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RESPONSE OF HEAT STRESSED BROILERS TO AMELIORATING EFFECTS OF SYNTHETIC VITAMIN C AND *Citrus sinensis* EXTRACT

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ABSTRACT: Heat stress is a critical environmental factor in the poultry industry, negatively impacting performance and causing economic losses. High temperatures reduce productivity and increase mortality. This study used 120 Abor Acre broilers (28 days old, 1.050 ± 0.4 kg) fed standard commercial feed. At day 35, 30 birds were randomly assigned to 4 treatments: T_1 (water as control), T_2 (30 mg/L synthetic vitamin C based on manufacturers' dosage), T_3 (30 ml/L orange extract), and T_4 (50 ml/L orange extract), each in three replicates. Birds were exposed to artificial heat stress (36 ± 2°C) for 15 days using an automated heater, with unrestricted access to feed and treatment solutions. Physiological data were recorded periodically, and blood samples were collected on days 0, 5, 10 and 15 for serum biochemical analysis. Data were analyzed using the SAS procedure for repeated and non-repeated measures. Results showed significantly higher final body weight (FBW) and average body weight gain (ABWG) in T_4 compared to other groups ($P < 0.05$). Average daily feed intake (ADFI) was highest in T_4 chickens, and feed conversion ratio (FCR) was lowest, indicating superior feed efficiency. Rectal temperature, body temperature, pulse rate, and respiratory rate were significantly highest in T_1 group ($P < 0.05$). Serum biochemical markers (AST, ALT, glucose, and creatinine) were highest in T_1, whereas T_4 recorded the lowest values. T_1 broilers had the highest external and internal thermal gradients, while T_4 had the lowest. Glycogen reserves were highest in T_4 group. The study concludes that sweet orange extract, especially at a 50% concentration, is a viable organic alternative to synthetic vitamin C (30 mg/L) for mitigating heat stress in broilers. It enhances growth performance, feed efficiency, and physiological stability under heat stress, making it a sustainable solution for poultry production in tropical climates.

Keywords: Broiler, Heat stress, Serum, Sweet orange extract, Vitamin C.

INTRODUCTION

Heat stress is one of the most significant stressors impacting poultry production having significant effects on the health and productivity of poultry. Excessive heat is a major threat in both hot climates and temperate regions (Hu et al., 2022; Sun et al., 2023). The broiler industry faces the challenge of heat stress which adversely impacts the growth rate, production performance, immune response, gut function and gut microbiota, redox balance, energy bioavailability in cells, and attainment of the body's homeostasis, culminating in huge economic losses to the poultry industry (Saeed et al., 2019; Uyanga et al., 2021).

A major decrease in feed efficiency, feed intake and body weight gain due to heat stress has been reported in many studies conducted on birds and other animals (Vandana et al., 2021; Ayoola et al., 2023; Chen et al., 2024). During stress conditions, the most important thing any living being must do is to survive rather than growth (Hawkins and Storey 2020; Akinmoladun et al., 2023). A study on broilers revealed that both recurring and continuous environmental stress (heat stress) significantly compromises growth performance by reducing protein digestibility up to 9.7%. Broilers under heat stress have shown increased metabolizable energy intake (20.3%) and heat production (35.5%) and decreased energy retention (20.9%) and lastly energy efficiency (32.4%) (De Souza et al., 2016).

Environment temperature is one of the factors that affect gut health including gut microbial ecosystem, gut health relating to nutrient absorption affect the numerous nutrient that is used for growth (Patra and Kar, 2021). Heat stress affects the physiological parameters in animals and the responses vary depending on the time and degree of thermal challenge (Ayoola et al., 2020).

Vitamins are essential nutrients that are required for various biochemical and physiological processes in the body (Johnson and Bales, 2022). Sweet orange (*Citrus sinensis*) is a main source of vitamin C but also contain ample amount of calcium, folic acid, potassium, thiamine, niacin and magnesium (Gupta et al., 2020). Dietary supplementation of vitamin C (ascorbic acid) aids in limiting stress metabolic signs, improved growth performance, enhanced immunological status and reduced mortality (El-Senousey et al., 2018, NCBI, 2021). Ascorbic acid serves as an antioxidant and has anti-

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inflammatory effects which is effective for poultry in cases of inflammation, oxidative stress, and infection (El-Senousey et al., 2018).

The current research trend in livestock production focuses on sustainable agriculture and application of organic products which involves using natural materials, drugs, or food supplements rather than synthetic products. In a heat stress environment, vitamin C which is an active ingredient in OE contributes to the energy supply of poultry birds by corticosterone biosynthesis (Oguntunji et al., 2019, Akinmoladun et al., 2023). The present study aims to evaluate the ameliorating effect of natural source of ascorbic acid (*Citrus sinensis)* extract on heat stress in broiler chicken as compared to the use of synthetic vitamin C based on manufacturers' dosage. The outcome of this study is expected to contribute to sustainable broiler production in the tropics.

MATERIALS AND METHODS

Study area

The study was carried out at the Poultry Unit, Teaching and Research farm, Bowen University, Iwo, Osun State. The laboratory work was conducted at the Animal Science laboratory, Bowen University, Iwo. The use and handling of animals were in accordance with the approval granted by the Bowen University ethics committee (BUEC). The study area is located in Derived Savanna Agro-Ecological Zone characterized with environmental temperature range of 15 to 28°C and mean annual rainfall of 1400 mm. The coordinate of the study area is Latitude 7⁰37'30" N and Longitude 4⁰11'40" E.

Birds and management

One hundred and twenty healthy abor acre broilers aged 28 days old, weighing 1.050±0.4 kg served as subjects of the study. The birds were raised in a standard poultry house and fed standard commercial feed from day-old. All necessary medications were administered to the birds. Vaccinations, medications, and other related management practices were done as recommended by the National Veterinary Research Institute (NVRI), Jos, Nigeria.

Ethical considerations

The methods/procedures used in this study were in concomitant with those outlined in the Animals ARRIVE guidelines and were carried out by the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines; EU Directive 2010/63/EU for animal experiments; or the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). The study was conducted with the approval of the Bowen University Ethics Committee.

Experimental unit

Sweet oranges were sourced from a local market and the juice was extracted. Fresh orange extract (OE) free of seeds and other foreign particles was stored in an air-tight jar and refrigerated for subsequent use. At age 35 days, thirty birds were randomly assigned to each treatment of oral supplementation as T_1 (control-water), T_2 (30 mg/L synthetic vitamin C based on manufacturers dosage), T_3 (30ml/L of OE), and T_4 (50ml/L of OE) in three replicates and birds were subjected to regulated artificial induced heat stress simulation pen maintained at 36 ± 2°C for 24 hours, 15 days using an electric automated heater. Throughout the trial, birds had unrestricted access to feed and treatment solution.

Data collection

Meteorological data

Meteorological data within the pen were monitored and recorded. Temperature within the pen was maintained at 38⁰C, while average relative humidity was 72.5% during the experiment. Feed intake, body weight, and feed conversion ratio were monitored weekly.

Physiological parameters

Data on physiological parameters were taken as described by Ayoola et al (2023) on days 0, 5, 10 and 15. Each parameter were measured and described as:

The respiratory rate (RR): Determined by counting the number of flank movements in one complete breathing in a minute or vent per minute using a stopwatch".

The skin temperature (ST°C): An infrared thermometer was placed at the shaved area under the wings. Nubee NUB8380, California, USA model was positioned at a 15 cm distance from the animal to and measurement was taken after the sound of alarm from the thermometer.

Rectal temperature (RT°C): A digital clinical thermometer was used in taking the measurement. The sensory tip of the thermometer was disinfected, and inserted via the vent of birds (depth of 1 cm). Reading on thermometer was taken after the indicator sound.

Panting rate (PR°C): This is a reading of times a bird panted within a minute using a stopwatch.

From the measured data: The body thermal gradients was calculated as:

Internal thermal gradient = (RT°C - ST°C); External thermal gradient = (RT°C – ambient temperature); Total thermal gradient = (ST°C – ambient temperature) (Yahav and Hurwitz, 1996)

Serum biochemical data

On days 0, 5, 10 and 15 of experiment, five birds were randomly selected from each replicate carefully to avoid stress. Blood samples were collected via wing veins from the selected birds. Blood samples for serum analyses were collected into plain bottles, centrifuged and serum was stored at -20°c until further analysis.

Statistical analysis

Data obtained for vitamin supplement on growth performance and muscle glycogen were analyzed with completely randomized design (CRD). The equation was of the form: $Y_{ij} = \mu + T_i + \mathcal{E}_{ij}$

Where: Yij = individual observation; μ = General mean; Ti = Treatment effect (i= 1……4); Ɛij = experimental error.

The interactive effects of vitamin supplements and periods on physiological and serum biochemical parameters were analyzed with a two factorial, 4 x 4 factorial experiment in a CRD.

The statistical model used was: $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \mathcal{E}_{ijk}$;

Where: Yij = individual observation; μ = population mean; Ai = Effect of factor A (i= 1……4); Bj = Effect of factor B (i= 1……4); (AB)ij = Interactive effect of factors A and B; Ɛijk = experimental error

The mean differences were determined using Duncan multiple range test (DMRT) at 5% probability

All statistical analyses were performed with the SAS-2002, version 16.

RESULTS

The feed composition fed to the experimental birds is as presented in table 1. As reported in table 2, the growth performance results showed no significant differences ($P > 0.05$) in initial body weight (IBW) among treatments. However, final body weight (FBW) and average body weight gain (ABWG) were significantly higher ($P < 0.05$) in the T_4 group (50% citrus-sweet orange) compared to T_1 (ordinary water). T_2 (synthetic vitamin) and T_3 (30% citrus-sweet orange) also outperformed T_1 but were not different from each other. Average daily feed intake (ADFI) was highest in T_4 and significantly higher ($P < 0.05$) than all other treatments, while the feed conversion ratio (FCR) was lowest in T_4, indicating the best feed efficiency. T_1 had the highest FCR, showing the poorest feed efficiency. No mortalities were recorded across treatments.

Table 1 - Gross composition of basal diet

Feed ingredients	Quantity (kg)
Maize	45
Palm kernel cake	8
Wheat bran	12
Groundnut cake	11
Soyabean meal	14
Fish meal	2
Oyster shell	1
Bone meal	3
Vitamin premix*	2.5
Salt	1.2
Methionine	0.15
Lysine	0.15
Total	100
Calculated values	
Metabolizable Energy (ME)	2800.5 kcal*kg^{-1}
Crude protein (CP)	20.1%
Crude fibre (CF)	4.98% n
Ether Extract (EE)	5.5%

*: Premix to provide the followings per kg of feed: Vitamin A: 500 iu, Vit.D3: 1200 mg, Vit.E: 11 mg, Vit.K3: 2 mg, Riboflavin: 20 mg, Nicotinic acid: 10 mg, Panthothenic acid: 7 mg, Cobalamin: 0.08 mg, Choline chloride: 900 mg, Folic acid: 1.5 mg, Biotin: 1.5 mg, Iron: 25 mg, Manganese: 80 mg, Copper: 2 mg, Zinc: 50 mg, Cobalt: 1.2 mg and Selenium: 0.1 mg.

Table 2 - Performance characteristics of broilers fed control and experimental diets for 15 days

Parameter	T_1	T_2	T_3	T_4	SEM
IBW (kg)	1.52	1.55	1.53	1.54	1.16
FBW (kg)	1.71^c	1.80^b	1.78^b	1.83^a	0.58
ABWG (kg)	0.19^c	0.25^b	0.25^b	0.29^a	0.21
ADFI (g)	256.55^c	303.13^b	308.27^b	331.25^a	2.12
FCR	1.88^a	1.69^b	1.72^b	1.59^c	-
Mortality (%)	0	0	0	0	-

^{abc} means with the same superscripts along a row are not significantly (P>0.05) different from each other. * IBW: initial body weight, FBW: final body weight, ABWG: average body weight gain, ADFI: average daily feed intake, FCR: feed conversion ratio. T_1 = control-water), T_2 = 30 mg/L Synthetic Vitamin C (based on manufacturers dosage), T_3 = 30ml/L of orange extract, and T_4 = 50ml/L of orange extract.

In table 3, rectal temperature (RTC) of birds in T_1 had the highest RTC by day 15 (43.76 ± 4.43°C), which was significantly higher ($P < 0.05$) than in T_2, T_3, and T_4. By day 5 onward, RTC in T_2, T_3, and T_4 decreased, with T_4 showing the lowest RTC at day 15 (39.06 ± 4.71°C). Body temperature (BTC) followed a similar trend, with T_1 showing significantly higher ($P < 0.05$) BTC by day 15 (41.65 ± 4.28°C), compared to T_2, T_3, and T_4. Birds on T_1 exhibited a significantly higher panting rate by day 15 (56.63 ± 14.16 breaths/min), compared to T_2, T_3, and T_4 ($P < 0.05$). T_2, T_3, and T_4 had consistently lower PR values, with T_4 showing the lowest panting rate (48.15 ± 10.51 breaths/min by day 15). Consequently, birds on T_1 had significantly higher RR from day 5 onward (25.81 ± 11.01 breaths/min at day 15) compared to the other treatments ($P < 0.05$). T_2, T_3, and T_4 had lower RR values, with no significant differences among them.

Table 4 reported the biochemical parameters measured in the study across treatments. AST levels, T_1 consistently exhibited the highest values, particularly at day 15 (177.5 ± 4.12 U/L), indicating greater liver stress. In contrast, treatments T_2, T_3, and T_4 resulted in significantly lower AST levels, with T_4 showing the most improvement (151.67 ± 3.77 U/L at day 15). ALT levels followed a similar trend. T_1 had significantly higher ALT levels by day 15 (10.65 ± 0.16 U/L), reflecting greater liver strain. The ALT levels in T_2, T_3, and T_4 were significantly lower, with T_4 and T_2 showing the best results. Glucose levels were also highest in T_1 (195.1 ± 32.0 mg/dL at day 15), showing a higher stress response in terms of energy metabolism. T_2, T_3, and T_4 significantly lowered glucose levels, with T_4 showing the greatest reduction (162.1 ± 32.0 mg/dL), indicating better glucose management and potentially reduced stress. Creatinine levels, indicative of kidney function, were significantly higher in T_1 (3.03 ± 1.06 mg/dL at day 7), suggesting kidney stress. T_2, T_3, and T_4 had significantly lower creatinine levels, with T_4 showing the lowest levels by day 15 (0.960 ± 1.10 mg/dL).

As reported in Figure 1, the ETG results show that T_1 experienced the highest heat stress, peaking at day 5 and gradually declining. T_2 and T_3 also peaked at day 5 but at lower levels than T_1. T_4 consistently exhibited the lowest ETG, with a smaller peak and faster reduction, demonstrating superior heat stress management. The ITG results showed in Figure 2, reported that T_1 had the highest initial ITG which gradually decreased over time, indicating poor internal heat regulation. T_2 exhibited a steady decline, with a sharp drop after day 10. T_3 showed a delayed reduction in ITG, peaking around day 10 before declining. T_4 consistently maintained the lowest and most stable ITG levels throughout the study, demonstrating superior internal heat management. The TTG results in Figure 3 show that T_1 had the highest peak around day 5, indicating the greatest heat stress, with a gradual decrease over time. T_2 and T_3 had similar trends, peaking slightly lower than T_1 and steadily declining, reflecting moderate stress reduction. T_4 displayed the lowest TTG, with a sharp decline after day 5, reaching near-zero by day 15, indicating the best heat stress management.

The glycogen levels in the treatments demonstrate significant differences ($P < 0.05$) in glycogen storage among the groups. For glycogen 1, T_1, T_2 and T_3 have comparable glycogen levels, ranging from 0.750 to 0.850, all marked with the same significance level. In contrast, T_4 significantly increased ($P < 0.05$) glycogen storage to 1.45, indicating superior glycogen accumulation.

Similarly, glycogen 2 results show T_1, T_2, and T_3 with similar glycogen levels ranging from 0.825 to 0.950. T_4 again demonstrated a significant increase in glycogen storage at 1.34 These findings suggest that higher supplementation of citrus-sweet orange enhances glycogen reserves in broilers, indicating its potential as an effective nutritional strategy for improving energy storage and overall performance in poultry production under heat stress conditions.

Table 3 - Effect of treatments (vitamin supplement) x periods on physiological parameters of heat-stressed broiler chickens

Parameters \ Treatment	Day	T_1	T_2	T_3	T_4
Rectal temperature (RTC ^{0}C)	0	41.09 ± 3.16	40.79 ± 3.23	40.10 ± 4.72	41.50 ± 3.54
	5	43.76 ± 4.43^a	42.10 ± 3.42^b	42.50 ± 4.18^b	42.41 ± 4.15^b
	10	42.70 ± 5.13^a	40.76 ± 3.33^b	40.76 ± 3.63^b	40.01 ± 4.23^b
	15	42.55 ± 4.17^a	40.26 ± 4.61^b	40.22 ± 4.53^b	39.06 ± 4.71^b
Body temperature (BTC ^{0}C)	0	38.78 ± 3.16	38.67 ± 4.23	38.53 ± 3.64	38.53 ± 4.20
	5	41.65 ± 4.28^a	40.03 ± 3.31^b	40.67 ± 3.11^b	40.45 ± 3.22^b
	10	40.67 ± 3.66^a	38.33 ± 4.33^b	38.51 ± 4.81^b	38.13 ± 3.42^b
	15	40.00 ± 3.52^a	38.11 ± 5.71^b	38.67 ± 4.91^b	38.14 ± 3.72^b
Panting rate (PR breath/min)	0	32.00 ± 1.00	33.21 ± 0.00	32.30 ± 0.00	32.00 ± 0.00
	5	56.63 ± 14.16^a	52.10 ± 11.26^b	52.63 ± 12.66^b	52.10 ± 12.81^b
	10	54.63 ± 12.11^a	51.21 ± 10.46^b	51.41 ± 11.32^b	51.23 ± 13.26^b
	15	52.81 ± 15.06^a	48.14 ± 11.16^b	48.13 ± 14.51^b	48.15 ± 10.51^b
Respiratory rate (RR breath/min)	0	20.63 ± 1.23	20.63 ± 1.16	20.63 ± 1.26	20.63 ± 0.16
	5	24.63 ± 7.22^a	22.93 ± 9.06^b	23.01 ± 7.06^b	22.20 ± 9.11^b
	10	25.81 ± 11.01^a	21.03 ± 10.01^b	21.34 ± 8.16^b	20.77 ± 7.56^b
	15	25.12 ± 12.36^a	21.23 ± 11.51^b	21.41 ± 8.04^b	20.64 ± 11.31^b

^{abc} means with the same superscripts along a row are not significantly ($P > 0.05$) different from each other. T_1 = control-water), T_2 = 30 mg/L Synthetic Vitamin C (based on manufacturers dosage), T_3 = 30ml/L of orange extract, and T_4 = 50ml/L of orange extract.

Table 4 - Effect of treatments (vitamin supplement) x periods on serum blood biochemical parameters of heat-stressed broiler chickens

Parameters \ Treatment	Day	T_1	T_2	T_3	T_4
AST (88-208 U/L)	0	135.2 ± 2.15	136.2 ± 2.15	134.5±2.15	134.9 ± 2.15
	5	177.5 ± 4.12^a	156.1 ± 4.15^b	157.5 ± 5.56^b	155.3 ± 4.71^{bc}
	10	170.2 ± 3.11^a	154.5 ± 4.17^b	152.5 ± 5.12^b	153.5 ± 3.62^b
	15	166.1 ± 4.49^a	152.3 ± 5.71^b	150.4 ± 5.88^b	151.67 ± 3.77^b
ALT (9-37 U/L)	0	4.18 ± 0.1	4.67 ± 0.1	4.53 ± 0.1	4.53 ± 0.16
	5	10.65 ± 0.16^a	6.03 ± 0.16^b	7.07 ± 0.16^{bc}	5.45 ± 0.16^{bc}
	10	8.03 ± 0.26^a	5.68 ± 3.12^c	6.0 ± 0^b	5.02 ± 1.44^c
	15	5.13 ± 0.18^a	4.18 ± 0.1^c	5.07 ± 0.16^{ab}	4.85 ± 0.16^c
Glucose	0	170.8 ± 32	170.5 ± 32	170.9 ± 32	171.2 ± 32.0
	5	195.1 ± 32^a	181.2 ± 26.2^b	183.1 ± 20.15^b	178.1 ± 17.25^c
	10	182.1 ± 32^a	177.2 ± 23.2^b	178.1 ± 18.13^b	169.0 ± 18.27^c
	15	175.1 ± 32.01^a	162.1 ± 31^b	163.1 ± 31^b	162.1 ± 32^b
Creatinine	0	0.86 ± 0.12	0.85 ± 0.12	0.82 ± 1.12	0.88 ± 1.12
	5	3.03 ± 1.06^a	2.63 ± 1.01^b	2.61 ± 1.06^b	2.60 ± 1.01^b
	10	1.60 ± 0.05^a	1.25 ± 0.05^b	1.25 ± 0.1^b	1.24 ± 0.10^b
	15	1.020 ± 0.1	0.950 ± 0.12	0.910 ± 1.11	0.960 ± 1.1

^{abc} means with the same superscripts along a row are not significantly ($P > 0.05$) different from each other; * AST: aspartate aminotransferase, ALT: alanine aminotransferase. T_1 = control-water), T_2 = 30 mg/L Synthetic Vitamin C (based on manufacturers dosage), T_3 = 30 ml/L of orange extract, and T_4 = 50 ml/L of orange extract.

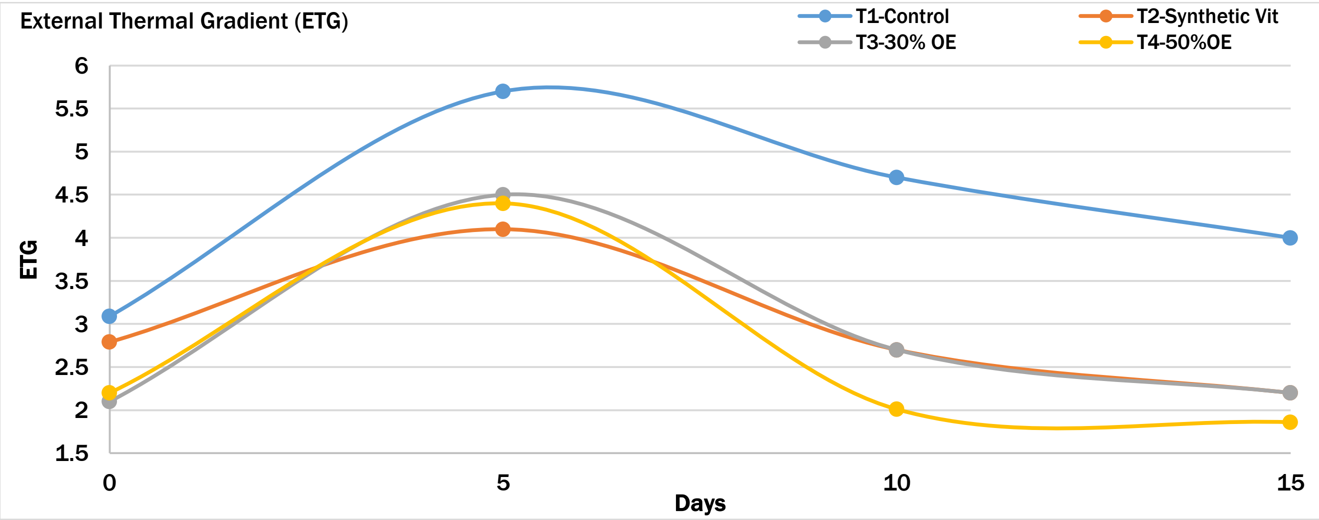

Figure 1 - Effect of treatments on external thermal gradient of experimental birds.

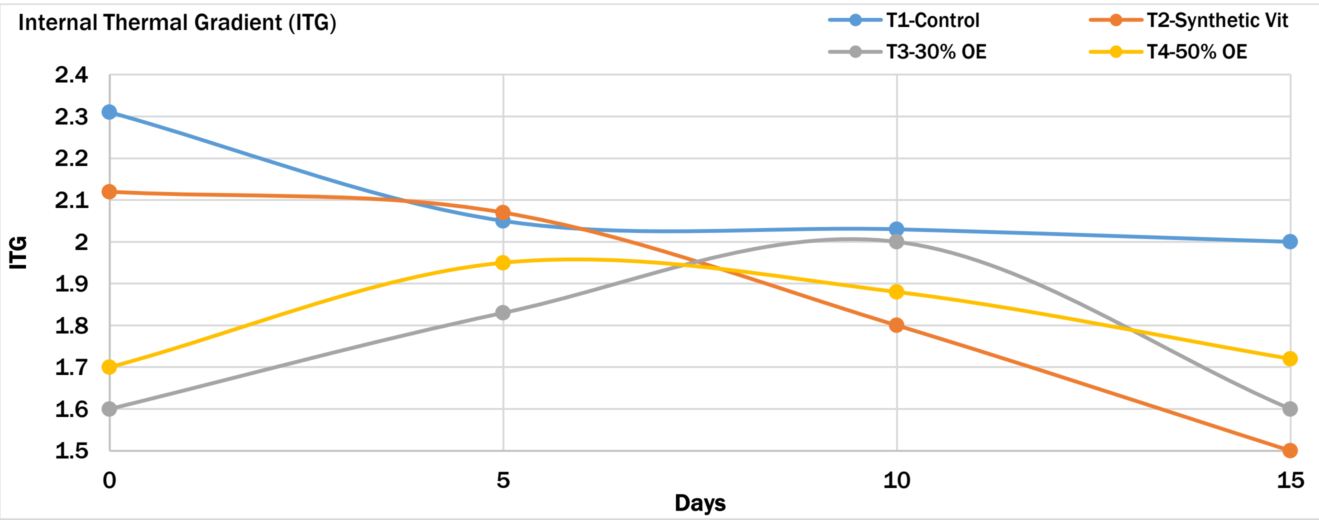

Figure 2 - Effect of treatments on internal thermal gradient of experimental birds.

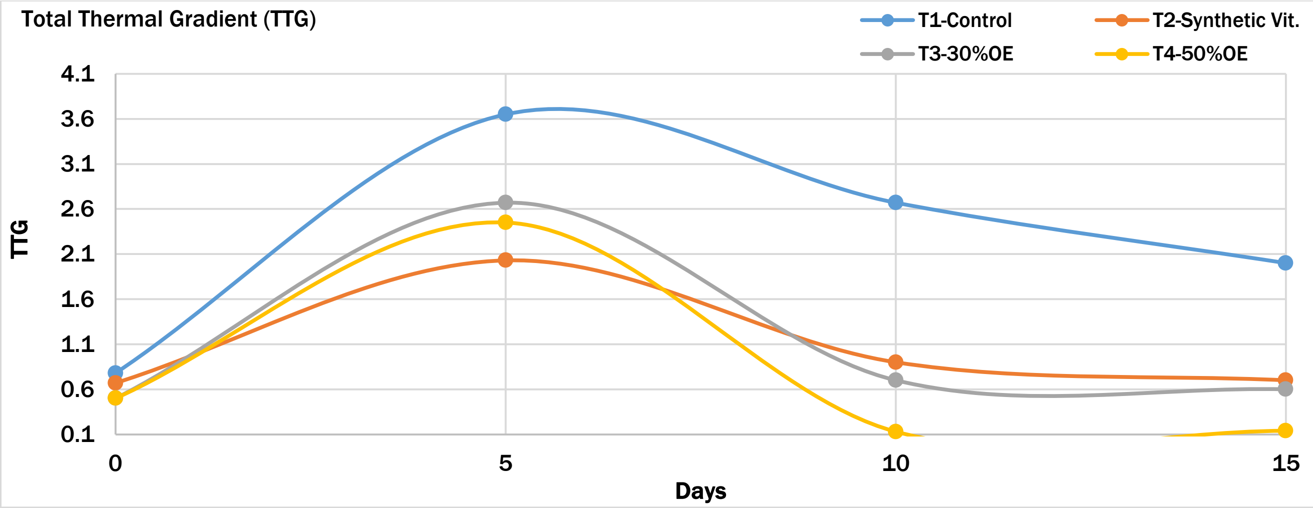

Figure 3 - Effect of treatments on total thermal gradient of experimental birds.

Table 5 - Muscle glycogen results of heat stressed broilers to ameliorating effects of synthetic vitamin C and *Citrus sinensis* in humid tropics.

Muscle glycogen parameters / Treatments	T_1	T_2	T_3	T_4
Glycogen 1	0.750 ± 0.2^{a}	0.800 ± 0.05^{a}	0.850 ± 0.05^{a}	1.45 ± 0.050^{b}
Glycogen 2	0.825 ± 0.25^{a}	0.870 ± 0.025^{a}	0.950 ± 0.02^{a}	1.34 ± 0.035^{b}

^{abc} Means along the same row with similar superscripts are not significantly (P > 0.05) different using Duncan's test as post hoc analysis. T_1 = control-water), T_2 = 30 mg/L Synthetic Vitamin C (based on manufacturers dosage), T_3 = 30ml/L of orange extract, and T_4 = 50ml/L of orange extract.

DISCUSSION

The present study investigates the use of organic materials, specifically sweet orange extract (OE), as an alternative to synthetic Vitamin C and its relationship with periods in mitigating heat stress in broiler chickens. The growth performance results showed that broilers on supplemented OE T_4 group, exhibited the best growth performance, indicating a positive influence of OE on feed intake and nutrient utilization. These findings are consistent with previous research that highlights the role of vitamin C in improving feed efficiency and growth performance under heat stress conditions (Sumanu et al., 2024; Mohamed et al., 2024). The improvement in growth performance in the OE-supplemented groups can be attributed to the antioxidant properties of sweet orange. Citrus fruits, including sweet orange, are rich in vitamin C, flavonoids, and carotenoids, which have been shown to reduce oxidative stress by scavenging free radicals (Ayoola et al., 2010). Under heat stress, oxidative stress is a major factor that impairs the physiological functions of poultry, leading to reduced feed intake and poor growth (Sahin et al., 2002). The antioxidant properties of sweet orange likely helped to mitigate this oxidative damage, allowing the birds to maintain better growth and nutrient absorption. Additionally, vitamin C plays a role in corticosterone biosynthesis, a hormone that supports energy metabolism during stress (Oguntunji et al., 2019, Ayoola et al., 2023). By enhancing energy metabolism, OE likely helped broilers maintain higher growth rates despite the heat stress conditions.

Heat stress leads to physiological changes in broilers, including elevated rectal temperature, heart rate, and respiratory rate, all of which indicate the birds' efforts to dissipate excess heat and maintain homeostasis (Yahav, 2009). The present study found that broilers on T_4 had significantly lower rectal temperatures, respiratory rates, and panting rates compared to the T_1, indicating improved thermal regulation. These results suggest that OE supplementation helped in reducing the heat load on the birds. The lower rectal temperatures in the OE groups may be due to the improved antioxidant defense provided by the bioactive compounds in sweet orange. Previous studies have shown that vitamin C supplementation reduces heat stress-induced hyperthermia in poultry by enhancing the bird's ability to dissipate heat (Akbarian et al., 2016). Additionally, vitamin C has been reported to enhance peripheral blood flow, which aids in heat dissipation by increasing heat loss through the skin (Alabi et al., 2021). Sweet orange extract, rich in vitamin C, likely reduced the metabolic and oxidative stress associated with heat, allowing the birds to maintain more normal respiratory function. The reduction in panting behavior in the OE groups could also be attributed to better hydration and electrolyte balance, which are often disrupted under heat stress (Sahin et al., 2002). Vitamin C has been shown to reduce respiratory alkalosis, a common consequence of excessive panting in heat-stressed birds, by buffering blood pH (Minka and Ayo 2011, Ayoola et al., 2023).

Biochemical markers of stress, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and creatinine, were also measured in this study to assess the effects of heat stress and the protective role of OE. Elevated levels of AST and ALT are indicative of liver stress and damage, while high creatinine levels suggest renal impairment, both of which are common under heat stress conditions (Sahin et al., 2002). The AST and ALT levels were significantly higher in the control group as compared to OE and synthetic vitamin, suggesting that OE helped to protect the liver from heat-induced damage. The protective effect of OE on liver function may be due to the antioxidant properties of vitamin C, which reduces oxidative stress and prevents liver cell damage (Majekodunmi et al., 2014). Studies have shown that antioxidants like vitamin C enhance the liver's capacity to neutralize free radicals, thereby preventing lipid peroxidation and maintaining normal liver function (Sahin et al., 2002).

Heat stress often leads to hyperglycemia as a result of increased corticosterone levels, which stimulate gluconeogenesis to provide additional energy for coping with stress (Yahav, 2009). The glucose levels in the control group were significantly higher (195.1 mg/dL) than those in the OE-supplemented and synthetic vitamin groups, indicating that the OE helped to regulate glucose metabolism and prevent excessive glucose release. The lower glucose levels in the treated groups suggest that the birds were under less stress, likely due to the antioxidant and anti-inflammatory effects of the supplements (Majekodunmi et al., 2014). Similarly, creatinine levels, which indicate renal function, were highest in the control group but significantly lower in the treatment groups, suggesting that vitamin supplements and OE also helped to protect kidney function under heat stress. Heat stress can lead to dehydration and reduced kidney function, but the presence of bioactive compounds in sweet orange likely improved the birds' hydration status and electrolyte balance, preserving renal function (Goto et al., 2022).

Glycogen storage is crucial for energy supply, especially under stressful conditions where energy demand is heightened (Minka and Ayo 2011, Oguntunji et al., 2019). The study found that the treated groups with vitamin supplement and OE had significantly higher glycogen reserves in both the liver and muscle tissues compared to the control group. This suggests that OE compared well with synthetic vitamin supplement, and helped to enhance energy storage, possibly by improving glucose utilization and reducing the energy cost of coping with heat stress. The antioxidant properties of vitamin C may have also contributed to preserving glycogen stores by reducing the oxidative breakdown of glycogen (Attia et al., 2011). The external thermal gradient (ETG) and internal thermal gradient (ITG) are measures of the bird's ability to regulate body temperature in response to environmental heat. The results showed that the T_1 had the highest ETG and ITG, indicating poor thermal regulation and greater susceptibility to heat stress. In contrast, T_4 group had the lowest ETG and ITG, demonstrating improved thermoregulation. This is consistent with previous research, which suggests that vitamin C enhances heat dissipation by improving blood circulation and reducing oxidative stress (Mujahid et al., 2009). According to Mormede et al. (2017), chickens generally recover from acute handling stress within 24 hours, therefore, the consistent results of measured parameters of experimental birds in this study can be attributed to the induced heat stress.

CONCLUSION

The findings of this study provide strong evidence that sweet orange extract, particularly at a concentration of 50%, is an effective organic alternative to synthetic vitamin C for mitigating heat stress in broilers. The OE-supplemented groups exhibited significantly better growth performance, lower physiological stress indicators, improved biochemical parameters, and greater energy storage compared to the control and synthetic vitamin C groups. The antioxidant, anti-inflammatory, and heat-mitigating properties of sweet orange likely contributed to these improvements. This study supports the use of organic supplements like sweet orange extract in sustainable poultry production, particularly in tropical climates where heat stress is a major challenge.

DECLARATION

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Author's contribution

Ayoola Mathew O. contributed to data collection and article write up, Aderemi Foluke A and Lawal Tunde .E. contributed to writing of manuscript, Alabi Olufemi M and Adeleye Bobola. E contributed to research design and data collection, Oguntunji Abel O. contributed to statistical analysis and result interpretation, Oladejo Opeyemi O contributed to data collection and laboratory analysis.

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Competing interest

The authors declare that there is no competing interest during the data collection or writing up of this article.

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