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Original Article

Thermoregulatory, libidinal and seminal responses of rabbit bucks to spice-supplemented diets

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ABSTRACT

This study evaluated the libidinal, seminal, and thermoregulatory effects of ginger, garlic, and onion supplementations in rabbit bucks. Fifty-five bucks were allotted to 11 experimental diets comprised of a basal diet with no spices and 9 others containing ginger, garlic, and onion at 5 g, 10 g, or 15 g/kg feed, with the eleventh diet supplemented with vitamin C at 400 mg/kg feed. In the wet and dry seasons, the libido, seminal parameters, respiratory rate (RR), and rectal temperature (RT) of the bucks were studied for 8 weeks. The temperature-humidity index (THI) of the rabbit pen was also monitored during the experiment. Data was analysed using multivariate analysis of the general linear model and probability with alpha set at <5% after separation by Duncan Multiple Range Test. Seasonal THI was significantly higher ($p<0.05$) in the dry season (30.22°C) than the wet season (28.92°C). Animals allotted to onion at 10 g/kg feed had the highest RR in the wet season, while the treatments had no significant effect on RR in the dry season. Bucks fed the lowest level (5 g/kg feed) of ginger and onion had significantly lower ($p<0.05$) RT in both the wet and dry seasons. Spice supplementation had a significant effect ($p<0.05$) on the buck's libidinal and sem1nal parameters in both wet and dry seasons. Season significantly ($p<0.05$) affected RR, RT, libidinal, and seminal parameters in spite of spice supplementation. The study concluded that spice supplementation improved the libidinal, seminal, and thermoregulatory responses of the rabbit bucks.

K E Y W O R D S : *Dry Season, Temperature - Humidity Index, Rectal temperature, Respiratory rate*

INTRODUCTION

Reproductive efficiency in domestic animals is fundamental to food security, optimum animal protein consumption and profit for livestock farmers. Rabbit production has the ability to reduce the animal protein consumption deficiency among the rural and urban population of Africa (Sikiru *et al*., 2024). The thermal environment is determined by temperature and humidity which is a controlling factor in energy metabolism and exchange. Warm blooded animals such as the rabbit have thermo-neutral zones beyond which loss of body heat is hampered leading to heat stress (Rahman *et al*., 2018). One of the indices that integrate temperature and humidity to adequately capture the thermal environment is the temperature - humidity index (THI). THI is inarguably the commonest thermal comfort index that was developed to assess the impact of the thermal environment on thermoregulatory status of animals (Ajao *et al.*, 2022; Daader *et al.*, 2018). Rabbit have limited capacity to lose body heat when ambient temperature and humidity are high and beyond the thermoneutral zones because of their

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thick fur and relatively non - functional sweat glands (Oladimeji *et al*., 2022). Due to this incapability, there is build up of heat inside the animal leading into heat stress. Nonetheless, increased panting and vasodilatation of the skin surface help the rabbit to lose excessive body heat (Ajao and Ola, 2021) though under chronic heat stress conditions body functions with reproduction coming first become impaired (Lenis *et al*., 2015). Rahman *et al*., 2018 reported that 32°C is the threshold beyond which physiological functions may occur in the animal but other studies have shown that ambient temperatures as low as 27°C (Marai *et al*., 2008) may be injurious to buck's fertility. Thus, heat stress, particularly under the hot and humid zones of the tropical and arid regions, is ranked as the most important problem facing rabbit production when compared to poor quality diets, diseases and parasites (Oladimeji *et al*., 2022). Heat stress initiate disturbance in rabbits' body temperature and hormonal secretions by disrupting protein and energy metabolism culminating in poor growth, loss of libido, reduced seminal volume, motility and viability, abnormalities and apoptosis in spermatozoa leading to reduced reproductive performance (Menegassi *et al*., 2016).

Rabbit farmers have used managerial, nutritional and physiological interventions to improve reproductive efficiency under heat stress conditions (Liang *et al*., 2022). However, the concern that aggregate of hormonal residues in the meat of the treated animals will be harmful to the consumer and the relative unavailability of such hormones discourages farmers from using them in enhancing rabbit buck fertility. In addition, vitamins C and E, common anti - stress medicaments have been reported to be less effective as plant bioactive substances in reducing heat stress (Pawar and Hugar, 2012). Hence, researchers have focused more on natural alternatives to synthetic drugs. For example, Daadeer *et al*. (2018) and Ogbuewu *et al*. (2013) studied the effect of natural plant - based antioxidants on heat stress alleviation and improvement of reproduction in the rabbit. These plants, especially ginger, garlic and onion contain phytochemicals that have pro- fertility and anti stress effects (Ramandeep *et al*., 2013). This study therefore investigated the dosages of ginger, garlic and onion that will improve thermoregulation and the reproductive efficiency of rabbit bucks under the tropical condition that is conducive to heat stress.

MATERIALS AND METHODS

Procurement and Processing of Test Ingredients

Sacks of fresh garlic, ginger and onion bulbs were obtained from the market. The spices were washed, peeled and sliced into chips. The chips were air-dried for 10 days and ground into powder by the use of an electric Binatone grinder. The powders were kept in polythene bags inside air – tight containers until incorporation into experimental diets.

Animal Procurement and Management

The study was conducted at the Teaching and Research Farm, Obafemi Awolowo University, Ile – Ife, Nigeria, situated at latitude 7° 33' 0" North and longitude 4° 34' 0" East. Five months old 60 rabbit bucks were procured from reputable farmers and individually housed in metal cages of the dimension 35cm x 42cm x 54cm. The bucks were fed forages and layers mash and provided with clean water.

Then 55 bucks were allotted to 11 treatments that correspond to the 11 experimental diets at average weight and age of 2kg and 8 months, respectively. The experimental diets comprised of a basal diet with no spices (control), nine others containing ginger, garlic and onion at 5g, 10g or 15g/kg feed and the positive control (supplemented with 400mg of vitamin C/kg feed).

Monitoring of Ambient Temperature and Relative - Humidity

Ambient temperature (AT) and relative – humidity (RH) of the rabbit pen were observed by means of a digital thermo-hygrometer to determine the Temperature – Humidity index (THI) for the entire duration of the study in each of the season. Temperature and humidity readings were recorded when the day was hot between12pm - 2pm. The weekly average of AT and RH values were used to compute THI of the animals' pen using Equation 1(Marai *et al*., 2002)

$$THI = t - [(0.31 - 0.31 \times RH)(t - 14.4)] \quad (1)$$

Where; RH = relative humidity, t(AT) = temperature (degree Celsius).THI values obtained in the experiment was categorized as observed for the tropics; <27.8°C = absence of heat stress, 27.8–28.9°C = moderate heat stress, 28.9–30°C = severe heat stress and above 30°C = very severe heat stress (Marai *et al*., 2002).

Determination of the Biophysical Thermoregulatory Response of Experimental Animals

The respiratory rate (RR) of the experimental animals was determined by counting the flank movements of a still animal for 1minute twice weekly at two days interval for 8 weeks. Immediately after determining the RR, a rectal thermometer was used to take the rectal temperature (RT) of the bucks by inserting the thermometer into their rectum. After the thermometer emitted a beeping sound, the value displaced on the LCD was then recorded as the RT. Data was collected in 3 animals per treatment.

Reproductive Evaluation of Experimental Animals

Determination of libidinal and seminal parameters

The bucks' libidinal and seminal responses were observed over a period of 8 weeks in the wet and dry seasons.

The collection of ejaculates and libidinal parameters started a week before the introduction of the experimental diets. Observations and samples were collected between 11am - 2pm when the THI was usually highest for the day. Ramandeep *et al*. (2013)'s procedure was modified to evaluate the libido of the rabbit bucks which consisted of mount latency (ML), number of mounts (NMT) and ejaculatory latency (EL). ML, NMT and EL were assessed at two days interval twice in a week between 11am and 1pm in both the wet and dry seasons. A doe was placed together with a buck in the buck's cage. Semen samples were collected from the bucks at weekly interval over the experimental period using a disposable artificial vagina (Ajao and Ola, 2021). Semen volume, sperm concentration, motility, viability and abnormality were determined according to the procedures of I.R.R.G. (2005).

Experimental design and data analysis

The animals were allotted to 11 treatments arranged in a 3 x 3 +2 factorial layout using completely randomized design. Data was subjected to analysis of variance using the general linear model. Means was separated by Duncan Multiple Range Test with alpha = <0 .05

RESULTS AND DISCUSSION

Ambient Temperature, Relative - Humidity and Temperature - Humidity Index observed during the Study

The mean temperature –humidity index (THI) recorded in this study was 28.92˚C and 30.22˚C (Table 1) in the wet and dry seasons, respectively. The seasonal difference in THI is consistent with the tropical climate and may be attributed to higher ambient temperature of the dry season (Ajao and Ola. 2021).

In addition, the prevalent THI in both wet and dry seasons implied that the rabbit bucks were severely heat stressed (Marai *et al.* 2002).

Table 1: Seasonal means of temperature, relative-humidity and temperature - humidity index recorded during the experiment Season

Parameter	Wet	Dry	SEM
Temperature (˚C)	30.73[a]	33.11[b]	0.12
Relative- humidity (%)	64.50[a]	50.40[b]	0.59
Temperature-humidity index(˚C)	28.92[a]	30.22[b]	0.10

[ab]*Means with different superscript within the row are significantly different (p<0.05)*

Biophysical Thermoregulatory Response of Experimental Rabbit Bucks

Respiratory rate (RR) (216.25c/m - 270.58c/m) in the wet and dry seasons was not significantly different among the experimental groups with the exception of the group fed onion at 15g/kg feed in the wet season. The wet seasonal RT (39.34°C) was significantly higher (p<0.05) than the dry seasonal RT (38.98°C). Animals allotted to ginger and garlic at 5g/kg feed had the lowest rectal temperature in the wet and dry seasons respectively. This result is similar to the findings of Zeweil *et al.* (2016) and could be attributed to the actions of the phytochemicals in the spices (El-Ratel *et al.*, 2022). The observed higher wet seasonal RT is different from the conclusions of Ajao *et al.* (2022) and may be due to the high relative-humidity of the wet season (Jimoh and Ewuola, 2018).

Table 2: Respiratory rate and rectal temperature of experimental rabbit bucks to spice – supplemented diets

Spice	Spice Level	Wet Season RR(c/m)	Dry Season RR(c/m)	Wet Season RT(°C)	Dry Season RT(°C)
Zero spice	0gram	236.25^{a}	297.17	39.29^{ab}	39.20^{b}
Ginger	5gram	224.92^{a}	212.67	39.24^{ab}	38.80^{a}
	10gram	247.83^{a}	219.33	39.31^{ab}	38.93^{ab}
	15gram	233.67^{a}	269	39.21^{ab}	39.01^{ab}
Garlic	5gram	216.25^{a}	227.33	39.07^{a}	38.93^{ab}
	10gram	258.75^{a}	266.83	39.46^{b}	38.94^{ab}
	15gram	247.17^{a}	274	39.45^{b}	39.22^{b}
Onion	5gram	239.25^{a}	242.83	39.32^{ab}	38.94^{ab}
	10gram	270.58^{b}	283.17	39.49^{b}	38.98^{ab}
	15gram	251.25^{a}	295.67	39.42^{ab}	38.92^{ab}
Vitamin C	400mg	254.17^{a}	288.5	39.50^{b}	38.93^{ab}
	±SEM	12.70	27.27	0.11	0.10
	Season Mean	243.64±3.83^{A}	261.50±8.22^{B}	39.34±0.03 ^{A}	38.98±0.03^{B}

^{ab}Means with different superscripts within the columns are significantly different (p<0.05), ^{AB}Means with different superscripts across the rows are significantly different (p<0.05); RR = Respiratory rate; RT = Rectal temperature, c/m = count per minute; SEM = Standard error of mean.

Reproductive Response of Experimental Animals to Spice Supplemented Diets

Libidinal parameters of experimental rabbit bucks fed spice – supplemented diets

Seasonal means of mount latency (ML) was not significantly different (p>0.05) among the wet (12.50s) and the dry seasons (20.05s) contrary to seasonal means of Ejaculatory latency (EL) (5.96s - 18.92s) and number of mounts (NMT) (17.50 c/3m - 8.98 c/3m) of the studied bucks. The recorded ML is higher than the report of Jimoh *et al*., 2021 which may be due to the higher temperature - humidity index prevalent this study. The elongated EL and lower number of NMT recorded in the dry season showed that the libido of the rabbit bucks was depressed and could be attributed to the higher heat stress in the dry season (Sabés-Alsina *et al*., 2015). ML (9.66s - 48.70s) and EL (4.04s - 62.27s) were lower in supplemented groups and the control in both seasons. Number of mounts (NMT) in the wet season was highest (21.10c/3m) in the group allotted to garlic at 5g/kg feed. The reduction of both ML and EL and the high NMT value implies spice supplementation improves the libido of the rabbit bucks which is similar to the findings of Ezike *et al*., 2023.

Table 3: Libidinal response of experimental rabbit bucks to spice – supplemented diets

Spice	Spice Level	ML1 (s)	ML2 (s)	EL1 (s)	EL2 (s)	NMT1 (c/3m)	NMT2 (c/3m)
Zero spice	0gram	9.66^{a}	13.39^{ab}	4.04^{a}	7.64^{a}	18.98^{bc}	11.54
Ginger	5gram	12.45^{a}	10.22^{a}	4.1^{a}	5.70^{a}	17.15^{bc}	8.67
	10gram	9.09^{a}	32.61^{ab}	4.63^{a}	31.56^{ab}	17.25^{bc}	7.71
	15gram	3.99^{a}	7.58^{a}	4.67^{a}	10.3^{a}	19.77^{bc}	11.21
Garlic	5gram	6.72^{a}	25.99^{ab}	3.81^{a}	29.36^{ab}	21.10^{c}	10.54
	10gram	17.57^{a}	16.68^{ab}	8.21^{ab}	14.78^{a}	15.13^{ab}	9.54
	15gram	14.57^{a}	13.01^{ab}	7.54^{a}	5.54^{a}	19.15^{bc}	11.50
Onion	5gram	8.85^{a}	19.72^{ab}	5.13^{a}	16.98^{a}	15.96^{b}	6.38
	10gram	3.98^{a}	9.49^{a}	3.65^{a}	7.65^{a}	16.71^{b}	9.58
	15gram	11.78^{a}	23.17^{ab}	5.21^{a}	16.39^{a}	19.31^{bc}	5.54
Vitamin C	400mg	38.87^{b}	48.70^{b}	14.6^{b}	62.27^{b}	11.98^{a}	6.54
	SEM	6.49	10.92	2.29	11.84	1.53	1.77
	Season Mean	12.50± 1.96	20.05± 3.29	5.96± 0.69^{A}	18.92± 3.57^{B}	17.50± 0.46^{A}	8.98± 0.54^{B}

Values with different superscript are significantly different within the column and the row (p<0.05); ML= Mount Latency; EL = Ejaculatory Latency; NMT = Number of mount per minute; 1= Wet season; 2= Dry season; s = seconds; c/3m= count per 3 minutes;

Seminal parameters of experimental rabbit bucks fed spice – supplemented diets

Semen parameters with the exception of semen volume are significantly lower ($p<0.05$) in the dry season (Table 4) which is not different from the findings of Sabés-Alsina *et al*., 2015.

Table 4: Effect of season on seminal parameters of experimental rabbit bucks

Seminal Parameters	Wet Season	Dry Season
Semen volume (ml)	0.64±0.02	0.67±0.02
Semen motility (%)	91.01±1.40[a]	73.13±1.61[b]
Live sperm cells (%)	91.22±1.28[a]	81.76±1.42[b]
Abnormal sperm cell (%)	11.60±0.38[a]	25.13±0.80[b]
Sperm concentration (10^6)	186.00±10.50[a]	251.00±14.70[b]

[ab]Means with different superscripts within the rows are significantly different ($p<0.05$)

In both the wet and dry seasons, semen volume (SV) (Figure 1) increased with levels of supplementation in supplemented groups though garlic and onion groups that had their lowest SV value at the highest level of supplementation. In the wet season, sperm motility (SM) among treatment groups (Figure 2) did not differ significantly ($p>0.05$). In the dry season, in ginger and garlic supplemented groups, sperm motility (SM) increased significantly ($p<0.05$) as spice inclusion levels increased while the opposite was the case in onion supplemented groups. These results is similar to the findings of Ezike *et al*. (2023) and Olojo *et al*.(2022) and may imply that the spices facilitated the production of seminal fluid and motility of sperm cells through their androgenic actions (Nagendra *et al.*, 2014).

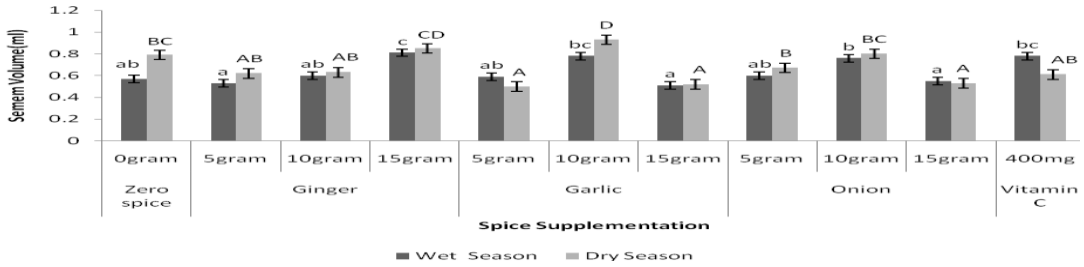


Figure 1: Semen volume of rabbit bucks fed spice - supplemented diets
[aA]*Bars with different letters are significantly different ($p<0.05$)*

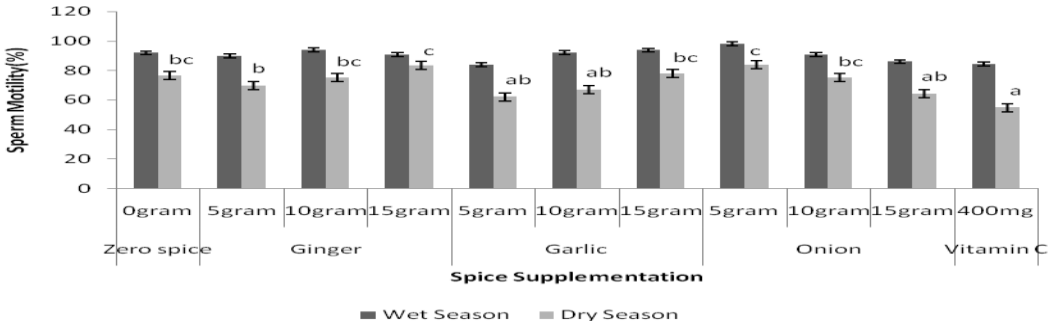


Figure 2: Sperm mortality of rabbit bucks fed spice - supplemented diets
[ab]*Bars with different letters are significantly different ($p<0.05$)*

In the wet season, live sperm cell (LSC) (Figure 3) was higher than 80% in all experimental groups. In the dry season, LSC was significantly lower ($p<0.05$) in the positive control group. This result is different from the report of Olojo *et al*. (2022).

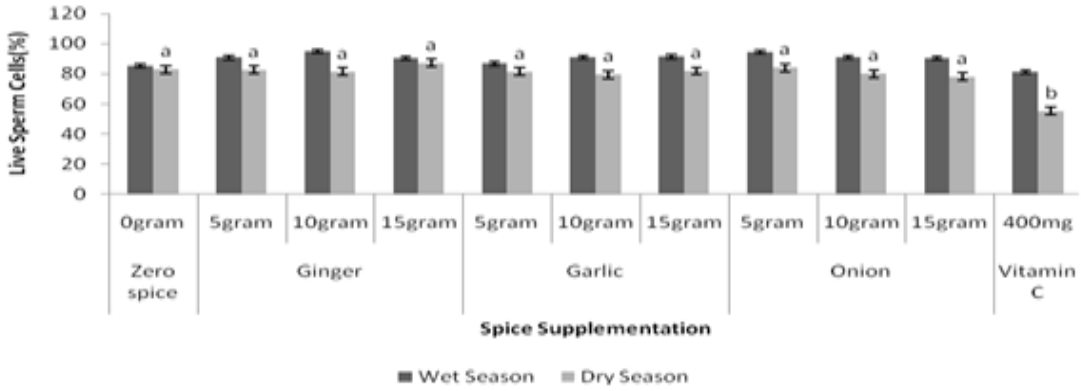


Figure 3: Percentage live sperm cell of rabbit bucks fed spice - supplemented diets
[ab]Bars with different letters are significantly different (p<0.05)

In the wet season, abnormal sperm cell (ASC) (Figure 4) was highest (13.35%) in groups allotted to garlic at 10g/kg feed (8.75%). In the dry season, high ASC values were observed in spice supplemented and control groups which were significantly different from ASC (16.42%) observed in the positive control. Spice supplementations and Vitamin C reduced sperm abnormality in agreement to the findings of El-Kholy *et al*., 2021 and might be attributed to their anti - perioxidation effects.

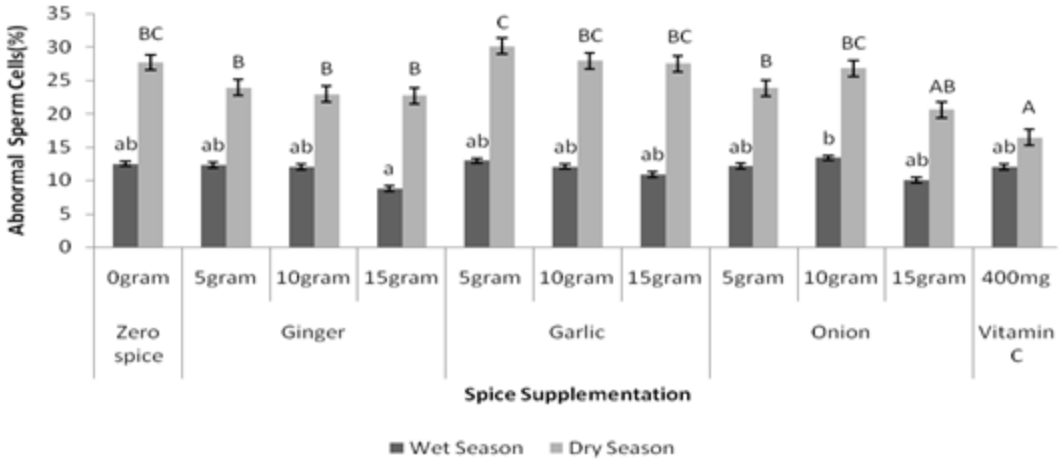


Figure 4: Percentage abnormal sperm cell of rabbit bucks fed spice - supplemented diets
[ab]Bars with different letters are significantly different (p<0.05)

Sperm concentration (SCN) (231 x 10^6 and 338 x 10^6) (Figure 5) in the wet season was not significantly different (p>0.05) among treatment groups contrary to the findings of Ezike *et al*. (2023). In the dry season, SCN (> 360 x 10^6) in groups allotted to the control, ginger and garlic supplementation was significantly higher (p<0.05) than the SCN observed in the groups allotted to positive control and onion supplementation similar to the conclusions of El-Kholy *et al*., 2021. The effect of the supplements might be attributed to their androgenic effect due to their phytochemical constituents (Ebeid *et al*., 2023).

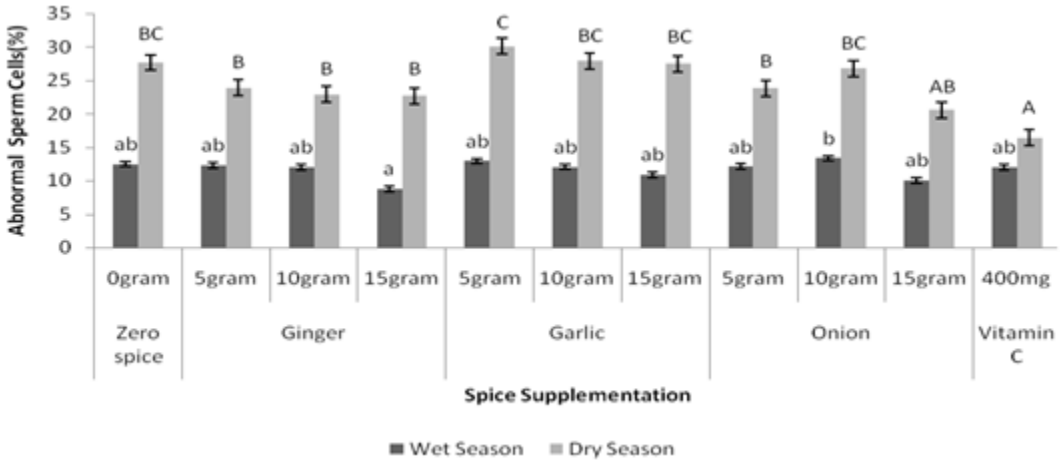


Figure 5: Sperm concentration of rabbit bucks fed spice - supplemented diets
[abc]Bars with different letters are significantly different (p<0.05)

CONCLUSION AND RECOMMENDATION

The temperature - humidity index monitored during the study showed that the rabbit bucks were raised under heat stress in both wet and dry seasons. Season and spice supplementation affected thermoregulation in the studied animals. Ginger and garlic at 5g/kg feed improved libidinal and seminal parameters of the rabbit bucks regardless of the impact of seasons. It is recommended that further research should be carried out on the effect of the spices on stress and reproductive hormones in rabbit bucks in order to understand the endocrinlogical mechanism by which application of dietary spices mitigate heat stress and improve fertility.

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Authors' Contributions

ABH was responsible for the management of the animals, sample collection and data analysis and writing the manuscript. OSI designed the experiment, assisted in data analysis and interpretation and reading and reviewing the manuscript.

Ethical Statement

The study was approved by the Postgraduate Research Committee of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

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