0.00	1.00
Brewry dry grain	15.00	15.00
Rice husk	30.00	30.00
Fish meal (72%)	3.00	3.00
Oyster shell	2.00	2.00
Bone meal	0.25	0.25
Vitamin premix*	0.25	0.25
Salt	0.25	0.25

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Lysine	0.15	0.15
Methionine	0.10	0.10
TOTAL	100.00	100.00
Calculated Nutrients		
CP (%)	16.20	16.06
Metabolizable Energy** (Kcal/kg)	2335.76	2336.24
CF (%)	13.75	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).

Construction of heat chamber (microclimate)

Ultraviolet radiation chamber by David (1997) was adapted for the construction of heat chamber (microclimate) except where otherwise stated.

Induction of heat stress

Heat stress was induced by introducing the experimental rabbits into the specially constructed heat chamber (microclimate) for a total of 2 weeks (weeks 4 and 5), between the hours of 7:00 to 8:00 a.m. (Shebl *et al.,* 2008) on an alternate day basis (i.e. three times weekly).

The temperature and relative humidity of the heat chamber (microclimate) were controlled to achieve thermal humidity index (THI) of 32.00 ±2.00. The experimental rabbits were taken out of the heat chamber for the rest 46 hours period and they were given freshwater *ad libitum* so as to be properly hydrated.

The temperature and relative humidity of the heat chamber were measured through the aid of a digital incubator thermo-hygrometer. The thermal humidity index (THI) was studied on 30 minutes basis. The THI of above 30 was considered to induce very severe heat-stress in the experimental rabbits (Marai *et al*., 2002). *Nigella sativa* was supplemented at 1.0% (Hammed and Amao, 2022)

THI= t-[(0.31-0.31 x RH) (t-14.4); where RH is relative humidity/100. t = ambient temperature

<27.8= absence of heat stress
27.8- 28.9= moderate heat stress;
29.0- 30.0= severe heat stress; and
>30.0 = very severe heat stress (Marai *et al.,* 2002).

Data collection

Hormonal assays

At the end of the experiment, blood was collected via the marginal veins of the rabbits for hormonal assay into plain bottles. The protocol for the assays (Luteinizing hormone, Testosterone, Follicle stimulating hormone and Inhibin B) was carried out according to the method described for the kits [Elabscience® ELISA Assay, USA].

Testicular morphometric

The rabbits were humanely sacrificed in accordance with the ethics and regulation guiding the use of research animals as approved by the Faculty of Agricultural Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria. Testes were carefully dissected from the sacrificed animals and trimmed off adhering tissues. Testis length, testis weight and testis volume were measured. The testis length was measured with the aid of a pair of vernier calipers, testis weight was determined using a sensitive digital scale and the testis volume was measured by water displacement according to Archimedes principle (Adu and Egbunike, 2010).

Semen collection and analysis

The semen was collected via the caudal epididymis according to the procedure described by Mesbah *et al*., 2007. The sperm count (round and elongated spermatids) and morphology was determined as described by Seed *et al*., 1996. Live sperm and sperm motility were determined using the procedures of Bjorndahl *et al*., 2003 and Cheng *et al*., 2006 respectively.

Statistical analysis

Data collected were subjected to One-way Analysis of Variance (ANOVA), using the procedure of Statistical Analysis System (SAS, 2006) and means were separated using Duncan's multiple range test (DMRT) of the same statistical package.

RESULTS AND DISCUSSION

The results of this experiment are presented in Tables 2, 3 and 4

The reproductive hormones of heat-stressed rabbit bucks fed black seed (*Nigella sativa*) supplemented diet is shown in Table 2.

Table 2: Reproductive hormones of heat-stressed rabbit bucks fed diets supplemented with black seed (*Nigella sativa*)

Hormones (ng/mL) (n=30)	T1 (+Control)	T2 (HS/-Control)	T3 (HSbNS)	T4 (HSdNS)	T5 (HSaNS)	SEM
Luteinizing hormone	1.80[b]	1.07[c]	3.47[a]	3.47[a]	3.49[a]	0.21
Follicle stimulating hormone	2.74[a]	1.76[b]	2.76[a]	2.76[a]	2.76[a]	0.08
Testosterone	0.90[b]	0.32[c]	1.40[a]	1.41[a]	1.43[a]	0.08
Inhibin B	0.63[b]	0.32[c]	2.99[a]	2.99[a]	2.99[a]	0.25

abc: Means on same row with different superscripts differ significantly (P<0.05)

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SEM: Standard Error of Mean. HS: Heat-stress. HSbNS: Heat-stress before *N.sativa*. HSdNS: Heat-stress during *N.sativa*. HSaNS: Heat-stress after *N.sativa*

Reproductive hormones of heat-stressed rabbit bucks fed diet supplemented with black seed (*Nigella sativa*) is showed on Table 2. The reproductive hormones were all significantly ($p<0.05$) influenced by the treatments. Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), testosterone and inhibin B concentrations were significantly ($p<0.05$) elevated at T3, T4 and T5 (*Nigella sativa* supplemented treatments) when compared to positive control (PC) and negative control (HS/-Control). The rabbit bucks on HS (-Control) had significantly low ($p<0.05$) concentration for these hormones compared to every other treatments. The FSH however had statistically similar values across the treatments except for HS/-Control that recorded a significantly low ($p<0.05$) concentration.

Activation of the hypothalamic-pituitary-adrenal axis and consequent increase in plasma glucocorticoid concentrations are two most important responses to heat-stress (Aggarwal and Upadhyay, 2013). Heat-stress imparts detrimental effects on male reproductive hormones partly by disrupting the normal release of gonadotropin releasing hormone (GnRH) from the hypothalamus as well as LH and FSH from the anterior pituitary gland (Aggarwal and Upadhyay, 2013).

Hansen, 2010; Aggarwal and Upadhyay, (2013) have indicated that heat-stress can lead to decline in the circulating of testosterone and LH but increased serum cortisol. Heat-stress also leads to leydig cell apoptosis and reduction in testosterone biosynthesis in adult rat testes (Li *et al*., 2016). The finding of the current experiment as heat-stressed rabbit bucks had a significantly suppressed LH, FSH testosterone and inhibin B concentration levels aligned with these latter reports. Moreover, increased testicular temperature which could be as a result of heat-stress, elevates the generation of ROS in the male reproductive tract by directly affecting cellular metabolism (Belhadj *et al*., 2014) and by influencing stress hormone levels (Megahed *et al*., 2008). The resulting increase in ROS production, in turn, damages testicular germ cells and other endocrine cells to disrupt the hormonal balance, thereby curbing male fertility (Aggarwal and Upadhyay, 2013).

Dietary supplementation of *Nigella sativa* at T3, T4 and T5 caused a significant elevation in the reproductive hormone concentration of bucks in these treatments compared to the control treatments (T1 and T2). Consistent with this notion, Gokce *et al*. (2010); Paradin *et al*. (2012) has demonstrated that *Nigella sativa* exceptionally elevates LH, FSH, testosterone and inhibin B concentration when administered to animals. Gokce *et al*. (2010) confirmed that thymoquinone has protective effect on testicular parameters and this enhances production of LH which stimulates the production of testosterone by leydig cells of the seminiferous tubules and indirectly stimulates spermatogenesis via testosterone. Also, *Nigella sativa* elevates inhibin B (Paradin *et al*., 2012) which is important for leydig cells, sertoli cells and germ cells proliferation and maturation. Inhibin B plays a key role in the regulation of hypothalamic-pituitary-gonadal hormone axis during animal sexual development (Chada *et al.*, 2003).

The Testicular and epididymal morphometric of heat-stressed rabbit bucks fed black seed (*Nigella sativa*) supplemented diet is shown in Table 3.

Table 3: Testicular and epididymal morphometric of heat-stressed rabbit bucks fed diets supplemented with black seed (*Nigella sativa*)

Parameters (n=30)	T1 (+Control)	T2 (HS/-Control)	T3 (HSbNS)	T4 (HSdNS)	T5 (HSaNS)	SEM
Mean testes weight (g)	1.04^b	0.67^c	2.40^a	2.40^a	2.41^a	0.15
Mean testes length (cm)	2.20^b	1.06^c	3.44^a	3.46^a	3.48^a	0.58
Mean testes width (cm)	0.75^b	0.56^c	0.98^a	0.98^a	0.98^a	0.35
Mean testes volume (cm^3)	0.21^b	0.16^c	0.38^a	0.37^a	0.37^a	0.02
Mean epididymis weight (g)	1.09^b	0.85^c	2.67^a	2.66^a	2.65^a	0.16
Mean epididymis length (cm)	10.52^b	0.67^c	17.52^a	16.98^a	17.60^a	1.34

abc: Means on same row with different superscripts differ significantly ($P<0.05$)

SEM: Standard Error of Mean. HS: Heat-stress. HSbNS: Heat-stress before *N.sativa*. HSdNS: Heat-stress during *N.sativa*. HSaNS: Heat-stress after *N.sativa*

The testicular and epididymal characteristics of heat-stressed rabbits as influenced by diet supplemented with black seed (*Nigella sativa*) is shown in Table 3. All the testicular variables evaluated were significantly ($p<0.05$) influenced by the treatments. *Nigella sativa* supplemented treatments (T3, T4 and T5) had significantly ($p<0.05$) higher values for mean testes weight, mean testes length, mean testes width, mean testes volume, mean epididymis weight and mean epididymal length than T1 and T2. However, negative control (T2) recorded a significantly ($p<0.05$) lower testicular and epididymal parameters when compared to other treatments.

The testicular and epididymal characteristics of heat-stressed rabbits in the present study was significantly low. This might

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be as a result of the adverse effect of induced heat-stress. This is however in agreement with the report of Marai *et al*. (2002), who reported that heat-stress caused deterioration of germinal epithelium and partial atrophy of the seminiferous tubules thus reduction in the testicular weight of heat-stressed animals. Also, de Krester (2004) reported that heat-stress increases temperature of the testes. This increasing temperature of the testes can prevent spermatogenesis, reduce testosterone secretion and cause degeneration of most testicular cells like leydig cells and cell of the seminiferous tubules beside the spermatogonia. Moreover, the trend in the epididymal morphometrics of heat-stressed rabbits is a reflection of the values for gonadal morphometrics. Heat-stress may be responsible for the reduction in the weight and length of both gonads and epididymis. Anoh, (2017) also attributed the reduction in gonads and epididymis weight to heat-stress caused by degeneration in the germinal epithelium and partial atrophy of the seminiferous tubules. Ngoula *et al*. (2020) further reported that weights of reproductive organs including testes and epididymis decreased in animal exposed to heat-stress compared to the control. Garrigue, (2017) also reported a decreased reproductive organ weight in mice exposed to high temperature for 60 days. The present study however disagree with the report of Ngoula *et al*. (2017) who reported that no significant difference in the relative organ weights of younger guinea pigs exposed to induced heat-stress.

Rodents such as guinea pigs and rabbits cannot produce sweat to regulate their body temperature under heat conditions (Fiala *et al*., 2005). They also lack ability to manufacture their own vitamin C (Michel *et al*., 2011). This condition make them susceptible to marginal increase in tolerable environmental temperature. The decrease in testicular weight could also be associated with decrease in number of sertoli and germ cells within the seminiferous epithelium (Nicolino *et al*, 2001). Exposure of animals to heat-stress can also result in overproduction of ROS, which impairs cell membrane and nucleic acids and subsequently induce apoptosis (Nicolino *et al*, 2001).

On the other hand, increased testicular and epididymal weights were observed in *Nigella sativa* supplemented groups of the present study which might be as a result of thymoquinone which is an active compound of *Nigella sativa* thus confirming the trend from the previous (Mukhalad *et al*., 2009; El-Tahomi *et al*., 2010; Paradin *et al*., 2012, Hammed and Amao, 2022) studies. These researchers argued that supplementing black seed (*Nigella sativa*) up to 1.0% enhanced the testicular and epididymal characteristics of rabbit bucks. Moreover, supplementing *Nigella sativa* could enhance the overall reproductive performance in growing rabbits. Paradin *et al.* (2012) further reported that thymoquinone (an active compound in *Nigella sativa*) enhances testosterone and other androgens. Moreover, the testes, epididymis and other reproductive organs are structurally and physiologically dependent on testosterone and other androgens. Basically, testosterone stimulates growth and secretory activities of reproductive organs (Nassar and Leslie, 2022). Therefore, a significant increase in these hormones could increase the number and function of somatic and germinal cells of testis thus result to an increased testes and epididymis weight.

The sperm characteristics of heat-stressed rabbit bucks fed diets supplemented with black seed (*Nigella sativa*) is shown in Table 4

Table 4: The sperm characteristics of heat-stressed rabbit bucks fed diets supplemented with black seed (*Nigella sativa*)

Parameters (n=30)	T1 (+Control)	T2 (HS/-Control)	T3 (HSbNS)	T4 (HSdNS)	T5 (HSaNS)	SEM
Sperm count ($\times 10^6$)	71.80^b	40.60^c	91.40^a	91.60^a	91.40^a	4.07
Motile sperm (%)	71.76^b	48.65^c	86.22^a	86.28^a	86.75^a	3.02
Non-motile sperm (%)	28.23^b	51.34^a	13.77^c	13.71^c	13.24^c	3.02
Normal sperm (%)	74.28^b	53.38^c	88.05^a	88.21^a	88.01^a	2.78
Abnormal sperm (%)	25.71^b	46.61^a	11.94^c	11.78^c	11.98^c	2.78
Live sperm (%)	82.96^b	61.98^c	85.81^a	86.13^a	85.88^a	1.92
Dead sperm (%)	17.03^b	38.01^a	14.18^c	13.86^c	14.11^c	1.92
Round spermatids	65.40^b	38.40^c	98.00^a	96.60^a	97.60^a	4.90
Elongated spermatids	59.20^b	37.20^c	97.40^a	98.80^a	98.00^a	5.22

abc: Means on same row with different superscripts differ significantly ($P<0.05$)

SEM: Standard Error of Mean. HS: Heat-stress. HSbNS: Heat-stress before *N.sativa*. HSdNS: Heat-stress during *N.sativa*. HSaNS: Heat-stress after *N.sativa*

The sperm characteristics of heat-stressed rabbit bucks as influenced by black seed (*Nigella sativa*) supplemented diet is presented in Table 4. Significant ($p<0.05$) differences were observed in all gonadal sperm variables evaluated. Significant

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(p<0.05) increases were recorded for sperm count, percentage motile sperm, percentage live sperm, round spermatids and elongated spermatids at the *N.sativa* supplemented treatments (T3, T4 and T5) when compared to the controls (T1 and T2). On the other hand, non-motile sperm, abnormal sperm and dead sperm were significantly (p<0.05) decreased at the *N.sativa* treated groups (T3, T4 and T5) when compared to the controls (T1 and T2). The HS/-Control (T2) had significantly (p<0.05) lowest values in terms of sperm count, percentage motile sperm, percentage live sperm, round spermatids and elongated spermatids when compared to other treatments. This treatment however, had significantly (p<0.05) higher values for percentage non-motile sperm, percentage abnormal sperm and percentage dead sperm in comparison to the rest of the treatments.

The gonadal semen analysis carried out in the present experiment revealed that fundamental sperm parameters-sperm count, sperm motility, sperm viability, and sperm morphology (W.H.O, 2010) were all significantly but adversely affected by heat-stress. The sperm count reflects semen quality and male reproductive potential whereas sperm motility, viability and morphology are able to predict fertility (Kompanje, 2013). However, exposure of animals to heat-stress could lead to thermo-dysregulation which might result to adverse significant changes in the sperm characteristics (Hjollund *et al*., 2002) as observed in the present study. Heat-stress can also result in testicular hyperthermia which thus causes adverse modification of sperm characteristics (reduced sperm count and overall poor semen quality) and overtime may result in infertility (Durairajanayagam *et al.*, 2014). Hyperthermia could elevate testicular and epididymal temperatures thus decrease the synthesis of sperm membrane coating protein, resulting in higher amount of morphologically abnormal sperm (Durairajanayagam *et al*., 2014). Sperm motility is also suppressed in hyper-thermic testes (Wechalekar *et al.*, 2010) Furthermore, heat-stress has been reported to suppress spermatogenesis hence decreasing sperm production (Durairajanayagam *et al*., 2014). Basically, exposure of animals to heat-stress causes deterioration of sperm morphology and impairs motility as well as sperm production which has a deleterious effect on the overall male fertility.

On the other hand, the present experiment also revealed significant increase in sperm count, sperm motility, sperm viability, and sperm morphology in *Nigella sativa* treated groups. The percentage increase of these parameters probably demonstrate the effect of *Nigella sativa* supplementation in the feed offered to these treatment groups of heat-stressed rabbits. This is consistent with the report of Al-Sa'aidi *et al*. (2009) that daily oral administration of *Nigella sativa* extract led to a clear improvement in sperm motility and overall fertility of male rats. Also, supplementing *Nigella sativa* led to increase testosterone level (Al-Sa'aidi *et al.*, 2009; Mclachlan *et al*., 2002). The increased testosterone can in turn enhance spermatogenesis and spermiogenesis in seminiferous tubules. Moreover, testosterone is responsible for epididymal function and sperm maturation (Haseena *et al*., 2015). This testosterone increment might however due to the effect of *Nigella sativa* on the main enzymes which affect metabolism and steroid secretion in the testes.

The result of the present experiment further agrees with previous studies (Haseena *et al*., 2015; Sevim *et al*., 2021) that *Nigella sativa* contains alkaloid and phenols which stimulates the secretion of testosterone and FSH. Paradin *et al*. (2012) showed that *Nigella sativa* seeds could increase fertility in male rats. This implies that *Nigella sativa* has the potential to protect rabbit's sperm characteristics from the adverse effect of heat-stress.

CONCLUSION

It can be concluded from this research that, black seed (*Nigella sativa*) supplementation in heat-stressed rabbit's bucks diet enhanced testicular and epididymal morphometric. Sperm count, sperm motility, sperm morphology, sperm viability, and reproductive hormone (LH, testosterone, FSH and inhibin B) concentration in heat-stressed rabbit bucks were improved by diet supplemented with *Nigella sativa*. *Nigella sativa* demonstrates a prophylactic and therapeutic effect on reproductive hormones, testicular morphometric and sperm parameters in heat-stressed rabbit bucks.

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