

Effects of Dietary Supplementation of Ginger, Garlic and Onion on Semen Profile, Haematological and Serum Antioxidant Status of Rabbit Bucks Raised in the Dry Season of the Humid Tropic

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ABSTRACT

Purpose: The study evaluated the semen, haematological and antioxidant status of rabbit bucks fed ginger, garlic and onion supplemented diets under a severely heat stressed condition. This is to evaluate the effect of heat stress and spice supplementation on seminal parameters, haematology and antioxidant capacity of rabbit buck raised in the tropics.

Research Method: Fifty - five bucks were allotted to 11 treatments comprising of control diet, three each of ginger, garlic and onion at 5g, 10g and 15g/kg feed plus positive control diet. Seminal, haematological and antioxidant traits of the bucks and their pen's Temperature – Humidity index (THI) were observed for 8 weeks.

Findings: THI (29.30 - 31.10°C) recorded during the study indicated the prevalence of severe to very severe heat stress. Heat stress impacted negatively more on sperm morphology than on other seminal parameters. Sperm cell motility, viability and abnormality were similar within supplemented groups and the controls. Semen volume and concentration were highest in animals fed garlic at 10 and 15g/kg feed respectively. Spice supplementation had no significant effect ($p > 0.05$) on the haematology and total antioxidant capacity (TAC) of the experimental animals.

Originality/value: The study concluded that spice supplementation mitigated heat stress effect on sperm cell morphology and was not deleterious on haematology and TAC of the bucks.

Keywords: Antioxidant, Seminal, Supplement, Temperature – Humidity index, Total antioxidant capacity

INTRODUCTION

In the tropics, high environmental temperatures alone or in combination with high humidity induce heat stress that hampers animal wellbeing and militate against animal productivity (Okab *et al.*, 2008). Hence, heat stress is one of the foremost constraint to rabbit production in Nigeria (Oseni, 2014) because rabbits exposed to elevated ambient temperatures are subjected to imbalances in their thermoregulatory mechanism (Habeeb *et al.*, 1999) leading to, disturbances in feed intake, feed utilization, water metabolism, blood parameters, enzymatic reactions and hormonal secretions.

In male animals, exposure to heat stress hampers spermatogenesis and decreases testosterone levels. Heat stress elevates oxidative stress due to increase in production of reactive oxygen species (ROS), which can lead to DNA damage disruption of cell membrane fluidity and apoptosis of spermatozoa (Hansen, 2009). Consequently, in the rabbit buck, heat stress decreases testosterone concentration, reduces

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libido, disrupts spermatogenesis, and causes apoptosis and morphological abnormalities of sperm cells thereby resulting in reduced or loss of fertility (Finzi *et al.*, 2000).

To attenuate the lethal effect of heat stress on rabbit production and reproduction, farmers have employed managerial, nutritional and physiological approaches (Ayyat *et al.*, 1997). In recent times, however, researchers have focused more on plants due to their antioxidant ability. This is because these plants contain bioactive phytochemicals such as flavonoid, alkaloid and organosulphur compounds which can ameliorate heat stress condition and improve productivity (Pawar and Hugar, 2012) through mopping up ROS and restoration of oxidative damages. The near availability all the year round, relative safety and cheapness of these plants make them a viable replacement to synthetic medicaments. Among the abundant flora in the tropics, *Allium sativum* (garlic), *Zingiber officinale* (ginger) and *Allium cepa* (onion) have shown to have pro growth, fertility (Nagendra *et al.*, 2014) and antioxidant effects (Pawar and Hugar, 2012). Though, these spices are widely used in African folk medicine to sustain human health and fertility, their use have not been well exploited in improving animals' production and health in general and in the rabbit in particular, especially in Nigeria.

Assessment of blood provides the opportunity to ascertain the health status of animals. As a result of this, haematological indices had proved to be an efficient assay of the adverse effect of stress that may emanate from poor nutrition, extreme weather and diseased conditions (Daramola *et al.*, 2005).

Total antioxidant capacity (TAC) is essentially the measure of the amount of free radicals scavenged by a test solution (Rubio *et al.*, 2016) and is indicative of oxidative repair or increased resistance to oxidative damage during circumstances of heat stress or disease (Koracevic *et al.*, 2001). Hence, the determination of TAC embodies an appropriate biochemical index for estimating overall antioxidant status (Nemec *et al.*, 2000).

This study examined the seminal, haematological and antioxidant responses of rabbits fed garlic,

ginger and onion supplemented diets. This is to investigate the efficacy of the spices in ameliorating the effect of heat stress in the rabbit in order to improve the welfare and productivity of the animal raised under the humid tropic which is conducive to heat stress.

MATERIALS AND METHOD

Experimental site and procurement of experimental animals

Fifty five rabbit bucks aged between 15 – 20 months with an average body weight of 1.5kg were sourced from reputable farms and the Rabbitary Unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile – Ife, Nigeria where the experiment was conducted. All the bucks were healthy.

Procurement and verification of spices

Fresh sacks of ginger, garlic and onion, which were the focal test materials were purchased from a vegetable market in Ilorin, Kwara State, Nigeria. The sample of garlic, ginger, and onion were identified and confirmed with vouchers numbered 17735, 17736 and 17737 respectively at the Herbarium, Department of Botany, Obafemi Awolowo University, Ile - Ife.

Experimental design

The animals sourced outside the Rabbitary Unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile – Ife were acclimatised for 2 weeks before the commencement of the study.

Fifty five of the rabbit bucks were individually housed in metal cages of the dimension 35cm x 42cm x 54cm. They were fed with 11 experimental diets which were the basal diet containing no spices (control) and ten others containing ginger, garlic and onion supplements at 5g, 10g or 15g/kg feed and the positive control supplemented with 400mg Vitamin C/kg (positive control) feed. Water and feed were provided ad libitum in concrete feeders and drinkers moulded with

openings to permit only the head of the animal to prevent contamination and waste. The experiment lasted for 8 weeks between the months of January and February, 2018 in the dry season.

Monitoring of ambient temperature and relative humidity

The ambient temperature and relative humidity was measured daily by means of a thermo-hygrometer from the beginning of the experiment until the end. Both temperature and humidity readings were taken between 11.00 am to 1.00 pm daily when the ambient temperatures were highest. The daily average of the recorded temperature and relative – humidity values for week 0, 4 and 8 were used to compute THI of the animals using the equation derived by Marai *et al.* (2002):

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

Where; RH = relative humidity, t = temperature (degree Celsius).

THI values obtained in the experiment was categorised 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).

Collection of seminal, haematological and total antioxidant parameters

A doe with a disposable artificial vaginal (AV) (Ola, 2016) positioned under it was used to collect semen samples from the selected bucks once a week for the experimental period. Samples were collected between 11am and 1pm. Semen characteristics were determined according to the procedures of I.R.R.G. (2005) and Al-Eissa *et al.* (2011).

The semen was collected into a graduated tube attached to the AV to measure the semen volume (SV). 10µL of the semen sample was diluted in ratio 1:100 and charged into the improved Neubauer counter to evaluate sperm count and

sperm concentration (SCN) of the ejaculate was calculated.

A drop of undiluted semen on a warmed glass slide (37°C) was observed under a light microscope at x400 magnification to determine percentage motility based on the intensity of the Brownian motion displayed by the sperm cells. A drop of undiluted sperm stained with eosin- negrosin was examined under a microscope at x1000 magnification for both live and dead sperm cells at 5 different microscopic fields and their number expressed in ratio. Similarly, a drop of semen mixed with haematoxylin stain was scrutinized under the microscope at a magnification of x1000 whereby spermatozoa with normal or abnormal morphology were counted from 5 different microscopic fields and expressed in percentages.

Two millilitres of blood samples were collected from 3 bucks from each of the experimental treatments. The samples for haematology were collected from the marginal vein of the bucks' ears into ethylene diamine tetra-acetic acid (EDTA) coated collecting tubes in week 0 and 8 of the experiment. Haematological parameters which include red blood cell, packed cell volume, haemoglobin concentration, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume were determined as described by Jain (1986). White blood cell count was determined by the method described by Ewuola and Egbunike (2008). Blood samples for antioxidant status were simultaneously collected from bucks into plain collecting tubes but also collected in week 5. Serum was extracted from the whole blood by centrifuging at 15, 000 rpm. TAC determination was carried out according to the method of Prieto *et al.* (1998).

Experimental model and statistical analysis

The experimental model below was used to evaluate the animals' responses in the study:

$$Yijklm = \mu + A_i + B_j + C_k + D_l + E_{ijklm}$$

Yijklm = response variables; μ = population mean; A_i = effect of ginger; B_j = effect of garlic;

Ck = effect of onion; D1 = effect of garlic; Eijklm = residual error

Data was analyzed using the Factorial Analysis Of Variance of the General Linear Model. Values of $P < 0.05$ were considered statistically significant and separated by Duncan Multiple Range Test. The IBM Statistical Package for the Social Sciences version 21.0 software was used for statistical analysis.

RESULTS AND DISCUSSION

Ambient temperature (AT), Relative-humidity (RH) and Temperature –Humidity Index (THI) fluctuated throughout the experiment (Table 01). AT and RH were significantly higher ($p < 0.05$) at the end of the experiment compared to the beginning contrary to RH which was lower.

Nonetheless, the fluctuations in ambient temperature and relative-humidity in the dry season were within the ranges that are characteristic of the tropics (Galvin, 2009; Trewin, 2014). THI range of $29.30^{\circ}\text{C} - 31.10^{\circ}\text{C}$ indicated that the studied bucks were very severely heat stressed according to the categorisation of Marai *et al.*, 2002. This observation was similar to the conclusions made by researchers in the

dry season in both Nigeria and Egypt (Akinsola, 2012; Nizza *et al.*, 2003).

Table 02 showed the seminal parameters of experimental rabbit bucks monitored at a weekly interval. Semen volume, sperm motility and viability were lower in the 1st week compared to the 2nd week under a very severe THI of 30.68°C . At the lower THI of the second week, these parameters improved. This observation is in agreement with the conclusions of Marai *et al.*, 2002 that lower THI was conducive for optimal seminal parameters. On the contrary, sperm concentration was reduced in the 2nd week despite the lowered THI but was statistically similar for the remaining period of the study.

However, seminal parameters with the exception of sperm morphology were sustained for most of the experimental period in spite of high THI in contradiction to the conclusions of El-Maghawry and Soliman, 2002 and Iraqi *et al.*, 2012. From this result, it is apparent that the severe heat stress did not affect spermatogenesis in variance with the observations of Ain-Baziz *et al.*, 2012 and Schneidgenová *et al.*, 2011 both of which reported low sperm parameters at high temperatures and high values at low temperatures.

Table 01: Ambient temperature, relative humidity and temperature -humidity index of the rabbit pen

Week	T ($^{\circ}\text{C}$)	RH (%)	THI($^{\circ}\text{C}$)
1	33.90 ^b	46.71 ^{ab}	30.68 ^d
2	31.94 ^a	51.43 ^{bc}	29.30 ^a
3	31.63 ^a	60.43 ^d	29.51 ^{ab}
4	32.21 ^a	55.23 ^c	29.73 ^b
5	33.65 ^b	48.71 ^{ab}	30.59 ^{cd}
6	33.70 ^b	48.29 ^{ab}	30.60 ^{cd}
7	33.33 ^b	47.43 ^{ab}	30.25 ^c
8	34.54 ^c	45.00 ^a	31.10 ^e
SEM*	0.20	1.51	0.13

Values within the column with different superscripts are significantly different at ($p < 0.05$). AT = Ambient temperature, RH = Relative-humidity, THI = Temperature –Humidity Index, SEM* = Pooled standard error of mean

Table 02: Weekly seminal parameters of rabbit bucks feed ginger, garlic and onion supplemented diets

Week	Volume (ml)	Motility (%)	Viability (%)	Sperm Conc. (10 ⁸)	Abnormality (%)	THI (°C)
1	0.60±0.05 ^a	54.17±4.03 ^a	89.39±2.20 ^d	7.00±1.21 ^a	14.77±1.79 ^a	30.68 ^d
2	0.83±0.05 ^c	77.65±4.22 ^b	89.64±2.10 ^d	3.71±1.21 ^b	17.95±1.56 ^a	29.30 ^a
3	0.71±0.05 ^{abc}	75.51±4.03 ^b	88.59±2.15 ^{cd}	3.18±1.24 ^b	23.66±1.59 ^b	29.51 ^{ab}
4	0.73±0.05 ^{abc}	79.17±3.97 ^b	81.52±2.14 ^{ab}	2.76±1.21 ^b	28.71±1.59 ^c	29.73 ^b
5	0.66±0.05 ^{ab}	78.56±3.93 ^b	80.57±2.19 ^{ab}	3.53±1.25 ^b	35.03±1.55 ^d	30.59 ^{cd}
6	0.76±0.05 ^{bc}	75.26±3.92 ^b	84.92±2.12 ^{bcd}	2.50±1.23 ^b	31.79±1.57 ^{cd}	30.60 ^{cd}
7	0.78±0.05 ^{bc}	82.15±3.95 ^b	84.45±2.19 ^{bc}	3.17±1.23 ^b	29.87±1.74 ^c	30.25 ^c
8	0.68±0.06 ^{ab}	82.55±4.04 ^b	74.56±2.21 ^a	3.43±1.22 ^b	31.69±1.72 ^{cd}	31.10 ^c
						0.13 [*]

Values with different superscript within the column are significantly different ($p < 0.05$), * = Pooled standard error of mean of Temperature - humidity index (THI).

This result might be attributed to the inherent adaptability of the studied animals and the experimental diets which might have made the animals' testes and reproductive accessory glands less sensitive to the deleterious effect of heat stress (El-Tarabany *et al.* 2015). Percentage abnormal sperm cells, contrary to other seminal parameters, increased subsequently at a weekly interval from the 2nd week till the 5th week (31.79%) and remained similarly high till the end of the experiment. This observation corroborates the findings of El- Sayed *et al.*, 2016 and could be attributed to the lethal effect of high THI on sperm cell morphology (Chenoweth, 2005; Marai *et al.*, 2002).

Semen volume (SV) increased with level of supplementation from 5g to 10g inclusion levels in garlic but was similar in onion and ginger groups (Table 03). This was comparable to the findings of El – Speiy and EL-Hanoun, 2013 and at variance with the findings of Ogbuewu *et al.*, 2013. Nonetheless, SV observed at 10g inclusion

level was not different in both garlic and onion and was similar to the value recorded in ginger at 15g/kg feed implying that garlic and onion inclusion levels were optimal at 10g/kg contrary to ginger which was optimal at 15g/kg. Values observed in these inclusion levels corroborated the findings of Pawar and Hugar, 2012 that spice supplementation due to their phytochemicals was effective in sustaining semen parameters under high ambient temperatures. Sperm motility and % live sperm cells (viability) were similar among the experimental animals in variance to the observations of Ogbuewu *et al.*, 2013. However, this result corroborated the observations of Shinkut *et al.*, 2016 who reported non-significant differences in SM and % live cells values between the control and the treatments. In garlic supplemented groups, with the exception of garlic at 15g/kg feed, SCN increased as level of inclusion increases similar to the report of Khaki *et al.*, 2009.

Table 03: Effect of ginger, garlic and onion supplemented diets on seminal parameters of rabbit

Treatment	TI(g)	Volume (ml)	Motility (%)	Viability (%)	Sperm Conc. (x10 ⁸)	Abnormality (%)
Control	0	0.79±0.06 ^{bcd}	76.54±4.51 ^{ab}	82.88±2.45	6.60±1.40 ^{abc}	27.67±1.80 ^{bc}
	5	0.69±0.06 ^{abcd}	76.26±5.06 ^{ab}	86.04±2.53	5.40±1.50 ^{abc}	26.05±1.93 ^{ab}
Ginger	10	0.63±0.06 ^{ab}	77.91±4.54 ^{ab}	81.46±2.45	4.24±1.40 ^{ab}	25.37±1.92 ^{ab}
	15	0.85±0.06 ^{cde}	83.58±4.51 ^b	87.08±2.45	3.99±1.40 ^{ab}	22.71±1.80 ^a
Garlic	5	0.55±0.06 ^a	64.58±4.97 ^a	86.21±2.70	2.74±1.50 ^a	31.19±2.07 ^c
	10	0.96±0.06 ^c	72.64±6.07 ^a	82.96±2.51	9.70±1.41 ^c	29.91±1.80 ^{bc}
	15	0.52±0.06 ^a	78.50±4.56 ^{ab}	85.64±2.47	2.21±1.40 ^a	28.77±1.84 ^{bc}
Onion	5	0.67±0.06 ^{abc}	81.25±4.51 ^b	84.00±2.45	3.27±1.40 ^{ab}	23.79±1.80 ^a
	10	0.80±0.06 ^{cde}	74.52±4.52 ^{ab}	80.13±2.45	3.31±1.40 ^{ab}	26.85±1.83 ^{ab}
Onion	15	0.59±0.06 ^a	65.37±4.64 ^a	85.07±2.50	2.23±1.52 ^a	24.55±1.99 ^a
	0.004	0.87±0.07 ^{de}	80.73±5.08 ^b	84.80±2.95	6.18±1.75 ^{bc}	26.66±2.46 ^{ab}

Values with different superscript within the column are significantly different ($p < 0.05$) TI= Treatment level

The low value observed at 15g/kg feed might be as a result of aggregations of some phytochemicals such as saponin of which toxic effect may impair spermatogenesis. SCN in ginger and onion supplemented groups were comparable regardless of level of inclusion and were also similar to the values observed in the control and positive control. This observation is similar to the conclusions of Shinkut *et al.*, 2016 while it was different from the findings of Ogbuewu *et al.*, 2013 who reported that ginger at high levels was deleterious to SCN. Nonetheless, despite the high THI under which this study was conducted, none of the treatments had an SCN lower than the normal range (1.50 - 5.00 x10⁸/ml) for the rabbit (Akpa *et al.*, 2012) which is indicative that the spices sustained spermatogenesis.

Ginger (15g/kg feed) and onion (15g/kg feed) supplemented groups were observed to have the lowest values of abnormal sperm cells which implied that ginger and onion were more effective, in comparison to garlic, in ameliorating the lethal effect of severe THI on sperm morphology. This is similar to the report of Khaki *et al.*, 2009, Ige and Akigbe, 2012 and El-Speiy and El-Hanoun, 2013 in similar researches.

However, the percentage of abnormal cells regardless of level of inclusion was high in garlic

supplemented groups comparable to the report of Omotoso *et al.* (2012) and was similar to the values observed in the control and the positive control. The general positive impact of spice supplementation on sperm parameters can be attributed to the spices' antioxidant properties and androgenic effects (Ramandeeep *et al.*, 2013; Morakinyo *et al.*, 2010; Hammami *et al.*, 2008) which may have enhanced sertoli cells activities in maintaining sperm production under the influence of heat stress (El-Tarabany *et al.*, 2015).

Table 04 and Table 05 showed the haematological parameters of the experimental bucks before and after the introduction of the spice - supplemented diets at week 0 and week 8 respectively. Red blood cell count (RBC) at the beginning and end of the experiment was not significantly different ($p > 0.05$) among the experimental groups in variance with the observations of Chineke *et al.*, 2006 who attributed the differences that they observed to disparity in breed. At the end of the study, compared to the beginning, RBC was depleted in all the treatments except in groups supplemented with onion at 5 and 10 g/kg feed. The reduction was similar to the findings of Shinkut, 2015 and may be due to the effect of heat stress as alluded by NseAbasi *et al.*, 2014. However, the numerically higher RBC value recorded in onion (5g and 10g inclusion levels)

supplementation was a pointer to improved RBC production which is in agreement with the reports of Najmul, 2015 and Onu and Aja, 2011. This could be attributed to the stimulatory activities of the phytochemicals on cytokines which are believed to be the initiator and regulator in the proliferation of RBC (Enitan *et al.*, 2012). PCV values were not significantly different ($p>0.05$) among the treatment and control groups at both the beginning and the end of the experiment similar to the findings of Ewuola *et al.* (2012) but at variance with the findings of Ugwu and Omale, 2011 who reported significant reduction of PCV in rats fed onion and garlic extracts. However, the mean PCV at the end of the study was higher than

the mean recorded at the beginning of the study implying that the experimental diets sustained the haematopoiesis process of the animals. Similar to PCV, haemoglobin concentration (Hbc) at both week 0 and 8 were not significantly different among the spice - supplemented groups and the control. The non- significance values of Hbc among the treatment and the control groups corroborated the result of Oluwole, 2001 and Najmul, 2015 in garlic and ginger treated rats and rabbits respectively, and differed with the conclusions of Enitan *et al.*,2012 who reported a significant increment in Hbc due to onion and garlic supplementations.

Table 04: Effect of ginger, garlic and onion on red blood cell count, packed cell volume, haemoglobin concentration and white blood cell count of rabbit bucks fed spice supplemented diets

Treatment	Treat Level	RBC ($10^6/\text{mm}^3$)		PCV (%)		Hbc (g/dl)		WBC ($10^3/\text{mm}^3$)	
		Wk 1	Wk 8	Wk 1	Wk 8	Wk 1	Wk 8	Wk 1	Wk8
Control	0	5.92 ^{ab}	3.67 ^a	28.67	31.50	15.33	15.67	6.37	5.63
	5g	4.36 ^a	3.89 ^a	25.33	29.17	14.00	15.33	5.05	7.53
Ginger	10g	5.90 ^{ab}	4.47 ^{ab}	24.17	31.83	15.00	15.33	6.00	6.17
	15g	6.00 ^{ab}	3.96 ^{ab}	32.00	29.83	15.67	15.00	7.55	6.12
	5g	5.45 ^{ab}	5.38 ^{ab}	29.33	29.50	16.00	16.00	5.52	6.22
Garlic	10g	6.01 ^{ab}	5.14 ^{ab}	26.17	31.50	15.67	15.67	5.23	7.89
	15g	6.66 ^{ab}	4.79 ^{ab}	32.67	32.00	16.33	16.00	6.03	5.87
	5g	5.54 ^{ab}	5.66 ^{ab}	26.50	31.00	15.67	15.33	8.83	6.57
Onion	10g	4.97 ^{ab}	6.00 ^b	23.67	32.00	14.33	15.00	8.58	6.63
	15g	6.48 ^{ab}	4.45 ^{ab}	28.00	30.00	16.00	13.67	5.93	5.78
Vitamin C	400mg	6.88 ^b	4.56 ^{ab}	29.83	31.67	16.00	15.67	5.08	7.30
SEM*		0.22	0.20	1.17	0.55	0.28	0.25	0.48	0.30
Weekly Mean		5.77	4.79	27.85 ^A	30.91 ^B	15.46	15.33	6.38	6.52
±SEM		±0.22	±0.19	±1.27	±0.62	±0.30	±0.27	±0.44	±0.33

Values with different superscript within the column or across the row are significantly different ($p<0.05$); RBC= Red blood cell; PCV= Packed cell volume; Hbc= Haemoglobin concentration; MCV= Mean corpuscular volume; Wk= Week, SEM* = Pooled standard error of mean

Table 05: Effect of ginger, garlic and onion on mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume of rabbit bucks fed spice supplemented diets

Treatment	Treat Level	MCH (pg)		MCHC (g/dl)		MCV (fl)	
		Wk 1	Wk 8	Wk 1	Wk 8	Wk 1	Wk 8
Control	0	25.95	43.94 ^b	49.81	49.89	48.54	87.39 ^b
	5g	32.78	40.19 ^{ab}	50.06	53.11	59.13	77.09 ^{ab}
Ginger	10g	25.96	34.56 ^{ab}	53.53	49.31	42.86	72.29 ^{ab}
	15g	26.79	39.05 ^{ab}	55.4	50.32	53.56	76.84 ^{ab}
	5g	30.62	29.97 ^{ab}	55.54	54.28	53.81	54.98 ^a
Garlic	10g	26.34	30.52 ^{ab}	57.13	49.79	43.64	61.38 ^{ab}
	15g	24.62	35.10 ^{ab}	58.35	50.10	49.21	70.72 ^{ab}
	5g	32.68	27.16 ^a	61.91	49.7	56.69	54.75 ^a
Onion	10g	30.25	28.80 ^a	68.82	47.22	47.37	59.73 ^a
	15g	25.18	30.63 ^{ab}	70.38	45.67	45.40	67.62 ^{ab}
Vitamin C	400mg	23.38	34.35 ^{ab}	73.5	49.84	42.95	69.41 ^{ab}
SEM*		1.14	1.39	3.3	0.97	2.63	2.72
Weekly Mean		28.05 ^A	33.53 ^{A*}	59.50 ^B	49.93 ^{B*}	50.06 ^C	67.38 ^{C*}
±SEM		±1.24	±1.40	±3.72	±1.06	±3.00	±2.77

Values with different superscript and superscript with asterisk within the column and across the row respectively are significantly different ($p < 0.05$).; TREAT= Treatment, MCH= Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration; WBC= White blood cell count .Haemoglobin concentration; MCV= Mean corpuscular volume; Wk= Week; SEM* = Pooled standard error of mean

It could however be concluded that the spices have no negative effects on Hbc since the values at both beginning and the end of the experiment were within the range for rabbit (Mitruka and Rawnsley, 1977). White blood cell was also not significantly different ($p>0.05$) among the experimental treatments both at the beginning and the end of the experiment. This result was similar to the report of Ugwu and Omale, 2011 who observed non – significant WBC values in rat fed onion and garlic extracts. Nonetheless, WBC observed in this study was within the normal range for the rabbit (Burke, 1994; Research Animal Resources, 2009). At the beginning and end of the study MCH, MCHC and MCV values were not significantly different ($p>0.05$) among the experimental animals, though their overall means were significantly higher at the end of the study with the exception

of MCHC with a lower overall mean. MCV (42.86 - 59. 13) at the beginning of the study was below the range observed for the rabbit (Research Animal Resources, 2009) suggesting that the experimental animals had subnormal sized RBC. At the end of the study, MCH and MCV values were within the established values for rabbit (Research Animal Resources, 2009) and also higher than the initial values which indicated that the diet with or without spice supplementation normalised the sizes of the RBC which is suggestive of the absence of anaemia in the animals (Enitan *et al.*, 2012; NseAbasi *et al.*, 2014). MCHC non-significant difference among the treatments corroborated the conclusions of Enitan *et al.*, 2012. However, despite MCHC lower overall mean at the end of the study, values among the experimental animals were still higher than the normal range reported by Olabanji

et al. (2007). In general, the haematological condition observed in the experimental animals could be due to adequate protein provided by the experimental diets and the antioxidant activities of phytochemicals in the ingested spices which maintained the haematopoietic and erythropoietic functions of the bone marrow (NseAbasi *et al.*, 2014) even under a severe heat stress condition.

Total antioxidant capacity (TAC) of the experimental animals ranged from 45.60 - 86.99mgAE/m at the beginning of the experiment. Animals allotted to garlic at 5g/kg feed had a significantly lower value and could be ascribed to the inherent nature of these animals since they had not been fed with the supplemented diets at the time. TAC at the middle (weeks 5) and end of the experiment (week 8) was not significantly different ($p>0.05$) among the experimental animals (Table 06). This result was similar to the findings of Ghalehkandi, 2014 who found that

TAC of garlic treated rats and the control was not significantly different regardless of the level of inclusion.

TAC fluctuated among the treatments groups with most groups having higher numerical TAC values at the end of the study than at the beginning though the weekly means were not significantly different. This implied that the ingested spices did not reduce the animals' TAC in agreement with the reports of Khaki *et al.*, 2012. Generally, the fluctuating TAC trend observed in this study could be attributed to the physiological adjustment of the experimental animals to maintain equilibrium (Moberg, 2000) in the production and eradication of reactive oxygen species under external environmental stressors one of which was sustained heat stress observed in this study.

Table 06: Effect of spice – supplemented diet on the total antioxidant capacity of the blood serum of rabbit bucks

Treatment	TRTL	TAC (mgAE/m)		
		Week 1	Week 5	Week 8
Control	0	79.29 ^b	85.66 ^b	84.60
	5g	73.58 _b	88.64 ^b	84.93
Ginger	10g	78.78 ^b	70.29 ^{ab}	75.04
	15g	82.27 ^b	62.99 ^a	78.07
	5g	45.60 ^a	79.76 ^{ab}	99.05
Garlic	10g	83.82 ^b	82.91 ^b	91.77
	15g	80.40 ^b	78.31 ^{ab}	88.12
	5g	83.39 ^b	85.37 ^b	86.31
Onion	10g	81.70 ^b	81.70 ^{ab}	84.29
	15g	86.99 ^b	82.23 ^{ab}	101.36
Vitamin C	400mg	79.67 ^b	74.95 ^{ab}	79.45
SEM*		2.48	1.93	2.87
Weekly mean±SEM		77.75±2.60	79.35±1.77	86.66±2.49

Values with different superscript within the column are significantly different ($p<0.05$), SEM* = Pooled standard error of mean; TRTL= Treatment level; TAC= Total antioxidant capacity SEM= Standard error of mean

CONCLUSIONS

The adverse effect of heat stress observed in this study was more apparent on sperm morphology than other semen parameters though ginger at 5g/kg feed ameliorated the impact most among spice supplementations. The finding of the

current study showed that heat stress and spices supplementation were not deleterious on the haematological parameters and the serum total antioxidant capacity of the experimental rabbit. Further research with the spices combined and at higher supplementation levels is recommended.

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