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TESTICULAR AND EPIDIDYMAL SPERM RESERVES AND SPERM PRODUCTION OF RABBIT BUCKS FED *Moringa oleifera* LEAF MEAL BASED DIETS SUPPLEMENTED WITH GARLIC, GINGER OR BLACK PEPPER

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ABSTRACT

This study was carried out to evaluate testicular and epididymal weights, morphometry, sperm reserves and sperm production of rabbit bucks fed *Moringa oleifera* leaf meal (MOLM), MOLM supplemented with garlic (GR), ginger (GG) or black pepper (BP). The diet was formulated to contain 6% MOLM and supplemented with GR, GG or BP at 700g/100kg diet. The study used 25 mature (age between 6 and 7 months and average weight of 2196±204 g) cross bred (mixture of crosses from California, Chinchilla, New Zealand White and Dutch rabbit bucks and lasted for three months. Parameters monitored was total testis volume, testes density, complete testis sperm reserve, complete caudal epididymides sperm reserve, daily sperm production (DSP) and daily sperm production per gram parenchyma (DSP/gP). A completely randomized design was used and the data obtained was analyzed using the general linear model procedure of SAS while means separation was done using pairwise difference (Pdiff) method. Total testis volume and testes density were significantly ($P< 0.01$) lower and higher respectively from the MOLM fed bucks than the supplemented groups. Complete testis sperm reserve was significantly ($P<0.05$) lowest in bucks fed MOLM + GG diet than the other groups. Complete caudal epididymides sperm reserve was also significantly ($P<0.01$) higher from MOLM fed bucks than the supplemented groups. DSP was significantly ($P<0.05$) lowest from MOLM + GR fed bucks. DSP/gP was significantly ($P<0.01$) highest from MOLM + BP fed bucks. It is recommended that to improve caudal epididymides, epididimydis sperm reserve and DSP/gP, rabbit bucks should be fed MOLM, MOLM + GG or MOLM + BP diets.

Keywords: Rabbit bucks**,** Testis**,** Epididymis, Sperm reserve, Sperm production

INTRODUCTION

Reactive oxygen species are a wide group of materials that oxidize DNA and cell membrane and cause infertility (Chi *et al*., 2008). Some amount of reactive oxygen species is needed for normal cell functions (Chandra *et al*., 2009). Excessive production of reactive oxygen species may overpower the body's natural antioxidant defence system, creating an environment unsuitable for normal physiological reactions, because oxidative stress is caused by an imbalance between pro-oxidants and antioxidants (Al-Gubory *et al*., 2010). Spermatozoa contain high levels of

polyunsaturated fatty acids which makes them vulnerable to reactive oxygen species (Hsieh *et al*., 2006). A correlation exists between excessive reactive oxygen species generation in semen and infertility (Hsieh *et al*., 2006). Antioxidants are a group of compounds that resist against oxidant formation (Erguder *et al*., 2007). Antioxidants mitigate or prevent generation of free radicals or reactive oxygen species (Ali *et al*., 2008). Determination of gonadal and extragonadal sperm reserves can assist in determining how often a male animal can be used for mating without having any depression in herd fertility (Orlu and Egbunike, 2009). Moereover, knowledge of the basic morphometric characteristics of the reproductive organs is of utmost importance for assessment and prediction of fertility (Gage and Freckleton, 2003).

The temperature comfort zone for rabbits is 15 to 20°C, as such rabbits can withstand cold than warm weather (Sabah *et al*., 2016). Rabbits are more susceptible to high than to low ambient temperatures especially in the tropics (Dyavolova *et al*., 2014). Temperatures above the comfort zone can cause oxidative stress which can affect the fertility and other physiological traits of rabbits (Askar and Ismail, 2012).

Research has shown that garlic is able to scavenge reactive oxygen species by amplification of intercellular antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase (Corzo-Martínez *et al*., 2007). Ginger is a strong antioxidant which may either mitigate or prevent generation of free radicals (Ali *et al*., 2008). Ginger powder exerted useful effect on spermatogenesis and sperm parameters (Shanoon, 2011), and has profertility properties (Morakinyo *et al*., 2008). Black pepper stimulates the digestive enzymes of the pancreas and intestines, increases biliary bile acid secretion and enhances the bioavailabilty of nutrients such as amino acids, vitamins, beta-carotene and selenium (Ahmad *et al*., 2006). It also increased gastric acid secretion (Manoharan *et al*., 2009), reduced oxidative stress (Muhtaseb *etal*., 2008) and enhanced performance of animals (Abou-Elkhair *et al*., 2014). Simultaneous administration of antioxidants has been reported to improve reproduction (Castellini *et al*., 1999). New initiatives in the livestock and pharmaceutical industries are seeking to promote the use of alternative materials that combine the effects of nutritional and medicinal properties, simultaneously (Esiegwu *et al*., 2014).

According to Birt (2006), phytochemicals work in synergy and their effects when served together are stronger than the sum of the effects of parts served separately. There are thousands of known phytochemicals (Shahidi and Naczk, 2004) but the most common and important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds (Ibrahim and Fagbohun , 2012). Most phytochemicals posse many properties which makes them vital to both plants and animals. Some of these properties include; anti-oxidant property, anti-microbial properties (Serge and David, 1999) and physiological activities (Leverin and McMatron, 1999). This study was therefore aimed at determining the effect of feeding MOLM, MOLM supplemented with either garlic (GR), ginger (GG) or black pepper (BP) on testicular and epididymal sperm reserves of cross bred rabbit bucks.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the Rabbitry Unit of the National Animal Production Research Institute (NAPRI), Shika, Zaria. NAPRI is located in the Northern Guinea Savanna ecological zone (10°11´ N, 7°8´E, 650 m above sea level). The area receives an annual rainfall of 1100 mm, spread from April to October (wet season). The mean minimum and maximum temperatures were 20 and 34°C. The mean relative humidity during the rainy season (May - October) was 72% and during the dusty harmattan period (December – February) it dropped to 21%.

Source and Processing of Dietary Test Ingredients

Fresh *Moringa* leaf, garlic (GR) and black pepper (BP) were obtained from Sokoto central market, while ginger (GG) was obtained from Sabon Gari market, Zaria metropolis**.** The fresh GR, GG and BP were then chopped into small pieces with the aid of a sharp knife. The *Moringa* leaf, GR, GG and BP were then spread separately on clean fabric mats in the sun. They were constantly dried until they became crispy to touch (considered dry). The dried test ingredients were then separately ground using a milling machine. Each of the ground test ingredient was then packed in air tight polythene bags and kept at room temperature until when needed for feed formulation.

Experimental Animals, Housing and Experimental Design

Twenty-five mature cross bred rabbit bucks (mixture of crosses from California, Chinchilla, New Zealand White and Dutch, age between 6 and 7 months) with average weight of 1706±106 g were used. The experiment lasted for three months. Each buck was housed in an individual metal cage of 0.6m x 0.5m x 0.4m. There were five treatments with five replicates per treatment. Each replicate had one buck arranged in a completely randomized design.

Management of Rabbit Bucks before the Onset of Experiment

The rabbits were quarantined for two weeks before the onset the experiment. During the quarantine period, 1g Oxytetracycline antibiotic powder and 1 g Vitalyte powder were administered to the rabbits via drinking water for five days against possible bacterial infections and to alleviate stress. Thereafter, 3 mg of ivermectin tablet per rabbit was dissolved in 5 ml of clean fresh water and a 5 ml syringe was used to drench the rabbits against internal and external parasites. Embaxin Forte ® was administered against coccidiosis at 0.6g per litre of water for three days.

Phytochemical Analyses

Alkaloids were determined according to the method of Harborne, (1973)., flavonoids according to the method of Kumaran and Karunakaran (2006)., phenols were determined according to the method of Hagerman *et al*. (2000). Saponins according to the method of Obdoni and Ochuko, (2001) and tannins were determined according to the method of Van-Burden and Robinson, 1981). The phytochemical components of the test ingredients are shown in Table 1.

Table 1: Phytochemical Components of MOLM, GR, GG and BP (mg/g)

Parameters	MOLM	Garlic	Ginger	Black Pepper
Alkaloids	4.17	1.52	11.1	6.26
Flavonoids	4.72	0.78	4.55	2.64
Phenols	1.34	2.60	0.77	4.50
Saponins	6.03	10.37	2.5	9.03
Tannins	15.23	2.97	9.4	2.49

Experimental Diets and Procedure

Five diets were formulated with diet one having no *Moringa oleifera* leaf meal (MOLM) or spices (Control), diet two had 6% MOLM in the diet, diet three had 6% MOLM in addition to 700g garlic (GR), diet four had 6% MOLM in addition to 700g ginger (GG) and diet five had 6% MOLM in addition to 700g black pepper (BP). The gross composition and calculated analyses of the experimental diets is shown in Table 2. The bucks were offered 150g feed/buckdaily in the

morning at 08.00 and 50 g/buck of *Brachiariadecumbens* hayin the evening. Left over feed was measured daily before feeding. Clean water was offered *ad libitum* daily. The rabbit bucks were weighed at the onset of the study and fortnightly.

Table 2: Feed Ingredient(s) Composition and Calculated Nutrient Analyses of diets fed the rabbit bucks

Ingredient (%)	**Control**	**MOLM**	**MOLM+GR**	**MOLM+GG**	**MOLM+BP**
Maize	48	50.5	50.5	50.5	50.5
Wheat offal	22.2	22.2	22.2	22.2	22.2
MOLM	-	6	6	6	6
Soya cake	18	17.5	17.5	17.5	17.5
Salt	0.25	0.25	0.25	0.25	0.25
Bone	2.8	2.8	2.8	2.8	2.8
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
*Mineral/vitamin premix	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
			+700g GR	*+700g GG*	*+700g BP*
Calculated Analyses					
ME (Kcal/Kg)	2707	2705	2705	2705	2705
CP (%)	18.35	18.46	18.46	18.46	18.46
Ether Extract (%)	3.61	4.10	4.10	4.10	4.10
Crude Fibre (%)	4.70	4.58	4.58	4.58	4.58
Calcium (%)	1.08	1.15	1.15	1.15	1.15
Available P (%)	0.66	0.67	0.67	0.67	0.67
Methionine (%)	0.52	0.52	0.52	0.52	0.52
Lysine (%)	1.13	1.13	1.13	1.13	1.13

*Bio-mix Broiler starter premix supplied per kg of diet: vitamin A: 5,000 I.U.; Vit D3: 1,000 I.U.; Vit E: 20mg; Vit K3: 1mg; Vit B1: 0.2mg; Vit B2: 2.4mg; Vit. B6: 2.4mg; Niacin: 16mg; Calcium Pantothenate: 4mg; Biotin:0.032; Vit B12: 0.01mg; Folic acid: 0.4mg; Choline Chloride: 120mg; Manganese: 40mg; iron: 5mg; Zinc: 18mg; Cobalt: 0.1mg; Iodine: 0.62mg; Selenium: 0.04mg. MOLM= *Moringa oleifera* leaf meal; GR= Garlic; GG= Ginger; BP= Black pepper. P= Phosphorous.

Determination of testicular and Epididymal Sperm Reserves and Sperm Production

Testicular and epididymal sperm reserves were determined as described by Rekwot *et al*. (1994), with slight modifications. Two bucks from each treatment were slaughtered and the testes removed, the length, weight and volume of each testis were determined using a

measuring tape, digital weight balance and water displacement method respectively and the values recorded. The *tunica albuginea* was carefully removed with a scalpel blade from each testis. The testicular spermatozoa number was determined by homogenization (Igboeli and Rakha, 1971; Egbunike *et al*., 1976). Each testis was homogenized in 25 ml of physiological saline solution using a scissors to mince. Antibiotics (Streptomycin sulphate 1mg/ml and Penicilin G 100 iu/ml) were added to the solution. The homogenate volume was measured after rinsing the scissors with 10 ml of physiologic saline solution and adding the effluent. 2.5 ml of the homogenate was transferred into a conical flask and further diluted with 40 ml of saline solution. The diluted testicular homogenate sample was stored overnight at 5˚C and filtered through guaze and the filtrate volume measured.

Spermatozoa/spermatids concentration was determined using Neubauer haemocytometer according to the method of Kwari and Waziri (2001). The portions of each epididymis was minced separately in 20 ml of normal saline with a sharp scissors and stored for 24 hours at 5˚C. The products were then filtered through guaze and the volume measured. Then 1ml of epididymal filtrate was placed in a test tube and further diluted with 2 ml of normal saline and the concentration of the sperm reserves was determined using Neubauer haemocytometer under a light microscope (Kwari and Waziri, 2001).

The daily sperm production was obtained using the formular:

Daily sperm production (DSP) = Testes sperm count (gonadal sperm reserve)/Time divisor.

The value of the time divisor for rabbits = 3.43.

Daily sperm production per gram parenchyma (testis) per animal was estimated by the formula of Amao *et al*., 2012).:

Daily sperm production/g parenchyma (DSP/gP) = gonadal sperm reserve/ Gross testes weight- tunica albuginea×1/3.43

Experimental Model and Data Analyses

A completely randomised design was used, with the following statistical model:

$X_{ij} = \mu + T_i + e_{ij}$.

Where: X_{ij} = individual observation on j^{th} rabbit in the i^{th} treatment.

μ = population mean

T_i = effect of the i^{th} treatment diet on testicular and epididymal sperm reserves

e_{ij} = random error associated with the X_{ij}.

Data obtained from the experiment were analyzed using the general linear model procedure of SAS, while means separation was done using pairwise difference (Pdiff) method (SAS, 2001).

RESULTS AND DISCUSSION

Testicular and Epididymal Sperm Reserves and Sperm Production of Rabbit Bucks fed MOLM, MOLM Supplemented with Garlic, Ginger or Black Pepper

Table 3 shows testicular and epididymal sperm reserves and sperm production of rabbit bucks. Significantly ($P<0.05$) higher complete testis sperm reserve concentration was obtained from the MOLM fed bucks compared with those supplemented with garlic, ginger or black pepper. This result does not agree with the work of Matthew (2009) that garlic increased testis sperm reserve. Also, this study does not agree with the findings of Morakinyo *et al*. (2010) that ginger stimulates spermatogenesis. These variations might be as a result of the level of garlic or ginger used to feed the animals. The lower complete testis sperm reserve obtained in this study from bucks fed MOLM + BP compared with those fed MOLM diet could be related to the study of Dalal *et al*. (2013) that black pepper has anti spermatogenic effect. Reduction in the weight of testes, regression of seminiferous tubules and as a whole spermatogenic arrest was seen in male animals fed black pepper (Ukoha *et al*., 2014). The significantly lower right, left and complete caput epididymides sperm reserves from the MOLM fed bucks compared with the control bucks does not agree with the work of Priyadarshani and Varma, (2014) who

found that lumen formation, an indication of the degree of spermatogenesis, was highly seen in male animals fed MOLM.

Significantly (P<0.05) lower left caput epididymide sperm reserve was obtained from the MOLM fed bucks compared with bucks fed MOLM + Garlic, Ginger or Black Pepper diets. This might mean that these spices had positive effect on spermatogenesis as a result of their added antioxidative properties. Antioxidants increased epididymal sperm concentration (Sonmez *et al*., 2005). In line with this, Matthew (2009) reported that garlic improved gonadal and extra gonadal sperm reserves while ginger stimulated spermatogenesis (Morakinyo *et al*., 2010). Increase in epididymal sperm concentration, spermatocyte counts, spermatid counts and weight of epididymis tubules was reported in male mice fed black pepper (Sutyarso *et al*., 2016). Significantly (P<0.05) lower right, left and complete corpus epididymides was obtained in the MOLM fed bucks compared with the control fed bucks. This result does not agree with the report of Priyadarshani and Varma, (2014) that MOLM increased spermatogenesis through increased lumen formation in the testis. This disparity might be due to the level of MOLM fed or the method of processing the MOLM. Significantly (P<0.05) highest right, left and complete corpus epididymides was obtained from the MOLM + GG fed bucks compared with bucks fed MOLM diet in this study, which could be related to the report of Morakinyo *et al*. (2010) that ginger stimulates spermatogenesis.

The right, left and complete caudal epididymides sperm reserves obtained from the MOLM fed bucks was significantly (P<0.05) higher than other treatments, indicating the potential of MOLM to enhance spermatogenesis. MOLM increased testicular lumen formation which enhanced spermatogenesis (Priyadarshani and Varma, 2014). The significantly (P<0.05) higher epididymidis sperm reserve obtained from MOLM fed bucks compared with the control fed bucks further indicates that MOLM has beneficial effect on spermatogenesis. Bucks fed MOLM + BP had significantly (P<0.05) lowest epididymidis sperm reserve compared with other treatments, indicating BP had some negative effects on spermatogenesis because of the presence of piperine in black pepper and also likely because of the level fed. D'cruz and Mathur (2005) reported that piperine from black pepper could damage epididymal environment.

Daily sperm production of bucks fed MOLM was significantly (P<0.05) lower compared with the control bucks indicating that the level of MOLM fed might not have been sufficient to elicit beneficial response in DSP. Cajuday and Pocsidio, (2010) reported that MOLM induced sperm production. Significantly (P<0.05) lowest daily sperm production obtained from MOLM + GR fed bucks might mean that garlic had some negative effect on daily sperm production. Garlic is also associated with the inhibition of leydig steroidogenic enzyme expression and sertoli cell markers, which are capable of inducing apoptosis in testicular germ cells; the spermatocytes and spermatids (Omotoso *et al*., 2009). Matthew, (2009) observed that garlic increased sperm output. This disparity might be as a result of the level of garlic used. However, in relation to this study, garlic was reported to have inhibitory effect on spermatogenesis (Dixit and Joshi, 1983; Hammami *et al.,* 2008).

Daily sperm production per gramme parenchyma was significantly (P<0.05) highest in rabbit bucks fed MOLM + BP diet, which could mean that black pepper enhanced efficiency of sperm production. This might have occurred possibly because black pepper actually maintains and enhances the levels and efficacy of important antioxidant compounds. It contains several powerful antioxidants and is thus one of the most important spices for preventing and curtailing oxidative stress (Vijayan and Thampuran, 2000). Black pepper also has high content of zinc (Bouba *et al*., 2012) and zinc helps in testicular growth, development of seminiferous tubules and as such enhancing spermatogenesis (Kumar *et al*., 2012).

The significantly lower right testis and right caput epididymides and highest left testis and left caput epididymide sperm reserves obtained from the MOLM fed bucks and other treatments indicate differences in spermatogenesis of the sides of the testis. The significantly lower complete caput epididymides sperm reserve obtained from the MOLM and MOLM + BP fed bucks compared with the control and other diets fed bucks did not reflect the assertion that MOLM or black pepper enhances sperm production as reported by Cajuday and Pocsidio, (2010). This might be as a result of the level of MOLM used or the types of other ingredients in the diet used or the ecotype of *Moringa* leaf used.

Table 3: Testicular and Epididymal Sperm Reserves and Sperm Production (×10^6/ml) of Rabbit Bucks fed MOLM, MOLM Supplemented with Garlic, Ginger or Black Pepper

[abcde]Means across rows with different superscripts are significantly (P ≤ 0.05) different. DSP = daily sperm production; DSP/gP = daily sperm production per gramme parenchyma. SE= Standard Error; P= Probability.

Parameter	Control	MOLM	MOLM+GR	MOLM+ GG	MOLM+ BP	SE	P
Testis:							
Right	28.50^a	18.50^b	8.50^d	13.00^c	16.50^b	1.27	0.02
Left	21.50^a	24.0^a	6.50^c	17.50^b	21.00^a	1.31	0.03
Complete	50.00^a	42.50^a	15.00^d	30.00^c	37.00^b	2.53	0.03
Caput Epididymides:							
Right	11.00^a	4.50^b	13.00^a	12.00^a	3.50^b	0.68	0.01
Left	15.00^a	6.50^c	16.50^a	10.00^b	8.50^b	0.80	0.03
Complete	26.00^b	11.00^d	29.50^a	22.00^c	12.00^d	1.47	0.03
Corpus Epididymides:							
Right	2.00^b	0.10^c	0.10^c	3.50^a	0.10^c	0.18	0.03
Lefts	3.00^b	0.10^d	3.50^b	11.00^a	0.50^d	0.45	0.02
Complete	5.00^b	0.00^d	3.50^b	14.50^a	0.50^c	0.61	0.02
Caudal Epididymides:							
Right	55.50^c	84.00^a	61.00^b	39.00^d	15.50^e	3.76	0.01
Left	47.00^c	79.50^a	64.50^b	45.50^c	20.50^d	3.71	0.02
Complete	103.00^c	164.00^a	126.00^b	84.50^d	36.00^e	7.46	0.01
Epididymidis	134^b	175^a	159^a	121^b	48.50^c	9.13	0.03
DSP	14.60^a	12.40^b	4.40^e	8.90^d	10.90^c	0.73	0.03
DSP/gP	4.40^b	4.00^b	1.20^d	2.40^c	5.60^a	0.43	0.01

CONCLUSION

It is concluded that 700g GR or BP to 6% MOLM die improved either caudal epididymides or epididimydis sperm reserves and or DSP/gP in cross bred rabbit bucks.

REFERENCES

Abou-Elkhair, R., Ahmed, H. A. and Selim, S. (2014). Effects of Black Pepper (*Piper nigrum*), Turmeric Powder (*Curcuma Longa*) and Coriander Seeds (*Coriandrum sativum*) and their Combinations as Feed Additives on Growth Performance, Carcass Traits, Some Blood Parameters and Humoral Immune Response of Broiler Chickens. *Asian-Australian Journal of Animal Science*, 27(6):847–854. doi: 10.5713/ajas.2013.13644.

Ali, B. H., Blunden,G., Tanira, M. O. and Nemmar, A. (2008). Some Phytochemical, Pharmacological and Toxicological Properties of Ginger (*Zingiber officinale* Roscoe): A review of Recent Research. *FoodChemical Toxicology*, 46(2):409–20.

Amao, O. A., Togun, V. A. and Adejumo, D. O. (2012) .Gonadal and Extra – Gonadal Sperm Characteristics of Rabbit Bucks Fed Cottonseed Cake – Based Diets Supplemented With Vitamin E. *Journal of Animal Science Advances*, 2(10): 793-802

Askar, A. A. and Ismail, E. I. I. (2012). Impact of Heat Stress Exposure on Some Reproductive and Physiological Traits of Rabbits Does. *Egyptian Journal of Animal Production.* 49(2):151-159.

Birt, D. A. (2006). Phytochemicals and Cancer Prevention: from Epidemiology to Mechanism of action. *Journal of the American Dietetic Association*., 106: 20 – 24.

Bouba, A. A., Njintang, H. S., Foyet, H. S., Scher, J., Montet, D. and Mbofung, C. M. F. (2012). Proximate Composition, Mineral and Vitamin Content of Some Wild Plants Used as Spices in Cameroon. *Food Nutrition Science*., 3: 423-432.

Cajuday L. A. and Pocsidio, G. (2010). Effects of *Moringa Oleifera* Lam. on The Reproduction of Male Mice (*Mus musculus*). *Journal of Medicinal Plant Research*. 4(12): 1115-1121.

Castellini, C., Lattaioli, P., Bernardini, M. (1999). Effect of Dietary Supplementation with α- Tocopheryl Acetate and Ascorbic Acid on Qualitative Characteristics and Fertiliszing Ability of Rabbit semen. *World rabbit science,* 7 (4): 217-220.

Chandra, A., Surti, N., Kesavan, S and Agarwal, A (2009). Significance of Oxidative Stress in Human Reproduction. *Arch Med*., ;5:528–542.

Chi, H. J., Kim, J. H., Ryu, C. S., Lee, J. Y., Park, J. S., Chung, D. Y., Choi , S.Y., Kim, M. H., Chun, E. K. and Roh, S.I(2008).Protective Effect of Antioxidant Supplementation in Sperm-Preparation Medium against Oxidative Stress in Human Spermatozoa.*Human Reproduction*, 23:1023-1028.

Corzo-Martínez, M., Corzo, N. and Villamiel, M. (2007). Biological Properties of Onions and

Garlic. *Trends in Food Science and Technology*, 18:609-625.

Dalal, P. K. Tripathi, A. and Gupta, S. K. (2013). "Vajikarana: Treatment of Sexual Dysfunctions Based on Indian Concepts," *Indian Journal of Psychiatry*, 55 (Suppl

2): S273 - S276.

D'Cruz, S. C. and Mathur P. P. (2005). Effect of Piperine on the Epididymis of Adult Male Rats. *Asian Journal of Andrology*, 7: 363-368.

Erguder, B., Avci, A., Devrim, E. and Durak, I. (2007). Effects of cooking techniques on

antioxidant enzyme activities of some fruits and vegetables. *Turkish Journal of Medical Science*, 37:151-156.

Esiegwu, A. C., Okoli, I. C., Emenalom, O. O., Esonu, B. O. and Udedibie, A. B. I. (2014). **The Emerging Nutricitical Benefits of the African Wonder Nut***(Garcinia Kola Heckel):***A Review. *Global Journal of Animal Scientific Research,* 2(2): 170-183.**

Gage, M. J. G. and Freckleton, R. P. (2003). Relative testis size and sperm morphometry across

mammals: No evidence for an association between sperm competition and sperm length. Proceedings of Biological Sciences, 22: 625-632.

Hsieh, Y., Chang, C. and Lin, C. (2006). Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *International Journal of Biological Sciences*, 2:23-29.

Ibrahim, T. A. and Fagbohun, E. D. (2012). Phytochemical and Nutritive Quality of Dried Seeds of Buchholzia Coriacea. *Greener Journal of Physical Sciences*, 2: 185-191.

Igboeli, G. and Rakha, A. M. (1971). Seasonal changes in the ejaculate characteristics of Angoni (short horn zebu) bulls. *Journal of Animal Science*, 33: 651-654.

Kumar, P., Yadav, B. and Yadav, S (2012). Effect of Zinc and Selenium Supplementation on Semen Quality of Barbari Bucks. Indian Journal of Animal Research, 48 (4): 366-369.

Kumaran, A and Karunakaran, R.(2006). Anti oxidant and free radical scavenging activity of an aqueous extracts of *Coleus aromaticus*. *Food chemistry*, 97: 109-114.

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

Leverin, G. and McMatron , H (1999). Alkaloids and Glycosides. *Clinical Microbiology Review*, 11: 156-250.

Matthew, S. (2009). Semen Characteristics, Gonadal Sperm Reserves and Haematological Parameters of Rabbit Bucks Fed Diets Supplemented with *Allium Sativum* (Garlic). A Dissertation Submitted to The School of Postgraduate Studies, Ahmadu Bello University, Zaria In Partial Fulfillment of The Requirements for The Award of The Degree of Master of Science In Theriogenology Department of Theriogenology and Production, Ahmadu Bello University, Zaria, Nigeria.

Morakinyo, A. O., Adeniyi, O. S. and Arikawe, A. P. (2008). Effects of *Zingiber officinale* on Reproductive Functions in the Male Rat. *African Journal of Biomedical Research*, 11: 329-334.

Morakinyo, O. A, Achema, P and Adegoke, O (2010). Effect of *Zingiber officinale* (Ginger) on Sodium Arseniteae"Induced Reproductive Toxicity in Male Rats. *African Journal of Biomedical Research.* 13(1):39-45.

Muhtaseb, M. S., El Talwar, D., Duncan, A., St J. O'reilly. D., Mckee, R. F., Anderson, J. H. and Foulisa, F. I. G. (2008). Free radical activity and lipid soluble anti-oxidant vitamin status in patients with long-term ileal pouch-anal anastomosis. *Colorectal Disease*,11: 67-72.

Omotoso, G., Oyewopo, A., Kadir, R., Olawuyi, S. and Jimoh, A. (2009). Effects ofAqueous Extract of *Allium sativum* (Garlic) On Semen Parameters In Wistar Rats.*The Internet Journal of Urology*, Volume 7 Number 2.

Orlu, E. E. and Egbunike, G. N. (2009). Daily sperm production of the domestic fowl (*Gallus domesticus*) as determined by quantitative testicular histology and homogenate methods. *Pakistan Journal of Biological Sciences*, 12: 1359-1364.

Priyadarshani, N. and Varma, M. C. (2014). Effect of *Moringa oleifera* leaf powder on sperm count, histology of testis and epididymis of hyperglycaemic mice Mus musculus. *American International Journal of Research in Formal, Applied & Natural Sciences*, 7(1): 7-13.

SAS (2001). Statistical Analysis user guide version 8. SAS institute Inc. Carry, North Carolina U.S.A.

Serge, A. and David, M. (1999). Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 2: 125−129.

Shahidi, F. and Naczk, M. (2004). Phenolics in food and nutraceuticals. In Sources, applications and health effects, (CRC Press: Boca Raton, Florida), 566pp.

Shanoon, A. K. (2011). Effects of *Zingiber officinale* Powder on Semen Characteristic

and Blood Serum Sex Hormones Concentration in Broilers Breeder Male.*International Journal of Poultry Science,* 10 (11): 863-866.

Sudha, P., Asdaq, S. M., Dhamingi, S. Sand Chandrakala, G. K. (2010). Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in animals. *Indian Journal of Physiology and Pharmacology*; 54(2): 133-40

Sutyarso, M. and Kanedi, M. (2016). The Effect of Fruit Extracts of Black Pepper on the Fertility Potential of Male Albino Mice. *American Journal of Medical and Biological Research*, 4(1):1-4. doi: 10.12691/ajmbr-4-1-1.

Toader, O. R. (2014). Study of The Effects of *Zingiber officinale* (Ginger) on Spermatogenesis In Mice. *Annals of West University of Timişoara, Ser. Biology.,* Vol XVII (2), Pp.145-152

Ukoha, U. U., Egwu O. A., Dimkpa, U., Maduka, S. O., Okafor, J. I., Ndukwe, G. U. and Ogonnadi, K. (2014). "Histological and weight changes in testes of male albino rats fed with diets containing yaji (A Local Meat Sauce)," *International Journal of Health Science Research*, 4 (8): 118-126.

Van-Burden, T. and Robinson, W. (1981). Formation of complexes between protein and Tannin acid. *Journal of Agriculture and Food Chemistry*, 1: 77 Viegi, L., Pieroni, A., Guarrera, P. M. and Vangelisti, R. (2003). A review of plants used in folk veterinarymedicine in Italy as basis for a data bank. *Journal of Ethnopharmacology*, 89, 221–224.

Vijayan, K. K. and Thampuran, R. V. A. (2000). Pharmacology, toxicology and clinical application of blacfk pepper. In: Black Pepper (Piper nigrum L.). (Ravindran, P.N. Ed.), Amsterdam: Harwood Academic Publishers. pp. 455–466.

Yanishlieva N.V. (2001): Inhibiting oxidation. In: Pokorny, J., Yanishlieva, N. V., Gordon H. (eds): Antioxidantsin Food – Practical Applications. Woodhead Publishing, Cambridge: 22–70. 38 - 42