EFFECTS OF GRADED LEVELS OF VITAMIN E AND SELENIUM ON GROWTH, BLOOD PROFILES AND SEMEN QUALITY OF RABBIT BUCKS

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ZARIA, NIGERIA

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DEPARTMENTOF ANIMAL SCIENCE,

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MARCH, 2019

DECLARATION

I hereby declare that the work in this thesis entitled **"Effects of Graded Levels of Vitamin E and Selenium on Growth, Blood Profiles and Semen Quality of Rabbit Bucks."**was carried out by me in the Department of Animal Science under the supervision of Dr. (Mrs) O.M. Daudu and Prof. (Mrs) G.T. Iyeghe-Erakpotobor.

Information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has previously been presented for the award of another degree or diploma at any University.

Onoja Blessing Asebe _____ _____

Signature Date

CERTIFICATION

This Dissertation titled **"Effects of Graded Levels of Vitamin E and Selenium on Growth, Blood Profiles and Semen Quality of Rabbit Bucks."**by Onoja, Blessing Asebe meets the regulations governing the award of the degree of Master of Science (Animal Science) of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

Two experiments were carried out to determine the effect of feeding graded levels of Vitamin E and Selenium on growth, blood profile and semen quality of rabbit bucks. In the first experiment, twenty mixed breed rabbit bucks aged between 6 – 8 weeks with an average weight of 861g were grouped into 4 groups of 5 bucks per groups and assigned to 4 dietary treatments in a Completely Randomized Design. The rabbits received graded levels of Vitamin E at 0 (which served as control), 20, 40 and 60 mg designated as treatments 1, 2, 3 and 4, respectively. The study lasted for 90 days. The results from the growth trial shows that there were significant ($P<0.05$) differences in final weight (1403.30-1870.00g), average daily gain (6.04-11.20g) and feed conversion ratio (7.74-12.17) of rabbits fed 20 mg/kg vitamin E inclusion. Average daily feed intake (73.52-87.86g) showed significant difference ($P<0.05$) at 0 mg/kg vitamin E inclusion. Packed cell volume (27.00-40.00%) and haemoglobin (9.10-13.20g/dl) were significantly ($P<0.05$) higher for rabbits fed 40 and 60 mg/kg vitamin E diets. Red blood cell count (4.70-7.00x10^{12}) was significantly ($P<0.05$) higher for rabbits fed 20, 40 and 60 mg/kg vitamin E diets. There were no significant ($P>0.05$) differences in glucose, total protein, albumin, aspartate transaminase, alanine transaminase and alkaline phosphatase across treatments. There were significant ($P<0.05$) difference in globulin (31.67-44.00g/l) and glutathione peroxidase (3.95-10.73μmol/l) for rabbits fed 40 mg of Vitamin E and plasma vitamin E (0.63-17.18mmol/l) showed significant ($P<0.05$) difference at inclusion rate of 60 mg vitamin E. Semen volume and pH were not significantly ($P>0.05$) different. Sperm motility (56.67-87.50%),sperm concentration (72-232x10^{6}), live to dead ratio (59.17-87.50%) and live to dead ratio (12.00-40.83%) were significantly ($P<0.05$)higher in rabbit bucks fed 60 mg/kg of vitamin E diet. Free tail (1.11-7.22%) was significantly ($P<0.05$) higher in rabbit bucks fed 20 mg/kg while coiled tail (0.67-4.98%) and bent tail (1.00-5.29%) were significantly ($P<0.05$) higher in rabbit bucks fed 20 and 60 mg/kg vitamin E diet. In the

second experiment, twenty mixed breed rabbit bucks aged between 6– 8 weeks with an average weight of 756g were divided into groups of 5 bucks pre group and the groups randomly assigned to four dietary treatments in a Completely Randomized Design. The rabbits received graded levels of Selenium at 0 (which served as control), 0.2, 0.4 and 0.6 mg for treatments 1, 2, 3 and 4, respectively. The results from the growth performance of rabbits on Selenium supplementation showed that there was significant ($P<0.05$) difference in final weight (1750.00-2052.50g), average daily feed intake (72.33-96.72g) and average daily gain (11.06-14.40g) for rabbit bucks fed diets with 0.4 mg/kg selenium inclusion Feed conversion ratio (6.27-6.72g) was significantly ($P<0.05$) different at 0 mg/kg selenium inclusion . Packed cell volume (34.00-42.00%) was significantly ($P<0.05$) higher at 0, 0.2 and 0.4 mg/kg selenium inclusion level compared to 0.6 mg/kg inclusion level. White blood cell count ($4.73\text{-}10.63x10^{9}$) was significantly ($P<0.05$) higher at 0 and 0.2 mg/kg selenium but statistically ($P>0.05$) similar at 0.2, 0.4 and 0.6 inclusion levels. Lymphocyte (74.67-85.00%) for rabbit bucks on 0 and 0.6 mg/kg was significantly ($P<0.05$) higher than that of rabbit bucks on 0.2 and 0.4 mg/kg diet which were both statistically ($P>0.05$) similar. Glucose, total protein, albumin, globulin, alanine transaminase and Glutathione peroxidase (GSH-Px) levels were not significantly ($P>0.05$) different. Aspartate transaminase (16.00-39.33IU/l) and alkaline phosphatase (102.00-286.00) were significantly ($P<0.05$) higher at 0.2 mg selenium inclusion level. Semen volume (0.35-1.55ml) was significantly ($P<0.05$) higher at 0.2 mg/kg selenium inclusion rate,sperm concentration ($42\text{-}182x10^{6}$) were significantly ($P<0.05$) higher at 0 and 0.2 mg selenium inclusion level. Live to dead ratio (59.83-83.13%) was significantly ($P<0.05$) higher at 0 and 0.2 mg selenium inclusion level. Bent tail (2.38-5.21%) was significantly ($P<0.05$) higher at inclusion rate of selenium0, 0.2 and 0.4 mg/kg selenium in the diets. Feeding graded levels of vitamin E (60 mg) and Selenium (0.2 mg) had positive effect on sperm volume, motility, and concentration of rabbit bucks.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Rabbits play an important role in the supply of animal protein to the Nigeria populace (Amaefula*et al*., 2005). Rastogi*et al.* (2000) reported that rabbits have a number of characteristics that might be advantageous to smallholder integrated farming system such as; small size, large litter size and short generation interval which allows for economic returns in short term (12-15 fryers/doe/year). Rabbits are also more efficient in extracting protein from forages than sheep and beef cattle since they subsist on waste materials from the vegetable garden, family kitchen and institutional canteens and cafeterias. Low investment is also required for establishing a small rabbitry with 3-5 breeding does among others (Rastogi*et al.*, 2000). Rabbit meat may be excellent for production of specific functional foods since its good properties such as the contents of minerals, vitamins and fatty acids can be further enriched via feeding (Ebeid*et al.*, 2013).

Reproductive performance of farm animals depends mainly on adequate level of vitamins and essential minerals due to their vital roles in cellular metabolism, maintenance and growth. It was reported that administration of vitamins especially A, D, E and K or supplementation with selenium improved reproductive performance in sheep (Tang *et al*., 1999; Gamal*et al*., 2014). The efficiency of sperm production, libido and quality of sperm 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 (Mahima*et al*., 2012).

Antioxidants have been known to scavenge for free radicals in the body (Amao*et al*., 2012). One effective antioxidant that is not expensive and is readily available is vitamin E (Tocopherol); It is the only significant lipid-soluble antioxidant present in animal blood (Amao*et al*., 2012). Vitamin E (α-tocopherol) is implicated in neurological and immune functions and protects the cells from potential deleterious effects of free radicals (Amao*et al*., 2012).

Vitamin E functions as an intra-cellular antioxidant scavenging for free reactive oxygen and lipid hydro peroxidases, and converting them to non-reactive forms, thus maintaining the integrity of membrane phospholipids against oxidative damage and peroxidation (Sinclair *et al*., 2000).Vitamin E is also involved in the control of enzyme activity to stabilize biological membrane cells (Feki*et al*., 2001).Vitamin E is essential for such body functions as growth, immune function enhancement, tissue integrity, reproduction, disease prevention, and antioxidant function in biological systems (Ebeid*et al.*, 2013).

Selenium is a micro-nutrient that is required for a number of biochemical functions in both humans and animals (Mahima*et al*., 2012). These include antioxidant, immune function, reproduction and thyroid hormone metabolism (Mclntoch, 2008). Selenium is an essential element for spermatogenesis, as it can protect the biological membranes from lipid peroxidation during spermatogenesis. In the seminal plasma, selenium can reduce the peroxidation of seminal lipids and enhance Glutathione peroxidase (GPx) activity leading to adequate viability of sperms to complete the fertilization process (Ebeid, 2009).

Selenium as a component of Glutathione peroxidase, can serve as the first line of defence against oxidative and nitrosative stress and probably protect testicular spermatozoa from toxic free radicals (Klotz *et al.,* 2003). Insufficient selenium intake by the organism is manifested by numerous biochemical changes, such as reduced Selenium concentration and Glutathione peroxidase activity in blood tissues, and increased activity of Aspartate

aminotransferase (AST) in serum due to muscular damage, increased production of reactive forms of oxygen and final products of lipid peroxidation in blood and tissues (Mirjana*et al.*, 2016). Insufficiency of selenium has been associated with reproductive complications and decreased sperm quality of rats, mice, chicken, pig, sheep and cattle (El-Shestawy*et al.*, 2014).

1.2 Justification of the study

Rabbits have a potential as meat-producing animals in the tropics, particularly on small scale production, thus the potential in alleviating poverty by minimizing the problem of animal protein supply in developing countries as it is considered a cheap alternative source of animal protein. The fertility of males and females in a particular herd/flock, as well as level of nutrition to a large extent, determines the rate of production in any livestock industry (Abdulrashid, 2014). However, variation in the seminal characteristics is known to be affected by many factors (genetic strain, feeding, health status, rearing condition, season, age and collection frequency), thus contributing to the large variability in semen traits (Alvariño, 2000). Furthermore, the complexity of semen evaluation is such that substantial variability among laboratories can be introduced in the evaluation of sperm parameters such as; sperm counts, motility and morphology (WHO, 1999).

However, many factors affect seminal traits (Boiti*et al.*, 2005) and thus it is crucial to define suitable ways to improve spermatozoa characteristics (Brun*et al.*, 2002). Numerous studies have reported useful effects of the antioxidant vitamin E on sperm parameters. The results confirm the protective and beneficial effects of vitamin E on semen quality and support their use in male infertility treatment (Muhammad *et al*., 2015).

Selenium has a biological function related to vitamin E and selenium deficiency induces oxidative stress and causes reduced fertility through reducing number and differentiation of germ cells as reported in mice (Sánchez-Gutiérrez *et al*., 2008). In spite of the promising

effects of dietary protein and selenium supplementations, further study with relatively higher protein levels plus slightly lower selenium level has been suggested (Abdulrashid, 2014). However, in Sub-Saharan region, much research has not been attempted on the effect of vitamin E and selenium supplementations on the reproductive traits of rabbits. Therefore, this study is aimed at evaluating the effect of graded levels of vitamin E and selenium on growth performance, blood profile and semen quality of rabbit bucks.

1.3 Objectives of the Study

The main objective of the study was to determine theeffect of graded levels of vitamin E and selenium on growth performance, blood profile and semen quality of rabbit bucks.

The specific objectives were evaluation of:

1. Growth performance of rabbit bucks fed graded levels of Vitamin E and Selenium inclusion.
2. Blood profile of rabbit bucksfed graded levels of Vitamin E and Selenium inclusion.
3. Semen quality of rabbit bucks fed graded levels of Vitamin E and Selenium inclusion.

1.4 Hypothesis

Null Hypothesis (H_o): Vitamin E and Selenium supplementations have no effect on growth performance, blood profile and semen quality of rabbit bucks.

Alternate Hypothesis (H_a): Vitamin E and Selenium supplementations have effect on growth performance, blood profile and semen quality of rabbit bucks.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brief history of Rabbits

The domestication of the major livestock species (cattle, sheep, and pigs) and the small species (poultry) were lost in the dawn of prehistory. However, rabbit domestication dates back no further than the present millennium (Moreki, 2007). Indeed, the wild rabbit *Oritolaguscuniculus*of Southern Europe and North Africa is thought to have been discovered by Phoenicians when they reached the shores of Spain about 1000 BC (Moreki, 2007). The Roman apparently spread the rabbit throughout the Roman Empire as a game animal. Like the Spaniards of that time, they ate foetuses or new born rabbits, which they called *laurices*, in their natural environment, rabbits are gregarious and prolific.

They are completely herbivorous (eat only plants) and most actively forage in the twilight or in the dark. The average lifespan of a rabbit is 5-10 years (potential life span of 15 years is possible). Rabbits had still not been domesticated, but Varron (116 to 27 BC) suggested that rabbits be kept in *leporaria*, stonewalled pens or parks, with hares and other wild species for hunting. These leporaria were the origin of the warrens or games parks that subsequently developed in the Middle Ages (FAO, 1997; Moreki, 2007)).

2.2 Breeds of Rabbits

Rabbit breeds are distinctively identified phenotypically by body size, shape and coat colour. Using this basis of classification, American Rabbit Breeders Association (2010) recognized 47 distinct breeds. The most common rabbit breeds in the tropics include: New Zealand White, Californian, Chinchilla, French Lop, Dutch, Checkered giant, Flemish giant, Angora and Rex. The two popular breeds for meat production are the New Zealand and the Californian. These breeds are most popular because they combine white fur (preferred by

processors) and good growth traits. New Zealand rabbits are slightly larger and weigh 4.1-5.9 kg than the Californian, 3.6-4.5 kg. The New Zealand rabbit has a completely white, red or black body, whereas the Californian is white with coloured nose (American Rabbit Breeders Association, 2010). The two most popular rabbits for fur production are the Rex and the American Chinchilla. The Rex is slightly smaller (3.2 kg) than the American Chinchilla (4.5 kg). There is a tendency for fur markets to be unstable, so one needs to ensure that market is available before starts of production (Moreki, 2007).

2.3 Production Characteristics of Rabbits

Medium-weight breeds (4.5 to 6kg) are able to start breeding at 6 to 7 months of age, with males maturing one month later than females (Cheeke 1986; Abdulrashid and Juniper, 2016).One buck can service about 10 does, but no more than two to three times a week. The female are usually placed in the buck's cage for breeding; if the buck is put in the cage of the doe she will fight to protect her territory. Mating should occur immediately, and the doe then returned to her cage (Cheeke 1986). The average gestation period is about 31days and if properly managed a doe can produce 5-6 litters in a year with an average of 6 kittens per litter (Cheeke 1986; Abdulrashid and Juniper, 2016). Twenty eight days after breeding a nest box is placed in the cage of the doe. According to Morimoto (2009), gestation length in different breeds of rabbits ranges between 28 to 36 days.

Abdulrashid and Juniper (2016) reported that rabbit does normally kindle 31-33 days of gestation and Nigeria does kindle 5-8 kits per litter, with an average 4-5 kits reach weaning stage.

Twenty eight days after breeding, a nest box is placed in the cage of the doe. The nest box can be removed 15 to 21 days after birth. The young are weaned in about 30 days so that the

doe will produce five litters a year (Pennsylvania State University 1994; Abdulrashid and Juniper, 2016)

2.4 Growth Performance of Rabbits

Growth performance of rabbits from researches in tropical countries is generally in the range of 10-20g per day, in contrast to 35-40g per day commonly observed in temperate regions. Daily body weight gains vary from 8-13g these are low values compared with values of 42g/day/rabbit obtainable in temperature climates (Abu *et al*., 2008). Weight gains reported on forage-concentrate diets under most tropical conditions range between 5-20g/day (Honthong*et al*., 2004; Abu *et al*., 2008) with a turn off target weight of 2.3-2.5 kg at 4-5 months of age. These weight gains depends on the type of rabbits used (weaners, 5-8 weeks old or growers, 9-15 weeks old), type of forage used, state of forage used (fresh forage or hay) and the environmental conditions (Abu *et al*., 2008).

2.5 Minerals Requirement of Rabbits

Minerals are grouped into two categories– macro minerals and micro minerals. Macro minerals include calcium, phosphorus, sodium, magnesium and potassium and are required in grams per day. Trace or micro minerals include copper, zinc, manganese, iron, iodine, selenium and cobalt, and are required in milligrams per day. (Amy, 2010). Perhaps the major concern on production relies on the reproductive status of the animal, which directly influences stock population increases and hence animal protein availability (Abdulrashid, 2014). Selenium has been shown to enhance the reproductive productivity of animals, particularly in hot summer environments (Maraiet *al*., 2009).

2.5.1 Selenium Requirement of Rabbit Bucks

Khanal, (2010) stated that the name selenium comes from the Greek word "Selene" which meant the moon owing to its duality in nature contradictory properties and roles Abdel-Azeem (2010). After its discovery, it was thought to be toxic to livestock with some suggestions of being carcinogenic (Abdel-Azeem, 2010). However, selenium was later shown to be an essential micronutrient and anti-carcinogenic substance Khanal (2010) Selenium exists in two chemical forms, organic and inorganic forms the inorganic forms could be selenite, selenate, selenide and even a metallic form, while organic selenium on the other hand are found bounded to different amino acids (Methionine and cysteine), in crops like grains, forages and in oil seed meals (Khanal, 2010).

Selenium can accept or give out electrons due to its existence in basal biological pH as an anion (Abdel-Azeem, 2010). It has been identified as a component of glutathione peroxidase (anti-oxidant enzyme), deiodinases, selenophospatesynthetase (Kohrle*et al*, 2008), thioredoxinreductases and other forms of selenoproteins are yet to be identified (Khanal, 2010). The minimal lethal oral dose of sodium selenium or sodium selenite has been set at 1.5-3mg Se/Kg body weight for rabbits, rats, cats and dogs (NRC, 1983; Abdel-Azeem, 2010). Khanal, (2010) reported that when administering the oral lethal dosage range for sodium selenite as 2.3 to 13mg Se/kg body weight for rabbits, guinea pigs, mice and rats could be enhanced.

Palacios and Lobinski (2007) affirmed that selenium is an essential trace element and may have antioxidant activity in biological systems. Khanal (2010) also reported selenium to be a component of seleno-proteins and is involved in immune and neuropsychological function in the nutrition of animals. Also, it is an essential component in selenium-dependant glutathione peroxides, which helps in regulating hydrogen peroxide damage to cell membranes (NRC, 2007). Palacios and Lobinski (2007) stated that in most species the role of selenium and

vitamin E are closely linked, but in the rabbit, it is less dependent on selenium for the removal of damaging peroxides produced during metabolic processes. Dietary supplementation of 0.1 ppm selenium in does has been observed to improve foetal and birth weights, but not with 0.3 ppm as stated by (Khanal*et al*., 2008). Deficiency symptoms include muscle and liver degeneration, impaired reproduction and immunity. Organic selenium sources are more effective in increasing tissue selenium concentration than inorganic selenium (Khanal*et al*., 2008). Young animals are easily affected by toxicity of selenium than adults (El-Sheshtawy*et al.,* 2014).

2.5.2 Role of selenium in reproduction

Selenium has been known to improve the performance in growing rabbits (Zhang *et al*., 2011). Scholl and Reilly, (2002) and Shaibu, (2014) reported that deficiency of selenium negatively influences reproduction in all animal species both in the male and female animals and leads to serious dysfunction in animal reproductive processes such as loss of embryo prior to implantation, still births, hampered intrauterine growth, prematurity, retention of the placenta and poor growth during the postnatal period. The importance of selenium in animal reproduction are brought to light with the increased incidence of retained foetal membranes, increased milk somatic cell counts, clinical mastitis and calf mortality as a result to selenium deficiency; and re-supplementation of selenium in the diets was shown to remedy these conditions (Abdel-Azeem, 2010)..

(Abdel-Azeem, 2010) suggested that selenium is been considered a nutraceutical (products isolated or purified from feeds that possess physiological role and imparts protection against diseases) due to its varieties of biological responses. In humans, unexplained infertility in women has been observed with lower selenium in follicular fluid than women with infertility arising from tubal or male factors (Gruptaet *al*, 2005).

Higher rate of conception observed in several studies and countered by others (Reviewed by Hostetler *et al*, 2003) have been attributed by Abdel-Azeem (2010) to the down-stream arising remedial effect of selenium supplementation on reduced retained placenta, reduced incidence of mastitis and lesser days to insemination and conception. The dissimilarities observed in litter sizes in these studies was also attributed to factors like maternal age, timing and duration of selenium supplementation, level of selenium supplementation in the diet and the sources of selenium (Hostetler *et al*., 2003).

In gilts however, selenium supplementation was found to have no impact (Svoboda *et al*, 2009). Gamal*et al.,* (2014) stated that selenium deficiency has been linked to reproductive problems in sheep and cattle and leads to largely immotile and a high incidence of sperm mid-piece defects. In males raised on a low selenium diet, male hypogonadism was found as well as reduced production and deteriorated semen quality Supplementation with selenium has been reported to improve reproductive performance in sheep (Gamal*et al.,* 2014).

2.6 Response of Reproductive Traits in Rabbit Bucks to Selenium Supplementation

Marai*et al* (2009), conducted a study in which rams were offered diets supplemented with 0.1 mg Se/kg DM as sodium selenite during the summer period and observed positive effect on reproductive and physiological traits, such as increased sperm concentration, sperm motility and decreased sperm abnormalities and dead spermatozoa. Thus, dietary selenium seems to have enormous potential with a positive impact on the thermoregulation and reproduction in animal production (Marai*et al.*, 2009). Abdulrashid and Juniper, (2016) stated that beneficial effects of selenium has been predominantly observed in the testes, with higher activity and deposition in the nucleus and mid piece of the sperm cells and subsequent positive impact on expression of genes related to the transcription of selenoproteins. In contrast, studies in rams by Marai*et al*., (2009), have shown that selenium could either directly affects the interstitial cells of the testes or serve as a structural component of the sperm cells.

Phospholipid GSHPx formerly known as sperm capsule selenoprotein have been shown to improve structure of the sperm cells in rats, whereas Selenoprotein and thioredoxinglutathionereductase are important in spermatogenesis (Kehr*et al.*, 2009). In addition, other reports in rats indicated that Selenoprotein was discovered in interstitial cells of the testes, and therefore selenium could have a positive effect in androgen (Testosterone) production, hence may influence semen quality (Abdulrashid and Juniper, 2016). However it has been indicated that, normal function of selenoenzymes in the testes could be adversely affected by stress factors, especially when oxidative stress overwhelmed antioxidant status, and this could subsequently inactivate or rather suppress normal function of Selenoprotein (Kehr*et al.,* 2009): the main transport mechanism for selenium in the body (Saxena and Jaiswal, 2007). In addition, Kehr*et al.,* (2009) reported that elevated activity of the selenoenzymes, phospholipid glutathione peroxidase (GSHPx) and selenoproteinin rat testes when fed 1.0mg Se/kg diet.

It has been reported that testicular mitochondria are sensitive to oxidative damage in sheep (Yan *et al*., 2010) and rats (Pino*et al.*, 2013), however, it was shown that seminal plasma has some degree of protection against oxidative damage, as it contains antioxidant enzymes such as catalase, superoxide dismutase and glutathione-transferase (Maia *et al.*, 2010). The activity of these enzymes has been shown to improve in the presence of selenium. Therefore, the depletion of endogenous selenium pools, particularly in hot summer periods, could result in spermatozoa and testicular tissues (seminiferous tubules, leydig and sertoli cells) to be more susceptible to damage by reactive oxygen species (ROS), due to the fact that lipid is a basic component of testicular and spermatozoa membranes and hence more susceptible to damage by free radicals (Gundogan*et al.*, 2010). Since, peroxidative damage to spermatozoa has been shown to be one of the major causes of male subfertility (BNF, 2001), it is thus considered expedient to exploit dietary selenium.

2.7 Vitamin Requirement of Rabbits

Vitamins are divided into two categories – fat-soluble and water-soluble. B vitamins and vitamin C are water soluble; vitamins A, D, E and K are fat-soluble. Fat soluble vitamins can be stored within the body in the liver and fat deposits, and if eaten in large amounts, are not needed on a daily basis. Water soluble vitamins are not stored within the body and any unused portions are excreted via the urine. However, the majority of B vitamins are synthesized by the hindgut bacteria and consumed during caecotrophy in rabbits and thus are not necessarily required in the diet (Amy, 2010).

2.7.1 Vitamin E requirement of Rabbit Bucks

Vitamin E refers to a group of potent chain-breaking antioxidants that are soluble in lipids and fats and made up of eight compounds includingtecopherols and tocotrienols which are identified with the prefixes alpha, beta, gamma and delta attached to the compounds respectively (Amy, 2010). Vitamin E was discovered by Herbert Mclean Evans and Katherine Scot Bishop in the year 1922 (Evans and Bishop, 1922) Evans and Bishop first isolated it in a pure form at the university of California,Berkely in 1935 by Gladys Andrew Emerson (Oakes, 2007). In 1938, it was first synthesized by Paul Karrer and later that year Widen Hover first used it as a therapeutic agent to induce normal growth in premature new-born infants (Amy, 2010). Numerous works have been done in the area of vitamin E and its nutritional physiological benefits since its discovery (Amy, 2010).

The most common form of vitamin E is the gamma tecopherol found in corn oil, soybean oil, margarine, dressings (Amao*et al*., 2012) but alpha tocopherol which has the most biologically active form of vitamin E is the second common form in diets but is found abundantly in wheat germ of sunflowers, and safflower oils (Amao*et al*., 2012). By its virtue as a fat soluble vitamin\antioxidant, it limits the production of ROS during fat oxidation

(National Institute of Health, 2009). In excessive amount (over 1,000mg i.e. 1,500IU, per day) (Vitamin E fact sheet), vitamin E can cause vitamin K deficiency and increase risk of bleeding a condition called “hypervitaminosis E”.

Vitamin E requirement can be defined as the amount of vitamin E required for the preventing peroxidation in the particular sub cellular membrane which is most susceptible to per oxidation (Koyuncu and Yerlikaya, 2007). Amao*et al*., (2012) reported that Vitamin E prevents oxidative damage to sensitive membrane lipids by suppressing hydroperoxide formation and protects cellular membranes thus maintaining membrane integrity and reducing oxidative stress. Culture of bovine embryos with vitamin E enhanced development and increase the percentages of embryos developed to early and expanded blastocysts also, blastocyst qualities of porcine somatic cell nuclear transfer and *in vitro* fertilization embryos were improved when embryo culture media was supplemented with vitamin E (Olson and Seidel, 2000). It has been recommended that 15 and 50 ppm of vitamin E is adequate for fryers and does, respectively (El-Sheshtawy*et al.,* 2014). Amy, (2010) stated that further supplementation is recommended for cases of impaired immunity or coccidiosis infection and that high doses of vitamin E (>200 ppm) improved the quality of rabbit meat after slaughter. Meats from rabbits fed high doses of vitamin E had greater stability, better colour, less dripping losses and longer shelf-life than cuts in the control animals (Amy, 2010).

Vitamins play an important role in animal’s reproductive performance (El-shahat and Abdel-monem, 2011). According to Shaibu, (2014) vitamin E deficiencies in several animal species have been known to negatively affect reproduction resulting in foetal death and resorption. Since it is established that ROS compounds and consequently oxidative stress affects female fertility (Moreira da‟ Silva *et al.,*2010) reported that vitamin E through its protection of cellular membranes, reduces oxidative stress and maintains the functional integrity of

membranes (Yaseen*et al.,* 2016), it might thus be deductively said that vitamin E through its role in abating oxidative stress in animal cells may affect the fertility status of such animal.

Shaibu, (2014) reported that weaning of kits was improved with extra vitamin E inclusion in the diet of does. This was however not in line with reports by Moreira da‟ Silva *et al.,*(2010) who reported no further response to extra dosage of vitamin E in performance of pregnant and lactating does, except in improvement in their litter size at birth. The inconsistency in result with vitamin E supplementation could be partly attributed to the way of introduction (diet, oral or injection), the experimental conditions (normal and stress), the intervals of supplementation (daily or weekly), the state of does (Multiparous) and more importantly is the level of inclusion. NRC(1977) recommends a level of 40mg vitamin E/kg diet to complete the perfect metabolic activity during pregnancy and breast feeding and that a rational supra-nutritional dosage(a little above the dietary recommendation) rather than mega dosage (excessive dietary inclusion) may be more essential. In this regard a daily oral supplementation with 30-50mg vitamin E kg/doe (Moreira da‟ Silva *et al.,* 2010) or weekly injection with 56mg vitamin E kg.doe (Abdel-Kafy, 2000) was more effective than a diet which had an inordinate level of Vitamin E (160mg/kg) Ukpai*et al*., (2011) reported that vitamin E supplementation either solely or in combination with Vitamin C has a positive influence on both performance indices and gonadal weight of male rabbits.

2.8Synergistic effects of Vitamin E and Seleniumon Reproduction

Vitamin E and mineral play an important role in the growth of animal and their reproductive performance (El-Sheshtawy*et al.,* 2014). The antioxidants have been defined as substances that delay or inhibit oxidative damage to cellular molecules (El-Sheshtawy*et al.,* 2014). Vitamin E and Selenium are among the selected nutrients that have complementary biological functions as antioxidants to minimize cellular damage caused by endogenous

peroxides (El-Sheshtawy*et al.,* 2014). Vitamin Efunctions in close relationship with selenium and is involved in the prevention of cell oxidation and maintaining the immune system. Deficiencies of either selenium or vitamin E results in muscular dystrophy (destruction of muscle tissue), infertility or fetalresorption (Gamal*et al*., 2014). There is physiological synergism between selenium and vitamin E, however, previous reports have suggested that vitamin E and selenium (Se) are important nutrients that act synergistically and can affect many biological processes including spermatogenesis and semen quality (Yousef*et al.*, 2003), reproduction (Koyuncu and Yerlikaya, 2007), metabolism, immunity, and protection against oxidative stress (Gamal*et al*., 2014). The association of vitamin E deficiency with impaired male reproduction was established more than three decades ago, and traditionally it is called the "antisterility vitamin". Recommended levels are based on either old data or on extrapolation from other species (Gamal*et al*., 2014).

Selenium has a biological importance that is common to vitamin E. Selenium is an essential compound of glutathione peroxide, the enzymes involved in the detoxification of hydrogen peroxide and lipid hydro peroxides. Moreover, selenium is a composition of selenoproteins and is involved in immune and neuro-psychological functions in the nutrition of animals (El-Sheshtawy*et al.,* 2014). Selenium deficiency plays a role in many economically important livestock diseases and has been implicated in problems like impaired fertility, abortion, retained placenta and neonatal weakness (Hernández 2008).

Preconception dietary administration with a combination of vitamin E and Selenium yeast had beneficial effects on alleviation of adverse effect of heat stress on the reproductive performance of doe rabbits in terms of increasing kindling rate, litter size and weight of kits at birth (Kalaba, 2012). Kalaba (2012) also reported the existence of a synergistic action between selenium and vitamin E, and both improved synergistic antioxidant effect. The role

of vitamin E and Selenium is seen as improving animal reproduction and their requirement in the reproductive tissues was found to be of great importance (Ebeid, 2012).

Gamal*et al.,* (2014) also reported that a diet supplemented with selenium and vitamin E improved sperm quality an effect possibly linked to the antioxidant properties of this vitamin. Vitamin E and selenium are essential nutrients with complementary biological functions as antioxidants for minimizing cellular damage caused by endogenous peroxides (Ebeid, 2012). Vitamin E prevents oxidative damage to sensitive membrane lipids by suppressing hydro peroxide formation and protects cellular membranes thus maintaining membrane integrity and reducing oxidative stress (Chow, 2001).

2.9 Reactive Oxygen Species (ROS)

Reactive oxygen Species (ROS) are defined as molecules of oxygen that are chemically reactive which include oxygen ions and peroxides produced alongside energy in the mitochondria with the potential to cause tissue damage. Abdel-Khalek*et al*, (2010) reported that ROS are by-product formed from the normal oxygen metabolism in the body and they have a pronounced role in cell signalling and homeostasis. The ROS levels rises greatly due to environmental stress such as UV or heat exposure (Rada and Leto, 2008) and other exogenous sources like ionizing radiation and this generated excessive levels of ROS lead to the damage of cellular structures.

The cells of the body can protect themselves from damage with the help of enzymes such as alpha-1-microglobulin, super oxide dismutase, catalases, lacto peroxidases, glutathione peroxidases and peroxiredoxins. Antioxidants of small molecular sizes also have pronounced properties in the cells and they include ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid (the most important antioxidant in human) and glutathione (Conner *et al.*, 2002). ROS has been said to cause apoptosis (programmed cell death) but also play a beneficial role in

the establishment of host defence against microbes and viral attack (Conner *et al.,* 2002). The harmful effect of ROS in the body includes; cardiovascular disease; DNA damage at the cellular level; oxidation of polyunsaturated fatty acids in lipids (lipid peroxidation), oxidation of amino acids in proteins and oxidative inactivating specific enzymes by oxidation of co-factors (Brooker, 2011). The ROS metabolites have also been suggested to cause dramatic damage to cellular structures, protein, lipids and DNA which consequently induce physiological and pathological conditions reducing performance and welfare of life animals and the quality of meat (Abdel-Khalek*et al*, 2010). Liu *et al.* (2012) implicated high ambient temperature conditions and possible associated ROS production and cellular destruction in decreased growth performance in rabbits.

2.10 Antioxidants and Their Roles in Rabbits.

Surai, (2016) reported that antioxidants are chemical substances that limit ROS production, scavenges existing free radicals and promote the repair of cells structures damaged by ROS. Various works have been done in the area of antioxidants and their protective mechanisms against the damaging effects of ROS such as singlet oxygen, oxide, peroxyl radicals, hydroxyl radical and peroxyl-nitrates (Moreira da Silva *et al*., 2010). According to Moreira da Silva *et al*, (2010) antioxidants are classified into the enzymatic forms which are natural antioxidants which functions by neutralizing excessive ROS and limiting damage to the cellular structures.

Glutathione peroxide (GSH-Px) and catalase (CAT) are the two main enzymes in the cytosol removing peroxides (El-Medany*et al.,* 2012) and GSH-Px in the rabbit liver which is about 6-12 times that found in cattle, sheep and cat implying their roles in ROS scavenging properties of antioxidants El-Medany*et al.,* (2012). The non-enzymatic antioxidants also called synthetic antioxidants or dietary supplement include Vitamin C, Vitamin E, Selenium, Zinc, taurine, hypotaurin, glutathione, beta carotene and carotene which influence the

complex antioxidant system of the body (Pierce *et al*., 2004). Vitamin E which must be obtained through dietary means functions as antioxidant by preventing lipid peroxidation and limiting agents that break oxidative chain reactions, usually by scavenging ROS before they can cause cellular damage (Wolf, 2005). The enzymatic and non-enzymatic antioxidants scavenge the attacking radicals rapidly and terminating their destructive pathways (Moreira da Silva *et al*., 2010).

2.10.1 Vitamin E as an antioxidant

Vitamin E functions as a peroxyl radical scavenger which limits the probation of free radicals in tissues through its reaction with them to produce tocopheryl radical which is reduced and returned to its original state by a hydrogen donor such as vitamin C (Traber and Steven, 2011). Vitamin E (α-tocopherol and its derivatives) limits lipid peroxidation by breaking oxidative chain reactions and also scavenging ROS thereby preventing them from causing damage to cell (Wolf, 2005). It is the major lipid soluble antioxidant in animal cells which protects cells from oxygen radicals both in-vivo and in-vitro and is regarded as the essential free radical scavenger in mammalian cell membrane (Olson and Seidel, 2000).The DNA damaged by free radicals caused to descendant distortions due to they contain sperm membranes in high concentration of unsaturated fatty acids, which are more susceptible to damage with types Reactive Oxygen Species (Sanchez *et al.*, 2008).

Vitamin E supplementation has become a common procedure to promote growth and health and improve the qualitative characteristics of farm animals, it has been demonstrated to be an efficient strategy for improving their reproductive function and germ cells are particularly vulnerable to oxidative damage and may require additional antioxidant protection (Hernández, 2008) Also vitamin E is quite an effective antioxidant which protects rabbit testis against lipid peroxidation (Yaseen*et al.*, 2016). It is the major lipids soluble antioxidant in animal cells which protects cells from oxygen radicals both in-vivo and in-vitro and is

regarded as the essential free radical scavenger in mammalian cell membrane (Yaseen*et al.*, 2016). Vitamin E helps to scavenge free radicals and eradicates them from the body which cause DNA damage (Naseem*et al.*, 2007).

Shaibu, (2014) reported that asides from the antioxidant properties of vitamin E, it also functions in enzymatic activities, gene expression and neurological activities. It has been suggested to play a prominent role in cell signalling though may not be associated with antioxidant metabolism (Azzi, 2007). Enzymatic activity of α-tocopherol inhibits protein kinase C (PKC) plays a role in the growth of smooth muscles. Alpha-tocopherol stimulates the dephosphorylation enzymes (protein phosphatase 2A) which cleaves phosphate group from the smooth muscle (Shaibu, 2014). Other roles of vitamin E include: Protection of lipids and production of polyunsaturated fatty acids, inhibits the aggregation of platelets (Atkinson, 2008), and the maintenance of the functionality of the Nervous system (Maller, 2010).

2.11 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 (Smith and Boyer, 2008).

In the rabbit doe, ovulation does not occur spontaneously, but has to be induced through a neuro-hormonal reflex, which is initiated during coitus and occurs 10-16 hours after coitus (Hafez and Hafez, 2000). Shinkut, (2013) reported that there have been some common incidences of failure to ovulate which appears to vary with the season of the year and when using artificial insemination, in the absence of a male, in such cases ovulation has to be induced by artificial methods which include treatment with luteinizing hormone, human chorionic gonadotropin injection (HCG), copper salts or other hormonal means.

The ovulation inducing method most frequently used is an intramuscular injection of gonadotropin releasing hormone or its synthetic analogues (Theau-Clement *et al.,* 1994). 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, however, the repeated injection of these hormones can result in failure of the does to ovulate due to antibody formation (Shinkut, 2013). Gonadotropin releasing hormone can be repeatedly injected without antibody formation (Khalifa*et al.,* 2000). Vasectomized males have been used to induce ovulation in does prepared for artificial insemination (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, reported 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. 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 Gonad Development and Puberty in Rabbits

Shinkut, (2013) reported that 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 (undifferentiated 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 (Nathalie *et al*., 2013). In the male, the Mesonephric ducts (Wolffian) duct becomes the current duct that is epididymis, vas deferens and vesicular glands. Two agents produced by the foetal testis are responsible for the male differentiation and development. Foetal androgens cause development of the male reproductive tract, Mullerian inhibiting substance, a glycoprotein, is responsible for suppression of the paramesonephric (Mullerian) ducts that is atrophy (Nathalie *et al*., 2013).

In the rabbits, the gonads begin to differentiate on the 16^{th} day of foetal life (Nathalie *et al.*, 2013). After birth the testes develop less quickly than the rest of the body but the age of five weeks they begin to grow very rapidly. The 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 of the reproductive age of rabbits and the testicular tubes become active at about 84 days with the first spermatozoa present in the ejaculate at about 110 days (Smith and Boyer, 2008).

Sexual maturity, is defined as the moment when daily spermatozoa production ceases to increase and is reached at 32 weeks of age in New Zealand White rabbits in temperate climates (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 behaviour appear at days 60 to 70 of age when the rabbit makes its first attempts at riding (Rodel and Bora, 2004). Coitus may occur for the first time at about the age of 100 days, but the viability of the spermatozoa is very weak or nil in the first ejaculates, so first mating should be timed for age 135 to 140 days (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 (Rodel and Bora, 2004).

Shinkut, (2013) reported that 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).

2.13 Spermatogenesis in Rabbit Bucks

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

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 (Garner and Hafez, 2000). 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).

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 (Garner and Hafez, 2000). 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 (Garner and Hafez, 2000), 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 (Parkinson, 2009). 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 (Garner and Hafez, 2000). 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 (Garner and Hafez, 2000). 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 remnant cytoplasmic droplet, expelled from the crypt of the Sertoli cell into the lumen of the seminiferous tubule (Parkinson, 2009).

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 (Pineda, 2003). The duration of the spermatogenic cycle can be calculated by multiplying that of the seminiferous 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).

Pineda, (2003) reported considerable changes occur to spermatozoa as they pass through the epididymis. The epididymis is highly androgen-dependent, thus, if androgen levels are suppressed, epididymal function is immediately impaired (Pineda, 2003). The protoplasmic remnant, which is initially sited close behind the sperm head, migrates distally to the end of the mid-piece, 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 (Parkinson, 2009).

Similarly, in the head of epididymis, 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 zonapellucida (Parkinson, 2009).

Parkinson, (2009) reported that spermatozoa take between 8 and 10 days to traverse the epididymis in rabbits. 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 little motility, but are rapidly active upon mixing with seminal plasma during ejaculation (Parkinson, 2009).

2.14 Evaluation of Semen in Rabbit Bucks

According to Ewuolaand Akinyemi.(2017) semen evaluation as an indicator of fertility provides significant information on breeding soundness of an individual animal. Semen is semi gelatinous suspension containing a mixture of spermatozoa and secretions from

epididymis and accessory sex glands that are released during ejaculation. The standard method of determining the fertility of a breeding male is by examination of semen through evaluation analysis (Hafez and Hafez, 2000). These authors further stated that, semen evaluation should be rapid and effective so that ejaculate samples are processed to preserve original quality and fertility (Ewuolaand Akinyemi,2017). Therefore, sensitive traits such as the motility test should be determined immediately, devoid of exposure to sudden temperature changes and mechanical damage to sperm cells during the making of smears.

According to Boiti*et al.,* (2005) Standard reference values of fresh rabbit semen are: Concentration (250-600 $x10^6$/ml), progressive motility (30-90%), volume (0.3-0.9ml) and pH (7.1). It has been established that one type of test may not provide an accurate assessment of ejaculate fertility, but when a variety of tests are carefully carried out and results combined, ejaculates with higher fertility potential could be identified (Seidel, Jr, 2012). Literally tests for fertility of male rabbits could be conducted in two major categories; Gross examination (visual examination) and microscopic examination.

Gross examination includes colour, volume and pH of the ejaculate, whilst microscopic examination would include assessments of sperm concentration, motility, live/dead and morphology. Since the duration of spermatogenesis in rabbits is about seven weeks, a minimum period of eight weeks is required for any exogenous factor to effect changes in the ejaculates, as such maximum period of ten weeks is recommended to determine the effect of external factors. Semen characteristics are influenced by the degree of sexual stimulation, frequency of ejaculation, age, testicular size and method of semen collection (Seidal, jr, 2012).

Season of the year and or temperatures also influences the physical and biochemical characteristics of semen, as well as blood hormone levels, sexual behaviour and fertility of

animals (Okab, 2007). Quality of semen that is acceptable as a standard, should be of such that the volume of ejaculate is 2mls with a colour ranging from milky to creamy. It should contain no foreign material such as hairs, debris or dirt and the percentage of progressively motile cells should exceed 50% and incidence of primary abnormalities should not exceed 10%. Some factors affects semen quality especially nutrition; nutrition is a modulator of reproductive function (Elmaz*et al*., 2007). It influences testicular growth, sperm production capacity and fertility, particularly in the tropics where there is seasonality in quantity and quality of feed. Under nutritional stress, protein supplementation decrease age at puberty of bulls and improve semen quality (Rekwot*et al*., 1987).

2.15 Haematology and Serum Biochemical Analysis

The assessment of the nutritional and health status of animal can be made by determining the concentration of certain blood metabolite such as non-esterified fatty acids (NEFA), βhydroxybutyrate (BHB), glucose and cholesterol (Ndlovu*et al.,* 2007). These metabolite concentrations indicate the extent of the metabolism of energy, proteins and other nutrients in the animals (Agenas*et al.,* 2006). The changes in the circulating nutrient-sensitive metabolite concentrations are important signals of the metabolic status of the animal and the organs function (Wettemann*et al.,* 2003). Other blood metabolites, such as total protein, globulin and albumin, serve as indicators of the protein status. Factors including the physiological status of an animal, health status, breed, nutrition, season and age may affect the concentration of these metabolites in the blood (Ndlovu*et al.,* 2007).

Haematological examination has been performed for a variety of reasons: as a screening procedure to examine general health of the animal, for assessing the nutritional status of the animal, as adjust to a patient‟s infection and to evaluate the progress of certain disease conditions (Friday *et al.,* 2014) and distinguish between normal and stress conditions

(Olabanji*et al.,* 2007). Haematological values provide baseline information for comparison in conditions of nutrient deficiency, physiology and health status of farm animals (NseAbasi*et al.,* 2014) and help in providing information on the relationship between blood characteristics and the environment (Isaac *et al.*,2013).

Packed cell volume or haematocrit is the percentage of the blood volume made up by erythrocytes. A decrease in haematocrit is anaemia, more specifically, anaemia can be a reduction in the numbers of red blood cells, the volume of red blood cells or the concentration of haemoglobin. Haemoglobin is the predominant protein in blood. Haemoglobin measurement are used as an estimate of the total red cell mass in an animal. (NseAbasi Et Al., 2014)

Red blood cells are the most numerous and longest lived of the circulating blood cells, it contains haemoglobin and are critical in tissue oxygenation and blood acid-base balance. The white blood cell count is the total number of white blood cells contained in a cubic millimetre or microliter of anticoagulated whole blood. Neutrophil, lymphocytes, monophils, eosinophils and basophil are components of the white blood cells and majorly they act as defence against infection or invader into the body. Blood glucose levels are performed to determine if the animal's blood glucose is in the normal range. Total protein test measures the combined amount of globulin and albumin in the blood; it gives information about kidney damage, liver damage and nutritional health of the animal (Isaac *et al.*,2013).

Albumin is a small protein made in the liver that constitutes the major protein in the blood serum. Albumin performs many functions in the body this includes nourishing tissues, transporting various substances through the body (hormones, vitamins, drugs and ions), and preventing blood fluid from leaking out of the blood vessels. Globulins are a group of proteins within the blood. They are produced by the liver and the immune system, globulins

have multiple different functions; the group includes immunoglobulins, enzymes, carrier proteins and complement (Isaac *et al.*, 2013).

Alkaline phosphatase is an enzyme- a protein that helps to bring about chemical reactions in the body, found mainly in the liver and bones. High levels of ALP in the blood may indicate bone or liver abnormalities. Alanine amino transferase is another enzyme found mainly in the liver, kidney, heart and muscles. High ALT may indicate acute hepatitis and aspartate amino transferase is an enzyme found mainly in the liver, heart and muscles. AST is released into the blood by injured liver or muscles cells but is used primarily to detect liver damage (Friday *et al.*, 2014).

Table 2.17: Normal Ranges of haematology and biochemistry parameters of rabbits

Parameters	**Ranges**
Haematological component	
Haemoglobin (g/dl)	8.0-17.5
Packed cell volume/ Haematocrit (%)	30.0-50.0
Red blood cell/ Erythrocytes(x 10^{12}/µl)	4.0-8.0
Mean corpuscular volume (FI)	58-67
Mean corpuscular haemoglobin (x 10g/ L)	29.0-37.0
Platelets (x 10^{3}/ µl)	112.0-795.0
White blood cell/ Leukocytes (x 10^{9}/µl)	5.0-16.5
Different count	
Neutrophil (%)	20.0-75.0
Lymphocytes (%)	25.0-85.0
Eosinophil (%)	1.0-5.0
Monocytes (%)	1.0-4.0
Biochemical components	
Cholesterol (mmol/L)	0.3-2.1
Total protein (g/L)	54-83
Albumin (g/L)	24-46
Globulin (g/L)	15-35
Alkaline phosphatase (IU/L)	17.0-192.0
Alanine aminotransferase (IU/L)	48.0-80.0
Aspartate aminotransferase (IU/L)	14.0-113.0

Adapted: Ahamefule*et al* (2008); Gambo*et al* (2011); Merck (2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The study was conducted at the Rabbit Research Unit of National Animal Production Research Institute (NAPRI), Shika, Zaria. Shika lies within the Northern Guinea Savannah Zone of Nigeria and is located on Latitude 11^0 12‟N and Longitude 7^0 33‟ E at an altitude of 691 m above sea level. Annual rainfall range is between 1100-1200 mm, while mean temperature is about 24.4 ^{0}C (14.5-39.3 ^{0}C). The lowest temperature occurs during the early dry season (November-January), while, the highest temperatures are experienced during late dry season between February-April. (Ovimaps, 2015)

3.2 Source and Processing of Test Ingredients

The concentrate was compounded at Zaria Feed Mill (Rock Seed Feed Mill), feed grade vitamin E and selenium were sourced from Hi Nutrient Limited Lagos and was incorporated into the compounded feed.

3.3 Experimental Design, Management of Experimental Animal and Data Collection

Two studies were carried out with a total of 40 mixed breed rabbit bucks aged 6-8 weeks and an average weight of 808 grams were used in both study. The rabbits were purchased from National Veterinary Research Institute Vom. Rabbits were weighed with a (weighing balance-hena :20kg- The big boss, China) at the onset of the experiment and allocated to two dietary groups in a Completely Randomized Design (CRD). Each diet had four levels with 5 rabbits allocated to each level. That gave a total of 20 rabbit per dietary group 0, 20, 40 and 60mg/kg Vitamin E and 0. 0.2, 0.4 and 0.6mg/kg of Selenium.

Five rabbits were housed in a metal cage, measuring 60x60 cm in dimension and well ventilated. Each cage was equipped with three round bottom earthen pot, two for feed and one to serve as drinker. The rabbits were administered an anti-stress (glucose via water), antibiotics (Tetracin; Vetinida pharmaceutical Limited India), and were treated against external and internal parasites using coccidiostat (Amprolium: Lonye Technology Limited Guangzhou, China) and Ivomec (Savorite Pharmaceutical Limited Baroda (Guj), India) during the two-week adjustment period. The rabbits were fed 200g of concentrate daily, forages and water given *ad libitum*. At week 6, the rabbits were separated into two per cage and at week 8 they were separated and housed individually in a cage. The rabbits were weighed weekly. The current weight was subtracted from previous weights to obtain weekly weight gain. The weekly intake and weight gains were used to compute the feed-to-gain ratio. Mortality was recorded during the experiment period.

3.4 Experimental Diets

The percentage composition of concentrate composition of experimental diets is presented in Table 3.1. Concentrate of known quantity (200g) were offered at morning and night *adlibitum*, besides dry forage (groundnut haulms) which was offered *adlibitum* to all rabbits. The forage was withheld so that the rabbit would consume much of the concentrate, thereafter consume less of the forage. Hence, experimental animals were given clean potable water at all times. All animals were managed in accordance with standard routine management procedure. Feed intake (FI) and body weight (BW) were recorded weekly in grams and kilograms, respectively. The inclusion levels were experiment one: 0 mg/kg Vitamin E, 20 mg/kg Vitamin E, 40 mg/kg Vitamin E and 60 mg/kg Vitamin and experiment two: 0 mg/kg Selenium, 0.2 mg/kg Selenium, 0.4 mg/kg Selenium and 0.6 mg/kg Selenium**.**

Table 3.1: Percentage Composition of the Experimental Diet Fed to Rabbit Bucks

	Inclusion levels of Vitamin E mg/kg			
Ingredients	0	20	40	60
Maize	16.00	16.00	16.00	16.00
Maize offal	43.00	43.00	43.00	43.00
Brewers dried grain	6.50	6.50	6.50	6.50
Groundnut seed cake	8.00	8.00	8.00	8.00
Soya cake	11.70	11.70	11.70	11.70
Rice offal	10.90	10.90	10.90	10.90
Limestone	1.20	1.20	1.20	1.20
Bone meal	2.00	2.00	2.00	2.00
Common salt	0.25	0.25	0.25	0.25
Biomix premix	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Calculated analysis	100	100	100	100
Crude Protein	15.05	15.05	15.05	15.05
Metabolizable Energy/kcal	2701	2701	2701	2701
Ether Extract	5.98	5.98	5.98	5.98
Crude fibre	11.28	11.28	11.28	11.28
Calcium	0.93	0.93	0.93	0.93
Available Phosphorus	0.32	0.32	0.32	0.32
Lysine	0.76	0.76	0.76	0.76
Methionine	0.30	0.30	0.30	0.30
Ash	3.22	3.22	3.22	3.22
Cysteine	0.22	0.22	0.22	0.22

***Biomix premix supplied per kg of diet: Vit.A, 10,000 iu; vit D_3, 2000 iu; vit E, 23 mg; vit.k, 2mg, vit B_1, 1.8; vit B_2, 5.5 mg; Niacin, 27.5mg; pantothenic acid,7.5mg; vit B_{12}, 0.015mg: Folic acid, 0.75mg; Biotin, 0.06mg; chloride, 300mg; cobalt, 0.2; Copper, 3mg; Iodine 1mg; Iron, 20 mg; Manganese, 40 mg; selenium, 0.2 mg; Zinc, 30 mg; Antioxidant, 1.25mg.(Manafactured by: Bioorganics Nutrient System Limited, IbafoOgun State, Nigeria*

3.5 Semen Collection

An artificial vagina (AV) suitable for use with breeding rabbits was assembled using plastic cone (main body), an elastic tissue (rubber latex/middle finger of the hand gloves) and rubber band (for holding/ tighten). The elastic tissue serves as inner rubber lining and contained liquid-glycerol (organic solvent and non-toxic), for cushioning and heat conservation. All materials needed for the exercise were sanitized using disinfectant, such as Dettol™. A mature weight rabbit buck produce sperm at maximum potential, since puberty is attained. Semen were collected using calibrated centrifuge tubes (graduated transparent test tubes), at four-week interval representing monthly records. The same animals were always used for semen collection. Prior to semen collection the temperature of the assembled AV was maintained at 40-42^{0}C by dipping into a beaker of warm water (45-55^{0}C). Lubrication of the inner sleeve was done immediately, using lubricating Jelly (Vaseline) (Zemjanis 1970).

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 (Zemjanis 1970).

3.5.1 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.

3.5.1.1 Volume

The volume of semen was measured directly from the calibrated tube used for the Collection.

3.5.1.2 Semen pH

This was determined by dipping a litmus paper into the ejaculate and corresponding colour changes were observed and recorded.

3.5.1.3 Gross motility

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

3.5.1.4 Spermatozoa concentration

This was determined using Neubauer haemocytometer (Model: 2080305, made by HEINZ Germany)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 (Binocular Microscope Model 500 by 3B Scientific) 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 × 10^6 (Azawi and Ismaeel, 2012).

3.5.1.5 Percentage live sperm cells

This was determined using the procedure 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 (Binocular Microscope Model 500 by 3B Scientific) and percentage of each estimated (Estesoet *al*., 2006).

3.5.1.6 Sperm abnormalities

This was determined by making a thin smear of the semen sample, on clean grease-free glass slide and stained with eosin-nigrosine. 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*., 1987).

3.6 Blood Sampling Procedure

Blood samples were taken from the marginal ear vein via venepuncture from three individual bucks, selected randomly from each treatment group, at twelfth week. The same animals were used for blood chemistry and haematology analysis. Prior to sampling the ear vein was palpated and then pierced with a needle (23GXII / 4: Jorita Jet- China). Samples for haematology were taken in tubes treated with either disodium salt of ethylene diamine tetra-acetic acid (EDTA) as anticoagulant which is used for haematological samples. Samples for routine biochemistry were taken in tubes that contained no anticoagulant.

Two millilitres (2 ml) of blood samples were collected through the marginal ear venepuncture using 25 gauge hypodermic needle. The blood samples were collected into ethylene diamine tetra acetic acid (EDTA) impregnated sample bottles which served as anticoagulant. This was carried out on each group on the twelfth week of the experiment.

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 Neubauerhaematocytometer (Model: 2080305, made by HEINZ Germany). (Friday *et al.*, 2014).

3.7 Serum Enzymes Assay

The sera gotten from the blood collected for haematology were thawed.Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Glutathione peroxidase 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.

Glutathione Peroxidase Assay: The serum sample was diluted with cumenehydroperoxide and added to 10 Standard wells set in the microtiter coated plate. Standard dilution with 100 µl and 50 µl was added to the first and the second well respectively and then mixed. From the first and second well, 100 µl was taken out and poured into the third and the forth well separately. Standard dilution with 50 µl was added to the third and the forth well and then mixed after which 50 µl was withdrawn separately and discarded. From the third and fourth well, 50 µl was separately withdrawn and added to the fifth and the sixth well. Standard dilution with 50 µl was added to the fifth and the sixth well and mixed after which 50 µl was withdrawn from them and added to the seventh and the eighth well. Standard dilution with 50 µl was added to the seventh and eighth well and mixed after which 50 µl was withdrawn from them and added to the ninth and the tenth well. Standard dilution with 50 µl was added to the ninth and the tenth well and mixed; 50 µl was withdrawn from them and discarded.

After dilution, 50 µl was kept in each well. Blank wells was set separately and Sample dilution with 40 µl as well as sample with 10 µl was added to the sample well then mixed gently. The closing plate with Closure plate membrane was incubated for 30 minutes at 37°C. The 30-fold wash solution was diluted with distilled water and reserved. The incubation mixture was removed by aspirating contents of the plate into a sink or waste container. Using a squirt bottle, each well was filled completely with wash solution and then the contents of the plate was aspirated into the sink. This procedure was repeated four times for a total of five washes. The plate was inverted and blotted after final wash.

Enzyme Conjugate reagent with 50 µl was added to each well, except the blank well and incubated for 30 minutes at 37°C after which it was manually washed. Substrate A and Substrate B with 50 µl was added to each well, covered and incubated for 15 minutes at 37°C. Stop Solution with 50 µl was added to each well and thoroughly mixed. The optical density of each well was determined within 15mins by a microplate reader. The analysis was carried out at Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria.

Alkaline Phosphatase (ALP), Alanine Transaminase (ALT) and Aspartate Transaminase

(AST) Assay: In a cuvette, 10 µl of sample was mixed with 500 µl of the reagent. The initial absorbance was read at 405 nm and subsequently over 3 minutes. The mean absorbance per minute was used in the calculation: ALP, ALT and AST activities (IU/l) =

2742 × ΔA 405 nm/min; Where: 2742 = Extinction coefficient; ΔA 405 nm/min = change in absorbance per minute for the homogenate sample.

Plasma Vitamin E Analysis: Anticoagulated blood with 0.5 ml was added to 10 ml of the solvent. The mixture was allowed to stand for 30 minutes before extraction. The mixture was then filtered and the filtrate was poured into 10 ml corvette which in turn separated the

vitamins into its various concentrations read on UV 2550 Spectrophotometer within 5 seconds (Chou*et al*.1985).

3.8 Statistical Analyses

All data collected were analysed using General Linear Model Procedure of SAS, (2008). Significant differences among means were compared using Dunnet‟s test/ least significance difference (Dunnet, 1964).

The following model was used for the analysis of productive, haematological and serum biochemistry as well as reproductive parameters:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = the observation on the j^{th} buck in Vitamin E or Selenium levels;

μ = overall mean;

T_i = Effect of j^{th} Vitamin E or selenium levels; e_{ij} = random error (All error terms were assumed to be random, normally distributed and independent with expectation equal to zero).

CHAPTER FOUR

4.0 RESULTS

4.1 EXPERIMENT 1: EFFECT OF GRADED LEVELS OF VITAMIN E ON GROWTH PERFORMANCE, BLOOD PROFILE AND SEMEN QUALITY OF RABBIT BUCKS.

4.1.1 Effect of Dietary Vitamin E on Growth Performance of Rabbit Bucks

Table 4.1 shows the result of the growth performance of rabbits fed different levels of vitamin E. There was significant ($P<0.05$) difference in final weight (1403.30 to 1870.00g),average daily weight gain (6.04 to 11.20g) and feed conversion ratio (7.74 to 12.17%) for rabbit bucks fed diets with 20 mg vitamin E/kg while average daily feed intake (73.52 to 87.86g) was significantly ($P<0.05$) higher at 0 mg vitamin E/kg.

4.1.2 Effect of Vitamin E on Blood profile of Rabbit Bucks fed with Vitamin E Supplements

Table 4.2 shows the blood profile of rabbit bucks fed vitamin E supplements at different levels. Packed cell volume (27.00 to 40.00%) and haemoglobin (9.10 to 13.20g/dl) were significantly ($P<0.05$) higher for rabbits fed 40 and 60 mg vitamin E/kg diets respectively. Red blood cell count (4.70 to 7.00 $x10^9$) was significantly ($P<0.05$) higher for rabbits fed 20, 40 and 60 mg vitamin E/kg diets respectively. There was no significant ($P>0.05$) difference in white blood cells, neutrophils, lymphocytes, monophils, and eosinophils across treatments.

4.1.3 Effect of Vitamin E on Serum Biochemical Profile of Rabbit Bucks.

Table 4.3 shows the effect of vitamin E on serum biochemical profile of rabbit bucks. There was no significant ($P>0.05$) difference on glucose, total protein, albumin, aspartate transaminase, alanine transaminase and alkaline phosphatase across treatments. The globulin concentration was significantly ($P<0.05$) higher at 20 and 40 mg vitamin E/kg inclusion levels then at 0, 20 and 60 mg vitamin E/kginclusion, which were statistically similar ($P>0.05$). Glutathione peroxidase was significantly($P<0.05$) higher at inclusion levels of 20, 40 and 60 mg vitamin E/kg but statistically ($P>0.05$) similar at 0, 20 and 60 mg vitamin

E/kginclusion level. Vitamin E concentration of rabbit bucks fed 60 mg vitamin E/kg of the feed was significantly (P<0.05) higher compared to other treatments.

4.1.4. Effect of Vitamin E on Semen Quality of Rabbit Bucks.

Table 4.4 shows the effect of vitamin E inclusion on semen quality of rabbit bucks fed different levels of vitamin E. Semen volume and pH were not significantly

(P>0.05) different. However, sperm motility and sperm concentration were significantly (P<0.05) had highest for rabbit bucks fed 60 mg vitamin E/kg of the feed while at 20 and 40 mg vitamin E/kg they were (P>0.05) similar values. Live % sperm at inclusion rate of 60 mg vitamin E/kg was significantly (P<0.05) higher at 60 mg/ kg vitamin E diet but at 0, 20 and 40 mg vitamin E/kg the live % was statistically (P>0.05) similar. The dead % of bucks‟ sperm observed was significantly (P<0.05) higher for bucks on 60 mg/kg vitamin E diet as compared to other inclusion levels. Inclusion of 60 mg vitamin E/kg gave the best results for sperm motility, sperm concentration, live and dead sperm.

4.1.5 Effect of Vitamin E on Spermatozoa Morphology of Rabbit Bucks

Table 4.5 shows the effect of vitamin E inclusion on sperm morphology of rabbit bucks spermatozoa fed different levels of vitamin E. The result showed that normal cells and detached head were not significantly (P>0.05) different. However, the percentage free tail, coiled tail and bent tail was significantly (P<0.05) higher in rabbits fed 20 and 60 mg vitamin E/kg in diets.

Table 4.1: Growth Performance of Rabbit Bucks fed Diets with Varying Levels of Vitamin E

Parameters	Inclusion Levels of Vitamin E (mg/kg diet)				SEM	P value
	0	20	40	60		
Initial weight (g)	861.00	862.00	860.00	860.00	0.58	0.11
Final weight (g)	1805.00[b]	1870.00[a]	1792.50[c]	1403.30[d]	0.41	<0.01
Average daily feed intake (g)	87.86[a]	86.73[b]	85.88[c]	73.52[d]	0.01	<0.01
Average daily gain (g)	10.49[b]	11.20[a]	10.36[b]	6.04[c]	0.03	<0.01
Feed conversion ratio	8.37[c]	7.74[a]	8.29[b]	12.17[d]	0.01	<0.01

[abcd]: Means with different superscripts in the same row are significantly (P<0.05) different

Table 4.2: Blood Profile of Rabbit Bucks fed Varying Levels of Vitamin E Inclusion

	Inclusion levels Vitamin E (mg/kg diet)					
Parameters	0	20	40	60	SEM	P value
Packed cell volume (%)	27.00^{c}	33.67^{b}	40.00^{a}	37.33^{ab}	1.43	0.00
Haemoglobin (g/dl)	9.10^{c}	11.30^{b}	13.20^{a}	12.33^{ab}	0.46	0.00
White blood cells ($x10^{9}$)	9.07	9.00	7.93	6.67	1.72	0.74
Red blood cells ($x10^{12}$)	4.70^{b}	6.17^{a}	7.00^{a}	6.47^{a}	0.31	0.00
Neutrophil (%)	15.67	15.00	8.67	13.67	2.45	0.25
Lymphocytes (%)	74.67	82.00	88.33	81.33	3.08	0.08
Monophils (%)	2.00	0.67	0.67	1.00	1.07	0.79
Eosinophils (%)	2.00	0.00	1.33	0.00	0.53	0.07
Basophils (%)	0.00	0.00	0.00	0.00	0.00	-

^{ab}: Means with different superscripts in the same row are significantly ($P<0.05$) different

Table 4.3: Serum Biochemical Profile of Rabbit Bucks fed Diets with varying levels of Vitamin E

	Inclusion Levels Vitamin E (mg/kg diet)					
Parameters	0	20	40	60	SEM	P value
Glucose (mmol/l)	3.30	3.03	2.63	3.10	0.39	0.63
Total Protein (g/dl)	65.67	68.00	65.33	60.00	3.76	0.47
Albumin (g/l)	34.00	28.33	21.33	28.00	3.33	0.11
Globulin (g/l)	31.67^{b}	39.67^{ab}	44.00^{a}	32.00^{b}	2.75	0.02
Aspartate transaminase (IU/l)	22.67	20.00	49.33	25.00	8.01	0.08
Alanine transaminase (IU/l)	26.00	18.33	38.00	23.00	7.54	0.31
Alkaline Phosphatase (IU/l)	168.67	217.33	232.00	118.00	27.13	0.06
Glutathione peroxidase (µmol/l)	3.95^{b}	8.94^{ab}	10.73^{a}	5.13^{ab}	1.74	0.05
Vitamin E (mmol/l)	0.63 ^{c}	2.00 ^{c}	7.79 ^{b}	17.18 ^{a}	0.96	<0.01

^{abc}: Means with different superscripts in the same row are significantly (P<0.05) different

Table 4.4: Semen Characteristics of Rabbit Bucks fed Varying levels of Vitamin E

	Inclusion levels of Vitamin E (mg/kg) diet					
Parameters	0	20	40	60	SEM	P value
Semen Volume (ml)	0.49	0.56	0.63	0.90	0.09	0.07
Sperm Motility (%)	68.33^{b}	56.67^{c}	62.78^{bc}	87.50^{a}	3.19	0.00
pH	7.87	7.00	7.22	6.75	0.47	0.43
Sperm Concentration ($x10^{6}$)	121^{b}	72^{c}	89^{bc}	232^{a}	9.70	<0.01
Live Sperm (%)	59.17^{c}	66.67^{b}	70.17^{b}	87.50^{a}	4.56	0.03
Dead Sperm(%)	40.83^{c}	33.33^{bc}	29.83^{b}	12.50^{a}	4.12	0.00

^{abc}: Means with different superscripts in the same row are significantly (P<0.05) different

Table 4.5: Sperm Morphology of Rabbit Buck Spermatozoa fed Vitamin E

	Inclusion levels of Vitamin E (mg/kg) diet					
Parameters (%)	0	20	40	60	SEM	P value
Normal cells	78.43	92.50	78.50	91.50	6.96	0.19
Detached head	5.83	2.44	5.67	4.00	1.22	0.26
Free tail	4.47^{c}	1.11^{a}	7.22^{d}	2.25^{b}	1.02	0.01
Coiled tail	4.98^{a}	0.67^{b}	4.17^{a}	1.25^{b}	0.37	0.00
Bent tail	5.29^{a}	2.28^{b}	4.44^{a}	1.00^{b}	0.39	0.00
Mid piece defect.	0.00	0.00	0.00	0.00	0.00	-

^{abcd}: Means with different superscripts in the same row are significantly (P<0.05) different

4.2 EXPERIMENT 2: EFFECTS OF SELENIUM SUPPLEMENTATION ON GROWTH, BLOOD PROFILEAND SEMEN QUALITY OF RABBIT BUCKS.

4.2.1 Effect of Dietary Selenium on Growth Performance of Rabbit Bucks

Table 4.6 shows the result of the growth performance of rabbits fed different levels of selenium. There was significant (P<0.05) difference in final weight (1750.00 to 2052.50g), average daily feed intake (72.33 to 96.72g) and average daily weight gain (11.06 to 14.40), for rabbit bucks fed diets with 0.4 mg selenium/kg diet while feed conversion ratio (6.27 to 6.72%)was significantly (P<0.05) higher at 0 mg selenium/kg inclusion rate.

4.2.2 The Effect of Selenium on Blood profile of Rabbit Bucks fed Selenium Supplementation

Table 4.7 shows the effect of selenium on blood parameters of rabbit bucks fed different levels of selenium. Packed cell volume had highest result at 0.2 and 0.4 mg selenium/kg inclusion level followed by 0 mg/kg selenium while 0.6 mg selenium/kg inclusion level had least values. White blood cell count was significantly (P<0.05) higher at 0 and 0.2 mg selenium/kg but statistically (P>0.05) similar at 0.2, 0.4 and 0.6 mg selenium/kg inclusion levels. It was observed that lymphocyte count for rabbit bucks on 0 and 0.6 mg/kg was significantly (P<0.05) higher than that of rabbit bucks on 0.2 and 0.4 mg selenium/kg which were both statistically (P>0.05) similar. However, haemoglobin, red blood cells, neutrophils, monocytes and eosinophils were similar (P>0.05) for all treatment.

4.2.3 Effect of Selenium on Serum Biochemical Profile of Rabbit Bucks.

Table 4.8 shows the result of the effect of selenium on serum biochemical profile of rabbit bucks. The glucose, total protein, albumin, globulin, alanine transaminase and Glutathione peroxidase (GSH-Px) showed no effects. Aspartate transaminase was significantly (P<0.05) higher at 0.2 mg selenium/kg inclusion level in the diets while alkaline phosphatase at 0.2 and 0.6 mg selenium/kg inclusion level was significantly (P<0.05) higher.

4.2.4 Effect of Selenium Inclusion on Semen Quality of Rabbit Bucks

Table 4.9 shows the result of the effect of selenium inclusion on semen quality of rabbit bucks. Semen volume showed highest result at 0.2 mg selenium/kg inclusion rate in the diet while other levels of inclusion indicated same effect. Sperm motility, pH and dead ratio showed no effect of selenium inclusion levels.Sperm concentration and live percentage sperm was significantly ($P<0.05$) highest at 0 and 0.2 mg selenium/kg inclusion rate in the diets.

4.2.5 Effect of Selenium on Sperm Morphology of Rabbit Bucks

Table 4.10 shows the result of the effect of selenium inclusion on sperm morphology of rabbit bucks. Normal cells, detached head, free tail and coiled tail were statistically ($P>0.05$) similar at 0, 0.2 and 0.4 mg/kg. Bent tail was significantly ($P<0.05$) higher at inclusion rate of 0.6 mg selenium/kg in the diets.

Table 4.6: Growth Performance of Rabbit Bucks fed Selenium Supplementation

Parameters	Selenium Inclusion Levels (mg/kg diet)					
	0	0.2	0.4	0.6	SEM	P values
Initial weight (g)	756.00	755.00	756.00	755.00	0.50	0.22
Final weight (g)	1905.00[c]	1750.00[d]	2052.50[a]	1960.30[b]	0.41	<.01
Average daily feed intake (g)	80.02[c]	72.33[d]	96.72[a]	89.64[b]	0.01	<.01
Average daily gain (g)	12.77[c]	11.06[d]	14.40[a]	13.39[b]	0.03	<.01
Feed conversion ratio	6.27[a]	6.63[b]	6.72[d]	6.69[c]	0.01	<.01

[abcd]: Means with different superscripts in the same row are significantly (P<0.05) different

Table 4.7: Haematological Parameters of Rabbit Bucks fed Selenium Supplementation

	Selenium Inclusion Levels (mg/kg diet)					
Parameters	0	0.2	0.4	0.6	SEM	P value
Packed cell volume (%)	37.67^{ab}	42.00^{a}	41.00^{a}	34.00^{b}	1.72	0.04
Haemoglobin (g/dl)	12.77	14.00	13.60	11.33	0.63	0.07
White blood cells($x10^{9}$)	10.63^{a}	7.13^{ab}	6.00^{b}	4.73^{b}	1.14	0.03
Red blood cells ($x10^{12}$)	6.53	7.17	6.90	5.80	0.41	0.18
Neutrophil (%)	10.00	16.67	10.00	12.67	1.62	0.06
Lymphocytes (%)	85.00^{a}	74.67^{b}	75.00^{b}	83.33^{a}	2.49	0.04
Monophils (%)	2.33	2.33	2.00	0.67	1.44	0.82
Eosinophils (%)	0.00	2.67	1.00	0.00	0.78	0.13
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00

^{ab}: Means with different superscripts in the same row are significantly ($P<0.05$) different

Table 4.8: **Serum Biochemical Profile of Rabbit Bucks fed Selenium Supplemental Diets**

	Selenium inclusion levels (mg/kg diet)					
Parameters	0	0.2	0.4 0.6	SEM	P value	
Glucose (mmol/l)	2.40	2.77	3.67	2.47	0.69	0.58
Total protein (g/dl)	60.00	56.67	67.33	56.67	5.94	0.57
Albumin (g/l)	28.00	29.33	39.67	26.00	3.76	0.12
Globulin (g/l)	32.00	27.33	27.67	30.67	2.75	0.59
Aspartate transaminase (IU/l)	16.00^{b}	39.33^{a}	22.00^{b}	23.00^{b}	2.26	0.01
Alanine transaminase (IU/l)	12.00	17.67	15.33	11.33	3.42	0.55
Alkaline Phosphatase (IU/l)	102.00^{c}	286.00^{a}	213.33^{b}	236.00^{b}	21.77	0.01
Glutathione peroxidase (µmol/l)	4.62	4.01	3.52	5.26	0.86	0.55

^{abc}: Means with different superscripts in the same row are significantly ($P<0.05$) different

Table 4.9: Semen Characteristics of Rabbit Buck fed Selenium

Parameters	Selenium Inclusion Levels (mg/kg diet) 0	0.2	0.4	0.6	SEM	P value
Semen Volume (ml)	0.83[b]	1.55[a]	0.64[b]	0.35[b]	0.19	0.01
Sperm Motility (%)	75.00	80.63	68.13	47.29	7.78	0.07
pH	7.00	7.13	7.00	7.08	0.15	0.92
Sperm Concentration.($x10^6$)	182[a]	176 [a]	68[b]	42[b]	21.63	0.00
Live (%)	60.00[c]	83.13[a]	69.25[b]	59.83[b]	7.16	0.08
Dead (%)	40.00[c]	16.87[a]	30.75[b]	40.17[c]	9.53	0.28

[ab]: Means with different superscripts in the same row are significantly (P<0.05) different

Table 4.10: Semen Morphology of Rabbit Bucks fed Selenium Supplementation

	Selenium inclusion levels (mg/kg diet)					
Parameters (%)	0	0.2	0.4	0.6	SEM	P value
Normal cells	86.77	85.64	86.68	79.41	7.09	0.11
Detached head	4.42	3.48	5.11	6.63	0.87	0.12
Free tail	2.58	4.75	2.95	4.39	0.84	0.26
Coiled tail	3.06	3.25	2.88	4.36	0.68	0.47
Bent tail	3.17^{b}	2.88^{b}	2.38^{b}	5.21^{a}	0.40	0.01
Midpiece defect	0.00	0.00	0.00	0.00	0.00	-

[ab]: Means with different superscripts in the same row are significantly (P<0.05) different

CHAPTER FIVE

5.0 DISCUSSION

5.1 EXPERIMENT 1: Effect of Vitamin E on Growth Performance, Blood Profileand Semen Quality of Rabbit Bucks.

5.1.1 Effect of Vitamin E Supplementation on Growth Performance of Rabbit Bucks.

Final weight, average daily gain and feed conversion ratio showed significant differences when 20 mg/kg vitamin E was included in the diet of rabbit bucks. The results in this study agrees with the findings of Amao*et al*. (2012) who recorded high final body weight (2015g) andaverage daily gain of (18.96g) in rabbits fed 30 mg vitamin E/kg supplementation.The average daily feed intake in this study increased at 20 mg/kg vitamin E inclusionwhich showed that growing rabbit performed better possible reasons are the natural antioxidants can protect intestinal mucosa against oxidative damage and pathogens and limit peristaltic activity in digestive disorders preventing diarrhoea Ebeid*et al*. (2013).The final weightresults in this study islow compared to the findings of Amao*et al*. (2012). This could be attributed to the level of inclusion of vitamin E and some environmental factors where the animals were raised such as temperature changes cold and hot weather.

5.1.2Effect of Vitamin E Supplementation on Haematological Parameters of Rabbit Bucks

Packed cell volume, haemoglobin and red blood cell count increased when 40 mg/kg vitamin E was included in the diet. This result is similar to the findings of Abdel-khalek*et al*. (2010) who reported an increased packed cell volume, haemoglobin and red blood cells in both pregnant and lactating rabbit does fed 40 mg dietary vitamin E/kg DM. However, Shaibu (2014) reported no significant difference in packed cell volume, haemoglobin and red blood cell in rabbit does on 40 mg/kg vitamin E supplementation. The values obtained in this study is within the reference range for rabbits: Packed cell

volume (35-50%), haemoglobin (9.4-14.9g/dl) and red blood cell count (4.8-7.4x10^9) (Black *et al*., 2009).

5.1.3 Effect of Vitamin E Supplementation on Blood Serum Biochemical Profile of Rabbit Bucks

The higher globulin level (39.67 and 44.00g/l) recorded for bucks on 20 and 40 mg vitamin E/kg supplementation level in this study shows that vitamin E may have improved protein status and improved the health of the liver of the rabbits (Shinde*et al*., 2007). Globulin is known to transport ions, hormones and lipid in the blood. The globulin values fall within the reference range for rabbits (32-49g/l) Black *et al*. (2009). The Glutathione peroxidase value observed in this study was higher at 20, 40 and 60 mg/kg inclusion level and this agreed with reports of Ebeid, (2012) and Shaibu (2014) who reported a significant difference in glutathione peroxidase in blood plasma of rabbit does at inclusion rate of 40 mg/kg diet which can reduce oxidative stress.

The higher serum plasma vitamin E at 60 mg/kg recorded in this study agreed with the findings of Abdel-Khalek*et al*. (2010) who observed that vitamin E increased plasma vitamin E in rabbit does. The results of this study suggests that vitamin E supplementation in rabbit bucks‟ diet to as high as 40 mg/ kg could improve protein anabolism and decrease protein catabolism (Gamal*et al.* 2014).

5.1.4 Effect of Vitamin E Supplementation on Semen Quality of Rabbit Bucks

The 60 mg/kg vitamin E supplementation resulted in higher sperm motility, concentration and percentage live sperm. This observation is similar to the report of Audet *et al.* (2004**)** who observed that feeding piglets with vitamin E led to increased sperm production. In addition, Ebeid (2009) suggested that vitamin E increases male fertility by aiding the development of spermatozoa, maturation and viability. The higher sperm motility, concentration and live sperm ratio recorded for bucks on 60 mg/kg compared to those on 0

mg/kg in this study agreed with the reports of Yaseen*et al.* (2016) who suggested that vitamin E provides biological stability to the spermatozoa plasma membrane. El-Sheshtawy*et al.* (2014) observed that vitamin E supplementation (1.35IU/kg injection) improved semen characteristics as indicated by increased sperm concentration, motility and live sperm ratio and decreased sperm abnormalities in bulls and rams. However, the increased sperm motility, concentration and percentage live sperm cells recorded in this study contrasted with the findings of Yousef*et al*. (2003) who observed a decreased sperm motility and increased seminal volume of rabbits fed vitamin E fortified diets. Sperm liveability of rabbit bucks could be improved by supplementing their diet with 60 mg/kg diet vitamin E.

5.1.5Effect of Vitamin E Supplementation on Sperm Morphology of Rabbit Bucks

Vitamin E supplementation at 20 and 60 mg/kg inclusion rate, sperm abnormalities like free tail, coiled tail and bent tail were observed in bucks in the treatment group. The morphological defects seen could be due to genetic traits, increased testicular temperatures, environmental condition and semen handling procedure (Cesare, 2013). This observation is in agreement with the findings of Echeverria-Alonzo *et al.* (2009) who reported morphological defects like free tail, coiled tail and bent tail for sperm of boars fed diet supplemented with 60 mg/kg vitamin E. From these reports, there is evidence that vitamin E may directly protect sperm cells from morphological damage thereby increasing motile sperm cell production (Marin-Guzman *et al*., 1997).

5.2 EXPERIMENT 2: Effects of Selenium Supplementation on Growth, Blood Profile and Semen Quality of Rabbit Bucks.

5.2.1 Effect of Selenium Supplementation on Growth Performance of Rabbit Bucks.

The findings of this study showed that final weight, average daily feed intake and average daily gain showed positive effect when 0.4 mg/ kgselenium was included in the diet of rabbit bucks.Feed conversion ratio was high at 0 mg/kg selenium inclusion this result is similar with the findings of Ebeid*et al*. (2013) who recorded 5.02g for rabbit bucks in control group of selenium inclusion. Therefore, it can be suggested that dietary selenium supplementation, has a potent trophic and morphogenic actions in rabbit bucks (Zhang *et al*., 2011).

5.2.2 Effect of Selenium Supplementation on Haematological Parameters of Rabbit Bucks

Blood parameters of animal might be influenced by several factors such as breeds, age, sex, nutrition, management, physiological factors and diseases (Merck Manual, 2012). In this study, packed cell volume, white blood cell and lymphocytes showed significant differences following inclusion of varying levels of selenium in rabbit buck's diet. The packed cell volume (37.67 to 42.00%) and white blood cells (10.63 and $7.13x10^9$) values recorded in this study showed that selenium improved blood concentration metabolites that are involved in protecting the body against infectious diseases and foreign invader (Meshreky and Shaheed, 2003). The values obtained in this study is in line with the normal range for rabbits packed cell volume and white blood cells (33-50%) and ($5\text{-}13x10^9$) (Etim*et al*., 2014).Valuesfor lymphocytes in this study agrees with the findings of Shaibu. (2014) who recorded (79.00%) for rabbit does on 40 mg vitamin E/kg. In the current study, values for lymphocytes obtained falls within normal range (43 to 80 %) for rabbits Etim*et al*. (2014) which is a good indicator of increasing immunity efficiency (Meshreky and Shaheed, 2003). The higher packed cell volume and lymphocytes in bucks under the

treatment group in this study suggests that selenium may have improved the immune-competence.

5.2.3 Effect of Selenium Supplementation on Blood Serum Biochemical Profile of Rabbit Bucks

Bucks on 0.2 mg/kg selenium had higher levels of serum aspartate transaminase, also, alkaline phosphatase were found to be higher on 0.2 mg/kg selenium treatment group. This result is in line with the findings of Ozardali*et al.* (2004) who observed increased activities of aspartate transaminase in blood plasma of rats administered selenium injection. However, Abdurashid (2014) observed decrease in aspartate transaminase and alkaline phosphatase concentrations in rabbits on 0.4 mg/kg dietary selenium supplementation contradicted the finding of this study. The higher aspartate transaminase and alkaline phosphatase in rabbits on 0.2 mg/kg inclusion rate may be due to an injury in either the liver or muscles of the bucks where these enzymes are known to be in higher circulation in liver and muscles (Ozardali*et al.* 2004).

5.2.4 Effect of Selenium Supplementation on the Semen Quality of Rabbit Bucks

The highest semen volume observed in the 0.2 mg/kg selenium supplemented group,

is an indication that this inclusion level potentiates androgenic activities (Yousef*et al.* 2003). The findings of this study agrees with Olson *et al*. (2005) who recorded high semen volume and concentration when selenium was included in the diet of rabbit buck. However, Marin-Guzman *et al*. (1997) did not find any effect of selenium on semen volume and sperm concentration of young boars. The result of the study shows that selenium supplementation up to 0.2 mg/kg of feed improves rabbit bucks‟ semen volume, concentration and live cells.

5.2.5 Effect of Selenium Supplementation on the Sperm Morphology of Rabbit Bucks

Bent tail increased at 0.6 mg/kg selenium inclusion level in the treatment group. This is an indication that increase in selenium inclusion level could cause damage to sperm cells. Ronald *et al*. (2009) reported that selenium supplementation at 0.5 mg/kg lowers morphology anomalies in rat and boars. From this result, it can be suggested that selenium supplementation in rabbit bucks caused minimal sperm morphology defects.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

1. Inclusion of Vitamin E at 20 mg/kg diet improved final weight and average daily gain.
2. Inclusion of Vitamin E at 40 and 60 mg/kg diet improved blood packed cell volume and haemoglobin in rabbit bucks.
3. Inclusion of Vitamin E at 20 and 40 mg/kg diet increased blood globulin of rabbit bucks.
4. Inclusion of Vitamin E at 60 mg/kg diet improved sperm motility, sperm concentration and semen live ratio of rabbit bucks.
5. Selenium inclusion of rabbit bucks improved final weight, average daily feed intake and average daily weight gain at 0.4 mg/kg selenium inclusion.
6. Selenium inclusion at 0.2 mg/kg increased alkaline phosphatase in blood serum biochemical profile of rabbit bucks.
7. Selenium inclusion at 0.2 mg/kg diet led to an increase in semen volume of rabbit bucks.
8. Inclusion of selenium at 0.6mg/kg diet of rabbit bucks bent tail defect was observed.

6.2 Recommendations

1. From the observed improved semen quality in the study, supplementation of the rabbit bucks diet with 60 mg/kg diet of Vitamin E can be recommended.
2. Inclusion of 0.2 mg/kg diet of Selenium is required for better semen morphology.

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