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EFFECTS OF DIETARY GARLIC SUPPLEMENTATION ON SPERM RESERVES, SERUM ANTIOXIDANT ENZYMES AND MALONDIALDEHYDE CONCENTRATION OF RABBIT BUCKS

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ABSTRACT

The aim of the study was to evaluate the effects of crude *Allium sativum*(garlic)in diets of rabbit bucks on sperm reserves, serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) concentration. Twenty-one bucks were used for the study and were randomly divided into 3 groups of 7 bucks each. Bucks in Group A served as control, while bucks in groups B and C were fed diets containing 2.5% and 5.0 % garlic respectively. The study lasted for 63 days after the period of acclimatization, Blood samples were collected on the last day of the study into collecting tubes without anticoagulant for serum antioxidant enzymes assay. Six bucks from each group were sacrificed and the testes and epididymis were harvested for sperm reserves determination. There were significant ($P<0.05$) increase in serum superoxide dismutase, catalase and glutathione peroxidase activities and a decrease in MDA concentration in serum of treated animals compared to bucks in the control. There were also significant ($P<0.05$) increase in sperm reserve of the left testis with increase in garlic dose. Both left and right cauda sperm reserves were significantly ($P< 0.05$) higher than the control in a dose dependent fashion. Testicular and epididymal reserves, showed increased number of sperm cells in the left testicles and epididymis than the right. Sperm reserve correlated positively with antioxidant enzymes and negatively with malondialdehyde concentration. From the result of the study it can be concluded that adding *Allium sativum* at 2.5% and 5.0 % in diets of rabbit bucks enhances serum antioxidant enzymes concentration, decreases MDA levels and improves sperm reserves concentration.

Keywords: Sperm reserves, Serum Antioxidant Enzymes,Malondialdehyde, Rabbit bucks, *Allium sativum*

INTRODUCTION

Rabbit production is a veritable means of alleviating animal protein deficiency in Nigeria (Ajala and Balogun, 2004).Rabbit meat is sixth after beef, fish, mutton, goat meat (chevon) and bush meat or game animals in the parametric assessment of meat animal production and consumption in Nigeria (Onifade *et al.*,1999).

Nigerians are yet to embrace rabbit production, however, rabbit production in Nigeria like other aspect of livestock production is confronted by many challenges among which includes: diseases and reproduction related problems especially infertility.

To enhance the production of healthy animal protein (low cholesterol and sodium) in Nigeria which rabbit meat is known for, there is a need to incorporate in rabbit feed botanicals reputed in disease prevention, high nutritive values and which may exert a positive effect on fertility in rabbit bucks. Drugs and synthetic hormones have been the mainstay for fertility boosting and control of reproductive related diseases in farm animals before now.There is paucity of information on the effect of garlic on reproduction in animals. The genus *Allium* contains the sulphur containing compounds which are medicinally active. As garlic is chopped or crushed the odour-free cysteine sulphoxide compounds are exposed to allinase enzymes and are converted quickly to thiosulphonate which gives the characteristics garlic aroma (Peter *et al*., 2008). The main thiosulphonate is allicin, the therapeutic value of garlic has also been attributed to this low molecular weight compound, although allicin is considered the main antioxidant and scavenging compound, studies reported that other compounds equally play key role (Chung, 2006).

Although a lot of work has been done to verify the medicinal properties of garlic, but very little has been said or done concerning its effect on reproduction.

MATERIALS AND METHODS

Study Area

The study was carried out at the Animal house of the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, situated in the Northern Guinea Savannah Zone of Nigeria and lying between latitudes 11˚3 N and 12˚N and between longitudes 7˚42 E and 8˚E at an elevation of 646 m above sea level.

Experimental Animals

Twenty one apparently healthy, domestic rabbit bucks (*Oryctolagus cuniculus*) between 10-12 months old with average body weight of 1.74 ± 0.11kg were used for the study. The bucks were screened and treated with ivermectin (Kepromec® Kepro Holland) broad-spectrum medication against ectoparasites and helminthes infestation before the experiment commenced water and feed were provided *ad libitum.* The bucks were housed in standard rabbit cages with dimension of 1.8×0.6m, one buck per cage.

Plant Sample

Allium sativum (garlic) bulbs were obtained from Sokoto main market, Sokoto State, Nigeria. The sample was confirmed with a voucher Number 423 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. Nigeria. The fresh bulbs were peeled and dried under shade, dried bulbs were then weighed and added to the feed raw materials and ground together to form the experimental diets (Table 1).

Table 1: Composition of the experimental diets (%)

Composition (%)	A	B	C
Crude *Allium sativum*	0.0	2.5	5.0
Dry Matter	93.6	91.77	91.68
Crude Protein	15.13	14.75	14.68
Ether Extract	11.51	13.23	9.86
Crude Fibre	19.65	19.39	17.77
Nitrogenfree extract	4.37	4.32	4.31
Ash	27.31	27.00	26.94
ME(kcal/kg)	1,647.26	1,752.98	1,494.83

Metabolizable energy calculated according to the formular of Pauzenga (1985):
ME = 37 × %CP + 81 × %EE + 35.5 × %NFE

Experimental Design

The rabbit bucks were randomly divided into three groups of seven each, designated as group A, B, and C. After 49 days of acclimatization in the cages, all rabbits were fed *ad libitum* diets corresponding to their group namely: Group A, B and C diets consisted of 0, 2.5% and 5.0% *Allium sativum*, respectively. The chemical composition of the experimental diets was determined by Weede Proximate Analysis Method and the result presented in Table 1. The period of feeding lasted for 63 days (i.e. through one spermatogenic cycle as reported by Swierstra and Foote, 1965; Oda and Waheeb, 2017).

Blood Sample Collection

Blood samples were collected by medial ear vein puncture using 21guage needle and syringe, then emptied into plain collection tubes tilted to facilitate the collection of serum. After clotting the samples were centrifuged at 1,000rpm for 10 minutes and sera collected into separate tubes and stored at -25 ˚C until the day of analysis.

Determination of TesticularSperm Reserves

Testicular sperm reserve was determined as described by Rekwot *et al.* (1994),with slight modifications. Six bucks from each group were humanely slaughtered and the testes removed and weight of each testis was determined using a digital weight balance and the values recorded. The *tunica albuginea* was carefully removed with a scalpel blade from each testis. The testicular spermatozoa number determined by homogenization (Egbunike *et al*., 1976) as follows: Each testis was homogenized in 25ml of physiological saline solution using a mortar and pestle with antibiotics (Streptomycin sulphate 1mg/ml and Penicilin G 100 iu/ml) added to the solution. The homogenate volume was measured after rinsing the mortar with 10ml of physiologic saline solution and adding the effluent. 2.5ml of the homogenate was transferred into a conical flask and further diluted with 40ml of saline solution. The diluted testicular homogenate samples were stored overnight at 5˚C and filtered through guaze and the filtrate volume measured. Spermatozoa concentration was determined using haemocytometer as follows:

Sperm Reserve = number of sperm cells counted in 25 small squares of haemocytometer × dilution factor × 10^4(Kwari and Waziri, 2001).

Determination of Epididymal Sperm Reserves

Epididymal sperm reserve was determined using the procedure described by Olukole *et al.* (2010). The epididymis were carefully removed from the testes with scalpel blade and weight of head, body and tail portions were determined using a digital weighing balance. These portions were minced separately in 20ml of normal saline with a sharp scissors and stored for 24 hours at 5˚C. The products were then filtered

through gauze and the volume measured. Then, 1ml of epididymal filtrate was then diluted with 2ml of normal saline and the concentration of the sperm reserves determined using Neubauer haemocytometer using light microscope.

Serum Antioxidant Enzymes Assay

Superoxide dismutase (SOD) was determined using Randox superoxide dismutase kit as described by Woolliams *et al.* (1983), absorbance was measured photometrically at 505nm. A standard curve was plotted for each standard against the percentage of inhibition which was used to determine the SOD activity (iu/L).

Catalase (CAT) activity was determined by Goth Method (Goth, 1991). Fifty microlitre (50 µL) of the serum sample was mixed with 1 mL of 65 mmol/mL, Hydrogen peroxide in 60 mmol/L Sodium Potasium phosphate buffer (pH 7.4) and incubated at 37˚C. One unit of Catalase represents the amount of enzymes that decomposes 1 µmol of Hydrogen peroxide per minute. The enzymatic reaction was terminated with 1 ml of 32.4 mmol/L ammonium molybdate. Hydrogen peroxide was measured at 405 nm wavelength using a spectrophotometer (Unico UV-2100 PC, Dayton, USA). The sensitivity was 0.001- 0.010units/ml, the intra and inter assay coefficient of variation were 3.8% and 9.9% respectively.

Glutathione peroxidase (GPX) detecting kit was purchased from Randox Laboratories Ltd UK. Reduction-Oxidation reaction took place between the serum glutathione reductase and Nicotinamide Adenosine Diphosphate (NADPH), oxide glutathione was reduced through change in oxidation of NADPH to $NADP^+$ at absorbance of 340nm wave length (Paglia and Valetine, 1987). The sensitivity was 0.01-0.10units/ml, the intra and inter assay coefficient of variation were 5.7% and 7.2% respectively.

Malondialdehyde (MDA) is a standard for determine free radicals damage in blood. A detection kit was purchased from Randox (Randox Laboratories Ltd United Kingdom). MDA in the serum was formed as an end product of lipid peroxidation and when treated with thiobarbituric acid (TBA) produced a colour product which was measured at 532nm (Placei*et al.,* 1966). The sensitivity was 0-0.10nmol/lit, the intra and inter assay coefficient of variation were 5.5% and 5.9% respectively.

Statistical Analyses

Data collected were expressed as mean ± standard error of mean (SEM) using Graphpad prism Version 5.0.One-way analysis of variance (ANOVA) was used to test for differences between groups, followed by Tukey's multiple comparison test, values of $P \leq 0.05$ were considered significant. Correlation analysis was use to relate oxidative stress biomarkers with sperm parameters.

RESULTS

The mean value of testicular weight and sperm reserves are presented in Table 2. The right, left and paired testes weights were not significantly different among the groups. However, the testicular sperm reserve was significantly ($P<0.05$) influenced by the treatments. Sperm reserve in left testicle (group C) increased significantly ($P<0.05$) compared to other groups.

Epididymal weight and sperm reserves of the rabbit bucks are shown in Table 3. The epididymal weights distribution were not significantly ($P>0.05$) different among the treatments. The sperm reserve in paired cauda epididymis were significantly influenced by the treatment compared to the paired caput and corpus. Also both left and right were influenced significantly by the treatment (group C and B).

Mean antioxidants enzymes (SOD, CAT, GPx) and MDA concentration are shown in Table 4. SOD concentration was significantly higher ($P<0.05$) in group C and B, compared to control (group A), likewise MDA concentration was significantly ($P<0.05$) lower in group C and B, compared to A.

Table 5 shows correlation analysis of sperm reserves [testicular sperm reserve (TSR), epididymal sperm reserve (ESR)] and oxidant/antioxidant biomarkers (SOD, CAT, GPx, MDA) in rabbit bucks. TSR correlated positively with SOD (r = 0.628) and strongly correlated with CAT (r = 0.994**)but negatively with MDA (-0.873). ESR correlated positively with CAT (r = 0.823), strongly correlated with SOD (r = 0.985**) and GPx (r = 0.919**) but negatively correlated strongly with MDA (r = 0.978**).

Table 2: **Mean (± SEM) values of testicular weight and sperm reserves of rabbit bucks fed diets containing 0, 2.5 and 5.0 % of garlic**.

Parameters	**Groups** **A (0%)** **n = 6**	**B (2.5%)** **n = 6**	**C (5.0%)** **n = 6**
Weight (g)			
Right Testis	1.51 ± 0.05	1.60 ± 0.02	1.85 ± 0.07
Left Testis	1.63 ± 0.05	1.67 ± 0.24	1.90 ± 0.01
Paired Testes	*3.140 ± 0.06*	*3.27 ± 0.04*	*3.75 ± 0.03*
TesticularSperm Reserve (× 10^6/g)			
Right Testis	136.50 ± 17.50	141.50 ± 20.50	186.00 ± 39.00
Left Testis	123.00 ± 22.00^a	143.50 ± 3.50^a	289.50 ± 26.50^b
TSR Paired Testes	*259.5±6.67^a*	*285±1.0^a*	*475.5±18.25^b*

Means with different superscripts letters along the row are statistically different(P < 0.05).

Table 3: **Mean (± SEM) values of epididymal weight (g), and sperm reserves (ESR × 10^6) of rabbit bucks groups (A, B and C) fed diets containing0%, 2.5% and 5.0% of garlic respectively**.

Treatment Parameters	**A (0%)**	**B (2.5%)**	**C (5.0%)**
Epididymal weight (g)			
Right epididymis	*0.17±0.05*	*0.57±0.01*	*0.51±0.04*
Left epididymis	*0.53±0.01*	*0.65±0.02*	*0.71±0.11*
Paired	*0.70±0.07*	*1.22±0.03*	*1.23±0.10*
Epididymal Sperm Reserves(× 106)			
Total caput	*86±0.22*	*73.5±0.12*	*79.0±0.02*
Total corpus	*34.5±0.02*	*98.5±0.03*	*77.0±0.08*
Total cauda	*461.5±9.08^a*	*876±10.17^b*	*1,015.5±12.5^b*
Right epididymis	*265.5±2.21^a*	*501.5±6.01^b*	*548.0±9.1^b*
Left epididymis	*316.0±5.06^a*	*546.5±7.05^b*	*623.5±4.05^b*
Paired ESR	*581.50±12.04^a*	*1,048±7.16^b*	*1,171.5±5.3^b*

Means with different superscripts letters along the columns are significantly (P < 0.05) different

Table 4: Mean (± SEM) values of antioxidant enzymes and malondialdehyde levels of rabbit buck groups fed diets containing 0, 2.5 and 5.0% of garlic.

S/N	Parameter	A (0%) n = 6	B (2.5%) n = 6	C (5.0%) n = 6
1	**SOD (iu/L)**	1.66 ± 0.08^{a}	2.06 ± 0.08^{b}	2.08 ± 0.11^{b}
2	**CAT(iu/L)**	40.83 ± 2.40	41.71 ± 1.02	44.57 ± 1.10
3	**GPX (iu/L)**	44.57 ± 1.31	49.29 ± 1.44	48.33 ± 1.41
4	**MDA (nm/L)**	1.81 ± 0.08^{a}	1.57 ± 0.07^{ab}	1.40 ± 0.07^{b}

Means with different superscripts letters along the row are significantly (P<0.05) different.

Table 5:**Correlation between biomarkers of oxidative stress (SOD, CAT, GPX & MDA) and paired sperm reserves (TSR & ESR) of rabbit buck fed diets containing 0, 2.5 and 5.0% of garlic.**

	SOD	***CAT***	***GPX***	***MDA***	***TSR***	***ESR***
SOD	*1.000*					
CAT	*0.712*	*1.000*				
GPX	*0.973***	*0.531*	*1.000*			
MDA	*-0.928***	*-0.923**	*0.816*	*1.000*		
TSR	*0.628*	*0.994***	*0.432*	*-0.873*	*1.000*	
ESR	*0.985***	*0.823*	*0.919***	*-0.978***	*0.753*	*1.000*

SOD= superoxide dismutase, CAT= catalase, GPX= glutathione peroxidase
MDA= malondialdehyde, TSR= testicular sperm reserve (paired), ESR= epididymal sperm reserves (paired).

DISCUSSION

The observed significant increase in left testes sperm reserves with increase in the level of garlic corroborates the report of Ekuma *et al.* (2017) that garlic has positive influence on sperm output in rabbit bucks. However, this contradicts the findings of Hammami *et al*. (2008) and Omotoso *et al.* (2009), which showed a decreased testicular sperm concentration in Wistar rats administered garlic. The difference with our findings may be due to the higher doses of *garlic* administered in those studies and species differences.

The changes in mean epididymal weights in all the groups is similar to the findings reported by Hammami *et al.* (2008) although Al-Bekairi*et al.* (1990) had earlier reported an increase in epididymal weight in garlic fed animals. There were no effects of garlic on the epididymal sperm reserves in the caput and corpus, but in the cauda a significantly (P<0.05) higher value was observed in a dose dependant manner in

both the right and left epididymis. Al-Bekairi *et al.* (1990) had reported an increase in epididymal sperm concentration in Wistar rats fed *A. sativum*, however, Hammami *et al.* (2008), reported a decrease in epididymal sperm concentration in Wistar rats fed graded levels of *A. sativum.*

In this study, we observed that in both testicular and epididymal sperm reserves, the left testes and epididymis had higher concentration of sperm cells than the right. One reason that could be adduced for the differences could be the slightly increased left testicular and epididymal weights, which probably was due to increased physiologic functions in the left as compared to the right. Similar variations in sperm concentrations between the right and left epididymis has been reported in other species for instance, Fadason (2009) reported increased sperm reserves in the right testes of Red Sokoto bucks. Ewuola while Egbunike (2010) reported increased sperm reserves in the right testes and epididymis in pubertal rabbits.

There were significant increases in the concentration of superoxide dismutase (SOD) in the sera of the treatment groups (B and C), in a dose dependent manner (Table 4). Flavonoid, one of the active constituents of garlic was reported to confer protection against the harmful effects of reactive oxygen species (ROS) (Shinkut, 2015). *In vitro* studies showed that flavonoid have potent antioxidant and free radical scavenging activities (Prochazkova *et al*., 2011; Attia *et al*., 2016; 2017). This may have been responsible for the elevated levels of antioxidant enzymes observe. Banerjee *et al*. (2002), who reported that chronic garlic administration in rats increased SOD concentration significantly. However, Omotoso *et al.* (2009), reported a decreased concentration of SOD in Wistar rats treated with aqueous garlic extract. We attributed the differences to doses.

The findings on Catalase concentration in this study between (group A) and groups (B and C), is similar with that of Banerjee *et al*. (2002), who reported no significant increase in concentration of Catalase in male rats treated with garlic compared to the control. However, it contradicts the findings of Reza *et al.* (2013), who reported a decreased concentration of Catalase in rats pretreated with garlic before exposure to Lead, the difference may be due to the differences in design of the study.

MDA is a product of lipid peroxidation in the body and it is used as a standard in assessing free radicals damage in the blood (Attia and Al-Harthi, 2015). The decreased concentration of MDA in the blood in this study in a dose-dependent manner may be an indication of an increased activity of antioxidant enzymes in the blood brought about by the administration of *A. sativum* to these groups (B and C). This finding is similar with that of Unsal *et al.* (2006), who reported a decrease in MDA levels in male rats pre-treated with *A. sativum* extract prior to testicular torsion and detorsion. Ghalehkandi (2014) also reported a significant decrease in MDA levels in semen of rats fed 120mg/kg aqueous extract of *A. sativum* for 35 days.

The increase in the activities of antioxidant enzymes indicates the response of the primary antioxidant system to act against free radicals, by preventing the depletion of sperm cells by ROS through the process of lipid peroxidation. The observed significant correlation between sperm reserves (TSR and ESR) and biomarkers of oxidative stress (SOD, CAT, GPx and MDA), shows there is a relationship between sperm reserves and biomarkers of oxidative stress. Therefore, this may be a confirmation of the beneficial role of dietary garlic as a good source of antioxidant.

CONCLUSION

Allium sativum at 2.5% and 5.0% inclusions in diets of rabbit bucks improved sperm reserves (testicular and epididymal), significantly increases the concentration of serum SOD with a corresponding significant decreases in serum MDA levels.

REFERENCES

Attia,Y. A., Al-Harthi, M. A. and Hassan, S. S.(2017).Turmeric (*Curcuma longa*Linn.) as a phytogenic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks.*Revista Mexicana de Ciencias Pecuarias,*8:11-21.

Attia, Y. A. and Al-Harthi, M. A. (2015).Nigella seed oil as an alternative to antibiotic growth promoters for broiler Chickens. *European Poultry Science,* 79: 80-96.

Attia, Y. A., Bakhashwain, A. A. and Bertu, N. K. (2016). Thyme oil (*Thymevulgaris*L.) as a natural growth promoter for broiler chickens reared under hot climate. *ItalianJournal of Animal Science,*16:1-8.

Ajala, M. K. and Balogun, J. K. (2004).Economics of rabbit production in Zaria, Kaduna state.*Tropical Journal of Animal Science,***7**: 1-10.

Al-Bekairi, A. M., Shah, A. H. and Qureshi, S. (1990). Effects of *Allium sativum* on epididymal spermatozoa, estradiol-treated mice and general toxicity. *Journal of Ethnopharmacology*,29: 117-125.

Banerjee, S. K., Maulik, A., Mancahanda-Dinda, A. K., Gupta, S. K. and Maulik, S. K. (2002).Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic.*Life Sciences*, 70: 1509-1518.

Chung, L. Y. (2006). The antioxidant properties of garlic compounds: Allylcysteine, alliin, allicin and allyldisulphide. *Journal of Medicinal Food, 9:205-213.*

Egbunike, G. N., Holtz, W. and Smidt, D. (1976). Reproductive capacity of German Landrace boar, II: sperm production rates as determined by quantitative testicular histology and from gonadal sperm reserves.*Reproduction in Domestic Animals,*11:35-37.

Ekuma, B. O., Amaduruonye, W., Onunkwo, D. N. and Herbert, U. (2017). Influence of garlic and Vitamin E on semen characteristics, reproduction performance and histopathology of rabbit bucks. *Nigeria Journal of Animal Production, 44: 117-128.*

Ewuola, E. and Egbunike, G. N. (2010). Gonadal and Extragonadal Sperm Reserves and Sperm Production of Pubertal Rabbits fed Dietary Fumonisin B. *Animal Reproduction Science,*119: 282-286.

Fadason, S. T. (2009). Testicular Functions, Testosterone Profile and Histopathological Changes Associated with Neutering in Red Sokoto Bucks. Ph.D dissertation, Ahmadu Bello University Zaria, Nigeria. Pages, 68-69.

Ghalehkandi, G. J. (2014). Garlic Juice Protects from Semen Oxidative Stress in Male rats exposed to Chromium Chloride. *Journal of Animal Reproduction,*11: 526-532.

Goth, L. (1991). A Simple Method for Determination of Serum CatalaseActivity and Revision of Reference range. *ClinicaChimicaActa*, 196:143-151.

Hammami, I., Nahdi, A. and Mauduit, C. (2008). The inhibitory effects on adult male reproductive functions of crude garlic (*Allium sativum*) feeding. *Asian Journal of Andrology,*10:593-601.

Kwari, H. D.and Waziri, M. A. (2001). Body weight, withers height, scrotal circumference, penis size and spermatozoa reserve of Balami rams. *SokotoJournal of Veterinary Science,*3:81-86.

Mansouri, O. and Abdennour, C. (2011). Efficacy of raw garlic on reproduction of domestic rabbit under Lead induced toxicity. *Annals of Biological Research, 2: 389-393.*

Nworgu, F. C. (2007). Economic importance and growth rate of broiler chicken served fluted pumpkin (*Telfariaoccidentalis*). *African Journal of Biotechnology,* 2**:**34-39.

Oda, S. S. and Waheeb, R. S. (2017). Ginger Attenuated Di (N-Butyl) Phthalate-induced Reproductive Toxicity in Pubertal Male Rabbits. *World Rabbit Science***,** 25: 387-393.

Olukole, S. G., Oyeyemi, M. O. and Oke, B. O. (2010). Gonadal and extragonadal sperm reserve of the domestic adult African greater cane rat (*Thryonomysswinderianus*). *Reproductive Biology*, 10: 155-158.

Omotoso, G., Oyewopo, A., Kadir, A., Olawuyi, S. and Jimoh, A. (2009). Effects of Aqueous Extract of *Allium Sativum*(garlic*)* On Semen Parameters in Wistar Rats. *The Internet Journal of Urology*,**7:**1-3.

Onifade, A. A., Abu, O. A., Obiyan, R. J. and Abanikannda, O. T. F. (1999). Rabbit Production in Nigeria: Some aspects ofcurrent status and promotional strategies. *World Rabbit Science*,**7:** 51-58.

Paglia, D. E. and Valetine, W. N. (1987). Studies on Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. *Journal of Laboratory and Clinical Medicine*, 70: 158-169.

Pauzenga. U. (1985). Feeding Parent Stock. Zootech, International. Pp.22- 25.

Peter, B. B., Patrick, M. F.andPina, L. (2008). Potential health benefits of garlic (Allium sativum): A Narrative Review. Journal of Complementary and Integrative Medicine, 5: 1-24.

Placei, Z. A., Cushman, L. L. and Johnson, B. C. (1966).Estimation of product of lipid peroxidation (malondialdehyde) in Biochemical Systems.*Annals of Biochemistry*,16: 356-364.

Prochazkova, D., Bousova, I. and Wilhelmova, N. (2011).Antioxidant and Prooxidant properties of Flavonoids.*Fitoterapia*,**4:**513-523.

Rekwot, P. I., Oyedipe, E. O. and Ehoche, O. W. (1994).The effects of feed restriction and realimentation on the growth and reproductive function of Bokoloji Bulls.*Theriogenology*, 42: 287-295.

Reza, A., Mehdi, A., Marzie, H., Hossein, T. and Neda, Z. (2013). Protective effects of garlic aqueous extract (*Allium sativum*), vitamin E and N-acetylcysteine on reproductive quality of male rats exposed to lead. *Veterinary Research Forum*, 4: 251-257.

Shinkut, M. (2015). Semen characteristics, gonadal sperm reserves and haematological parameters of rabbit bucks fed diets with *allium sativum* (garlic). M.Sc dissertation, Ahmadu Bello University, Zaria, Nigeria, Pages, 84-85.

Swierstra, E. E. and Foote, R. H. (1965). Duration of spermatogenesis and Spermatozoa Transport in the Rabbit based on Cytological Changes, DNA Synthesis and Labeling with Tritiated Thymidine. *Development Dynamics*, 116: 401-411.

Unsal, A., Eroglu, M., Avci, A., Cimentepe, E., Guven, C., Derya-Balby, M. and Durak, I. (2006). Protective role of natural antioxidant supplementation on testicular tissue after testicular torsion and detorsion. *Scandenevian Journal of Urology and Nephrology*, 40: 17-22.

Vernet, P., Aitken, R. J. and Drevet, J. R. (2004).Antioxidant Strategies in the Epididymis. *Molecular Cell Endocrinology*, 216: 31-39.

Woolliams, J. A., Wiener, G., Anderson, P. H. and McMurray, C. H. (1983). Improve method for the determination of blood Glutathione. *Research in Veterinary Sciences*, 34: 253-256.