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Effect of dietary inclusion of vitamin E as anti-oxidant on the semen characteristics of local cocks

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Abstract

The study was conducted to determine the effect of dietary inclusion of vitamin E as anti-oxidant on the semen characteristics of local cocks. Avian spermatozoa are subject to oxidative stress, causing male infertility because of its high polyunsaturated fatty acids content. Therefore, it was justified to research and report on the use of vitamin E as an antioxidant on the semen characteristics of local cocks. A total of 50 mature local cocks of uniform sizes (weighing about 1.52-1.53kg) were used for the study. Their exact ages were not known due to the lack of adequate record keeping by the traditional household keepers. The birds were divided into two treatment groups with 25 birds per treatment. Data generated were analyzed using independent group sample t-test. Each treatment had five replicates with 5 birds per replicate. Feed fed to T_1 birds contained 1000 IU vitamin E kg^{-1} feed, while T_2 (control) feed had no vitamin E. From the results obtained, sperm progressive motility, live: dead, normal and abnormal sperm cell morphologies were found to be highly significant ($p<0.01$), while semen volume and sperm concentration were significant ($p<0.05$). T_1 birds that fed diet containing vitamin E had the highest percentage values for semen volume, sperm progressive motility, live sperm cells, normal sperm cells and sperm cell concentration compared to control birds (T_2). Percentage values for dead and abnormal sperm cells were highest in control (T_2). In conclusion, the use of vitamin E as anti-oxidant to improve semen qualities was recommended because of higher values of sperm progressive motility, semen volume, live sperm cells, normal sperm cells and sperm cell concentration recorded in T_1.

Keywords*: vitamin E, cock, semen, spermatozoa, reactive oxygen species*

Description of problem

Fertility of poultry (local or exotic) is chiefly related to semen quality with respect to semen volume, sperm concentration, sperm motility, sperm viability, sperm fertilizing aptitude and the sperm forward progression. The above semen qualities are generally tested to examine and predict the male fertility in poultry (1, 2). However, semen quality can be affected by oxidative stress. Oxidative stress has been frequently implicated in the cause of infertility in male chickens (3, 4).

However, during the usual process of respiration, oxygen is increasingly abridged to yield water. Nevertheless, the partial diminution of oxygen (0_2) to yield water (H_{20}) during the process of respiration leads to the creation of chemical entities with potent oxidizing properties and are called reactive oxygen species (ROS). In vivo, reactive oxygen species (ROS) are continually produced during the course of physiological metabolism in living tissues. However, when the reactive oxygen species (ROS) is in excess of the available antioxidant capacity in the animal cells, oxidative stress is said to have occurred. In other words, oxidative stress is caused by the imbalance between pro-oxidants and anti-

oxidants at either cellular or individual level (5).

In poultry, oxidative stress may occur due to numerous factors such as: elevated concentration of polyunsaturated fatty acids in the feed, contamination of feed with fungal toxins, feed deficient in antioxidants (6, 7), environmental factors such as heat, high stocking density, transportation (8, 9), and pathological conditions such as fatty liver haemmorrhagic disease syndrome, ascites, coccidiosis and arthritis (10, 11). The production of reactive oxygen species (ROS) are increased during stressful conditions.

According to (12) avian spermatozoa are subject to reactive oxygen species, causing male infertility because of its high polyunsaturated fatty acids content. According to (13) reactive oxygen species can cause various types of gene mutations and polymorphosim and thereby resulting to decreased semen quality. Reactive oxygen species are involved in many physiological functions of spermatozoa, but excessive production of the same may result in oxidative stress (14). Oxidative stress reduces gamete numbers, decreases sperm motility and increases percentage of dead cells in male semen (15, 16)

Therefore, these negative effects of oxidative stress on the fertility of poultry males calls for a nutritional protocol that will help in alleviating these negative effects by boosting their anti-oxidant capacity at both cellular and individual level. However, one of the nutritional protocols that can be used to boost the body anti-oxidant capacity against the negative effects of oxidative stress on the fertility (semen) of male poultry is the dietary inclusion of vitamin E.

Vitamin E is receiving considerable attention in poultry nutrition due to its functional role as a dietary antioxidant in combating negative effects of oxidative stress. Vitamin E is the primary component of antioxidant system of spermatozoa. According to (17) vitamin E is a group of eight fat soluble compounds that include four tocopherols and four tocotrienols. Vitamin E is the main natural lipid soluble antioxidant contained in cell membranes which debar free radical stimulated per oxidation and is capable of improving quality of semen and its fertilizing capacity (18).

Vitamin E plays vital roles in the prevention of infertility in male poultry by restraining reactive oxygen species (free radicals) production (19). Vitamin E inclusion in a balanced poultry ration significantly supports reproductive functions, including sperm concentration, sperm motility, semen volume, sperm viability, and sperm cell integrity in avian species (20,18). Vitamin E enhances the functions of sperm mitochondria and increases sperm membrane integrity (21), thereby enhancing sperm qualities such as motility percentages and viability. Vitamin E added to the diet of quails increased semen volume, sperm cell concentration, viability, and percentage motility of sperm cells, as well as decreasing the percentages of dead and abnormal sperm cells (22). Feeding Lohmann Brown breeder roosters a diet supplemented with vitamin E enhanced semen volume, sperm cell concentration, and forward motility percentage (23). Vitamin E was added to the diet of cockerels and high percentages of live and normal spermatozoa were recorded (24). Having known the various negative effects of oxidative stress on semen quality, it was therefore, considered just to embark on this research with the aim to investigate the effect of vitamin E as anti-oxidant on the semen characteristics of local cocks. The positive outcome of this research finding will help to reduce farmer's constant complaints on poor reproductive performance of male birds used for breeding.

Materials and Methods

Location and duration of study

This experiment was conducted at the Poultry unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka Enugu State. It is located on latitude on $6^0$25N and $07^0$24E (25) and at an altitude of 430m above sea level (26) in the derived savanna region of South Eastern Nigeria. The climate of this study is a typical humid tropical type with a relative humidity range of 56.01-103.83%. Average diurnal minimum temperature ranges from 22^0C -24^0C. The rainfall ranges from is 1567.05mm-1846.98mm (Metrological Center, Crop Science Department, University of Nigeria, Nsukka). The study lasted for 16 weeks (4 months).

Table 1: Percentage composition of experimental diet

Maize	46.97
Soy bean meal	15.34
Wheat offal	20.13
Palm kernel cake	7.56
Vitamin premix	0.25
Bone meal	3.00
Lime stone	6.00
Methionine	0.20
Lysine	0.20
Salt	0.25
Antisalmonella	0.10
Total	**100**
Proximate compositions of experimental diet	(%)
Crude protein	16.50
Ether extract	6.50
Moisture	15.50
Metabolizable Energy Kcal.kg^{-1}	2530

Sources of experimental materials

Vitamin E that was used for this study was purchased from a reputable drug/supplement dealer in Ogige market Nsukka Enugu State. Feed ingredients used were purchased from Agubata Stores at Orie Orba in Udenu Local Government Area, Enugu State.

Experimental birds and management

A total of Fifty (50) mature local cocks of uniform sizes (weighing about 1.52-1.53kg) were used for the experiment. They were purchased from a local market called Orie orba in Udenu Local Government, Nsukka North, Enugu State. They were reared and managed in a deep litter system. Water and feed were provided *ad libitum*. After stabilizing them together for two weeks, they were randomly assigned into two treatments (T_1 and T_2). Each treatment had 5 replicates with 5 birds per replicate. Each treatment was fed 2kg feed daily. Treatment one diet contained 1000 IU vitamin E kg^{-1} feed (well mixed), while control (T_2) diet had no vitamin E. On arrival, anti-stress drug (vitalyte) was administered to the birds via drinking water. During the pre-experimental period, they were vaccinated against common bacterial and viral infections such as fowl pox disease and also dewormed after two weeks using anti-helminthes drug piperazine. Subsequently, broad spectrum anti-biotic was used intermittently.

Experimental diet

The compositions of trial diets and proximate analysis of the trial diets are shown in **Table 1** The diet was formulated and mixed to contain 16.50% crude protein and metabolizable energy content of 2530Mcal/kg ME respectively.

Semen collection

From each replicate in both treatments, 3 cocks were randomly selected for semen collection two times per week for evaluation. Semen was collected by massage technique

as described by (27). From the semen collected, Semen volume, sperm progressive motility, live spermatozoa, dead spermatozoa, normal sperm cell, abnormal sperm cell and sperm cell concentration were measured and determined. For the semen collection, each cock was held by one person who massaged the dorsal-abdominal region of the bird, while another person carefully waited for ejaculation to collect the semen with clean calibrated test tube.

Method of Semen Analysis

- **Sperm progressive motility**

The motility of spermatozoa was determined by a subjective microscopic method as described by Wishart and Bakst (28).

- **Sperm concentration**

The visual sperm cells count was determined by using Improved Neubauer Haemocytometer and the method described by (29).The Improved Neubauer counting chamber has an area of $1mm^2$ and a depth of 0.1mm.The central area ($1mm^2$) is divided into 25squares,each with an area of $0.04mm^2$ and each of these is further marked into 16 square. The volume of diluents confined between the central square and the cover glass is 0.1 which is equivalent to 0.1 µl (30). The following formula was used as stated as follows:

Sperm concentration $= \frac{N \times DF}{A \times D} \times 10^6$

Where N= Number of cells counted

DF=Dilution Factor= 20= $\frac{Semen+Ethyl\ Alcohol+Sodium\ Citrate}{Semen} = \frac{0.05+0.20+0.75}{0.05}$

A=Area of chamber counted=$0.2mm^2$ and D=Depth of chamber= 0.1mm (30)

- **Live and dead, normal and abnormal spermatozoa**

Live and dead, normal and abnormal spermatozoa were determined by differential staining of fresh semen with eosin-negrosin mixture as described by (31).

- **Semen volume**

Semen was collected with a calibrated glass centrifuge tube and the volume read off directly and recorded in ml

Statistical analysis

The experimental lay out was an independent group sample t-test at probability level of 0.01 and 0.05

Results and Discussion

Table 4 shows the results the effect of dietary inclusion of vitamin E as anti-oxidant on the semen characteristics of local cocks. From the results, values for sperm progressive motility, live sperm cells, dead sperm cells, normal sperm cell and abnormal sperm cells were highly significant ($p<0.01$), while semen volume and sperm concentration values were significant ($p<0.05$). T_2 birds (control) significantly had the highest values for dead sperm cells, abnormal sperm cells and lowest values for semen volume, sperm progressive motility, live sperm cells, normal sperm cells and sperm cell concentration, while T_1 birds significantly recorded the lowest values for dead sperm cells, abnormal sperm cells and the highest values for semen volume, sperm progressive motility, live sperm cells, normal sperm cells and sperm concentration.

Table 4: Effect of the dietary inclusion of vitamin E as anti-oxidant on the characteristics of semen of local cocks.

Parameters	T_1	T_2	P-values
Semen volume(ml)	0.43±0.04	0.32±0.01	0.02*
Sperm progressive motility (%)	100.00±0.00	83.33±2.10	0.00**
Live sperm cells (%)	85.00±2.24	68.33±3.07	0.00**
Dead sperm cells (%)	15.00±2.24	31.67±3.01	0.00**
Normal sperm cells (%)	68.33±3.07	51.67±3.04	0.00**
Abnormal sperm cells (%)	31.67±3.05	48.33±3.04	0.00**
Sperm concentration ($x10^{12}$)	1.85±0.48	1.53±0.00	0.04*

*Mean significant ($p<0.05$), ** means highly significant ($p<0.01$)

Significant increase in the values of abnormal sperm cells, dead sperm cells and reduced values for semen volume, sperm progressive motility, live sperm cells, normal sperm cells and sperm concentration recorded in T_2 birds (control) could be attributed to the low vitamin E in their diet which would have ameliorated or countered the negative activities of reactive oxygen species (ROS) by making sure that the reactive oxygen species (ROS) which are products of body metabolism did no overwhelm or reach a threshold that would have resulted to oxidative stress which is known to affect semen quality negatively (13). However, as far as an animal continues to live, the production of reactive oxygen species which are products of body metabolism is unavoidable, but when the effects and production of these reactive oxygen species are not countered by boosting the animal's anti-oxidant mechanism using anti-oxidant like vitamin E, oxidative stress which brings about damages in semen quality will occur. However, the lower the inclusion of anti-oxidants like vitamin E in diet of an animal, the faster the reactive oxygen species (ROS) rising to that threshold that will result to oxidative stress. Poor semen quality observed for control birds agrees with the documentation in the literatures that oxidative stress reduces gamete numbers, decreases sperm cell motility and increases percentage of dead sperm cells in male poultry semen (15,16) and it occurs when reactive oxygen species (ROS) is in excess of the available antioxidant capacity in the animal cells.

Birds on diet with vitamin E inclusion (T_1) significantly had the highest values for progressive motility, live sperm cells, normal sperm cells, semen volume, sperm cell concentration and lowest values for dead and abnormal sperm cells. This could be attributed to increased level of vitamin E in their diet which to an extent assuaged or countered the negative activities of reactive oxygen species (ROS) which may have triggered oxidative stress that is known to have detrimental effects on semen quality. It is well documented in the literatures that vitamin E inclusion in a balanced poultry ration supports reproductive functions, including sperm cell concentration, sperm cell motility, semen volume, sperm cell viability, and sperm cell integrity in avian species (20,18). The improved semen qualities recorded in this study agrees with the work of (22) that included vitamin E to the diet of male quails and recorded improved percentage fertility due to increased semen volume, concentration, viability, and percentage motility of sperm

cell, as well as decreasing the percentages of dead and abnormal sperm cells. Feeding Lohmann Brown breeder roosters a diet supplemented with vitamin E which eventually enhanced the semen volume and sperm concentration (23) agrees also with the better semen qualities recorded in this current work. Enhanced semen quality recorded in this current work also agree with the work of (24) who added vitamin E to the diet of cockerels and observed that the vitamin E was capable of increasing the percentage of live and normal spermatozoa cells.

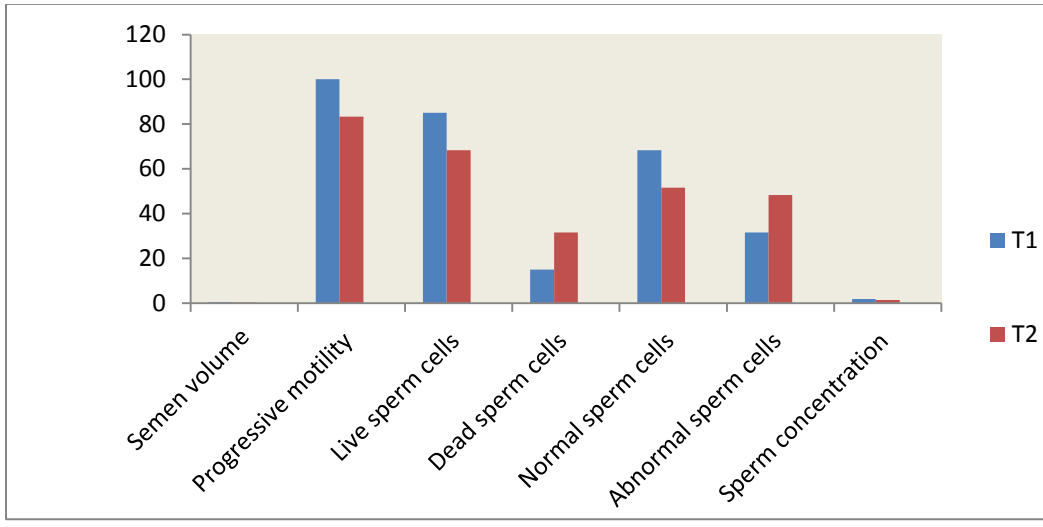

Fig. 1: Effect of vitamin E on semen qualities of local cocks

Conclusion and Applications

1. Birds on vitamin E diet (T_1) had better semen qualities compared to those on control (T_2).
2. Treatment one was recommended because of enhanced progressive motility, live sperm cells, normal sperm cell, and semen volume and sperm concentration were recorded.
3. Also, dead and abnormal sperm cells were significantly lower than that of the control. The better semen qualities recorded with vitamin E inclusion in diet of local cocks suggests that vitamin E can be used by farmers to enhance semen quality in local cocks.

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