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RESEARCH ARTICLE

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Early-age thermal manipulation and supplemental antioxidants on physiological, biochemical and productive performance of broiler chickens in hot-tropical environments

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ABSTRACT

Heat stress has been ranked as a critical environmental issue confronting chicken farmers worldwide because of its detrimental effect on the growth, performance and health of the birds. This study evaluated the effects of early-age thermal manipulation (EATC) and supplemental antioxidants on the physiological responses of broilers in a hot tropical environment. A total of 300 day-old Ross broiler chicks were allocated to five thermal and dietary treatments, having 5 replicates of twelve birds each. The treatments were: chicks reared using the conventional method (CC), chicks exposed to early thermal manipulation with a temperature of 38 °C at day 5 with no antioxidant supplementation (TC), TC plus vitamin E at 250 mg/kg of feed (TV), TC plus selenium at 0.5 mg/kg of feed (TS) and the combination of TS and TV(TVS). The experiment was laid out in a Completely Randomized Design and data collected were analyzed using SAS (2008). The results showed that TVS broilers had significantly higher ($P < 0.05$) body weights at the finisher phase than the other treatment groups. The feed conversion ratio of TVS broilers was comparable to the TV group but lower ($P < 0.05$) than the other treatments. Reduced levels ($P < 0.05$) of heterophil, lymphocytes and hetrophil and lymphocyte ratio were recorded in the TVS compared to TV, TS and TC broilers. On day 42, the rectal temperature was significantly higher in CC than those in other treatment groups, which were comparable. TVS birds had higher ($P < 0.05$) weights of spleen, liver and lower abdominal fat than other treatments. The lowest concentration of plasma malondialdehyde and the highest activity of superoxide dismutase and glutathione peroxidase were recorded in TV and TVS birds. The study concluded that the growth performance and oxidative status in broilers were improved by the combination of EATC with supplemental Se and vitamin E (TVS).

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1. Introduction

Climate change has threatened the survivability of broiler production, particularly in the tropics, due to high ambient temperatures, causing heat stress (Abd El-Hack et al., 2020; Letcher, 2019). The concept of heat stress is divided into two: acute heat stress (AHS) and chronic heat stress (CHS) (Lara & Rostagno, 2013; Oke et al., 2022). Acute heat stress occurs as a result of exposing the birds to a very high temperature for a short period, while chronic heat stress involves exposing birds to prolonged heat for a long time (usually several weeks) (Kpomasse et al., 2021; 2023). The effects of heat stress on birds' performance have been reported to be detrimental (Oke et al., 2017; Oyelola et al., 2023). Prolonged heat stress affects the metabolism of chickens as well as their body composition (Zeferino et al., 2016). The adverse effect can be evident in the performance of broilers, especially weight gain, and meat quality (De Souza et al., 2016). Heat stress could elicit an increase in the level of malondialdehyde, which is a byproduct of lipid oxidation (Mujahid et al., 2009) and also causes vitamin (A and E) and mineral (zinc, iron and selenium) levels in tissue to decrease, leading to a reduction in the oxidative capability of the birds (Kelman et al., 2014). Excessive heat stress leads to the buildup of free radicals in the body, which produces reactive oxygen species, leading to an imbalance in oxidation and reducing the effectiveness of antioxidant defence mechanisms, thereby causing oxidative damage to DNA, protein, and other biological molecules as well as lipid peroxidation (Akosile, Majekodunmi, et al., 2023; Akosile, Sogunle, et al., 2023; Lin, Decuypere, et al., 2006).

Different approaches have been used to alleviate the detrimental effects of heat stress on chickens; they include biological (such as genetics, thermal manipulation, and diet) and environmental (such as air conditioning, intensive ventilation, and humidification) interventions (Lin, Jiao, et al., 2006). The use of housing equipment to relieve the birds from the stress is expensive and not economically friendly to most farmers in developing countries; therefore, nutrition has been suggested

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as a potential alternative for dealing with the problem of heat stress. Antioxidants like Vitamin A, E, C and selenium in the chicken system have been reported to aid in the reduction of free radicals and the prevention of lipid peroxidation, both of which protect cells from reactive oxygen species (Grashorn, 2007). Vitamin E is a key chain-breaking enzyme and selenium is an essential trace element and a crucial part of the enzyme selenium-dependent glutathione peroxidase (Surai, 2002; Yoon et al., 2007). Maini et al. (2007) found that birds fed a diet enriched with Vitamin E and Selenium performed better. The authors recorded a 10% increase in weight gain and a 15% increase in the feed conversion ratio in the chickens administered these antioxidants. In a study by Swain et al. (2000), the immune system of young chickens was found to be impaired by a lack of Vitamin E, Selenium, or both. The authors indicated that when Selenium levels were low, vitamin E requirements increased, establishing a synergistic link between vitamin E and Selenium. Therefore, when selenium and vitamin E are combined, a cell's defensive mechanism against free radicals is formed (De Almeida et al., 2012).

For broilers under heat stress, administering a combination of vitamin E (250 mg/kg food) and selenium (0.5 mg/kg feed) has been shown to reduce FCR and lipid peroxidation and also increase enzyme activity (GPx and SOD), indicating oxidative stability (Habibian et al., 2014; Harsini et al., 2012). Additionally, it has been shown that the combined administration of tocopherol and selenium yielded positive results (Skrivan et al., 2008). The two antioxidants have been found to aid in improving chicken health and performance and the quality of their meat (Sevciková et al., 2006).

Early-age thermal manipulation, which involves subjecting young chicks to a temperature of 38 + 1 °C for 24 h, has also been shown to lower the body temperature and improve long-term broiler heat stress tolerance without compromising growth or feed conversion ratio (Marandure et al., 2011**)**. Although earlier studies have focused on the separate use of early-age thermal manipulation (Meteyake et al., 2020; Oke et al., 2020) and supplementation of antioxidants (Oke, 2018; Uyanga et al., 2022; 2023), there is, however, a scarcity of information on the combined effects of the two strategies. It is hypothesized that broiler chickens thermally conditioned at an early age and also administered selenium and tocopherol could have added advantage, with better thermotolerance and growth performance under hot tropical conditions. Therefore, this study aimed to determine the separate or combined effects of early-age thermal manipulation and dietary vitamin E and selenium supplementation on broilers raised in hot environments.

2. Materials and methods

2.1. Experimental animals and management

Three hundred day-old broiler chicks (Ross), purchased from a reputable hatchery, were used for this study. A deep litter management system was used in raising the birds in an open-sided poultry housing system (where the environmental elements were not controlled during the hot season). The average ambient temperature and relative humidity of the poultry house were 31.7 °C and 82%, respectively. The pen was thoroughly washed and disinfected before the arrival of the birds. The drinkers and feeding troughs used for the experiment were washed before they were placed in the pens. Wood shavings were used as litter materials at a depth of 5 cm. The lighting schedule was continuous in the first twenty-four hours, followed by six hours of darkness and eighteen hours of light until the end of the experiment. The conventional protocol was followed for the vaccination of the birds.

2.2. Experimental design

On arrival, the chicks were placed in the pen and were brooded for the first four days at a temperature of 34 °C (Oke et al., 2020). On day 5, the birds were assigned to five treatment groups; four groups were thermally conditioned and this was done by exposing them to a temperature of 38 ± 1 °C for 24 h, following the description of earlier studies (Marandure et al., 2011) and a group served as control.

The experiment comprised five treatments and they were; birds raised using the conventional method (CC), birds exposed to thermal manipulation and fed diet with no antioxidant (TC), birds exposed to thermal manipulation and fed diet supplemented with Vitamin E (TV), birds exposed to thermal manipulation and fed diet supplemented with selenium (TS), birds exposed to thermal manipulation and fed diet supplemented with Vitamin E and Selenium (TVS). Each treatment had five (5) replicates of twelve birds.

Vitamin E and Selenium were purchased from a reputable pharmacy and were administered to the birds through the feed. The dosage for administration singly and when combined for vitamin E was 250 mg/kg of feed; for selenium, it was 0.5 mg/kg of feed (Habibian et al., 2014, 2015). The feeding of the antioxidants commenced immediately after the birds were exposed to early-age thermal manipulation on day five and were administered till the end of the experiment (day 42). The chickens were reared an open-sided poultry house. Commercial feed was used throughout the period of the experiment. Water was also supplied to the birds *ad libitum*. The composition of the diet is shown in Table 1.

2.3. Data collection

2.3.1. Growth performance

The body weights, feed intake, feed conversion ratio and mortality of the birds were monitored across the treatment in each replicate throughout the experiment. A weighing scale (model: Camry EK 5055-005, Zhongshan Camry Electronic Co. Ltd., Zhongshan, Guangdong, China) was used to take the weights.

2.3.2. Feed intake

Feed intake was measured at the end of each week. The birds were given a known quantity of feed, and the leftover for each replicate was measured.

Table 1. Diets fed to the chickens at different ages.

Ingredient	Starter	Grower	Finisher
Corn (7.34% CP)	46.00	48.5	52.60
Soybean meal (45.43% CP)	42.00	38.5	33.6
DCP*	1.63	1.60	1.60
Limestone	1.30	1.07	0.84
DL-methionine	0.45	0.42	0.38
L-lysine HCL	0.24	0.16	0.15
L-threonine	0.08	0.05	0.03
Salt	0.30	0.30	0.30
Soy oil	7.00	8.40	9.5
0.5% premix#	0.50	0.50	0.50
Bile acid/emulsifier	0.50	0.50	0.50
Total	100	100	100
Nutrition levels			
ME, kcal/kg	3.02	3.12	3.20
Crude protein, %	23.06	21.55	19.50
Methinine, %	0.74	0.69	1.15
Methionine + Cysteine, %	1.05	0.99	0.63
Lysine, %	1.44	1.29	0.90
Threonine, %	0.97	0.88	0.78
Trypsin, %	0.26	0.24	0.22

*DCP: $CaHPO_4{\cdot}2H_2O$.
#The premix supplied thefollowing per kg of diets: phytase 2,000 U/g, Se 0.3 mg, I 0.4 mg, Mn 80 mg, Zn 75 mg, Fe 80 mg, Cu 10 mg, choline 300 mg, folic acid 1 mg, pantothenic acid 12 mg, nicotinic acid 40 mg, biotin 0.15 mg, vitamin B_{12} 0.02 mg, vitamin B_6 4 mg, vitamin B_2 3.6 mg, vitamin B_1 4 mg, vitamin K_3 2 mg, vitamin E 20 IU, vitamin D_3 3,000 IU, vitamin A 8,000 IU.

Total feed intake (g) = Total feed given to birds (g) – feed leftover (g)

Average feed intake=Total feed intake (g) – Total feed leftover (g)

Number of birds

2.3.3. Body weight

At the start of the experiment, the body weights were taken using a weighing scale and this was repeated at the end of each week till the end of the experiment.

2.3.4. Weight gain

The weight gain of the birds was determined by the difference in their weekly weight.

2.3.5. Feed conversion ratio

Records of weekly feed consumption were used to calculate the feed conversion ratio (FCR).

$$\text{FCR} = \frac{\text{Average feed intake}(g)}{\text{Average weight gain}(g)}$$

2.3.6. Mortality

Mortality records were maintained throughout the experimental period and were expressed as a ratio of the number of dead birds to the total number of birds contained in each pen at the beginning of the study expressed as a percentage. This was calculated on a replicate basis.

Mortality=total number of birds – total number of dead bird

$$\%\text{Mortality} = \frac{\text{number of dead birds} \times 100}{\text{total number of birds}}$$

2.3.7. Blood parameters

On day 42, 10 birds were selected from each treatment and blood samples were collected. The Blood parameters considered were haematological indices, serum biochemical indices, superoxide dismutase, glutathione peroxidase, malondialdehyde (MDA) content and triiodothyronine.

2.3.8. Haematological parameters

For Hematological parameters, two birds were selected from each replicate and blood samples were collected from the birds by venipuncture (wing vein). Each bird was selected in the morning between 6 and 7 am from each replicate in the sixth week of the experimental period using a 3 ml syringe with a 25 G needle and carefully moved into EDTA tubes (0.5 mL). Haematological parameters were determined using hematology analyzer (Sysmex, Kobe, Japan).

White Blood Cells (WBC), Red Blood Cells (RBC) and packed cell volume (PCV) were evaluated using Wintrobes microhaematocrit and Calorimetry procedures (Lamberg & Rothestein, 1977). Blood was collected in an ethylene diamine tetraacetic acid (EDTA) container and spun for 5 min at 11000 rpm in a microhaematocrit centrifuge (Becton, Dickinson and Co. New Jersey, USA). The PCV values were obtained by measuring the total blood column height using a microhaematocrit reader. The RBC count was determined after diluting the blood sample with 0.9 percent NaCl and was gently mixed. According to Yalçm et al. (2005), leucocyte differentials (heterophils, lymphocytes, eosinophils, monocytes, and basophils) were counted for each smear.

2.8.9. Serum biochemistry

For Serum biochemistry, 2 mL of blood samples were collected from two birds per replicate in the sixth week of the experiment using a 3 mL syringe with a 25 G needle; the blood was collected in a plain bottle and was allowed to settle to its distinctive components for about fifteen minutes for the serum to separate. The serum was removed from the residual cellular components by using a pipette to suck out the serum from the plain tubes for serum biochemical parameters. Total protein, albumin, globulin, alanine aminotransferase (ALT) and aspartate transaminase (AST) were determined using an automated chemistry analyzer (LabmaxPlenno, Labtest, Lagoa-Santa, Brazil).

2.9. Determination of antioxidant enzymes activity

2.9.1. Glutathione peroxidase (GPx)

The activity of GPx in samples was determined using a modified method of Paglia and Valentine (1976). In brief, 100 µL of plasma was added to 200 µL of GSSGR (5 units/mL), 50 µL of glutathione (40 mM) and 620 µL of K-P buffer (0.25 M). To this mixture, 10 µL of 20Mm NADPH (in 1% Na2CO3) and 20 µL of 15 mM cumene hydroperoxide were added. Changes in absorbance were observed at 340 nm for 3 min with a spectrophotometer and one unit of GPx was defined as µmol NADPH oxidized/minute.

Table 2. Effect of early age thermal manipulation and supplemental antioxidants on growth performance of broiler chickens at the finisher phase.

Parameter	CC	TC	TS	TV	TVS	SEM	*P*-value
Final body weight (Kg)	1.70^{c}	2.02^{b}	2.05^{b}	2.11^{b}	2.28^{a}	0.044	0.0001
Weight gain (Kg)	1.66^{c}	1.99^{b}	2.01^{b}	2.07^{b}	2.25^{a}	0.07	0.0001
Feed intake (Kg)	3.33^{b}	4.05^{a}	4.00^{a}	4.04^{a}	4.12^{a}	0.045	0.0001
FCR	2.00^{ab}	2.04^{a}	1.99^{ab}	1.94^{ab}	1.83^{b}	0.023	0.0255
Mortality	0	0	0	0	0	0	0

Note: ^{abc} means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: FCR: feed conversion ratio; CC: Control; TC: thermal conditioning; TV: Thermal conditioning with vitamin E; TS: thermal conditioning with selenium; TVS: thermal conditioning with vitamin E and selenium.

2.9.2. Superoxide dismutase (SOD)

The activity of superoxide dismutase was evaluated using the methods of Antolovich et al. (2002). A mixture of 10ul plasma, 3 mL Tris-HCL buffer, and 6.1 µL the pyrogallol (50 mM in 10 mM HCL) was observed immediately using a spectrophotometer (Schimadzu-RF5000, Kyoto, Japan) within 1 minute at 325 nm to monitor change in absorbance. One unit of SOD activity was defined as the amount of the enzyme inhibiting autoxidation by 50%.

2.9.3. Malondialdehyde (MDA)

MDA was determined by the thiobarbituric acid (TBA) method, as described by Placer et al. (1996). Cayman's chemicals TBARS assay kit (Item No. 10009055) was used for this assay. 2.5 mL Trichloroacetic acid (20%) and 1 mL TBA (67%) were mixed with 0.5 mL of plasma and then placed in a hot water bath (95 °C) for 30 min. After cooling, 4 mL of butanol was added and mixed; then it was centrifuged at 2000×*g* for 10 min using Thermo Scientific (Osterode am Harz, Germany). After the collection of the supernatant, the optical densities were measured spectrophotometrically at 532 nm.

2.9.4. Rectal temperatures

The rectal temperature was taken with the use of clinical digital thermometer (HI98501, Hanna Instruments, Inc., Laval, Quebec, Canada), which was gently inserted into the cloaca of the bird and was placed at the side of the rectum; a sound was made by the rectal thermometer signaling that the temperature has been recorded. Then, the rectal thermometer was removed and the value on it was recorded.

2.10. Carcass evaluation

At the end of the experiment (42 days of age), two birds with body weights close to the treatment average body live weight were selected for carcass traits evaluation. The birds were subjected to 12 h of fasting prior to being killed. After fasting, each bird was weighed, slaughtered, bled, de-feathered, and eviscerated. The birds were processed and the weight of the cut parts, which included drumstick, shank, gizzard, thigh, breast meat, wing, neck, head, intestinal organ (heart, liver, pancreas, and proventriculus) and lymphoid organs (spleen) was recorded and expressed as a percentage of the bird's live weight at slaughter.

2.11. Statistical analysis

All the data collected was expressed as means with One-way ANOVA and Tukey's test was used to analyze differences among treatments by using SAS statistical program. The significance level was set at $P < 0.05$.

3. Results

3.1. Growth performance

The effect of early-age thermal manipulation and supplemental antioxidants on the growth performance of broiler chickens at the finisher phase is shown in Table 2. The initial weight of the birds was not significantly different in all treatment groups. The final weight of birds in TVS was significantly higher ($P < 0.05$) than those in other treatments. Those in TV, TS and TC were similar and significantly higher than those in CC. The weight gain of broilers in TVS was significantly higher ($P < 0.05$) than those in TV, TS and TC, which were comparable, while those in CC had the least weight gain. The feed intake in TVS, TV, TS and TC were similar and significantly higher ($P < 0.05$) than in CC. The feed conversion ratio of birds in TC, CC, TS and TV were similar and significantly higher ($P < 0.05$) than those in TVS.

3.2. Haematological parameters at finisher phase

The effect of early-age thermal manipulation and supplemental antioxidants on blood haematological indices of broiler chickens at the finisher phase is shown in Table 3. Heterophil level in CC was significantly higher ($P < 0.05$) than TC and TS, which were similar and significantly higher than those in TV and TVS. Lymphocytes in CC, TC and TS were comparable and significantly higher ($P < 0.05$) than those in TV and TVS, with TVS as the lowest. The ratio of Heterophils to Lymphocytes in CC and TC was significantly higher ($P < 0.05$) than those in other treatment groups; those in TS were significantly higher ($P < 0.05$) thanTV and TVS, which were similar.

3.3. Plasma proteins and lipid profile of broilers at the finisher phase

The effect of early-age thermal manipulation and supplemental antioxidants on plasma proteins and lipid profile of broiler chickens at the finisher phase is shown in Table 4. The levels of total protein, albumin and globulin in TVS and TV were similar and significantly higher ($P < 0.05$) than those in other treatment groups. Glucose in CC was significantly higher than others; those in TC and TS were similar and higher ($P < 0.05$) than those in TV and TVS. Cholesterol in CC was significantly higher than in other treatment groups;

those in TC were comparable to TS, with TVS as the least. Triglyceride in CC was significantly higher ($P < 0.05$) than in other treatment groups, which were similar. The uric acid level in CC was significantly higher ($P < 0.05$) than in other treatment groups; those in TS and TC were comparable and higher ($P < 0.05$) than those in TVS and TV. Sodium in TVS was significantly ($P < 0.05$) higher than others; those in TV, TS and TC were similar but higher ($P < 0.05$) than CC. The level of calcium in all treatment groups was not significant. The ratio of albumin to Globulin in CC was significantly higher ($P < 0.05$) than in the other treatment groups, which were comparable.

Table 3. Effect of early age thermal manipulation and supplemental antioxidants on haematological indices of broiler chickens at finisher phase.

Parameter	CC	TC	TS	TV	TVS	SEM	*P*-value
PCV (%)	33.00	33.75	33.75	36.75	35.00	0.55	0.2204
Hb (g/dL)	11.05	11.72	11.20	12.35	11.38	0.2	0.2505
RBC ($\times 10^6$/µL)	2.72	3.00	2.92	3.12	2.82	0.09	0.7392
WBC ($\times 10^9$/µL)	14.17	13.85	14.57	15.05	15.05	0.24	0.4318
HET (%)	34.50^{a}	30.00^{b}	28.00^{bc}	25.50^{cd}	23.70^{d}	0.89	0.0001
LYM (%)	70.00^{a}	66.50^{ab}	66.25^{ab}	64.75^{bc}	62.00^{c}	0.69	0.0004
EOS (%)	0.50	0.25	0.5	0.25	0.50	0.11	0.9052
BAS (%)	0.75	0.25	0.75	1.00	0.75	0.13	0.4884
MONO (%)	0.00^{b}	0.25^{b}	0.5^{ab}	1.00^{a}	0.00^{b}	0.11	0.0043
MCV (%)	123.22	113.38	116.31	117.86	126..99	3.02	0.6654
MCH (g/dL)	41.20	39.34	38.53	39.66	41.24	0.93	0.8859
MCHC (g/dL)	33.41	34.72	33.19	33.67	32.51	0.29	0.1587
HET/LYM	0.49^{a}	0.45^{ab}	0.42^{bc}	0.39^{c}	0.38^{c}	0.01	0.0001

^{abc} means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: PCV: Packed cell volume; Hb: hemoglobin; RBC: Red blood cell; WBC: white blood cell; HET: heterophil; LYM: lymphocyte; EOS: eosinophils; BAS-: basophils; MONO: monocytes; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; CC: Control; TC: thermal manipulation; TV: Thermal manipulation with vitamin E; TS: thermal manipulation with selenium; TVS: thermal manipulation with vitamin E and selenium.

3.4. Rectal temperature

The effect of early age thermal conditioning and antioxidant supplementation on the rectal temperature of broilers is shown in Table 5. There was no difference in the rectal temperature of the birds at day 21 was. At day 28, the rectal temperature in CO birds was significantly higher ($P < 0.05$) than those in other treatments; those in TC were higher ($P < 0.05$) than TCVS, TCV and TCS. The rectal temperature was significantly higher ($P < 0.05$) in CO with TCVS as the least in day 35. At day 42, the rectal temperature was significantly higher ($P < 0.05$) in CO than those in other treatment groups which were comparable.

3.5. Liver and renal function indices of broiler chickens at finisher phase

The effect of early-age thermal manipulation and supplemental antioxidants on broiler chickens' liver and renal function indices at the finisher phase is shown in Table 6. Aspartate aminotransferase level in CC was significantly higher ($P < 0.05$) than others; those in TC were higher than TS and TV, with TVS as the least. The level of Alanine transaminase in CC was significantly higher ($P < 0.05$) than those in other treatments, with those in TVS as the lowest. The ratio of aspartate aminotransferase to Alanine transaminase in TVS was comparable to TV and significantly higher ($P < 0.05$) than in other treatment groups.

3.6. Antioxidant indices of broiler chickens at finisher phase

The effect of early-age thermal manipulation and supplemental antioxidants on antioxidant indices of broiler chickens at

Table 4. Effect of early age thermal manipulation and supplemental antioxidants on plasma proteins and lipid profile of broiler chickens at finisher phase.

Parameter	CC	TC	TS	TV	TVS	SEM	*P*-value
T.PROT#	5.30^{c}	6.10^{b}	6.20^{b}	6.85^{a}	7.20^{a}	0.11	0.0001
Albumin#	3.72^{c}	4.07^{b}	4.10^{b}	4.47^{a}	4.75^{a}	0.08	0.0001
Globulin#	1.57^{c}	2.02^{b}	2.10^{b}	2.37^{a}	2.45^{a}	0.13	0.0001
Glucose#	245.33^{a}	228.14^{b}	221.01^{b}	198.22^{c}	187.11^{c}	4.91	0.0001
Cholesterol#	144.92^{a}	138.24^{b}	132.87^{bc}	131.42^{c}	112.67^{d}	2.53	0.0001
Triglyceride#	161.35^{a}	129.80^{b}	120.80^{b}	127.50^{b}	126.77^{b}	3.44	0.0001
Uric acid (ng/ml)	14.77^{a}	12.60^{b}	12.57^{b}	11.37^{c}	11.05^{c}	0.31	0.0001
Na (mmol/L)	121.50^{d}	137.95^{c}	136.25^{c}	153.65^{b}	176.50^{a}	4.39	0.0001
K (mmol/L)	3.47^{c}	4.15^{b}	4.25^{b}	4.27^{b}	5.10^{a}	0.12	0.0001
Ca (mg/dL)	14.97	14.45	14.60	14.45	14.97	0.23	0.9264
ALB/GLB	2.37^{a}	2.01^{b}	1.95^{b}	1.94^{b}	1.88^{b}	0.28	0.0021

Note: $^{abcd:}$ means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: T.PROT: Total protein; Na: Sodium; K: Potassium; Ca: Calcium; CC: Control; TC: thermal manipulation; TV: Thermal manipulation with vitamin E; TS: thermal manipulation with selenium; TVS: thermal manipulation with vitamin E and selenium; #mg/dL. ^{a,b}: means differ along the row.

Table 5. Effect of early age thermal conditioning and antioxidant supplementation on the rectal temperature (°C) of broiler chickens.

AGE	CO	TC	TCV	TCS	TCVS	SEM	P-Value
Day 21	41.50	41.25	41.35	41.73	41.43	0.082	0.4631
Day 28	42.85^{a}	42.53^{b}	41.75^{c}	41.75^{c}	41.5^{c}	0.1218	0.0001
Day 35	42.78^{a}	42.45^{b}	42.13^{c}	42.18^{c}	41.68^{d}	0.0869	0.0001
Day 42	42.18^{a}	41.43^{b}	41.35^{b}	41.25^{b}	41.25^{b}	0.1036	0.006

Note: ^{abc} means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: CO: Control; TC: thermal conditioning; TCV: Thermal conditioning with vitamin E; TCS: thermal conditioning with selenium; TCVS: thermal conditioning with vitamin E and selenium.

Table 6. Effect of early age thermal manipulation and supplemental antioxidants on liver and renal function indices of broilers at finisher phase.

Parameter	CC	TC	TS	TV	TVS	SEM	P-Value
AST (U/L)	169.25[a]	149.25[b]	130.75[c]	132.00[c]	115.00[d]	4.32	0.0001
ALT (U/L)	35.50[a]	31.25[b]	27.75[c]	24.25[d]	20.00[e]	1.25	0.0001
AST/ALT	4.75[b]	4.78[b]	4.75[b]	5.45[ab]	5.78[a]	0.11	0.0010

Note: [abcde] means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: AST: Aspartate aminotransferase; ALT: Alanine transaminase; CC: Control; TC: thermal manipulation; TV: Thermal manipulation with vitamin E; TS: thermal manipulation with selenium; TVS: thermal manipulation with vitamin E and selenium, [a,b,c,d]: means differ along the row.

Table 7. Effect of early age thermal manipulation and supplemental antioxidants on antioxidant indices of broiler chickens at finisher phase.

Parameter	CC	TC	TS	TV	TVS	SEM	P-Value
SOD (ng/ml)	1.95[d]	3.15[c]	3.22[c]	4.60[b]	6.10[a]	0.33	0.0001
GPX[#]	8.47[c]	11.75[b]	12.37[b]	14.05[a]	15.05[a]	0.53	0.0001
MDA*	4.33[a]	3.86[b]	3.52[c]	3.18[d]	3.04[d]	0.11	0.0001

Note: [abcd] means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: SOD: superoxide dismutase; GPX: glutathione peroxidase; CC: Control; TC: thermal manipulation; TV: Thermal manipulation with vitamin E; TS: thermal manipulation with selenium; TVS: thermal manipulation with vitamin E and selenium, *(nmol/ml), [#]nmol NADPH/min/mg, [a,b,c,d:] means differ along the row.

Table 8. Effects of early age thermal manipulation and supplemental antioxidants on carcass traits of broiler chickens.

Parameter	CC	TC	TS	TV	TVS	SEM	P-Value
Live weight (g)	1892.50[d]	2025.00[c]	2050.00[bc]	2137.50[b]	2300.00[a]	32.16	0.0001
Carcass (g)	1692.50[d]	1842.5[c]	1885.00[bc]	1962.50[b]	2125.00[a]	33.85	0.0001
Wings (%)	8.32	8.07	8.14	8.09	8.11	0.06	0.8033
Liver (%)	2.19[d]	2.32[cd]	2.52[cb]	2.72[b]	2.97[a]	0.06	0.0001
GIT (%)	5.82	5.85	4.95	5.08	5.03	0.15	0.1151
Spleen (%)	0.07[c]	0.15[b]	0.14[b]	0.17[ab]	0.19[a]	0.009	0.0001
Breast Muscle	19.37	19.68	20.04	20.23	20.11	0.21	0.7386
Thigh (%)	10.59[c]	12.23[bc]	13.69[ab]	11.83[bc]	15.15[a]	0.41	0.0003
Drum Stick (%)	9.56	8.5	8.31	8.26	8.36	0.31	0.7044
Neck (%)	3.71	4.00	3.68	3.4	3.72	0.20	0.9466
Shank (%)	1.85	1.63	1.46	1.99	1.8	0.08	0.3241
Gizzard (%)	2.64	2.32	3.22	2.75	3.02	0.13	0.2828
Heart (%)	0.46	0.53	0.54	0.44	0.49	0.02	0.6616
Proventriculus (%)	0.59	0.59	0.59	0.58	0.56	0.03	0.9967
Kidney (%)	0.02	0.01	0.01	0.01	0.01	0.001	0.8683
Abd fat (%)	2.30[a]	1.75[b]	1.53[bc]	1.41[cd]	1.18[d]	0.08	0.0001

Note: [abcd] means value having different superscript are significantly different ($p < 0.05$).
Abbreviations: GIT: gastrointestinal tract; BRT MUS: breast muscle; DRM STK: drum stick; PROVEN: proventriculus; ABD FAT: abdominal fat; CC: Control; TC: thermal manipulation; TV: Thermal manipulation with vitamin E; TS: thermal manipulation with selenium; TVS: thermal manipulation with vitamin E and selenium, [a,b,c]: means differ along the row.

the finisher phase is shown in Table 7. The level of Superoxide dismutase in TVS was significantly higher than in other treatment groups, with CC as the least. Glutathione Peroxidase in TVS and TV were similar and significantly higher than others; those in TS and TC were similar and higher than CC.

3.7. Carcass traits

The effect of early-age thermal manipulation and supplemental antioxidants on carcass traits of broiler chickens is shown in Table 8. The live weight of birds in TVS was significantly higher ($P < 0.05$) than those in other treatments. Those in TV and TS were similar and significantly higher ($P < 0.05$) than those in TC, while those in CC had the lowest live weight. The carcass weights of birds in TVS, TV and TS were significantly higher ($P < 0.05$) than those in CC. The weight of the liver was significantly higher ($P < 0.05$) in TVS; those in TV and TS were comparable and higher than those in TC and CC, with CC as the least. The weight of the spleen in TVS and TV were similar and significantly higher ($P < 0.05$) than those in TS and TC; those in CC have the lowest ($P < 0.05$) weight. The thigh of birds in TVS and TS were comparable and significantly higher ($P < 0.05$) than those in other treatment groups. Those in TV and TC were similar and significantly higher ($P < 0.05$) than those in CC. The weight of the abdominal fat was significantly higher ($P < 0.05$) in CC; those in TC and TS were comparable but higher ($P < 0.05$) than those in TV and TVS, and those in TVS had the lowest ($P < 0.05$) weight. The weight of the wings, intestine, breast muscle, drumstick, neck, shank, gizzard, heart, proventriculus and kidney were not significantly different ($P > 0.05$) in all the treatment groups.

4. Discussion

Early age thermal manipulation (EATC) in broiler chickens is a unique management tool that helps fast-growing broiler chickens cope under harsh environmental conditions in the tropics (Oke et al., 2020; Yahav, 2000). This is a sensitive process that takes advantage of the immature nature of the neonatal chicks by inducing thermotolerance at an early age (Yahav et al., 2004). Marandure et al. (2011) described EATC as exposing chicks below the age of 7 days to a temperature of 38 + 1 °C for 24 h, and this has been reported to help strengthen broiler chicks' resilience to heat stress during the finishing period (Nyuiadzi et al., 2017). Additionally, it has been discovered that vitamin E and selenium can aid in enhancing broiler

performance in tropical environments, and beneficial results have been documented in different reports with different doses of the antioxidants (Habibian et al., 2015; Harsini et al., 2012).

The body weight, weight gain and feed conversion ratio of TVS birds in this study were improved. This observation could be attributed to the beneficial synergistic effect of early-age thermal conditioning and Vitamin E and selenium supplementation in the birds' diet. The early-age thermal conditioning has been reported to help in building the muscles of birds when exposed to a high ambient temperature later in life (Halevy et al., 2006), and administration of Selenium and Vitamin E helped to improve the oxidative stability of the meat, thereby leading to the production of healthy birds with good body mass. This agrees with the results of earlier studies (Habibian et al., 2014, 2015; Harsini et al., 2012; Yalçm et al., 2005; Yoon et al., 2007). The performance of TV, TS and TC birds was similar, reflecting that early-age thermal conditioning had an effect on the birds, but administering antioxidants singly had little or no effect on their performance. This is in line with the studies of Yoon et al. (2007), Niu et al. (2009b) and Rama Rao et al. (2011), who revealed that vitamin E and Selenium did not affect the performance of heat-stressed birds when used separately. The better growth performance of the TC than the CC in this study aligns with earlier reports (Meteyake et al., 2020; Oke et al., 2020). Moreover, the poor performance of CC birds can be attributed to their poor thermotolerance. This is in line with the studies of Sun et al. (2015), who reported a decline in the growth performance of birds undergoing heat stress. The Feed conversion ratios of the TVS and TV birds were similar and better than others, showing that the birds made efficient use of the feed they were given. This result is, however, in contrast with the findings of Niu et al. (2009b), and Ncho et al. (2021), who reported that there was no difference in the feed conversion of birds fed antioxidants under heat stress.

A report from the blood examination revealed a decreased level in heterophil and lymphocyte levels in TVS chickens, resulting in a decreased heterophil/lymphocyte (H:L) ratio. The findings of Khan et al. (2002) reveal that exposure to heat stress caused a reduction in the number of lymphocytes by a reduction in the size of lymphatic organs such as the spleen, thymus and bursa. However, there was a similarity in the lymphocyte of the chickens in the control, TC and TS in this study. Also, broilers experiencing heat stress have been reported to have an elevated level of heterophil and heterophil/lymphocyte ratio (Yalçin et al., 2003). Gross and Siegel (1983) have revealed that the H:L ratio is a reliable measure of stress in broilers. Furthermore, Thiam et al. (2022) reported that the robustness and state of the immune system of chickens can be indicated by the heterophil/lymphocyte (H/L) ratio. The result obtained showed that TVS birds were not affected by heat stress, and their immune system was efficient. However, TV, TS and TC birds had similar levels of Heterophil and H/L ratio, although lesser than CC birds but higher than TVS birds; this signified that the birds could not actively resist stress. This result was in contrast with the findings of Leshchinsky and Klasing (2001), who observed no difference in H/L ratio when fed supplemental vitamin E at 100 and 200 mg/kg in the diet of broilers.

The decrease in total protein, albumin and globulin levels in CC birds suggests that they were adversely affected by the hot environment where they were reared. This is consistent with the reports of Liu et al. (2016), who reported a low level of total protein and albumin in heat-stressed birds. Moreover, higher blood glucose, triglycerides and total cholesterol concentrations were recorded in CC birds, suggesting that they had lower tolerance to heat stress. An increase in the levels of blood glucose occurs as a result of the rise in the secretion of noradrenalin, adrenalin and glucocorticoids, which birds need to survive under stressful situations (Ognik & Sembratowicz, 2012). The glucocorticoids influence metabolism by triggering gluconeogenesis from muscle tissue proteins, lymphoid and connective tissues. They also control various aspects of glucose homeostasis. The result of this study is supported by the findings of Beckford et al. (2020) and Livingston et al. (2022), who reported that the production of glucose increases under heat stress. The slight increase in the glucose levels of TC and TS birds reflects the lipolytic condition in the birds. The birds were making use of stored fat to maintain their energy level, while a decrease in TVS and TV birds implies that the birds had the required level of energy needed to maintain homeostasis, hence no need for a breakdown of stored glucose. Also, cholesterol and triglyceride increased in CC birds, showing a higher stress level. The higher blood cholesterol and triglyceride recorded in CC birds in this study agree with the findings of Shim et al. (2006) and Lu et al. (2019), who reported a surge in the cholesterol and triglycerides of heat-stressed broilers. A lower blood cholesterol level recorded in TVS birds suggests that the heat resilience of the chickens was improved. Some authors (Hosseini-Mansoub et al., 2010) have reported that dietary selenium supplementation helped to lower the serum concentration of LDL cholesterol and enhance the concentration of HDL cholesterol in heat-stressed birds.

Also, the uric acid level in CC birds is higher than in the other groups. This has a nexus to the oxidative stress caused by free radicals released during heat stress. This agrees with the findings of Livingston et al. (2022), who reported an increase in acid concentration in heat-stressed birds. TVS chickens recorded a relatively lower concentration of uric acid in the study, suggesting that this treatment benefited the chickens. Plasma potassium and sodium levels have been reported to decrease when broilers are exposed to heat stress (Beckford et al., 2020). Potassium and sodium levels were reduced in CC birds. At the same time, those in TVS increased, indicating an enhanced level of electrolytes in the systems of the birds, thereby placing them in a good state regarding their health and wellbeing. The reduced level in CC birds revealed that the birds were experiencing heat stress and were trying to reduce the heat load by consuming excess water, leading to hemodilution and a drop in the level of this electrolyte. This observation aligns with the findings of Borges et al. (2004).

The physiological state of broilers has been reported to be affected under heat stress (Borges et al., 2004). The higher rectal temperature recorded in birds raised under the conventional method in this study suggests a lower ability to dissipate heat as a result of a poor thermoregulatory system and

the reduced rectal temperature recorded in the birds that were exposed to thermal conditioning and fed vitamin E and selenium indicated that the chickens had a better tolerance ability that has been acquired as a result of the early age thermal conditioning, leading to a general rise in the adaptation degree of birds when they were re-exposed to heat later in life (Chen et al., 2013; Yahav et al., 2004).

Higher levels of AST and ALT in CC birds imply that the broilers were more affected by environmental stress. This result corroborates the findings of Liu et al. (2016) and Luo et al. (2018), who reported that the level of AST and ALT increased under heat stress. The increment could be associated with liver damage, resulting in reduced weight. TC birds revealed that EATC could not help the birds to have reduced levels of AST and ALT, but treatment fed with antioxidants; TS, TV and TVS possessed low AST and ALT levels, signifying a decrease in stress and with the TVS birds having the best result.

Vitamin E and/or Selenium have been reported to improve antioxidant enzyme activity with decreased lipid peroxidation (Gouda et al., 2015). SOD, GPx and MDA are the vital oxidative stress parameters in broilers. In this study, the concentration of SOD was higher in TVS birds. This result is congruent with the findings of Harsini et al. (2012), who reported that supplementation of Vitamin E and Selenium had a combined effect on the Cu/Zn-SOD activity, but when Selenium was administered alone, no effect was recorded; this explains the low concentration that was recorded in TS birds. However, TV birds had a better result than TS; this proves the chain-breaking ability of free radicals of Vitamin E. Also, the GPx concentrations in TVS and TV birds were enhanced than in other treatments. This observation corroborates the findings of Sahin et al. (2002), who reported that administering 250 mg/kg of Vitamin E increased the anti-oxidative abilities of broilers. Additionally, Jang et al. (2014) revealed that Selenium is required for the activity of GSH-Px. The authors revealed that adequate Selenium is needed to complement the effects of Vitamin E, a chain-breaking antioxidant, in the diet of heat-stressed birds. The combination of the vitamin and Selenium on the thermal-conditioned chickens in this study elicited a better antioxidant status observed in TVS birds. This report is supported by the findings of Harsini et al. (2012), Niu et al. (2018) and Ibrahim et al. (2019), who found that dietary supplementation of Selenium and/or vitamin E enhanced SOD and GPx levels. Furthermore, Habibian et al. (2015) revealed that Vitamin E and Selenium helped prevent lipid peroxidation under heat stress. Lipid peroxidation can be measured in plasma malondialdehyde (MDA) concentration. In this study, TVS and TV birds had reduced levels of MDA, reflecting a decrease in lipid peroxidation in the birds. This suggests a beneficial action of vitamin E, as it is known as an antioxidant of the membrane that helps in reducing the negative impacts of free radicals and reactive oxygen species that would lead to the oxidation of crucial sulphydryl groups and phospholipids (Cinar et al., 2014). This result is similar to the findings of Voljč et al. (2013) and Leskovec et al. (2019). This outcome could be explained by vitamin E's capacity to combat free radicals and lessen lipid peroxidation (Pompeu et al., 2018). The findings of Elgendey et al. (2022) asserted that the dietary addition of selenium and vitamin E increased the accumulation of antioxidants and decreased MDA in the liver tissue of broilers.

The weight of the liver in TVS birds was the highest, and the least weight was found in CC. The reduced liver weight in CC birds suggests that the birds were attempting to respond to the adverse effects of heat stress, hence the need to overwork the liver. According to Jastrebski et al. (2017), the liver is a critical organ in the adaptation processes of broilers and is associated with the synthesis of fatty acids in avian species; it has a significant function in regulating glucose storage and flow and in maintaining the entire metabolism of thermally stressed bird (Manoli et al., 2007). The observation in this study aligns with the findings of Zhang et al. (2021), who reported that the liver is an essential organ in maintaining homeostasis. The liver weights of CC birds in this study are in tandem with the report of Shim et al. (2006), who reported that the relative weights of the liver in broiler chickens decreased when experiencing heat stress. The spleen is an essential lymphoid organ needed to fight antibacterial in birds' systems. Under heat stress, it has been reported that birds have low immune responses as a result of the reduced weight of the spleen (Hirakawa et al., 2020). Habibian et al. (2014) reported that administering the antioxidants (Vitamin E and Selenium) separately to the birds did not affect the lymphoid organ weights. However, in this study, it was observed that TV and TS birds fed vitamin E and Selenium had a better immunity status when compared to the control group (CC). Increased spleen weight in TVS birds indicates they had higher immunity than other treatment groups. This result is supported by Habibian et al. (2015), who reported that the combined supplementary levels of vitamin E and Se (250 mg/kg vitamin E and 0.5 mg/kg Se) improved the health and immunity of broilers reared under heat stress. Furthermore, the thigh of birds in TVS had more mass than those of CC, TC, and CC, indicating a better structural development. This may be ascribed to the trace element properties that Selenium possesses that help augment calcium release and improve skeletal muscle development. This result is supported by the findings of Zaboli et al. (2013), who reported that Selenium helped build a better skeletal system.

A low weight of abdominal fat was recorded in TC, TS, TV and TVS birds, which signified that the birds had a low build-up of fat. Fat accumulation is caused by the rise in the plasma corticosterone concentration, which results in fat formation in the thighs, cervical region, abdominal region, and adipose tissues, which results in poor carcass appearance and lowers the birds' metabolic rates, which eventually results in decreased physical activity (Jiang et al., 2008).

Feeding of vitamin E and Selenium has been reported to help decrease the release of plasma corticosterone in the system of birds (Jiang et al., 2008). However, administering Selenium or vitamin E singly to TS or TV could not elicit a better reduction of fat accumulation. However, when combined, a relatively lower accumulation was observed in the TVS chickens, which reveals a cumulative advantage on the birds. This result aligns with the findings of Kucuk et al. (2003)

and Habiban et al. (2016), who reported that antioxidants and microelements helped reduce the production of abdominal fat. However, the findings in this study are in contrast with the observations of Niu et al. (2009a, 2009b) and Zeferino et al. (2016), who reported that dietary supplementation did not affect the weights of the lymphoid organs and carcass traits in heat-stressed broilers. Also, there is evidence from other studies that the administration of vitamin E and Se improved the specific and nonspecific arms of the immune system (Niu et al., 2009a, 2009b).

5. Conclusion

To conclude, our findings in the present study have shown a positive cumulative effect of supplemental vitamin E and Selenium plus thermal manipulation on the growth performance of broilers in hot tropical environments. Moreover, the antioxidant status of the chickens was upregulated by the treatments, particularly those treated with the combined antioxidants. Overall, the thermotolerance of the chickens was improved by the combination of supplemental antioxidants and thermal manipulation.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data accessibility statement

Data cannot be shared publicly at present because it forms part of an ongoing study.

Animal welfare statement

The Animal Experimental Board of the Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria, approved the experimental protocol.

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