Effect of dosage of vitamin E on physiological and blood parameters in growing Arbor acre pullets under humid tropical conditions

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Abstract

One hundred and forty-four growing Arbor acre broiler breeder pullets were used to determine the effect of vitamin E (dL- α -tocopheryl acetate; VE) on physiological and blood responses. The birds aged 21 days were randomly assigned to 4 treatments comprising 0, 50, 100, 150mg VE/kg of diet, with 3 replicates per treatment till day 84. Data obtained for rectal temperature (RT), heart rate (HR), respiratory rate (RR), skin temperature under wing (STW) and on the breast (STB), haematology and plasma biochemistry were subjected to analysis of variance. In female chickens, VE significantly ($p < 0.05$) affected HR, STW, WBC, heterophil, lymphocyte and H/L ratio but did not affect RT, RR, STB, PCV, RBC, Hb concentration, eosinophil, monocyte, basophil, MCV, MCH, MCHC and biochemical parameters. Only pullets on 100mg/kg VE had lower HR than the control group. For STW, 50mg/kg VE had lower value than the control. VE, irrespective of dosage, increased ($p < 0.05$) WBC and lymphocyte in pullets compared to control group. Except in 100mg/kg feed group, heterophil and H/L ratio in pullets on 50 and 150mg/kg feed recorded lower values than the control. VE administration of at least 50mg/kg in the diet of growing broiler breeder chickens might help in decreasing skin temperature, improving immunity and reducing stress under hot conditions.

Keywords: tocopherol; heat stress; broiler breeder; blood; haematology; plasma biochemistry

Introduction

It is well established that stress has detrimental effects on growth, immunity and performance of poultry species (Ajakaiye *et al.*, 2010; Olubodun *et al.*, 2015). Heat stress (HS), which ensues under high environmental temperature, leads to economic losses. Reports on detrimental effects of HS on different classes of chickens are commonly found in literatures (Ayo *et al.*, 2011; Fouad *et al.*, 2016; Zaboli *et al.*, 2019). However, growing broiler breeder chickens had received little attention among other classes of chickens. Broiler breeder chickens are known to be more susceptible to HS than strains of chickens. HS generates a lot of

free reactive oxygen species (ROS) called radicals. Free radicals react with polyunsaturated fatty acids (PUFAs) in biological bi-layered membranes to initiate a chain-reaction process known as lipid peroxidation in living systems thereby compromising the membrane integrity (Estévez, 2010). Vitamin E, a good chain-breaking natural antioxidant is reputed for scavenging the ROS that cause oxidation in the biological membranes. Unlike other vitamins, it is not synthesized in chickens. Its use has been reported in various classes of chickens (Sahin *et al.*, 2010; Guetchom *et al.*, 2012), but little is known of its effects in young broiler breeder chickens reared under tropical environment. Therefore, the

present study aimed at determining the effect of dietary vitamin E on physiological and blood parameters in growing broiler breeder chickens under humid tropical conditions.

Materials 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 7° 13'N; longitude 3° 26'E (Google Earth, 2017) 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 using formula of Tao and Xin (2003).

$THI = 0.85 T_{db} + 0.15 T_{wb}$, where THI is temperature-humidity index; T_{db} is the dry-bulb temperature in °C and T_{wb} is the wet-bulb temperature °C. Effective temperature was read off psychrometric chart (Ferdeber and Houghten, 1941).

Animals and management

One hundred and forty-four, 21 day-old *Arbor acre* female broiler breeder birds managed according to the manual from the industry were allotted to four treatment groups. Birds in Treatment I received diet containing no vitamin E (dL- α -tocopheryl acetate; VE) while Treatment II, III and IV received dietary treatment with 50, 100 and 150mg VE/kg feed, respectively in an experiment that last till day 84. There were three replicates and 12 birds per replicate. Commercial diet was used for the birds at different stages of growth. Water was made available *ad libitum*.

Data collection

Physiological responses

Rectal temperature (RT) of birds (n=2) from each replicate was measured with *Jorita*

digital thermometer (model: ECT-5; 0.1°C accuracy). Respiratory rate (RR) of the birds was taken as the number of breaths per minute by counting the flank movement. Stethoscope was used to take the heart rate (HR) of the birds. Non-contact infra-red thermometer was used to take the skin temperature under wings (STW) and on the breast (STB) within 15cm from the bird. All measurement of physiological responses was taken twice weekly from d21 – d84 between 14.00 and 16.00 h.

Blood sampling and analyses

Blood samples were obtained from the chickens on d28, 56 and 84 via the wing web into heparinized and blank tubes for haematology and plasma biochemistry, respectively. Parameters determined include PCV, RBC, Hb concentration, WBC, differential count, H/L ratio, total plasma protein, albumin, globulin, uric acid, creatine kinase and creatinine. MCV, MCH and MCHC were calculated.

Statistical analyses

Data collected on body temperature, respiratory, heart rates, haematological and plasma biochemical parameters were subjected to one-way analysis of variance using SAS (2002) computer statistical package. Means that are significantly different were separated with Tukey's Studentized range test.

Results and discussion

Table 1 shows the summary of the meteorological observations during the experiment. The average pen temperature, relative humidity, THI and effective temperature was 28.2°C, 79.6%, 80 and 33°C, respectively. Result of physiological responses in growing broiler breeder pullets as affected by vitamin E treatment is presented in Table 2. VE did not significantly ($p>0.05$) affect RT, RR and STB in pullets. However, HR in pullets was significantly ($p<0.05$) affected by VE.

Pullets on 100mg/kg VE had lower HR than the control group. Birds on 50mg/kg VE had lower STW than the control and 150mg/kg VE in pullets, though similar to 100mg/kg VE.

Table 1: Summary of meteorological observations during the experiment

Climatic factor	0800h	1300h	1700h	Average
Ambient temperature (°C)	25.7±1.76	30.9±2.57	28.8±2.81	28.2±3.20
Relative humidity (%)	80.3±7.48	62.6±7.65	66.3±9.41	79.6±8.41
Temperature-humidity index	75.8	81.2	78.9	80.0
Effective temperature (°C)	27	35	32	33

Table 2: Effect of vitamin E on the physiological parameters in growing broiler breeder pullets under humid tropical conditions

Parameter	Vitamin E (mg/kg feed)				sem	p
	0	50	100	150		
Rectal temperature (°C)	41.2	41.1	41.1	41.1	0.05	0.627
Heart rate (beats/min)	238.8 ^a	234.5 ^{ab}	230.3 ^b	235.1 ^{ab}	1.86	0.015
Respiratory rate (breaths/min)	42.7	43.4	40.9	42.5	0.86	0.203
Skin temperature on breast (°C)	37.0	37.0	37.3	37.1	0.14	0.526
Skin temperature under wings (°C)	40.5 ^a	40.1 ^b	40.3 ^{ab}	40.5 ^a	0.04	0.011

^{a,b}Means within the same row with similar superscripts differ significantly (P<0.05)

Table 3 and Figures 1-3 show the effect of vitamin E on haematological parameters in growing breeder pullets. WBC, heterophil, lymphocyte and H/L ratio were significantly (p<0.05) affected by vitamin E in female birds. VE increased (p<0.05) WBC and lymphocyte in pullets compared to control group. Except in 100mg/kg feed group, heterophil and H/L ratio in 50 and

150mg/kg feed groups recorded higher values than the control. Effect of vitamin E on biochemical parameters in growing breeder pullets is shown in Table 4. Plasma biochemical responses were not significantly (p>0.05) affected by different dosages of vitamin E. Similar results were obtained in broiler breeder pullets for plasma biochemistry.

Table 3: Effect of vitamin E on the haematological parameters in growing broiler breeder pullets under humid tropical conditions

Parameter	Vitamin E (mg/kg feed)				Sem	P
	0	50	100	150		
Packed cell volume (%)	34.2	35.1	36.2	35.3	0.90	0.903
Red blood cell (x10 ¹² /l)	3.17	3.30	3.33	3.54	0.10	0.634
Haemoglobin conc. (g/dl)	11.6	12.0	12.0	12.0	0.29	0.968
White blood cell (x10 ⁹ /l)	1.93 ^b	4.24 ^a	4.40 ^a	4.18 ^a	0.80	0.016
Eosinophil (%)	1.83	2.28	2.28	1.82	0.120	0.327
Monocyte (%)	2.67	2.33	3.00	2.29	0.141	0.255
Basophil (%)	0.11	0.11	0.17	0.12	0.053	0.979
MCV (fL)	112.3	127.3	120.8	107.9	6.86	0.767
MCH (pg)	38.0	45.3	39.1	33.2	2.48	0.412
MCHC (g/dL)	34.1	35.0	34.1	34.0	0.69	0.951

^{a,b}Means within the same row with similar superscripts differ significantly (P<0.05)

Effect of dosage of vitamin E on physiological and blood parameters

^{a,b}Means represented by the bar with different superscripts differ significantly ($P<0.05$)

Figure 1. Effect of vitamin E on heterophil in broiler breeder pullets

^{a,b}Means represented by the bar with different superscripts differ significantly ($P<0.05$)

Figure 2. Effect of vitamin E on lymphocyte in broiler breeder pullets

^{a,b}Means represented by the bar with different superscripts differ significantly ($P<0.05$)

Figure 3. Effect of vitamin E on heterophil-lymphocyte ratio in broiler breeder pullets

Table 4: Effect of vitamin E on the biochemical parameters in growing broiler breeder pullets under humid tropical conditions

Parameter	Vitamin E (mg/kg feed)				Sem	P
	0	50	100	150		
Total protein (g/dl)	5.3	5.8	5.1	5.8	0.23	0.622
Albumin (g/dl)	3.9	4.4	3.6	4.2	0.22	0.536
Globulin (g/dl)	1.4	1.4	1.5	1.5	0.05	0.829
Uric acid (mg/dl)	6.4	6.9	5.8	6.3	0.43	0.837
Creatine kinase (U/l)	0.76	0.86	0.83	0.80	0.062	0.950
Creatinine (mg/dl)	1.49	1.45	1.65	1.58	0.157	0.974

The mean climatic measurements during the experimental period showed that the birds were continually under stressful conditions. Broiler chickens have the thermal comfort zone within 18 and 22°C (Charles, 2002). Meanwhile, the present study was carried out under mean temperature which is 6.2°C above the upper critical limit, which is sufficient to subject the birds to heat stress. Changes in blood cells, in terms of morphological and quantity, are associated with heat stress, which reflect in packed cell volume, leukocyte count, red blood cell count and haemoglobin concentration (Roll *et al.*, 2010). Laboratory analysis changes in hematological parameters of heterophil/lymphocyte ratio (H/L) serves as a good indicator of stress factors (Altan *et al.*, 2000; Huff *et al.*, 2005). The heterophil-lymphocyte ratio ranging from 0.72 and 1.17 is a good pointer. Birds with heterophil to lymphocyte ratio of about 0.2, 0.5 and 0.8 are known to be under low, optimal and high degrees of stress, respectively in chickens (Gross and Siegel, 1983). Vitamin E supplementation in diet reduced the heart rate in growing female breeder chicken. Birds on 100mg VE per kg feed recorded 3.7% lower heart rate than the group that received no VE. Vitamin E is a major chain-breaking anti-oxidant in living systems, functioning in biological membranes (Surai *et al.*, 2016). Its involvement in thermoregulation and the cardio-vascular responses in growing breeder chickens have not been fully understood. However, it has

been reported that accumulation of free radical may lead to endothelial damage in cells of the heart (Arab *et al.*, 2006), causing the heart to work more. It is known as well that one of the responses of chicken to heat stress is the stimulation of cardiovascular system, leading to vasodilatation. Skin temperature under wings is closely related with the core body. Lowered skin temperature in birds fed vitamin E is an indication of efficient thermoregulation in female birds that received 50mg VE/kg feed. Supplemental vitamin E had no effect on PCV, RBC, Hb concentration, red blood cell derivatives, and some differential counts eosinophil, monocyte and basophil of both pullets and cockerels. Similar results were obtained in broiler chickens by other authors (Tras *et al.*, 2000). WBC, heterophil, lymphocyte and H:L ratio were affected by vitamin E. Vitamin E is known to reduce cytotoxic action caused by free radicals in the living cells and improves the phagocytic activity of macrophages in broiler chickens (Leshchinsky and Klasing, 2001). Leukocytes and the differentials are usually affected by high temperature and immunity of chickens is compromised under heat stress episodes. Heterophil in avian class, as neutrophil in mammals, has the phagocytic function (Qureshi *et al.*, 1998). This multiplies and proliferates during heat spell while lymphocyte decline in number. The use of vitamin E in the present study reduced heterophil count and increase lymphocyte, thereby lowering the

H:L ratio. In another study similar to the present one, VE raised WBC and lymphocyte, and lowered heterophil, H:L ratio, in Control group of layer hens (Ajakaiye *et al.*, 2010). HS promotes the release of catecholamines and corticosteroids that induce lipid peroxidation of membranes, including membranes of T and B lymphocytes (Silva *et al.*, 2011). Antioxidant supplementation in the diets stimulates immunity in avian class. In the present study, VE did not affect any of the plasma biochemical parameters considered. This is contrary to the previous reports. However, most of the reports were not in growing breeder chickens.

Conclusion

Vitamin E supplementation of at least 50mg/kg feed in the diet of growing broiler breeder chickens increased WBC and lymphocytes and decreased skin temperature, heterophil and H:L ratio and so may help improved immunity during hot period in the humid tropical environment.

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