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Effects of *Allium sativum* and *Allium cepa* on semen characteristics, sperm reserves and haematology of rabbit bucks

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Abstract

The effect of dietary inclusion of garlic and onion on semen characteristics, gonadal, extragonadal sperm reserves and haematology of rabbit bucks were evaluated. Twenty-four rabbit bucks of average age and weight 10 ± 2.0 months and 1.47 ± 0.01 kg respectively, were used for the study. They were randomly assigned into four groups of six bucks each. Group A served as control, while Groups B, C and D received dietary inclusion of 5% garlic, 5% onion, 2.5% garlic + 2.5% onion, respectively. The fresh bulbs of garlic and onions were peeled, air-dried, and the dried bulbs were weighed, added to the feed and grounded together to form experimental diets. Before dietary supplementation, semen samples were collected to serve as baseline values, followed by weekly collections for another 9 weeks using an artificial vagina. Haematological parameters were examined according to the standard procedure, while testosterone profile was conducted using the ELISA method. At the termination of the experiment, two bucks from each group were euthanised, and the testes were harvested to evaluate gonadal and extragonadal sperm reserves. No significant ($p > 0.05$) difference was recorded in the live weight, sperm concentration, and sperm abnormality of the rabbit bucks. A significant ($p < 0.05$) difference was observed in the ejaculate volume, gross motility, pH, reaction time, and percentage of live spermatozoa. The epididymal sperm reserves in group B, was significantly ($p < 0.05$) higher in the right than left. Testosterone profile showed significant ($p < 0.05$) difference at 9 and 10 a.m. On haematology, there was a significant increase in PCV, RBC count, haemoglobin concentration and WBC count in groups B, C and D by week 9 compared to the control. In conclusion, the dietary inclusion of garlic and onion effectively improved the spermiogram of rabbit bucks.

Keywords: Garlic, Haematology, Onion, Rabbit buck, Spermiogram, Testosterone

Introduction

In Nigeria, the demand for livestock products is expanding due to the growing population, with more preference lately for rabbit meat. In line with this, there has been an increased demand for rabbit meat, particularly due to its high protein, low fat, cholesterol and sodium levels (Adedeji *et al*., 2015), leading to a decline in rabbit population, thus creating the need to improve the population of rabbits. Garlic (*Allium sativum L.*) and onion (*Allium cepa L.*) are among the oldest of all cultivated plants with their origin in Central Asia (Ali *et al*., 2000). These edible *Allium* species have long been used as food ingredients and medicine (Atmaca, 2003; Khaki *et al.*, 2009; Kim & Kim, 2011; Khaki *et al.*, 2012). Garlic and onions are rich sources of a wide variety of phytochemicals (Hassanpour *et al*., 2011), which can effectively scavenge free radicals (Khaki *et al*., 2011). Garlic powder has been administered as tablets with a meal to lower serum lipid levels in humans (Jain *et al*., 1993), a mixture of garlic and cinnamon oils in paraffin oil to be effective in treating rabbits injured by *Sarcoptec scabei* (El-Sawy *et al*., 2016) while onion juice remedied sperm parameters (Khaki *et al*., 2016) and crude garlic was used as inclusion rates in rabbits' ration to improve sperm qualities (Shinkut *et al*., 2016). Supplemental onion and garlic inclusion levels above 3.009 g per 600 g Dry Matter feed diets was reported to enhance reproductive efficiency in Koekoek breeder cocks (Okoro *et al*., 2016). A wide number of plants-derived botanical products are now used in traditional medicine because of their beneficial properties in improving fertility in animals (Iqbal & Bhanger, 2007; Yama *et al*., 2011). Garlic and onion are natural seasoning that contains high amounts of potent antioxidants and are used in traditional medicine globally to treat several diseases. Antioxidants have an essential effect on sperm health parameters; however, there is limited information on the effect of dietary inclusions of garlic and onion on the reproductive functions of rabbits (Adeleye *et al*., 2020). This study investigated the effects of garlic and onion as dietary inclusions in rabbit rations on spermiogram, gonadal and extragonadal sperm reserves, testosterone profile and haematology.

Materials and Methods

Plant clove and bulb

Allium sativum (Garlic) and *Allium cepa* (Onion) bulbs were obtained from Sokoto's main market, Sokoto State, Nigeria. They were taken to the Herbarium, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, for authentication and identification with the voucher numbers 423 and 01408, respectively. The fresh bulbs of the garlic and onions were peeled and dried under shade. The dried bulbs were then weighed and added to the feed's raw material (Maise grain, wheat offal, soya cake), which was now grounded together to form the experimental diets (Table 1).

Experimental animals

Twenty-four apparently healthy male domestic rabbits (*Oryctolagus cuniculus*) of average age 10 ± 2.0 months and average body weight of 1.47 ± 0.01 kg were purchased from the Animal house, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were screened for ectoparasite and haemoparasite then routinely treated using Ivermectin (Kepromec®) injection subcutaneously prior to commencement of the study. Each buck was housed in standard rabbit cages. Feed and water were provided *ad libitum.*

Table 1: Composition of the experimental diets (%)

	Groups (n=6)			
Ingredient (kg)	Normal control	5% garlic	5% onion	2.5% garlic + 2.5% onion
Maize grain	49.2	51.0	50.0	50.5
Wheat offals	29.05	23.0	22.55	22.45
Soya cakes	18.0	17.25	18.7	18.30
Crude *Allium sativum*	0.00	5.0	0.00	2.5
Crude *Allium cepa*	0.00	0.0	5.0	2.5
Bone Meal	2.8	2.8	2.8	2.8
Methionine	0.2	0.2	0.2	0.2
Lysine	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
Total (100 kg)	100.00	100.00	100.00	100.00

Phytochemical analysis

The crude garlic and onion were subjected to phytochemical analysis using standard methods to test for alkaloids, tannins, flavonoids, saponins, steroids and cardiac glycosides (Evans, 2009).

Experimental design

The rabbit bucks were randomly divided into four groups of six bucks each after four weeks of acclimatisation. Group A served as control while those in groups B, C and D were fed with 5% garlic, 5% onion, and 2.5% garlic + 2.5% onion, respectively. The bucks were fed isocaloric and isonitrogenous diets with garlic and onion as additives (Table 2), and the period of feeding lasted for 9 weeks. Prior to dietary supplementation, semen samples were collected from the rabbits using an artificial vagina to serve as baseline references, followed by weekly collections for another 9 weeks. Nine semen samples were therefore assessed for each buck, giving a total of 216 samples all through the experimental period. At the end of the 9th week, two bucks in each group were sacrificed by jugular venesection. The testes were exteriorised for gonadal and extragonadal sperm/spermatid reserves examination.

Measurement of live weight

All the bucks were weighed at the beginning (pre-treatment), week 4 and the end of the feeding trial (week 9) using Mettler's XP6002S (capacity: 6100 g, readability: 10 mg, linearity ± 0.020) weighing balance. Changes in weight were determined and recorded.

Semen collection

The bucks were trained for semen collection during the acclimatisation period and semen was collected into a calibrated tube, using a specially designed artificial vagina (AV) for rabbits. The AV was assembled thus: a short plastic cone was obtained; the middle finger of a surgical glove was cut off at the base. The other end was also cut open to make it patent and serve as the rubber liner. A rubber band was used to fix one end of the liner over the plastic cone, glycerol was administered into the space between the cone and the rubber liner. The other end was folded over the plastic cone and fixed with rubber band. The assembled AV was placed in a beaker with warm water at 40^{o}C, causing the expansion of the liner while the glycerol provided the necessary pressure and required temperature. The traces of water on the AV were cleaned, a short test-tube was attached to the narrow end of the AV and the wider end lubricated with bland petroleum jelly for easy penetration. To collect the semen from the rabbit bucks, the collector was gloved and a rabbit doe was usually put into the buck's cage to serve as a teaser. The buck was monitored closely and as it mounted the doe, the AV was placed gently at the vulva of the doe so as to direct the penis into the AV for penetration and eventual ejaculation (Shinkut *et al*., 2016).

Semen evaluation

The semen samples were maintained in a waterbath at 37˚C, and each ejaculate was evaluated as described by Rekwot *et al*. (1987).

Volume**:** The volume of the semen was immediately measured directly from the calibrated tube used for the collection.

Semen pH**:** This was determined by dipping a litmus paper (Merck KGaA, 64271 Darmstadt, Germany) into the ejaculate and colour changes with corresponding values were observed and recorded (Shinkut *et al*., 2016).

Gross sperm motility: Semen samples were examined for motility by placing a drop of the undiluted semen on a warm glass slide and examined with a cover slip under ×10 magnification. A descriptive scale ranging from very poor (0-19), poor (20-40), fair (41-50), good (51-80), very good (81-90) and excellent (91-100) was recorded (Rekwot *et al*., 1987).

Table 2: Proximate composition of experimental diets supplemented with *A. sativum* and *A. cepa*

Composition (%)	Group A (n = 6)	Group B (n = 6)	Group C (n = 6)	Group D (n = 6)
Crude Protein %	24.13	22.78	23.54	22.13
Ether Extract %	2.53	2.65	2.45	2.57
Crude Fibre %	9.13	6.78	7.54	8.13
Ash %	4.44	4.58	3.61	4.28
NFE (Nitrogen Free Extract) %	59.77	63.21	62.86	62.89
Dry Matter %	89.58	89.12	88.84	88.82

n = number of rabbits

Spermatozoa concentration: This was determined using a Neubauer haemocytometer as described by Azawi & Ismaeel (2012). Micropipette was used to aspirate the semen to 0.5 mark and physiological saline was aspirated to make it up to 1.0 mark, hence diluting the semen. Outside of the pipette was wiped to remove any adhering semen. The first three drops were discarded whereas two drops were placed at either margin of the haemocytometer, and cover slipped. The haemocytometer was carefully placed in a pre-wetted chamber and the lid closed and left for 5 minutes. The haemocytometer was carefully removed and then examined using a microscope at × 40 magnification. The sperm cells were counted in five squares of the chamber (i.e., four corners and the centre squares). The semen concentration was calculated using the formula of Azawi & Ismaeel (2012) as follows:

Concentration (sperm cells/mL) = Number of sperm cells counted in the twenty-five small squares × dilution factor × 10^6.

Reaction time (libido): A matured doe (teaser) was introduced to the buck prior to semen collection and observed for sex drive. The time in seconds it took the buck to sniff, groom and mount the female was recorded.

Percentage live sperm cells: This was determined using the method described by Esteso *et al*. (2006). A thin smear of the semen was made on a clean grease-free slide and stained with eosin-nigrosin stain. One hundred sperm cells were counted using light microscopy at × 40 magnification. Dead spermatozoa were stained pink or reddish while live spermatozoa remained colourless. When the slides were dried, one hundred (100) sperm cells were counted and the percentage of stained and unstained sperm cells were evaluated.

Sperm morphological abnormalities: This was determined by making a thin smear of the semen sample on a clean grease-free glass slide and stained with eosin-nigrosin. One hundred sperm cells (normal cells and different types of morphological sperm defects) per slide using hand counter under light microscopy at × 40 magnification, were counted and recorded (Rekwot *et al*., 1987). This procedure was repeated by two technicians and the average recorded.

Determination of gonadal sperm/spermatid reserves

The gonadal sperm/spermatid reserves were determined as described by Rekwot *et al*. (1994) and Ogunlade *et al.* (2006) with slight modifications. Two bucks from each group were sacrificed and the testes exteriorised, with the length, weight and volume of each testis determined using a measuring tape, digital weight balance (essae®) and water displacement method, respectively, and the values recorded. The *Tunica albuginea* was carefully dissected with a scalpel blade and removed from both left and right testis. Each testis was homogenised in 25 ml of physiological saline solution using a mortar and pestle and to this was added streptomycin sulphate (1 mg/ml) and Penicilin G (100 iu/ml) to prevent bacterial growth. The homogenate volume, after rinsing the mortar with 10 ml of physiological saline solution and adding the effluent, was measured. A volume of 2.5 ml of the homogenate was transferred into a conical flask and further dilution was made with 40 ml of physiological saline solution. The diluted homogenate sample was stored overnight at 5^{o}C to allow sperm cell to ooze out of the testicular tissue and filtered through gauze. Thereafter, the filtrate volume was measured. The gonadal sperm/spermatid concentration were determined using a haemocytometer according to the method of Kwari & Waziri (2001).

Determination of epididymal sperm reserves

Evaluation of epididymal sperm reserves was done as described by Olukole *et al*. (2010). The epididymis was carefully removed from the testis with scalpel blade and the length and weight of the head, body and tail portions were determined using a measuring tape and digital weighing balance (essae®). These portions were minced separately in 20 ml of normal saline with sharp scissors and stored for 24 hours at 5^{o}C to allow sperm cell to ooze out of the epididymal tissue. The product was then filtered through gauze and the volume was measured. Then 1 ml of epididymal filtrate was diluted with 2 ml of normal saline and the concentration of the sperm reserves was determined using Neubauer haemocytometer under a light microscope (Shinkut *et al*., 2016). Sperm cells and spermatids were counted diagonally from top left to bottom right in five large squares (Ogunlade *et al*., 2006).

Testosterone assay

At the end of the experiment, blood samples were collected thrice, by 8 am, 9 am, and 10 a.m. through the marginal ear venipuncture from bucks in each group into a non-ethylene diamine tetra-acetic acid sample bottles using 25 gauge hypodermic needle and allowed to clot at room temperature for an hour. The blood was centrifuged at 3000 g for 15 minutes and the serum was harvested and stored at -20^{o}C until used for serum testosterone assay. The assay

was by enzyme-linked immunosorbent assay (ELISA) technique. The test was carried out according to the manufacturer's instructions using testosterone kits (Accu-bind®). The reagent was constituted as described by the manufacturer, and the procedure was carried out thus: The serum reference for each Streptavidin-coated micro-well was assayed in duplicate. The serum sample (10 µl) was pipetted into the assigned wells with a precision ≥ 1.5%. The testosterone enzyme reagent-horseradish peroxidase (50 µL), was added to all the micro-well and the microplate were swirled gently for 30 seconds. Testosterone biotin reagent- purified rabbit IgG (50 µL) was also added and the microplates swirled gently for 30 seconds. The microplates were covered with plastic wrap and incubated for 60 minutes at room temperature, 25°C. The contents of the microplates were discarded by decantation and the micro-wells were blotted using absorbent paper. Wash buffer (350 µL) was aspirated into the micro-wells thrice and discarded. Working substrate solution (100 µL) was added to all wells and incubated for 15 minutes at room temperature. Stop solution (50 µL) was added to each well and was gently mixed for 20 seconds. The absorbance was read in each well within 30 minutes at 450 nm using a reference wavelength of 620-630 nm to minimise well imperfections in the microplate reader (SpectraMax plus 384®).

Haematological examination

Blood samples were collected from all the bucks at pre-treatment, week 4 and week 9 of the feeding period. The blood samples were collected into sample bottles containing ethylenediamine tetra-acetic acid (EDTA). Packed cell volume (PCV) was determined by the microhematocrit method as described by Coles (1986); red blood cell count, haemoglobin concentration and white blood cell counts were carried out using Neubauer haematocytometer as described by Schalm *et al*. (1975).

Data analysis

Data were expressed as mean ± standard deviation (SD) for Tables and mean ± standard error of mean (SEM) for Figures. One-way analysis of variance (ANOVA) repeated measure followed by Tukey's *post hoc* test for multiple comparisons was used to test for differences between groups. GraphPad Prism version 8.0.2 for Windows (GraphPad software San Diego, California, USA) was used for the analysis. Values of $p < 0.05$ were considered significant.

Results

The qualitative analysis showed the presence of carbohydrates, saponins, flavonoids, and steroids in *Allium cepa* and *Allium sativum*, with cardiac glycosides, terpenoids, alkaloids and tannins in *Allium sativum* (Table 3). The mean live weight (kg) gains did not differ significantly ($p > 0.05$) among the treatment groups of 5% garlic, 5% onion and 2.5% garlic + 2.5% onion in relation to control before treatment at week 4 and week 9 of the feeding trials (Figure 1).

At week 2, there was significant ($p < 0.05$) difference in the mean ejaculate volume (ml) between 5% garlic (1.75 ± 0.70) when compared to the control (0.50 ± 0.07), 5% onion (0.65 ± 0.09) and 2.5% garlic + 2.5% onion (0.70 ± 0.19). Also, at week 9, the mean ejaculate volume was significantly ($p < 0.05$) higher in 2.5% garlic + 2.5% onion (1.20 ± 0.39) when compared to control (0.48 ± 0.09), 5% garlic (0.35 ± 0.04) and 5% onion (0.62 ± 0.08) (Figure 2). There was significant difference ($p < 0.05$) in mean pH recorded by week 1 (7.67 ± 0.42) and week 4 (8.00 ± 0.45) in the group fed 2.5% garlic + 2.5% onion group when compared with other groups (Figure 3).

At week 2, the mean value of the gross sperm motility (%) in 5% onion group (75.83 ± 6.11) was significantly ($p < 0.05$) higher than the control (50.83 ± 9.95). Also, there was significant difference ($p < 0.05$) between the control (54.17 ± 4.90), 5% garlic (80.83 ± 2.39), 5% onion (74.17 ± 4.20) and 2.5% garlic + 2.5% onion (75.83 ± 5.23) by week 4 (Figure 4). The mean sperm concentration (10^6/ml) showed no significant difference when all the groups were compared throughout the feeding period (Figure 5). There was no significant difference ($p > 0.05$) in the reaction time (seconds) recorded in all the treatment and control groups throughout the experimental period except for week 2 where 5% garlic (36.66 ± 2.75) and 5% onion (50.50 ±

Table 3: Qualitative Phytochemical Screening

S/n	Constituents	Tests	*A. sativum*	*A. cepa*
1	Carbohydrate	Molisch	+	+
2	Anthraquinones	Bontragers	-	-
3	Alkaloids	Wagner's Reagent	+	-
4	Cardiac glycosides	Kelle-Killiani	+	-
5	Flavonoids	Sodium Hyroxide	+	+
6	Saponin	Frothing	+	+
7	Steroids	Liberman Buchard	+	+
8	Triterpene	Liberman Buchard	+	-
9	Tannins	Iron chloride	+	-

Present: +; absent: -

2.50) were significantly ($p < 0.05$) higher than control (5.17 ± 1.30) and 2.5% garlic + 2.5% onion (4.83 ± 0.08) (Figure 6). From pre-treatment to week 7, no significant difference ($p > 0.05$) was observed in the mean sperm livability (%) all the groups. At week 8, the mean percentage sperm livability was significantly ($p < 0.05$) higher in 5% onion (75.83 ± 3.00) when compared with control (56.66 ± 5.72) 5% garlic (49.16 ± 4.28) 2.5% garlic + 2.5% onion (34.16 ± 4.40) (Figure 7).

There was no significant ($p > 0.05$) difference observed in the mean percentage sperm morphological abnormalities (%) in all the groups throughout the study period (Figure 8). The mean gonadal weight (kg) was not significantly different ($p > 0.05$) between 5% garlic (3.65 ± 0.17) and 2.5% garlic + 2.5% onion (3.03 ± 0.89) but were significantly ($p < 0.05$) lower when compared with control (4.88 ± 0.53) and 5% onion (4.49 ± 0.49). The mean gonadal length (cm) was significantly lower in 5% garlic (5.15 ± 0.63) and 2.5% garlic + 2.5% onion (4.75 ± 0.21) when compared to the control (5.85 ± 0.64) and 5% onion (5.65 ± 0.77). 5% onion (7.00 ± 2.80) alone showed significant differences ($p < 0.05$) in the mean gonadal volume (ml) and mean gonadal sperm reserve ($\times 10^6$/g) when it was compared with other groups (Table 4). However, the mean epididymal sperm reserves ($\times 10^6$/g) in 5% garlic (24.00 ± 3.20), was significantly ($p < 0.05$) higher in the right epididymis when compared to control (9.50 ± 0.50) 5% onion (15.00 ± 1.50) and 2.5% garlic + 2.5% onion (14.00 ± 1.00) (Table 5). At 8 a.m. there were no significant ($p > 0.05$) differences observed in the mean testosterone profile (ng/ml) all the groups. There was significant difference ($p < 0.05$) recorded by 9 a.m. with 5% garlic (2.44 ± 0.21), 5% onion (2.54 ± 0.01) and 2.5% garlic + 2.5% onion (2.52 ±

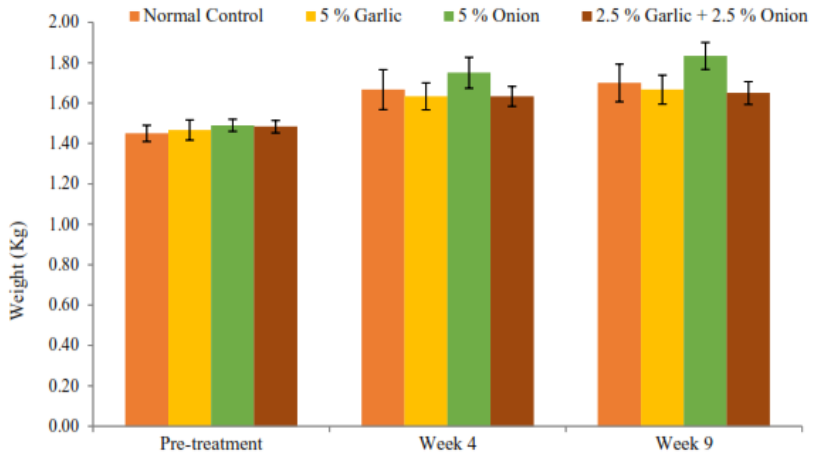

Figure 1: Mean live weights of rabbit bucks fed *Allium sativum* and *Allium cepa*
Values are expressed as mean ± SEM

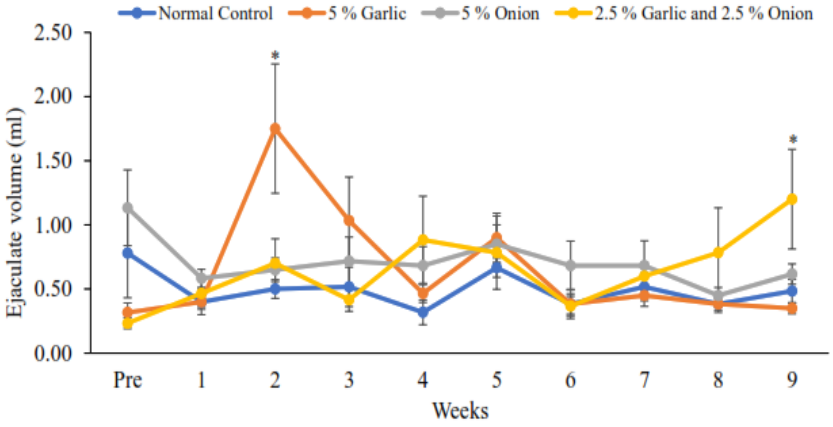

Figure 2: Mean ejaculate volume of rabbit bucks fed *Allium sativum* and *Allium cepa*
Values are expressed as mean ± SEM. *p < 0.05, significantly different when compared to the control. Pre; pre-treatment

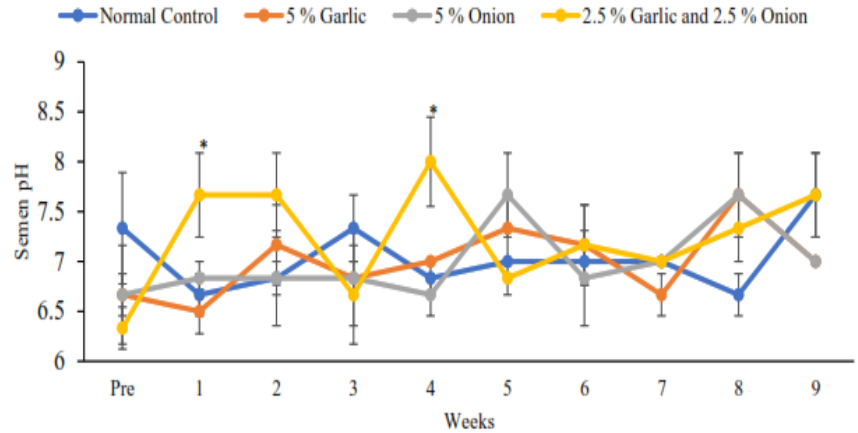

Figure 3: Mean semen pH of rabbit bucks fed *Allium sativum* and *Allium cepa*
Values are expressed as mean ± SEM. *p < 0.05, significantly different when compared to the control. Pre; pre-treatment

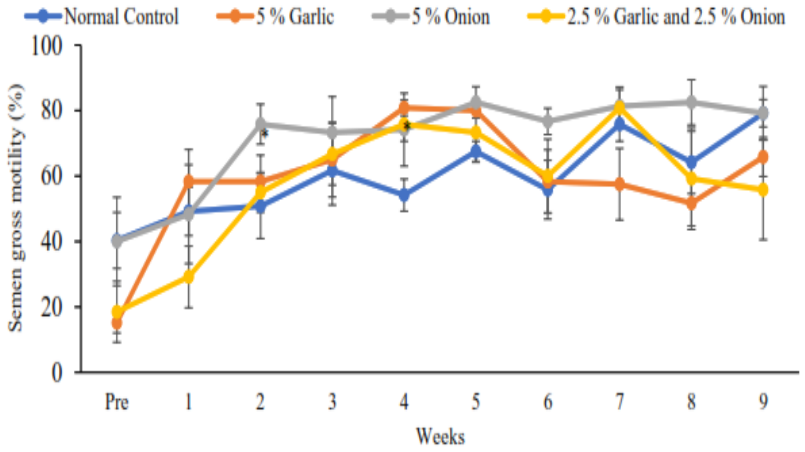

Figure 4: Mean semen gross motility of rabbit bucks fed *Allium sativum* and *Allium cepa*

Values are expressed as mean ± SEM. *p < 0.05, significantly different when compared to the control. Pre; pre-treatment

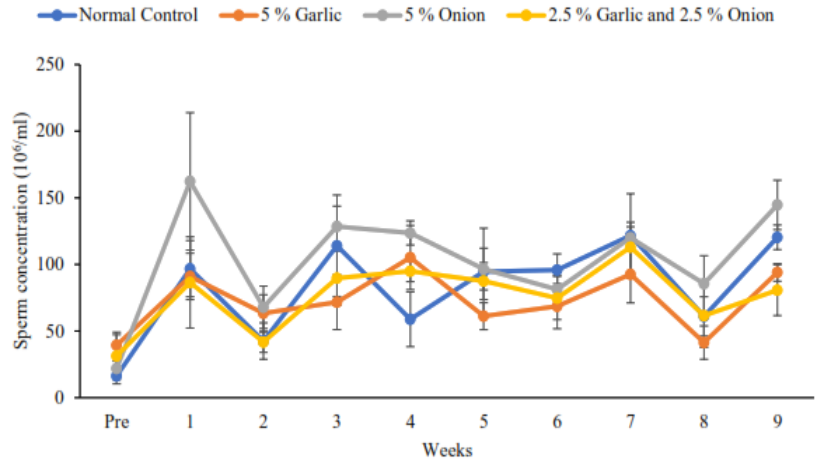

Figure 5: Mean sperm concentration of rabbit bucks fed *Allium sativum* and *Allium cepa*

Values are expressed as mean ± SEM. Pre; pre-treatment

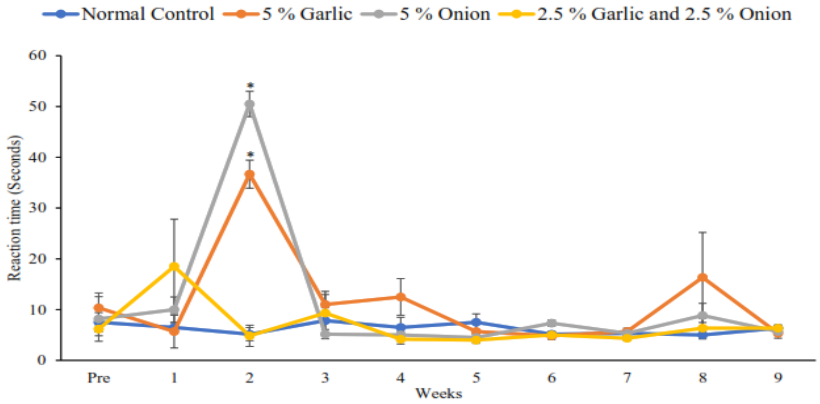

Figure 6: Mean reaction time of rabbit bucks fed *Allium sativum* and *Allium cepa*

Values are expressed as mean ± SEM. *p < 0.05, significantly different when compared to the control. Pre; pre-treatment

0.20) when compared with the control (2.06 ± 0.19). By 10 a.m., there were significant ($p < 0.05$) differences between 5% garlic (1.43 ± 0.09), 5% onion (1.80 ± 0.05) and 2.5% garlic + 2.5% onion (2.15 ± 0.07) when compared to control (1.06 ± 0.08) (Figure 9). At pre-treatment and week 4, there were no significant differences ($p > 0.05$) in the means RBC ($x10^{12}$/L) of the control, 5% garlic, 5% onion and 2.5% garlic + 2.5% onion. At week 9, there was significant ($p < 0.05$) increase in RBC count in groups 5% garlic (6.84 ± 0.34), 5% onion (6.39 ± 0.39), and 2.5% garlic + 2.5% onion (6.79 ± 0.32); compared with the control (4.93 ± 0.25). There was no significance ($p > 0.05$) in the means haemoglobin concentration (g/dl) of the control, 5% garlic, 5% onion and 2.5% garlic + 2.5% onion at pre-treatment and week 4. By week 9, there was significant increase ($p < 0.05$) in haemoglobin concentration values of groups 5% garlic (13.82 ± 0.46), 5% onion (13.00 ± 0.40), 2.5% garlic + 2.5% onion (12.72 ± 0.49); compared with control (10.88 ± 0.49). At pre-treatment and week 4, there were no significant differences ($p > 0.05$) between the PCV (%) means of the control, 5% garlic, 5% onion and 2.5% garlic + 2.5% onion. By week 9, there was significant increase ($p < 0.05$) in the PCV between groups 5% garlic (39.43 ± 0.61), 5% onion (40.27 ± 0.68), 2.5% garlic + 2.5% onion (39.55 ± 0.49); when compared with control (34.45 ± 0.69). At pre-treatment, there were no significant increase ($p > 0.05$) in the WBC counts ($x10^9$/L) between control (5.55 ± 0.35), 5% onion (5.41 ± 0.39) 2.5% garlic + 2.5% onion (5.03 ± 0.25). At week 4, there were no significant difference ($p > 0.05$) between control (5.40 ± 0.25), 5% onion (5.51 ± 0.44) and 2.5% garlic + 2.5%

onion (5.08 ± 0.25). At week 9, there were significant increase ($p < 0.05$) between groups fed with 5% garlic (6.35 ± 0.42), 5% onion (6.30 ± 0.40), 2.5% garlic + 2.5% onion (5.84 ± 0.45); when compared to control (5.22 ± 0.38) (Table 6).

Discussion

The phytochemical analysis of garlic showed the presence of carbohydrates, alkaloids, cardiac glycosides, flavonoids, saponins, triterpenes, and phenolic steroids, while onions had carbohydrates, flavonoids, saponins, and phenolic steroids. This result agrees with those reported in previous research (Carotenuto *et al*., 1999; Shinkut *et al*., 2016; Pareek *et al*., 2017; Grzelak-Blaszczyk *et al*., 2018). The non-significance in the live weight is in concert with a previous study (Omotoso *et al*., 2012). This showed that at the level of the percentage inclusions used in this study, garlic and onion does not possess growth-promoting abilities. The ejaculate volume of this study is in concert with Campos *et al*. (2014). However, the increase in mean ejaculate volume of the group fed 2.5% garlic + 2.5% onion at weeks 7 to 9 could be attributed to possible additive or synergistic effect of garlic and onion mixture due to the reactive oxygen species (ROS) scavenging characteristics of onion and garlic in the sex accessory gland (Banihani, 2019). Our findings agreed with those from other studies in which rabbit buck diets were

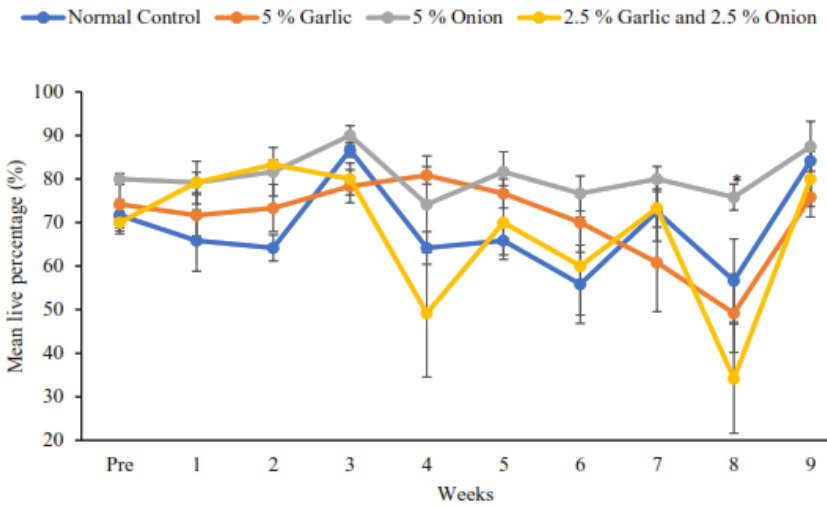

Figure 7: Mean percentage live spermatozoa of rabbit bucks fed *Allium sativum* and *Allium cepa*

Values are expressed as mean ± SEM. *p < 0.05, significantly different when compared to the control. Pre; pre-treatment

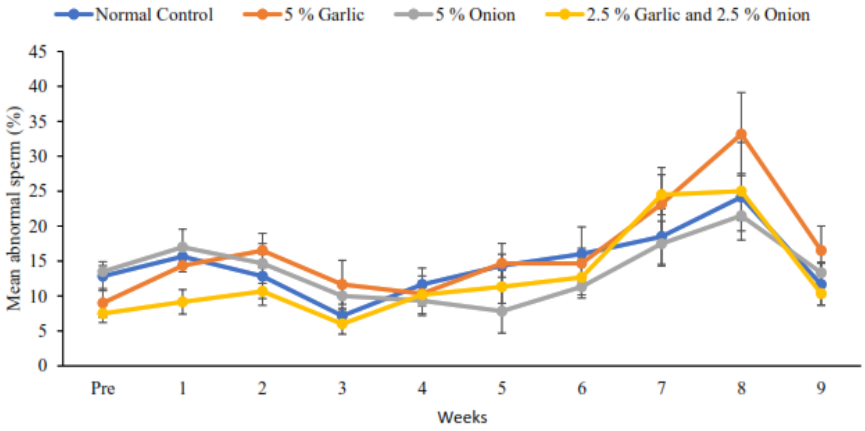

Figure 8: Mean percentage sperm morphological abnormalities of rabbit bucks fed *Allium sativum* and *Allium cepa*

Values are expressed as mean ± SEM. Pre; pre-treatment

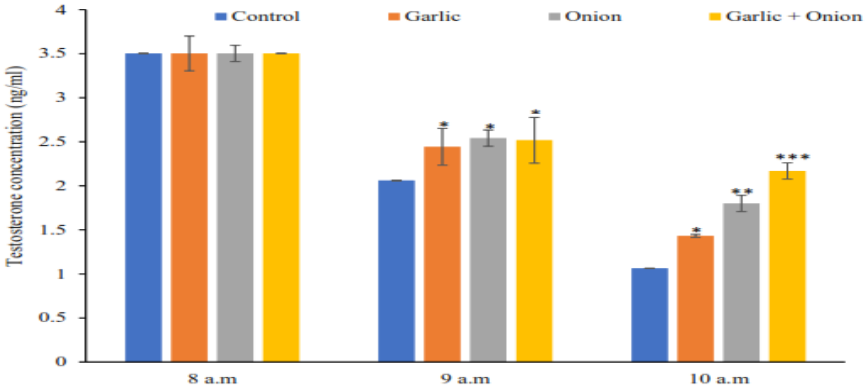

Figure 9: Mean testosterone concentration of rabbit bucks fed *Allium sativum* and *Allium cepa*

Values are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 significantly different when compared to the control

Table 4: Gonadal weight, length, volume and sperm reserve of rabbit bucks

Parameters	Normal Control	5% Garlic	5% Onion	2.5% Garlic + 2.5% Onion
Weight (g)				
Right Testis	2.35 ± 0.22	1.83 ± 0.01	2.23 ± 0.39	1.49 ± 0.34
Left Testis	2.53 ± 0.52	1.82 ± 0.23	2.26 ± 0.30	1.54 ± 0.55
Paired	4.88 ± 0.53^{b}	3.65 ± 0.17^{a}	4.49 ± 0.49^{b}	3.03 ± 0.89^{a}
Length (cm)				
Right Testis	2.85 ± 0.50	2.45 ± 0.07	2.75 ± 0.63	2.40 ± 0.14
Left Testis	3.00 ± 0.14	2.70 ± 0.56	2.90 ± 0.14	2.35 ± 0.07
Paired	5.85 ± 0.64^{a}	5.15 ± 0.63^{b}	5.65 ± 0.77	4.75 ± 0.21^{b}
Volume (cm^3)				
Right Testis	3.34 ± 2.38	3.00 ± 1.40	2.50 ± 0.71	3.00 ± 0.00
Left Testis	2.75 ± 1.77	4.00 ± 1.40	2.50 ± 0.71	3.50 ± 0.71
Paired	6.09 ± 4.15	7.00 ± 2.80^{a}	5.00 ± 1.42^{b}	6.50 ± 0.71^{a}
Gonadal Sperm Reserve ($\times 10^6$/g)				
Right Testis	6.00 ± 1.00	11.00 ± 2.00^{a}	4.50 ± 0.50	8.50 ± 0.50
Left Testis	6.00 ± 3.00	6.00 ± 1.00	5.00 ± 2.00	9.50 ± 2.50
Paired	12.00 ± 4.00^{b}	17.00 ± 3.00^{b}	9.50 ± 2.50^{a}	18.00 ± 3.00^{b}

Values are expressed as mean ± SD. ^{a,b}Means across rows with different letter superscripts are significantly different ($p < 0.05$)

supplemented with onion and garlic (Okoro *et al*., 2016; Shinkut *et al*., 2016; Banihani, 2019). This suggests that these sperm reproductive parameters were influenced by the range of onion and garlic supplemental inclusion levels used in the study; hence, the reproductive organ's attempt to adjust to the onion and garlic supplemental levels. The mean semen pH value of slightly alkaline to slightly acidic in the group fed 2.5% garlic + 2.5% onion when compared to the control, 5% garlic, 5% onion, by weeks 1 and 4 could be as a result of garlic potentiating the effect of onion; which has been reported to increase the pH of the sperm by previous researches (Shinkut *et al*., 2016; Sabir *et al*., 2019). The mean gross sperm motility of control and 5% onion group agrees with previous work by Khaki *et al*. (2009), but disagreed with the findings of Sabir *et al*. (2019), who reported no difference in the percentage spermatozoa motility of rabbits fed graded doses of the crude *A. cepa*. This could be due to differences in breed, age, and, more importantly, the source and variety of the onion species used. The non-significance in the control and 2.5% garlic + 2.5% onion group also disagree with the work of Okoro *et al*. (2016), who reported that daily supplementation with 2.5 g onion and 2.5 g garlic per 600 g DM feed reduced progressive motile cells in Koekoek breeder cocks. This was attributed to species differences and the variety of onion and garlic used for this study.

The observation of no significant difference in the mean sperm concentration in 5% onion when compared to control disagrees with the work of Khaki *et al*. (2009), who reported that administration of 4 g/kg of freshly extracted onion juice for 20 consecutive days in rats significantly affected sperm concentration in comparison to the control. This could be as a result of the different extraction methods and also species differences. The physiological explanation for higher mean sperm concentration in the group fed 5% Onion when compared to 5% garlic, and 2.5% garlic + 2.5% onion is not clear and merits further investigation. However, some researchers have shown that onion contains exogenous and endogenous antioxidants such as selenium, glutathione, vitamins A, B, and C and flavonoids such as quercetin and isorhamnetin (Griffiths *et al*., 2002; Khaki *et al*., 2009). These antioxidants protect sperm DNA from oxidation and damage and could improve sperm concentration in rabbit bucks. Also, the presence of antioxidants, such as vitamin C, and the flavonoids- quercetin in onion may have a protective effect against free radicals and protect sperm from oxidative damage (Khaki *et al*., 2009). The significant decrease in the mean sperm concentration in 2.5% garlic + 2.5% onion in comparison to group A in this study disagree with the work of Okoro *et al*. (2016), who reported that daily supplementation increases the sperm concentration in Koekoek breeder cocks. The observation of a significantly higher percentage of live spermatozoa in 5% onion group when compared to the control, 5% garlic and 2.5% garlic + 2.5% onion is consistent with

Table 5: Epididymal weight, length and sperm reserves of rabbit bucks fed *Allium sativum* and *Allium cepa*

Parameters	Groups	Caput			Corpus			Cauda		
		Right	Left	Paired	Right	Left	Paired	Right	Left	Paired
Weight (g)	Normal Control	0.26 ± 0.01	0.26 ± 0.01	0.52 ± 0.02	ND	ND	ND	0.35 ± 0.01	0.35 ± 0.04	0.70 ± 0.05
	5% Garlic	0.34 ± 0.11	0.27 ± 0.13	0.61 ± 0.24	ND	ND	ND	0.28 ± 0.01	0.27 ± 0.01	0.55 ± 0.02
	5% Onion	0.33 ± 0.06	0.32 ± 0.03	0.65 ± 0.09	ND	ND	ND	0.36 ± 0.12	0.33 ± 0.06	0.69 ± 0.18
	2.5% Garlic + 2.5% Onion	0.29 ± 0.06	0.30 ± 0.11	0.59 ± 0.17	ND	ND	ND	0.32 ± 0.05	0.31 ± 0.07	0.63 ± 0.12
Length (cm)	Normal Control	1.05 ± 0.07	1.00 ± 0.00	2.05 ± 0.07	1.65 ± 0.21	2.25 ± 1.06	3.90 ± 1.27	1.80 ± 0.28	1.65 ± 0.35	3.45 ± 0.63
	5% Garlic	1.15 ± 0.07	1.10 ± 0.14	2.25 ± 0.21	1.75 ± 0.35	1.85 ± 0.35	3.55 ± 0.70	1.40 ± 0.14	1.10 ± 0.00	2.50 ± 0.14
	5% Onion	0.95 ± 0.07	1.10 ± 0.14	2.05 ± 0.21	2.20 ± 0.57	2.25 ± 0.35	4.45 ± 0.92	1.40 ± 0.14	1.55 ± 0.50	2.95 ± 0.64
	2.5% Garlic + 2.5% Onion	1.25 ± 0.21	1.00 ± 0.00	2.25 ± 0.21	1.85 ± 0.49	1.80 ± 0.56	3.65 ± 1.05	1.25 ± 0.21	1.25 ± 0.21	2.50 ± 0.42
Epididymal sperm reserve ($\times 10^6$/g)	Normal Control	3.00 ± 0.46	3.50 ± 0.50	6.50 ± 0.96	3.30 ± 1.00	4.08 ± 0.40	7.38 ± 1.40	9.50 ± 0.50	10.00 ± 1.00	19.50 ± 1.50
	5% Garlic	4.00 ± 1.00	4.50 ± 1.50	8.50 ± 2.50	4.20 ± 0.50	3.50 ± 0.50	7.70 ± 1.00	24.00 ± 3.20	11.50 ± 4.50	35.50 ± 7.70[a]
	5% Onion	4.50 ± 1.20	3.00 ± 0.50	7.50 ± 1.70	4.70 ± 0.50	4.10 ± 1.00	8.80 ± 1.50	15.00 ± 1.50	11.00 ± 2.00	26.00 ± 3.50[b]
	2.5% Garlic + 2.5% Onion	6.00 ± 0.40	4.00 ± 1.00	10.00 ± 1.40	5.02 ± 0.20	3.50 ± 1.50	8.52 ± 1.70	14.00 ± 1.00[b]	15.00 ± 2.00	29.00 ± 3.00[b]

Values are expressed as mean ± SD. [a,b,c]Means along columns different letter superscripts are significantly different ($p < 0.05$), ND=Not determined

the work of Morales *et al*. (2006), who showed that onion contains quercetin, a bioactive flavonoid compound which increases the expression of metallothionein, a stress protein reported to offer protection against oxidative stress, and damage, therefore could improve sperm livability. It is believed that the left testicles are bigger than the right, and it produces more spermatozoa. Shinkut *et al*. (2016) reported that garlic was more potent on the left testicle, but in our study, we discovered it was the right testicle that garlic had more effect on. This could be due to the modification ability of the phytochemical constituents present in garlic to act more on the right testicles.

Testosterone hormone is pulsatile and episodic in nature and tends to decrease in concentration by the hour (Rekwot *et al*., 1997). At 9 a.m. to 10 a.m., the observation of a significant increase in testosterone concentration is consistent with the findings of Sabir *et al*. (2019), who suggested that powdered form of onion at 400mg OP/kg in the diet compared to control increases testosterone concentrations in rabbit bucks. However, at 10 a.m., we observed a significant increase in testosterone concentration of groups fed with 5% garlic, 5% onion and 2.5% garlic + 2.5% onion when compared to control. The highest value of testosterone was found in 2.5% garlic + 2.5% onion group, which suggest the possible synergistic effect of a mixture of 2.5% garlic and 2.5% onion in the diet of

Table 6: Haematological profile of rabbit bucks fed *Allium sativum* and *Allium cepa*

	Normal Control	5% Garlic 5	5% Onion	2.5% Garlic + 2.5% Onion
PCV (%)				
Pre-treatment	32.6 ± 0.88	32.05 ± 0.80	33.17 ± 0.77	31.23 ± 1.05
Week 4	32.15 ± 0.60	31.7 ± 0.74	32.05 ± 0.84	31.42 ± 0.92
Week 9	34.45 ± 0.69	39.43 ± 0.61[a]	40.27 ± 0.68[a]	39.55 ± 0.49[a]
HB (g/dl)				
Pre-treatment	11.33 ± 0.46	11.10 ± 0.58	11.23 ± 0.56	11.00 ± 0.50
Week 4	11.13 ± 0.54	10.92 ± 0.53	10.92 ± 0.57	10.58 ± 0.45
Week 9	10.88 ± 0.49	13.82 ± 0.46[a]	13.00 ± 0.40[a]	12.72 ± 0.49[a]
RBC ($\times10^{12}$/L)				
Pre-treatment	4.99 ± 0.28	4.93 ± 0.34	4.72 ± 0.43	4.54 ± 0.56
Week 4	5.02 ± 0.27	4.89 ± 0.30	4.68 ± 0.39	4.60 ± 0.41
Week 9	4.93 ± 0.25	6.84 ± 0.34[a]	6.39 ± 0.39[a]	6.79 ± 0.32[a]
WBC ($\times10^{9}$/L)				
Pre-treatment	5.55 ± 0.35	5.19 ± 0.34	5.41 ± 0.39	5.03 ± 0.25
Week 4	5.40 ± 0.35	5.12 ± 0.30	5.51 ± 0.44	5.08 ± 0.25
Week 9	5.22 ± 0.38	6.35 ± 0.42[a]	6.30 ± 0.40[a]	5.84 ± 0.45[b]

Values are expressed as mean ± SD. [a,b]Mean with different letters across rows are significant ($p < 0.05$). PCV; Packed cell volume, HB; Haemoglobin concentration, RBC; Red blood cell, WBC; White blood cell count

rabbit bucks.

The increase in the values of RBC count, Hb concentration, PCV and WBC counts at week 9 were similar to previous works by Samson *et al*. (2012) and Suha (2014), who reported an increase in RBC, PCV, WBC and Hb concentration in rats administered aqueous extracts of garlic and onion, in a dose-dependent manner, when compared to the control. However, it disagrees with the work of Adebolu *et al*. (2011), who observed a significant decrease in PCV and WBC count in rats administered 1ml of crude garlic extracts daily when compared with the control. They adduced the decrease to the administration of crude garlic extracts for a prolonged period of 7 weeks in the albino rats. The general increase in RBC, PCV, WBC, and Hb of rabbits fed garlic and onion supplemented diets indicates that garlic and onion may contain blood-forming factors that may have stimulated more blood production by the rabbits fed supplemented diets than those fed non-supplemented diets (Samson *et al*., 2012). Garlic and onion compounds seem to have a stimulatory effect on some haematopoetic growth factors (cytokines), which interact with specific receptors on the surface of haematopoietic cells, regulating the proliferation and differentiation of progenitor cells and the maturation and functioning of mature cells (Samson *et al*., 2012). Phytochemical nutrients of garlic and onion seem to act as ROS scavengers; compete with haemoglobin in the RBC for oxygen resulting in tissue hypoxia which in turn stimulates the kidney directly to cause formation and secretion of erythropoietin or that the end product of metabolism of garlic and onion in the body may stimulate Hb synthesis and RBC production by their indirect effect on erythropoietin (Samson *et al*., 2012; Tende *et al*., 2012).

In conclusion, this study was able to establish an increase in gross sperm motility, reaction time and percentage of live spermatozoa as a result of diet supplementation with onion. Garlic, however showed increased gonadal/testicular and extragonadal/epididymal sperm reserves more to the right testis and epididymis than left, while the combination of onion and garlic showed elevated levels of ejaculate volume from weeks 7 to 9 of the experimental period.

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Conflict of interest

The authors declare that there is no conflict of interest.

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