



## EFFECT OF VITAMIN E ON SPERM AND OXIDATIVE STRESS PARAMETERS OF WEST AFRICAN DWARF GOAT BUCKS

[EFECTO DE LA VITAMINA E EN EL ESPERMA Y EL ESTRÉS OXIDATIVO PARÁMETROS DE MACHOS CABRIOS DE LA RAZA ENANA DEL OESTE DE ÁFRICA]

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### SUMMARY

The aim of the present study was to determine the effect of supplementation of vitamin E on sperm and oxidative stress parameters in West African Dwarf goat bucks. The bucks were allocated to 4 treatments consisting of 0 mg, 15 mg, 30 mg and 45 mg of vitamin E. At the end of 30 days consecutive administration of vitamin E, semen and blood samples were collected and evaluated for sperm and oxidative stress parameters. The results showed that sperm motility, acrosome integrity, membrane integrity and live spermatozoa; seminal Malondialdehyde (MDA), arginase activity and leukocytes; and serum MDA and testosterone were similar in all the levels of inclusion and comparable to the control. However, higher arginase activities were observed in all the levels of vitamin E compared to the control while 15 mg of vitamin E had higher percentage of sperm abnormality compared to other treatments and the control. The findings indicated that the levels of vitamin E used did not have beneficial effect on the sperm and oxidative stress parameters. Further study of higher levels of vitamin E is therefore necessary to ascertain the appropriate level required for optimal improvement in these parameters.

**Keywords:** Antioxidant; buck; sperm viability; stress

### INTRODUCTION

West African Dwarf (WAD) goats (*Capra hircus*) are widely distributed in the South-Western part of Nigeria with a prevailing tropical climate. The area is

### RESUMEN

El objetivo del presente estudio fue determinar el efecto de la suplementación de vitamina E en los parámetros del esperma y el estrés oxidativo en machos de la raza de cabras enanas del África occidental. Los machos fueron asignados a 4 tratamientos que consistieron en: 0 mg, 15 mg, 30 mg y 45 mg de vitamina E. Al final de 30 días de administración consecutiva de la vitamina E, el semen y muestras de sangre fueron recogidos y evaluados los parámetros espermáticos y de estrés oxidativo. Los resultados mostraron que la movilidad de los espermatozoides, la integridad del acrosoma, la integridad de la membrana y espermatozoides vivos; Malondialdehído seminal (MDA), la actividad de la arginasa y leucocitos; y MDA en suero y la testosterona fueron similares en todos los niveles de inclusión y comparable con el control. Sin embargo, se observaron actividades de la arginasa más altos en todos los niveles de vitamina E en comparación con el control mientras que el nivel de 15 mg de vitamina E tenía un mayor porcentaje de espermatozoides anormales en comparación con otros tratamientos y el control. Los resultados indicaron que los niveles de vitamina E utilizado no tenían efecto beneficioso sobre los parámetros del esperma y el estrés oxidativo. Por lo tanto, más estudios de los niveles más altos de vitamina E son necesarios para determinar el nivel apropiado para la mejora óptima en estos parámetros.

**Palabras clave:** Antioxidante; machos; viabilidad espermática; estrés

particularly noted for unfavourable environment, which is humid and favours high prevalence of diseases. The goat production in this zone makes a major contribution to the agrarian economy. As the demand for animal protein in the sub region is

constantly high, the prospects for increasing the numbers and productivity of this breed of goat need to be utilized. Therefore, improvement of this important breed of goat presents a great potential to alleviate the problem of protein malnutrition in the region.

Oxidative stress is one of the major factors that affect male fertility (Agarwal and Prabakaran, 2005) and its effect on sperm malfunctions through induction of lipid peroxidation to bio-membranes has been reported (Arabi and Seidaie, 2008). Goat spermatozoa in particular are sensitive to oxidative stress due to high content of unsaturated fatty acids in phospholipids of plasma membrane and low antioxidant capacity of goat seminal plasma (Donghue and Donoghue, 1997; Watson, 2000). The effect of reactive oxygen species (ROS) on sperm motility and the toxicity of the fatty acid peroxides generated by their attack on the membrane phospholipids were earlier reported (Jones *et al.*, 1978). There are two potential sources of ROS: the leukocytes and the spermatozoa themselves (Aitken *et al.*, 1992). Antioxidant molecules which can counteract the cellular damage due to ROS are present in seminal plasma and spermatozoa. Seminal plasma has been reported to contain  $\alpha$ -tocopherol (Moilanen *et al.*, 1993) and enzymatic systems that reduce oxidative damage (Zini *et al.*, 1993). Vitamin E ( $\alpha$ -tocopherol) is well known lipid-soluble, chain breaking antioxidant (Burton and Ingold, 1986; Luo *et al.*, 2011) found in cells (Cheeseman *et al.*, 1988) where it contributes to their integrity and function. As a naturally occurring antioxidant, vitamin E is located in biological membranes where it acts to protect the membrane polyunsaturated fatty acid from oxidation and attenuate oxidative damage to cellular membranes (Sugiyama, 1992). Esterbauer *et al.* (1991) reported that vitamin E was effective in preventing lipid peroxidation and other radical-driven oxidative events. Vitamin E plays important role in reproductive function and previous report indicated increase in total sperm output and sperm concentration during dietary supplementation in boars (Brzezinska-Slebodzinska *et al.*, 1995), rabbits (Yousef *et al.*, 2003) and rams (Luo *et al.*, 2004; Yue *et al.*, 2010). Impairment of mammalian fertility was also attributed to vitamin E deficiency. Brzezinska-Slebodzinska *et al.* (1995) observed that supplementation with vitamin E increased the concentration of spermatozoa in semen; an effect possibly linked to the antioxidant properties of this vitamin. However, the chain-breaking antioxidant of vitamin E for preserving the functional competence of spermatozoa has not been reported to be present in WAD goat bucks. Information is scarce on the effect of vitamin E on sperm and oxidative stress parameters of West African Dwarf goat bucks that are managed in this zone associated with less favourable environment.

The present study was therefore designed to evaluate the effect of vitamin E on sperm and oxidative stress parameters of West African Dwarf goat bucks.

## MATERIALS AND METHODS

### Animal and management

The study was carried out at the Goat Unit of Teaching and Research Farm, Federal University of Agriculture, Abeokuta which falls within 7° 10' N and 3° 2' E and altitude 76 m above sea level. It lies in South-Western part of Nigeria with a prevailing tropical climate, a mean annual rainfall of 1,037 mm and average temperature of 34.7 °C. Twenty (20) WAD goat bucks aged 2.5-3 were used. The animals were kept under an intensive management system and maintained under a uniform and constant nutritional regimen with concentrate feed at 300g/body weight and supplemented with guinea grass (*Panicum maximum*).

### Trial and Data Collection

The animals were divided into 4 groups of 5 animals each and each group was randomly assigned one of the experimental treatments consisting of 0, 15, 30 and 45 mg/BW of vitamin E administered via oral route to the animals for 30 days consecutively. Thereafter, semen samples were collected with the aid of artificial vagina. Two replicates each of semen and blood samples were collected from the bucks 3 times at an interval of 15 days post vitamin E administration. Sperm and oxidative stress parameters were determined and values obtained during the 3 times collections were pooled for each parameter.

### Evaluation

#### Subjective microscopic evaluation of sperm motility

Sperm motility was determined as described by Bearden and Fuquay (1997). Briefly, semen was placed in Clifton Water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37 C and accessed for sperm motility at a magnification of 400× with Celestron PentaView LCD digital microscope. A 5  $\mu$ L sample of semen was placed directly on a heated microscope slide and overlaid with a 22 x 22 mm cover slip. For each sample, five microscopic fields were examined to observe progressive sperm motility and the mean of the five successive evaluations was recorded as the final motility score.

### **Sperm concentration**

The concentration was determined by the use of a Minitube Photometer [SDM1 Ram 12300/0106, Germany].

### **Acrosome Integrity**

The percentage of spermatozoa with intact acrosomes was determined according to Ahmad *et al.* (2003). Briefly, 50  $\mu$ L of each semen sample was added to a 500  $\mu$ L formalin citrate solution (96 mL 2.9 % sodium citrate, with 4 mL 37 % formaldehyde) and mixed carefully. A small drop of the mixture was placed on a microscope slide and a total of 200 spermatozoa were counted in at least three different microscopic fields for each sample at a magnification of 400 $\times$  with Celestron PentaView LCD digital microscope. Intactness of acrosome characterized by normal apical ridge of spermatozoa was assessed.

### **Sperm Membrane Integrity**

Hypo-osmotic swelling test (HOST) assay as described earlier (Jeyendran *et al.*, 1984) was used to determine sperm membrane integrity and this was done by incubating 10  $\mu$ L semen in 100  $\mu$ L hypo-osmotic solution (fructose and sodium citrate) at 37  $^{\circ}$ C for 30 min, 0.1 mL of the mixture was spread over a warmed slide, covered with a cover slip and observed at a magnification of 400 $\times$  with Celestron PentaView LCD digital microscope. Two hundred spermatozoa (200) were counted for their swelling characterized by coiled tail, indicating intact plasma membrane.

### **Sperm abnormality and live sperm**

Sperm morphological abnormalities and live sperm were determined as described by Bearden and Fuquay (1997) with the use of eosin-nigrosin smears. A thin smear of mixture of semen and eosin-nigrosin solution was drawn across the slide and dried. Sperm cells that did not absorb eosin-nigrosin solution were recorded as live sperm cells. The percentage of morphologically abnormal spermatozoa with defects in the head, midpiece and tail were observed (400 $\times$  magnification) with Celestron PentaView LCD digital microscope.

### **Malondialdehyde (MDA) concentration**

Malondialdehyde concentration as index of lipid peroxidation in the semen samples was measured in a thiobarbituric acid reactive substances (TBARS) according to Yagi (1998). For this assay, 0.1 mL of semen sample was incubated with 0.1 mL of 150 mM Tris-HCl (pH 7.1) for 20 min at 37  $^{\circ}$ C. Subsequently, 1 mL of 10 % trichloroacetic acid (TCA) and 2 mL of

0.375 % thiobarbituric acid were added followed by incubation in boiling water for 30 min. Thereafter, it was centrifuged for 15 min at 3000 g inside the blank tube and the absorbance was read with UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 532 nm. The concentration of MDA was calculated as follows: The concentration of malondialdehyde MDA (nmol/mL) =  $AT - AB / 1.56 \times 10^5$ ; Where: AT = the absorbance of the sample serum, AB = the absorbance of the blank,  $1.56 \times 10^5$  molar absorptivity of MDA

### **Seminal leukocytes**

Peroxidase test adapted from Endtz (1974) and as recommended by WHO (1992) was used. A stock solution was prepared by mixing 50 mL distilled water with 50 mL 96 % ethanol plus 125 mg benzidine. The working solution was obtained by adding 5  $\mu$ L 30 %  $H_2O_2$  to 4 mL of stock solution. Twenty microliters of working solution was mixed with 20  $\mu$ L of semen sample in a small test tube. After incubation for 5 min at room temperature, 20  $\mu$ L of working solution was mixed with 20  $\mu$ L of phosphate-buffered saline. Then, 10  $\mu$ L was placed in a haemocytometer, and peroxidase-positive cells (dark brown round cells) were counted.

### **Arginase activity**

A 0.1 g bovine serum albumin (BSA) as standard in 10 mL of water was used in tubes containing 1 mL alkaline copper reagent (a mixture of copper sulfate reagent, sodium dodecyl sulfate solution, and sodium hydroxide solution (1:2:1) and 0.1 mL semen samples mixed and incubated for 10 min at room temperature (Lowry *et al.*, 1951). After this, 4 mL folin Ciocalteu's phenol reagent was added to the tubes, mixed and incubated for 5 min at 55 $^{\circ}$ C. The absorbance of the samples was recorded at 650 nm in spectrophotometer (UV spectrophotometer, SW7504 model by Surgifriend Medicals, England). The results were expressed as units/mg of protein (specific activity).

### **Serum testosterone**

Blood samples were collected through jugular vein, centrifuged and serum harvested and stored at -20 $^{\circ}$ C for testosterone assay. Testosterone was determined by ELISA (enzyme-linked immunosorbent assay) using testosterone kit, catalog number 2000 from Dialab (UK) and read with Robonic 11 ELISA Reader (UK).

### **Statistical analysis**

Data obtained were subjected to analysis of variance in a completely randomized design and means

separated by Duncan Multiple Range Test (Duncan, 1955). The results analyzed using a one-way analysis of variance in SAS 1999 package were expressed as the means  $\pm$  SEM in SAS 1999 package using the model below:

$$Y_{ijk} = \mu + A_i + L_j + \sum_{ijk}$$

Where,  $Y_{ijk}$  = Dependent variables;  $\mu$  = Population mean;  $A_i$  = effect due to  $i^{\text{th}}$  vitamin E treatments;  $L_j$  = effect due to  $j^{\text{th}}$  level of inclusion,  $j = 0, 15, 30, 45$ ;  $\sum_{ijk}$  = Experimental error

## RESULTS

The means of sperm characteristics of WAD buck goats administered with different levels of vitamin E are presented in Table 1. The results showed that sperm concentration, sperm motility, acrosome integrity, membrane integrity and live spermatozoa were similar in all the levels of inclusion and

comparable to the control. However, 15 mg of vitamin E had increase ( $P < 0.05$ ) percentage of sperm abnormality compared to other treatments and the control.

The means of seminal oxidative stress parameters of WAD buck goats administered with different levels of vitamin E are presented in Table 2. The results showed that seminal MDA, arginase activity and leukocytes were similar in all the levels of inclusion and comparable to the control.

The means of serum oxidative stress parameters of WAD buck goats administered with vitamin E are presented in Table 3. The results showed that serum MDA and testosterone were similar in all the levels of inclusion and comparable to the control. However, higher arginase activities were observed in all the levels of vitamin E compared to the control.

Table 1: Means ( $\pm$ SEM) sperm characteristics of WAD buck goats administered with vitamin E

Parameters	0 mg	15 mg	30 mg	45 mg
Sperm concentration ( $\times 10^8/\text{mL}$ )	22.05 $\pm$ 152.43	22.74 $\pm$ 210.35	18.43 $\pm$ 262.63	19.87 $\pm$ 196.43
Motility (%)	94.67 $\pm$ 1.28	92.33 $\pm$ 4.17	94.67 $\pm$ 3.38	96.67 $\pm$ 1.20
Acrosome integrity (%)	93.67 $\pm$ 2.32 <sup>a</sup>	84.83 $\pm$ 3.15 <sup>b</sup>	89.50 $\pm$ 1.66 <sup>ab</sup>	91.75 $\pm$ 1.84 <sup>ab</sup>
Membrane integrity (%)	91.67 $\pm$ 2.78	83.58 $\pm$ 2.09	89.71 $\pm$ 3.32	92.33 $\pm$ 1.56
Live sperm (%)	92.08 $\pm$ 3.25	87.92 $\pm$ 5.12	93.25 $\pm$ 2.47	97.50 $\pm$ 1.91
Abnormality (%)	1.37 $\pm$ 0.22 <sup>b</sup>	2.66 $\pm$ 0.18 <sup>a</sup>	1.25 $\pm$ 0.25 <sup>b</sup>	1.79 $\pm$ 0.22 <sup>b</sup>

<sup>a,b</sup> Values within rows with different superscripts differ significantly ( $P < 0.05$ )

Table 2: Means ( $\pm$ SEM) seminal oxidative stress parameters of WAD buck goats administered with vitamin E

Parameters	0 mg	15 mg	30 mg	45 mg
Seminal MDA (nmol/mL)	0.04 $\pm$ 0.01	0.02 $\pm$ 0.01	0.04 $\pm$ 0.02	0.04 $\pm$ 0.01
Seminal arginase activity (units/mg protein)	1.13 $\pm$ 0.06	1.10 $\pm$ 0.21	1.25 $\pm$ 0.12	1.22 $\pm$ 0.25
Seminal leukocytes ( $\times 10^3/\text{mL}$ )	0.52 $\pm$ 0.09	0.63 $\pm$ 0.08	0.48 $\pm$ 0.06	0.43 $\pm$ 0.08

Table 3: Means ( $\pm$ SEM) serum MDA, arginase activity and testosterone of WAD buck goats administered with vitamin E

Parameters	0 mg	15 mg	30 mg	45 mg
Serum MDA (nmol/mL)	0.18 $\pm$ 0.02	0.18 $\pm$ 0.01	0.18 $\pm$ 0.01	0.18 $\pm$ 0.01
Serum arginase activity (units/mg protein)	1.44 $\pm$ 0.08 <sup>b</sup>	1.76 $\pm$ 0.03 <sup>a</sup>	1.85 $\pm$ 0.16 <sup>a</sup>	1.75 $\pm$ 0.03 <sup>a</sup>
Serum testosterone (ng/mL)	0.40 $\pm$ 0.10	0.40 $\pm$ 0.06	0.40 $\pm$ 0.06	0.37 $\pm$ 0.09

<sup>a,b</sup> Values within rows with different superscripts differ significantly ( $P < 0.05$ )

## DISCUSSION

The present study indicated that the levels of vitamin E used did not have beneficial effect on the sperm and oxidative stress parameters. Pasqualotto et al. (2000) reported an association between high seminal reactive oxygen species levels and reduced sperm count and motility. The protective effects of vitamin E against oxidative damage of sperm cells become more significantly observable when hygienic conditions are poorly controlled such as increased incidence of infections/inflammations of reproductive apparatus (Luo et al., 2011). During inflammation, the antioxidant defence of reproductive system downplays and generates an oxidative stress (Potts and Pasqualotto, 2003), which may impair testis function and affect negatively semen characteristics (O'Bryan et al., 2000). Testicular tissue becomes one of the targets for oxidative stress due to high content of polyunsaturated membrane lipids (Mishra and Acharya, 2004). Furthermore, vitamin E was reported to play a role in association with antioxidant enzymes, for preserving the functional competence of spermatozoa subjected to an oxidative attack (Therond, et al., 1996). Vitamin E has also been observed to increase sperm viability and reduced lipid peroxidation when subjected to oxidative stress inducer (Bansal and Bilaspuri, 2009). However, the animals used in this present study were apparently healthy and were not subjected to oxidative stress; the possible reason why the protective effect of vitamin E was not observed. Moreover, the WAD goat is hardy and thrives in harsh conditions of management (Adeloye, 1998) which is largely possible because of the innate adaptogenic power of the animal to react to a particular quantum stressor (Adeloye and Daramola, 2004). In contrast, high tocopherol concentrations could cause ineffective antioxidant action even becomes a pro-oxidant (free radicals) that precisely reproduce the formation of radicals (Wahjuningsih and Rachmawati, 2012). The type of phenolic antioxidants (such as tocopherol) in excessive concentration will lose its effectiveness as an antioxidant and even to form a pro-oxidant (Wahjuningsih and Rachmawati, 2012). Changes in antioxidant function become pro-oxidant or free radicals cause more unsaturated fatty acids that are subjected to free radicals (Wahjuningsih and Rachmawati, 2012). This situation further accelerates and expands the incidence of lipid peroxidation of sperm plasma membrane damage due to loss of some essential unsaturated fatty acids making up the membrane (Nur et al., 2005). This tendency is reinforced by the fact that the content of MDA as a lipid peroxidation product that is toxic to spermatozoa is found higher at a high dose of tocopherol (Wahjuningsih, 2010). This body of evidence support the present study that the levels of vitamin E used was neither enough for antioxidative effect nor in excess

to elicit pro-oxidative effect on sperm and oxidative stress parameters. In contrast, vitamin E as antioxidant (Yue et al., 2010) was observed to prevent membrane damage mediated by free radicals (Gurel et al., 2005). In addition, Kutlubay et al. (2007) reported reduced oxidative stress in the testis following administration of vitamin E. Oxidative stress is one of the major factors that affect male fertility (Agarwal and Prabakaran, 2005). Oxidative stress is known to play a major role in the sperm malfunctions through induction of lipid peroxidation to biomembranes (Arabi et al., 2001]. The difference in observation in the present study and those reported could be due to species of animal or methods of administration. This discrepancy may also be caused by different doses and treatment periods (Chattopadhyay et al., 2001). Although the amount of nutrient intake was not measured in this study but the administration of vitamin E did not adversely affect reproductive functions of the bucks. In a study of effects of feeding different levels of vitamin E in creep feeds on calf creep performance and subsequent 28 days feedlot performance, Gunter et al. (2004) established a linear decrease as the concentration of vitamin E increased in creep feed, and observed that dry matter consumption average was not significant among treatments. Furthermore, studies indicated that vitamin E supplementation had no significant effect on dry matter intake of dairy cows (Bell et al., 2006), dry matter intake and feed intake of Aohan fine wool sheep (Liu et al., 2013).

A positive correlation has been reported between sperm motility and arginase activity in seminal plasma and spermatozoa (Elgun et al., 2000; Eskiocak et al., 2006). The main role of arginase in testis is regulation of nitric oxide (NO) concentration (Nathan, 1997). Increased arginase activity generally results in lower NO concentration and subsequently leads to increased sperm motility (Elgun et al., 2000). This action could be the possible reason for the slight higher serum arginase activity with vitamin E in this study. However, this effect was not reflected in the seminal arginase activity.

Contaminating leucocytes and immature spermatozoa are the major sources of reactive oxygen species in semen (Aitken et al., 1992; Agarwal et al., 2003; Garrido et al., 2004). Excessive reactive oxygen species levels present in sperm cells' environment can either be produced in large amounts by leukocytes or the spermatozoa themselves, the process that results in decreased membrane fluidity of both plasma and organelle membranes and, consequently causes damage to membrane function (Sikka et al., 1996). Sequence to loss of membrane function, spermatozoa loses the ability to function properly and therefore, fertilization is impaired (Riffo and Parraga, 1996). Although contaminating leucocytes can be a critical

factor for sperm survival, the phagocytic role of leukocytes in eliminating defective spermatozoa is beneficial to sperm concentration, motility and acrosome reaction and may even stimulate sperm functions through the release of ROS (Kaleli, *et al.*, 2000; Henkel, 2011). Notwithstanding, high concentration of leukocytes, particularly activated leukocytes, are still harmful to sperm functions (Henkel, 2011). The similar concentration of leukocytes following addition of vitamin E in this study further indicated that the levels of vitamin E used in the present study did not play the role of antioxidant in reducing defective spermatozoa or excessive ROS produced by leukocytes.

### CONCLUSION

The findings indicated that the levels of vitamin E used did not have beneficial effect on the sperm and oxidative stress parameters. Further study on higher levels of vitamin E is therefore necessary to ascertain appropriate level that can have beneficial effect on these parameters.

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