



PHYSIOLOGICAL RESPONSES IN BROILER CHICKENS ADMINISTERED LYCOPENE DURING THE HOT-DRY SEASON

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ABSTRACT

This study evaluates the effects of lycopene administration on body weight, mortality, cloacal temperature, and haematological responses, in broiler chickens exposed to heat stress. 40 day-old broiler chicks were divided into control and lycopene groups, 20 chickens each. Each bird in the control group received olive oil (1 ml.kg⁻¹), and lycopene at 10 mg.kg⁻¹ mixed with olive oil was given to each bird in the lycopene group by oral gavage once daily for 28 days. The dry- and wet-bulb temperature of the broiler chickens' pen was recorded three times daily from days 8 to 28. The body weights were measured weekly. The incidence of mortality was recorded. The cloacal temperatures were measured on days 14, 21 and 28. The blood samples were collected for haematological analyses, on day 28. The results indicated a high overall temperature-humidity index (31.24 ± 0.43 °C) of the thermal micro-environment of the broiler chickens. There was no significant difference ($P > 0.05$) in body weight. The percentage mortality in the control group was relatively higher compared to the lycopene group. Lycopene reduced the cloacal temperature responses and the daily fluctuations in broiler chickens. The decreased ($P < 0.05$) heterophil:lymphocyte ratio and percentage erythrocytes

haemolysis were recorded in the lycopene group. In conclusion, lycopene administration reduced mortality and improved cloacal temperature and haematological responses without exerting any significant beneficial or negative effects on the body weight in broiler chickens exposed to heat stress.

Key words: cloacal temperature; erythrocytes fragility; heat stress; heterophil:lymphocyte ratio; mortality; temperature fluctuations

INTRODUCTION

Genetic selection of commercial broiler lines has produced birds with fast growth rates, low immune status, and highly susceptible to heat stress [38]. The health and productivity of chickens may be adversely affected by high ambient temperature (AT) and high relative humidity (RH) [32]. The hot-dry season in the Northern Guinea Savannah zone of Nigeria is characterised by high AT and high RH, which causes heat stress in broiler chickens, consequently leading to economic loss including mortality [4]. Heat stress increases body temperature in birds and disrupts body temperature rhythm which may lead to adverse effects on physiological functions [9]. To enable optimal

body functions during stressful conditions, broiler chickens' body temperature, like those of mammals, must be kept within a certain physiological range [22]. Heat stress decreases erythrocytes and immune functions and blood parameters are a useful tool in evaluating the health status of broiler chickens exposed to stressful conditions [13].

Due to climate change, finding feeding and management strategies to lessen the effects of heat stress on poultry production is crucial, not just in tropical countries, but for many other areas of the world as well. The negative effects of heat stress on broiler chickens have been alleviated by the application of a variety of mitigation management methods, including nutritional supplements [17, 40]. One of the potent dietary carotenoids for preventing the damaging effects of heat stress on cells and tissue is lycopene [36]. It is highly effective in removing reactive oxygen species (ROS), particularly singlet oxygen [25]. Lycopene is beneficial for enhancing growth responses in broiler chickens exposed to heat stress [2, 26, 39]. Lycopene's health benefits may be linked to its antioxidant properties, which enhance the numerous physiological reactions in broiler chickens [20, 35].

This study aims to evaluate body weight, mortality, cloacal temperature and haematological responses in broiler chickens exposed to heat stress during the hot-dry season and the ameliorative effects of lycopene.

MATERIALS AND METHODS

Location

The study was conducted in the poultry house of the Veterinary Teaching Hospital, Ahmadu Bello University, Samaru (11° 10' N, 07° 38' E), Zaria. The experiment was carried out from March to April, during the hot-dry season of the zone [10].

Experimental flock and management

Forty day-old, male, *Arbor acres* broiler chicks with an average weight of 38.65 grams per bird were obtained from a reputable sales outlet. On arrival at the pens, they were assigned to two different groups, that is, the control and lycopene groups. The chicks were raised on a deep litter system. The birds were fed a standard diet from day one to day 28. Proximate analysis of commercial feed was conducted as indicated in Table 1. Clean drinking water

was provided *ad libitum*. Biosecurity measures were followed throughout the experiment.

Table 1. Nutrient contents of the broiler chickens feed

Nutrient contents	Amount
Metabolisable energy (kcal.kg ⁻¹)	2800
Proximate analysis* (%):	
i) Dry matter	97.50
ii) Crude protein	24.74
iii) Crude fibre	4.61
iv) Oil	3.40
v) Ash	4.50
vi) Nitrogen-free extract	62.75

* Analysed in the biochemical laboratory, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

Ethical approval

This study with reference number ABU-CAUC/2021/025 was approved by the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria, Nigeria.

Experimental design and groupings

Forty day old broiler chicks were divided into two groups of 20 chickens each, using simple random sampling. Each bird in the control group received olive oil (1 ml.kg⁻¹), and lycopene at 10 mg.kg⁻¹ mixed with olive oil was given to each bird in the lycopene group by oral gavage once daily for 28 days. A gelatine capsule containing 10 mg of lycopene (General Nutrition Corporation, Pittsburgh, U.S.A.) was reconstituted in olive oil (Goya en espana, S.A.U., Sevilla, Spain) to a suitable working concentration [23]. Commencing at 7:00 h (GMT+1), each bird in the groups was administered with either olive oil only (1 ml.kg⁻¹) or mixed with lycopene by oral gavage once daily for 28 days. Lycopene was administered at 10 mg.kg⁻¹ [39]. Body weights were measured weekly. The incidence of mortality was recorded. Cloacal temperature of each bird in the groups was measured on days 14, 21 and 28. On day 28, three ml of blood per bird was collected from 7 broiler chickens per group via wing vein into potassium ethylenediaminetetraacetate (K₂EDTA) sample bottles. The blood collected was used for haematological analyses and erythrocytes osmotic fragility test analyses.

Thermal micro-environmental parameters

The dry- and wet-bulb temperatures of the micro-environment of the poultry pen were measured three times daily using a dry- and wet-bulb thermometer (Aura Labtech, India) at 07:00 h, 13:00 h and 19:00 h (GMT+1) from day 8 to 28. From the data, RH on each day was calculated using an online relative humidity calculator (www.1728.org/relhum.htm). The temperature-humidity index (THI) on each day was determined using the formula as described by Tao and Xin [37]:

$$\text{THI} = 0.85 \times \text{DBT} + 0.15 \times \text{WBT}$$

(where DBT = Dry-bulb temperature, and WBT = Wet-bulb temperature)

Body weight measurement

The birds were weighed singly using a weighing scale balance on days 7, 14, 21, and 28.

Cloacal temperature measurement

A digital clinical thermometer (Hartmann Digital Thermometer, Paul Hartman AG, Heidenheim, Germany) was used to take the cloacal temperature. It was placed into the cloaca about 2 cm and in direct contact with the mucosal wall. After the thermometer sounded an alert to signal that the reading had stabilized, the value was recorded.

Determination of haematological parameters

The pack cell volume was determined using the micro-haematocrit method as described by Cheesbrough [7]. The concentrations of haemoglobin (Hb) were obtained by dividing the corresponding pack cell volume values by three. For the white blood cell and differential leucocyte count, blood smears were stained with Giemsa stain as described by Cheesbrough [7]. Briefly, the blood films (methanol pre-fixed) were covered with the diluted stain (1 in 20) for 25 minutes. The slides were finally washed off the stain with tap water, air-dried and observed microscopically under oil immersion. A total of 100 leucocytes were counted on each slide, including heterophils, lymphocytes and monocytes.

Determination of erythrocyte osmotic fragility

Erythrocyte Osmotic Fragility (EOF) was determined according to the method described by Faulkner and King [14]. Briefly, 0.02 ml of blood from each bird in the groups were added to tubes containing phosphate-buffered

sodium chloride (NaCl) solution at pH 7.4 at increasing concentrations (0, 0.1, 0.3, 0.5, 0.7, and 0.9 %). After being gently mixed, the tubes were incubated for 30 minutes at room temperature (25–26 °C). After mixing the contents of each tube and centrifuging it at 400 ×g for 10 minutes (Hettich Centrifuge, Germany), the supernatant was decanted. The optical density of the supernatant was determined using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, England) at 540 nm. Haemolysis in each tube was expressed as a percentage, with 100 % being the haemolysis in distilled water (0 % NaCl). The percentage haemolysis was calculated using the formula:

$$\text{Percentage haemolysis} = \frac{\text{Optical density of test solution} \times 100}{\text{Optical density of standard solution}}$$

Data analyses

The data obtained were expressed as a percentage and mean ± standard error of the mean (Mean ± SEM). DBT, RH and THI were analysed using one-way analyses of variance (ANOVA). The body weights and cloacal temperatures were analysed using repeated-measures two-way ANOVA. The ANOVA tests were followed by Tukey's post-hoc test to compare the mean. The haematological parameters and erythrocyte osmotic fragility were analysed using the Student t-test. Values of $P < 0.05$ were considered significant. These analyses were carried out using GraphPad 5 for Windows (San Diego, CA, USA).

RESULTS

Thermal environment parameters inside the broiler chickens' house

Thermal environment parameters inside the broiler chickens' house during the study period are presented in Table 2. There was a significant increase in DBT and THI values at 13:00 h compared to 7:00 h and 19:00 h. A significantly ($P < 0.05$) higher RH value was recorded at 7:00 h than at 13:00 h and 19:00 h, although the differences between the values recorded at 13:00 h and 19:00 h were insignificant.

Table 2. Thermal environment parameters inside the broiler chickens' house

Parameter	Hour of the day			Overall
	7:00 h	13:00 h	19:00 h	
DBT (°C)	24.61 ± 0.49c (19–29)	39.86 ± 0.67a (32–45)	32.39 ± 0.51b (27–38)	32.29 ± 0.42 (27.33–35.67)
RH (%)	79.10 ± 1.69a (47.40–92.00)	46.86 ± 2.41b (17.90–93.80)	52.27 ± 3.05b (16.70–79.90)	59.41 ± 1.74 (41.40–78.90)
THI	24.21 ± 0.48 c (18.85–28.55)	38.29 ± 0.65a (31.10–43.20)	31.23 ± 0.45b (25.95–35.75)	31.24 ± 0.43 (26.68–34.47)

^{ab}Means for the same parameter having different superscript letters are significantly different ($P < 0.05$).
 Values in parentheses are the minimum and maximum. DBT: Dry-bulb temperature; RH: Relative humidity;
 THI: Temperature-humidity index; n = 21 days.

Body weight and mortality

Body weights and mortality of broiler chickens administered lycopene during the study period are presented in Table 3. There was no significant ($P > 0.05$) difference in the body weights, between the control and lycopene groups. Percentage mortality of 15 % recorded in the control group was relatively higher compared to 5 % recorded in the lycopene group.

Table 3. Body weight and mortality of broiler chickens administered lycopene

Parameters	Days	Control	Lycopene
Body weight (g per bird)	1	38.63 ± 0.13	38.67 ± 0.13
	7	87.49 ± 2.19	90.67 ± 0.67
	14	155.50 ± 0.63	154.6 ± 2.51
	21	316.10 ± 3.39	304.80 ± 9.70
	28	701.70 ± 12.61	697.40 ± 23.54
Mortality (%)	-	15	5

Daily fluctuations in cloacal temperature responses

Daily fluctuations in cloacal temperature responses in broiler chickens administered lycopene during the study period are presented in Table 4. In each group, an increase ($P < 0.05$) in the cloacal temperature values was recorded at 13:00 h compared to 7:00 h and 19:00 h. A decrease ($P < 0.05$) in the value was obtained in the lycopene group compared to the control group at 13:00 h on days 21 and 28. Whereas an increase ($P < 0.05$) was observed in the lycopene group compared to the control group at 7:00 h on days 21 and 28, and at 19:00 h on day 28.

Daily range of fluctuations in cloacal temperature responses

A decrease ($P < 0.05$) was observed in the daily range of fluctuations in cloacal temperature responses in broiler chickens administered lycopene compared to the control group on days 14, 21 and 28 (Table 5).

Table 4: Daily fluctuations in cloacal temperature (°C) responses in broiler chickens administered lycopene

Days	Hour of the day	Control	Lycopene
Day 14	07:00 h	40.46 ± 0.052 (40.10–40.90)	40.54 ± 0.072 (39.90–41.10)
	13:00 h	41.37 ± 0.101 (40.60–42.30)	40.95 ± 0.111 (39.50–41.70)
	19:00 h	40.44 ± 0.082 (39.70–40.90)	40.60 ± 0.062 (40.20–40.90)
Day 21	07:00 h	40.61 ± 0.072b (40.20–41.00)	41.14 ± 0.062a (40.80–41.70)
	13:00 h	41.64 ± 0.051a (41.10–42.10)	41.40 ± 0.031b (41.20–41.60)
	19:00 h	41.41 ± 0.101 (40.00–41.70)	41.00 ± 0.031 (40.70–41.20)
Day 28	07:00 h	40.52 ± 0.083b (39.90–41.00)	41.13 ± 0.063a (40.80–41.90)
	13:00 h	42.05 ± 0.081a (41.60–42.80)	41.72 ± 0.061b (41.10–42.20)
	19:00 h	41.06 ± 0.062b (40.70–41.40)	41.53 ± 0.092a (41.00–42.50)

^{abc12} Means for the same parameter having different superscript letters across the row are significantly ($P < 0.05$) different; Values in parentheses are the daily minimum and maximum cloacal temperatures.

Table 5. Daily range of fluctuation in cloacal temperature (°C) responses in broiler chickens administered lycopene

Days	Control	Lycopene
Day 14	1.05 ± 0.12a (39.70–42.30)	0.64 ± 0.09b (39.50–41.70)
Day 21	1.09 ± 0.08a (40.00–42.10)	0.61 ± 0.08b (40.70–41.70)
Day 28	1.53 ± 0.14a (39.90–42.80)	0.77 ± 0.09b (40.80–42.50)

^{ab} Means for the same parameter having different superscript letters across the row are significantly ($P < 0.05$) different; Values in parentheses are the daily minimum and maximum cloacal temperatures.

Haematological responses

Haematological values in broiler chickens administered lycopene are presented in Table 6. There was no significant ($P > 0.05$) difference in the values of PCV, Hb, white blood cell and differential leucocyte count between the two groups. An increase ($P < 0.05$) in the values of heterophil:lymphocyte (H:L) ratio was recorded in the lycopene group.

Erythrocyte osmotic fragility

At 0.7 %, the percentage haemolysis of 8.80 ± 1.14 % obtained in the control group was significantly ($P < 0.05$) higher than the corresponding value of 3.72 ± 0.45 % recorded in the lycopene groups (Fig. 1). There was no significant difference between both groups in the percentage haemolysis at 0.9 %, 0.5 %, 0.3 % and 0.1 %.

Table 6. Haematological responses of broiler chickens administered lycopene

Parameters	Control	Lycopene
PCV (%)	34.30 ± 1.53	30.50 ± 1.95
Hb (g.dl ⁻¹)	8.15 ± 0.55	6.64 ± 0.64
WBC ($\times 10^3 \mu\text{l}$)	7.86 ± 0.61	6.12 ± 1.57
Heterophil ($\times 10^3 \mu\text{l}$)	1.22 ± 0.12	0.65 ± 0.26
Lymphocyte ($\times 10^3 \mu\text{l}$)	5.87 ± 0.49	5.38 ± 1.31
Monocyte ($\times 10^3 \mu\text{l}$)	0.13 ± 0.07	0.07 ± 0.04
H/L Ratio	$0.41 \pm 0.09a$	$0.11 \pm 0.03b$

^{ab} Means for the same parameter having different superscript letters across the row are significantly ($P < 0.05$) different; PCV = Pack cell volume; Hb = Haemoglobin concentration; WBC = White blood cell count; H/L ratio: heterophil:lymphocytes ratio.

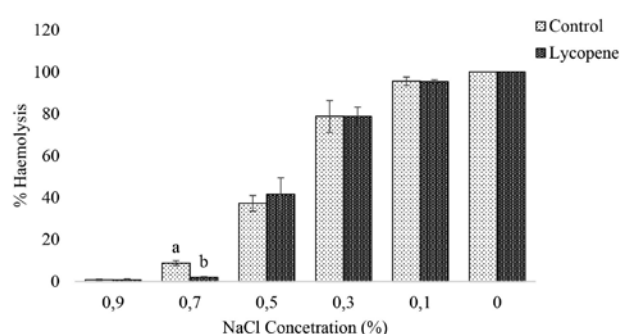


Fig. 1. Erythrocytes osmotic fragility (EOF) in broiler chickens administered lycopene

^{ab} Means for the same parameter having different superscript letters differ significantly ($P < 0.05$)

DISCUSSION

Chickens, as homeotherms, have an optimal environmental temperature range which is considered the thermo-neutral zone [3, 24]. The DBT values at 13:00 and 19:00 were 39.86 °C and 32.39 °C, respectively, which were outside the known thermoneutral zones of 18–26 °C for broiler chickens raised in tropical climates [19, 29]. The high RH was between 79.10 % and 46.86 % higher than the standard for broiler chickens grown in tropical climates, which is between 30 and 40 % [18, 40]. The high RH was between 79.10 % and 46.86 % higher than the usual range for broiler chickens grown in tropical climates, which is between 30 and 40 % [24]. Due to the difficulties in heat dissipation through evaporative cooling, broiler chickens suffer from heat stress when the ambient temperature and relative humidity are high [30, 41]. THI is an index of heat stress that is used to assess the combined impacts of both ambient temperature and RH [37]. The overall THI of 31.24 °C obtained in the present study is above 30.10 °C, reported by S u m a n u et al. [33]. The broiler chicks' thermal microenvironment may be uncomfortable given the high THI. The hourly THI values fluctuated between 24.21 ± 0.48 at 7:00 h and 38.29 ± 0.65 at 13:00 h. The fluctuation range is higher than the THI values ranging between 25.55 and 35.30, reported by S u m a n u et al. [33]. The high range of fluctuations in the THI indicates that the birds are more susceptible to the harmful effects of heat stress.

The body weight of birds in the group not administered lycopene was 701.70 ± 12.61 g per bird at day 28. This value was below 873.0 ± 1.0 g per bird at 4 weeks recorded by E g b u n i w e et al. [12] in broiler chickens during the hot-dry season in the zone. The body weight value is also below 783.7 ± 27.2 g per bird reported by S u m a n u et al. [34]. The decrease in body weight may be due to the effects of heat stress which is known to impair productivity in broiler chickens K h a n et al. [17]. In the present study, lycopene administration did not exert any significant beneficial or adverse effects on the body weight of broiler chickens. Lycopene supplementation at 200–400 mg.kg⁻¹ of diet has been shown to provide optimal protection in broiler chickens [26, 27]. In the present study, lycopene was administered at 10 mg.kg⁻¹ per day based on our earlier study indicating its beneficial effects in apparently healthy Wistar rats [23]. The value was lower than

40 mg.kg⁻¹ used by Sun et al. [35], who demonstrated the beneficial effects of lycopene on chick birth weight. The findings indicate that administering 10 mg.kg⁻¹ of supplemental lycopene for 28 days may not improve the body weight of broiler chickens. This agrees, in part, with that of Wang et al. [39] who did not observe any significant effect of 10 mg.kg⁻¹ of lycopene on the body weight of broiler chickens fed graded levels of dietary lycopene from days 1–21.

Previous reports suggest that such high DBT, RH and THI values may cause increased mortality in chickens [4]. Thus, higher mortality was recorded in the broiler chickens that were not administered lycopene and percentage mortality was lower in the broiler chickens administered 10 mg.kg⁻¹ of supplemental lycopene. Although this study was conducted under hot-dry conditions, the result is in agreement with Fathi et al. [13] who observed that lycopene supplementation alleviates adverse effects of cold stress on mortality in broiler chickens fed diets supplemented with lycopene, and attributed it to the ability of lycopene to enhance antioxidant enzyme activities. The beneficial effects of low levels (10 mg.kg⁻¹) of lycopene administration on the mortality of broiler chickens exposed to heat stress require further investigation in a large sample size.

The distinct and similar pattern of daily fluctuations of the cloacal temperature responses observed in both control and lycopene groups indicates that the temperature rhythm of the broiler chickens was maintained for normal physiological processes [9, 22]. Thus, the cloacal temperatures recorded are within the normal reference range of 40.0 to 42.0 °C, established for poultry [21, 31]. This finding suggests that the *Arbor acres* chickens have adapted to the hot-dry season in the zone. The cloacal temperatures were decreased at 13:00 h and increased at 7:00 h and 19:00 h in birds administered lycopene. These findings suggest that lycopene may be stabilizing the body temperature responses by modulating the variation through an increase in the morning and evening and decreasing temperature in the afternoon period. According to Ayob et al. [5], lycopene reduces cloacal temperature responses in broiler chickens, particularly at 12:00 h and 15:00 h. Also, decreased range of fluctuation in cloacal temperature was recorded in broiler chickens administered lycopene. Egboniwe et al. [11] reported that the wider the range of fluctuations, the more heat stress occurs and the greater the increased

chances of negative impacts. By decreasing the range of fluctuations, lycopene may, thus, mitigate the effects of heat stress on the thermoregulatory mechanisms required to maintain homeothermy.

The result shows that lycopene administration reduced the effects of heat stress in broiler chickens as evidenced by decreased H:L ratio. This was due to an increase in heterophil counts and a reduction in lymphocyte counts, causing an increase in the H:L ratio [36]. The findings agree with the report of Hosseini – Vashan [16] who recorded a decreased H:L ratio in broiler chickens fed diets supplemented with dried tomato pomace. H:L ratio is a well-known stress indicator for birds and has been known to rise in response to heat stress [6]. Depressed immune system has been associated with elevated H:L ratio [28]. The decreased H:L ratio observed in the present study suggests that lycopene may ameliorate the damaging effects of heat stress on broiler chickens' immune systems. The decreased H:L ratio seen in the present study implies that lycopene might mitigate the damaging effects of heat stress on the broiler chickens' immune systems.

Because their membranes contain high contents of unsaturated fatty acids, erythrocytes are particularly vulnerable to the damaging effects of heat stress [1, 8]. Lycopene administration significantly enhanced the integrity of the erythrocyte membrane of the broiler chickens by decreasing the percentage haemolysis. The effects of lycopene may be reducing lipoperoxidation of erythrocyte membranes via scavenging of ROS *in-vivo*. The findings of the present study are in line with the observation that increased antioxidant activity in broiler chicken erythrocytes decreased lipid peroxidation and cell membrane fragility [31]. It suggests that the antioxidant effects of lycopene may enhance erythrocyte membrane integrity by decreasing the effects of ROS on erythrocyte membranes [15]. Thus, lycopene exerts an ameliorative effect as indicated by its beneficial role in reducing the impact effects of heat stress on erythrocytes membrane fragility.

CONCLUSIONS

In conclusion, lycopene administration did not exert any significant effects on the body weights of broiler chickens. It reduced mortality and mitigates the impact of exposure to heat stress on cloacal temperature and dai-

ly fluctuations. Lycopene ameliorated the effects of heat stress by decreasing the H:L ratio and enhancing erythrocyte membrane integrity. Thus, lycopene administration reduced mortality and improved cloacal temperature and haematological responses without exerting any significant beneficial or negative effects on body weight in broiler chickens.

CONFLICT OF INTEREST

None.

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