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ORIGINAL RESEARCH ARTICLE

Effects of tocopherol and age on physiological and blood parameters in growing broiler breeder cocks under humid tropical conditions

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Abstract

This study evaluated the effect of tocopherol and age on the physiological and blood parameters of growing broiler breeder cocks. Day-old chicks (n=36) were randomly assigned to 4 treatments consisting 0, 50, 100, 150 mg tocopherol/kg feed. The experiment lasted for 12 weeks, phased into three (1-4, 5-8 and 9-12 weeks). Blood sampling for haematology and plasma biochemistry was done at age 4, 8 and 12 weeks. Data obtained were subjected to ANOVA in 4x3 factorial arrangement. Tocopherol had no significant (P>0.05) influence on heart (HR), respiratory rate (RR) and body temperature. However, age affected HR (P<0.001), RR (P<0.001), and breast (BT; P<0.001) and wing temperature (WT; P<0.05). Older cockerels had higher HR than the younger ones. RR decreased as age increased. Cockerels of 1-4 weeks had higher BT and WT than 5-8 weeks. Eosinophil (EOS) and monocyte were significantly (P<0.01) affected by tocopherol. EOS was lower in 50 mg/kg than others, except 100 mg/kg. Tocopherol decreased monocyte in dose-dependent manner. Age had significant effect on PCV (P<0.001), WBC (P<0.01) and EOS (P<0.001). Younger (4 weeks) cockerels exhibited higher PCV than older ones (8 and 12 weeks). WBC was higher in 4-week-old than in 12-week-old cockerels. Eight weeks cockerels had higher EOS than others. There was no significant (P>0.05) effect of dietary tocopherol on plasma biochemical parameters in cockerels but age affected plasma total protein (P<0.05), albumin (P<0.05), uric acid (P<0.001), creatine kinase (P<0.001) and creatinine (P<0.001). Eight-weeks-old cockerels had lower TP, ALB and CRE than 12-weeks-old. However, higher URI and CK activity were recorded in 8 than in 12-weeks-old. Interaction between tocopherol and age was not (P>0.05) significant in the physiological, haematological and biochemical parameters measured. In conclusion, 50mg tocopherol/kg feed may be efficacious in reducing skin temperature. Age as a factor affects thermoregulation and blood constituents in growing broiler breeder cockerels.

Keywords: Broiler breeder; vitamin E; heat stress; blood metabolites

INTRODUCTION

Responses exhibited by animals under high ambient temperature when the capacity to maintain body temperature is lost is known as heat stress (HS). This is a major recurring problem in poultry production under tropical conditions. High ambient temperature in the tropics is further exacerbated by climate change. One of the most important indicators of the climate change is the global warming (IPCC, 2007). It is expected that by 2050 the average temperature of the globe would have increased by 2°C. The last three hottest years in Africa (2010, 2016, and 2017) occurred in the last decade (ACMAD, 2017). Subjecting chickens to high environmental temperature above thermo-neutral zone causes negative influences including poor health and welfare, digestive inefficiency, reduced growth rate, lowered meat quality, lowered immunity and poor productivity in chickens (Lara and Rostagno, 2013; Song and King, 2015). Heat stress increases body temperature, respiration/panting rate, heart rate and causes changes in leucocyte number and other haematological parameters (Garriga et al., 2006).

Besides, HS has been reported to result in generation of large amount of reactive oxygen species (ROS; known as free radicals) that overwhelm the natural antioxidant systems in chickens (Mujahid et al., 2007). 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, 2015). Supplying tocopherol (also known as vitamin E), a well-known fat-soluble, natural chain-breaking antioxidant in biological systems and living cells has been found to be beneficial. Its use and efficacy in combating oxidative stress had been reported in chickens (Sahin et al., 2010; Guetchom et al., 2012). Researchers had reported much on the usefulness of tocopherol on immunity, egg production, fertility and hatchability in laying breeder chickens and other poultry species (Sahin et al., 2002a; 2010). Zduńczyk et al. (2013) reported during 10 weeks experiment involving tocopherol and selenium that the body weights of hens and egg production were not affected by dietary treatments. Higher selenium content of diets in the above study (Zduńczyk et al. (2013) contributed to a significant increase in egg weight. However, tocopherol significantly increased serum α-tocopherol concentrations, superoxide dismutase (SOD) activity, the ferric reducing ability (FRAP) and immunoglobulin A (IgA) concentrations. Numerous studies exist in literature involving the use of dietary tocopherol in commercial broiler chickens but little is known of its effects in young broiler breeder chickens reared under tropical environment. Of the eight forms of tocopherol (tocopherol and tocotrienol with four presentations (α, β, γ, δ) each) in nature, α-tocopherol is the most biologically active metabolite in living cells (Dalólio et al., 2015). A molecule of α-tocopherol can trap two hydroxyl molecules. Tocopherol is not synthesized in chickens. Addition of tocopherol to the conventional diet of young broiler chickens was found to increase plasma tocopherol and mildly decrease the number of damaged fibers in the pectoral muscle (Guetchom et al., 2012). However, there is dearth of literature report on its use in growing breeder birds under HS conditions. It is only of recent that a work was published on the effect of dietary tocoperol on the broiler breeder pullet of 3 to 12 weeks of age (Abioja et al., 2019a). Intensive genetic development recently obtained in broiler breeding has led to emergence of strains of chickens that attain market weight in shorter time span but with less thermotolerance and poor antioxidant status (Brossi et al., 2009). Infertility emanating from male line has been reported to contribute to low egg fertility and hatchability. However, the question of toxicity level of tocopherol in the diet of chickens may be raised. Literatures had reported no toxicity level in breeder chickens up to 1000 mg/kg feed, except the hypervitaminosis reported by Sünder et al. (1999), when laying hens were dosed 100,000 and 200,000 mg/kg feed for 20 weeks. Therefore, the present study aimed at determining the effect of dietary tocopherol on physiological and blood parameters in growing broiler breeder cockerels under humid tropical conditions.

MATERIALS AND METHODS

Experimental location and meteorological observation

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 and altitude 76 m above sea level). The location falls within the humid tropical rain forest of the South-western Nigeria. Pen temperature during brooding was maintained at 32°C on day-old and decreased gradually to 25°C on day 21. Thereafter, the birds were reared in open-sided pens. Data on pen temperature and relative humidity in different units were monitored using thermo-hygrometer at 08:00, 13:00 and 17:00h daily from days 21 to 84. Temperature-humidity index (THI) for the period under consideration was read off from THI table of USDC-ESSA as modified. The thermal environment is taken to be stressful on the birds when the calculated THI is greater than or equal to 72.

Animals and management

The experiment was carried out during hot-dry season under tropical conditions in an open-sided poultry pens. The pens were adequately and hygienically prepared before the arrival of the chicks. Thirty-six male *Arbor acres* broiler breeder birds obtained from a reputable hatchery, managed according to the manual from the industry, were allotted to four treatment groups. Birds in Treatment I received diet containing no

tocopherol (dL-α-tocopheryl acetate) while Treatments II, III and IV received dietary treatment with 50, 100 and 150mg tocopherol/kg feed respectively in an experiment that last till d 84. Brooding was done for 21d. There were three replicates and 3 birds per replicate. Commercial diet was used for the birds at different stages of growth. Water was made available *ad libitum*. The liveweight gain was not monitored because feed restriction was practised to regulate obtained weight in accordance to the manufacturer's manual. The birds were kept in cages during the experiment. There was no mortality throughout the experimental period.

Data collection

***Physiological responses*:** Physiological responses (heart rate (HR), respiratory rate (RR), skin temperature on the breast (BT) and under wings (WT), and rectal temperature (RT)) were taken in all the birds 2 days in each week during the experimental period between 13:00 and 14:00 h. The birds were handled gently without much agitation during data collection. Stethoscope was used to take the HR of the birds. The stethoscope was placed on the chest region of the birds to monitor the heart beat in a specified time. The reading was taken for 15 seconds then multiplied by 4 to give the value per minute. Respiratory rate of the birds was taken as the number of breaths per minute by counting the flank movement. Non-contact infra-red thermometer was used to take the BT and WT within 10 cm from the bird. Rectal temperature of the birds was measured with *Jorita* digital thermometer (model: ECT-5; 0.1˚C accuracy) inserted into the rectum (colon) of the birds till it beeped. The temperature was read off on the visual display unit.

***Blood sampling and analyses**:* Blood samples were obtained from all the chickens on days 28, 56 and 84 via the wing web into heparinized and blank tubes for haematology and plasma biochemistry respectively. Plasma from the heparinized tubes was harvested using centrifuge. The plasma samples were stored at -20˚C for further analyses. Plasma biochemical parameters for birds aged 4 weeks could not be analysed because of some logic reasons.

Haematological parameters: Packed cell volume (PVC), erythrocyte counts (RBC), leukocyte cell count (WBC) and haemoglobin concentration (Hb) were determined using the method of Dacie and Lewis (1991). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration were determined from the data obtained for PCV, RBC and Hb concentration. Differential counts of leukocytes (heterophil, lymphocyte, monocyte, basophil and eosinophil) were obtained by making a differential smear stained with Wright's stain (Conn, 1969; Clark, 1972). Ratio of heterophil to lymphocyte was calculated.

***Plasma biochemistry**:* Total plasma protein (TP) was determined according to Colowick and Kaplan (1995) while plasma albumin (ALB) was determined using bromocresol purple method of Varley et al. (1980). Globulin (GLO) was taken as the difference between total protein and albumin. Plasma concentration of uric acid (URI) and creatine kinase (CK) activity were measured by commercial colorimetric diagnostic kits (Lin et al., 2004). Creatinine was analysed as described by Ochei and Kolhatkar (2000).

Statistical analyses

The 12-week experimental period was phased into 3 age groups of 4 weeks each (1-4, 5-8 and 9-12 weeks). Data on physiological and blood parameters were subjected to a two-way analysis of variance using SAS computer statistical package (SAS, 2008) in 4×3 factorial arrangement with tocopherol treatment and age group as factors. For biochemistry, it was in 4 x2 factorial arrangement.

Model: $Y_{ijk} = \mu + T_i + A_j + TA_{ij} + \Sigma_{ijk}$, where Y_{ijk} is the parameter of interest, μ is the population mean, T_i is the i^{th} effect of tocopherol (i = 0, 50, 100, 150), A_j is j^{th} effect of age group/age of birds (j = 1-4, 5-8, 9-12 for physiological parameters; j = 4, 8, 12 weeks for haematological parameters; j=8,12 for biochemical parameters), TA_{ij} is the interactive effect between tocopherol and age, and Σ_{ijk} is the residual error. Means that are significantly different were separated with Tukey's HSD test. Means were considered significantly different at $P < 0.05$.

RESULTS

Table 1 shows the summary of the meteorological observations during the experimental period. The average pen temperature, relative humidity and temperature-humidty index (THI) were 28.2˚C, 79.6% and 80.0 respectively. Ambient temperature increased gradually from 08:00 to 13:00 h before declining as evening set in by 17:00 h. Reverse pattern was observed in relative humidity, which declined before rising up again in the evening. Result on effect of tocopherol on physiological parameters in growing broiler breeder cockerels is presented in Table 2. Dietary tocopherol did not significantly (P>0.05) affect average heart rate (HR), respiratory rate (RR), skin temperature on the breast (BT), under the wings (WT) and rectal temperature (RT). Effect of age on physiological parameters in growing broiler breeder cockerels under humid tropical conditions is shown in Table 3. Age had significant effect on HR (P<0.001), RR (P<0.001), BT (P<0.001) and WT (P<0.05) but not (P>0.05) on RT. Cockerels at 9-12 weeks had higher HR than younger birds. The difference between 1-4 and 5-8 week old cockerels was not significant. Respiratory rate decreased significantly with increasing age. Birds of 1-4 weeks had higher BT and WT than older ones, except that WT in 1-4 weeks was similar to 9-12 weeks. Interaction between tocopherol and age on physiological responses was not significant (P>0.05).

Table 4 shows the effect of tocopherol on haematological parameters of growing broiler breeder cockerels. Only EOS and MON of all the haematological parameters considered were significantly (P<0.01) affected by dietary tocopherol. EOS was lower in 50 mg/kg than others, except 100 mg/kg.

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

Table 2: Physiological parameters in growing broiler breeder cockerels under humid tropical conditions fed different doses of tocopherol

Parameter	**Tocopherol (mg/kg)**					**P value**
	0	**50**	**100**	**150**	**SEM**	
Heart rate (beats/min)	241.3	240.3	241.5	239.7	2.38	0.941
Respiratory rate (breaths/min)	43.8	42.2	41.2	42.8	1.22	0.470
Skin temperature on breast (˚C)	37.3	37.2	37.3	37.2	0.13	0.944
Skin temperature under wings (˚C)	40.6	40.1	40.5	40.6	0.19	0.247
Rectal temperature (˚C)	41.3	41.2	41.3	41.2	0.06	0.673

Means on the same row with different superscripts differ significantly (P<0.05)

Table 3: Effect of age on physiological parameters in growing broiler breeder cockerels under humid tropical conditions

Parameter	**Age (weeks)**			**SEM**	**P value**
	1-4	**5-8**	**9-12**		
Heart rate (beats/min)	235.3^{b}	232.6^{b}	254.3^{a}	1.79	0.000
Respiratory rate (breaths/min)	50.5^{a}	43.1^{b}	33.9^{c}	0.92	0.000
Skin temperature on breast (˚C)	38.6^{a}	36.3^{c}	36.9^{b}	0.10	0.000
Skin temperature under wings (˚C)	40.7^{a}	40.1^{b}	40.6^{ab}	0.15	0.014
Rectal temperature (˚C)	41.3	41.1	41.3	0.05	0.058

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

Control birds had more monocytes than 100 and 150 mg/kg birds but similar to the proportion in monocyte in 50mg tocopherol group. Effect of age on haematological parameters in broiler breeder cockerels is shown in Table 5. Age of the birds significantly affected PCV ($P<0.001$), WBC ($P<0.01$) and EOS ($P<0.001$). Birds aged 4 weeks old (49.1%) had higher PCV than the older groups (33.1 and 35.1%). There was no difference between PCV in 8-week and 12-week cockerels. For WBC, higher value was recorded in birds of 4 weeks compared to 12 weeks old cockerels, though either was not different from the 8 weeks old cockerels. Birds of 8 weeks recorded higher EOS than the other two age groups. Eosinophil increased significantly from 0.88% in 4-weeks-old birds to 2.49% in 8-weeks-old cockerels. EOS in 12-week-old cockerels was similar to the 4-week-old group. There was no significant ($P>0.05$) interactive effect between tocopherol and age on haematology of the growing cockerels. Effect of tocopherol on plasma biochemical parameters considered in growing broiler breeder cockerels shown in Table 6.

Table 4: Haematological parameters in growing broiler breeder cocks as affected by tocopherol

Parameter	**Tocopherol (mg/kg feed)**				**SEM**	**P value**
	0	**50**	**100**	**150**		
Packed cell volume (%)	38.5	38.8	39.2	40.0	1.89	0.949
Red blood cell ($\times 10^{12}$/l)	2.91	3.56	3.24	3.80	0.455	0.560
White blood cell ($\times 10^{9}$/l)	2.79	2.68	2.83	2.94	0.714	0.995
Haemoglobin conc (g/dl)	11.2	11.1	11.8	9.7	1.10	0.611
Heterophil (%)	44.3	43.3	45.5	42.0	2.36	0.744
Lymphocyte (%)	50.2	52.7	50.0	54.0	2.42	0.525
Eosinophil (%)	1.83^{a}	0.83^{b}	1.67^{ab}	1.83^{a}	0.210	0.003
Monocyte (%)	3.50^{a}	3.17^{ab}	2.67^{b}	2.17^{b}	0.245	0.001
Basophil (%)	0.17	0.00	0.17	0.00	0.007	0.122
H-L ratio	0.95	0.85	0.94	0.84	0.083	0.696
MCV (fL)	148.6	117.3	153.6	105.2	26.6	0.525
MCH (pg)	42.7	33.7	44.1	26.0	7.30	0.305
MCHC (g/dl)	29.1	28.8	30.7	23.8	2.97	0.420

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

H-L ratio: heterophyl-lymphocyte ratio; MCV: Mean corpuscular column; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

Table 5: Effect of age on haematological parameters in growing broiler breeder cocks

Parameter	**Age (weeks)**			**SEM**	**P value**
	4	**8**	**12**		
Packed cell volume (%)	49.1^{a}	33.1^{b}	35.1^{b}	1.63	0.000
Red blood cell ($\times 10^{12}$/l)	3.17	3.70	3.26	0.394	0.604
White blood cell ($\times 10^{9}$/l)	4.78^{a}	2.49^{ab}	1.16^{b}	0.619	0.005
Haemoglobin (g/dl)	0.13	0.13	0.00	0.100	0.619
Heterophil (%)	40.8	44.1	46.5	1.98	0.139
Lymphocyte (%)	55.6	50.0	49.6	2.03	0.062
Eosinophil (%)	0.88^{b}	2.25^{a}	1.50^{b}	0.176	0.000
Monocyte (%)	2.63	3.00	2.38	0.206	0.142
Basophil (%)	0.13	0.13	0.00	0.057	0.282
H-L ratio	0.77	0.96	0.95	0.070	0.084
MCV (fL)	159.5	93.8	140.3	23.10	0.160
MCH (pg)	45.8	29.0	35.1	6.32	0.208
MCHC (g/dl)	28.6	30.4	25.3	2.58	0.395

*^{a,b}Means within the same row with different superscripts differ significantly ($P<0.05$)*H-L ratio: heterophyl-lymphocyte ratio; MCV: Mean corpuscular volumn; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration

Table 6: Plasma biochemical parameters in growing broiler breeder cocks under humid tropical conditions as affected by tocopherol

Parameter	Tocopherol (mg/kg feed)				SEM	P value
	0	50	100	150		
Total protein (g/dl)	5.55	6.03	5.18	4.44	0.782	0.557
Albumin (g/dl)	3.89	4.57	3.80	3.13	0.714	0.587
Globulin (g/dl)	1.67	1.46	1.38	1.31	0.235	0.730
Uric acid (mg/dl)	5.02	5.67	5.65	4.96	0.502	0.636
Creatine kinase (U/l)	0.74	0.67	0.76	0.77	0.100	0.879
Creatinine (mg/dl)	1.54	2.09	1.47	1.21	0.219	0.106

Table 7: Effect of age on plasma biochemical parameters in growing broiler breeder cockerels under humid tropical conditions

Parameter	Age (weeks)			SEM	P value
	4	8	12		
Total protein (g/dl)	ND	4.34^{b}	6.25^{a}	0.553	0.040
Albumin (g/dl)	ND	2.91^{b}	4.78^{a}	0.505	0.031
Globulin (g/dl)	ND	1.43	1.47	0.166	0.877
Uric acid (mg/dl)	ND	7.65^{a}	3.01^{b}	0.355	0.000
Creatine kinase (U/l)	ND	1.09^{a}	0.38^{b}	0.071	0.000
Creatinine (mg/dl)	ND	0.58^{b}	2.58^{a}	0.155	0.000

^{a,b}Means within the same row with different superscripts differ significantly (P<0.05)
ND-Not determined

There was no significant (P>0.05) effect of tocopherol on biochemical parameters. However, effect of age shown in Table 7 was significant on TP (P<0.05), ALB (P<0.05), URI (P<0.001), CK (P<0.001) and CRE (P<0.001). Cockerels that are 8 weeks old had lower TP (P<0.05), ALB (P<0.05) and CRE (P<0.001) than those that are 12 weeks old. However, higher URIC and CK were recorded in 8 weeks old cockerels than in 12-weeks old. There existed no interactive effects of tocopherol and age on plasma biochemical parameters in growing cockerels.

DISCUSSION

From practical experience, broiler breeder chickens are more susceptible to stress than other classes of chickens. Exposure of young broiler breeder chickens to vagaries of climatic variables commonly experienced in the open-sided pens in the tropics, especially high pen temperature causes a lot of stress responses that affect the welfare of the birds negatively (Leeson and Summers, 2000). The mean ambient temperature, humidity and THI (28.2°C, 79.6% and 80.0) respectively) obtained during the experimental period show that the birds were continually under stressful conditions. Broiler chickens prefer environmental temperature in the range between 18 and 22°C and will perform maximally within this (Charles, 2002; de Souza et al., 2015). Meanwhile, the present study was carried out under mean temperature which was 6.2 °C above the upper critical limit and high humidity. High relative humidity during thermal stress further aggravated the detrimental effect of high temperature on the chickens (Lin et al., 2005). THI value greater than or equal to 72 is indicative of thermal stress (Abioja et al., 2014).

No differences were observed in heart rate of the birds that received different dosage of vitamin E. Reports on the effect of tocopherol on heart rate in chickens is scarce in literature. Contrary to the present finding, honey in water was found to reduce heart rate in broiler (Abioja et al., 2012) and laying chickens (Adekunle et al., 2017). Injection of 200 nmol melatonin intracerebroventricularly in chickens decreased heart rate (Taati et al., 2019). Increased heart rate is one of the responses of chickens to heat stress. This is to ensure an increased blood flow into the heart to ensure enough oxygen supply to different parts of the body. The average respiratory rate and body (skin and rectal) temperature in chickens were similar in the four treatment groups. Skin temperature under wings is closely related with the rectal temperature in broiler chickens (Abioja

et al., 2019b). The response of skin and rectal temperature in this study contradicts the report of Sinkalu et al. (2008) that vitamin E administered orally lowered body temperature in black Harco pullets during hot-dry season. The authors asserted that this resulted because of the hypothermic effect of vitamin E in chickens and that vitamin E became effective in thermolysis 4 hours post-administration. The reason for the differences in results may be because the birds in the present study were 1 to 12 weeks of age while authors reportedly used 11-week-old pullets just for three days. Its effectiveness may also be age-dependent.

Abioja et al. (2019a) however, discovered that tocopherol administration in feed did not change the rectal temperature but at 50mg/kg feed tocopherol reduced the skin temperature in broiler breeder pullets. Other physiological parameters were not influenced by dietary tocopherol. Tocopherol is a major chain-breaking anti-oxidant in living systems, functioning in biological membranes Khan et al., 2012; 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 broiler chickens by other authors (Tras et al., 2000) who reported no differences in PCV, RBC, WBC of broiler chickens offered levels of vitamin E in feed. Attia et al. (2017) in an experiment with probiotics showed that vitamin E alone did not elicit any improvement in PCV, RBC, WBC, Hb concentration, differential leukocyte counts (except basophil) and H:L ratio in treated group above chronic heat stress group. Though tocopherol is known to reduce cytotoxic action caused by free radicals in the living cells and improve 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 (Genovese et al., 2013). This type of cell multiplies and proliferates during heat spell while lymphocyte decline in number. The use of tocopherol in the present study neither affect lymphocyte count nor H:L ratio. This disagrees 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 (Mustaf et al., 2009). Its mechanism of action on the coronary circulatory system is yet to be fully elucidated, as well. Similar to the present study, addition of KCl to drinking water of broiler chickens did not affect respiratory rate (Yosi et al., 2017).

Blood cells have numerous functions in biological systems that are essential for the homeostasis and health of animals. Roll et al. (2010) reported that the number and morphological changes in different blood cells and components have been taken to be important indicators of physiological responses of chickens to heat stress. Any supplement or additive that ensures stability and integrity of different cell types in the circulatory system is recommended during stress scenarios. Supplemental tocopherol had no effect on packed cell volume, haemoglobin concentration, red blood cell derivatives, white blood cell count, heterophil, lymphocyte, basophil and heterophil-lymphocyte ratio in cockerels in the present study. Only eosinophil and monocyte showed significant difference. Similar findings were reported in with the finding of Campo and Dávila (2002) in heat stressed broiler chickens offered α-tocopherol in diet at 250ppm that reported a positive influence on lymphocyte and H:L ratio. Navid et al. (2010) reported that combining 100mg tocopherol with 50mg Zn per kg feed for growing broiler chickens may not, in all cases, have effect on the haematological parameters kept under thermoneutral zone. But it had significant effect on heterophil-lymphocyte ratio under HS situation. da Silva et al. (2009) reported that vitamin E lowered heterophil-lymphocte ratio in broiler chickens. In another study, tocopherol raised WBC and lymphocyte, and lowered heterophil, H:L ratio, in control group of layer hens (Ajakaiye et al., 2010). Heat stress promotes the release of catecholamines and corticosteroids that induce lipid peroxidation of membranes, including membranes of T and B lymphocytes. de Souza et al. (2011) had earlier demonstrated that heat stress reduced circulatory lymphocytes in chickens. Tocopherol, in turn, stimulates the enzyme glutathione peroxidase activity of

circulating neutrophils and macrophages and also promotes increased activity of T lymphocytes (Silva et al., 2011). Antioxidant supplementation in the diets stimulates immunity in avian class (Mohiti Asli et al., 2007; Sahin et al., 2010).

The ratio of eosinophil in the blood of growing breeder cockerels was lowered by tocopherol at 50mg/kg dose. Actually, stress induces increase in the eosinophil proportion in the blood. Ajakaiye et al. (2010) had reported an increase in eosinophil in Shika Brown laying chickens during and after road transportation. Dietary tocopherol at 50 mg/kg feed caused a reduction in number of circulating eosinophil and monocyte in this study. This agrees with the report of Abd El-Hack et al. (2019) that tocopherol at 250 mg/kg feed lowered eosinophil in laying hens during summer. Monocyte is one of the non-lymphoid parts of the immune system that provide nonspecific immunological defence in animals. They are the biggest macro-phagocytic white blood cells formed in bone marrow to ward off intruding pathogens. Heat stress leads to increase in mobilisation of monocytes. Cytotoxicity experienced in heat-stressed animals is caused by the innate immune system, which is comprised mostly of monocytes and macrophages. Imasuen and Ijeh (2017) similar to the present finding had reported that two natural sources of antioxidants, ginger and black pepper powder applied either singly or combined reduced circulating monocytes in chickens.

In the present study, tocopherol did not affect any of the plasma biochemical parameters considered. This is contrary to the previous reports (Navid et al., 2010; Sahin et al., 2001; 2002b). However, most of the reports were not in growing breeder chickens. Sahin et al. (2001) observed that addition of 250mg/kg dietary tocopherol reduced uric acid and increased total protein and albumin in broiler compared to the control group without tocopherol in feed. The present study was limited to 150 mg tocopherol/kg feed, which had no effect on blood proteins and uric acid. Uric acid is considered an important antioxidant (Simoyi et al., 2002; Hare and Johnson, 2003). Creatine kinase is an intracellular enzyme and its plasma concentration has been shown to rise markedly during acute heat stress (Whitehead and Keller, 2003). The hyperthermia-associated myopathy is manifested by the increased activities of isoenzyme creatine kinase (Daroit and Brandelli, 2008). Eliagib et al. (2012) reported gender differences in plasma uric acid in three indigenous strains of chickens in Sudan.

Birds of different ages in this study exhibited different heart rate, respiratory rate, skin and rectal temperatures. Variations in physiological parameters were expected since thermoregulatory mechanisms differ between perinatal, neonatal and post natal periods in chickens (Takahashi et al., 2005). Heart rate is influenced by several factors which include method and instrument used, season, age, sex, health status and exercise (Mutibvu et al., 2017). Contrary to the finding of the present study, heart rate declines with age in human (Zhang, 2007). In support of lower respiratory rate in older chickens in the present study, Baéza et al. (2012) reported that higher proportion of older broiler chickens were observed panting than younger ones. Younger animals do have higher body temperature than older ones (Collins et al., 1985). Mutibvu et al. (2017) reported that the rectal temperature in broiler chickens reared intensively increased with age from week 1 to 4. But the rectal temperature was not affected by age in the present study. This might be due to limited duration of the study. Probably if the period had been extended beyond 12 weeks to 24 weeks, differences would have been observed. Skin temperature changes considerably and rapidly with changes in environmental conditions surrounding chickens. It is dependent on the blood flow to the body surface (vasodilatation) and away from the surface (vasoconstriction) experienced during high environmental temperature and thermoneutral conditions respectively. In the present study, skin temperature decreased with age in growing cockerels.

This present findings on increased PCV and WBC with age agree with the report of Onyishi et al. (2017) on haematological parameter dynamics that there was a decrease in PCV between 4 and 8 weeks of age. The authors found out that packed cell volume and white blood cell count were affected by age. It was reported that age accounted for 20 and 36% of variations in PCV and WBC respectively. Guinea fowls of 10 weeks had higher PVC than those of 26 weeks (Ali et al., 2019).

Variation observed in eosinophil is contrary to the report of Albokhadaim (2012) that white blood cell count and its differentials were not affected by age in Saudi indigenous chickens during summer season. The author used chickens of 1 and 3 months old. This rhymes with the age considered (4, 8 and 12 weeks) in the present study. There was no difference in eosinophil between cockerels of 4 and 12 weeks of age. Total protein and albumin values were lower in younger chickens than older ones. This disagrees with the report of Albokhadaim (2012) that there was no difference in total protein and albumin in young and old chickens irrespective of the sex. Creatinine values were lower in younger chickens than older ones. However, higher URIC and CK were recorded in 8 weeks old cockerels than in 12-weeks old. This is disagreement with the report of Albokhadaim (2012).

CONCLUSION

Dietary tocopherol at 50 mg/kg feed might be efficacious in reducing skin temperature. Increasing dosage of tocopherol beyond 50 mg/kg feed may elevate core body temperature of chickens. Age as a factor does affect thermoregulation and some blood constituents in growing broiler breeder cockerels.

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