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Evaluation of the synergistic influence of selenium and vitamin E on juvenile growth, antioxidant status, and physiological responses of heat-stressed broiler chickens

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Abstract

Heat stress is a growing concern in broiler production and this has been exacerbated by climate change. This study involved 300-one-day-old male Ross 308 broiler chicks, which were divided over five treatments with five replicates of 12 chickens each. The chickens were assigned in a completely randomised design as follows: conventionally reared chicks (CC), chicks subjected to early-age thermal conditioning (EATC) at 38 °C for 24 h on day 5 (TC), EATC-treated chicks supplemented with vitamin E at 250 mg kg^{-1} feed (TCV), EATC-treated chicks supplemented with selenium at 0.5 mg kg^{-1} feed (TCS), and a combination of TCS and TCV (TCVS). Growth performance data and blood samples were collected and analysed at the end of the third week of the experiment. The results showed that TCVS chickens had higher body weights than CC chickens. The plasma concentration of triiodothyronine (T3) was significantly ($p < 0.05$) higher in the TCVS chickens. Higher levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were detected in the TCVS, TCV and TCS groups. The plasma malondialdehyde (MDA) concentration was the lowest ($p < 0.05$) in the TCVS chickens. The results demonstrated that EATC combined with supplemental vitamin E and selenium (TCVS) improved performance and oxidative status in broiler chickens.

Keywords: oxidation, performance, poultry, thermal challenge, thermotolerance

1 Introduction

Poultry production in subtropical and tropical regions has been reported to suffer from heat stress due to the combined influence of elevated relative humidity and temperature, especially during the hot season (Oke *et al*., 2017; Ayo & Ogbuagu, 2021; Kpomasse *et al*., 2021; Ajayi *et al*., 2022; Akosile *et al*., 2023a, b; Kpomasse *et al*., 2023a, b; Uyanga *et al*., 2023). Broiler chickens are the most susceptible to the detrimental effects of heat stress because they cannot dispiate metabolic heat due to their rapid metabolism and lack of sweat glands (Nawaz *et al*., 2021). Thus, broiler chickens suffer from the negative effects of heat stress (Hamdy, 2020). Heat stress is a major environmental factor that has been exacerbated by the increase in global temperature associated with climate change; these effects are expected to be maximised in the future (Letcher, 2019; Oke *et al*., 2022; Uyanga *et al*., 2022). A chicken is outside the thermoneutral zone if it cannot balance thermogenesis and thermolysis (Alagawany *et al*., 2017). The ideal temperature for rearing broilers is 18–22 °C; higher temperatures can cause cellular damage and disrupt physiological functions (Shakeri *et al*., 2020; Oke *et al*., 2021). Chicks respond to high temperatures by decreasing feed intake, increasing water intake, increasing panting and sleeping behaviour, and raising their wings (Mack *et al*., 2013).

High ambient temperatures alter blood parameters, immunity, hormones, feed intake, feed conversion ratio (FCR),

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water intake, meat quality, and marketing weights, resulting in economic losses (Lara & Rostagno, 2013; Zhu *et al*., 2017). Temperatures above 26 °C contribute to a 1.5 % decrease in appetite with each increase in temperature, thus negatively affecting growth and development (Zhang *et al*., 2017). High temperatures increase the excretion and mobilisation of vitamins and minerals for immunological responses rather than to meet metabolic needs (Aengwanich, 2008; Oke *et al*., 2024). Heat stress causes a rise in respiration rate and a loss of CO2, causing respiratory alkalosis that impacts the growth and welfare of chickens (Saeed *et al*., 2019) and increases anaerobic glycolysis, which lowers the pH of the meat (Gonzalez-Rivas *et al*., 2020).

Vitamin E is an important cellular enzymatic activity regulator, which protects against lipoperoxidative damage (Gao *et al*., 2010; Traber & Stevens, 2011). According to Perez-Carbajal *et al*. (2010), Vitamin E exerts immunomodulatory functions, improving the proliferation, and survival of macrophages, lymphocytes, and plasma cells. Thus, adding Vitamin E to the diet of stressed chickens strengthens their immune system (Attia *et al*., 2016). Supplementing broiler chickens with tocopherol at 250 mg kg^{-1} is a preventative measure to mitigate the effects of heat stress, as tocopherols are associated with improved meat quality and growth performance traits (Jiang *et al*., 2013; Habibian *et al*., 2014). In laying hens, Vitamin E is used at 125-250 mg kg^{-1} to improve egg production, feed conversion, and the immune system (Jiang *et al*., 2013; Attia *et al*., 2016). In addition, selenium, a crucial component of glutathione peroxidase, is an essential trace element, that functions in protein composition, antioxidant properties, fat, protein, vitamin, and carbohydrate metabolism, basal metabolic rate, and improves the immunological state (Scheideler *et al*., 2001; Yoon *et al*., 2007; Saleh & Ebeid, 2019; Oni *et al*., 2024). Selenium supplementation at 0.5 mg kg^{-1} improves broiler performance (Harsini *et al*., 2012).

Tocopherol and selenium work synergistically in poultry as they act as priming antioxidants to prevent oxidative damage during heat stress (Ziaei *et al*., 2013). Swain *et al*. (2000) showed that a deficiency of Vitamin E, selenium, or both adversely affected the immune system of chicks, and that when selenium levels were low, Vitamin E requirements increased. Therefore, the combination of Vitamin E and selenium provided a defence mechanism against free radicals (De Almeida *et al*., 2012). Supplementation of broilers with Vitamin E (250 mg kg^{-1} feed) and selenium (0.5 mg kg^{-1} feed) reduced the feed conversion ratio and lipid peroxidation. It increased body weight, weight gain, and glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity, indicating oxidative stability (Habibian *et al*., 2014). These two antioxidants have been found to improve chicken health and performance, as well as meat quality (Ševčiková *et al*., 2006).

Thermal manipulation is used to abate the adverse effects of thermal challenge on chickens. Different authors have applied diverse methods for early-age thermal manipulation. Yahav & McMurtry (2001) suggested that a temperature between 36 and 37.5 °C on day 3 is optimal for thermal manipulation. De Basilio *et al*. (2003) suggested exposing broilers to 38 to 40 °C for 24 hours. However, Marandure *et al*. (2011) reported that exposing chicks younger than 7 days to 38 ± 1 °C for 24 hours was more beneficial. According to Yahav *et al*. (2004), thermal conditioning is a sensitive process that takes advantage of the immature nature of neonatal chicks by inducing thermotolerance at an early age.

Although earlier studies have focused on the separate use of early-age thermal manipulation (Yahav & McMurtry, 2001; Oke *et al*., 2020) or supplementation with synthetic or phytogenic antioxidants (Surai, 2000; Habibian *et al*., 2014; Voemesse *et al*., 2019; Oke, 2018; Saiz del Barrio*et al*., 2020; Tokofai *et al*., 2020, 2021, 2023), there is a need for additional information on the combined impact of these two strategies. The combined administration of tocopherol and selenium has yielded positive results (Surai, 2000; Sahin *et al*., 2001; Skrivan *et al*., 2008; Habibian *et al*., 2014). It has been hypothesised that broiler chickens thermally conditioned at an early age and supplemented with selenium and tocopherol have better thermotolerance and growth performance in a hot tropical environment (Onagbesan *et al*., 2023). Therefore, we aimed to determine the separate and combined effects of early-age thermal manipulation and dietary Vitamin E and selenium supplementation on the detrimental effects of heat stress in broilers reared in tropical environments.

2 Materials and methods

2.1 Experimental chickens and management

Three hundred male one-day-old broiler chicks (Ross 308) were used for the study. On arrival, the chicks were placed in pens and brooded for the first four days at 34 °C (Oke *et al*., 2020). On day 5, the chickens were randomly allocated to five groups (with five replicates of twelve chicks) for each treatment; four groups were thermally conditioned (TC) by exposing them to 38 ± 1 °C for 24 hours after De Basilio *et al*. (2003) and Marandure *et al*. (2011) procedure, and the fifth group served as a negative control (CC). Apart from the treatment protocol, the chickens had ad libitum drinking water and feed. The feeding program had two phases: starter (days 1 to 15), and grower (days 16 to 21). Diets

were crumbled for the starter phase, pelletised for the grower phase, and formulated to meet the nutrient requirements of Ross 308 (Aviagen, https://aviagen.com/).

The treatments used were: the CC group was reared conventionally (negative control), the TC group was thermally conditioned and fed a diet without antioxidant supplementation (positive control), the TCV group was thermally conditioned with Vitamin E supplementation (250 mg kg^{-1} diet), the TCS group was thermally conditioned with selenium supplementation (0.5 mg kg^{-1} diet), and the TCVS group was thermally conditioned with Vitamin E and selenium supplementation (250 and 0.5 mg kg^{-1} diet). The powdered form of the additives was thoroughly mixed with the feed. The antioxidant supplementation started after the thermal manipulation on the fifth day of life until the end of the experiment (day 21). Water was provided to the chickens without restriction. The chickens were housed according to the standard protocol in an open-sided deep litter system (with wood shavings as the bedding material), with an average temperature and relative humidity of 32.7 °C and 55.6 %, respectively, measured with thermo-hygrometers. The study focused only on the juvenile growth phase only (0-21 days).

2.2 Data collection

2.2.1 Growth performance

Initial body weight (BW) was measured at the beginning of the experiment. Each chicken was weighed weekly using a weighing scale with an accuracy of 0.1 g (model: camry eK 5055-005, Zhongshan camry electronic co.ltd., Zhongshan, Guangdong, China), until the end of the experiment. Weight gain (WG) was calculated by subtracting the initial weight from the final weight. Feed samples with known weights were provided daily to the chickens and the leftover including the wastage (spillage) collected for each replicate. Feed intake (FI) was measured at the end of each week as follows:

$$\text{Feed intake (g)} = \frac{\text{Total feed given to birds (g)} - \text{Feed leftover (g)}}{\text{Number of birds}} \quad (1)$$

The feed conversion ratio (FCR) was calculated as follows:

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \quad (2)$$

The mortality rate (MR) was measured by recording chicken death throughout the experimental period.

On day 21, two chickens from each replicate were randomly selected from each replicate and blood samples were collected by venipuncture (wing vein) using a 3 ml syringe with a 25G needle. Two millilitres of blood were collected in ethylenediaminetetraacetic acid (EDTA) tubes and subjected to analysis of haematological parameters, such as white blood cell (WBC) count, red blood cell (RBC) count, and packed cell volume (PCV), assessed using the Wintrobes microhematocrit and calorimetry procedures (Lamberg & Rothestein, 1997).

Two millilitres of blood were collected in plain bottles and allowed to settle for 15 minutes to separate the serum. The samples were pipetted into clean tubes, and biochemical parameters such as total protein, globulin, albumin, uric acid, triglycerides, alanine aminotransferase (ALT), and aspartate transaminase (AST) were determined. Analysis was performed using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.2.2 Determination of antioxidant enzyme activity

The activity of glutathione peroxidase (GPx; μmol NADPH oxidized/min) was evaluated following a modified description of Chafik *et al.* (2018). Briefly, 100 μl of plasma was added to 200 μl of GSSGR (5 units ml^{-1}), 50 μl of glutathione (40 mM), and 620 μl of K-P buffer (0.25 M). Then, ten microliters of 20 mM NADPH (in 1 % Na_2CO_3) and 20 μl of 15 mM cumene hydroperoxide were added to the mixture. The absorbance was measured at 340 nm for three minutes.

SOD activity was assessed by colorimetry (Antolovich *et al.*, 2002). A mixture of 10 μl of plasma, 3 ml of Tris-HCL buffer, and 6.1 μl of pyrogallol (50 mM in 10 mM HCL) was examined at 325 nm for one minute.

Malondialdehyde (MDA) was determined using the thiobarbituric acid (TBA) method following Rababah *et al.* (2006). This test was conducted using a Cayman's assay kit. A total of 0.5 mL of plasma was combined with 2.5 mL of trichloroacetic acid (20 %) and 1 mL of TBA (67 %) and submerged in a hot water bath (95 °C) for 30 min. After cooling, 4 mL of butanol was added . The supernatant was discarded after centrifugation at 2000 × g for 10 minutes. The optical density at 532 nm was determined spectrophotometrically.

2.3 Data analyis

The data collected were analysed using one-way ANOVA, and Tukey's test was used to analyse differences between treatments using the Statistical Analysis System (SAS, 2008) statistical programme. A P value of 0.05 was used.

3 Results

3.1 Juvenile growth performance

The results of antioxidant supplementation and early-age thermal manipulation on the growth performance of broiler

Table 1: *Effect of early-age thermal manipulation and antioxidant supplementation on the growth performance of broiler chickens in the juvenile growth phase (0–21 days).*

Parameter	*CC*	*TC*	*TCS*	*TCV*	*TCVS*	*SEM*	*P-value*
Initial BW (g)	36.5	37.0	35.7	35.0	35.3	0.507	0.7432
Final BW (g)	765.0^{b}	775.0^{ab}	780.0^{ab}	787.5^{ab}	790.0^{a}	2.945	0.0293
WG (g)	728.5^{b}	738.0^{ab}	744.3^{ab}	752.5^{ab}	754.8^{a}	3.152	0.0296
FI (g)	1027.5	982.5	1005.0	960.0	995.0	9.414	0.2225
DFI (g)	48.9	46.8	47.9	45.7	47.4	0.448	0.2225
FCR	1.41	1.33	1.35	1.28	1.32	0.016	0.1116
Mortality	0	0	0	0	0	0	0

^{ab} mean values with different letters differ significantly ($p < 0.05$); FCR = feed conversion ratio, FI = feed intake, DFI = Daily feed intake, WG = weight gain, BW = body weight; CC = control, TC = thermal manipulation, TCV = thermal manipulation with Vitamin E, TCS = thermal manipulation with selenium, and TCVS = thermal manipulation with Vitamin E and selenium.

Table 2: *Effect of early-age thermal manipulation and antioxidant supplementation on the blood hematological parameters of broiler chickens at the juvenile growth phase (0–21 days).*

Parameter	*CC*	*TC*	*TCS*	*TCV*	*TCVS*	*SEM*	*P-value*
PCV (%)	35.25	35.5	37.5	37.25	35.25	0.72	0.7901
Hb ($g\,dl^{-1}$)	11.55	11.72	12.35	12.65	12.07	0.27	0.7577
RBC ($\times 10^{12}\,l^{-1}$)	3.5	3.35	3.50	3.60	3.02	0.14	0.7821
WBC ($\times 10^{9}\,l^{-1}$)	14.47	14.45	14.62	16.42	14.17	0.28	0.0756
HET (%)	30.75	30.00	29.25	28.50	29.50	0.38	0.4721
LYM (%)	67.75	69.00	69.00	70.50	69.00	0.44	0.4606
EOS (%)	0.25	0.00	0.50	0.25	0.00	0.09	0.4146
BAS (%)	0.50	0.75	0.50	0.00	0.75	0.15	0.5732
MONO (%)	0.75	0.25	0.75	0.75	0.50	0.11	0.5732
MCV (fl)	109.65	107.73	107.73	104.50	121.54	5.38	0.9036
MCH (pg)	35.87	35.71	35.47	35.36	41.63	1.83	0.8199
MCHC ($g\,dl^{-1}$)	32.75	33.05	32.90	33.86	34.25	0.23	0.1907
HET/LYM	0.45	0.43	0.42	0.42	0.40	0.01	0.4501

MCHC = mean corpuscular hemoglobin concentration, MCH = mean corpuscular hemoglobin, MCV = mean corpuscular volume, MONO = monocyte, BAS = basophil, EOS = eosinophil, LYM = lymphocyte, HET = heterophil, WBC = white blood cell, RBC = red blood cell, Hb = hemoglobin, PCV = packed cell volume; CC = control, TC = thermal manipulation, TCV = thermal manipulation with Vitamin E, TCS = thermal manipulation with selenium, and TCVS = thermal manipulation with Vitamin E and selenium.

chickens at the juvenile growth phase (day 0–21 days) are presented in Table 1. There was no significant difference in the initial weight of the chickens. Only the final weights of the chickens in the TCVS treatment were significantly ($p < 0.05$) greater than those in the CC group. Feed consumption, and feed conversion ratio of the chickens were not significantly different.

3.2 Haematological indices

Table 2 shows the the haematological parameters of broiler chicks at the juvenile growth (day 21) phase as affected by early-age thermal manipulation and antioxidant supplementation. The haematological parameters were similar across the treatments.

3.3 Plasma proteins and lipid profile

The impact of early-age thermal manipulation and antioxidant supplementation on plasma proteins and lipids during the juvenile growth phase (day 21) is shown in Table 3. Total protein, albumin, and globulin were not significantly different between the treatments. The uric acid was significantly ($p < 0.05$) higher in CC than in other treatments, while TC and TCV were similar and higher than in TCS and TCVS, with TCVS being lowest. Triglyceride levels in CC were higher ($p < 0.05$) than in the other treatments, while TC and

Table 3: *Effect of early-age thermal manipulation and antioxidant supplementation on the plasma protein and lipid profiles of broiler chickens at the juvenile growth phase (day 21).*

Parameter	*CC*	*TC*	*TCS*	*TCV*	*TCVS*	*SEM*	*P-value*
T.PROT(g dl^{-1})	7.75	6.22	6.32	7.25	7.37	0.21	0.0703
ALB (g dl^{-1})	4.87	4.22	4.02	4.57	4.15	0.11	0.0739
GLOB (g dl^{-1})	2.87	2.00	2.30	2.67	3.22	0.14	0.0582
U.A (mg dl^{-1})	12.50^{a}	9.95^{b}	8.40^{cd}	9.22^{bc}	7.47^{d}	0.41	0.0001
TRIG (mg dl^{-1})	132.70^{a}	114.00^{b}	111.338^{b}	84.97^{c}	83.38^{c}	4.49	0.0001
Sodium (mg dl^{-1})	120.62^{b}	146.57^{a}	150.28^{a}	138.55^{a}	152.95^{a}	3.00	0.0001
Potassium (mg dl^{-1})	6.72^{c}	9.42^{b}	10.20^{b}	10.75^{ab}	12.25^{a}	0.44	0.0001
Calcium (mg dl^{-1})	12.62	10.77	12.7	10.42	10.47	0.47	0.3213
ALB/GLB	1.77	2.14	1.79	1.74	1.34	0.09	0.0967

^{ab} mean values with different letters differ significantly ($p < 0.05$); T.PROT = total protein, ALB = albulin, GLOB = globulin, U.A. = uric acid, TRIG = triglyceride, ALB/GLB = albumin-to-globulin ratio; CC = control, TC = thermal manipulation, TCV = thermal manipulation with Vitamin E, TCS = thermal manipulation with selenium, and TCVS = thermal manipulation with Vitamin E and selenium.

Table 4: *Effect of early-age thermal manipulation and antioxidant supplementation on liver enzymes in broiler chickens in the juvenile growth phase (day 21).*

Parameter	*CC*	*TC*	*TCS*	*TCV*	*TCVS*	*SEM*	*P-value*
AST (U/L)	119.25^{a}	103.75^{b}	104.75^{b}	105.25^{b}	95.25^{c}	1.92	0.0001
ALT (U/L)	41.00^{a}	29.50^{b}	28.00^{b}	31.75^{b}	25.25^{b}	1.42	0.0002
AST/ALT	2.91	3.60	3.79	3.31	3.89	0.13	0.1027

^{ab} mean values with different letters differ significantly ($p < 0.05$); ALT = alanine transaminase, AST = aspartate aminotransferase; CC = control, TC = thermal manipulation, TCV = thermal manipulation with Vitamin E, TCS = thermal manipulation with selenium, and TCVS = thermal manipulation with Vitamin E and selenium.

TCS were comparable and significantly ($p < 0.05$) higher than those of TCV and TCVS. A lower ($p < 0.05$) level of sodium was found in CC than the other treatment groups. Potassium in TCVS was significantly higher than those in other treatments, although similar to TCV, and TC and TCS were comparable, while CC was the lowest. Calcium levels and albumin / globulin ratio were similar between the treatments.

3.4 Liver enzymes

Table 4 shows the impact of early-age thermal manipulation and antioxidant supplementation on liver indices of broiler chickens at the juvenile growth phase. AST level in CC was higher ($p < 0.05$) than others, while TC, TCS, and TCV were similar but higher than TCVS. ALT in the CC treatment was significantly ($p < 0.05$) greater than values recorded in the other treatments.

3.5 Thyroid hormones

The impacts of early-age thermal manipulation and antioxidant supplementation on thyroid hormone levels at the juvenile growth phase are presented in Table 5. TCVS had the highest level of triiodothyronine and differed significantly ($p < 0.05$) from the other treatments; the levels of TCV and TCS were greater than those of TC and CC, with CC having the lowest level. Thyroxine in TCVS, TCV, and TCS was higher ($p < 0.05$) than TC and CC, with CC being the lowest. The ratio of triiodothyronine to thyroxine in the CC was higher ($p < 0.05$) and comparable to that in the TCVS, TC, TCS, and TCV treatments.

3.6 Antioxidant indices

The levels of SOD in the TCVS, TCV, and TCS groups were similar and significantly ($p < 0.05$) greater than those in the TC and CC groups, with that in the CC group being the lowest (Table 6). GPx in TCVS and TCV were similar and higher ($p < 0.05$) than others; TCS and TC were similar but significantly ($p < 0.05$) higher than CC. The level of MDA in CC was high and similar to TC, while TCV and TCS had higher values than the TCVS chickens.

Table 5: *Effect of early-age thermal manipulation and antioxidant supplementation on the thyroid hormone levels of broiler chickens in the juvenile growth phase (day 21).*

Parameter	*CC*	*TC*	*TCS*	*TCV*	*TCVS*	*SEM*	*P-value*
T3 (ng ml^{-1})	1.10^{d}	1.35^{c}	1.54^{bc}	1.63^{b}	1.93^{a}	0.06	0.0001
T4 (ng ml^{-1})	75.02^{c}	98.00^{b}	123.17^{a}	121.25^{a}	131.37^{a}	5.11	0.0001
T3/T4	0.017^{a}	0.010^{b}	0.010^{b}	0.010^{b}	0.015^{ab}	0.00	0.0165

^{ab} mean values with different letters differ significantly ($p < 0.05$); T3 = triiodothyronine, T4 = thyroxine, T3/T4 = ratio of triiodothyronine to thyroxine; CC = control, TC = thermal manipulation, TCV = thermal manipulation with Vitamin E, TCS = thermal manipulation with selenium, and TCVS = thermal manipulation with Vitamin E and selenium.

Table 6: *Effect of early-age thermal manipulation and antioxidant supplementation on the antioxidant indices and MDA concentration of broilers at the juvenile growth phase (day 21).*

Parameter	*CC*	*TC*	*TCS*	*TCV*	*TCVS*	*SEM*	*P-value*
SOD (U/L)	2.82^{c}	4.85^{b}	6.60^{a}	6.90^{a}	7.45^{a}	0.41	0.0001
GPX (U/L)	8.80^{c}	10.82^{b}	10.87^{b}	11.92^{ab}	12.55^{a}	0.31	0.0001
MDA (U/L)	4.03^{a}	3.81^{ab}	3.66^{b}	3.48^{b}	2.81^{c}	0.10	0.0001

^{ab} mean values with different letters differ significantly ($p < 0.05$);
MDA = malondialdehyde, SOD = superoxide dismutase, GPX = glutathione peroxidase; CC = control, TC = thermal manipulation, TCV = thermal manipulation with Vitamin E, TCS = thermal manipulation with selenium, and TCVS = thermal manipulation with Vitamin E and selenium.

4 Discussion

The body weights of the CC, TCV, TCS, and TC chickens were similar at the end of the juvenile growth phase; these results are in agreement with those of Guo *et al*. (2001) and Yoon *et al*. (2007), who reported that dietary selenium or Vitamin E had no effect on the weight gain of chicks during the juvenile growth phase. This may be because the birds were still in their developmental state. At this stage, they could actively regulate their body temperature to avoid heat stress; so the effect of the antioxidants was not yet pronounced. The higher body weight of TCVS chickens than the control group suggests that this treatment had a beneficial effect on the chickens. These results align with those of Halevy *et al*. (2006) and Oke *et al*. (2020), who reported that thermal manipulation at an early age helps to build up the muscles of chickens and increase their thermotolerance ability.

Blood examination revealed that early-age thermal manipulation and antioxidant supplementation did not affect haematological parameters during the juvenile growth phase. An increase in uric acid and triglyceride levels recorded in the chickens reared under the conventional method (CC) in the present study, indicates a high build-up of acid in the chickens. However, a lower concentration was recorded in TCVS and TCS chickens. This observation can be attributed to the ability of selenium to reduce toxicity in the body. These results are congruent with those of Livingston *et al*. (2022), who reported increase in uric acid and triglyceride levels in heat-stressed chickens. Higher levels of AST and ALT were recorded in CC chickens than in the other chickens, which implied that the broilers were affected by environmental stress. These findings corroborate those of Liu *et al*. (2016) and Luo *et al*. (2018), who reported that under heat stress, the levels of AST and ALT increase in broilers. This increase could be due to liver damage, resulting in reduced weight. The high concentration recorded in TC chickens revealed that early-age thermal manipulation could not help the chickens reduce AST and ALT, but supplemented chickens (TCS, TCV, and TCVS) had reduced AST and ALT, signifying a decrease in stress in the TCVS chickens.

TCVS, TCV, and TCS chickens possessed high levels of triiodothyronine (T3) and thyroxine (T4). An increase in these parameters led to a decrease in their ratio, whereas a reduced concentration of these thyroid hormones was recorded in CC chickens. Thyroid hormones have been reported to play vital roles in regulating metabolic processes (Quinteiro-Filho *et al*., 2012); reduced T3 and T4 concentrations indicated that the chickens were not in good condition. This result agrees with that of Etches *et al*. (2008), who showed that the concentration of T3 was reduced in chickens experiencing heat stress, while T4 level was inconsistent. The reduction in T3 could be ascribed to the reduced peripheral deiodination of T4 to T3. This leads to a decrease in heat production so that homeothermy can be maintained

(Leksrisompong *et al*., 2007). According to Vinoth *et al*. (2016), early-age thermal manipulation helps improve the levels of T3 and T4 in heat-stressed chickens, and Dalolio *et al*. (2015) reported that dietary Vitamin E and selenium increased the production of these hormones.

Malondialdehyde, glutathione peroxidase, and superoxide dismutase are vital parameters of oxidative stress in broilers. The higher concentrations of SOD and GPx and the lower concentration of MDA in the TCVS, TCV, and TCS chickens indicated the antioxidative effects of the supplement on the chickens during the juvenile growth phase. This result was congruent with that of Harsini *et al*. (2012), who indicated that Vitamin E and selenium had combined effects on Cu/Zn-SOD activity; however, when selenium was administered alone, no effect was recorded. This finding could explain the low concentration that was recorded in TCS chickens. However, the results were better in the TCV chickens than in the TCS chickens, which confirmed the chain-breaking ability of free radicals that Vitamin E possessed. The glutathione peroxidase concentrations in chickens exposed to thermal manipulation and fed Vitamin E and selenium and chickens exposed to thermal manipulation and fed Vitamin E alone were higher than in the other groups in the present study. These results corroborate the findings of Sahin *et al*. (2002), who reported that the administration of 250 mg Vitamin E kg^{-1} feed increased the antioxidative abilities of broilers. Surai *et al*. (2000) and Jang *et al*. (2014) revealed that selenium is required for the activity of GSH-Px; the authors demonstrated that adequate selenium is needed to complement the effects of Vitamin E, a chain-breaking antioxidant, in the diet of heat-stressed chickens.

5 Conclusions

The synergistic combination of selenium and Vitamin E can be used as a preventive measure to mitigate the effects of thermal stress in broilers.

Conflict of interest

The authors declare there is no conflict of interest of any kind in this study.

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