SEMEN CHARACTERISTICS, GONADAL SPERM RESERVES AND HAEMATOLOGICAL PARAMETERS OF RABBIT BUCKS FED DIETS SUPPLEMENTED WITH *ALLIUM SATIVUM* (GARLIC)

BY

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DECLARATION

I declare that the work in this dissertation entitled "**Semen Characteristics, Gonadal Sperm Reserves and Haematological Parameters of Rabbit Bucks fed Diets Supplemented with *Allium sativum* (Garlic)**" has been performed by me in the Department of Theriogenology and Production, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Matthew SHINKUT ……. ……..

Signature Date

CERTIFICATION

This dissertation entitled **"SEMEN CHARACTERISTICS, GONADAL SPERM RESERVES AND HAEMATOLOGICAL PARAMETERS OF RABBIT BUCKS FED DIETS SUPPLEMENTED WITH *ALLIUM SATIVUM* (GARLIC)"** by Matthew SHINKUT meets the regulations governing the award of the degree of Master of Science in Theriogenology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

My life, my achievements, my attainments, my belongings, my success, my crowns, my legacies, my all… I dedicate to my Lord Jesus Christ, in You I live, move and have my being. I also dedicate this Thesis to my Dear wife Mrs Mercy Maisabo-Matthew and lovely daughters, Marvel Kubai Matthew and Matilda Kushiyak Matthew.

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ABSTRACT

This study was designed to evaluate the effects of crude *Allium sativum* (garlic) in diets of rabbit bucks on semen characteristics, haematological parameters, serum and antioxidant enzymes, sperm reserves and histopathology. Twenty one (21) 10.0 ± 2.1 months old bucks, with average body weight of 1.74 ± 0.1 kg were used for this study. Bucks were randomly divided into 3 groups of 7 bucks each. Group A were fed normal rabbit diet without garlic and served as control, group B and C received 2.5% and 5.0% garlic in diets respectively. The bucks were allowed to acclimatize for 49 days, before the commencement of the study, during which semen, blood samples and live weight were taken to establish a base line data. During the study, which lasted for 63 days, semen samples were collected weekly between the hours of 8.00-10.00am using artificial vagina (AV) for evaluation. Blood samples were also collected weekly for haematological evaluation. On the 62nd 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 and antioxidant enzymes assay. Three bucks from each group were humanely slaughtered and the testes and liver samples were harvested for sperm reserves and histological examinations. 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. Values of $P < 0.05$ were considered significant. There were no significant ($P > 0.05$) differences in mean live weight among the groups of bucks. The results of semen analysis showed, no significant ($P > 0.05$) difference in volume, pH, motility, % live spermatozoa and % abnormal sperms between control (group A) and treatment groups (B and C). However, the mean spermatozoa concentration significantly ($P < 0.05$) increased in the treated

groups in a dose dependent manner from week 3-9 of the study. Packed Cell Volume (PCV), Haemoglobin concentration and Red Blood Cell (RBC) counts were reduced, while blood protein were slighly increased in the treatment groups (B and C) in a dose dependent manner. Serum Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline phosphatase (ALP) levels were (39.33 ± 3.92^{a}; 29.00 ± 4^{b}; 27.71 ± 3.23^{b}), (42.50 ± 11.19^{a}; 21.86 ± 4.01^{b}; 18.43 ± 5.04^{b}) and (41.57 ± 3.79^{a}; 37.67 ± 3.69^{a}; 26.26 ± 3.31^{b}) respectively. Similarly, antioxidant enzymes showed a dose dependent increase in Superoxide dismutase (1.66 ± 0.08^{a}; 2.06 ± 0.08^{b}; 2.08 ± 0.11^{b}), Catalase (40.83±2.40; 41.71±1.02; 44.57±1.10) and Glutathione Peroxidase (44.57±1.31; 49.29±1.44; 48.33±1.41) respectively, while Malondialdehyde levels were (1.81 ± 0.08^{a}; 1.57 ± 0.07^{ab}; 1.40 ± 0.07^{b}) respectively. Testicular and epididymal, weight and length did not differ significantly ($P > 0.05$) among the groups. However, there was significant ($P < 0.05$) increase in the sperm reserves of the left testis (123.00 ± 22.00^{a}; 143.50 ± 3.50^{a}; 289.50 ± 26.50^{b}). Both left (244.50 ± 16.5^{a}; 472.50 ± 117^{b}; 538.5 ± 89.5^{b}) and right (216.50 ± 12^{a}; 403.50 ± 74^{b}; 477.00 ± 32^{b}) epididymal sperm reserves were significantly ($P < 0.05$) different. In both testis and epididymis reserves, we observed an increased number of sperm cells in the left than the right, also there were increased number of sperm cells within the lumen of seminiferous tubules in the treatment groups compared to the control . There was no obvious histopathological lesion observed in both liver and testes of the control and treatment groups. It was concluded from the study that *A. sativum* at 2.5 and 5.0 % inclusion rates improved sperm output in rabbit bucks.

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ABBREVIATIONS

A. sativum	*Allium sativum*
AGE	aged garlic extract
SAC	S-allylcysteine
SAMC	S-allyl mercaptocysteine
LD_{50}	Lethal Dose 50
DAS	diallyl disulphide
DADS	dially disulphide
DATS	diallyl trisulphide
LDL	Low density Lipoprotein
NSAID	Non steroidal anti-inflammatory drug
HDL	Higher Density Lipoprotein
DNA	Deoxyribonucleic Acid
USDA	United States Department for Agriculture
RHD	Rabbit Haemorrhagic Disease
RCD	Rabbit Calicivirus disease
VHD	Viral Haemorrhagic disease
RHDV	Rabbit Haemorrhagic Disease
HCG	Human Chorionic Gonadotrophin

HPG	Hypothalamic-Pituitary-Gonadal axis
LH	Luteinizing Hormone
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
HPGL	Hypothalamic-Gonadal-liver axis
PSG	Pregnant Serum Gonadotrophin
SC	Scrotal Circumference
BCS	Body Condition Score
OIE	International Office of Epizootics
ME	Metabolizable Energy
CP	Crude Protein
CSF	Cerobrospinalspinal Fluid
IGF-I	Insulin-like Growth factor I
AV	Artificial Vagina
˚C	Degree Centigrade
EDTA	Ethylenediamine tetraacetic acid
PCV	Packed Cell Volume
RBC	Red Blood Cell
WBC	White Blood Cell

ALT	Alanine Transaminase
AST	Aspartate Transaminase
ALP	Alkaline Phosphatase
SOD	Superoxide dimutase
CAT	Catalase
GPX	Glutathione Peroxidase
MDA	Malondialdehyde
TBA	Thiobarbituric acid
NADPH	Nicotine Adenosine diphosphate
H & E	Haematoxylin Eosin
SEM	Standard Error of Mean
ANOVA	One Way Analysis of Variance
EGSR	Extragonadal Sperm Reserves
SPZ	Spermatozoa
IC	Interstitial Cell
ST	Seminiferous Tubules
L	Lumen
ROS	Reactive Oxygen Species

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Garlic belongs to the kingdom: Plantae; Phylum: Magnoliophyta; Class: Liliopsida; Order: Asparagales; Family: Alliaceae; Genus: *Allium*; Species: *sativum*. Other plants of the same genus as garlic include: Onions, Leaks and Chives. Garlic belongs to one of the largest plant genera on earth (White and Zellner, 2008). The name garlic comes from Anglo Saxon word "garlec" meaning "spear" in reference to its spear-shaped leaves. It is believed that the plant originated from a wild ancestor *Allium longicuspis*, in South Central Asia, an area occasionally referred to as "garlic crescent" (Estes, 2000).

Garlic has been analysed for moisture, carbonhydrates, protein, fat, minerals, energy, ash, pH, and essential oil contents (Haciseferogullari *et al*., 2005). Protein content was found to be higher than that in other plants such as bean and pea (Cemeroglu and Acar, 1986), but crude oil content was considerably low. Garlic is known to contain high levels of Potassium, Phosphorus, Magnesium, Sodium, Calcium and Iron, with the amount of these minerals in the bulb depending on the soil content of the respective minerals where the bulb is grown. Vitamins like riboflavin, thiamine, nicotinic acid, vitamin C and vitamin E are important chemical constituents of garlic (Alejandra *et al.*, 2010).

Garlic (*Allium sativum*) has enjoyed wide patronage and extensive researches from ages past, due to wide spread belief on its medicinal and health benefits. More than a thousand publications over the past decade alone revealed the widespread interest on this plant (Harunabu *et al.*, 2001). Many of the *Allium* plants have been shown to reduce health risks and modulate body metabolism to favour the prevention of diseases. Garlic

is considered to be the best disease preventive plant among the *Alliums* because of its potent and widespread effect (Harunabu *et al*., 2001).

In ancient times, the Babylonians, Egyptians, Phoenicians, Vikings, Chinese, Greeks, Romans and Hindus used garlic frequently, as a remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of ageing and other ailments (Block, 1985). It was used to treat the wound surface of soldiers during the World War ll (Essman, 1984).

Garlic (*Allium sativum*) is one of the most researched plants, with a long history of medicinal use (Block, 1985). Many of its constituents contribute to its medicinal properties and potential to lower the risk of diseases (Banerjee *et al*., 2002). Various formulations of garlic exist, which include raw garlic homogenate (Isaacson *et al.*, 1998), garlic powder, aged garlic extract and steam-distilled garlic (Banerjee *et al.*, 2002). The important components, among others, include allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) which is thought to be the chief bioactive compound present in garlic; allyl methyl thiosulfonate, 1-propenyl allyl thiosulfonate and γ-L-glutamyl-S-alkyl-L-cysteine (Aqel, 1991).

Some studies have reported that the use of high concentrations or prolonged administration of garlic may cause undesirable effects. A significant loss of the normal cellular architecture of the heart, liver and kidneys after 30 days feeding of garlic homogenate at a dose of 1g/kg/day was also reported by Banerjee *et al*. (2001, 2002). Chronic administration of garlic powder (50mg/day) also resulted in inhibition of spermatogenesis in rats, reflecting the antiandrogenic nature of garlic (Dixit and Joshi, 1982). It was observed that excessive consumption of garlic compromised some male reproductive functions, such as spermatogenesis and testosterone levels which are

essential for reproduction (Hammami *et al.,* 2008, Hammami *et al.,* 2009). Administration of aqueous extract of garlic at different doses also caused a reduction in the percentage of morphologically normal spermatozoan as well as sperm concentration in wistar rats (Omotoso *et al*., 2010). On the other hand, Yuriko *et al*. (2001), reported that garlic supplementation increases testicular testosterone concentration in rats. Al-Bekairi *et al*. (1990), reported a significant increase in epididymal sperm count in mice administered aqueous garlic extract and also Salah *et al*. (2014) reported a significant increase in semen concentration by garlic in his study.

An acute toxicity study carried out showed that after subcutaneous administration of graded doses of garlic in experimental rabbits, LD_{50} 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. In another study, Ari *et al*. (2012), used inclusion rates (0, 10, 15 and 20%) daily supplementation of garlic per kilogram of feed at both starter and finisher diets in broilers and reported a corresponding reduction in cholesterol deposition, increased broilers performance as the inclusion levels increased, with no obvious signs of toxicity.

Rabbit *(Oryctolagus cuniculus)* is a micro livestock species appearing to be one of the cheapest and fastest means of producing high quality animal protein and fast-growing livestock (Vietmeyer, 1985). Rabbits possess a number of features that might be of advantage in the small holder subsistence type integrated farming in developing countries. Rabbit production is a veritable means of alleviating animal protein deficiency in Nigeria (Abdulmalik, 1994; Hassan and Owolabi, 1996; Ajala and Balogun, 2004).

Rabbit production in Nigeria could be described as rudimentary or primitive as compared to countries such as France, Hungary, China and the United State. This is evident from the small rabbit keeping population in which gender bias and sociological status of rabbit farmers, weak inventory of rabbit keeping infrastructure, low consumption rate of rabbit meat, absence of an organized or thriving market for rabbit meat products and lack of governmental and institutional support limit the expansion of rabbit production (Onifade *et al.,* 1999).

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).

Aduku and Olukosi (1990) and Onifade *et al.* (1999) reported that rabbit production has a lot of benefits among which includes its high adaptability, easiness to handle and manage, high growth rate, high efficiency in converting forage to meat, short gestation period and very high prolificacy. It also provides high returns on investment, high quality meat products and it contains high protein level of about 20.8%, low sodium, low fat and cholesterol levels which compares favourably with the local bush meat. Its use for laboratory processes and as pets and its faeces is a good source of manure, and its consumption is 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 as most non ruminant species cannot (Taiwo *et al.,* 1999). Costs of beef, chevon, mutton, chicken and frozen fish are high compared to rabbit meat (Aduku and Olukosi, 1990; Ajala, 1990).

1.2 Statement of Research Problem

Ironkwe and Amaefule (2007), reported between 83% and 140% returns on capital investment in rabbit farming but despite this attractive prospect, Nigerians are yet to appreciate rabbit production. 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 (Nworgu, 2007).

To boost the production of healthy animal protein (low cholesterol and sodium) in Nigeria which rabbit meat is reputed for, there is a compelling need to incorporate in rabbit diet/feed botanicals known for its reputation in prevention of diseases, high nutritive values and which may exert a positive effect on sperm production in rabbit bucks.

1.3 Justification of the Study

Globally, there is a strong campaign towards organic livestock production to prevent the deleterious effects of drug residues and hormones from edible animal tissues to humans. 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 particularly 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 reproduction.

Therefore, the outcome of this study will also constitute a source of baseline data, for advising the ethno-medical practitioners and the public on the consumption and usage of garlic.

1.4 Aim of the Study

The aim of the study is to examine the semen characteristics, haematological parameters, serum and antioxidant enzymes assay, gonadal/extragonadal sperm reserves and histopathology of rabbit bucks fed diets with graded levels of garlic (*Allium sativum)*

1.5 Objectives

i. To determine the semen characteristics of rabbit bucks fed diets with graded levels of garlic
ii. To determine the haematological parameters of rabbit bucks fed diets with graded levels of garlic
iii. To determine the serum and antioxidant enzymes levels of rabbit bucks fed diets with graded levels of garlic.
iv. To determine the gonadal and extragonadal sperm reserves of rabbit bucks fed diets with graded levels of garlic
v. To determine the testicular and liver architecture of rabbit bucks fed diets with graded levels of garlic

1.6 Research Questions

i. Does feeding graded levels of garlic in diets have varied effects on semen characteristics, sperm reserves and testicular architecture of rabbit bucks?
ii. Does feeding graded levels of garlic in diets have varied effects on haematological parameters, serum and antioxidant enzymes of rabbit bucks?

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal Plants

Medicinal plant is any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1993). Several plants have been used in traditional medicine for many years, but no sufficient scientific data to confirm their efficacy (Sofowora, 1993).

The plant kingdom having been explored for compounds of medicinal value for many years, still undoubtedly has many species of plants containing substances of medicinal value that are yet to be discovered (Trease and Evans, 1989).

Plants have been known to provide a rich source of raw materials for traditional medicine in Africa and other parts of the world. About 80% of the population in developing countries are unable to afford pharmaceutical drugs and most of them rely on traditional medicine to meet their primary health care needs (Suresh *et al.,* 2013).

2.2 Medicinal Plants with Anti Fertility Properties

Medicinal plants still enjoy wide spread patronage across the globe. However, several species have been described as antifertility plants (Lin, 1992, Al-Ruwais, 2002). The practice of traditional medicine for the control of fertility in most parts of the world has been based on the use of medicinal plants for many years (Priya *et al.,* 2012). Several medicinal plants have been used as dietary adjunct and in treatment of numerous diseases thereby, inducing infertility without proper knowledge of their functions (Marles and Farnsworth, 1994). Although, several medicinal plants pose different types of antifertility activities such as anti implantation, abortifacient, oestrogenic and

spermicidal, as shown (Table 2.1). A large number of medicinal plants pose some degree of toxicity (Marles and Farnsworth, 1994).

Table 2.1 List of Medicinal Plants with Their Anti Fertility Activities.

S/N	Common name	Botanical name	Family	Plant part used
Antiestrogenic activity				
1	Turmeric	*Carum cervi*	Apiaceae	Rhizome
2	Wild carrot	*Daucus carota*	Apiaceae	Dry seed
3	Varuna	*Crataeva nurvala*	Capparidaceae	Stem bark
4	Papaya	*Carica papaya*	Caricaceae	Fruits, Seeds
5	Mukajhuri	*Acalypha indica*	Euphorbiaceae	Whole plant
6	Bhutala	*Croton roxburghii*	Euphorbiaceae	Bark
7	Fenugreek	*Trigonella foenum gracum*	Fabaceae	Seed
8	Honey suckle mistletoe	*Dendrophthoe falcate*	Loranthaceae	Aerial parts
9	Abuta	*Cissampelos pareira*	Menispermaceae	Leaves
10	Lotus	*Nelumbo nucifera*	Nymphaceae	Seeds
11	Betel pepper	*Piper betel*	Pedaliaceae	Petiole
12	Knot weed	*Polygonum hydropiper*	Polygonaceae	Root, Powder
13	Sour Chinese date	*Zizyphus jujube*	Rhamnaceae	Bark
14	Caster	*Curcuma aromatic*	Zingiberaceae	Rhizome
15	Haldi	*Curcuma longa*	Zingiberaceae	Rhizome
Antiimplantation activity				
1	Aaghada	*Achyranthus aspera*	Amranthaceae	Root
2	Papaya	*Carica papaya*	Caricaceae	Fruits, seeds
3	Common sesban	*Sesbania sesban*	Fabaceae	Seeds
4	Golden shower	*Cassia fistula*	Fabaceae	Pods, seeds
5	Calliandra brevipes	*Derris brevipes*	Fabaceae	Powder root
6	Hore hound	*Bullota undulate*	Labiatae	Leaves, Flower
7	Onion	*Allium cepa*	Liliaceae	Bulb
8	Honey suckle mistletoe	*Dendrophthoe falcate*	Loranthaceae	Aerial parts
9	Malai vembu	*Malia azedarach*	Meliaceae	Seed
10	Ground cherry	*Physalis alkekengi*	Piperaceae	Plants
11	Witches weed	*Striga orobanchioides*	Serophulariaceae	Plants
12	Perumaram	*Ailanthus excels*	Simaroubaceae	Leaf, stem
Abortifacient activity				
1	Aaghada	*Achyranthus aspera*	Amranthaceae	Root
2	Calliandra brevipes	*Derris brevipes*	Fabaceae	Powder root
3	Honey suckle mistletoe	*Dendrophthoe falcate*	Loranthaceae	Aerial parts
4	Perumaram	*Ailanthus excels*	Simaroubaceae	Leaf, stem
5	Desert date	*Balanites roxburghii*	Zygophyllaceae	Fruits
Antiovulatory activity				
1	Haemorrhage plant	*Aspilia Africana*	Asteraceae	Leave
2	Dhak-ki-be	*Rivea hypocrateriformis*	Convolvulaceae	Aerial parts
3	Snake gourd	*Trichosanthus cucumerina*	Cucurbitaceae	Plant

Contraception activity				
1	Sage leaf alangium	*Alangium salvifolium*	Alangiaceae	Stem bark
2	Wild caper	*Capparis aphylla*	Capparidaceae	
3	Thunder god vine	*Trypterygium wilfordii*	Celastraceae	Root
4	Tree spinach	*Cnidoscolous aconitifolius*	Euphorbiaceae	Leaves
5	Goonj	*Abrus precatorius*	Fabaceae	Leaves
6	Pudina	*Mentha arevensis*	Lamiaceae	Leaves
7	Nirmali	*Strychnos potatorum*	Loganiaceae	Root
8	Tult	*Rumex steudeli*	Polygonaceae	Root
9	Desert date	*Balanites roxburghii*	Zygophyllaceae	Fruits
10	Ylang	*Cananga adorata*	Annonaceae	Root, Bark
11	Cumin	*Cuminum cyminum*	Apiaceae	Seed
12	Yarrow	*Achillea millefolium*	Asteraceae	Flowers
13	Goonj	*Abrus precatorius*	Fabaceae	Leaves
14	Tulasi	*Ocimum gratissimum*	Labiataceae	Leaves
15	Indian squirrel tail	*Colebrookia oppositifolia*	Lamiaceae	Leaf
16	Neem	*Azadirachta indica*	Meliaceae	Seed
17	Devils claws	*Martynia annua*	Pedaliaceae	Roots
18	Long pepper	*Piper longum*	Piperaceae	Seed
19	Black pepper	*Piper nigrum*	Piperaceae	Fruit powder
20	Bilva	*Aegle marmelos*	Rutaceae	Leaf
21	Common rue	*Ruta graveolens*	Rutaceae	Plant powder
22	Brahmi	*Bacopa monnieri*	Serophulariaceae	Plant
23	Surinam wood	*Quassia amara*	Simaroubaceae	Bark, leaves

Source: (Priya *et al.*, 2012).

2.3 Mechanism of Action of Antifertility Plants

Information concerning the mechanisms by which chemicals or plant parts or their extracts exhibit their antifertility properties is not clearly understood. However, attempts were made from studies carried out in commonly used Laboratory animals such as rats, mice, guinea pigs and rabbits to shed more light in understanding the possible course of action (Farnsworth *et al*., 1975[a]). The reproductive cycles of these species differ among themselves and as such this explains the varying responses sometimes exhibited by a given anti-fertility substance when tested in different species. The antifertility agents may interrupt reproductive functions at some point (Udor and Kehinde, 1991; Pathak *et al*., 2000). In both male and female the Hypothalamo-Pituitary-Gonadal axis as well as other parts of the reproductive tracts, may be affected. Thus, a given compound or plant extract may exert its antifertility effects in more than one of these areas by different pharmacologic mechanisms. Conversely, different compounds or extract may act in the same way to inhibit fertility but in some cases by different mechanisms. There are other antifertility substances that affect other stages e.g. implantational and post implantational mechanism (Verma and Chinoy, 2002; Ekanem and Okoronkwo, 2003).

During spermatogenesis, preleptotene and leptotene spermatocytes differentiate from type B spermatogonia and spermatogonia residing near the basal lamina translocate across the blood testes barrier in the inter-Sertoli compartment of the seminiferous epithelium, entering the adlumina compartment for further development (Kretser and Kerr, 1988). The timely movement of the developing germ cells across the epithelium, coupled with the continual attachment of germ cells onto the Sertoli's cells throughout their development are conceivably essential for the completion of spermatogenesis. The

cellular events of germ cells movement and germ cells attachment are also potential target for antifertility agents (Kretser and Kerr, 1988).

2.4 Classification of Garlic and Description

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Liliopsida

Order: Asparagales

Family: Alliaceae

Genus: *Allium*

Species: *A. sativum*

(Source: White and Zellner, 2008).

2.5 Description and Distribution of Garlic (*Allium sativum*)

The name Garlic may have originated from Celtic word '*all*' meaning pungent. Cultivated practically world wide, garlic appears to have originated in central Asia and then spread to China, the near East and the Mediterranean region before moving west to central and southern Europe, Northern Africa (Egypt) and Mexico (Singh and Singh, 2008). Today garlic is cultivated in most regions of the world.

Allium sativum (cultivated) garlic, is a species of monocot, bulb-forming perennial. Relatives include onions (*Allium cepa)*, and leeks (*Allium ampeloprasum*). Garlic is not found in the wild and '*sativum*' means 'cultivated' but appears to have originated in mountainous regions in Central Asia (Brewster, 2008).

Garlic has been cultivated for over 5000 years. Diverse representations in Mesopotamian writings from 3000 B.C. and Egyptian art dating to 2700 B.C suggest that garlic was already widely used (Block, 2010). Hippocrates, Galen, Pliny the Elder, and Dioscorides recommend garlic for conditions including parasitic infestations, respiratory problems, poor digestion. Garlic is also mentioned in the Bible (Block, 2010).

Garlic bulbs contain separate fleshy sections (cloves), each covered with a papery skin (tunic). The plants produce a leafless flower stem (a scape), but the flowers are sterile and produce bulbils (small cloves) rather than seeds; the species is propagated clonally from cloves and bulbils (Block, 2010). Hundreds of cultivars are divided into two subspecies: 1) Hardneck garlic (*A sativum* ssp. *ophioscorodon*) and 2) Softneck garlic (*Allium sativum ssp. sativum)*, (Block, 2010). Garlic produces various sulfur compounds that together with their breakdown products yield a characteristic pungent taste and odor, which may persist on the breath and body for up to 30 hours as garlic is metabolized (Block, 2010).

2.6 Phytochemistry of Garlic

Garlic has been analysed for moisture, carbohydrates, protein, fat, minerals, vitamins, energy, ash, pH, acidity and essential oil contents as shown (Table 2.2), (Haciseferogullari *et al*, 2005). The protein content was found to be considerably higher than that in other vegetables such as bean and pea (Cemeroalu and Acar, 1986), but crude oil content was considerable lower. Garlic moisture was also low, compared to caper bud and caperberries fruits (Ozcan and Akgul, 1998; Ozcan, 1999) and other vegetables (Cemeroglu and Acar, 1986). In terms of minerals content, garlic is known to contain high levels of potassium (21 g/kg), phosphorous (6 g/kg) followed by magnesium (1 g/kg), sodium (532.78 mg/Kg), calcium (363.61 mg/Kg) and iron (52.91

mg/Kg). In addition, garlic also contains some quantities of selenium and germanium. The amount of these minerals in the bulb depends on the content of the respective minerals in the soil where the bulb is grown. Vitamins like riboflavin, thiamine, nicotinic acid, vitamin C and vitamin E are important chemical constituents of garlic (Alejandra *et al.*, 2010).

The biological effects of some of these constituents in intact garlic, such as lectins (the most abundant proteins in garlic), prostaglandins, fructan, pectin, adenosine, vitamins B_1, B_2, B_6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids and essential amino acids, have been studied for over several decades (Fenwick and Hanley, 1985). Recently, special attention has been given to certain steroids, saponins and sapogenins such as β-chlorogenin. Several studies have demonstrated the importance of their biological and pharmacological activities such as antifungal, antibacterial, antitumor, anti-inflammatory, antithrombotic and hypocholesterolemic properties (Matsuura, 2001; Lanzotti, 2006). Since β-chlorogenin is bioavailable *in vivo* and detected in blood, this indicates that β-chlorogenin may be a bioactive compound in garlic. Other characteristic chemical constituents of garlic include allixin and organo-selenium compounds. These chemical compounds are reported to exhibit several biological effects, including cholesterol reduction and cancer prevention (Amagase, 2006).

However, despite the fact that the above mentioned compounds contribute in part to garlic bioactivity, evidence from several investigations suggests that the biological and medicinal functions of garlic are mainly due to their high content in organo-sulphur compounds (Augusti and Mathew, 1974; Wargovich *et al.*, 1988), which likely work synergistically with other compounds such as organo-selenium compounds.

2.6.1 Phytochemical constituents of garlic and their effects

Phytochemical screening is the method employed to detect the present and isolation of accumulated natural plants products or secondary metabolites of plants such as saponins, flavonois, carbohydrates, triterpenes, alkaloid e.t.c. in the plant extract through simple chemical tests. These secondary metabolites give plants their therapeutic properties and thus referred to as active constituents (Farnsworth, 1966).

Saponins are steroids in large number of plants and are products of human and animal nutrition. Several biological effects are ascribed to it, these include: immunostimulant, anticarcinogenic and improve libido and spermatogenesis (Francis *et al.,* 2002). Haemolysis of RBC results from saponin ability to form complexes with cell membrane cholesterol leading to pores formation and cell permeability. It also caused alteration in negatively charged carbohydrates portions on cell surface (Gauthier *et al*., 2009).

Flavonoids are one of the many molecules that are used by cells for the protection against the harmful effect of Reactive Oxygen Species (ROS). *In vitro* studies showed that flavonoids have potent antioxidant and free radical scavenging activities (Prochazkova *et al.*, 2011).

Triterpenes are known to exhibit cytotoxicity against a variety of tumour cells as well as anticancer efficacy in pre clinical animal models (Anupam *et al.*, 2011). Alkaloid in form of glucosinolate present in cruciferous vegetables is activator of liver detoxification enzymes, protecting against carcinogenesis, mutagenesis and other forms of toxicity and ROS (Fahey *et al*., 1997).

Table 2.2 Nutritional Value and Properties of Garlic. Values Expressed per 100 g of raw Garlic.

Properties	Values	Minerals	Values	Vitamins	Values
Energy	I19kca	Potassium	446 mg	Thiamin(Vit.B_I)	0.1 6 mg
Moisture	70 %	Phosphorus	134mg	Riboflavin (Vit. B_2)	0.02 mg
Protein	4.30 g	Magnesium	24.1 mg	Niacin (Vit. B_3)	1.02 mg
Carbohydrate	24.3 g	Sodium	19 mg	Piiridoxin (Vit. B_6)	0.32 mg
Fibre	1.20 g	Calcium	17.8mg	Folic acid	4.8 µg
Fat	0.23 g	Iron	1 .2 mg	Ascorbic acid (Vit- C)	14 mg
Ash	2.30%	Zinc	1.1 mg	Carotenoids (β-carotenes))	5 µg
pH	6.05	Iodine	4.7 µg	Vitamin A	Traces
Acidity	0.17%	Selenium	2µg	Vitamin E (Tocopherols))	0.011 µg

Source: Alejandra *et al*. (2010).

Intact garlic cloves contain only a few medicinally active compounds (Block, 1992; Lawson, 1993). The primary sulphur-containing constituents in whole garlic are the S-alk (en) yl-L-cysteine sulphooxides (CSs, 1.8%) and γ~glutamyl-S-alk(en)yl-L-cysteine peptides (0.9%), both non-volatile and therefore, odour-free sulphur compounds. It has been estimated that S-allyl-L-cysteine sulphoxide (alliin) and S-methyl-L-cysteine sulphoxide (methiin), the major CSs in garlic, together with S-(2-carboxypropyl) glutathione, γ -glutamyl-S-allyl-L-cysteine, γ-glutamyl-S-(trans-1 -propenyl)-L-cysteine and γ-glutamyl-S-allyl-mercapto-L-cysteine, make up more than 82% of the total sulphur content of whole garlic (Fenwick and Hanley, 1985; Sendl, 1995). The γ-glutamylcysteine peptides are biosynthetic intermediates for corresponding CSs (Lancaster and Shaw, 1989).

On prolonged storage or during germination, the enzyme γ-glutamyl transpeptidase acts on γ- glutamylcysteine peptides to form thiosulfinates (Sendl, 1995) such as S-allyl-cysteine (SAC), which is also present in intact garlic and contributes heavily to the health benefits of some garlic preparations (Amagase *et al.,* 2001). The thiosulfinates other than SAC (e.g. allicin) as well as other oil-soluble components such as ajoenes (e.g. E-ajoene and Z-ajoene), vinyldithiins (e.g. 2-vinyl-(4H)-l,3-dithiin and 3-vinyl-(4H)-l.2-dithiin), and sulphides (e.g. diallyl sulphide, DAS, diallyl disulphide, DADS, and diallyl trisulphide, DATS), provide to garlic its characteristic odour and flavour as well as most of its biological properties (Lanzotti, 2006), but they are not naturally occurring compounds in intact garlic. When garlic is cut, crushed, chewed, dehydrated or otherwise processed, the vacuolar enzyme, alliinase, is released and rapidly lyses the cytosolic CSs (mainly alliin), which are converted into hundreds of organo-sulphur compounds in a short period of time. First, it forms the reactive intermediate allylsulfenic acid (R-SOH), which immediately condenses to form the odoriferous alkyl

alkane- thiosulfinates, among which, allicin represents 70-80% of total. Then, allicin (allyl 2-propene thiosulfinate) and other thiosulfinates such as allyl methane thiosulfinate, which are very unstable products, instantly undergo a number of transformations, giving rise to other sulphur-compounds derivatives, depending on environmental and processing conditions as temperature, pH and solvent polarity (Block, 1985; Reuter and Sendl, 1995; Amagase *et al.,* 2001). Sulphur-containing compounds in *c*ommercial garlic preparations vary, depending on the manufacturing processes. Likewise, the variety of garlic determines' the composition and quantity of each CS identified, which, in turn, determine the odour, flavour variation and biological activities observed.

2.7 Medicinal and Pharmacologic Properties of Garlic

Early men of medicine such as Hippocrates, Pliny and Aristotle attributed a number of therapeutic uses for this botanical (Murray, 2005). According to the US Food and Drug administration survey of 900 people, garlic stands as the second most utilized food supplement behind Echinacea (Cone flower), with almost 17% of the population using garlic in the preceding 12 months (Timbo *et al*., 2006). Most of the garlic consumed today comes from China, South Korea, India, Spain and United States. In addition to its reputation as a healthy food, garlic has antiviral, antibacterial, antifungal, and antioxidant capacities. Additionally, anti-atherosclerotic and anti-cancer properties have also been demonstrated (Peter *et al.,* 2008).

The genus *Allium* contains the sulphur compounds which are medicinally active (Peter *et al*., 2008). When garlic is chopped or chrushed the odour-free cysteine sulphoxide are exposed to allinase enzymes and quickly convert to thiosulphonates which give off garlic characteristics aroma. The main thiosulphonate is allicin, which has a half life of

16 hours at room temperature or two and half days when kept as juice or crushed form. Other thiosulphonates include alliin, allyl cysteine and allyl disulphate. The allinase enzyme responsible for the thiosulphonate conversion is inactivated at pH below 3.5 or when heated. Microwave radiation will destroy allinase activity within one minute (Pedrazza-Cheverri *et al*., 2006)

Until recently, the therapeutic value of garlic has been attributed to the low molecular weight thiosulphinates (allicin). Although, allicin is considered the major antioxidant and scavenging compound, studies have shown that other compounds may play a vital role (Chung, 2006). Research findings have characterized some polar compounds of phenolic and steroidal origin which proffer various pharmacologic properties. These latter compounds in contrast to the thiosulphinates are without odour and are also heat stable (Lanzotti, 2006). Furthermore, some scavenging properties of garlic are not affected by heating or cutting (Pedraza-Chaverri *et al*., 2006).

2.7.1 Effects on cardiovascular diseases

Approximately 30% of cardiovascular patients who use herbal supplements take garlic (Yeh *et al.*, 2006). Known risk factors for cardiovascular disease include inflammation, high cholesterol, high hymocysteine, high blood pressure, diabetes and dementia including its most common form, Alzheimer's disease. As early as in the 1930's studies have shown the beneficial effects of garlic on cardiovascular diseases (Taubman, 1934, Rahman, 2001). Garlic is known to scavenge oxidants, increase superoxide dismutase, catalase, glutathione peroxidase and glutathione levels, as well as inhibit lipid peroxidation and inflammatory prostaglandins (Peter *et al.*, 2008). Garlic also reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-coA. It has been shown to inhibit Low Density Lipid (LDL) oxidation, platelet aggregation, arterial plaque

formation, decrease homocysteine, lower blood pressure and increase microcirculation which is important in diabetes, where microvascular changes increase heart disease and dementia risks (Peter *et al.,* 2008). Garlic may also help to prevent cognitive decline by protecting neurons from neurotoxicity and apopsis, thereby preventing ischemia or reperfusion related neuronal death, theirby improving learning and memory retension (Borek, 2006).

2.7.2 Effects on Alzheimer's disease

This is a neurological disorder in which death of brain cells causes memory loss and cognitive decline. It also results in a neurodegenerative type of dementia and often starts mildly and gets progressively worse. Garlic is known for its neuroprotective abilities *in vitro* (Peng *et al.,* 2002), aged garlic has been looked to for multiple benefits that some researchers believed may address a number of underlying mechanisms which contributes to classic Alzheimers beta-amyloid plaque. Garlic produces a cumulative benefit and also exhibits enhanced neuroprotection by virtue of being "natural NSAID, natural anti-oxidant" natural anti-apoptotic agent and memory enhancer (Chauhan, 2006).

2.7.3 Effects on atherosclerosis and hyperlipidemia

This is a medical condition associated with high levels of cholesterol and lipids in the blood above normal, leading to plaque deposits along the arterial vessels, consequently the arterial vessels are narrowed, impeding normal blood flow through the vessels.

Lau. (1987) found that aged garlic extract was effective in lowering serum cholesterol and triglycerides. Thirty (30) volunteer individuals with blood cholesterol levels higher than 245 mg/dl were involved in a study to investigate the effect of garlic on blood cholesterol levels (Mahmoodi *et al.,* 2006), the subjects ingested 5g garlic twice a day

for 42 days and then refrained from garlic for next 42 days. After 42 days of garlic consumption the mean total blood cholesterol levels and triglycerides were reduced significantly, while higher density lipoprotein (HDL) increased. It was concluded that consumption alone can decrease serum lipids and may be effective in mild cases of these conditions and as a prophylactic.

2.7.4 Effects on diabetes

Garlic is known to reduce blood glucose in streptozotocin induced as well as alloxan-induced diabetes mellitus in rats and mice (Thomson *et al*., 2007; Peter *et al*., 2008). Administration of garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate aminotransferase and alanine amino transferase levels, while it increased serum insulin in diabetic rats but not in normal rats (Peter *et al*., 2008).

Interestingly, comparism was made between the action of garlic extract and glibenclamide, a well known antidiabetic drug. The antidiabetic effect of garlic was more effective than that observed with glibenclamide (Eidi *et al*., 2006). Unfortunately, the effect of garlic on humans with diabetic condition is not well documented, as the few studies carried out have conflicting results (Jain *et al*., 1993; Zhang *et al.*, 2001).

2.7.5 Effects on homocysteine

Elevated plasma homocysteine concentrations have been associated with an increased risk of vascular disease as high homocysteine is known to inhibit endothelial cell proliferation (Weiss *et al*., 2006; Sayar *et al.*, 2007) and contribute to atherothrombotic events. Aged garlic extract has been reported to decrease plasma total homocysteine concentration by 30% (Yeh and Yeh, 2006).

2.7.6 Effects on hypertension

Garlic is reportedly used in complementary therapy for blood pressure control by approximately 50% of patients with hypertension (Capraz *et al*., 2006). An *in vitro* study has comfirmed that garlic has vasoactive sulphur compounds and that red blood cells can convert garlic's organic polysulphides into hydrogen sulphide, a known endogenous cardioprotective vascular cell signaling molecule (Benavides *et al*., 2007).

2.7.7 Effects on thrombosis

It has been reported that daily consumption of one fresh garlic clove for six months resulted in 80% decrease in serum thromboxane B_2 in middle-aged men (Ali and Thomson, 1995). The exact mechanism by which these compounds (allyl propyl disulphide, diallyl disulphide and other sulphur compounds present in garlic essential oil) alter platelet function is not known, but *in vitro* studies suggest that they may act via inhibition of platelet lipooxygenase enzymes which in turn suppresses the production of thromboxane B_2 (Rajaram, 2003).

2.7.8 Anti cancer effect

Inhibition of the growth of neoplastic cells is perhaps the most notable functions or actions of garlic (Peter *et al*., 2008). Various forms of garlic, including fresh garlic extract, aged garlic, garlic oil and a number of organosulphur compounds appear to offer protection against some cancers. Garlic likely has several synergistic biological effects that either prevent or possibly may fight cancer. The chemopreventive activity has being attributed to the ability to modulate the activity of several metabolizing enzymes that activate (Cytochrome P450s) or detoxify (gluthathione S- transferase) carcinogens and inhibit the formation of DNA adducts in several target tissues (Hassan, 2004). Garlic has been shown to stimulate immune effector cells including T- and

natural killer cell number and activity. Numerous epidemiological, clinical and laboratory studies have demonstrated the role of garlic in cancer prevention (Fleischauer and Arab, 2001; Milner, 2001; Setiawan *et al*., 2005; Galeone *et al*., 2006) especially in relation to digestive tract cancers like oesophageal and stomach cancers (Gao *et al*., 1999).

2.7.9 Anti microbial

During the World War I, the success of garlic in treating wounds and dysentery was well known. However, its antimicrobial properties remained a mystery until Sandoz Pharmaceuticals isolated a compound alliin, when garlic is chopped, chrushed or bruised the alliin is converted by an enzyme allinase to the bioactive component allicin (Peter *et al.,* 2008). Garlic exerts a broadspectrum antimicrobial activity against many species of bacteria, virus, parasites, protozoan and fungi (Adetumbi and Lau, 1983; Hughes and Lawson, 1991; Koch, 1993). Garlic oil, powder and other constituents have been shown to exert potent antibacterial effects on *Helicobacter pylori* (You *et al*., 1998; Sivam, 2001).

2.7.10 Antifungi infections

Ajoene is an active compound in garlic that plays a pivotal role as a topical fungal agent (Ghandi and Gohekhar, 1988 Ledezma *et al*., 2006). Garlic inhibited the growth of fungi along with the drug Ketoconazole, when tested on the fungi *Malassezia furfur*, *Candida albicans* as well as 35 srains of various dermatophyte species (Shams-Ghahfarokhi *et al*., 2006).

2.7.11 Antistress effect

Garlic has the capacity to protect against the ravages of stress that affects the autonomic nervous and neuroendocrine systems. In a study, rats that were trained with endurance exercises to physical fatique enjoyed improved parameters of aerobic glucose metabolism, attenuated oxidative stress and vasodilation when given garlic at a dosage of 2.86 g/kg 30 minutes before exercise (Morihara *et al*., 2006).

2.7.12 Side effects

The main side effect associated with garlic intake is breath odour, especially when taken raw. According to Mitchell (2003), when garlic is taken before meals the odour is decreased. Nausea and vomiting are other major side effects (Peter *et al*., 2008)

Although garlic generally has little in terms of safety issues, there are isolated cases of topical garlic burns (Friedman *et al*., 2006) and anaphylaxis (Yin and Li, 2007). Although rare, garlic allergy has been attributed to the protein alliin lyase, which has induced IgE mediated hypersensitivity response from skin prick testing (Kao *et al*., 2004). Consumption of garlic has been reported to be associated with decreased platelet aggregation and bleeding events (Chagan *et al*., 2004). However, caution must be exercised in the use of garlic while undergoing anti coagulant therapy (Saw *et al.,* 2006).

2.8 Rabbit Production in Nigeria

Rabbit *(Oryctolagus cuniculus)* is a micro livestock species and is one of the cheapest and fastest means of producing high quality animal protein and fast-growing livestock (Vietmeyer, 1985). Rabbits possess a number of features that might be of advantage in the small holder subsistence type integrated farming in developing countries. Rabbit

production is a veritable means of alleviating animal protein deficiency in Nigeria (Abdulmalik, 1994; Hassan and Owolabi, 1996; Ajala and Balogun, 2004).

Rabbit production in Nigeria could be described as rudimentary or when compared to countries such as France, Hungary, China and the United State. This is evident from the small rabbit keeping population in which gender bias and sociological status of rabbit farmers, weak inventory of rabbit keeping infrastructure, low consumption rate of rabbit meat, absence of an organized or thriving market for rabbit meat products and lack of governmental and institutional support limit the expansion of rabbit production (Onifade *et al.*, 1999).

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).

Aduku and Olukosi (1990) and Onifade *et al.* (1999) reported that rabbit production has a lot of benefits among which includes its high adaptability, easiness to handle and manage, high growth rate, high efficiency in converting forage to meat, short gestation period and very high prolificacy. It also provides high returns on investment, high quality meat products (it contains high protein level of about 20.8%, low sodium, low fat and cholesterol levels which compares favourably with the local bush meat). Its use for laboratory processes and as pets and its faeces is a good source of manure, and its consumption is without cultural and religious bias (Biobaku and Oguntona, 1997; Omole *et al.*, 2005). The presence of caecal microbes enables the rabbit to digest large amounts of fibrous feed as most non ruminant species cannot (Taiwo *et al.*, 1999). Costs of beef, chevon, mutton, chicken and frozen fish are high compared to rabbit meat (Aduku and Olukosi, 1990; Ajala, 1990).

Rabbit farming in Nigeria like other livestock production is faced with many challenges, resulting in gross shortage of meat to meet up the growing demands (Nworgu, 2007). The growth rate of the Nigerian agricultural sector is below the potentials of natural and human resources due to high cost of agricultural inputs, poor funding of agriculture, inadequate functional infrastructural facilities, inconsistencies of government agricultural policies, inadequate private sector participation, poor mechanized farming and little or no adoption of some simple agricultural technologies developed by scientists (Nworgu, 2007).

Ironkwe and Amaefule (2007), reported between 83% and 140% returns on capital investment in rabbit farming but despite this attractive prospect, Nigerians are yet to appreciate rabbit production, consequently resulting to its neglect.

2.9 Breeds of Rabbits

The United States Department for Agriculture (USDA) has classified rabbits according to size, weight and type of skin. Small rabbit breeds weigh about 1.4 - 2.0 kg at maturity, medium breeds; 4.0 - 5.4 kg, and large breeds; 6.4 - 7.3kg (USDA, 1972). Based on this classification, there are two most popular breeds for meat production which includes the New Zealand White and the Californian. These breeds are most popular because they combine white fur and good growth characteristics. The New Zealand rabbits are slightly larger than the Californian, 4 - 5.9 kg and 3.6 - 4.5 kg, respectively. The New Zealand rabbit has a completely white, red or black body, whereas the Californian is white with colored nose, ears and feet. The two most popular rabbits for fur production are the Rex and the American Chinchilla. The Rex is slightly smaller than the American Chinchilla, 3.2kg and 4.5kg (USDA, 1972). At present there are many breeds of rabbit being used for both meat and skin production in developing

countries. In Brazil the breeds are: New Zealand White, Californian, Chinchilla, Palomino, Hollander, Rex, Dalmation, Flemish Giant, New Zealand Red, Barboleta, Champaigne, d'Argent; in Ecuador, the breeds are: New Zealand White, Blue Viennese, Silver German and Angora; in Malawi, the breeds are: New Zealand White, Californian, Angora, Rex; in Nepal the breeds include the Californian Hybrids, while in Ghana, the breeds are the Thuringa, Blue Viennese, Flemish Giant, Checkered Giant, Lop, Californian, Alaska, and the Yellow Silver (USDA, 1972). In Nigeria, the most common breeds include the New Zealand White, Californian, Angora, Rex amongst others. All of these breeds of domestic rabbits are descendants of the European wild rabbit, *Oryctolagus cuniculus* (Aduku and Olukosi, 1990). Although many of these are breeding successfully in various countries, the most popular breeds are the New Zealand White and the Californians. These two breeds are also the most popular in commercial rabbit industries in the developed countries. The various production traits such as fertility, growth and feed conversion rates when considered, under commercial conditions, New Zealand Whites and Californians are amongst the breeds available for meat production (Bombeke *et al.*, 1975).

2.10 Problems of Rabbit Production in Nigeria

Heat is one of the most important climatic factors which may affect rabbit production in the tropics. The rabbit is very largely dependent on respiratory evaporation for the regulation of its body temperature and this confers only a limited power of adaptation to hot climates. Heat is also dissipated by radiation and convection, but these are somewhat restricted by the rabbit's furry covering. Johnson *et al.* (1957) reported that short hair and larger ears helped the cooling process in New Zealand White rabbits.

2.11 Diseases of Rabbits

2.11.1 Non infectious conditions

The common non infectious conditions in rabbits include cannibalism, sore hocks, dental malocclusion, bloat and fur eating. However, cleanliness and good management, which do not necessarily have to be sophisticated or involve the use of expensive drugs, can be extremely effective in the prevention of diseases (Stewart, 1974).

2.11.2 Infectious diseases

2.11.2.1 Viral diseases

Infectious myxomatosis is a fatal disease transmitted by mosquitoes, biting flies and by direct contact (Aduku and Olukosi, 1990). The virus of infectious myxomatosis of rabbits (Sanarelli) induces multiple lesions in the skin, lymph glands, tunica vaginalis, epididymis, testicle, spleen, and lungs. The virus causes growth and destruction of cells in the epidermis overlying the myxomatous masses leading to the formation of vesicles. The disease is also characterized by conjunctivitis, listlessness, anorexia with high temperature. In severe outbreaks animals die within 48 hours after manifesting signs. The disease can be prevented by vaccination (Rivers, 1930).

Rabbit hemorrhagic disease (RHD), also known as rabbit calicivirus disease (RCD) or viral hemorrhagic disease (VHD) is a highly infectious and fatal disease affecting the wild and domestic rabbits. The infectious agent for the disease is rabbit hemorrhagic disease virus (RHDV), or rabbit calicivirus (RCV). The virus infects only rabbits (Mercks, 2006).

Other viral diseases include fibroma, herpes virus infection, viral enteric disease, oral papilloma, rabbit pox and papillomatosis both of which affect the skin and integuments (Aduku and Olukosi, 1990).

2.11.2.2 Bacterial diseases

Pasteurellosis is a highly contagious disease caused by *Pasteurella multocida.* The disease manifests as inflammation of the mucus membranes of the air passages and lungs. Pasteurella specie can also cause abscesses which may be found on any part of the body (Mcnitt *et al.,* 2000). Mastitis also known as "blue breast disease" is caused by Streptococcus or Staphylococcus in lactating does. The mammary glands become hot, reddened and swollen and the doe may have fever as high as 40°C or more (Aduku and Olukosi, 1990). Enterotoxaemia is caused by Gram positive bacteria, *Clostridium perfringes Type E, Clostridium spiriforme* and *Escherichia coli* which produce powerful enterotoxins. These bacteria normally reside in the gut of healthy rabbits in dormant states but are rapid multiplication and production of enterotoxins under ideal conditions causing enterotoxaemia. Rabbits exhibit clinical signs of enterotoxaemia, acute diarrhea, lethargy, reduced feed intake, rough hair coat, soiling of the perineum, collapse and sudden death within 24 to 48 hours after the first symptoms are noticed (Mcnitt *et al.,* 2000).

Colibacillosis is a common bacterial disease of young rabbits and other species. It is caused by the enteropathogenic strains of *Escherichia coll* in the small intestine and caecum. *E coli* is usually present in the digestive tract of healthy rabbits and does not normally cause diarrhea. Enteropathogenic strains of *E coli* could be transferred from the doe to her kids through faecal contact. Proliferation of pathogenic strains *of E coli*

strains is triggered by factors such as concurrent diseases, stress and low fiber diets (Cheeke *et al.*, 1987; Mcnitt *et al.*, 2000).

Rhinitis (Snuffles or nasal catarrh) is an acute, subacute or chronic inflammation of the mucous membrane of the air passages and lungs induced primarily by Pasteurella but Pseudomonas, *Bordetella bronchiseptica,* staphylococcus and streptococcus have also been isolated. Initial sign is a thin serous exudates from the nose and eyes that later becomes purulent (Cheeke *et al.*, 1987; Mcnitt *et al.*, 2000).

Otitis media or interna ("wry neck" or head tilt) results from *Pasteurella multocida* or *Encephalitozoon cuniculi* infection. There is an accummulation of pus or fluid in the middle or inner ear causing the rabbit to twist its head (Mcnitt *et al.*, 2000; Mercks, 2006). Listeriosis is a sporadic septicaemic disease characterized by sudden death or abortions and it is most common in does in advanced pregnancy caused by *Listeria monocytogenes.* It spreads through the blood to the liver, spleen and gravid uterus (Mcnitt *et al.*, 2000). Other bacterial diseases are pneumonia, conjunctivitis (weepy eye), spirochetosis (vent disease), salmonellosis, staphylococcosis, orchitis, septicaemia and enteritis.

2.11.2.3 Fungal diseases

Trichophyton and Microsporum are group of fungi that infect rabbits and cause disease of the skin and fur under certain conditions. Ringworm is caused by *Trichophyton mentagrophytes* and is characterized by lesions which may occur in any area of the rabbit's skin with loss or thinning of fur. The affected area may be inflamed or capped with white bran-like flaky material (Mercks, 1998).

2.11.2.4 Parasitic diseases

Coccidiosis is a major disease affecting rabbit production this disease is caused by protozoan parasite *Eimeria stidae, E magna, E irresiduna, E media,* or *E perforans.* Affected rabbits exhibit diarrhea, anorexia, rough hair coat and unthriftiness (Aduku and Olukosi, 1990). Mange is also a major cause of poor production in the rabbit industry. The disorder may be caused by *Sarcoptes scabie* or *Notoedres cati.* Pruritis and alopecia are major clinical signs (Cheeke *et al.,* 1987; Mercks, 2006).

Encephalitozoonosis caused by Encephalitozoon cuniculi is a protozoan parasite and is the cause of a mild but long standing disease in rabbits (Cheeke *et al*., 1987). Only one roundworm presents a problem in domestic rabbits. Several more have been reported in wild rabbits but these rarely occur in domestic rabbits. The pinworm, *Passalurus ambiguous,* is a very common parasite of domestic rabbits, but it does not affect other animals or people (Cheeke, *et al.,* 1987; Mercks, 2006). Tapeworms also occur in rabbits as adults in the intestine and as larval forms in the liver and abdominal cavity. The rabbit tapeworm *Citotaenia variablis* is uncommon in domestic rabbits. Diarrhea and emaciation manifest in severe tape worm infestation while rabbits harboring a few tapeworms show no signs of the disease (Cheeke *et al*., 1987). Rabbits are intermediate hosts to some intestinal tapeworms such as *Taenia pisiformis* and *Multiceps seralis*. Also pinworm and ear mite infections are common to rabbits (Rai, 1988). Babesiosis in rabbits is transmitted by ticks such as the *Amblyomma* specie and *Dermacentor* specie in wild rabbits. Babesia species are erythrocytic protozoan parasite of wild lagomorphs worldwide. Babesia species pathogenic to rabbits are *Babesia bovis* (Yabsley *et al.,* 2006) lagomorphs often serve as reservoirs host for the parasite, which normally infects cattle and humans (Hoffman and Smith, 2005).

2.12 Reproduction in Rabbits

The male rabbit has an oval-shaped testes within the scrotum which remain in communication with the abdominal cavity, the testicles descend at about two months after birth (Hoffman and Smith 2005; Kemppainen, 2007; Smith and Boyer, 2008).

In the rabbit doe, ovulation does not occur spontaneously, but has to be induced through a neurohormonal reflex, which is initiated during coitus and occurs 10-16 hours after coitus (Jones *et al.,* 1976; Hafez, 1993). There have been some common incidences of failure to ovulate which appears to vary with the season of the year (Adams, 1972). When using artificial insemination, in the absence of a male, ovulation has to be induced by artificial methods which include treatment with luteinizing hormone, human chorionic gonadotrophin injection (HCG), copper salts or other hormonal means (Adams, 1972). The ovulation inducing method most frequently used is an intramuscular injection of Gonadotropin releasing hormone or its synthetic analogues (Foote *et al.,* 1963; Michelmann and Paufler, 1973; Battaglini *et al.,* 1982; Donnez *et al.,* 1989; Theau-Clement *et al.,* 1990). This has been shown to induce ovulation in rabbit does with results similar to those obtained by natural mating. Alternatively, luteinizing hormone or human chorionic gonadotropin can be used to induce ovulation in rabbit does (Foote *et al.,* 1963), however, the repeated injection of these hormones can result in failure of the does to ovulate due to antibody formation (Adams, 1972). Gonadotropin releasing hormone can be repeatedly injected without antibody formation (Adams, 1981). Vasectomized males have been used to induce ovulation in does prepared for artificial insemination (Khalifa, 1994; Khalifa *et al.,* 2000), but the method is time-consuming and some females may refuse to mate, but ovulate when given luteinizing hormone or gonadotropin releasing hormone.

Giojalas *et al.* (2004), comparing human and rabbit spermatozoa, suggested that the timing and duration of the capacitation is programmed according to the egg availability in the oviduct, long in periodic ovulators and short in induced ovulators, such as rabbits. Indeed, Brackett *et al.* (1982), reported that *in vitro* capacitation of raw rabbit semen is long and difficult whereas Percoll-selected spermatozoa (without granules) show a faster rate of capacitation. This circumstance maximizes the possibilities that an ovulated egg would meet spermatozoa in the best functional state.

2.12.1 Gonad development and puberty in rabbits

In mammals, the gonads arise primarily from two germinal ridges on the dorsal side of the abdominal cavity medial to the kidneys. A group of granulated yolk sac cells invade the germinal ridges and this invasion leads to the formation of the primary sex cords (undifferentiation gonad). The undifferentiated gonads consist of cortex and medulla that give rise to the ovary and testis respectively (Nathalie *et al*., 2013). Closely attached are the glomeruli and mesonephric tubules that give rise to the renal system. Associated with the renal system are the paramesonephric (Mullerian) and Mesonephric (Wolffian) ducts, giving rise to female and male tubular genital systems respectively. In the male, the Mesonephric ducts (Wolffian) duct becomes the excurrent duct ie epididymis, vas deferens and vesicular glands. Two agents produced by the fetal testis are responsible for the male differentiation and development. Fetal androgens cause development of the male reproductive tract, Mullerian inhibiting substance, a glycoprotein, is responsible for suppression of the paramesonephric (Mullerian) ducts ie atrophy (Nathalie *et al*., 2013).

In the rabbits, the gonads begin to differentiate on the 16th day of fetal life (Nathalie *et al.,* 2013). After birth the testes develop less quickly than the rest of the body. From the

age of five weeks they begin to grow very rapidly. Accessory glands undergo a similar development, but at a more even rate and are less precocious (Nathalie *et al.*, 2013). Spermatogenesis begins between days 40 and 50, the testicular tubes become active at about 84 days and the first spermatozoa are present in the ejaculate at about 110 days (Hoffman and Smith, 2005; Kemppainen, 2007; Smith and Boyer, 2008).

Sexual maturity, defined as the moment when daily spermatozoa production ceases to increase, is reached at 32 weeks by New Zealand White rabbits in temperate climates (Lombardi and Fernandez, 2003; Rodel and Bora, 2004). However, a young buck in these same conditions can be used for reproduction from the age of 20 weeks. Indeed the first manifestations of sexual behavior appear at days 60 to 70 when the rabbit makes its first attempts at riding. Coitus may occur for the first time at about 100 days, but the viability of the spermatozoa are very weak or nil in the first ejaculates, so first mating should be timed for age 135 to 140 days (Kemppainen, 2007; Smith and Boyer, 2008). The onset of puberty varies from breed to breed, but environmental conditions in the rabbitry also play an essential role, particularly feeding, which is even more important than climate (Lombardi and Fernandez, 2003; Rodel and Bora, 2004).

Does generally reach puberty when they have grown to 70 to 75 percent of their mature weights. However, it is usually preferable to wait until they reach 80 percent of their mature weights before breeding them. These relative weights should not be considered absolute thresholds for all rabbits, but rather limits applicable to the population as a whole. Sexual behaviour (acceptance of mating) appears long before the ability to ovulate and bear a litter (Rodel and Bora, 2004).

2.12.2 Spermatozoa production

Reproductive inefficiency is the most limiting constraint to efficient rabbit production in the tropics (Gbadamosi and Egbunike, 1999). The efficiency of sperm production, libido and quality of spermatozoa tend to remain uniform throughout the reproductive life of an animal but may be significantly altered by age, nutrition, environment, health status, drugs, and chemicals (Togun and Egbunike, 2006). Among these factors nutrition is the most prominent management factor affecting sexual maturity, and improved feeding can optimize spermatozoa cell production and the survival of an animal (Salisbury *et al.*, 1978; Rekwot *et al.*, 1987a, 1988). The unavailability of grains and high cost of imported feed ingredients have contributed to the high cost of commercial feeds and this is a serious constraint to the expansion of commercial livestock production in Nigeria.

Undernutrition poses adverse effects on the reproductive capacity of males, therefore, successful reproduction requires complete provisions of macro and micronutrients, including Zinc (Hidiroglou and Knipfel, 1984), Vitamin A (retinol) (Chung *et al*., 2009), Vitamin B_{12}, Vitamin B_9, and Vitamin E (Abdu, 2008), Vitamin D (Chinoy and Ahmedabad, 1998), Folate (Ebisch, *et al.*, 2007), Selenium (Hawkes and Turek, 2001), Nickel (Yokoi *et al.*, 2003), Manganese (Lee *et al*., 2006), Chromium and Copper (Tuormaa, 2000), Fatty acid (Aksoy *et al*., 2006), Protein (Zambrano *et al.*, 2005), Arginine (Wu *et al.*, 2009), and Camitine (Ng *et al.*, 2004).

Nutrition is a major factor in the effectiveness of reproductive function and can affect the efficiency of related hormone production and the growth of reproductive organs. In addition, delayed appearance of motile sperm in the ejaculate, retarded testicular growth, decreased size of Leydig cells and low differentiation of the seminiferous tubules and of the interstitial tissue are observed in the male gonads of underfed

maturing bull-calves (Almeida *et al.*, 2007). Restricted diets significantly decrease the number of cells of the spermatogenic series at all stages and the number of Sertoli cells. Thus, it is apparent that the cellularity of the tubular epithelium is significantly lower in low nutrition animals compared to those on high nutrition (Almeida *et al*., 2007).

Vertebrate male reproductive endocrine systems commonly comprise hypothalamus, anterior pituitary gland and testes, which form the hypothalamic-pituitary-gonadal (HPG) system. The male reproductive tract mainly consists of testes, epididymis and ductus deferens (Foley, 2001). The major function of the male reproductive tract is to produce spermatozoa and hormones. The anterior pituitary gland produces two hormones: follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Emanuele and Emanuele, 1998).

The hypothalamic-pituitary-gonadal axis (HPG axis) refers to the effects of the hypothalamus, pituitary gland, and gonads as if these individual endocrine glands were a single entity as a whole (Emanuele and Emanuele, 1998). The hypothalamic-pituitary-gonadal axis is a critical part in the development and regulation of the body's systems, such as the reproductive and immune systems. Changes in the activities of these glands may result in a corresponding change in the levels of hormones produced by these glands and thereby causing a local or widespread effect on the body (Emanuele and Emanuele, 1998).

This axis controls development, reproduction, and aging in animals. The hypothalamus produces gonadotropin-releasing hormone (GnRH). The anterior portion of the pituitary gland produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and the gonads produce estrogen and testosterone (Emanuele and Emanuele, 1998).

The hypothalamus is located in the brain and secretes GnRH (Millar *et al.*, 2004). GnRH travels down the anterior portion of the pituitary through the hypophyseal portal system and binds to receptors on the secretory cells of the adenohypophysis (Charlton, 2008). In response to GnRH stimulation these cells produce Luteinizing Hormone and Follicle Stimulating Hormone, which travel into the blood stream (Vadakkadath and Atwood, 2005). These two hormones play an important role in communicating with the gonads. In females Luteinizing Hormone and Follicle Stimulating Hormone, act primarily to activate the ovaries to produce estrogen and inhibin and to regulate the estrous cycle (Vadakkadath and Atwood, 2005). Estrogen forms a negative feedback loop by inhibiting the production of GnRH in the hypothalamus. Inhibin acts to inhibit activin, which is a peripherally produced hormone that positively stimulates GnRH-producing cells (Meethal *et al.*, 2009). In males luteinizing hormone stimulates the interstitial cells located in the testes to produce testosterone, and follicle stimulating hormone plays a role in spermatogenesis. Only small amounts of estrogen are secreted in males

One of the most important functions of the HPG axis is to regulate reproduction by controlling the uterine and ovarian cycles (Katja and Marieb, 2007). In females, the positive feedback loop between estrogen and luteinizing hormone help to prepare the follicle in the ovary and the uterus for ovulation and implantation. When the egg is released, the ovary begins to produce progesterone to inhibit the hypothalamus and the anterior pituitary thus stopping the estrogen- luteinizing hormone positive feedback loop. If conception occurs, the placenta will take over the secretion of progesterone therefore the dam cannot ovulate again. If conception does not occur, decreasing secretion of progesterone will allow the hypothalamus to restart secretion of GnRH (Katja and Marieb, 2007).

2.12.3 Spermatogenesis

Spermatogenesis is a multi-step process of germ cell expansion and development which occurs within the seminiferous tubules of the testis that determines male fertility (Hafez, 2000). Spermatogenesis comprises three phases: stem cell renewal and germ cell proliferation, meiosis and differentiation and lastly spermiogenesis (Hunter, 1980; Rekwot *et al.,* 1994; Hafez, 2000). Stem cells located along the basement membrane of the serminiferous tubules divide, resulting in another stem cell and a committed cell called a spermatogonium (Sharpe, 1994). The spermatogonia undergo a species-specific number of mitotic divisions, with the final division resulting in differentiated type B spermatogonia (Hafez, 2000). The type B spermatogonia then divide to form preleptotene spermatocytes that detach from the basement membrane as they undergo meiosis to form round spermatids. After undergoing extensive differentiation (spermiogenesis) the differentiated elongated spermatids (now spermatozoa) are released into the tubule lumen (spermiation) (Sharpe, 1994; Parkinson, 2009).

In the mammalian male, the germinal epithelium is located within the seminiferous tubules. The precursor cells of either male or female gametes, called *gonocytes*, originate from extra-embryonic endodermal tissue. These migrate to the gonadal zone where they differentiate either to oogonia or spermatogonia (Hafez, 2000).

Spermatogenesis begins with the mitotic division of spermatogonia in close proximity to the basement membrane and proceeds toward the lumen. Spermatogonia are divided into A, intermediate and B classes, with each class further subdivided according to morphology and degree of differentiation. Spermatogonia are activated to form active, type A spermatogonia (Parkinson, 2009). In the rabbit, five spermatogonial generations exist: A1, A2, Intermediate-1, Intermediate-2 and B (Rex and Luiz, 2008).

A-series spermatogonia are the least differentiated and form the reservoir of stem cells within the seminiferous tubule. It is likely that stem cells are regenerated by asymmetrical divisions of early A-series spermatogonia, with one daughter cell remaining as an uncommitted stem cell, the other being committed to undergo further mitotic and meiotic divisions. Al spermatogonia remain in contact with the basement membrane, but as the final mitotic division of spermatogonia gives rise to the primary spermatocytes, the cytoplasm of the Sertoli cells starts to intervene between the basement membrane and the primary spermatocytes. DNA synthesis occurs during mitotic divisions and then, to its greatest extent, during the formation of tetraploid nuclei during meiosis (Hochereaude-Rivers *et al.,* 1990). RNA synthesis occurs during preleptotene and late pachytene (Parkinson, 2009). The first meiotic division then proceeds through the highly sensitive zygotene and pachytene stages. The pachytene stage is particularly sensitive to noxious damage, such as by high testicular temperature and inadequate maintenance of spermatogenesis by inappropriate gonadotrophin levels. During the first meiotic division, the cells move deeper into the seminiferous epithelium and the tight cell junctions of the Sertoli cells form beneath the spermatocytes, this Sertoli-Sertoli cells junction form the blood-testis barrier, which helps to protect the developing germ cells from potentially harmful blood borne chemicals (Parkinson, 2009).

Thus, the progeny of the first meiotic division, the secondary spermatocytes, move from the basal to the apical compartment of the seminiferous epithelium and are thereafter separated from the general tissue fluid compartment. The second meiotic division produces spermatids, which do not divide further. The spermatids thereafter differentiate into spermatozoa (Parkinson, 2009). At the end of meiosis, spermatids are round cells with round nuclei, which have to then undergo the very marked changes in

cell function and morphology that occur during spermiogenesis. Immediately after completion of meiosis, the spermatids undergo a period of RNA synthesis, which is then followed by the beginning of nuclear chromatin condensation (Garner and Hafez, 2000).

Simultaneously, acrosomal contents are synthesized in the Golgi, whose vesicles progressively fuse to form the acrosome. As the nucleus condenses and elongate the acrosome forms over the basal pole of the nucleus (Courtens and Delpech, 1979), while at the opposite pole the flagellum starts to form from one of the centrioles. A transient microtubular structure, the manchetter appears during the formation of the flagellum in the postnuclear cytoplasm of the elongating spermatid. The function of the manchetter is unknown and it disappears after the flagellum is formed (Zirkin, 1971; Faweett, *et al.*, 1973). The last stage of flagellum formation is the development of the mid-piece, when a helix of mitochondria condenses around the proximal part of the flagellum. During formation of the acrosome and flagellum, the cytoplasm of the spermatid is deeply invaded by a process of the Sertoli cell that extends between the forming flagellum and the residual cytoplasm. It is suggested that this process is responsible for the reduction in cytoplasmic volume of the spermatid that occurs during spermiogenesis. Finally, the remaining cytoplasm is engulfed by the Sertoli cell as the formed spermatozoon, with its reminant cytoplasmic droplet, expelled from the crypt of the Sertoli cell into the lumen of the seminiferous tubule (Fouquet, 1972).

Various researchers have classified the cellular associations in the cycle of the seminiferous epithelium into distinct stages. The duration of the cycle of the seminiferous epithelium and the duration of the spermatogenic cycle in the rabbit are 10.9 and 43.6 days respectively (Swierstra and Foote, 1965). The duration of the spermatogenic cycle can be calculated by multiplying that of the serminiferous epithelium by 4 because spermatogenesis extends over four consecutive cycles of the

seminiferous epithelium (Pineda, 2003). Epididymal transit takes a further 8-10 days. Thus, the interval between the most sensitive stage of spermatogenesis, meiotic prophase, and ejaculation, is approximately 30 days (Amann and Walker, 1983). Hence, the interval between damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 50 days, depending upon the site of damage (Amann and walker, 1983).

The seminiferous epithelium appears as concentric layers of spermatogonia, spermatocytes and spermatids, with characteristic associations between generations of cells throughout the depth of the seminiferous epithelium. Each generation of seminiferous cells is linked by cytoplasmic bridges, so that developmental stages are synchronous within each generation and substantial areas of seminiferous epithelium exhibit cells at a similar stage of development (Parkinson, 2009).

Although the Sertoli cells are the only non-germinal cells in the seminiferous epithelium, they are fundamental to normal spermatogenesis. Cytoplasmic processes from the Sertoli cells surround clusters of germinal epithelial cells. This allows the Sertoli cells to receive and convey signals and metabolic products from the extratubular environment through the basement membrane to the meiotically dividing germinal cells (Tiba *et al.*, 1994).

Considerable changes occur to spermatozoa as they pass through the epididymis (Hammerstedt and Parks, 1987). The epididymis is highly androgen-dependent, thus, if androgen levels are suppressed, epididymal function is immediately impaired. The protoplasmic remnant, which is initially sited close behind the sperm head, migrates distally to the end of the midpiece, before being finally shed in the tail of the

epididymis. Sperm are immotile in the head of the epididymis, but they acquire the capacity for motility as they pass through its body (Hammerstedt and Parks,. 1987).

Similarly, in the head of epidiymis, sperm do not have the ability to fertilize, but this is acquired during passage in the epididymal body. Less obvious, but of equal or greater importance to the morphological changes exhibited by sperm during their passage at the epididymis, are the changes in their plasma membrane, to which surface glycoproteins are added or modified by epididymal secretions and luminal cells. It is likely that this act to stabilize the acrosome while the sperm is within the female genital tract, to reduce the surface immunogenicity of the sperm and to enhance the ability of the sperm membrane to bind to the zona pellucida (Hammerstedt and Parks, 1987).

Spermatozoa take between 8 and 10 days to traverse the epididymis in rabbits (Swierstra and Foote, 1965). Pineda, (2003) reported that in the ram, sperm take 5 days to pass through the head and body of the epididymis and a further 4-7 days to traverse the epididymal tail. The transit time of the head and body are fixed, but the tail of the epidiymis has dual functions of both a site for maturation and storage, so that, in periods of high ejaculation frequency, the passage time of the tail may be reduced and relatively immature sperm ejaculated (Parkinson, 2009). Although sperm held in the tail of the epididymis have the capacity for motility, motility is not itself acquired until the time of ejaculation. Thus, sperm within the epididymis exhibit litle motility, but are rapidly active upon mixing with seminal plasma during ejaculation (Parkinson, 2009).

2.12.4 Factors affecting reproductive performance of the male

Reproductive performance must be considered in the light of management system and genetics of the breed (Menzies, 2011). Semen quality, like other phenotypic expressions consist of genetic component, environmental component and a variety of interactions

between the two. A number of factors may affect semen production, characteristics and quality (breed, feeding, health status, rearing condition, season and collection frequency, etc.) causing a wide variety in semen traits (Alvarino, 2000; Boiti *et al.*, 2005).

2.12.4.1 Breed (Genetics)

Breed affects the fertility of farm animals in a variety of ways. Some animals may be genetically infertile. Reproductive traits have low heritabilities, a discrete phenotypic expression and are expressed only in sexually mature ewes leading to low selection intensities and long generation intervals (Petrovic *et al.*, 2007). Selection of breed can greatly influence reproductive performance, particularly prolificacy and age at first lambing. Sheep breeds are very diverse in performance and there is the need to be familiar with the traits of the more popular ones (Menzies, 2011).

In the bovine species, *Bos indicus* was reported in Africa to have had higher sperm production, sperm concentration and sperm viability than *Bos taurus* (Brito *et al*., 2002). The higher sperm quality reported for *B indicus* could have been due to better adaptation to tropical environment (Brito *et al*., 2002). There were observed differences in semen characteristics of male animals from different breeds exposed to similar protocols of rearing (Brun *et al.*, 2002; Viudes *et al.*, 2004; Moce *et al.*, 2005).

2.12.4.2 Libido

Libido (sex drive) is a critical component of fertility. It is independent of scrotal circumference, Semen quality, body weight, growth rate or masculinity. A reliable method of measuring libido is to measure serving capacity. Social interactions affect breeding performance and social rank is related to age and seniority in the herd/flock as the case may be. The most dominant male tends to complete the highest number of

services. In this situation, the number of females serviced may be related more to social dominance than libido as measured by a serving capacity test (Blezinger *et al.*, 1999).

2.12.4.3 Scrotal circumference

Scrotal circumference (SC) is a very important trait in male animal reproduction, which can be used as a measure of fertility (Vilakazi, 2003). According to Lasley (1978), SC has a heritability estimate of 50%, which means that is it influenced mainly by genetic rather than environmental factors. SC is positively correlated to age of the animals (Brito *et al.,* 2002). Several researchers have reported a high positive correlation between scrotal circumference and sperm production as well as semen quality (Palasz *et al.,* 1994; Valefilho *et al.,* 1997; Elmaz *et al.,* 2007, Akpa *et al.*, 2012). Hoogenboezem and Swanepoel. (2000) reported that SC is positively correlated with the overall potential breeding efficiency and seminal characteristics. They also noted that semen quality and scrotal circumference are affected by factors related to under development of the testes and testicular degeneration.

Small scrotal circumference size may also result from lack of testicular development (King, 1993), due to thermal and nutritional factors which interfere with the endocrine regulation of spermatogenesis (Bearden and Fuquay, 1997).

2.12.4.4 Live weight and body condition score

Effect of body condition scores (BCS) and live weight changes before mating, during mating and after mating period, on reproductive efficiency of different breeds of sheep in the different rearing systems have been reported (Aliyari *et al., 2012).* Most researchers have reported that BCS and live weight have great impact on sheep reproductive efficiency (Gunn *et al.,* 1983; Koycegiz *et al.,* 2009). A correlation exists between BCS, live weight and amount of reserve body fat (Oregui *et al.,* 1997). BCS is very suitable for prediction or determination of liveweights of different genotypes of

sheep (Zygoyiannis *et al.,* 1997). Live weight is a combination of skeleton size and BCS and is not a good representative to evaluate the reproductive efficiency. Adult weight is affected by factors such as skeleton scheme (body size), fullness or hunger trap (filled or empty digestive tract) (Adedeji and Gbadamosi, 1999; Koycegiz *et al.,* 2009). Many researchers have reported that, fertility may be affected by BCS (Koyuncu, 2005; Madani *et al.,* 2009).

Akpa *et al.* (2012) reported that sperm production and quantity can be affected by both animal size and physiological status. There is a positive and significant correlation between testicular dimensions and body measurements. These suggest that males with larger scrotal size might possess larger body size and good reproductive ability. Testicular length and circumference are measures of testicular size which had been found to be significantly correlated with body weight (Adedeji and Gbadamosi, 1999; Bratte *et al.,* 1999). The strong positive correlation between BCS and scrotal circumference indicates that an increase in body condition would increase testicular size particularly scrotal circumference which may invariably improve the fertility of the animal. Similar findings were reported for works conducted on stallions (Naden *et al.,* 1990; Blanchard *et al.,* 2001).

2.12.4.5 Age

Age has been reported to be a major cause of variation of semen quality in male animals due to physiological changes that occur as they grow to sexual maturity (Coe, 1999). Sexual maturity occurs at about 5 months (depending on the breed) and semen quality generally decreases in older rabbits bucks. Recently, it was observed that the lowest percentage of spermatozoa with damaged chromatin (1.7-2.4%) was observed between 6 and 16 months of age. Decreased spermatozoa chromatin stability was

found in ejaculates taken from male rabbits less than 5 months and than in more than 20 months of age (Gogol *et al*., 2002).

In general, sexual development of ram Iamb appears to be more closely associated with body growth than with chronological age (Dyrmundsson and Lees, 1972). David *et al.* (2007) found that in Lacaune and Manech tete rousse sheep, sperm motility and concentration were higher up to the age between 2-3 years then begins to decline gradually. Okukpe *et al.* (2001) reported that semen characteristics were significantly affected by age of Yankasa rams. They observed that higher sperm concentration, motility and live spermatozoa were observed in adult rams. Semen production efficiency was also highest in adult rams. Similar findings were reported in rams by Colas *et al.* (1990) and Salhab *et al*. (2001). Serrano (1984) indicated that bulls from 2-5 years of age produce larger volumes of ejaculate compared to bulls less than 2 years of age and bulls older than 5 years of age. Several other researchers reported that the volume of ejaculate, sperm concentration and motility improved with age of the bull (Almquist and Amann, 1976; Everett and Bean, 1982; Shannon and Vishwanath, 1995; Chenoweth *et al.,* 1996; Garner *et al.,* 1996, Mathevon *et al.,* 1998; Coe, 1999 and Hassan *et al.,* 2009).

An increase in the frequency of abnormal spermatozoa has been reported with advancing age in bulls (Rao, 1971, Wolfe *et al.,* 2000). A similar finding was obtained in humans by Kidd *et al.* (2001) who reported an increase in ejaculate volume of 3 to 22 %, sperm motility of 3 to 37 % and normal sperm cells of 4 to 18 % to be associated with an increase in age. Gustafsson and Sekoni (1980) reported that old bulls (5 to 8 years of age) had higher incidence of sperm abnormalities than young bulls (1.5 to 2 years of age) and younger bulls less than 13 months had higher incidence of head abnormalities, proximal and cytoplasmic droplets than in bulls of 1.5 to 2 years of age.

2.12.4.6 Environment

The major contribution to semen variation is environment (Curtis, 1983; Chandler *et al.*, 1985; Cupps, 1991; Bearden and Fuquay, 1997). The environment could be defined in terms of all factors surrounding the animal, which includes environmental temperature, nutrition, season and management of the animal (Bonsma, 1980).

Effects of temperature on semen characteristics**:** Ambient temperature is one of the most important factors affecting reproduction in domestic animals (King, 1993). The effect of temperature on semen quality should be treated with caution because it often affects feed intake in animals and thus, changes ascribed to temperature may be due to altered nutrition. Furthermore, temperature and photoperiods are positively correlated particularly in temperate regions of the world and under natural conditions; it might be difficult to differentiate which portion of the effect is due to temperature and which is due to photoperiod (McDowell, 1972).

In the tropics, sperm production and semen quality decrease during the hot season (Fields *et al.*, 1979; Kumi-Diaka *et al.*, 1981; Rekwot *et al.*, 1987[b]). However, seasonal variations cannot be attributed only to greater ambient temperature. Reproduction can be affected by heat stress; under high ambient temperature and/or humidity, body thermoregulalory mechanisms are unable to increase body heat loss and internal temperature increases above physiological limits (Chemineau, 1994). Direct exposure of the testis at high temperatures, causes changes in certain critical stages of spermatogenic cycle, which is also directly related to the quality of the ejaculate (Alejandro *et al.*, 2014).

Heat stress can decrease semen quality in bulls (Johnston *et al.*, 1963) and rams (El-Darawany, 1999; Mohammed and Abdelatif, 2010; Mohammed *et al.*, 2012). Waites (1970) and Setchell *et al*. (1994) reported thermo-regulation of the testis to be essential

for semen production and quality. The detrimental impact of high ambient temperature on sperm quality in breeder cocks was reported by Rekwot *et al.* (2005) in the Northern Guinea Savannah zone of Nigeria.

However, semen characteristics are not immediately affected by changes in testicular temperature because damaged spermatogenic cells do not enter ejaculates immediately after heat stress (Hansen, 2009). Increase in the frequency of abnormal cells was found to be associated with extreme temperatures such as heat stress (Rathore, 1970; Stephan *et al.,* 1971). During a period of heat stress, degenerative changes in the seminiferous tubules (Kumi-Diaka *et al.,* 1981), testicular degeneration (Mamabolo, 1999) and abnormal scrotal thermogram (Lunstra and Coulter, 1997) were reported in bulls. Skinner and Louw, (1966) reported a reduction in sperm motility, percentage live spermatozoa and an increase in the number of morphologically abnormal spermatozoa in the ejaculates of bulls exposed to a temperature of 40°C for 6 to 8 weeks.

Effects of season on semen characteristics**:** The influence of season on semen characteristics of domestic animals reared in the temperate regions of the world has been documented **(**Mahmoud *et al.,* 1996; Penfold *et al.,* 2000). It has been established that the tropical environment of high ambient temperature and relative humidity have adverse effects on livestock and poultry species (McDaniel *et al.,* 1995; 1996; Ayo *et al.,* 1996; Ayo and Sinkalu, 2007). Onuora (1982) reported that the hot humid environment of South-Eastern Nigeria has negative effects on semen characteristics in the guinea fowl. The detrimental impact of high ambient temperature on sperm quality in breeder cocks was reported by Rekwot *et al.* (2005) in the Northern Guinea Savannah zone of Nigeria. Ahmad and Noakes (1995), Ibrahim (1997) and Mathevon *et al.* (1998) reported that the months and seasons of year have a significant effect on semen characteristics. Areas with marked seasonal variation in environmental temperatures

during the summer months, resulted in lower semen quality, testicular degeneration and abnormal scrotal thermogram in bulls (Curtis, 1983).

Taylor *et al.* (1985) reported that sperm production in Holstein bulls (ejaculate volume, sperm concentration and total sperm number) was greater during the summer than winter in temperate environments. Higher semen quality in dairy *Bos taurus* bulls during the summer was also reported (Chandler *et al.,* 1985; Soderquist *et al.,* 1996; 1997). However, Mathevon *et al.* (1998) reported that Holstein bulls produced more sperm (higher sperm concentration and total sperm number) with greater motility during the winter and the spring. Godfrey *et al.* (1990) reported that in a temperate climate, *Bos indicus* (but not *Bos taurus)* bulls suffered from cold stress that was reflected in decreased sperm production and semen quality during the winter.

In the tropics and semi-tropics, sperm production and semen quality decreased during the hot season only in *Bos taurus* and crossbred bulls, but *Bos indicus* bulls were not affected (Fields *et al.,* 1979; Kumi-Diaka *et al.,* 1981). However, some authors showed that in Africa, sperm production (ejaculate volume, sperm concentration and total sperm number) and percentage of normal sperm cells decreased during the hot season in *Bos indicus* bulls (Igboeli and Rakha, 1971; Rekwot *et al.,* 1987[b]).

Moghaddam and Pourseif, (2012) reported on the seasonal variations in seminal characteristics of the two crossbred rams Ghezel X Baluchi and Arkharmerino X Ghezel in Iran. The effect of season and/or photoperiod on semen quality and quantity has been previously studied in different breeds of rams (Amir *et al.,* 1986; Karagiannidis *et al.,* 2000; Kafi *et al.,* 2004; Zamiri and Khodaei, 2005) and other seasonal breeding animals such as buck (Karagiannidis *et al,* 1999; Barkawi *et al.,* 2006) and stallion (Janett *et al.,* 2003). There is a report of significant effect of season on spermatozoa progressive motility, percentage of live spermatozoa and abnormal spermatozoa, semen pH and

semen index (Chemineau *et al*., 1992). These seasonal variations in both semen quality and quantity are mainly due to the changes in daylight length throughout the year (Chemineau *et al.,* 1992).

Malejane *et al.* (2014) reported that there was significantly higher semen quality in Dorper rams in South Africa during summer, autumn and spring than during winter. Similarly, Moghaddam *et al* (2012) reported that the best semen is produced during late summer up to the second month of autumn. Similar finding was reported in Poyaya bucks in Spain by Zarazaga *et al.* (2005).

In Nigeria, Nayak and Misra (1991) reported that the hot-dry season, characterized by elevated ambient temperature and high relative humidity causes a temporary decrease in sperm production and fertility than in the cold-dry season. Obidi *et al.* (2008) reported that sperm concentration per ml in the Shikabrown breeder cocks varied with season, and that the highest spermatozoa concentration was obtained during the rainy season.

2.12.4.7. Diseases

It is widely known that inflammation of the male reproductive organs (O'Bryan *et al.,* 2000) influence various testicle functions and seminal characteristics by affecting biosynthesis of pro- inflammatory eicosanoids (prostaglandins and leukotriens) and cytokines (Knapp, 1990).

The effect of diseases on fertility in male animals may be temporal or permanent depending on the type of infectious agent (virus, fungi, bacteria, and protozoan) and the lesions produced on reproductive tracts. A large number of microorganisms have been isolated from semen and the prepuce (Pefa *et al.,* 2011). The direct effect of pathogens has been mainly focused on the testicles and glands forming part of the reproductive tract. Infection could be limited to a single organ (seminal vesicles) or spread extensively to other organs such as the epididymis, seminiferous tubules, prostate,

bulbourelhral glands and urethra and could reach the urinary bladder, ureter and kidneys. The inflammatory processes producing these infections are complex and difficult to differentiate amongst the affected organs, they have thus been brought together under the term seminal vesiculitis syndrome (McCauley, 1980).

The World Animal Health Organization (OIE, 2011) has listed several diseases as having proven important in transmission through semen. Such diseases have been grouped into two groups according to whether their transmission through semen has been demonstrated. The first group are diseases whose presence and transmission through semen has already been demonstrated and these include; foot and mouth disease, vesicular stomatitis, infectious bovine rhinotracheitis (IBR), bovine virus diarrhoea (BVD), papillomatosis, leptospirosis, tuberculosis, paratuberculosis, mycoplasmosis, anaplasmosis, brucellosis, campylobacteriosis and trichomoniasis (OIE, 2011). The second group comprise of diseases whose presence through semen has been demonstrated but not their transmission and they include; babesiosis, leucosis (when there is contamination with blood) and trypanosomiasis (OIE, 2011). The most studied agents have been brucellosis and tuberculosis, possibly because they have been involved in eradication programmes (Thibier and Guerin, 2000). Brucellosis is produced by a facultative intracellular, gram-negative coccobacillary bacteria; it does not form a capsule or spores and is not mobile, *B. abortus, B.ovis and B, melitensis* are species affecting cattle and small ruminants (Seleem *et al.*, 2010). *Brucella ovis* is the primary cause of lowered fertility in rams in most multi-sired breeding systems (Kimberling and Parson, 2007).

The lesions produced by *B. abortus* directly affects the testicular parenchyma, the cells of the genital tract, produce erythritol which promotes the growth of the pathogens. Thus it preferred localization (Givens and Marley, 2008). It is an important cause of

vesiculitis in regions having a high disease incidence (McCauley, 1980). Pathological lesions caused include orchitis and epididymitis and are accompanied by fibrosis of the vaginal tunic and the presence of abscesses (Nicoleti, 1980). *Brucella ovis* affects sheep but not goats, causing epididymitis, orchitis and impaired fertility in rams (Nicoleti, 1980). Initially, only poor quality semen may be seen; later, lesions may be palpable in the epididymis and scrotum. Epididymitis may be unilateral or, occasionally, bilateral. The testes may undergo atrophy, some rams shed *B ovis* for long periods without clinical apparent lesions (Givens and Marley, 2008). Trypanosomosis causes reproductive disorders such as testicular degeneration, scrotal inflammation, penile protrusion, posthitis, epididymitis, abnormal spermatogenesis and deterioration of semen characteristics (Sekoni *et al.,* 1990, Adamu *et al.,* 2007, Mbaya *et al.,* 2011, Victor *et al.,* 2012, Okubanjo *et al.,* 2014). Okubanjo *et al.* (2014) reported epididymal damage and depletion of epididymal sperm reserves in Yankasa rams experimentally infected with *T. congolense.* Similar findings were earliar reported in *Trypanosoma evansi* infected bucks (Boly *et al.,* 1993), *Trypanosoma brucei* infected gazelles (Mbaya *et al.,* 2011), *T. congolense* infected bulls (Sekoni *et al.,* 1990) and *T. vivax* infected bulls (Adamu *et al.,* 2007). Decrease or lack of epididymal sperm reserves is a consequence of testicular degeneration (Omekea and Igboeli, 2000). Trypanosomes are known to localize in nutrient-rich gonads where they drain nutrients and damage testicular parenchyma (Shehu *et al.,* 2006). However, other researchers have stated anorexia due to anaemia, immunological factors, physical swelling, production of active and toxic substances by trypanosomes and increased vascular permeability (Omekea and Onuora, 1992) as other mechanism involved in the degenerative changes (Okubanjo *et al.,* 2014).

Pasteurellosis, a bacterial infection caused by *Pasteurella multocida* is common in domestic rabbits. It is highly contagious and is transmitted by direct contact, although transmission by coughing or sneezing may also occur. Signs of pasteurellosis include rhinitis (stuffy, runny nose), pneumonia, abscesses, reproductive tract infections and inflammation of the reproductive tract which is usually seen in adult rabbits (Mercks Veterinary Manual, 2011). Does are more often infected than bucks, if both horns of the uterus are affected, often the doe becomes sterile, if only one horn is involved, a normal litter may develop in the other. The only sign of an infection in the uterus may be a thick, yellowish gray vaginal discharge. Bucks may discharge pus from the urethra or have an enlarged testicle. Long term infection of the prostrate and seminal vesicles is likely. Infection can be passed during breeding and so infected animals should not be bred (Mercks Veterinary Manual, 2011). Ketosis (pregnancy toxaemia) is a rare disorder that may result in death of does 1 to 2 days before giving birth. The disease is more common in first-litter does (Mercks Veterinary Manual, 2011). Treponematosis (vent diseases, rabbit syphilis) is a venereal disease of rabbits caused by Treponema bacteria. It occurs in both sexes and is transmitted through coitus and from the doe to its offspring. It is closely related to the organism causing human syphilis, the bacteria is not transmissible to other domestic animals or humans. It is characterized by small blisters or slow healing sores which become covered with heavy scabs. These sores are generally confined to the genital region but the lips and eyelids may be involved. Infected rabbits should not be mated (Mercks Veterinary Manual, 2011).

Listeriosis is a bacterial infection of the blood characterized by sudden death or abortions, seen occasionally. The bacteria causing the disease spread by way of the blood to the liver, spleen and uterus. It can infect many animals including humans (Mercks Veterinary Manual, 2011).

2.12.4.8 Management

Management plays a key role in determining the animal's performance for a given reproductive trait (Petrovic *et al.,* 2007). Since conception rates are a combination of the fertility of the male and female, it is critical to evaluate the male component of reproduction.

Semen collection frequency: Semen collection frequency may have an impact on sperm quality. Long abstinence periods (Pascual *et al*., 1993) and successive ejaculations (Ollero *et al.,* 1994) have been associated with membrane alterations of spermatozoa. A decrease in semen volume and sperm concentration with successive ejaculations has been reported in several studies (Ollero *et al.,* 1994; Kaya *et al.,* 2002). Ollero *et al*. (1994) reported that the maximum proportion of viable cells was obtained in the second ejaculate after an abstinence period of 3 days. The authors concluded that the use of the second and or a mixture of second and third ejaculates would improve the results in artificial insemination. The general recommendation is to establish a routine of semen collection, for example of two-three times per week (two collections per day/per animal) on different and non-consecutive days, independently of the use of the semen obtained. Increased semen collection frequency may have an effect on sperm quality and the composition of the seminal plasma (Kaya *et al.,* 2002), although it remains to be determined whether this has an impact on field fertility. In this sense, the procedure of taking one or two collections per day from each animal during the working week (Monday-Friday), with a 2-day rest period during the weekend, has been described in Ireland (Gordon, 1997).

Hygienic conditions: Semen collection in farm animal species is not a sterile procedure, and some degree of contamination with bacteria cannot be avoided (Clement *et al.,* 1995; Althouse *et al.,* 2000; Thibier and Guerin, 2000; Althouse and Lu, 2005; Aurich

and Spergser, 2007; Bielanski, 2007; Yaniz *et al*, 2010). In rams, semen is usually collected with an open-ended artificial vagina (Santolaria *et al.,* 2011) or electroejaculator with open-ended funnel, which may be contaminated with bacteria from the surface of the penis and prepuce, collection area, equipment and people. As a consequence, bacteria might compromise semen quality during storage and contaminate the female's reproductive tract. Ram semen is normally colonized by a variety of microorganisms that may reduce semen preservation and fertility (Yaniz *et al.,* 2010). In particular, the contamination of ram semen with enterobacterial species reduced sperm quality during storage at 15 °C in a concentration-dependent manner (Santolaria *et al.,* 2011).

2.12.4.9 Nutrition

Nutrition occupies a very strategic and critical position in livestock reproduction and health. If feed availability falls below a threshold according to species, type or status of animal, it could affect the breeding and fertility potentials of an animal. Moreover, changes in feed availability can influence seasonal breeding patterns (Martin and Walkden-Brown, 1995). This effect has been extensively studied in sheep and marked differences in sensitivity to feed availability have been described. Generally speaking, the effect of feed availability on seasonal breeding is much stronger in breeds originated from Mediterranean or tropical climates than in breeds originated from temperate latitudes (Martin and Walkden-Brown, 1995; Boukhliq *et al.*, 1996; Bielli *et al.,* 1999; Zarazaga *et al.*, 2005). Conversely, feed availability or even some increase in nutrient availability can be the proximate factor triggering breeding activity. Luzi *et al.* (1996) showed that a restricted dietary protocol reduces libido and some seminal traits. However, the most important factor is not the amount of feed given but its chemical constituents.

Testicular spermatozoa/spermatid reserves and epididymal sperm reserves in bulls on the high plane of nutrition were significantly higher than those for the bulls on the low and medium planes of nutrition (Rekwot *et al.,* 1994). Feeds high in calorific value through its specific dynamic action may contribute to the thermoregularity mechanism of the animals during acute winter months, resulting in enhanced number of spermatozoa and improved progressive motility (Salisbury *et al.,* 1978).

According to Bhosrekar and Rajdan (1973) bulls fed on 100% dried crude protein level had the least abnormalities and dead spermatozoa. Different dried crude protein level (80%, 100% and 120%) with constant energy level did not cause any, significant difference in the quality of semen. However, different periods of feeding in different seasons have been shown to significantly affect the semen quality and composition (Bhosrekar and Rajdan, 1973).

Diets with varying, energy levels (80, 100 and 120%) did not influence morphological and biochemical qualities of semen whereas, 120% energy level caused significant increase in primary spermatozoa abnormalities (Bhosrekar and Rajdan, 1973). Specific recommendations on feeding rations for rabbit bucks are not available, and only some specific requirements have been established (de Bias and Wiseman, 1998).

The interaction between nutrition and reproduction has long been known to have important implications on reproductive performance (Martin *et al.,* 2004). Nutrition is known to affect many aspects of the reproductive processes and much research effort has been devoted to puberty, gamete production and hormonal regulation (Martin *et al.,* 2004). The inter-relationships between nutrition, growth rate and age at puberty in the male are very similar to those in the female. Thus, males reared on high as opposed to low planes of nutrition reach puberty at earlier ages and heavier body-weights (Brown,

1994). Similarly, in female nutritional influences are much more pronounced, if they occur in early life rather than post weaning (Pruitt *et al.*, 1986). Feed restriction of Merino ram lambs prevented them attaining puberty in their first potential breeding season (Martin and Walkden-Brown, 1995). Rams growing at 130 g/day during the re-alimentation period reached puberty 4 weeks earlier and sustaining higher sperm concentrations than those growing at 80 g/day. Alkass *et al.* (1982) and Amann. (1987), noted a reduction in sperm concentration and output, following feed restriction of rams to 75% of their estimated maintenance needs for periods in excess of the 5-6 weeks required to develop sperm from spermatids in the germinal epithelium to fully mature spermatozoa in the distal cauda epididymidis.

Semen quality and quantity can adversely be affected by nutrition (Brown, 1994). Reduction in feed intake in animals exposed to high temperatures may lead to a deficient protein and energy intake (Vilakazi, 2003). There is evidence that the effect of nutrition on semen characteristics is mediated by the effect of dietary constituents on the hypothalamo-pituitary axis, although there are also some indications that dietary changes affect the testis growth indirectly (Brown, 1994). Diet restriction is associated with a decrease in the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn affects semen production and quality through their effect on testicular size and function (King, 1993). Similar observations were made by Martin *et al.* (1994a) where the level of nutrition was associated with a reduction in testicular size. During periods of nutritional stress, the animal body secretes stress hormone-glucocorticoids which lower the circulation or secretion of FSH and LH and hence causes inefficient spermatogenesis and poor semen quality (Bearden and Fuquay, 1997). Growth and subsequent body weight appears to be a better guide to the onset of puberty in rams than age (Dunn, 1955; Watson *et al.*, 1956; Moule, 1970; Dyrmundsson, 1973;

Rattray, 1977). Ragab *et al.* (1966) and Pretorius and Marincowitz, (1968), reported that rams reared on higher planes of nutrition attained puberty (age at which sperm first appeared in the ejaculate) at younger ages and at heavier body weights than rams on lower levels of feeding. Similarly, Krishnan (2014) observed that the level of nutrition markedly influences puberty in livestock. The faster an animal grows the earlier it reaches sexual maturity. In cattle, puberty occur at a particular live weight or body size than at a fixed age.

Krishnan (2014) reported that energy deficiency in young animals affects age at puberty and sexual development due to reduced endocrine activity. When mature animals are fed low energy diets for long, libido and testosterone production are negatively affected which subsequently affect semen quality (Krishman, 2014). Obesity or overfeeding reduces libido and sexual maturity in male animals and it has been observed that excess dietary energy may adversely affect sperm production and semen quality due to fat deposition in the scrotum, thereby reducing heat radiated from the scrotal skin and increasing scrotal and testicular temperatures. In another study, bulls fed high-nutrition diets had greater scrotal circumference than those fed medium-nutrition diets, but paired testes weights were the same (Coulter and Kastelic, 1999). Since testicular weight was greater in bulls fed high nutrition, perhaps fat deposition in the scrotum increased scrotal circumference in these bulls.

Furthermore, the low-plane rations considerably delayed descent of the testicles, penile development and first appearance of spermatozoa in the ejaculate (Petrovic *et al.*, 2007). As these processes are androgen-dependent, it was concluded that restricted feeding had inhibited gonadotrophic hormone release which resulted in a decrease in androgen activity and well as semen quality (Pretorius and Marincowitz, 1968).

Energy: Energy balance is probably the single most important nutritional factor related to poor reproductive function in animals (Scramuzzal and Matin, 2006). High-plane feeding of boars leading to leg weakness and lack of libido prompted the recommendation of lower planes of nutrition for boars in Dutch artificial insemination centres (Bindari *et al.,* 2013). Kemp *et al.* (1989) showed that maintenance feeding regiments, without a feed increment for the energy cost on the day of mating (1.3 MJ metabolizable energy (ME)), reduced spermatozoa numbers, but not their quality, by 32 and 41 % compared with those receiving twice and thrice maintenance rations.

Conversely, low nutrition in calves during young age suppressed LH secretion before 25 weeks, delayed puberty, and reduced testicular development at maturity (Kastelic, 2013). The initial suppression of testicular development and delayed puberty was not corrected in bulls fed reduced nutrition before approximately 26 weeks of age and subsequently placed on the high-nutrition regimen. Therefore, it is not possible to compensate for the effects of low nutrition early in life by subsequently giving supplemental feed (Kastelic, 2013),

Protein: The effect of dietary protein on reproduction is complex (Funston, 2007). Prolonged inadequate protein intake has been reported to reduce reproductive performance. More recently it has been found that reproductive performance may be impaired if protein is fed in amounts that greatly exceed the bull's requirements (Bindari *et al.,* 2013).

Feeding a low protein diet to adult bulls for about 1 year, resulted in smaller testes and epididymides in bulls and their gonadal and extragonadal reserves of spermatozoa were reduced to 70-75% of that for control bulls (Laszezka *et al.,* 1969). Though the volume of ejaculates and the percentage of live spermatozoa were also in decline after 8 months on this diet, there were no apparent morphological changes in the spermatozoa

(Laszezka *et al.,* 1969). Similarly, Rekwot *et al*. (1987a) and Rekwot *et al.* (1988) found that bulls fed low protein diets had poorer semen quality than those fed high protein diets. Jibril *et al.* (2011) reported an increase in semen concentration in rams fed high protein diets. They observed that even though an increased protein intake above the minimum requirement (12% CP) enhanced spermatogenesis, higher levels of proteins in diets resulted in excess urea and more available ammonia which might have influenced the physiology and reproduction often associated with decline in fertility as reported by other workers (Jordan and Swanson, 1979; Kaim *et al.,* 1983; Canfield *et al.,* 1990; Elrod and Butler, 1993).

Fats: The impact of fats on reproduction is a focus of considerable research (Clark and Henry, 1999). Because fatty acids and cholesterol are substrates for hormone synthesis, increasing fat in the diet may increase levels of reproductive hormones (testosterone, progesterone, prostaglandins) or fats may act directly on the reproductive axis (Martin *et al.,* 2004). Ruminant diets usually contain less than 2 to 5 % fat (Brandt and Anderson, 1990). Supplementing fat to improve reproduction was initially attempted to increase the energy density in the diet, high fat diets for cattle contain 6% to 8 % fat and exceeding these dietary fat levels could impairs rumen functions (Marx, 2000).

Obesity is a major health problem contributing to increased infertility in males, as well as increased susceptibility for diseases leading to a decline in testosterone production with age (Landry *et al.*, 2013).

Minerals and Vitamins: There is high zinc content in semen and this element plays an importance role in spermatogenesis, coupled with the synergistic roles it plays in the uptake of vitamin A by the spermatozoa. Vitamin A on the other hand, plays an essential role in the attainment of puberty and in the maintenance of both libido and the integrity of the testicular germinal epithelium (Hurley and Doane, 1987).

Cupps (1991) reported that deficient nutrient intake such as Iodine, Zinc, Cobalt and Vitamins A and E are associated with a reduction in semen quality in terms of morphology, concentration and motility. In general, all the vitamins are essential for reproduction due to their specific roles in cellular metabolism maintenance and growth. Vitamin A and E are of great importance because of their role in maintaining cellular integrity and antioxidant property in the semen respectively. Vitamin A is necessary for normal epithelial development in all species and failure of epithelial development negatively impacts spermatogenesis (Krishnan, 2014).

A deficiency of selenium affects the morphology and motility of the spermatozoa and may be linked with infertility in many domestic livestock (Scott *et al.,* 1998). The spermatozoa membranes are attacked by the increasing formation of reactive oxygen species which lower the viability of the spermatozoa (Irvine, 1996). Selenium increases formation of the anti-oxidant glutathione peroxide, which in turn decreases the reactive oxygen species and hence increases spermatozoa viability and fertility (Bray *et al.,* 1997).

2.13 Nutritional Mechanisms of Control of Reproduction

Nutrition is the most important factor affecting reproduction and the responses to it can be divided into short-term effect, that act mainly on the neuroendocrine system controlling testicular activity (Martin *et al.,* 1994a) and long-term effects, that act on testicular growth and sperm production (Oldham *et al.,* 1978).

The beneficial effects of nutrition on reproduction in sheep are well known (Blache *et al.,* 2007). In particular, nutrition is one of the main factors affecting ovulation rate and spermatogenesis. Generally, sheep could be grazed on grass for half of a year, however during winter or at lambing, they may be housed or given supplements feed. It is

important to get the nutrition right to prevent loss of body condition (Martin *et al.*, 2004).

2.13.1 Effect on the hypothalamo-pituitary axis

The most fundamental driver of reproductive function is a group of neuroendocrine cells in the preoptic-hypothalamic continuum that synthesizes GnRH (Martin *et al.*, 2004). These cells secrete discrete pulses of GnRH into the portal blood system, leading to the anterior pituitary gland where they elicit pulses of LH and a relatively continuous stream of FSH. Both LH and FSH are involved in the regulation of the production of both spermatozoa and hormones by the testis (Martin *et al.*, 2004). The frequency of GnRH pulses, and consequently LH pulses, is the code used by the nervous system to control testicular function. Direct measurement of the activity of GnRH cells is difficult, but usually unnecessary because LH pulses are easily detected in peripheral blood and their frequency can be used as a bioassay of GnRH cellular activity (Thiery and Martin, 1991). The network of neurones that controls GnRH secretion is not yet defined, but it is thought to be the final common pathway through which many factors influence gonadal activity, including metabolic status (Martin *et al.*, 1994b).

Glucose is the principal source of metabolic energy for the testis (Setchell and Hinks 1967) and for the brain (Pell and Bergman, 1983). Insulin was implicated because, in rats, it can cross the blood-brain barrier into the cerebrospinal fluid (CSF), from where it is taken up by neural tissues (Schwartz *et al.*, 1990), presumably those that contain insulin receptors, such as the median eminence and mediobasal hypothalamus (Van Houten *et al.*, 1980; Baskin *et al.*, 1987). These areas are important in the control of GnRH release in sheep (Thiery and Martin, 1991). In rams, the lupin supplement increases insulin concentrations in peripheral plasma as well as celebrospinal fluid (CSF), where the values are about 10% of those in plasma (Miller, 1995; Martin *et al.*,

1994b). In both plasma and CSF, there is a tendency for glucose concentrations to increase too, but they generally differ little from control values, presumably due to the regulatory influence of the extra insulin.

Martin and Walkden-Brown (1995) tested the role of the glucose-insulin system by intravenous and intra-abomasal infusions of glucose. These treatments greatly increased the circulating concentrations of both glucose and insulin, but had no effect on gonadotrophin secretion or testicular growth (Boukhliq *et al.*, 1991; 1992). Intracerebral route for administration of insulin by injecting it into the third ventricle increased LH pulse frequency in mature Merino rams on a maintenance diet (Miller *et al.*, 1995).

Bergman (1990) reported that 70% of the energy requirement of ruminants is met by the volatile fatty acids, primarily acetate, propionate and butyrate that are produced by fermentation in the rumen and are rapidly absorbed into the rumenal epithelium, where most of the butyrate is metabolised. Propionate is the only glucogenic volatile fatty acid and virtually all of it is absorbed immediately from the portal bloodstream by the liver where it is used for the production of glucose. Most of the acetate enters the general circulation and is used directly us an energy substrate by most peripheral tissues (Bergman, 1990). The possibility that volatile fatty acids could act as a nutritional signal was suggested by Murray *et al.* (1990) who observed a tendency for testicular growth to increase in rams fed a mixture of volatile fatty acids as a dietary supplement. This effect was subsequently confirmed by Boukhliq *et al.* (1992) who also found that this treatment increased LH pulse frequency and FSH concentrations. Intra-abomasal infusion of Casein did not affect testicular growth in Merino rams, whether in the presence or absence of exogenous glucose (Boukhliq *et al.*, 1992), but it did increase FSH secretion (Martin *et al.*, 1992) and tended to increase LH pulse frequency.

When low nutrition was accomplished by restricted feed intake, hypothalamic and pituitary function were suppressed, with LH secretion most severely affected (Brito *et al.,* 2006). It appeared that insulin-like growth factor I (IGF-I) was a possible signal to the central "metabolic sensor" involved in translating body nutritional status to the gonadotropin releasing hormone (GnRH) pulse generator (Brito *et al.,* 2006). Nutrition also affected blood testosterone concentrations, indicating effects on the number and/or function of Leydig cells. Age-related increases in physiological and GnRH-stimulated serum testosterone concentrations occurred earlier in bulls receiving high nutrition but were delayed in bulls receiving low nutrition, these effects were probably mediated by both LH and IGF-I (Brito *et al.,* 2006). Circulating leptin and insulin apparently have only permissive roles on GnRH secretion but may enhance testicular development. Growth hormone concentrations decreased concomitantly with increasing IGF-I concentrations during sexual development in bulls, suggesting the testes could contribute considerable amounts of circulating IGF-l (Brito *et al*., 2006).

Leptin is a hormone produced by adipose tissue whose production increases with the amount of body fat (Landry *et al.,* 2013). Several studies have supported a relationship between increased leptin production and regulation of reproductive function (Kiess *et al.,* 1998; Clark and Henry, 1999; Tena-Sempere *et al.,* 1999; Tena-Sempere and Barreiro, 2000). Indeed, leptin acts at all levels of the hypothalamus-pituitary-gonadal (HPG) axis in males. However, most of the obese individuals become insensitive to increased endogenous leptin production and develop a functional leptin resistance (Tena-Sempere and Barreiro, 2000). This deregulation of leptin signaling might result in abnormal endocrine and reproductive functions. Altered leptin dynamics may contribute to male infertility in different ways, leading to hypogonadism. These include leptin

resistance or leptin insufficiency at the hypothalamus and leptin modulation of testicular physiology (Landry *et al.,* 2013).

The mechanisms whereby leptin regulates reproductive function are multifaceted and likely involve actions at different levels of the hypothalamic-pituitary-gonadal axis. It is well established that the hypothalamus, a key element in the control of feed intake and neuroendocrine regulation of reproductive function, is the primary target of leptin (Casanueva and Dieguez, 1999; Ahima *et al.,* 2000). Indeed, Ob-R gene is expressed in specific hypothalamic nuclei (Couce *et al.*, 1997; Zamorano *et al.,* 1997), and the ability of leptin to modulate the expression of several hypothalamic neuropeptides is well documented (Casanueva and Dieguez, 1999; Ahima *et al.,* 2000). In the context of reproductive control, the hypothalamic effects of leptin likely involve stimulation of GnRH release, as predicted by experimental studies using hypothalamic tissue and immortalized GnRH-producing GT1-7 neurons *in vitro* (Yu *et al.,* 1997; Magni *et al.,* 1999). This action likely accounts for the stimulatory effects of systemic administration of leptin upon serum gonadotropins (Nagatani *et al.,* 1998). Besides its primary actions at the hypothalamic level, the initial characterization of the pattern of Ob-R distribution and biological effects of leptin in different *in vitro* systems indicated the possibility of additional actions of leptin (Landry *et al.,* 2013). Leptins slightly but significantly increased basal and GnRH-stimulated LH secretion from hemi-pituitaries of normally fed male rats (Yu *et al.,* 1997), although absence of stimulatory effects has also been reported (Tena-Sempere and Barreiro, 2000). Interestingly, such effects may depend on the prevailing metabolic status and the domain of leptin molecule tested, as inhibitory LH responses were observed in feed-restricted animals and after exposure to the leptin fragment (Tena-Sempere *et al.,* 1999; Tena-Sempere and Barreiro, 2000).

2.13.2 Effect on the testis

Change in nutrition alters not only the total amount of testicular tissue, but also the efficiency with which the gametes are produced by that tissue (Oldham *et al.,* 1978). Oldham *et al.* (1978) also reported that a 25% increase in testicular size led to an 81% increase in production of spermatozoa in rams, while Cameron *et al.* (1988) found that an 86% increase in testicular size led to a 250% increase in production of spermatozoa in rams. The number of ejaculated spermatozoa was not affected until 7 weeks after a change in diet, suggesting that the effects on spermatogenic efficiency are exerted after the last spermatogeni**c** division. This is similar to the effects of stimulatory photoperiod in more seasonal breeds of sheep, where efficiency is increased by reducing the rate of degeneration of germ cells following the mitotic and meiotic divisions of the spermatogenic cycle (Hochereau-de Reviers *et al.,* 1985). There is a need for detailed cytological analysis to determine whether nutrition operates by this mechanism, and also to determine the relative roles of FSH, testosterone and LH in mediating these responses at testicular level. Histological studies show that nutrition markedly affects the diameter of the seminiferous tubules (Setchell *et al.,* 1965), the relative proportion of the testis occupied by the seminiferous tubules and the proportion of the seminiferous tubule occupied by the seminiferous epithelium (Oldham *et al.,* 1978).

If changes in production of spermatozoa result primarily from alterations in Sertoli cell function, it is peculiar that peripheral inhibin concentrations do not seem to be affected by diet in mature rams, despite clear effects on FSH concentrations and testicular mass (Martin *et al.,* 1994[a]). The effects of nutrition on the activity of the interstitial tissue should be reflected in rates of production of testosterone (Martin and Walkden-Brown, 1995). However, in mature rams, significant changes in testicular mass induced by nutritional treatments were not associated with changes in the size of the testosterone

response to LH (Ritar *et al.*, 1984; Martin *et al.*, 1994[a]). Tena-Sempere and Barreiro (2000), reported the direct effects of leptin at the gonadal level. Initial evidence for an inhibitory role of leptin on ovarian function was presented (Spicer and Francisco, 1997; Zachow and Magoffin, 1997). These studies paved the way for the characterization of the biological effects and mechanisms of action of leptin upon the testis (Tena-Sempere and Barreiro, 2000). On the basis of evidence showing the expression of the Ob-R gene in rat testis (Zamorano *et al.*, 1997), characterization of direct testicular actions of leptin was undertaken. Tena-Sempere and Barreiro (2000) evaluated the effect of leptin upon testicular testosterone secretion *in vitro.* Leptin was found to inhibit testosterone secretion by the Leydig cells. The inhibitory effect of leptin upon testosterone secretion was mapped to a domain of the native leptin molecule comprised between amino acid residues 116-130, as the active fragment, leptin116-I30 amide, was able to mimic the inhibitory response to leptin (Tena-Sempere and Barreiro, 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 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. 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).

3.2 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 experiment was carried out under controlled ambient temperature. 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.

3.3 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.

Plate I: *Allium sativum* (garlic) cloves.

3.4. Extraction of Plant

Fifty (50) grams of garlic powder was poured into a conical flask, distilled water was added in the ratio of 1:5 w/v. During extraction the mixture was shaken continuously for two hours, the suspension was filtered with Whatman's filter paper and the filtrate concentrated by using vacuum rotary evaporator. The extract obtained was stored at 4˚C for the phytochemical analysis (Assayed *et al.,* 2008).

3.5 Phytochemical Analysis

3.5.1 Test for carbohydrades

3.5.1.1 Molisch Test

To a small portion of the garlic's extract in a test tube, few drops of Molisch reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A reddish coloured ring at the interphase indicated presence of carbohydrates (Evans, 1996).

3.5.1.2 Fehling Test:

To a small portion of the garlic's extract in a test tube, 5ml of an equal mixture of Fehling solution A and B was added and boiled on a water bath. A brick red precipitate indicated the presence of reducing sugar (Evans, 1996).

3.5.2 Test for free anthracene derivatives (Bontrager's test)

To a portion of the garlic's extract in a dry test tube, 5 ml of chloroform was added and shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution. A bright pink colour in the aqueous (upper) layer indicated the presence of free anthraquinones

3.5.3 Test for cardiac glycosides (Keller-kiliani test)

A portion of the garlic's extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution, this was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. It was observed carefully at the interphase for purple- brown ring. This indicated the presence of deoxy sugars and a pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides (Evans, 1996).

3.5.4 Test for saponin (Frothing test)

About 10 ml of distilled water was added to a portion of the extract and shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honey comb froth that persisted for 10-15 mins indicated presence of saponins (Evans, 1996).

3.5.5 Test for unsaturated steroids and triterpenes (Liebermann-Bucchard test)

To a portion of the extract equal volume of the acetic acid anhydride was added, mixed gently and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immedietly and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour indicated the presence of triterpene (Evans, 1996).

3.5.6 Test for tannins (Ferric chloride test)

To a portion of the extract, 3-5 drops of 1% Ferric chloride solution were added, a blue-black precipitate indicated presence of tannins.

3.5.7 Test for flavonoids (Sodium hydroxide test)

Few drops of 10 % sodium hydroxide were added to the extract. Yellow coloration indicated presence of flavonoid (Evans, 1996).

3.5.8 Test for alkaloids (Mayer's test)

To a portion of the extract, few drops of Mayer's reagent were added and the development of turbidity or precipitate indicated the presence of alkaloids (Evans, 1996).

3.6 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). Blood and semen samples were collected before and during the feeding period. In the course of feeding, blood and semen samples were collected once a week for evaluation. At the end of feeding, three bucks from each group were humanely slaughtered and organs such as the testes and liver were harvested for histopathological examination.

Table 3.1 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 *Allium sativum*	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 3.2 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
Nitrogenfree extract	4.37	4.32	4.31
Ash	27.31	27.00	26.94

3.7 Measurement of Live Weight Changes

Live weights (Kg) were measured for each buck in each group using weighing scale (Camry model). This was carried out weekly during the feeding period and changes in weights were recorded.

3.8 Semen Collection and Evaluation

3.8.1. Assembling the artificial vagina

The bucks were trained for semen collection during the acclimatization period and semen collection was done using a specially designed artificial vagina for rabbits as shown (Plate II).

The artificial vagina, (AV) was assembled as follows: a short plastic cylinder was obtained, a latex condom was used as a liner, whose end was cut off to allow both ends opened. A rubber band was used to fix the liner on the cylinder at one end, then glycerol was administered into the space between the cylinder and the rubber liner and the other end of the cylinder was fixed with another rubber band to assemble the AV. The assembled AV was placed in a beaker of warm water at 40˚C, the warm water caused expansion of the glycerol within the liner and also provided the necessary pressure and temperature. Traces of water was cleaned from the AV, a short test tube was attached at one end of the AV and the other end lubricated with non perfumed petroleum jelly for easy penetration.

To collect the semen from the bucks, it was ensured that the collector was properly gloved and a rabbit doe was introduced to the buck's cage to serve as a teaser. The buck was watched 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.

3.8.2 Semen evaluation

The ejaculate obtained was evaluated as described by Zemjanis (1970). This included the visual or gross evaluation of the ejaculate soon after collection for volume, pH and colour as well as microscopic examination for motility, concentration, percentage live spermatozoa and morphological abnormalities.

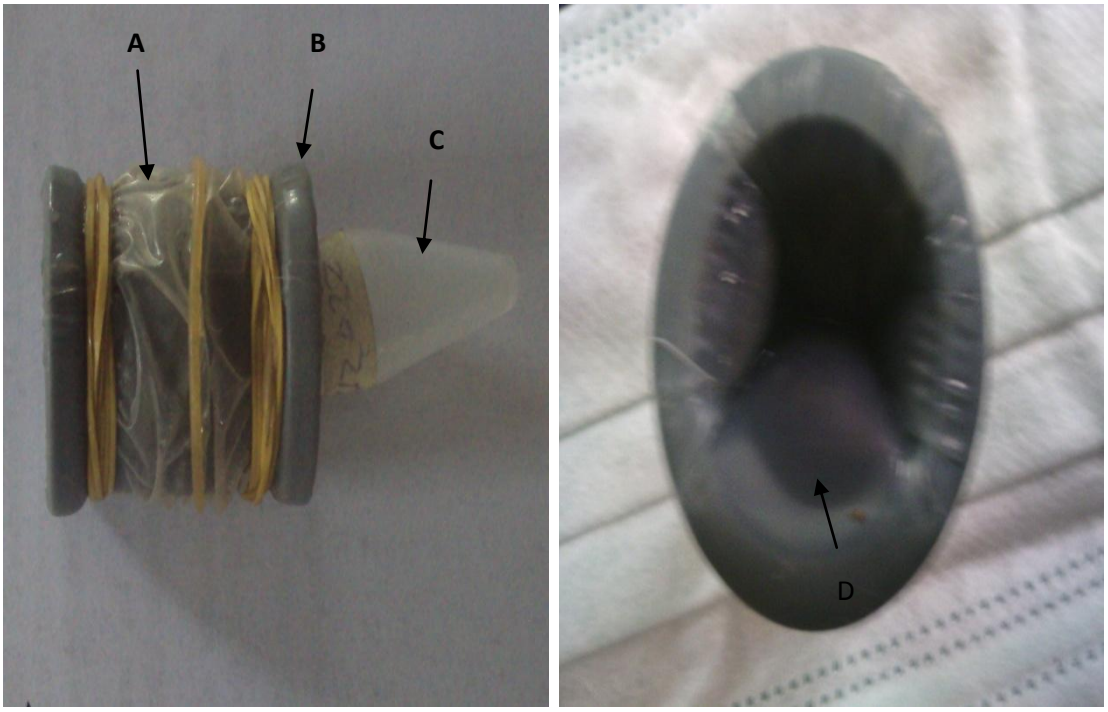

Plate II: Artificial Vagina adapted from IMV Technologies 2911 model (AV) for rabbits. **A**. rubber liner fixed on both ends by rubber bands. **B.** Plastic cylinder. **C**. Short test tube for collection of ejaculates. D. Expanded glycerol within the liner, narrowing the lumen of the AV.

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

3.8.2.2. Colour: The three colour categories of milky, creamy and watery designated 1, 2 and 3 were used for scoring the semen as described by Zemjanis (1970).

3.8.2.3. Semen pH: this was determined by dipping a litmus paper into the ejaculate and corresponding colour changes were observed and recorded.

3.8.2.4. Gross motility: was examined as quickly as possible after collection, by placing a drop of the semen sample on a pre-warmed glass slide, cover sliped and examined at ×10 magnification.

3.8.2.5. Spermatozoa concentration: were determined using Neubauer haemocytometer as described by Azawi and Ismaeel (2012). Micropipette was used to aspirate 25 µl of semen and diluted with 5 ml of 3 % NaCl in a test tube, dilution factor of 5000. The exterior of the pipette was wiped to remove any adhering semen. A cover slip was placed on the haemocytometer and two drops of the diluted semen was placed under the cover slip on each side of the haemocytometer. The haemocytometer was carefully placed in a pre-wetted chamber and the lid closed and left for 5 minutes. It was then examined using a microscope at ×40 magnification and the sperm cells were counted in five Thoma squares of the chamber (ie four corner and the centre squares). The semen concentration was calculated as follows:

Concentration (sperm cells/mL) = Number of sperm cells counted in the twenty five small squares × dilution factor × 10^4 (Azawi and Ismaeel, 2012).

3.8.2.6. Percentage live sperm cells: this was determined as 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. This technique was based on the principle that eosin-nigrosin penetrates and stains dead sperm cells while live sperm cells repel the stain. Dead spermatozoa stained pinkish or reddish while live spermatozoa remained colourless. One hundred (100) stained and unstained sperm cells were counted when the slides were dried, using light microscopy at ×40 magnification and percentage of each estimated (Esteso *et al.,* 2006).

3.8.2.7. Sperm abnormalities: was determined by making a thin smear of the semen sample, on clean grease-free glass slide and stained with eosin-negrosin. One hundred sperm cells were counted per slide using hand counter under light microscopy at ×40 magnification. Five cell types were recorded: normal cell, detached head, free tail, coiled tail and bent tail (Rekwot *et al.,* 1987b).

3.9 Determination of Gonadal Sperm/Spermatid Reserves

Gonadal sperm/spermatid reserves was determined as described by Rekwot *et al*. (1994), with slight modifications. Three bucks from each group were humanely 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 determined by homogenization (Igboeli and Rakha, 1971; Egbunike *et al*., 1976). Each testis was homogenized in 25 ml 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 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 haemocytometer according to the method of Kwari and Waziri (2001).

3.10 Determination of Epididymal Sperm Reserves

This 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 portion were determined using a measuring tape and digital weighing balance. These portions were 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 then diluted with 2 ml of normal saline and the concentration of the sperm reserves was determined using Neubauer haemocytometer under a light microscope.

3.11 Blood Sampling for Haematological Examination.

Two mililitres (2 ml) of blood samples were collected through the marginal ear veno-puncture using 25 guage hypodermic needle. The blood samples were collected into ethylenediamine tetraacetic acid (EDTA) impregnated sample bottles. This was carried out on each group on a weekly basis. Packed cell volume (PCV) was determined by Microhematocrit method. Blood protein was determined by Refractometer method, while complete RBC and WBC count were carried out using Neubauer haematocytometer.

3.12. Serum Enzymes Assay

The sera were thawed, the Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) where 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.

Superoxide dismutase detecting kit (Cat no SD125) was purchased from Radox, Radox Laboratories UK). In the presence of superoxide dismutase, xanthine and xanthine oxidase react to generate superoxide radicals which react with 2-(4-iodophenyl)-3(4-nitrophenol)-5phenyltetrazolium chloride (I.N.T) to form a red formazan dye measured at 505nm (Woolliams *et al*., 1983).

Catalase 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 wave length using a spectrophotometer (Unico UV-2100 PC, Dayton, USA).

Glutathione peroxidase detecting kit was purchased from Radox Laboratories Ltd UK. Reduction-Oxidation reaction took place between 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).

Malondialdehyde is a standard for determine free radicals damage in blood. A detection kit was purchased from Radox (Radox Laboratories Ltd United Kingdom). Malondialdehyde 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)

3.13 Histological Examination

At the termination of the experiment, three bucks from each group were humanely slaughtered and testes and liver samples were taken for examination. Histopathology examination was carried out as described by Zahid *et al.* (2002). Harvested organs were placed in bouin solution for four days for fixation. After fixation, the tissues were dehydrated, infiltrated with liquid paraffin and embedded in paraffin blocks sectioned at 5 microns thickness using a rotatory microtome. Each section was stained with Haematoxylin and Eosin (H&E) using standard staining procedures according to Luna (1968) and Humason (1972). Each slide was examined under the light microscope at ×400.

3.14 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.

CHAPTER FOUR

RESULTS

4.1 Phytochemical Analysis of Garlic

The aqueous extract of *Allium sativum* bulbs showed the presence of some classes of Phytochemicals (Carbohydrates, Cardiac glycosides, Saponin, Triterpene, Flavonoids, Alkaloids). The result of the qualitative analysis is shown in Table 4.1.

Table 4.1. Qualitative Phytochemical Screening of Garlic (*A. sativum*)

S/n	Constituents	Tests	Inference
1	Carbohydrate	Molisch	+
2	Anthraquinones	Bontragers	_
3	Cardiac glycosides	Kelle-Killiani	+
4	Saponin	Frothing	+
5	Steroids	Liberman Buchard	_
6	Triterpene	Liberman Buchard	+
7	Tannins	Iron Chloride	_
8	Flavonoids	Sodium Hydroxide	+
9	Alkaloids	Mayers Dragendorff	+

Key

+ = Presence

□ = Absence

4.2 Baseline Data

The baseline mean values for live body weight, semen colour, ejaculate volume, pH, gross spermatozoa motility, concentration, live sperm percentage and abnormal sperm percentage are presented in Table 4.2

Table 4.2: Mean (± SEM) values of semen characteristics, body weight of the rabbit bucks in groups A, B and C during the 7 weeks of acclimatization.

Parameters	**A**	**B**	**C**
	n = 6	**n = 6**	**n = 6**
Body Weight (Kg)	1.71 ± 0.09	1.80 ± 0.05	1.73 ± 0.06
Colour	Milky	Milky	Milky
pH	6.71 ± 0.14	6.97 ± 0.03	6.65 ± 0.20
Volume (ml)	0.28 ± 0.03	0.24 ± 0.02	0.26± 0.03
Motility (%)	69.32 ± 3.73	72.45 ± 2.15	72.95 ± 2.25
Concentration ($\times 10^6$/ml	76.20 ± 12.26	87.98± 14.85	100.90 ± 18.86
Live spermatozoa (%)	83.26 ± 2.08	78.63 ± 2.03	79.29 ± 2.08
Abnormal sperm cells (%)	27.89 ± 4.5	24.55 ± 2.11	20.95 ± 1.71

4.3 Live Weight Changes

Figure 4.1 presents the mean (± SEM) values of live weight of rabbit bucks fed varying levels of garlic. The mean body weight did not differ significantly ($P>0.05$) among the groups.

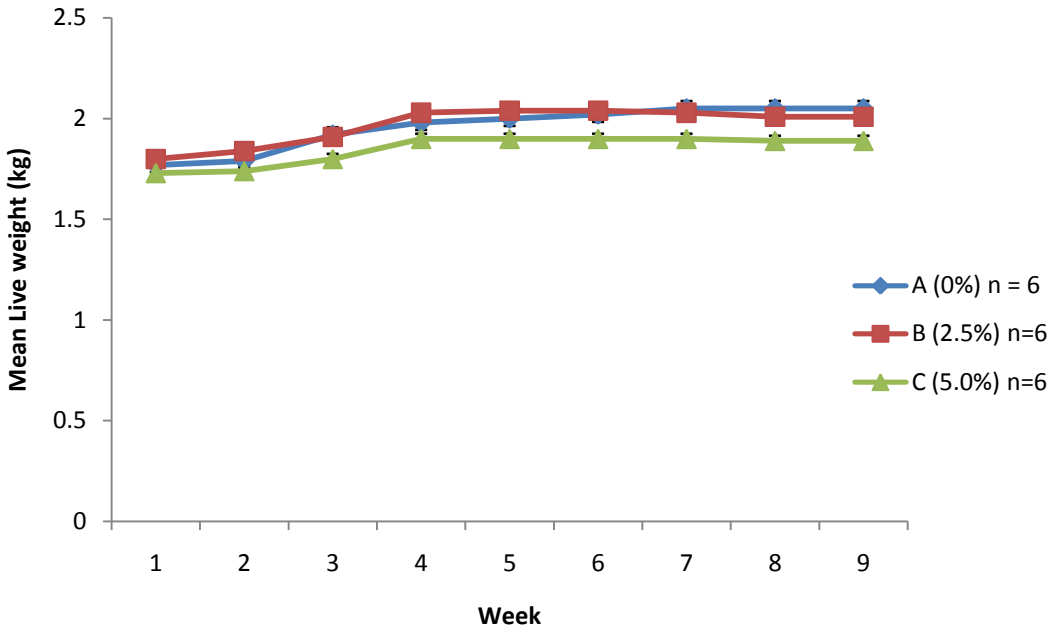

Figure 4.1 Mean live weight (kg) of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic respectively.

4.4 Semen Characteristics

The mean values of ejaculate volume, pH, gross sperm motility, sperm concentration, live sperm percentage and abnormal sperm percentage of rabbit bucks fed diets with 0, 2.5 and 5.0 % garlic are presented in Figures 4.2-4.7.

4.4.1. Ejaculate volume:

There were significant ($P < 0.05$) differences in the mean ejaculate volume at week 8, between group A (1.02 ± 0.75) and the experimental groups B (0.3571 ± 0.13) and C (0.2167 ± 0.08), as shown in Figure 4.2.

4.4.2. Mean pH:

Mean pH of group A (8.33 ± 0.42) was slightly higher than that of groups B (6.857 ± 0.14) and C (6.571 ± 0.20) at week 7 of the feeding period (Figure 4.3). It was observed that group A maintained a slightly alkaline pH values, while groups B and C maintained a slightly acidic pH values. However, there were no significant ($P > 0.05$) differences in mean pH values among the groups.

4.4.3. Gross motility

There were no significant ($P > 0.05$) difference in gross spermatozoa motility among the three groups. (Figure 4.4).

4.4.4. Sperm concentration

There were significant ($P < 0.05$) difference in sperm concentration ($\times 10^6$/ml) between group A and C from week 3 (A, 42.75 ± 21.25; C, 169.70 ± 31.32); week 4 (A, 46.80 ± 13.41; C, 148.30 ± 28.96); week 5 (A, 42.40 ± 16.50; C, 148.3 ± 28.96); week 6 (A, 63.00 ± 32.74; C, 178.50 ± 36.75); week 7 (A, 61.00 ± 9.59; C, 138.40 ± 30.95); week 8

(A, 62.40 ± 17.18; C, 106.00 ± 8.04) and week 9 (A, 64.17 ± 6.31; C, 123.50 ± 8.62) while, group A differed significantly ($P < 0.05$) with B at week 5 (A, 42.40 ± 16.50; B, 98.17 ± 13.18); week 7 (A, 61.00 ± 9.59; B, 110.70 ± 36.80); week 8 (A, 62.40 ± 17.18; 108.00 ± 9.24) and week 9 (A, 64.17 ± 6.31; C, 125.57 ± 7.79) of the study. However, to a greater extend there were significant ($P < 0.05$) differences in the mean values in a dose-dependent manner among the groups (Figure 4.5).

4.4.5. Percentage live spermatozoa

There were no significant ($P > 0.05$) differences in the percentage live spermatozoa among the groups (Figure 4.6).

4.4.6. Percentage sperm abnormality

A high percentage of normal sperm cells in all the groups were observed (Plate III). However, the common morphological abnormalities observed were: Detached head (Plate IV); Free tail (Plate V); Bent tail (Plate VI) and tightly coiled tail of sperm cell (Plate VII). There were no significant ($P > 0.05$) differences in mean percentage abnormal sperm cells among the groups (Figure 4.7).

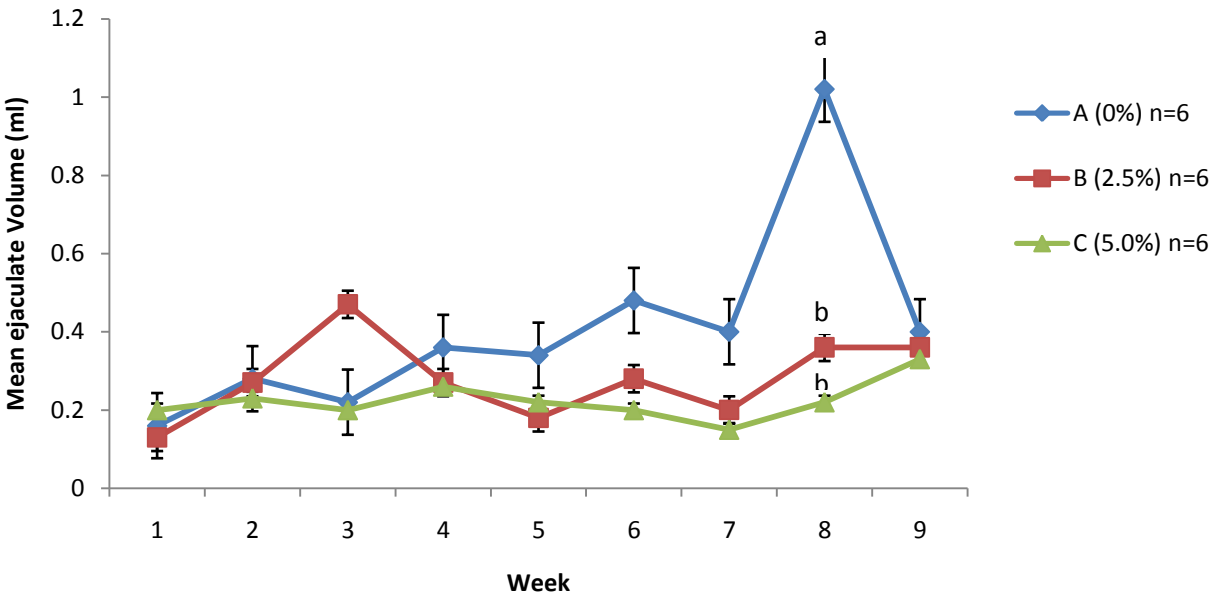

Figure 4.2 Mean ejaculate volume of rabbit bucks fed diets containing 0, 2.5 and 5% inclusion of garlic (*A. sativum*).

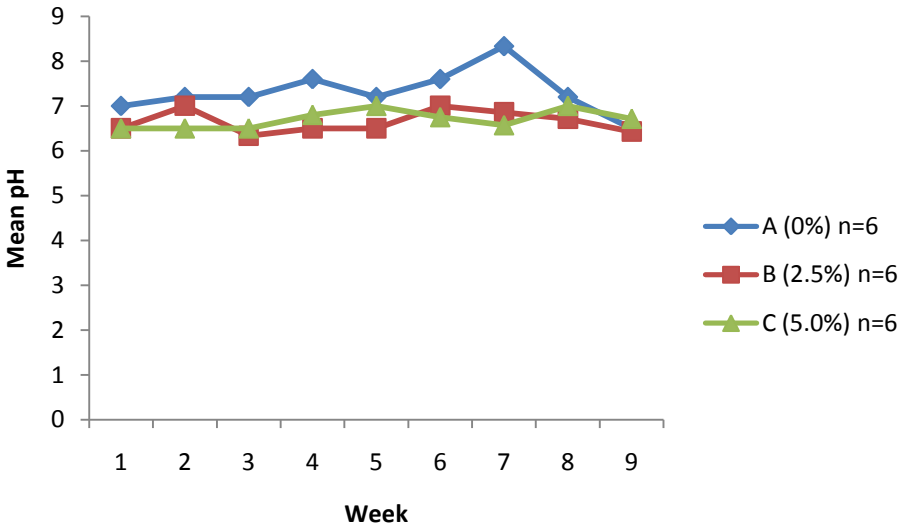

Figure 4.3 Mean pH values of rabbit bucks semen fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).

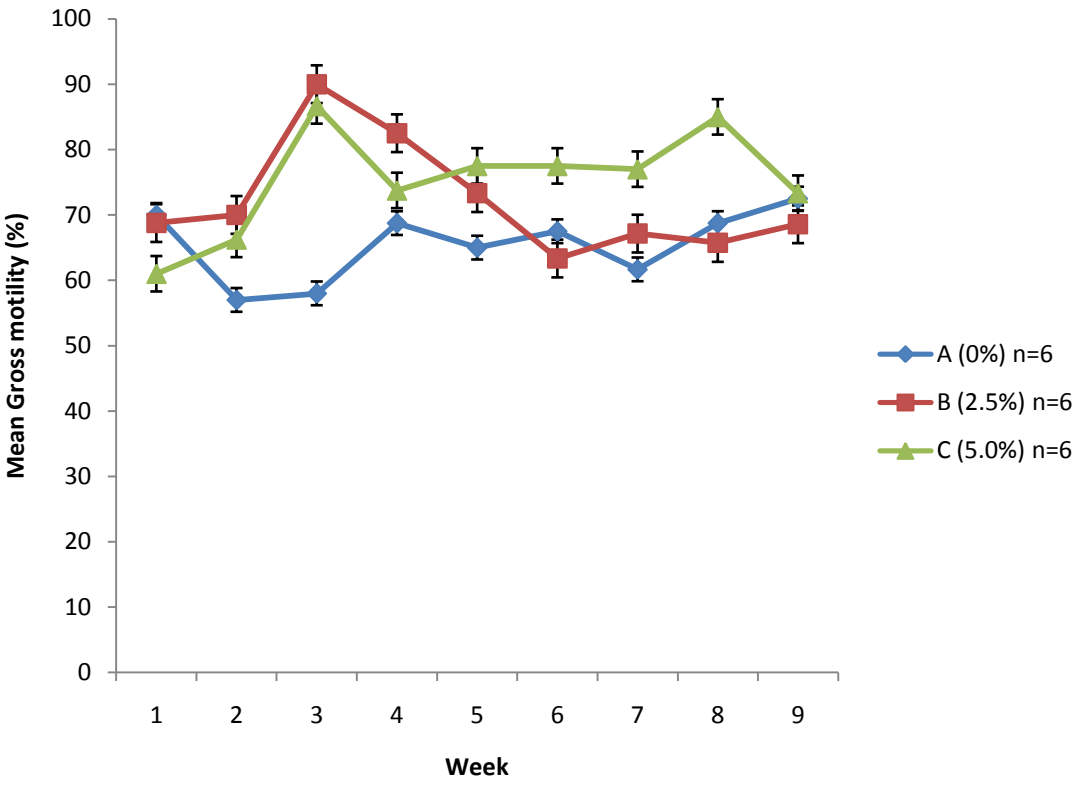

Figure 4.4 Mean semen gross motility of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).

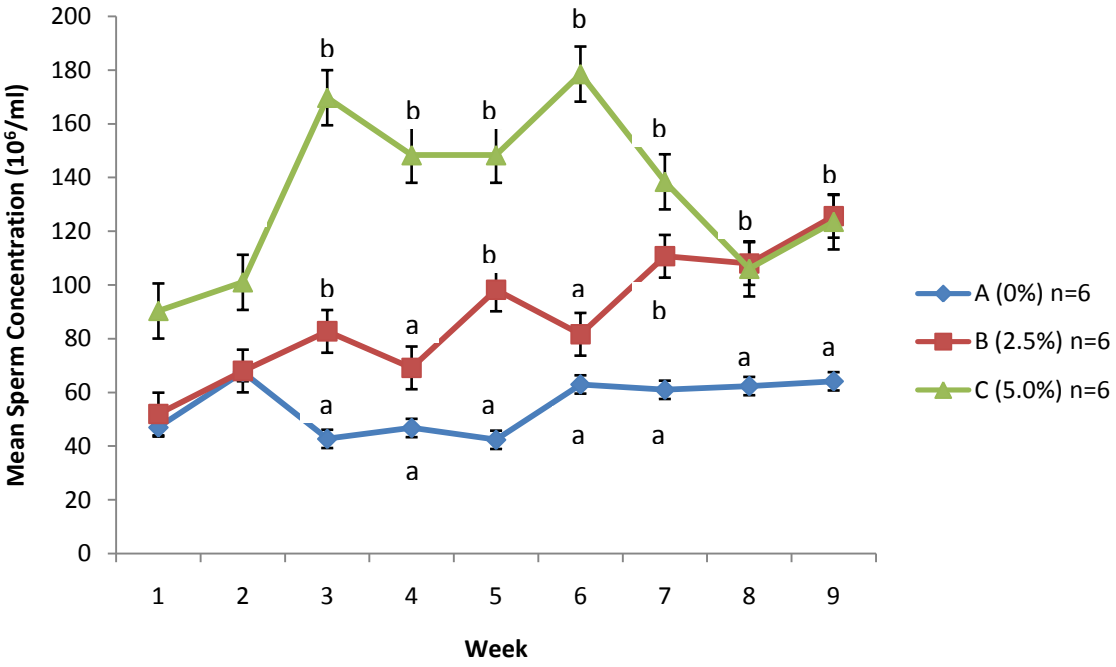

Figure 4.5 Mean sperm concentration (× 10^6/ml) of rabbit bucks semen fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).

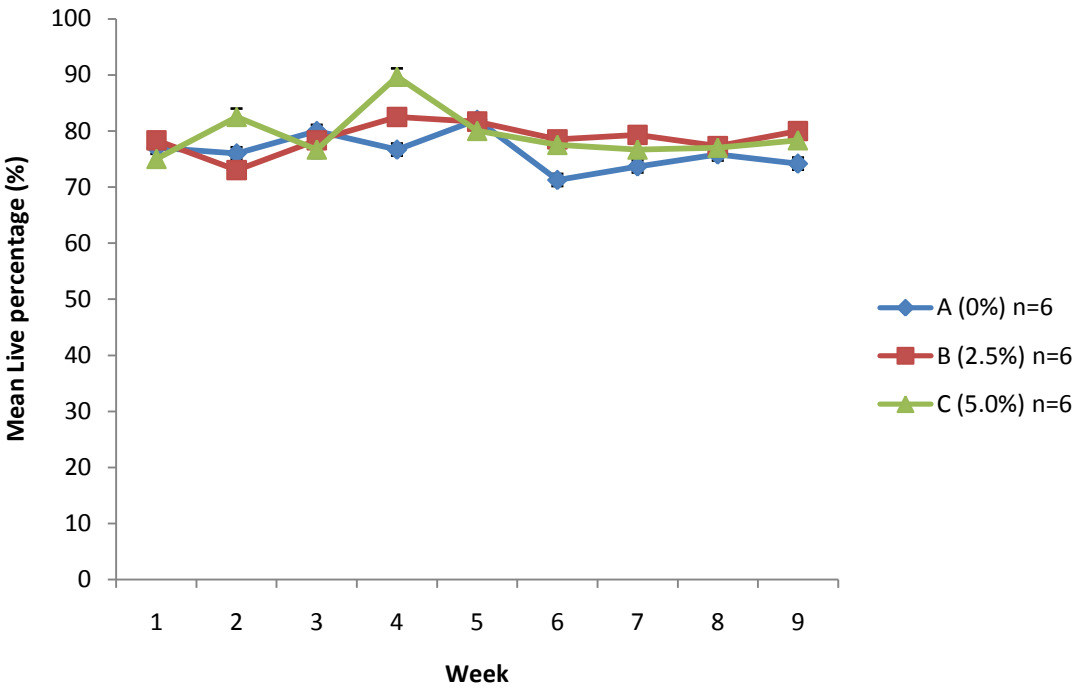

Figure 4.6 Mean percentage live spermatozoa of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).

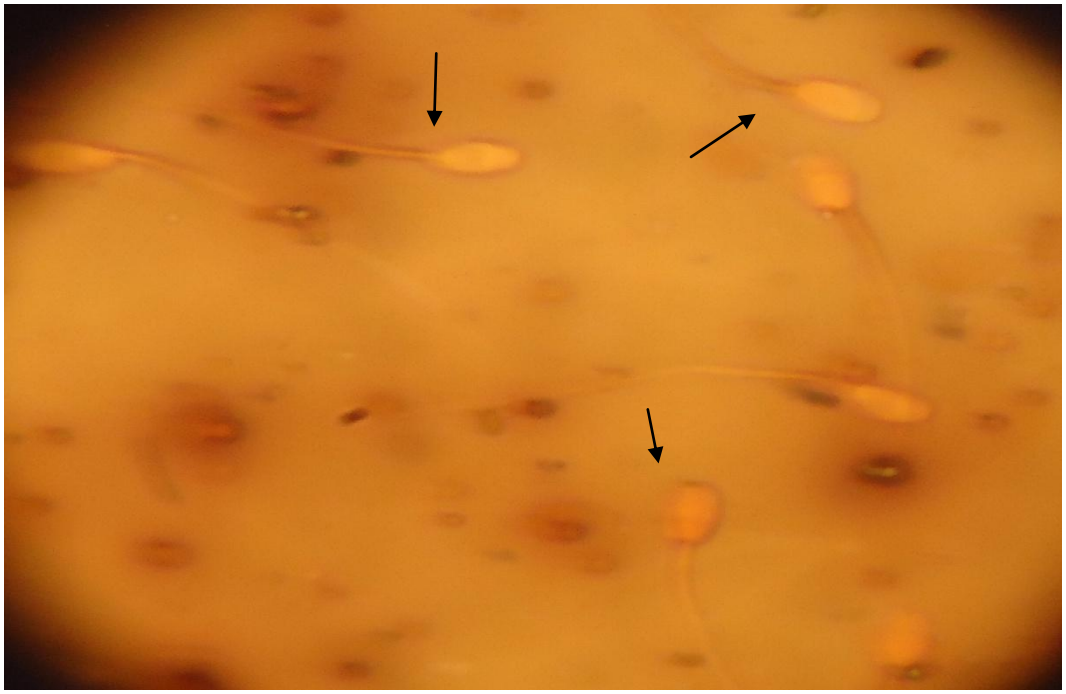

Plate III: Morphologically normal sperm cells of rabbit buck fed diet supplemented with *A. sativum*.

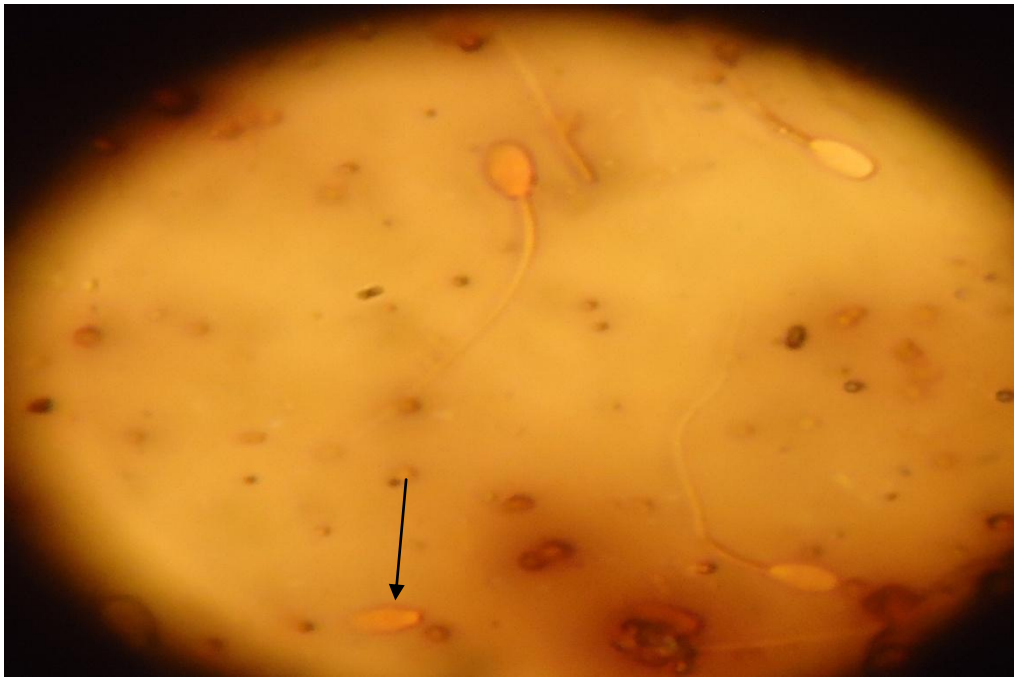

Plate IV: Detached sperm cell head of rabbit buck fed diet supplemented with *A. sativum*.

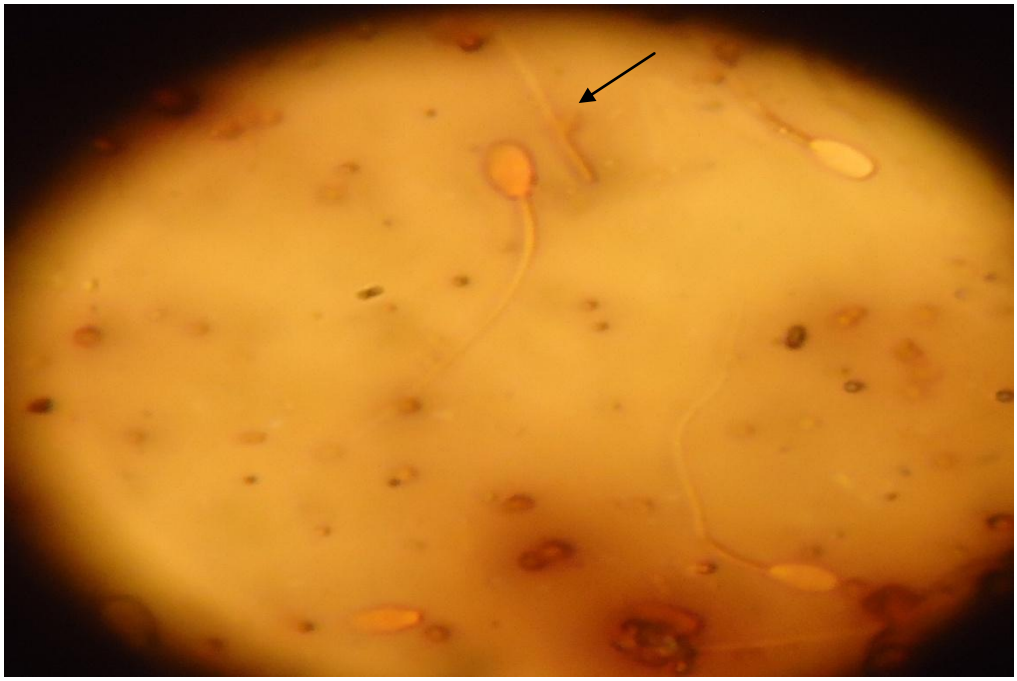

Plate V: Free sperm cell tail of rabbit buck fed diet supplemented with *A. sativum*.

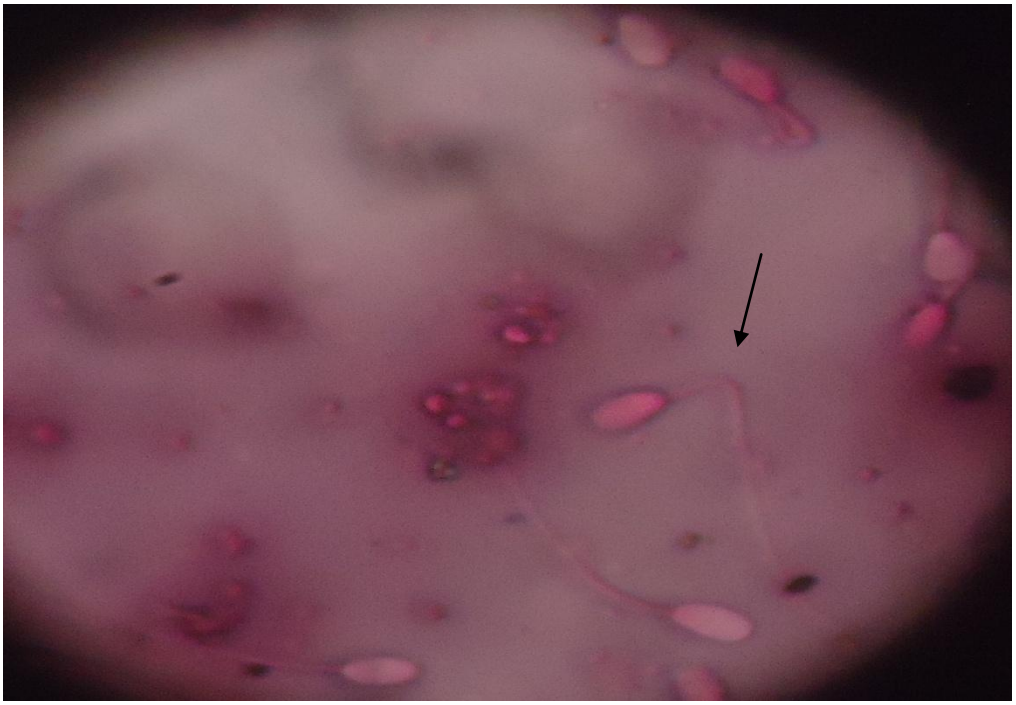

Plate VI: Bent sperm cell tail of rabbit buck fed diet supplemented with *A. sativum*.

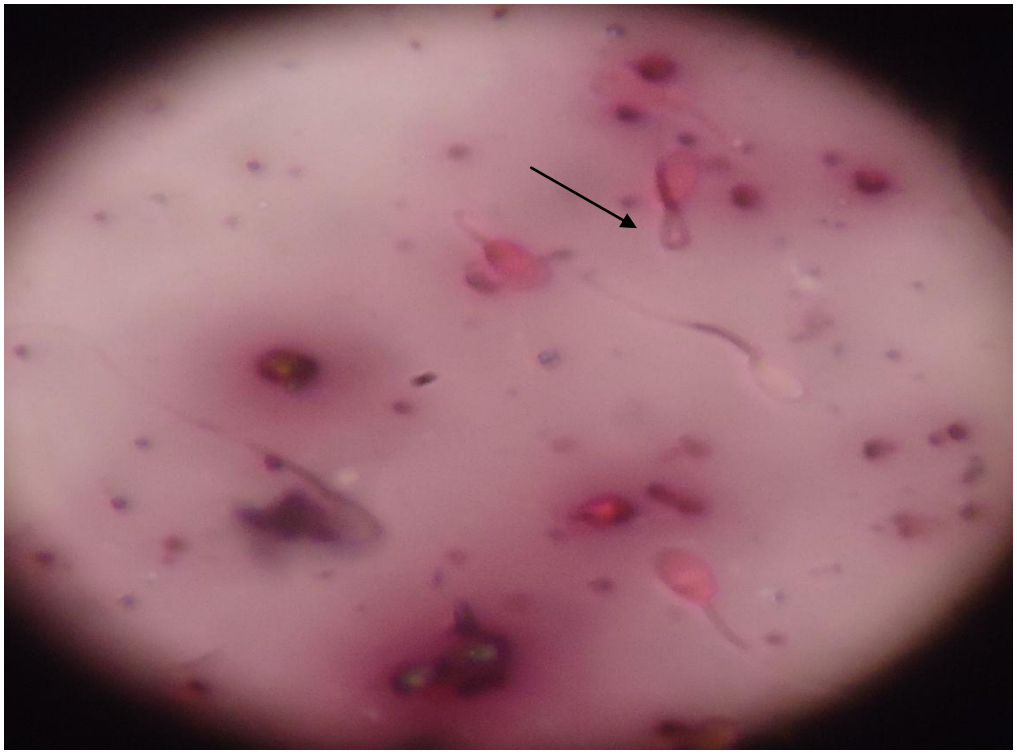

Plate VII: Tightly coiled sperm cell tail of rabbit buck fed diet supplemented with *A. sativum.*

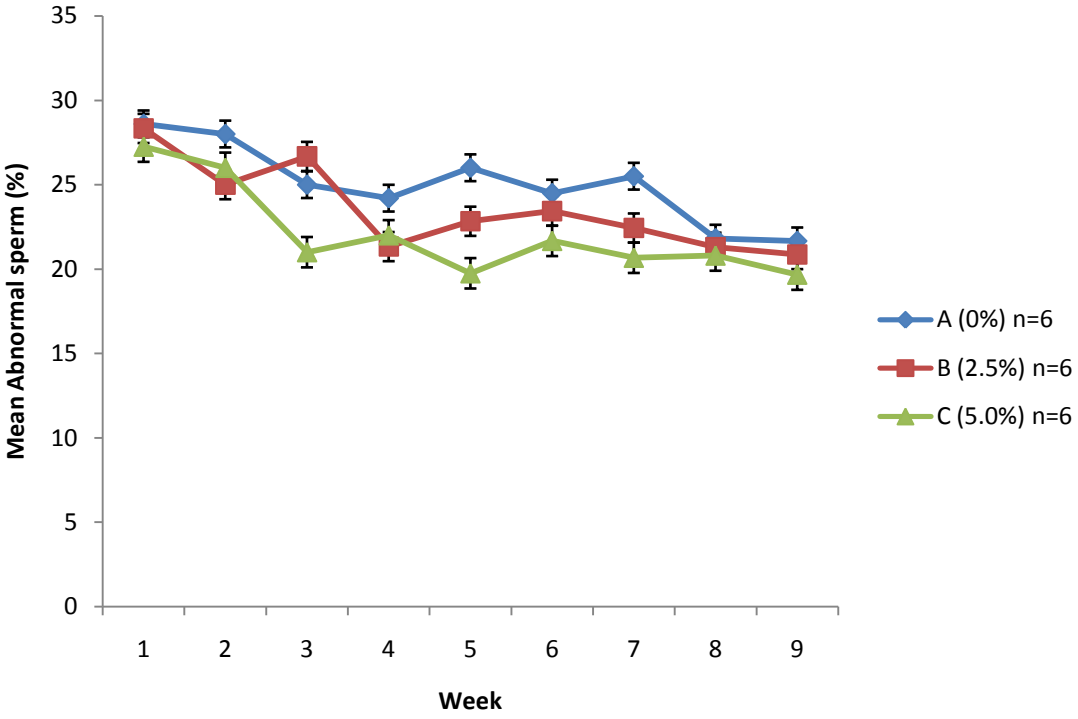

Figure 4.7 Mean percentage sperm abnormality of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic.

4.5 Haematological Parameters

The mean (± SEM) of packed cell volume (PCV), haemoglobin concentration, total protein, red blood cell (RBC) count and white blood cell (WBC) count are presented in Figures 4.8-4.12.

Figure 4.8 shows the weekly mean (± SEM) values for PCV of rabbit bucks in groups A, B and C fed 0, 2.5 and 5.0 % garlic respectively. There was significant difference ($P<0.05$) at week 6 between groups A (30.83 ± 2.02) and C (21.86 ± 2.75) of the study.

Figure 4.9 shows the mean (±SEM) vaues for haemoglobin concentration in g/dL of rabbit bucks in groups A, B and C fed 0, 2.5 and 5.0 % garlic respectively. There was a significant ($P< 0.05$) difference in the mean haemoglobin concentration at week 6 of the feeding period, between group A (10.30 ± 0.65) and C (7.29 ± 0.92).

Figure 4.10 shows the mean (±SEM) values of total blood protein in g/dL of rabbit bucks in groups A, B and C fed 0, 2.5 and 5.0 % garlic respectively. There were significant ($P<0.05$) differences in the mean total blood protein values between groups A and C at week, 8 (A, 6.50 ± 0.17; C, 7.19 ± 0.09) and 9 (A, 6.68 ± 0.17; C, 7.33 ± 0.08) of the feeding period.

Figure 4.11 shows the mean (±SEM) values for RBC counts of rabbit bucks in groups A, B and C fed 0, 2.5 and 5.0 % garlic respectively. RBC values were higher in the non treated group through out the duration of the study compared to the treated groups. Generally, there was significant ($P<0.05$) decrease in RBC count in a dose-dependent manner among the groups.

Figure 4.12 shows the mean (±SEM) values for WBC counts of rabbit bucks in groups A, B and C fed 0, 2.5 and 5.0 % garlic respectively. There were no significant ($P>0.05$) differences between the groups.

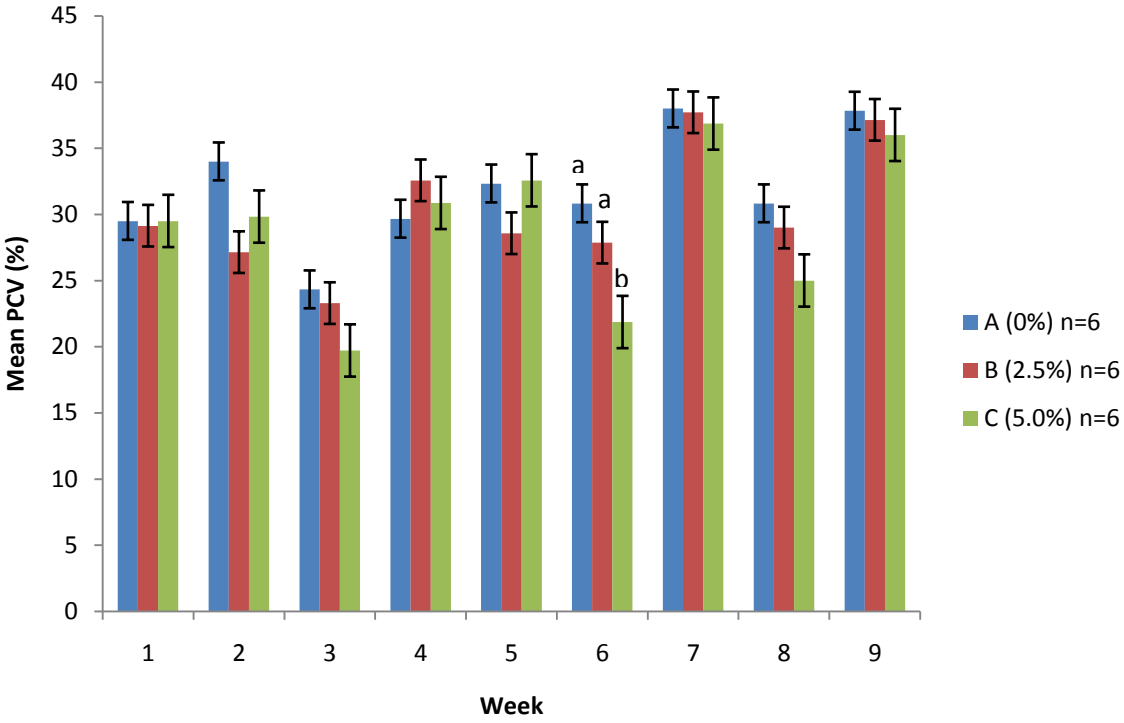

Figure 4.8 Mean Packed cell volume (PCV) of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic (*A. sativum*).

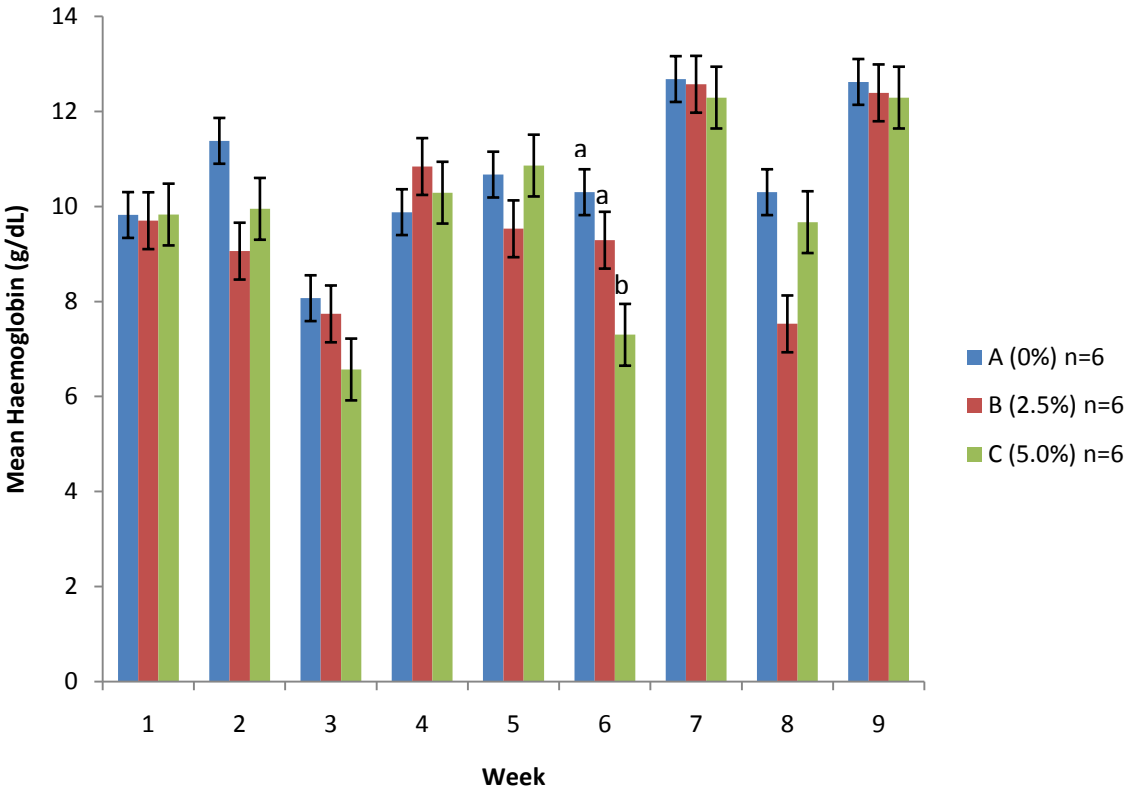

Figure 4.9 Mean haemoglobin concentration (g/dL) of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic.

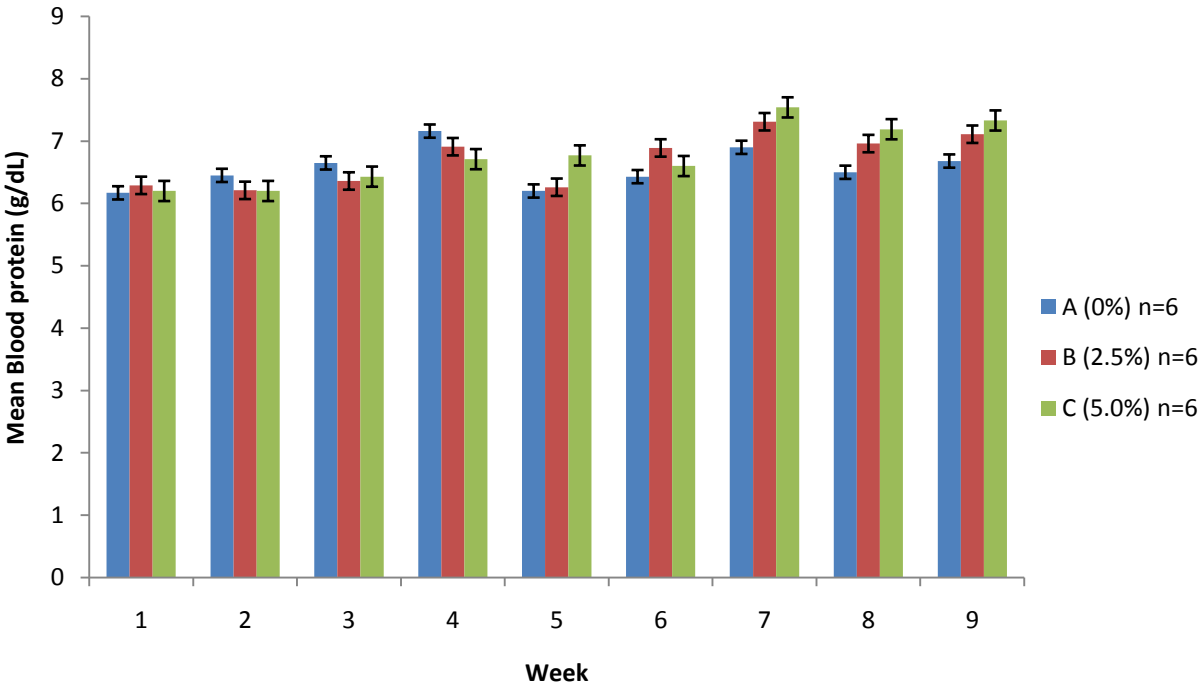

Figure 4.10 Mean total protein concentration (g/dL) of rabbit bucks fed diets with 0, 2.5 and 5.0% garlic.

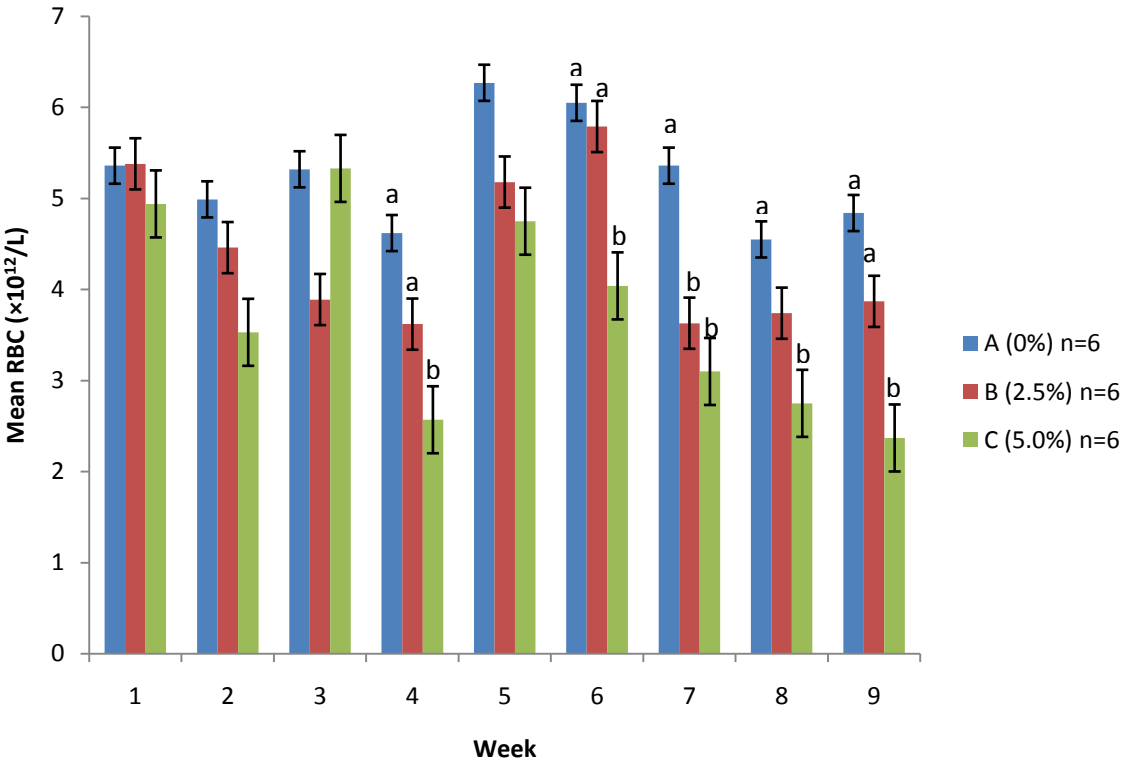

Figure 4.11 Mean red blood cell (RBC) count ($\times 10^{12}$/L) of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic

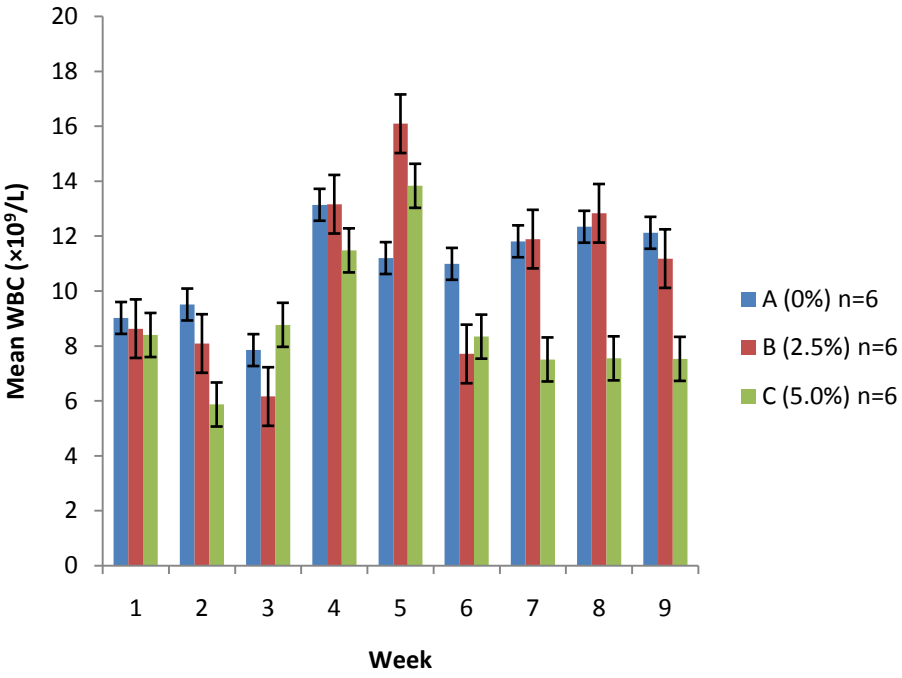

Figure 4.12 Mean white blood cell (WBC) count ($\times 10^9$/L) of rabbit bucks fed diets containing 0, 2.5 and 5.0% of garlic.

4.6 Serum and Antioxidant Enzymes Assay.

There were significant ($P < 0.05$) difference in mean Aspartate Transaminase (AST) levels between group A control (42.50 ± 11.19) and the treatment groups (B, 21.86 ± 4.01; C, 18.43 ± 5.04). Similarly, Alanine Transaminase (ALT), levels in group A (39.33 ± 11.19) differed significantly ($P < 0.05$) with that of the treatment groups (B, 29.00 ± 2.20; C, 27.71 ± 3.23). However, Alkaline Phosphatase (ALP) levels in groups A (41.57 ± 3.79) and B (37.67 ± 4.01) differed significantly ($P < 0.05$) with that of group C (26.29 ± 3.31). (Table 4.3).

There were significant ($P < 0.05$) differences in the levels of superoxide dismutase (SOD) between group A (1.66 ± 0.08) and the treatment groups (B, 2.06 ± 0.08; C, 2.08 ± 0.11). While Catalase and Glutathione levels, though higher in the treatment groups (B and C) were not significantly ($P > 0.05$) different with the control (group A). The mean malondialdehyde (MDA) levels in group A (1.81 ± 0.08) were higher significantly ($P < 0.05$) with that of group C (1.40 ± 0.07). (Table 4.3).

Table 4.3: Mean (± SEM) values of Serum and antioxidant enzymes 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	**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}
4	**SOD (iu/L)**	1.66 ± 0.08^{a}	2.06 ± 0.08^{b}	2.08 ± 0.11^{b}
5	**CAT(iu/L)**	40.83 ± 2.40	41.71 ± 1.02	44.57 ± 1.10
6	**GPX (iu/L)**	44.57 ± 1.31	49.29 ± 1.44	48.33 ± 1. 41
7	**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.

4.7 Gonadal and Extragonadal Weight, Length and Spermatids/Sperm Reserves.

Table 4.4 Shows the gonadal weight, length and sperm reserves of rabbit bucks groups fed diets with 0, 2.5 and 5.0 % of garlic respectively. There were no significant ($P > 0.05$) differences oberved in the gonadal weight and length between the groups. The sperm reserves in the left was significantly ($P < 0.05$) higher in group C (289.50 ± 26.50^{b}) compared to group A (123.00 ± 22.00^{a}) (Table 4.4).

Table 4.5 Shows the mean (±SEM) values for epididymal weight, length and sperm reserves of rabbit bucks groups fed diets with 0, 2.5 and 5.0 % of garlic respectively. There were no significant ($P > 0.05$) difference in the epididymal weight and length among the groups. However, the right extragonadal sperm reserves (EGSR), was significantly ($P < 0.05$) lower in group A (216.50 ± 11.50^{a}) compared to the treated groups (B, 403.50 ± 73.50^{b}; C, 477.00 ± 32.00^{b}). Like wise, the left EGSR was significantly ($P < 0.05$) lower in group A (244.50 ± 16.50^{a}) compared to groups (B, 472.50 ± 117.00^{b}; C, 538.50 ± 89.50^{b}) (Table 4.5). Interestingly, it was observed that the left testes and epididymis had higher sperm/spermatids reserve, compared to the right in this study.

Table 4.4: Mean (± SEM) values of Gonadal weight, length and sperm reserves of rabbit buck groups fed diets containing 0, 2.5 and 5.0% of garlic.

Parameter	**Groups**		
	A (0%) n = 2	**B (2.5%) n = 2**	**C (5.0%) n = 2**
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
Length (cm)			
Right Testis	3.15 ± 0.15	2.65 ± 0.35	2.90 ± 0.10
Left Testis	3.05 ± 0.05	2.95 ± 0.05	2.85 ± 0.05
Gonadal Sperm Reserve ($\times 10^6$/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]

Means with different superscripts letters along the row are significant ($P < 0.05$) different

Table 4.5: Mean (± SEM) values of Extragonadal Weight (g), Length (cm) and sperm reserves (EGSR × 10^6) of rabbit buck groups (A, B and C) fed diets containing 0%, 2.5% and 5.0% of garlic respectively.

Parameters	Groups n = 3	Caput		Corpus		Cauda	
		right	left	right	left	right	left
Weight (g)	A (0%)	0.13 ± 0.05	0.17 ± 0.06	0.01 ± 0.00	0.04 ± 0.01	0.03 ± 0.02	0.32 ± 0.01
	B (2.5%)	0.16 ± 0.04	0.17 ± 0.03	0.02 ± 0.01	0.04 ± 0.02	0.39 ± 0.05	0.36 ± 0.16
	C (5.0%)	0.17 ± 0.10	0.22 ± 0.03	0.03 ± 0.02	0.06 ± 0.05	0.31 ± 0.03	0.44 ± 0.04
Length(cm)	A (0%)	1.50 ± 0.50	1.00 ± 0.10	3.40 ± 0.70	3.95 ± 0.25	1.75 ± 0.25	1.03 ± 0.65
	B (2.5%)	1.40 ± 0.10	1.00 ± 0.00	2.20 ± 0.20	3.05 ± 0.15	1.85 ± 0.25	1.75 ± 0.28
	C (5.0%)	1.10 ± 0.40	1.05 ± 0.05	2.50 ± 0.50	2.90 ± 0.10	1.15 ± 0.15	1.40 ± 0.10
EGSR(10^6)	A (0%)	34.00 ± 4.0	52.00 ± 34.	15.00 ± 3.0	19.50 ± 1.50	216.50 ± 12^a	244.5 ± 16.5^a
	B (2.5%)	38.5 ± 1.50	35.00 ± 27.	59.50±4.50	39.00 ± 29.0	403.50 ± 74^b	472.5 ± 117^b
	C (5.0%)	33.00 ± 22	46.00 ± 24	38.00±19.0	39.00 ± 14.0	477.00 ± 32^b	538.5 ± 89.5^b

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

4.8 Histopathological Changes

The hepatic histopathological features of the liver of rabbit bucks in the different treatment groups are shown in Plates VIII and IX, respectively. There were no pathological lesions observed in all the groups.

The testicular histopathological changes in the testicles of groups B and C are shown in Plates X and XI respectively. There were no pathological changes observed in all the groups. However, the seminiferous tubules were better organized and were more clearly defined in groups B and C, compared to group A (control). Further more, the lumen of the seminiferous tubules in the treatment groups had higher density of sperm cells in a dose dependent manner.

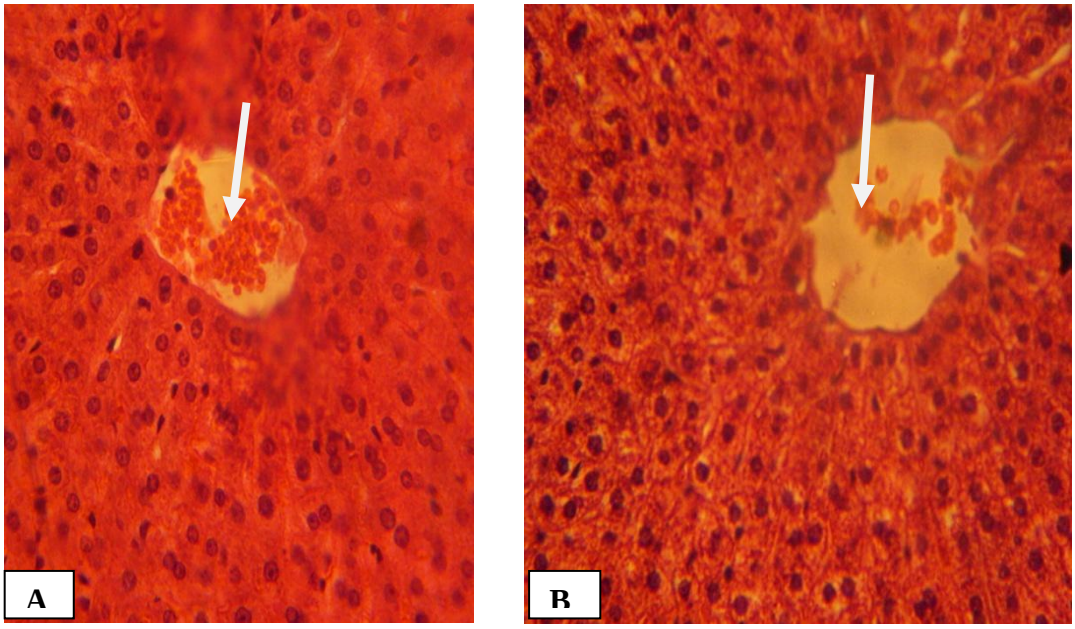

Plate VIII: Photomicrograph of the liver of rabbit bucks. **A**. Normal architecture of the liver of a rabbit in the control (group A), normal hepatic architecture with hepatocytes evenly distributed in the hepatic tissue, white arrow showing central vein. **B**. Histology of liver of a rabbit in group B, Liver devoid of any pathologic changes, with hepatocytes uniformly distributed in the tissue, white arrow showing central vein. (H & E × 400).

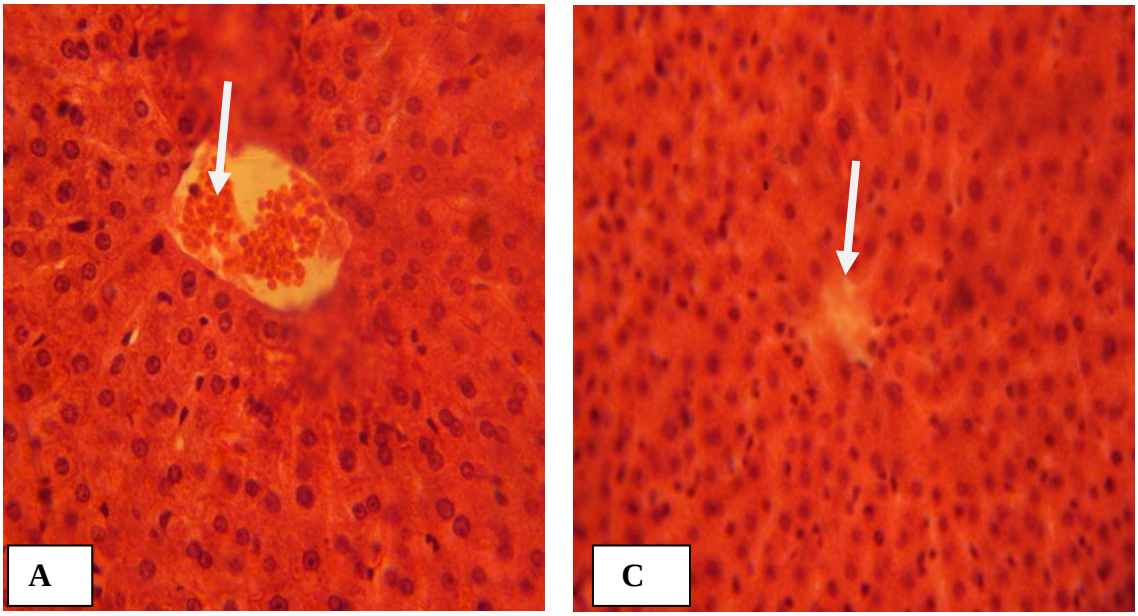

Plate IX: Photomicrograph of the Liver of rabbit bucks. **A**. Normal architecture of the liver of a rabbit in the control (group A). showing normal hepatic architecture with hepatocytes evenly distributed in the hepatic tissue, arrow showing central vein. **C**. Histology of the liver of a rabbit in group C. Devoid of any pathologic changes, showing hepatocytes uniformly distributed in the tissue, white arrow showing central vein. (H & E × 400).

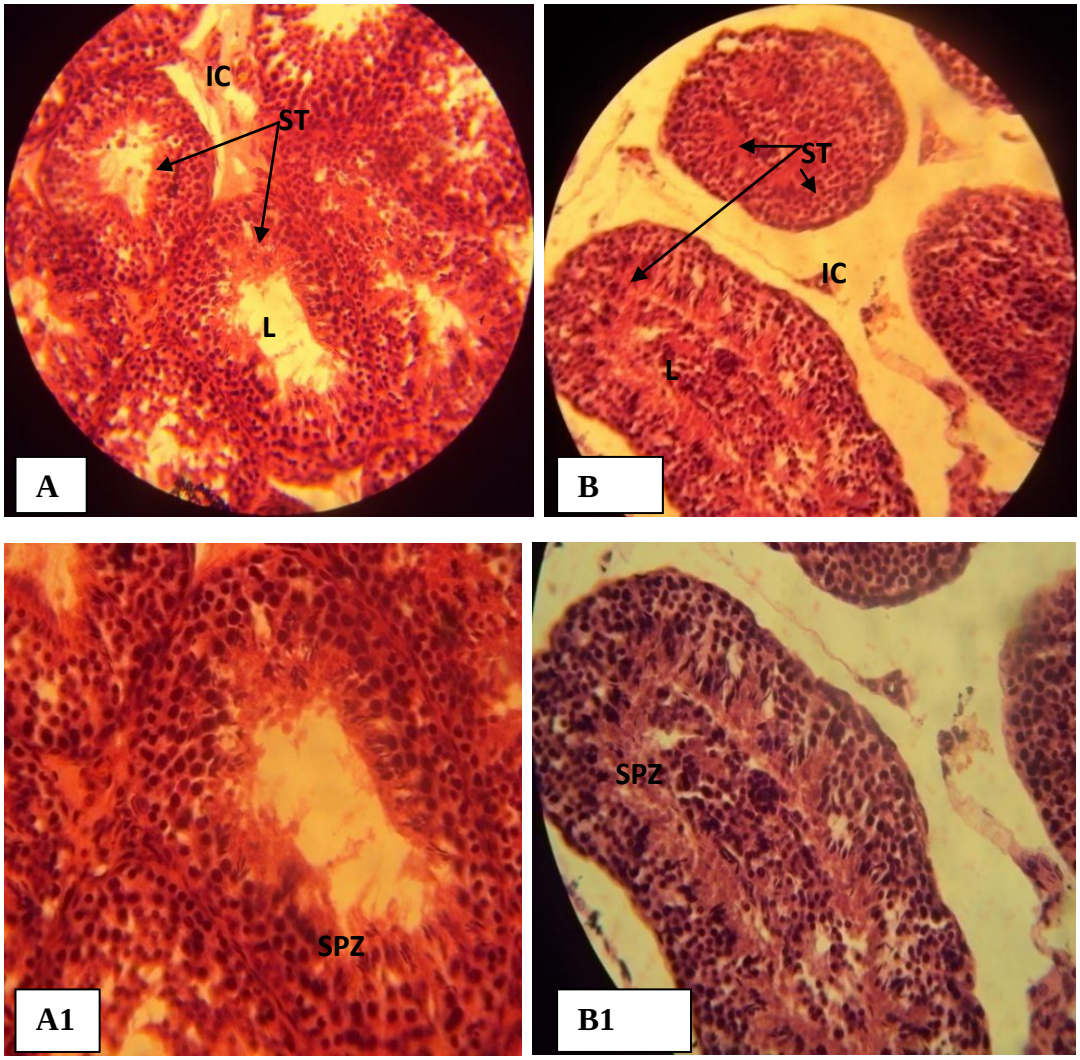

Plate X: Photomicrograph of the testes of rabbit bucks. **A**: Histologic section of the testis of a rabbit in group A. Note the seminiferous tubules (ST) not clearly separated, interstitial cells (IC) not clearly separated from spermatogenic cells surrounding the ST. **A1**. Shows spermatozoa (SPZ) sparcely distributed in a relatively spaceous 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. **B1**. Shows spermatozoa (SPZ) distributed within the lumen (L) of ST with no obvious space in the lumen. (H & E × 400).

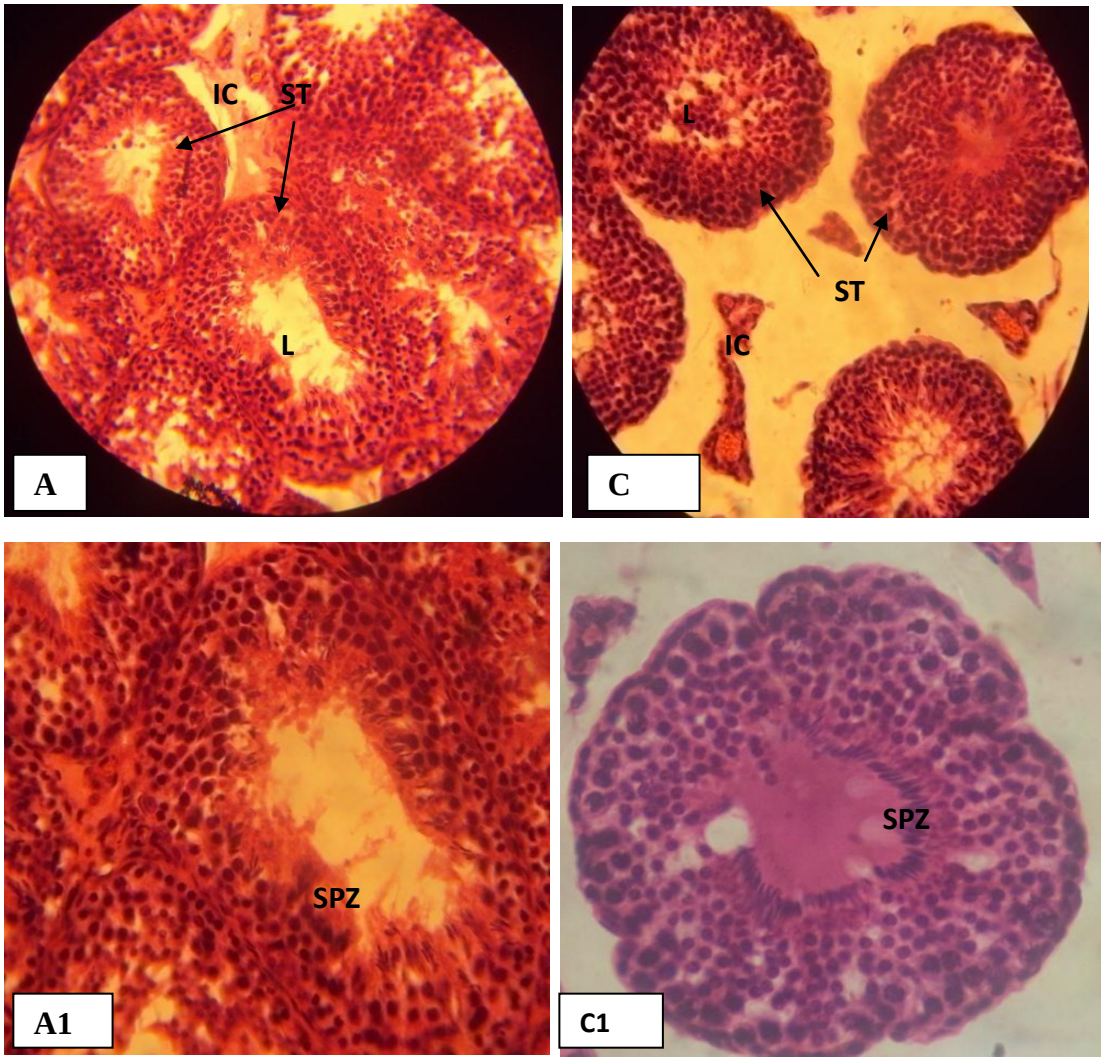

Plate XI: Photomicrograph of the testes of rabbit bucks. **A**: Histologic section of the testis of a rabbit in group A. Note the seminiferous tubules (ST) not clearly separated interstitial cells (IC) not clearly separated from spermatogenic cells surrounding the ST. **A1**. Shows spermatozoa (SPZ) sparcely distributed in a relatively spaceous lumen (L) of the ST. **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. **C1**. Shows densely packed spermatozoa (SPZ) well arranged within the lumen (L) of ST (H & E × 400).

CHAPTER 5

DISCUSSION

The observation on phytochemical analysis is similar with that of Gazuwa *et al*. (2013), who obtained similar results except for saponin that was absent in his findings. However, Mikail (2010) reported the presence of saponins, carbohydrate, cardiac glycosides and anthraquinone. The slight variation in the phytochemical constituents may be due to species variation, different environments in which the garlic was grown, methods of handling and processing of the garlic.

The findings of no significant differences in live body weights in all the groups, is consistent with the findings by Al-Bekairi *et al*. (1990), Yuriko *et al*. (2001) and Omotoso *et al*. (2012). They all reported a no significant increase in body weight in their respective studies. However, our findings contradict that of Hammami *et al*. (2008), who reported a significant decrease in mean body weight of adult male rats administered 15% and 20% inclusion rate of *A. sativum.* The difference could be due to the higher inclusion rates used by Hammami *et al*., (2008), which may have affected the acceptibility and consequently the rate of intake of the diets, leading to a resultant decrease in body weight. The higher dose of garlic may also have affected the body fat metabolism or glucose metabolism in the animals.

The observation of increased mean ejaculate volume of the control compared to experimental groups at week 8 of the study could be due to possibility of contamination of the semen by urine as highlighted by Jordi *et al*. (2005) who reported a 13% chance of contamination of rabbits' semen by urine during collection with artificial vagina. The normal ejaculate volume for rabbit bucks as reported by Campos *et al*. (2014) ranges between 0.3-0.6 ml, this is far below the abrupt increase we observed at this week of the study.

The absence of a significant difference in the mean gross sperm motility between the control and experimental groups, contradicts the findings of Omotoso *et al.* (2012), who observed a decrease in percentage spermatozoa motility in wistar rats treated with graded doses of *A. sativum*. This difference may be due to variety of garlic used, dose administered and also species differences.

Observation on the effect of garlic on sperm concentration has been varied in the present study. There were gradual increases in mean sperm concentration in the treatment groups (B and C), with a significant difference between group A and group C from week 3 to 9 of the study. This corroborates the study of Al-Bekairi *et al*. (1990), who evaluated the effect of 100 mg/kg/day of aqueous extract of *A. sativum* on epididymal spermatozoa in mice treated for 3 months and found out that the sperm count was significantly increased in the treatment groups compared to the control. Salah *et al*. (2014) reported a significant increase in sperm concentration in mice treated with aqueous and alcoholic extracts of *A. sativum*. However, this contradicts the findings of Hammami *et al.* (2008), who found out that after the administration of crude garlic at inclusion rate of 5.0%, 10%, 15% and 30% to wistar rats for 30 days, observed a reduction in sperm concentration at 10%, 15% and 30% doses. Omotoso *et al*. (2009) reported a decreased sperm concentration in wistar rat treated with 500 mg/kg/day and 1000 mg/kg/day of aqueous garlic extract for 30 days in a dose dependent manner. Saponin which is one of the constituents of garlic was reported to have some positive effects on libido and spermatogenesis (Francis *et al*., 2002) this probably may have had an effect on the sperm concentration in the treated groups. It is possible to attribute the differences of other studies with our finding to higher inclusion rates/doses of the *A. sativum* used.

The absence of significant differences in percentage live spermatozoa among the groups, contradicts the findings of Omotoso *et al.* (2012), who observed a decreased percentage live spermatozoa in wistar rats, treated with higher doses of garlic.

The observation on percentage abnormal spermatozoa between the groups also disagress with the findings of Omotoso *et al*. (2012), who observed a marked increase in percentage abnormal spermatozoa in a dose dependent manner in wistar rat treated with garlic. The several reasons adduced for differences in observations by different investigators could necessitate more intensive studies to determine the optimal inclusion rates, varieties of the plant and other physicochemical properties necessary to advance the use of garlic in a more beneficial manner.

The observation on Packed cell volume (PCV) and haemoglobin concentration, are similar with the study carried out by Ugwu and Omale (2011), which they administered 0.5 mg/kg, 1.0 mg/kg and 1.5 mg/kg of aqueous garlic extract to wistar rats for 28 days and reported a decreased PCV in a dose dependent fashion. Tende *et al*. (2014), administered 20 mg/kg and 40 mg/kg of aqueous *A. sativum* extract to wistar rats and reported a slight decrease in PCV and haemoglobin concentration of the treatment group compared to the control. Gauthier *et al*. (2009) reported that saponin has ability to form complexes with cell membrane cholesterol leading to formation of pores and cell permeability resulting in haemolysis of red blood cells this probably was responsible for the decreased PCV and Haemoglobin concentration observed. However, findings in the present study differ from that of Suha (2014), in Iraq administered 1.0% and 5.0% garlic in feed, reported a slight increase in PCV and haemoglobin concentration in a dose dependent fashion in rabbits. The possible reasons for the differences in results, may be due to the variety of garlic used, location where the garlic was grown and storage

condition, as all these may affect the concentration of garlic active components, their stability and benefit to be derived (Alejandra *et al.*, 2010).

The observation on blood protein levels is similar with that reported by Ayoola and Uzoamaka (2013), who administered graded levels of *A. sativum* (1.0 g, 1.5 g, 2.0 g and 2.5 g) in feed to *Clarias gariepinus* juvenile and observed a higher blood protein in the group that received 2.5 g of *A. sativum*.

There was a general decrease in red blood cells (RBC) counts in the treatment groups with group C having the lowest values. Despite these inconsistencies in observation, this corroborates the study of Al-Bekairi *et al.* (1990), Ayoola and Uzoamaka (2013), who reported a significant higher RBC count in the control groups of their study compared to the treatment groups. We attribute our observations to possibly be due to the effect of saponin on RBC as reported by Gauthier *et al.* (2009). However, our findings differ with that of Suha (2014), who reported a slightly higher RBC count at 5.0% inclusion of *A. sativum*, the possible reasons for this differences being as a result of variety of *A. sativum* used, environment grown and storage condition.

The observation of no significant differences in the mean WBC count in both the control (group A) and treatment group (B and C) is similar with the findings by Ugwu and Omale (2011), Tende *et al.* (2014) who reported no significant difference in WBC count in both control and treatment groups. However it contradicts the findings of Suha (2014), who reported a significant increase in total WBC count at 5.0% inclusion of *A. sativum* compared to the control, the differences could be due to *A. sativum* variety used, environment grown and storage condition.

Serum enzymes or Diagnostic enzymes are usually found in low concentration in blood but in the event of damage or disease to an organ the enzymes present in such organ

(within the cells) leak out into the blood. Aspartate Transaminase (AST) is an enzyme mostly found in the heart and liver and to some extent in muscles. When these organs are diseased or injured this enzyme is released into the blood circulation. In this study, the treatment groups (B and C) were observed to have a significantly lower concentration of AST in the blood compared to the control. Alanine Transaminase (ALT) is a transaminase enzyme found in serum and bodily tissues but is commonly associated with the liver. Significantly elevated levels of ALT suggest a possible medical condition like liver cirrhosis and hepatitis (Kumar and Clark, 2001). In this study, ALT concentration was significantly low in the treatment groups (B and C) compared to the control (A). Both AST and ALT activities are reliable test for liver function status. Our findings contradict that of Ayoola and Uzoamaka (2013), who reported increased AST, ALT and Alkaline Phosphatase (ALP) concentration in the serum of *Clarias gariepinus* administered 2.5g of garlic for 90 days. The possible reasons for the differences with our study could be due to species, dose differences and duration of the study.

There were significant increases in the concentration of superoxide dismutase (SOD) in the blood of the treatment groups (B and C), in a dose dependent manner. Flavonoid, one of the active constituents of garlic was reported to confer protection against the harmful effects of ROS, *in vitro* studies showed that flavonoid have potent antioxidant and free radical scarvenging activities (Prochazkova *et al*., 2011) this may have been responsible for the elevated levels of antioxidant enzymes observed in the study. This study corroborates the study of Banerjee *et al*. (2002), who reported that chronic garlic administration in rats increased SOD concentration significantly. However, this contradicts the findings of Omotoso *et al.* (2009), who reported a decreased

concentration of SOD in wistar rats treated with aqueous garlic extract. Findings in the present study can be attributed to the dose used in the study.

The findings on Catalase concentration in this study between (group A) and treatment 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 design of the study.

The absence of no significant differences in the mean values of Gluthathione peroxidase (Gpx) assayed between the control (group A) and treatment groups (B and C) is similar to that of Banerjee *et al*. (2002) and Reza *et al*. (2013) who reported no significant changes in wistar rats exposed to lead and administered garlic. Ghalehkandi (2014), also reported no changes in Gpx concentration in wistar rats treated with *A. sativum*.

MDA is a product of lipid peroxidation in the body and it is used as a standard in assesing free radicals damage in the blood. The decreased concentration of MDA in the blood in this study in a dose-dependent manner is 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 pretreated with *A. sativum* extract prior to testicular torsion and detorsion. Ghalehkandi (2014) also reported a significant decrease in MDA levels in semen of rats administered 120mg/kg aqueous extract of *A. sativum* for 35 days.

Sperm plasma membrane, being rich in poly-unsaturated fatty acids makes it highly susceptible to Reactive Oxygen Species (ROS) attack. To mitigate the harmful effects

of ROS, testes are naturally equipped with a powerful antioxidant defence system involving enzymes like SOD and catalase (Vernet *et al*. 2004). Superoxide dismutase is considered the first line of defense against deleterious effects of Oxygen radicals in the cell by catalyzing the dismutation of superoxide anions radicals to Hydrogen peroxide, which is readily degraded by catalase. In a biological system, the antioxidant enzyme catalase protects SOD inactivation by hydrogen peroxide, while the SOD reciprocally protects catalase against inhibition by superoxide anion. Therefore, balance of this enzyme system is essential to eliminate superoxide and peroxide radicals generated in the tissues (Reza *et al*., 2013). In this study, administration of *A. sativum* in feed of the rabbit bucks increased the activities of SOD and catalase, concurrently decreasing the levels of lipid peroxidation in the testes. The increase in the activities of antioxidant enzymes indicates the response of the primary antioxidant system to act against free radicals, their by preventing the depletion of sperm cells by ROS through the process of lipid peroxidation.

The observation of no significant differences in mean gonadal/testicular weight and length in all the groups is consistent with the findings of Hammami *et al*. (2008), who reported similar result in wistar rat fed graded levels of *A. sativum.* Omotoso *et al*., (2009), also reported similar results in wistar rat administered graded doses of aqueous extract of *A. sativum*). There was significant increase in sperm reserves as the dose increased in the left testis. This contradicts the findings of Hammami *et al*. (2008) and Omotoso *et al*. (2009) who both reported a decreased testicular sperm concentration in wistar rats administered *A. sativum*. The difference with our findings may be due to the high dose of *A. sativum* administered in those studies and species differences.

There were no changes in mean epididymal weights and lengths in all the groups in this study. This is consistent with the findings of Hammami *et al*. (2008) but differs with

that of Al-Bekairi *et al*. (1990), who reported an increase in epididymal weight in garlic fed animals. There were no effects on the epididymal sperm reserves in the caput and corpus, but in the cauda and a significantly higher value was observed in a dose dependant manner in both the right and left epididymis. This agrees with the study of Al-Bekairi *et al*. (1990) who reported an increase in epididymal sperm concentration in wistar rats administered *A. sativum* and disagrees with Hammami *et al.* (2008), who reported a decrease in epididymal sperm concentration in wistar rats fed graded levels of *A. sativum.*

In this study, we observed that in both gonadal and extragonadal sperm reserves, the left testes and epididymis had higher values of sperm/spermatids reserves than the right. Fadason (2009) reported increased sperm reserves in the right testes of red Sokoto bucks. Ewuola and Egbunike (2010) reported increased sperm reserves in the right testes and epididymis in pubertal rabbits in their study. One reason that could be adduced for the differences with our study could be the slightly increased left testicular and epididymal weights in our study, which probably was due to increased physiologic functions in the left as compared to the right, may have been responsibly for the increased sperm reserves on the left.

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 by diets 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.

The testis is a vital, well vascularised and very sensitive organ in the body and this therefore might explain the swift response of the testis to changes within and outside the body. Thus, it is strategically located in an "outpocketing" of the abdominal cavity called the scrotum. It is richly endowed with polyunsaturated fatty acid, which explains its high susceptibility to oxidative stress caused by Reactive Oxygen Species (ROS). *A. sativum*, is known to increase the blood levels of antioxidant enzymes (SOD, CAT, Gpx) which scavenge the ROS, thereby protecting the sperm cells against depletion caused by oxidative stress. Banerjee *et al*. (2002), observed that *A. sativum* at low dose could significantly increase SOD activity, while at higher dose, SOD activity was decreased. In this study, there was increased concentration of these enzymes with treatment which translates to preventing or reduction of oxidative stress in the testes.

This is indicated by a significant decrease in the concentration of MDA with treatment in this study. This could also be the reason why there was higher spermatozoa concentration in the treatment groups compared with the control. Histological features of the treated rabbit bucks showed seminiferous tubules with lumen filled with spermatogenic 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 distorsion in testicular architecture at ≥ 10% inclusion, with empty spaces in the lumen of the seminiferous tubules, devoid of spermatogenic cells.

It is important to note that administration of *A. sativum* may have brought about increased antioxidant activities in the rabbit bucks as indicated by the elevated antioxidant enzymes levels and decreased MDA concentration. 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 output compared to the control.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The Phytochemical analysis of *A. sativum* indicated a number of compounds present which may have acted singly or in synergy with one another to produce the effects observed. *A. sativum* in diets of rabbit bucks had a positive effect on sperm output but decreased some haematological parameters which were within the normal range. *A. sativum* showed positive effects on some antioxidant enzymes with improvements in both gonadal and extragonadal sperm reserves.

6.2 Conclusions

Based on our findings the following conclusions were made**:**

1. Carbohydrate, Cardiac glycosides, saponin, Triterpene, Flavonoids and Alkaloids are present in *A. sativum* used in the study.
2. *A. sativum* inclusion at 2.5 and 5.0 % in the diets of rabbit bucks improved sperm concentration till week 9 of the study.
3. *A. sativum* reduced PCV, haemoglobin concentration and RBC count but slightly increased blood proteins levels.
4. *A. sativum* gradually decreased AST, ALT and ALP concentration and elevated antioxidant enzyme (SOD) concentration with corresponding decrease in MDA levels.

v. *A. sativum* increased gonadal/testicular and extragonadal/epididymal sperm reserves in a dose dependent manner, more in the left testes and epididymis than the right.

vi. *A. sativum* is not toxic to liver and testes but improved sperm output.

6.3 Recommendations

Based on the results of this study, it is recommended that:

i. *A. sativum* at 2.5-5.0% improved sperm output, gonadal and extragonadal sperm reserves in the treated groups and should be considered in feed formulation.
ii. Further studies should be carried out on the effects of *A. sativum* on male reproductive hormones and acrosomal enzymes to complement the results obtained in the present study.
iii. More studies should be carried out on the effects of *A. sativum* on female reproduction and hormones, to complement the results obtained in the present study.

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