

BLOOD PROFILE AND PHYSIOLOGICAL RESPONSE OF BROILER CHICKENS TO SWEET ORANGE PEEL SUPPLEMENTED WATER IN A HOT HUMID ENVIRONMENT

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ABSTRACT

This study investigated the effect of sweet orange peel powder (SOPP) supplemented water on the rectal temperature (RT), respiratory rate (RR) and blood profile of broiler chickens raised in a hot humid environment. Day-old Ross broiler chickens ($n = 96$) were randomly allotted into four treatment groups: Control – without SOPP, 2 g SOPP, 4 g SOPP and 6 g SOPP per litre of water. Three replications of eight birds each per treatment in a completely randomized design were performed. Rectal temperatures were comparable ($p > 0.05$) among the treatments across the weeks except the third week. Birds on 6 g SOPP had a significantly ($p < 0.05$) higher value ($41.32\text{ }^{\circ}\text{C}$) compared with birds in the control ($40.90\text{ }^{\circ}\text{C}$) and 4 g SOPP ($41.00\text{ }^{\circ}\text{C}$). Respiratory rate was not significantly different ($p > 0.05$) among treatments across the weeks. Significant ($p < 0.05$) variations were observed in the red blood cell (RBC) and mean corpuscular haemoglobin (MCH) values at week 4. However, at week 7, heterophil (H), lymphocyte (L), heterophil:lymphocyte ratio (H/L) and RBC were significantly ($p < 0.05$) different among the treatments. The H/L values were 0.42, 0.40, 0.38 and 0.36 for Control, 2 g SOPP, 4 g SOPP and 6 g SOPP, respectively. Serum biochemical indices examined were comparable among the treatment groups across the weeks. Inclusion of SOPP into drinking water of broiler birds did not adversely affect the birds as observed in the RT, RR and blood profile. Rather it helps the birds in coping with the environmental stress, as observed in the reduced H/L.

Key words: rectal temperature; respiratory rate; serum biochemistry; sweet citrus peel; water supplementation; haematology

INTRODUCTION

One of the main limiting factors to the production efficiency of livestock animals especially broiler chickens in the tropical environment is heat stress. Nigeria and other tropical areas are typically hot and humid during most part of the year, as such broiler chickens are often raised in an ambient temperature higher than the thermo-neutral zone of 18 to $24\text{ }^{\circ}\text{C}$ (Cahaner *et al.*, 2008;

Soleimani *et al.*, 2008), which is required for the maintenance of normal physiological responses. As chickens are homeotherms, stability of the body temperature requires loss of excessive body heat and the rate of exchange depends on the ability of the environment to accept heat and vapour as well as the animal production status (Maya-Soriano, 2012). Birds maintain homeostasis under stress conditions through several strategies such as, biochemical, physiological and behavioural. Heat exchange could be

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evaluated directly from physiological measurements such as rectal and skin temperatures, respiratory rate, panting, heat production and growth rate (Renaudeau *et al.*, 2012).

Sweet orange peel has been reported to be a good source of antioxidants as it contains high concentrations of phenols (Manthey, 2004), flavonoids (Vlaicu *et al.*, 2020) and vitamins, especially vitamin C (Yang *et al.*, 2011), which plays key role in the mitigation of heat stress. Efficacy of sweet orange peel in water in ameliorating the negative effect of heat stress on growth performance of broiler chickens has been reported by Majekodunmi *et al.* (2021). Economic losses in poultry, as a result of heat stress, are consequences of marked deviation in the level of blood biochemical and growth rate. The blood profile is a good reflection of the health status of an animal, therefore, blood examination is a good way of screening the health status of an animal to investigate the effect of diet on it. Alteration in values of triglyceride, serum cholesterol, packed cell volume, total white blood cells and haemoglobin by heat stress has been reported by Dinu *et al.* (2007). This research aimed at investigating the effect of sweet orange peel powder (SOPP) in drinking water of broiler chickens reared during the hot season in south-west Nigeria on rectal temperature, respiratory rate, haematology and selected serum biochemistry.

MATERIAL AND METHODS

Experimental site and meteorological observations: The research was conducted at the Poultry unit of the Teaching and Research farms, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria which falls within 7° 10' N and 3° 2' E and altitude 76 m above sea level. The experiment was carried out in an open sided naturally ventilated poultry house during the hot season between the month of March and April for a period of seven weeks. Ambient temperature and relative humidity of the poultry house microclimate were recorded daily at 8:00, 14:00 and 20:00 hours using thermo-hygrometer. Temperature-humidity index (THI) was calculated according to Marai *et al.* (2001) using mathematical model: $THI = T^{\circ}C - ((0.31 - 0.31 \times RH/100) \times (T^{\circ}C - 14.4))$.

Sourcing and processing of test ingredient: Sweet orange peels were obtained from orange retailers' in open markets within Abeokuta, Ogun state, Nigeria. They were sorted out to remove dirt and thereafter sundried. The crispy peels were then milled, sieved and stored in an air tight container at room temperature until when needed. Phytochemical analysis of SOPP was carried out and the result had earlier been reported by Majekodunmi *et al.* (2021).

Animals, experimental design and management: Ninety-six one-day-old Ross 308 broiler birds were brooded for a period of two weeks, thereafter weighed and randomly assigned to four treatments of three replicates of eight birds each in a completely randomized design: Treatment 1 – Control (received drinking water without SOPP), Treatments 2, 3 and 4 received 2 g, 4 g and 6 g of SOPP per litre of water, respectively. Birds were housed in a deep litter compartments with wood shavings as bedding. Starter and finisher diets were formulated according to NRC (1994) recommendations to meet the nutrients requirement of each feeding phase and provided *ad libitum*. The detailed composition of experimental starter and finisher diets have been reported earlier by Majekodunmi *et al.* (2021). Freshly prepared SOPP water according to the quantity per treatment was provided daily to the birds *ad libitum* from day 15 to day 49 of the experiment. Routine vaccinations and other medications were administered.

Physiological data: Rectal temperature (RT) in °C and the respiratory rate (RR) in breath/minute of two birds per replicate were recorded thrice a week, while the weekly average was calculated for each pen. The RT was measured using a digital thermometer, which was inserted into the rectum, reading was taken when the thermometer beeped. With each bird lightly and calmly restrained, the abdominal region was observed to count respiratory movements within 1 min with the aid of a stopwatch to determine the respiratory rate.

Blood collection and analyses: At day 28 and 49 of the experiment, three randomly selected birds per replicate were bled at the jugular vein and blood samples were collected into heparinized and plain vacutainer tubes without EDTA for haematological study and selected serum biochemical analysis, respectively.

Haematological indices: Packed cell volume (PCV) and haemoglobin (Hb) concentration were determined by micro-hematocrit and cyanomethaemoglobin methods, respectively (Schalm *et al.*, 1975). Red blood cell (RBC) and white blood cell (WBC) counts were estimated using haemocytometer (Schalm *et al.*, 1975). Blood smears were stained using May-Grunwald and Giemsa stains approximately 4 hours after methyl alcohol fixation. Leucocyte differentials (heterophils, lymphocytes, eosinophils, monocytes and basophils) were counted for each smear and heterophil-lymphocyte ratio (H/L) was calculated according to Yalcin *et al.* (2005). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from Hb, PCV and RBC (Jain, 1986).

Serum biochemistry

Biuret method, described by Kohn and Allen (1995), was used to determine the serum total protein. Albumin was determined using Bromocresol green (BCG) method (Peters *et al.*, 1982). Globulin concentration was obtained by subtracting albumin from the total protein value. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by spectrophotometric method, as described by Rej and Holder, (1983). Serum malondialdehyde (MDA) concentration was evaluated as described by Janero (1990).

Statistical analysis

Data generated were analysed using General Linear Model (GLM) of SAS software (SAS, 1999). Treatment means were compared using the Duncan option of the software.

RESULTS

The weekly and average temperature humidity index (THI) in the poultry house during the experimental period is shown in Table 1 and Figure 1, respectively. The ranges of weekly THI reported at 08:00, 14:00 and 20:00 hr were 25.38–26.92, 30.06–31.88 and 27.50–28.84, respectively. Average THI of 25.89, 30.58 and 28.24 were recorded at 08:00, 14:00 and 20:00 hr, respectively, during the experimental period.

Rectal temperature (RT) and respiratory rate (RR)

Average weekly RT of broiler chickens given water supplemented with sweet orange peel powder is presented in Table 2. No significant ($p > 0.05$) variations were observed among the treatments at weeks 4, 5, 6 and 7. At week 3, birds in the control group had similar ($p > 0.05$) RT value with birds on 2 g SOPP and 4 g SOPP. However, birds on 6 g SOPP had a significantly ($p < 0.05$) higher RT value (41.32 °C) compared with birds in the control group (40.90 °C) and 4 g SOPP (41.00 °C). The overall average results of the RT showed no significant ($p > 0.05$) differences among the treatment groups.

Table 3 shows the RR of the broiler chickens given water supplement with sweet orange peel powder. RR rate was not significantly different ($p > 0.05$) among treatments across the weeks. The average overall RR (Figure 2) ranged from 50.79 to 56.53 breath/minute with birds on 6 g SOPP having the highest value and the control group had the lowest RR value.

Table 1. Weekly THI inside the poultry house during the experimental period

Week	THI (08:00 hr)	THI (14:00 hr)	THI (20:00 hr)
3	25.48	30.06	27.50
4	25.38	30.72	28.84
5	25.51	30.44	27.71
6	26.59	31.88	28.67
7	26.92	30.13	28.25

THI: Temperature Humidity Index

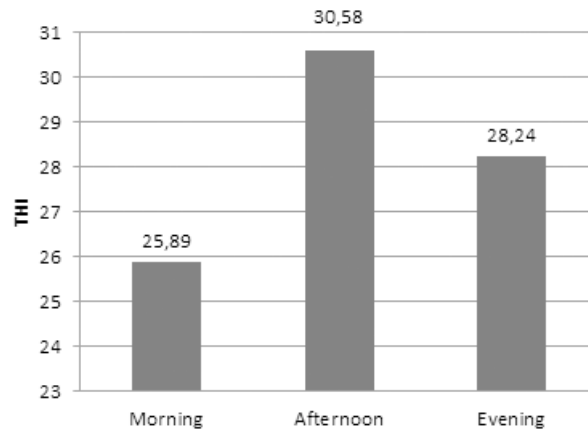


Figure 1. Average Temperature humidity index of the poultry house

Table 2. Rectal temperature of broiler chickens given water supplemented with sweet orange peel powder in a hot humid environment (n = 6)

Week	Control	2 g SOPP	4 g SOPP	6 g SOPP	SEM
3	40.90 ^b	41.08 ^{ab}	41.00 ^b	41.32 ^a	0.58
4	41.10	40.97	41.18	41.20	0.08
5	41.38	41.53	41.27	41.72	0.09
6	41.80	41.72	42.02	42.05	0.10
7	41.96	42.22	41.98	41.70	0.10
Average Temperature (°C)	41.42	41.50	41.49	41.59	0.09

^{a,b} Means on the same row with different superscripts are significantly different ($p < 0.05$), SEM – Standard error of mean, CONTROL – Control, 2G SOPP – 2 g SOPP, 4G SOPP – 4 g SOPP, 6G SOPP – 6 g SOPP

Table 3. Respiratory rate of broiler chickens given water supplemented with sweet orange peel powder in a hot humid environment (n = 6)

Week	Control	2 g SOPP	4 g SOPP	6 g SOPP	SEM
3	57.33	62.67	59.33	58.67	1.35
4	46.00	52.67	47.33	54.00	1.96
5	49.33	48.00	53.33	60.00	1.74
6	51.33	50.67	54.67	53.33	1.90
7	50.00	55.33	58.00	56.67	2.82

^{a,b} Means on the same row with different superscripts are significantly different ($p < 0.05$), SEM – Standard error of mean, CONTROL – Control, 2G SOPP – 2 g SOPP, 4G SOPP – 4 g SOPP, 6G SOPP – 6 g SOPP

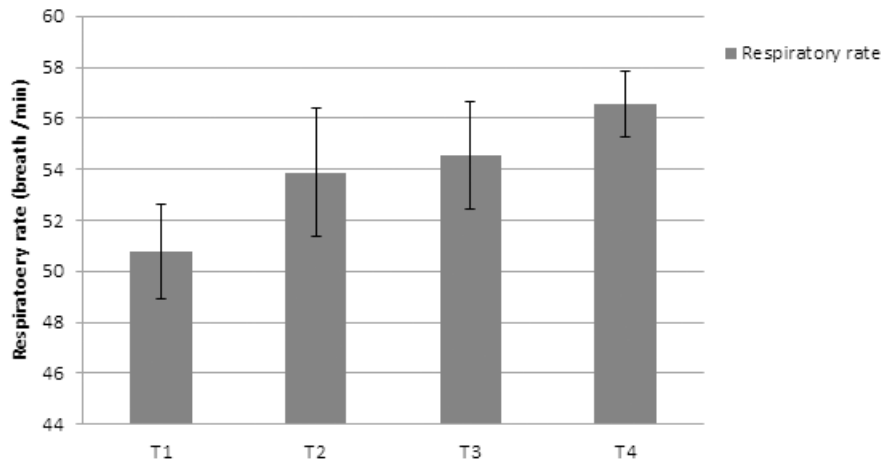


Figure 2: Average Respiratory rate of broiler birds given water supplemented with sweet orange peel powder

Table 4. Hematological parameters of broiler chickens given water supplemented with sweet orange peel powder at week 4 (n = 9)

Parameters	Control	2 g SOPP	4 g SOPP	6 g SOPP	SEM
Packed cell volume (%)	31.67	32.00	31.83	32.00	0.45
Haemoglobin (g/dL)	10.77	11.07	10.93	10.73	0.15
Red blood cell ($\times 10^{12}/L$)	2.83 ^a	2.38 ^b	2.58 ^{ab}	2.45 ^{ab}	0.07
MCV (fL)	112.87	134.36	125.21	132.01	3.71
MCHC (g/dL)	34.14	34.50	34.37	33.57	0.33
MCH (pg)	38.40 ^b	48.49 ^a	43.11 ^{ab}	44.31 ^{ab}	1.31
White Blood Cell ($\times 10^9/L$)	18.58	18.32	18.10	18.62	0.20
Heterophil (%)	25.83	24.67	27.00	27.17	0.55
Lymphocyte (%)	72.33	73.50	71.33	70.33	0.61
H/L	0.36	0.34	0.38	0.39	0.01
Monocyte (%)	1.00	0.66	0.66	0.83	0.15
Eosinophil (%)	0.50	0.50	0.33	1.00	0.12
Basophil (%)	0.33	0.66	0.66	0.66	0.12

^{a,b} Means on the same row with different superscripts are significantly different ($p < 0.05$), MCV – Mean corpuscular volume, MCHC – Mean corpuscular haemoglobin concentration, MCH – Mean corpuscular haemoglobin, SEM – Standard error of mean

Haematological parameters

The haematological parameters of the broiler chickens at week 4 are presented in Table 4. There were no significant ($p > 0.05$) differences in the parameters measured except the RBC and MCH. Birds in the control group had significantly ($p < 0.05$) higher values of RBC ($2.83 \times 10^{12}/L$) compared with birds on 2 g SOPP ($2.38 \times 10^{12}/L$). However, the RBC value of

birds on 2 g SOPP was similar ($p > 0.05$) to the values recorded for birds on 4 g SOPP ($2.58 \times 10^{12}/L$) and 6 g SOPP ($2.45 \times 10^{12}/L$). On the other hand, the MCH value of birds on 2 g SOPP (48.49 pg) was significantly ($p < 0.05$) higher compared to the birds in the control group (38.40 pg). The MCH values recorded for birds on 4 g SOPP (43.11 pg) and 6 g SOPP (44.31 pg) were similar ($p > 0.05$) to the Control and 2 g SOPP values.

Table 5. Hematological parameters of broiler chickens given water supplemented with sweet orange peel powder at week 7 (n = 9)

Parameters	Control	2 g SOPP	4 g SOPP	6 g SOPP	SEM
Packed cell volume (%)	30.50	31.33	32.17	28.83	0.52
Haemoglobin (g/dL)	10.23	10.52	10.77	9.72	0.18
Red blood cell (x10 ¹² /L)	2.57 ^{ab}	2.62 ^{ab}	2.72 ^a	2.40 ^b	0.05
MCV (fL)	118.87	119.80	118.48	120.12	0.42
MCHC (g/dL)	33.53	33.55	33.47	33.70	0.10
MCH (pg)	39.87	40.20	39.65	40.47	0.18
White Blood Cell (x10 ⁹ /L)	13.00	11.13	11.60	12.82	0.44
Heterophil (%)	28.50 ^a	27.67 ^{ab}	26.67 ^{ab}	25.50 ^b	0.43
Lymphocyte (%)	67.83 ^b	69.00 ^{ab}	70.00 ^{ab}	71.67 ^a	0.55
H/L	0.42 ^a	0.40 ^{ab}	0.38 ^{ab}	0.36 ^b	0.01
Monocyte (%)	1.17	1.33	1.00	1.33	0.13
Eosinophil (%)	1.33	1.33	1.50	0.67	0.15
Basophil (%)	0.83	0.67	0.83	0.67	0.11

^{a,b} Means on the same row with different superscripts are significantly different ($p < 0.05$), MCV – Mean corpuscular volume, MCHC – Mean corpuscular haemoglobin concentration, MCH – Mean corpuscular haemoglobin, SEM – Standard error of mean

The Table 5 shows haematological parameters at week 7. Significant variations ($p < 0.05$) were observed in the RBC, Heterophils, Lymphocytes and Heterophils-lymphocyte (H/L) ratio among the treatments. Birds on 4 g SOPP showed a significantly ($p < 0.05$) higher value of RBC ($2.72 \times 10^6 \text{ mm}^3$) compared to the birds on 6 g SOPP ($2.40 \times 10^6 \text{ mm}^3$). Heterophil percentage significantly ($p < 0.05$) varied among the treatments with birds in the control group having the highest value (28.50 %) that is significantly ($p < 0.05$) higher than 25.50 % recorded for birds on 6 g SOPP. However, the opposite was the case for the Lymphocyte percentage among the treatment groups: birds in the control group had a significantly ($p < 0.05$) lower lymphocyte value (67.83 %) compared with birds on 6 g SOPP (71.67 %). The H/L ratio of birds in the control group (0.42) was significantly ($p < 0.05$) higher than those recorded for birds on 6 g SOPP (0.36).

Serum biochemistry

Tables 6 and 7 show the results of the selected serum biochemical parameters of the experimental birds at weeks 4 and 7, respectively. The parameters measured (Glucose, Total protein, Globulin, Albumin, ALT, AST and MDA) showed no significant ($p > 0.05$) differences among the treatment groups at both week 4 and 7.

DISCUSSION

Thermal comfort indices, such as temperature-humidity index (THI), are species-dependent and have been developed to assess the impact of the thermal environment on thermoregulatory status of animals in enclosure. Marai *et al.* (2001) established stages of thermal comfort values as: absence of heat stress (< 27.8), moderate heat stress (27.8–28.8), severe heat stress (28.9–29.9) and very severe heat stress (> 30.0). The THI results recorded during this experiment indicate the absence of heat stress in the morning (08:00 hr), but severe heat stress in the afternoon (14:00 hr) and evening (20:00 hr) indicating that the birds were subjected to continuous heat stress for most part of the experimental period.

Rectal temperature is a useful measure of thermal stress (Spiers *et al.*, 2004) as it can give a good prediction of homeostatic condition of an animal. The high rectal temperature recorded at week 3 in 6 g/L SOPP treatment group, could be attributed to the higher phytochemical concentration in the SOPP dosage, which triggered an initial increase in metabolic activities resulting in increased metabolic heat production. In a similar experiment, Majekodunmi *et al.* (2021) revealed an increase in feed intake and weight gain of broilers given 6 g/L

Table 6. Selected serum biochemistry parameters of broiler chickens given water supplemented with sweet orange peel powder at week 4 (n = 9)

Parameters	Control	2 g SOPP	4 g SOPP	6 g SOPP	SEM
Glucose (g/dL)	151.95	142.57	166.53	171.35	6.07
Total protein (g/dL)	4.07	5.20	4.08	4.62	0.24
Globulin (g/dL)	1.15	1.52	1.02	1.17	0.11
Albumin (g/dL)	2.93	3.70	3.08	3.47	0.16
ALT (i.u/L)	11.33	11.17	12.50	13.00	0.65
AST (i.u/L)	82.33	74.67	84.50	80.33	2.56
MDA (U/LX10 ⁻¹⁰)	6.74	6.01	6.28	8.08	0.56

AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase, MDA – Malondialdehyde, SEM – Standard error of mean

Table 7. Selected serum biochemistry parameters of broiler chickens given water supplemented with sweet orange peel powder at week 7 (n = 9)

Parameters	Control	2 g SOPP	4 g SOPP	6 g SOPP	SEM
Glucose (g/dL)	154.67	145.97	153.75	150.17	7.84
Total protein (g/dL)	6.12	6.35	5.90	6.13	0.12
Globulin(g/dL)	2.13	2.25	3.25	2.43	0.25
Albumin(g/dL)	3.98	4.08	4.46	3.62	0.24
ALT(i.u/L)	31.50	31.33	32.83	33.50	0.52
AST(i.u/L)	129.00	126.00	143.00	137.17	6.51
MDA(U/LX10-10)	4.12	8.07	6.27	3.14	1.03

AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase, MDA – Malondialdehyde, SEM – Standard error of mean

SOPP at week 3. Increase in feed intake especially during high temperature period is known to cause increased metabolic heat production, which could account for the high rectal temperature recorded at week 3. However, according to Medeiros (2001), rectal temperature of broiler chickens ranges from 40.6 to 43.0 °C with an upper critical limit of 45 °C, above which survival is threatened. This shows that even the high rectal temperature recorded in 6 g/L SOPP group at week 3 is still within the normal range for broiler chickens. The respiratory rate recorded in this study is within the range of 40-60 breath/min observed in commercial broiler strains by Nascimento *et al.* (2012). Thus, inclusion of SOPP did not jeopardize the homeothermy of the chickens, as they were able to efficiently thermo-regulate and maintain their core temperature and respiratory rate within the normal range even at

temperature above the thermo-neutral zone.

Haematological parameters could monitor an animal's health condition, including their response to environmental and nutritional stress (Iyaode *et al.*, 2020). Red blood cell counts of broilers given 2 g/L and 4 g/L SOPP increased by 22.5 % and 9.2 %, respectively, from week 4 to 7, while it decreased by 10.1 % in control chickens. High RBC values indicate an increase in oxygen carrying capacity of the blood, which is necessary to avoid a reduced oxygen condition called hypoxia. The slight reduction in RBC in 6 g/L group by 2.1 % implies that inclusion of SOPP at 2–4 g/L is sufficient to boost the red blood cell counts, while higher dose tends to gradually reduce it. This study agrees with the result of Amaga *et al.* (2019), who reported significant influence of maize replacement with soaked sweet citrus peel on RBC of broilers but not on haemoglobin concentration,

packed cell volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration at 9 weeks. Similarly, Seidavi *et al.* (2015) in their report stated, that inclusion of orange peel extract into broiler drinking water at 1000–1250 mg/L for 42 days increased the volume of red blood cells and thereby recommended orange peel as a cheap supplement to improve blood constituents. Haemoglobin (Hb) values, obtained in this study, are similar to 7.40–13.10 g/dl range reported for normal birds by Mitruka and Rawnsley (1977). Likewise, the PCV obtained was similar to control and also within the reference range of 22.61–33.41 % and 24.71–33.76 % reported by Kongpechr *et al.* (2020) for male and female ROSS 308 broilers at 35 days. The PCV value obtained thereby shows that the broilers were not anaemic. The MCH values recorded in this study are within the range reported by Mitruka and Rawnsley (1977), Bounous and Stedman (2000) and Orayaga *et al.* (2016). The observed RBC values indicate no adverse effect of SOPP on the absorption of nutrients needed for blood formation and function (Orayaga *et al.*, 2016).

Heterophil: Lymphocyte (H/L) ratio is a good indicator of stress (Hindi *et al.*, 2012). In this study, H/L ratio decreased as the dosage of SOPP increased, with the highest dose of SOPP (6 g/L) having the lowest heterophil, highest lymphocytes and overall lowest H/L ratio. This report agrees with those of Pourhossein *et al.* (2015), that the application of orange extract in the diet of broiler chickens reduced heterophil percentage and (H/L) ratio. The lower H/L in broilers given SOPP water could be influenced by the flavonoid constituent in SOPP, which plays a role in quenching oxygen radicals by exerting antioxidant effect in the birds (Iskender *et al.*, 2016). According to Azizi *et al.* (2018), flavonoids in dried orange extract could enhance immunity by increasing IgG and IgM antibodies of chickens. Abdulameer (2018) also stated that sweet orange peel is a potential anti-stress agent that can improve the blood profiles of broilers during heat stress. Insignificantly reduced malondialdehyde (MDA) concentration was also observed from week 4 to week 7 despite the prevailing THI. Lower H/L ratio and MDA are indicators of reduced stress. Hence, inclusion of SOPP into drinking water of broilers can help the chickens in coping with the environmental stress.

Absence of significant differences in the serum parameters between the control and SOPP treatment groups in this study suggests that the dosage of SOPP did not adversely affect the serum indices and organ function. This finding agrees with the report of Behera *et al.* (2019), who did not find significant ($p \leq 0.05$) difference in glucose, TP, ALB, and globulin level of broilers fed 2.5 to 7.5 % of citrus waste for 7 weeks. Likewise, Abbasi *et al.* (2015) reported no effect of dried *citrus sinensis* pulp in the diet of broiler birds on the serum enzyme activities (ALT and AST), which is an indication of no organ toxicity.

CONCLUSION

The result of this study shows that the inclusion of SOPP into drinking water of broiler chickens, raised during the hot dry season, had no effect on the rectal temperature, respiratory rate, serum indices and haematological parameters, except heterophil, lymphocyte and H/L ratio at week 7 of the experiment. Heterophil: lymphocyte ratio reduced with increased inclusion of SOPP into drinking water. Hence, inclusion of SOPP up to 6 g/L to the water could be beneficial in the mitigation of heat stress in broiler chickens raised during the hot dry period.

Animal welfare statement

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

AUTHOR CONTRIBUTIONS

Conceptualization: Majekodunmi, B. C.

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Project administration: Majekodunmi, B. C., Adekunle, E. O.

All authors have read and agreed to the published version of the manuscript.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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