

Serum Enzymes and Histopathology of Rabbit Bucks fed Diets Supplemented with *Allium sativum* (Garlic)

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Abstract: This study was designed to evaluate the effects of crude *Allium sativum* in diets of rabbit bucks on serum enzymes and histopathology. Twenty one (21) 10±2 months old, 1.8±0.2 kg average body weight rabbit bucks were used for this study. These bucks were allowed to acclimatize for 49 days (for at least one spermatogenic cycle) before they were randomly divided into 3 groups of 7 bucks each. Group A served as control, group B and C received 2.5% and 5.0% garlic in diets respectively for a period of 62 days. On the last day of the study, 3mls of blood was collected through the marginal ear venepuncture from each buck into a non EDTA sample bottle for serum enzymes assay. Three of the bucks from each group were humanely sacrificed and samples of testes and liver obtained were fixed in bouin's solution and later processed and stained with haematoxylin and eosin for histopathology. The data generated were analysed using Graph Pad Prism version 5.0, repeated measure one way analysis of variance (ANOVA) was used to test for differences between groups, followed by Turkey's multiple comparison test and values of $P < 0.05$ were considered significant. It was observed that the serum levels of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) decreased in the treatment groups in a dose-dependent manner. The histopathology showed no changes in both liver and testes of the control and treatment groups. However, there was an increase in the number of sperm cells within the lumen of seminiferous tubules in the treatment groups in a dose-dependant manner. It was concluded that *A. sativum* at 2.5 and 5.0 % inclusion rate is not toxic to the liver and testicles but improved sperm concentration.

Keywords: Serum Enzymes, Histopathology, Rabbit Buck, Diets, *Allium sativum*

I. Introduction

Garlic (*Allium sativum*) has enjoyed wide patronage and extensive researches, due to wide spread belief on its medicinal and health benefits. Numerous investigations on the plant have been published during the last decade, indicating the widespread scientific interest (Harunabu *et al.*, 2001). Garlic is considered to be one of the best disease preventive plants among the *Alliums* because of its potent and widespread effect (Harunabu *et al.*, 2001) and eaten as an ingredient of a variety of many dishes worldwide.

However, the use of high concentrations or prolonged administration of garlic may cause undesirable effects. It has been reported that a significant loss of the normal cellular architecture occurred in the heart, liver and kidneys after 30 days feeding of garlic homogenate at a dose of 1g/kg/day (Banerjee *et al.*, 2001; 2002). Chronic administration (Dixit and Joshi, 1982) or excessive consumption (Hammami *et al.*, 2008, Hammami *et al.*, 2009) of garlic have been shown to result in the inhibition of spermatogenesis, and could compromise some other functions of the male reproductive system including testosterone production. The administration of aqueous extract of garlic at different doses also caused a reduction in sperm concentration and the percentage of morphologically normal spermatozoa in wistar rats (Omotoso *et al.*, 2010).

On the other hand, Yuriko *et al.* (2001) observed that garlic supplementation increases testicular testosterone concentration in rats. Apart from this hormonal change, significant increase in epididymal sperm count in mice administered aqueous garlic extract increase had been reported (Al-Bekairi *et al.*, 1990; Salah *et al.*, 2014).

An acute toxicity study carried out showed that after subcutaneous administration of graded doses of garlic in experimental rabbits, LD₅₀ was found to be 3034 mg/kg and maximum tolerated dose was 2200mg/kg. Signs of toxicity induced by the extract include: loss of appetite, depression, partial paralysis and death at higher doses of 3200mg/kg and 4200mg/kg (Mikail, 2010). Palmer *et al.* (1999), tested garlic inclusion levels (0.5, 2.5 and 5%) on 3 week-old-piglets for 5 weeks and reported no signs of toxicity.

Rabbit production is a veritable means of alleviating animal protein deficiency in Nigeria (Abdulmalik, 1994; Hassan and Owolabi, 1996; Ajala and Balogun, 2004), since the meat is sixth after beef and other sources

in the parametric assessment of meat animal production and consumption in the country (Onifade *et al.*, 1999). Rabbit production also provides high returns on investment, high quality meat products with high protein level of about 20.8%, low sodium, low fat and cholesterol levels which compares favourably with the local bush meat (Aduku and Olukosi, 1990; Onifade *et al.*, 1999). The consumption of rabbit meat, its use for laboratory investigations, as pets and application of faeces as a good source of manure are without cultural and religious biases (Biobaku and Oguntona, 1997; Omole *et al.*, 2005).

The presence of caecal microbes enables the rabbit to digest large amounts of fibrous feed better than most non ruminant species (Taiwo *et al.*, 1999). It is for this reason that the costs of beef, chevon, mutton, chicken and frozen fish are higher compared to rabbit meat (Aduku and Olukosi, 1990; Ajala, 1990). Irrespective of the attractive prospect, Nigerians are yet to embrace rabbit production. Like other livestock production systems in the country, rabbit production is confronted by many challenges including diseases and reproduction related problems, especially infertility (Nworgu, 2007).

Drugs and synthetic hormones have been the mainstay for fertility boosting and control of reproductive related diseases in farm animals before now, with its attendant problems including withdrawal period. To enhance the production of healthy animal protein (low cholesterol) in Nigeria rabbit meat appears to be the best option. It is necessary therefore, to incorporate in rabbit feed plant products which are known to have healing or preventive effects on prevalent diseases, high nutritive values and which may exert a positive effect on sperm production. This is in line with the global paradigm shift to organic livestock production to prevent the deleterious effects of drug residues and hormones from edible animal tissues to humans.

There is paucity of information on the effect of garlic on reproduction in rabbits. Although a lot of work has been done to verify the medicinal properties of this plant, very little has been said or done concerning its effect on reproductive of rabbits. This study was designed to determine the levels of serum enzymes and pathology in the testes and livers of rabbit bucks fed diets supplemented with *Allium sativum* (garlic).

II. Materials And Methods

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. The mean annual rainfall in the area is 1100 mm lasting from May to October (816 mm/month). Mean daily temperature during the wet season is 25°C and mean relative humidity of 72%. The dry season last from November to April, the mean daily temperature ranging from 14 to 36°C and relative humidity of 20-30% (www.world66.com).

Experimental Animals

Twenty one (21) apparently healthy, domestic rabbit bucks (*Oryctolagus cuniculus*) 10 ± 2.0 months old with average body weight of 1.74 ± 0.1 kg were used for the study. The bucks were screened and treated with broadspectrum medication (Kepromec®) against endoparasites and helminthes infestation before the commencement of the experiment, while water and feed were provided *ad libitum*. The bucks were housed in standard rabbit cages, one buck per cage.

Plant Sample

Allium sativum (Garlic) bulbs were obtained in June, 2014 from Sokoto main market, Sokoto State, Nigeria. The sample was identified, confirmed with a voucher Number 423 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria. The fresh bulbs were peeled and dried under shade. The dried bulbs were then weighed and added to the feed raw materials and ground together to form the experimental diets.

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, all rabbits were fed diets corresponding to their group as indicated in Table 3.1. The diets were of isonitrogenous and isocaloric values, consisted of maize, soyabean meal, rice offals, crude *Allium sativum*, vitamin premix, palm oil, bone meal, methionine and salt. Group A, B and C diets consisted of 0, 2.5% and 5.0% *Allium sativum*, respectively. The chemical composition of the feed is presented in Table 3.2, the period of feeding lasted for 63 days (ie through one spermatogenic cycle). At the end of feeding, blood samples were collected into non EDTA sample bottle for sera and three bucks from each group were humanely slaughtered and organs such as the testes and liver were harvested for histopathological examination.

Table 1 Composition of the Experimental Diets (%)

Ingredients(kg)	A	B	C
Maize	30.16	29.29	28.57
Soyabean meal	28.12	27.40	26.64
Rice offals	35.32	34.41	33.46
Crude <i>Allium sativum</i>	0.0	2.5	5.0
Vitamin premix	0.5	0.5	0.5
Palm oil	1.0	1.0	1.0
Bone meal	4.0	4.0	4.0
Methionine	0.4	0.4	0.4
Salt	0.5	0.5	0.5
Total (%)	100	100	100

Table 2 Chemical Composition of Experimental Diets

Item (%)	A	B	C
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
Nitrogen free extract	4.37	4.32	4.31
Ash	27.31	27.00	26.94

Serum Enzymes Assay

The sera were thawed, the Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) were assayed using the Audiocomb Serum Auto-analyser (Bayer Express Plus, Bayer Germany, Serial Number 15950) in the Chemical Pathology Laboratory, Ahmadu Bello University Teaching Hospital (ABUTH), Shika.

Histological Examination

At the termination of the experiment, three bucks from each group were humanely sacrificed and testes and liver samples obtained were immediately fixed in bouin's solution, for not less than four days. After fixation, the tissues were processed and sectioned at 5µ thickness using standard procedures (Luna, 1968; Humason, 1972). Each section was stained with haematoxylin and eosin (H&E) and examined with a light microscope as described by Zahid *et al.* (2002).

Data Analysis

Data collected were expressed as mean ± standard error of mean (SEM) using Graphpad prism version 5.0. Repeated measure one-way analysis of variance (ANOVA) was used to test for differences between groups, followed by Tukey's multiple comparison Test. Values of P < 0.05 were considered significant.

III. Results

Table 3: Mean (± SEM) values of Serum enzymes of rabbit buck 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	AST (iu/L)	42.50 ± 11.19 ^a	21.86 ± 4.01 ^b	18.43 ± 5.04 ^b
2	ALT (iu/L)	39.33 ± 3.92 ^a	29.00 ± 2.20 ^b	27.71 ± 3.23 ^b
3	ALP (iu/L)	41.57 ± 3.79 ^a	37.67 ± 3.69 ^a	26.29 ± 3.31 ^b

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

Histopathological Changes

The histopathological examination of the liver of rabbit bucks in the different treatment groups showed no observable changes (Plates 1). Similarly, there were no microscopic changes in the testes from bucks in all the groups. However, the seminiferous tubules in the treatment groups had more sperm cells in a dose dependent manner (Plate 2).

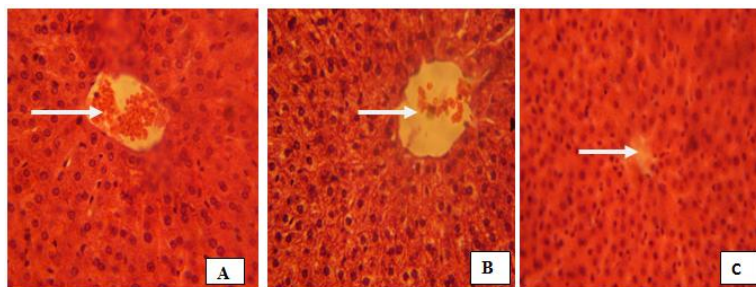


Plate 1: Photomicrograph of the livers from rabbit bucks. **A** (Control), **B** (Group B) and **C** (Group C) showing normal hepatocytes around central vein (arrow). H & E stain. $\times 400$.

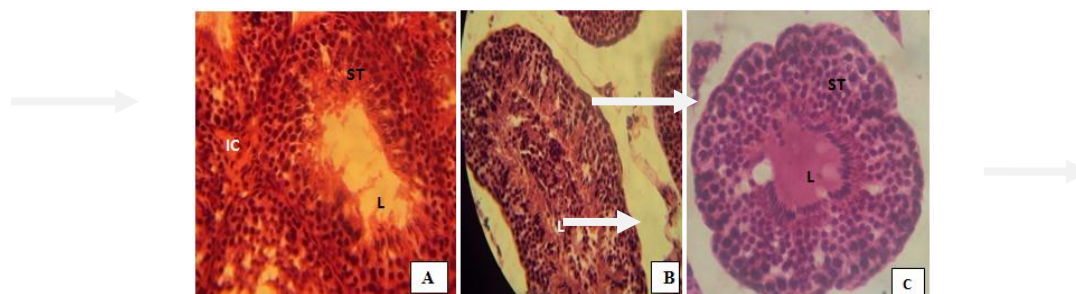


Plate 2 Photomicrograph of the testes of rabbit bucks. **A:** Histologic section of the testis of a rabbit in group A. Note the seminiferous tubules (ST) not clearly separated, interstitial cells (IC) not clearly separated from spermatogenic cells surrounding the ST, Spermatozoa (SPZ) sparsely distributed in a relatively spacious lumen (L) of the ST. **B:** Histologic section of the testis of a rabbit in group B testis. Note the ST clearly defined with thick layers of spermatogenic cells, interstitial cells (IC) clearly defined, Spermatozoa (SPZ) distributed within the lumen (L) of ST with no obvious space in the lumen. **C.** Histologic section of the testis of a rabbit in group C. Note the ST very clearly defined with thick layers of spermatogenic cells, interstitial cells (IC) clearly defined, packed Spermatozoa (SPZ) well arranged within the lumen (L) of ST (H & E $\times 400$).

IV. Discussion

Histological features of the liver showed a normal cytoarchitecture with the hepatocytes and central vein clearly seen in all groups. ALT, AST and ALP levels were lowered in this study. Paul (2009) reported that fluctuation in ALT activity is normal over the course of the day and ALT can increase in response to strenuous exercise. Ozcan (2012) reported the normal range values of serum enzymes as follows (AST, 6.00-20.00 iu/L; ALT, 6.00-9.00 iu/L; ALP, 12-26 iu/L).

In our study, histopathological finding showed a preserved structural integrity of the liver tissues. Alkaloid which is contained in garlic is reported to be an activator of liver detoxification enzymes providing protection against all forms of toxicity (Fahey *et al.*, 1997) this probably was responsible for the observations on serum enzymes and normal architecture of the liver. This further suggests a non deleterious effect of 2.5% and 5.0% of *A. sativum* in liver of the rabbits. Tende *et al.* (2014), administered 20 mg/kg and 40 mg/kg aqueous extract of *A. sativum* for 28 days to wistar rats and reported a normal cytoarchitecture of the liver, devoid of pathologic changes. This however, differs with the findings of Banerjee *et al.* (2001), who studied the morphological alterations in rat liver and kidney induced by garlic at 250 mg/kg, 500 mg/kg and 1000 mg/kg and reported large areas of necrosis, haemorrhage and neutrophils infiltration in the liver of the group that received the highest dose (1000 mg/kg). This would indicate that *A. sativum* toxicity can only occur at a very high dose or inclusion rate, implying a very wide margin of safety.

Histological features of the treated rabbit bucks showed seminiferous tubules with lumen filled with sperm cells with no apparent space in the lumen. The seminiferous tubules were clearly defined with very obvious interstitial cells in a dose dependent manner. It was also observed that the H & E stains were lightly taken by the cells of the treated bucks. Our findings are similar with that of Hammami *et al.* (2008) who used *A. sativum* inclusions of 0, 5.0%, 10%, 15% and 30% in male rats for 30 days and observed a distortion in testicular architecture at $\geq 10\%$ inclusion, with empty spaces in the lumen of the seminiferous tubules, devoid of sperm cells.

It is important to note that administration of *A. sativum* may have brought about increased antioxidant activities in the bucks this probably protected the sperm cells from depletion due to lipid peroxidation caused by ROS within the testes. This could be the reason why bucks of the treated groups had improved sperm concentration within the lumen of ST compared to the control.

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