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EFFECT OF VITAMIN E SUPPLEMENTATION ON SPERM MORPHOLOGY AND SEMEN CHARACTERISTICS OF RABBIT BUCKS

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ABSTRACT

Twenty mixed breed rabbit bucks aged between 6–8 weeks with an average weight of 861g were randomly assigned to four (4) dietary treatments with five (5) bucks per treatment in a Completely Randomized Design to determine the effect of feeding graded levels of Vitamin E on sperm morphology and semen characteristics of rabbit bucks. The rabbits received graded levels of vitamin E/kg feed (0mg/kg, 20 mg/kg, 40 mg/kg and 60 mg/kg for treatments 1, 2, 3 and 4, respectively). Feed and water were given to the rabbit bucks *ad libitum* during the experiment. The semen collected was taken to laboratory for sperm morphology and semen characteristics and the data collected were analysed using general linear model procedure of statistical analysis system (SAS). The results revealed that the mean number of normal cells, detached head and mid piece defect were not significantly (P>0.05) different across the treatments. However, the percent abnormal defects percent of free tail and coiled tail were significantly (P<0.05) lower for rabbits fed 20 and 60 mg/kg and the least bent tail was obtained in 60 mg/kg diet. The results for semen characteristics had significant (P<0.05) differences in all the parameters except for semen volume and pH. The results showed that those fed 60 mg/kg of vitamin E diet gave significantly (P<0.05) higher sperm motility, sperm concentration, live ratio and low dead ratio (87.50 %, 232x10^6, 87.50 % and 12.00 %) respectively. Therefore, supplementation of vitamin E at 60mg/kg in the diet of rabbit bucks is recommended.

Keywords: Bucks, Rabbit, Semen, Sperm Morphology, Vitamin E

INTRODUCTION

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 small holder subsistence type 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). 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. 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).

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. Vitamin E reduces the production of prostaglandins such as thromboxane and it is also vital for the formation and normal functioning of red blood cells and muscles (Bassey *et al*., 2021)

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). Some studies have reported useful effects of 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). However, in Sub-Saharan region, there is insufficient information or research on the effect of vitamin E supplementations on sperm morphology and semen characteristics of rabbit bucks. Therefore, this study was aimed at evaluating the effect of vitamin E supplementation on sperm morphology and semen characteristics of rabbit bucks.

MATERIALS AND METHODS

Experimental site

The study was conducted at the National Animal Production Research Institute (NAPRI), Shika, Zaria. Shika lies within the Northern guinea savannah Zone of Nigeria and located on latitude 11^0 12'N and longitude 7^0 33' E with 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), with the lowest temperature occurring during the early dry season (November-January), while, the highest temperatures are experienced during late dry season between February-April. (Ovimaps, 2015).

Source and processing of test ingredients

The concentrate was compounded at Zaria feed mill, feed graded vitamin E and selenium were sourced from Hi nutrient limited in Lagos and was incorporated into the compounded feed.

Experimental diet

The percentage composition of concentrate composition of experimental diets is presented in Table 1. Concentrate was offered *ad libitum* and much later dry forage (groundnut haulms) was offered *ad libitum* to all the rabbits. The forage was withheld so as the rabbit would consume much of the concentrate, thereafter consume less of the forage. The inclusion levels were: 0 mg/kg Vitamin E inclusion, 20 mg/kg Vitamin E inclusion, 40 mg/kg Vitamin E inclusion, 60 mg/kg Vitamin E inclusion**.**

Table 1: Percentage composition of the experimental diet fed to rabbit bucks

	Inclusion levels of vitamin E mg/kg diet			
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 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
Vitamin/mineral 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
Total	**100**	**100**	**100**	**100**
Calculated analysis				
Crude Protein	15.05	15.05	15.05	15.05
Metabolizable Energy (kcal/kg)	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; vitamin E, 23 mg; vitamin 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, Ibafo Ogun State, Nigeria.

Experimental animal, design and management

A total of 20 mixed breed rabbit bucks aged 6-8 weeks of average weight 861 grams were used in this study. The rabbits were purchased from National Veterinary Research Institute Vom. Rabbits were weighed with a weight balance. The rabbits were grouped into four dietary treatments with five rabbit bucks in each of the treatments in a Completely Randomized Design (CRD). Each rabbit was housed in a metal cage, measuring 60x60 cm in dimension and well ventilated. Each cage was equipped with two round bottom earthen pot, one for feed and one to serve as drinker. The rabbits were administered an anti-stress (glucose via water), antibiotics and were treated against external and internal parasites. All animals were kept, maintained and treated in accordance with standard routine management**.**

Semen collection

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 (Zemjanis, 1970). 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).

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.

Volume

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

Semen pH

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

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 magnifications.

Spermatozoa concentration

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

Percentage live sperm cells

This was determined as described by Esteso *et al*. (2006). A thin smear of the semen was made on a clean grease free slide and stained with eosin-nigrosin stain. This technique was based on the principle that eosin-nigrosin penetrates and stains dead sperm cells while live sperm cells repel the stain. Dead spermatozoa stained pinkish or reddish while live spermatozoa remained colourless. One hundred (100) stained and unstained sperm cells were counted when the slides were dried, using light microscopy at ×40 magnification and percentage of each estimated (Esteso *et al*., 2006).

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 magnifications. Five cell types were recorded: normal cell, detached head, free tail, coiled tail and bent tail (Rekwot *et al*., 1987).

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.

The model:

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

Where;

Y_{ij} = The observation on the j^{th} buck in i^{th} levels of Vitamin E

μ = Overall mean;

T_i =Treatment effect

e_{ij} = Random error (All error terms were assumed to be random, normally distributed and independent with expectation equal to zero).

RESULTS AND DISCUSSION

Table 2 shows the result of the effect of vitamin E supplementation on sperm morphology of rabbit bucks. The result showed that normal cells, detached head and mid piece defect were not significantly (P>0.05) different. There were significant (P<0.05) differences across the treatments for free tail, coiled tail and bent tail. However, the lower percent abnormal defects (free tail and coiled tail) were observed in those rabbit bucks fed 20 mg/kg vitamin E while lower value for bent tail was found in those in 60 mg/kg vitamin E group. The lower free bent tail at rabbit bucks in 20 mg/kg vitamin E followed by those at 60 mg/kg vitamin E means that vitamin E which is antioxidant reduces defect in sperm. The higher morphological defects seen could be due to genetic traits, increased testicular temperatures, environmental condition and semen handling procedure. This observation is in agreement with the findings of Echeverria-Alonzo *et al.* (2009) who reported higher morphological defects like free tail, coiled tail and bent tail for sperm of boars fed diet supplemented with vitamin E at different levels with more defects with zero inclusion of vitamin E. The implication with higher defects means that it will affect the ability of the sperm to reach and penetrate an egg. The lower defects in the treatment groups simply means that vitamin E may directly protect sperm cells from morphological damage thereby increasing motile sperm cell production (Marin-Guzman *et al.*, 1997).

Table 3 shows the result of the effect of vitamin E supplementation on semen characteristics of rabbit bucks. Semen volume and pH were not significantly (P>0.05) different. However, sperm motility, sperm concentration and live ratio were significantly (P<0.05) higher for rabbit bucks fed 60 mg/kg of vitamin E diets. There was significant (P<0.05) difference across the treatments for dead ratio but the dead ratio percent decreases as the vitamin E inclusion increases. Inclusion of 60 mg/kg vitamin E gave the best results for sperm motility, sperm concentration, live and dead sperm.

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 vitamin E compared to those on 0 mg/kg vitamin E 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. The higher motility observed in rabbit fed 60 mg/kg vitamin E also implies that it is important in fertility because sperm need to move through the female reproductive tract to reach and fertilize egg. Poor sperm motility can be a cause of male factor infertility. In line with this, 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. The results implies that the addition of vitamin E in the diet of rabbit bucks increased, motility, progressive motility, viability and normal spermatozoa. The observations in this study indicates that the vitamin E was able to protect sperm conditions. Natural antioxidants such as vitamin E, inhibits cell impair by binding to the free radical and neutralizing its unpaired electron mediated by a tocopheryl-quinone' formation (Hamed *et al*., 2013). The significantly lower value of dead sperm cells obtained in the treatment with higher vitamin E (60 mg/kg) compared with control group (0 mg/kg) which indicates that vitamin E reduces the production of dead sperm cells. The lower dead sperm cells found in rabbit bucks fed higher vitamin E (60 mg/kg) means that it involves in the maintenance of higher functional integrity of epididymis (Awojobi and Oyeyemi, 2001).

Table 2: Effect of Vitamin E Supplementation on Sperm Morphology of Rabbit Bucks

	Inclusion levels of vitamin E (mg/kg diet)					
Parameters (%)	**0**	**20**	**40**	**60**	**SEM**	**P-value**
Normal cells	77.80	72.50	78.50	95.33	6.96	0.19
Detached head	5.83	2.44	5.25	4.00	1.22	0.26
Free tail	5.47^{c}	1.11^{a}	7.22^{d}	2.25^{ab}	1.02	0.01
Coiled tail	3.98^{b}	0.67^{a}	4.17^{b}	1.25^{a}	0.37	0.00
Bent tail	4.29^{b}	1.22^{a}	4.44^{b}	1.00^{a}	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, SEM= Standard error of mean

Table 3: Effect of Vitamin E Supplementation on Semen Characteristics of Rabbit Bucks

	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 ratio (%)	69.25^{b}	66.67^{b}	64.17^{b}	87.50^{a}	4.56	0.03
Dead ratio (%)	45.83^{c}	38.47^{bc}	28.50^{b}	12.00^{a}	4.12	0.00

abc: Means with different superscripts in the same row are significantly (P<0.05) different, SEM= Standard error of mean

CONCLUSION AND RECOMMENDATION

Dietary supplementation of vitamin E does not have significant effect on mean normal sperm cell, detached head, mid piece defect, semen volume and pH.

Supplementation of vitamin E at 60mg/kg in the diet of rabbit bucks showed improved semen characteristics and is therefore recommended.

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