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Semen quality and sperm characteristics in broiler breeder cockerels fed vitamin E during hot season

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ABSTRACT. Thirty-two *Arbor Acres* (AA) cockerels aged 27 weeks were used to determine the effect of vitamin E (VE) on the semen characteristics and sperm quality during hot season. The birds were fed diets containing 0, 50, 100 and 150mg VE kg^{-1} feed. There were 4 replicates and two birds per replicate. Semen collection was done thrice a week for three weeks in all birds. Dietary vitamin E significantly ($p < 0.05$) increased the semen volume (SV) and number of sperm cells (NS) in AA cockerels. CONTROL birds (0.71 mL) had lower SV than birds fed 150 mg VE (0.94 mL). Birds on 100 and 150 mg VE recorded similar NS, which was higher than 0 and 50 mg VE groups. The least NS was in the CONTROL group. Sperm abnormality gradually ($p < 0.05$) decreased as dietary VE increased from 0 to 150 mg. Significant ($p < 0.01$) effect of dietary VE was recorded in percentage live sperm (PLS). Semen from cockerels on VE treatment had higher PLS than the CONTROL. In conclusion, it may be beneficial to supplement the broiler breeder cockerel diet with VE up to 150 mg kg^{-1} feed during hot season as it increased SV, NS and PLS and decreased sperm abnormality.

Keywords: cockerel; heat stress; spermatogenesis; sperm abnormality; tocopherol.

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Introduction

Prevailing temperature in the tropics is usually too high for productivity in chickens (Abioja et al., 2012). High environmental temperature negatively affects spermatogenesis in both mammalian (Bah, Chaughari, & Al-Amin, 2001; Ordas, Vahedi, Seidavi, & Rahati, 2015; Garcia-Oliveros et al., 2020) and avian species (Karaca, Parker, & McDaniel, 2002; Attia, El-Naggar, Abou-Shehema, & Abdella, 2019) during heat stress (HS). Poor fertility in broiler breeder eggs is mostly related to male birds, even though females may be responsible for the flock fertility decline (Hocking & Bernard, 2000; Habibian, Ghazi, Moeini, & Abdolmohammadi, 2014). The optimum expression of genetic potential in male breeders requires a thermo-neutral zone of 20-22°C for optimum performance (Cassuce et al., 2013), which is extremely difficult to sustain in poultry pens in the tropics. Fouad et al. (2016) listed reduction in testicular weight, semen volume, sperm concentration, sperm output, spermatids, spermatocytes, spermatogonia, normal and live sperm count and sperm motility as a result of testicular injury caused by overwhelming reactive oxidative species (ROS) generated during HS in poultry species. Polyunsaturated fatty acids present in poultry spermatozoa are extremely susceptible to lipid peroxidation, generating a lot of ROS which are harmful (Ahsan et al., 2014; Surai, 2016). Sperm physiology requires presence of ROS in minimal quantity for proper functioning of acrosome reaction, capacitation and fertilization. However, excessive generation of ROS, such as witnessed under HS conditions portends danger to the membrane of sperms (Khan, Rahman, Javed, & Muhammad, 2012). Sperm output and semen characteristics are affected by large accumulation of ROS. The balance between ROS and scavengers in semen influence the membrane integrity, sperm viability and fertilising ability (Partyka, Lukaszewicz, & Niżański, 2012; Rui et al., 2017). In mammals, excessive levels of ROS have been significantly correlated with decreased sperm motility (Alahmar, 2019). Poultry sperm motility is affected in the same manner when birds experience oxidative perturbations. Meanwhile, sperm motility is an indication of semen quality and sperm viability in male breeder chickens (Sun et al., 2019).

Vitamin E, a group of eight fat soluble compounds comprising four (α, β, γ, δ) tocopherols and four (α, β, γ, δ) tocotrienols (Górnaś, 2015) is a well-known antioxidant. Of the eight vitamin E molecules, α-tocopherol is the richest in nature and one α-tocopherol molecule can neutralize two peroxyl radicals responsible of lipid oxidation (Vincenzo & Vito, 2016). It is therefore considered as the basic part of antioxidants in sperm and acts as the important protection to reduce the production of ROS (Vincenzo & Vito, 2016). Vitamin E could act as a transcriptional regulator in the process of lipid metabolism and oxidation in biological systems, thereby exerting its function (Li, Zhao, Chen, Zheng, & Wen, 2009). Surai and Ionov (1992) stated that the first discovery of Vitamin E (α-tocopherol) in the semen of avian species (turkey) was in 1981; 85% of which is located within the sperm cells and small proportion in the seminal plasma.

Low vitamin E in the body system of poultry may cause damages to reproductive organs involved in spermatogenesis, result in testicular dysfunction seminiferous tubules shrinkage and deformation in spermatozoa. Testicular degeneration in poultry, rats and hamster was reported by Todorovic, Jovic and Davidovic (2004). Surai, Fisinin and Karadas (2016) reported vitamin E deficiency caused lower production of germ cells. Inadequate vitamin E disrupts spermatogenesis because of degeneration of the seminiferous tubules (Wilson et al., 2003). Dietary vitamin E supplementation is reputed to be a common way of reducing lipid peroxidation in tissue and blood plasma of layers (Zduńczyk et al., 2013) and seminal plasma of broiler breeder males (Eid, Ebeid, & Younis, 2006; Surai, Kochish, Romanov, & Griffin, 2019). It is an excellent biological chain-breaking antioxidant that protects bio-membranes from peroxidative injury caused by ROS. Sperm membrane is made up of lipids, which are high in polyunsaturated fatty acids, as constituent. These lipids play major roles in sperm maturation, capacitation and acrosome reaction. The involvement of lipids in these vital reproductive processes generates large amount of ROS. Vitamin E works as an antioxidant by restrictin and preventing chain formation thereby keeping the chain length of the free radicals as short as possible (Surai, 2003). It effectively scavenges the ROS producing stable ROOH groups and reduced susceptibility of sperm cells lipid peroxidation caused by FE^{2+} (Lin, Chang, Yang, Lee, & Hsu, 2005). In a review on vitamin E in poultry species, Rengaraj and Hong (2015) stated that vitamin E in feed of male poultry helps in maintenance of sperm quality through decreasing lipid peroxidation in semen. Zduńczyk et al. (2013) used 30 and 60 mg kg^{-1} feed in laying chickens and recommended that the dose should be increased. Wang, Wang, Barton, Murphy, and Huang (2007) stated that antioxidant enzymes like superoxide dismutase and glutathione peroxidase, which act as ROS scavengers, are increased by vitamin E. Intramuscular injection of vitamin E with gonadotropin releasing hormone analogue was reported to improve reproductive performance and serum testosterone in post-molt male broiler breeder chickens (Hezarjaribi, Rezaeipour, & Abdollahpour, 2016). However, prolonged usage of vitamin E and ultra-high dosage may pose a threat and exhibit marks of toxicity. Danikowski, Sallmann, Halle and Flachowsky (2002) that fed chickens for 12 months with vitamin E in the upward of 20,000 IU α-tocopherol kg^{-1} feed reported that the reproductive performance of cocks was negatively influenced by high doses of vitamin E although decreased TBARS indicated rising oxidative defence. Low sperm count, sperm quality and testicular weight were observed in male chicken fed high level of vitamin E. Panda and Cherian (2014) supplemented feed with dosage of vitamin E After the dosage of 125 mg kg^{-1} feed, the efficiency of vitamin E There are lot of information about the use of vitamin E in various doses through drinking water as antioxidants in breeder birds (Wang et al., 2007). However, there are scanty investigations on dietary inclusion of vitamin E as an antioxidant for *Arbor acres* breeder birds hence the amount incorporated in the commercial feeds does not always yield the desired result. Moreover, birds may need more supplemental antioxidants during hot season when the stress threshold is higher. Therefore, the present study aimed at determining the appropriate dosage of vitamin E for broiler breeder cockerels during hot dry season.

Material and methods

Experimental location and meteorological observations:

The study was carried out at the Poultry Unit of University Farms, Federal University of Agriculture, Abeokuta, Nigeria (latitude 7º 13'N; longitude 3º 26'E (Google Earth, 2020) and altitude 76 m above sea level). Data on pen temperature and relative humidity in different units were monitored using thermo-hygrometer at 08:00, 13:00 and 17:00h daily. Temperature-humidity index (THI) was calculated.

Animal welfare statement

The experimental procedure has been approved by the Animal Experimental Board of the Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta Nigeria. Also, the Guideline for Animal Research of Nigeria Institute of Animal Science (NIAS) was followed.

Animals and management:

Thirty two *Arbor acres* broiler breeder cockerels managed according to the manual from the industry were allotted to four treatment groups of 8 birds per treatment. Birds in Treatment I received diet containing no extra vitamin E (dL-α-tocopheryl acetate; VE) while Treatment II, III and IV received dietary treatment with additional 50, 100 and 150 mg VE kg^{-1} feed respectively, according to Biswas, Mohan and Sastry (2009) that recommended 150 mg tocopherol per kg feed. The composition of experimental diet is shown in Table 1. Two cockerels were placed in each battery cage unit. Water was made available *ad libitum*.

Table 1. Composition of experimental diet.

Ingredient	Composition
Maize	47.8
Fish meal (72% CP)	1.3
Soybean	5.5
Wheat offal	41
Bone meal	1.6
Limestone	1.8
Lysine	0.2
Methionine	0.2
*Premix	0.3
Salt	0.3
Total	100
Calculated:	
Crude protein (%)	14.01
Metabolizable energy (Kcal)	2749
Crude fat (%)	3.68
Crude fibre (%)	4.8
Calcium (%)	1.13
Phosphorus (%)	0.32

*1 kg of feed contains: Vit A: 6,000 IU; Vit D3: 2,000 IU; Vit E: 10 mg kg^{-1}; Vit K3: 1.6 mg kg^{-1}; Vit B1: 1.4 mg kg^{-1}; Vit B2: 4 mg kg^{-1}; Vit B6: 2 mg kg^{-1}; Vit B12: 0.01 mg kg^{-1}; Niacin: 30 mg kg^{-1}; Folic acid: 11 mg kg^{-1}; Choline: 0.24 g kg^{-1}; Zn: 50 mg kg^{-1}; Fe: 50 mg kg^{-1}; Mn: 50 mg kg^{-1}; Cu: 100 mg kg^{-1}; I: 1.2 mg kg^{-1}; Se: 0.2 mg kg^{-1}.

Semen collection

Before the commencement of semen sampling, the cockerels were trained for a period of two weeks for semen collection. Semen sampling by abdominal massage method took place when the birds were 27 weeks of age. This was done thrice a week for four weeks consecutively into labelled *Eppendorf* tubes by 08.00h. Laboratory examination of the semen was done immediately.

Semen analyses

Semen volume was measured with graduated tubes. **S**emen concentration was measured using the direct cell count method with haemocytometer. Semen was diluted with normal saline with dilution rate of 1:250 and loaded into the counting chambers of haemocytometer. The counting chambers are 0.1 mm in depth and have ruled area on the bottom of the chambers that is 1.0 squared mm, the square is sub-divided into 25 smaller squares. Semen concentration was taken as the product of number of sperm counted, dilution rate and constant (50,000) as described by Peters et al. (2008). The sperm count was taken as a product of semen volume and concentration. Semen sample (5 µL) was placed directly on a heated microscope slide and overlay with a 22x22 mm cover slip and viewed under light microscope (*Celestron PentaView* microscope, LCD-44348, RoHS, China) x400 magnification. Each sample was viewed twice (2 slides per sample) and four microscopic fields were examined to observe progressive sperm motility (sperm that move forward essentially in a straight line) and progressively motile spermatozoa was counted repeatedly and the mean of five successive evaluations was recorded as the final motility score according to Ax, Dally, Didion, and Lenz (2000). Semen malondialdehyde (MDA) concentration was measured in a thiobarbituric acid reactive substance (TBARS).

Sperm sample (0.1 mL) was incubated with 0.1 mL of 150 mM Tris HCl (pH 7.1) for 20 minutes at 37°C. Subsequently, 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.375% thiobarbituric acid (TBARS) was added. The mixture was incubated in a boiling water bath for 30 minutes and centrifuged for 15 minutes at 3000rpm inside the blank tube and the absorbance level was read with a spectrophotometer (SW7504 model by Surgifriend Medicals, England) at 532 nm. MDA concentration was taken as the difference between absorbance of semen sample and the blank divided by molar absorptivity of MDA ($1.56x10^5$). Arginase activity is spectrophotometrically determined using the protein concentration method according to the procedure of Lowry, Rosebrough, Farr and Randall (1951).

A thin smear of mixture of semen and eosin-nigrosin solution was drawn across slides for the respective treatments and dried. The percentage of morphologically abnormal spermatozoa with defects in the head, mid piece and tail was observed under Celestron PenView LCD microscope (x400 magnifications) and recorded. The calculation of sperm abnormality is based on the percentage of morphologically abnormal spermatozoa through differential staining with eosin-nigrosin stain (Blesbois, 2007). A thin smear of mixture of semen and eosin-negrosin solution was drawn across the slide and dried. The samples were observed under Celestron PenView LCD microscope (x 400 magnifications) for live and dead spermatozoa. Spermatozoa that appeared white were regarded as live while those that picked up the stain were regarded as dead spermatozoa. The calculation of viable sperm is based on the percentage of live spermatozoa through differential staining with eosin-negrosin stain (Blesbois, 2007). Hypo-osmotic Swelling Test (HOST) assay was used to determine sperm membrane integrity. This was done by incubating 10 µL semen in 100 µL Hypo-osmotic solution (7.35 g sodium citrate [0.0285M] and 13.5 g fructose [0.075M]) at 37°C for 30 minutes; 0.1 mL of the mixture was spread over a warmed slide, covered with slip and observed under Celestron PenView LCD digital microscope (400 x magnifications). 200 spermatozoa were counted and the percentage of spermatozoa positive to HOST (characterized by curled tails, indicating intact plasma membrane) was determined and those with no swelling (characterized by uncurled tails) were classified as spermatozoa with abnormal membrane integrity.

Statistical analyses:

Data collected on sperm motility, semen concentration, malondialdehyde concentration and arginase activity, semen volume, number of sperm cells, total, head, mid-piece and tail abnormality and percentage live sperm were subjected to one-way analysis of variance using Statistical Analysis System [SAS] (2002) computer statistical package. Means that are significantly different were separated with Tukey's Studentized range test.

Results

Table 2 shows the summary of climatic observations in the pens during the experiment. The mean temperature, relative humidity and THI were 31.4°C, 80.7 and 98.8% respectively. Effect of dietary vitamin E on semen characteristics and oxidative status is presented in Table 3. Sperm motility, semen concentration, malondialdehyde concentration and arginase activity were not significantly ($p > 0.05$) affected by vitamin E treatment. Dietary vitamin E significantly ($p < 0.05$) increased the semen volume (SV) and number of sperm cells (NS) in AA cockerels. CONTROL birds (0.71 mL) had lower SV than birds fed 150 mg VE (0.94 ml). Birds on 100 and 150 mg VE recorded similar NS, which was higher than 0 and 50 mg VE groups. The least NS was in the CONTROL group.

Table 2. Summary of climatic observations during the experiment.

Factor	Average
Minimum temperature (°C)	26.3
Maximum temperature (°C)	36.5
Mean temperature (°C)	31.4
Relative humidity (%)	80.7
Temperature-humidity index	98.8

Table 4 shows the effect of dietary vitamin E on the sperm abnormality and quality in broiler breeder cockerels. Sperm abnormality gradually ($p < 0.05$) decreased as dietary VE increased from 0 to 150 mg. CONTROL birds and those on 50 mg VE had similar abnormality of head, mid-piece and tail. These were lower than the obtained values in 150 mg VE group. Similar result is observed in total abnormality, where the values

followed the pattern: 0 = 50 < 100 < 150 mg VE. Significant ($p < 0.01$) dietary VE effect was recorded in pencentage live sperm (PLS). Semen from cockerels on VE treatment groups had higher PLS than the CONTROL. Effect of VE was not significant ($p > 0.05$) on sperm membrane integrity. There was no significant ($p > 0.05$) effect of dietary vitamin E on sperm membrane integrity.

Table 3. Effect of dietary vitamin E on semen characteristics and oxidative status in *Arbor Acres* cockerels.

Parameter	Vitamin E (mg kg^{-1} feed)			
	0	50	100	150
Motility (%)	79.1± 2.50	81.0±1.74	81.3±1.73	84.0±1.36
Semen volume (mL)	0.71±0.090^{b}	0.83±0.065^{ab}	0.90±0.034^{ab}	0.94±0.082^{a}
Concentration (x10^{9} mL^{-1})	2.0±0.21	2.18±0.25	2.67±0.55	2.68±0.40
Number of spermatozoa	1.42±0.081^{c}	1.81±0.106^{b}	2.40±0.058^{a}	2.52±0.070^{a}
MDAconcentration (nmol mL^{-1})	2.01±0.58	1.55±0.27	1.40±0.26	1.48±0.25
Arginase activity (nmol mL^{-1})	1.86±0.41	1.63± 0.31	1.51±0.21	1.32±0.26

^{a,b,c}Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Effect of dietary vitamin E on sperm abnormality and quality in *Arbor Acres* cockerels.

Parameter	Vitamin E (mg kg^{-1} feed)			
	0	50	100	150
Abnormal head (%)	16.4±1.53^{a}	15.9±1.49^{a}	9.9±1.10^{b}	7.9±0.82^{b}
Abnormal mid-piece (%)	10.2±1.01^{a}	10.2±0.89^{a}	7.8±0.85^{ab}	5.7±0.61^{b}
Abnormal tail (%)	6.0±0.80^{a}	5.9±1.01^{a}	4.8±0.76^{ab}	3.3±0.60^{b}
Total (%)	11.0±0.82^{a}	10.3±0.99^{a}	7.8±0.75^{b}	5.6±0.59^{c}
Live sperm (%)	86.8±2.34^{b}	95.4±2.42^{a}	96.1±2.46^{a}	96.9±2.45^{a}
Membrane integrity (%)	47.7±4.12	49.0±3.90	50.4±3.86	52.3±4.21

^{a,b}Means in the same row with different superscripts differ significantly ($P<0.05$)

Discussion

In this study, the mean temperature and THI recorded (31.4 and 98.8°C respectively) were high because the experiment took place during hot season (Abioja et al., 2020). Elevated environmental temperature imposes stress on chickens (Al-Fataftah & Abu-Dieyeh, 2007).

In the present study, dietary vitamin E supplementation up to 150 mg kg^{-1} feed did not elicit any improvement in sperm motility in young male chickens. Mohamad Asrol and Abdul Rashid (2017) obtained similar result in gross and individual sperm motility in Kampong roosters even with supplemental vitamin E up to 400 IU (180 mg). Vitamin E did not improve sperm motility in Indian reared Kadaknath cockerels (Biswas et al., 2009). This is in contrary to the finding in *in vivo* studies in human reported by Keskes-Ammar et al. (2003) that supplementation with vitamin E increased motility of sperms caused by ROS. Surai et al. (2016) reported that dietary vitamin E increased sperm motility in chickens. The insignificant effect of vitamin E on progressive sperm motility may be adduced to low dosage used in this study.

Semen volume increased with increase in dosage of vitamin E in the diet of cockerels in present study. However, the concentration was similar in the treatment group. Semen comprises of spermatozoa and seminal fluid. Seminal fluid in chicken, a protein-rich fluid from testis, rudimentary epididymis and cloacal gland (Alvarez-Rodriguez et al., 2020), mixes with the sperm cells to give the semen its volume. It shows that increase in volume was not due to increase in seminal plasma but in the number of sperm cells. Sperm count was increased in dosage-dependent manner by supplemental dietary vitamin E. This disagrees with the report of Biswas et al. (2009) that vitamin E did not change semen volume and sperm concentration in Kadaknath cockerels. Increase in sperm count may be taken as an indication of elevated spermatogenic efficiency in the treated group. The level of plasma testosterone in male quails was increased by dietary vitamin E (Abedi, Vakili, Mamouel, & Aghaei, 2016).

There was similarity in the semen MDA concentration and arginase activity in all the treatment groups. Malondialdehyde is the end production of lipid peroxidation. The obtained result in this experiment is contrary to the findings that the reduction in MDA is elicited by vitamin E in the feed of cocks (Surai et al., 2016). Sahin, Sahin, Orderci, Yaralioglu and Kucuk (2001) and Surai et al. (2016) reported in contrast to the present finding that vitamin E decreased semen MDA concentration. The reason for no significant result may be because of strain differences. Other reasons may be because the highest dosage of vitamin E employed in this study (150 mg kg^{-1} feed) was too low to elicit a significant response in the concentration of

malondialdeyde. Semen arginase activity is related to sperm count and motility in humans (Elgün, Kaçmaz, Sen, & Durak, 2000), rams (Gür & Kandemir, 2012) and goats (Türk, Gür, Kandemir, & Sönmez, 2011; Daramola & Adekunle, 2017). Oxidative perturbations exhibited by administration of dexamethasone in ram caused a decreased arginase activity in ram semen (Kaya et al., 2020). In fact, arginase activity in seminal plasma of mammalian males could be used as a biochemical criterion to indicate the quality of semen. However, its involvement in avian semen is not fully known for there is a dearth of information on this. In the present study, unlike in mammals, dietary vitamin E did not affect seminal plasma arginase activity. Therefore, possibility of dietary vitamin E in influencing arginase activity in chicken semen is doubtful. Vitamin E in feed of broiler breeder cockerels gradually decreased sperm abnormality in dose-dependent manner in the present study. Similar to this, Biswas et al. (2009) found that vitamin E in feed lowered abnormality in sperm cells of Kadaknath cockerels. Vitamin E has a broad spectrum of function in animal body systems with crucial efficiency in reproductive success. Its deficiency has been noted to cause high percentage of morphologically abnormal sperm cells in male animals (Rengaraj & Hong, 2015). Mohamad Asrol and Abdul Rashid (2017) reported that administering 400 IU vitamin E in feed of Kampong roosters lowered sperm tail abnormality after 4 weeks of administration. Percentage live sperm in semen was improved by supplementation with vitamin E. This falls in line with the report of Mohamad Asrol and Abdul Rashid (2017), though other author, Biswas et al. (2009) did not obtained significant difference in percentage live sperm. It is known that the availability of Vitamin E enhances the sperm mitochondrial functions and causes reduction in sperm membrane lipid peroxidation thereby increasing sperm membrane integrity (Asl, Shariatmadari, Sharafi, Torshizi, & Shahverdi, 2018). However, supplementation with 150 mg vitamin E per kg feed had no effect on the sperm membrane integrity. The reason may be that the cockerels still have capacity to utilize higher doses of vitamin E.

Conclusion

In conclusion, it may be beneficial to supplement the diet of broiler breeder cockerels with VE up to 150 mg kg^{-1} feed during hot season as it increased semen volume, number of sperm cells in ejaculate and percentage live sperm and decreased total, head, mid-piece and tail abnormality in spermatozoa.

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