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Inclusion of Curcumin in the Diet Combats Heat Stress in Laying Hens and Improves Physiological Efficiency and Productive Performance

Noura K. Al-Suwailem [1], Abdulaziz A. Alaqil [1], Gouda F. Gouda [1], Haytham H. Abdelwahid [1], Nancy N. Kamel [2], Osama A. El-Sayed [3] and Ahmed O. Abbas [1,*]

[1]Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia
[2]Department of Animal Production, National Research Centre, El Buhouth St., Dokki, Giza P.O. Box 12622, Egypt
[3]Department of Poultry breeding, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza P.O. Box 12611, Egypt
***Corresponding author:** aabbas@kfu.edu.sa

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ABSTRACT

Certain medicinal plant products have significantly improved poultry production and health aspects, particularly under thermal stress. This research examined the impact of including curcumin (CURC) in laying hens' diets on the productive aspects, inflammatory cytokines, stress indicators, lipid profile, and immunological indices under thermoneutral and heat stressed (HS) conditions. For a 10-week trial period, 288 commercial 40 weeks old HY-Line Brown laying hens were equally distributed into 2×4 treatment groups based on HS and CURC treatments. Hens were split into two HS treatments: a regular temperature (24°C) or HS-exposure (35°C; from 10:00 to 18:00 daily). Each cohort of hens was further divided into four CURC subgroups that received a standard diet complemented with 0, 100, 200, and 300mg of CURC per kilogram of feed, respectively. Supplementing laying hens' diets with curcumin (CURC) up to 200mg/kg significantly enhanced egg weight, daily egg production, feed intake, and feed efficiency under both thermoneutral and HS conditions. In heat-stressed hens, CURC at 200mg/kg notably reduced stress biomarkers and inflammatory cytokines. Additionally, CURC markedly improved the lipid profile in plasma, egg yolk, and liver, and enhanced key immunological markers. These findings suggest that dietary CURC supplementation at 200mg/kg can effectively support layers performance as improves egg production and quality under thermal stress conditions.

Key words: Curcumin, Laying hens, Productive performance, Stress indicators, Inflammation markers, Immunological parameters.

INTRODUCTION

Understanding how animals react to environmental stressors may benefit all species (Nassar et al. 2023; Ahmed 2025; Tesakul et al. 2025). Poultry are more vulnerable to high thermal stress compared to other animals, because of their restricted ability to dispense body heat (Deeb and Cahaner 2002). According to Tůmová and Gous (2012), laying hens do best in environments that are between 20 and 25°C. However, signs of heat stress may appear as the temperature rises over 30°C (Yardibi and Tuerkay 2008). Several studies reported that laying hens experiencing heat stress have considerable adverse impacts on many performance metrics, including body weight, gut development, egg quality, feed consumption, feed digestibility, feed efficiency, and egg production (Mashaly et al. 2004; Lara and Rostagno 2013; Abd El-Hack et al. 2017a; Abbas et al. 2022; Al-Otaibi et al. 2022). Decreased blood leukocytes, a specific decrease in lymphocytes coupled with an increase in heterophils circulation, and an inhibition of cellular and humoral immunity are all signs of impaired immunological function in heat-stressed layer (Abd El-Hack et al. 2017a; Borzouie et al. 2020). Additionally, heat-stressed laying hens exhibited elevated circulating levels of corticosterone and pro-inflammatory cytokines (Deng et al. 2012; Abbas et al. 2022; Kim and Lee 2023). Furthermore, avian cells under heat stress conditions produced excessive reactive oxygen species and free radicals, which induced lipid peroxidation (Yang et al. 2010).

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Curcumin (CURC) is a predominant polyphenol found in turmeric (*Curcuma longa*), a herbal plant from the ginger family (Priyadarsini 2014). Asian nations have traditionally used this plant as a therapeutic herb because to its antioxidant, anti-inflammatory, anti-mutagenic, antibacterial, and anti-cancer properties (Hewlings and Kalman 2017; Iweala et al. 2023; Saha 2024; Jain et al. 2025). Supplementation of CURC has been used to improve food metabolism and digestibility and to avoid biliary problems and anorexia in both animals and human (Chattopadhyay et al. 2003; Alsultan and Gameel 2004). Furthermore, CURC supplementation significantly reduces blood sugar, triglycerides and LDL levels, while enhancing hepatic function (Gandhi et al. 2011; Cui et al. 2022; Muhshi et al. 2024; Wu et al. 2024).

Birds exposed to heat stress expend substantial energy to maintain homeostasis and survival, resulting in considerable economic losses in poultry production (Quinteiro-Filho et al. 2012). Consequently, dietary strategies incorporating antioxidant-rich medicinal plant extracts, such as curcumin derivatives, have been utilized to alleviate the unfavorable impacts of heat stress (Iweala et al. 2023; Udayakumar et al. 2024; Fouad et al. 2025). Numerous avian species, such as quails (Silva et al. 2018; Liu et al. 2024), broilers (Guo et al. 2023; Chen et al. 2024; Gharib et al. 2025), and laying hens (Arshami et al. 2013; Li et al. 2024), have shown improved productivity when given CURC. CURC has successfully lessened the undesirable impacts of several environmental stressors on chicken performance (Liu et al. 2020; Nawab et al. 2020; Hafez et al. 2022; Eleiwa et al. 2023; Hernández-García et al. 2025). Yet, research on curcumin's impact on heat-stressed poultry production is still in its infancy, and more study of physiological mechanisms is required. This study investigates how dietary CURC supplementation at different dosages affects heat-stressed hens' laying performance, immunological parameters, inflammatory cytokines, stress indicators, and cholesterol profile.

MATERIALS AND METHODS

Experimental design

Two hundred eighty-eight commercial laying chickens of the HY-Line Brown breed (40 weeks old and 1940.2±10.3g average weight) were kept in separate cages (40 x 40 x 50cm³) with an ecologically controlled system (144 cages per room). One week before the beginning of the experiment, the two rooms were provided with identical environmental conditions (24±1°C, 50% relative humidity, 30Lux LED illuminations, and a daily photoperiod of 17h light and 7h dark). All hens were housed in the same environmental conditions from 40 to 50 weeks of age, except for temperature; one room was maintained at 24°C (thermoneutral) while the other was heated to 35°C from 10:00 to 18:00 daily (HS). All hens had free access to both diets and fresh water. Based on the amount of dietary curcumin (CURC-05811, ≥98.0% purity by HPLC, Sigma-Aldrich, MA, USA) supplementation, the hens in each room were sub-grouped into 36 hens per supplemented group given 0, 100, 200, or 300mg CURC per kg of basal diet. The basal diet was prepared to cover the nutritional requirements for commercial HY-Line Brown layers. The chemical analysis of the formulated diet was carried out using AOAC methodologies (Latimer Jr. 2023) and presented in Table 1.

Table 1: Ingredients and nutritional analysis of the basal diet

Ingredients	g/kg as fed
Yellow corn	566.5
Soybean meal (44% CP)	275.0
Wheat bran	10.0
Soybean oil	30.0
Bone meal	30.0
Limestone	80.0
Salt (NaCl)	4.0
Premix [1]	3.0
DL-Methionine	1.5
Calculated nutrients	(per kg diet)
Metabolizable energy	1.26MJ
Calcium	40.2g
Available phosphorus	5.2g
Determined nutrients	(per kg diet)
Crude protein	167.5g
Crude fat	66.0g
Crude fiber	47.0g

[1]Premix (content per kg of the experimental diet)): 8000IU vitamin A; 1500IU vitamin D; 4mg riboflavin; 10µg cobalamin; 15mg vitamin E; 2mg vitamin K; 500mg choline; 25mg niacin; 60mg manganese; 50mg zinc

Productive performance

Every day throughout the 40–50-week period, the number and weight of eggs laid by each bird were documented as well as daily feed intake (FI). Additionally, the average egg weight (EW) and egg production (EP%) were calculated per hen. The egg mass (EM) per hen was computed by multiplying the number of eggs by their average weight, and the feed conversion ratio (FCR) was computed by dividing the total FI by EM per hen.

Sample collection

At the completion of the trial, blood samples (n=12; per group) were obtained from hens' brachial vein using heparinized tubes (Romero and Reed 2005). Blood plasma was separated by centrifugation for 10 minutes at 4°C at 2000×g. The separate plasma was collected and kept at -20°C to examine inflammatory cytokines, stress indicators, and cholesterol levels. Another, blood samples (n=12; per group) were drawn in heparinized tubes to evaluate the immunological parameters. Twelve eggs were taken from each treatment group and cracked to isolate the yolks. To acquire liver specimens, twelve hens per each group were killed via cervical dislocation. Liver and egg yolk samples were preserved in liquid nitrogen to examine the cholesterol profile.

Inflammatory cytokines

Plasma levels of inflammatory cytokines were assessed according to the previous protocol's directives (Al-Otaibi et al. 2022) via chicken-specific ELISA kits (MyBioSource Inc., San Diego, California, USA; IL-1β: MBS2024496; IL-6: MBS2021018 and TNF-α: MBS2031870). The data were collected using an absorbance microplate reader (ELx808TM BioTek Instruments, Winooski, Vermont, USA).

Stress indicators

The stress markers were measured in plasma samples by poultry-specific ELISA kits (MyBioSource Inc., San

Diego, California, USA) following the manufacturer's directions, including the ceruloplasmin (CP; MBS1609488) (Al-Otaibi et al. 2022), malondialdehyde (MDA; MBS260816), and corticosterone (CORT; MBS2567948) (Abbas et al. 2022).

Cholesterol profile

The cholesterol levels in liver and egg yolk samples as well as plasma total cholesterol (TCH), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were evaluated using specific kit (ab65390; Abcam, Cambridge, MA, USA) and procedures outlined by Alaqil et al. (2020). Egg yolk and liver cholesterols were calculated per dL of 10mg tissue extracted solution.

Immunological parameters

Total white blood cell count (TWBC), were calculated using brilliant cresyl blue and hemocytometer slide according to (Gehad et al. 2008). An additional 10µL of the blood was applied to a glass plate and fixed with methanol to calculate the heterophil/lymphocyte (H/L) ratio according to Zhang et al. (2009) using Hema-3 dye solutions (Fisher Scientific, Pennsylvania, USA). Oil immersion was employed to identify and classify up to 200 white blood cells using a 1000× magnification microscope.

To determine the T- and B-lymphocyte stimulation index (SI), the peripheral blood mononuclear cells (PBMCs) were isolated from blood samples and SI was calculated as previously described (Alaqil et al. 2020).

Statistical analysis

The general linear model (GLM) within SPSS software (IBM Corp., Armonk, NY, USA) was used. A two-way analysis of variance (ANOVA) was conducted to evaluate the impacts of HS (two levels), CURC (four levels), and the HS×CURC interaction on immunological parameters, stress indicators, inflammatory cytokines, cholesterol profile metrics, and productive performance characteristics. A significant criterion of $P<0.05$ was used to differentiate between the variable means using the Tukey's post hoc test.

RESULTS

Productive performance

Table 2 enumerates the hens' production characteristics influenced by HS, CURC, and their interactions. HS exerted a significant deleterious impact on the production characteristics of laying hens, causing the feed conversion ratio (FCR) to rise by 14.6%. In contrast, egg production, weight and feed intake decreased by 13.3 percentage points (p.p.), 5.3%, and 8.1%, respectively. When CURC levels rose to 200mg/kg, hens' productive performance significantly enhanced ($P<0.05$). Administering CURC at 200mg/kg to heat-stressed hens displayed significant interaction effect ($P<0.05$) on their production characteristics.

Inflammatory cytokines

Table 3 indicates that the plasma inflammatory cytokines levels were significantly elevated with HS exposure. Meanwhile, a reduction in plasma inflammatory cytokines was observed ($P<0.05$) in response to dietary CURC supplementation following a dose dependent manner. Notably, the rise in IL-6 and IL-1β levels in hens under heat stress was decreased ($P<0.05$) by dietary therapy with 200mg/kg CURC.

Stress indicators

Table 4 delineates the impacts of HS, CURC, and their interactions on the stress indicators of the hen. The findings indicated that plasma CORT, MDA, and CP levels were significantly elevated due to HS experience. Conversely, stress indicators were decreased ($P<0.05$) when hens were fed dietary CURC supplementation at increasing dosages. There was a significant interaction effect ($P<0.05$) for HS×CURC on the stress indicators of the hens. Administration of 200mg/kg of CURC diminished ($P<0.05$) the plasma stress markers in heat-stressed hens.

Table 2: Effect of dietary *curcumin* (CURC) supplementation on the productive performance of laying hens exposed to cyclic heat stress conditions

Items	Thermoneutral (24°C)[1]				Heat stress (35°C)				SEM	*P-value*		
	0	100	200	300	0	100	200	300		HS	CURC	HS × CURC
EP, %	91.5^{b}	91.3^{b}	96.4^{a}	93.5^{ab}	72.0^{e}	81.0^{d}	85.1^{c}	81.7^{d}	0.69	<0.001	<0.001	<0.001
EW, g	63.8^{ab}	63.3^{bc}	64.6^{a}	64.1^{ab}	60.6^{de}	59.2^{e}	62.5^{c}	60.2^{d}	0.24	<0.001	<0.001	<0.001
FI, g	107^{c}	110^{b}	113^{a}	112^{a}	100^{f}	101^{e}	104^{d}	101^{e}	0.25	<0.001	<0.001	<0.001
FCR	1.83^{e}	1.90^{de}	1.82^{e}	1.87^{de}	2.29^{a}	2.14^{b}	1.97^{cd}	2.07^{bc}	0.03	<0.001	<0.001	<0.001

[1]Treatment groups: the laying hens were maintained at 24°C as a thermoneutral or exposed to a cyclic heat stress of 35°C (HS), the hens were further divided into four subgroups supplemented with curcumin (CURC) at 0, 100, 200, and 300mg/kg of the diet. EP: egg production; EW: egg weight; FI: feed intake; FCR: feed conversion ratio. Means within the same raw with different superscripts significantly differ ($P<0.05$). SEM: standard error of means.

Table 3: Effect of dietary *curcumin* (CURC) supplementation on the inflammatory cytokines of laying hens exposed to cyclic heat stress conditions

Items	Thermoneutral (24°C)[1]				Heat stress (35°C)				SEM	*P-value*		
	0	100	200	300	0	100	200	300		HS	CURC	HS × CURC
TNF-α, pg/mL	97.1	93.7	91.4	92	167.8	163.9	156	159.3	0.25	<0.001	<0.001	0.274
IL-6, pg/mL	4.1^{d}	3.6^{d}	3.4^{d}	3.5^{d}	9.6^{a}	9.4^{a}	5.0^{c}	7.4^{b}	0.24	<0.001	<0.001	<0.001
IL-1β, pg/mL	266^{d}	260^{d}	241^{d}	247^{d}	823^{a}	735^{b}	597^{c}	692^{b}	0.69	<0.001	<0.001	<0.001

[1]Treatment groups: the laying hens were maintained at 24°C as a thermoneutral or exposed to a cyclic heat stress of 35°C (HS), the hens were further divided into four subgroups supplemented with curcumin (CURC) at 0, 100, 200, and 300mg/kg of the diet. TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; IL-1β: Interleukin-1β. Means within the same raw with different superscripts significantly differ ($P<0.05$). SEM: standard error of means.

Table 4: Effect of dietary *curcumin* (CURC) supplementation on the stress indicators of laying hens exposed to cyclic heat stress conditions

Items	Thermoneutral (24°C)[1]				Heat stress (35°C)				SEM	*P-value*		
	0	100	200	300	0	100	200	300		HS	CURC	HS × CURC
CORT, pg/mL	5.5^{d}	5.5^{d}	3.6^{e}	4.9^{d}	11.4^{a}	10.7^{a}	7.4^{c}	8.7^{b}	0.21	<0.001	<0.001	<0.001
MDA, nmol/mL	2.4^{d}	2.2^{d}	1.8^{d}	2.0^{d}	5.3^{a}	4.8^{ab}	3.9^{c}	4.4^{bc}	0.14	<0.001	<0.001	0.015
CP, ng/mL	945^{e}	949^{e}	858^{f}	892^{ef}	1515^{a}	1418^{b}	1113^{d}	1305^{c}	18.5	<0.001	<0.001	<0.001

[1]Treatment groups: the laying hens were maintained at 24°C as a thermoneutral or exposed to a cyclic heat stress of 35°C (HS), the hens were further divided into four subgroups supplemented with curcumin (CURC) at 0, 100, 200, and 300mg/kg of the diet. CP: ceruloplasmin; MDA: malondialdehyde; CORT: corticosterone; H/L ratio: heterophil to lymphocyte cell ratio. Means within the same raw with different superscripts significantly differ (P<0.05). SEM: standard error of means.

Table 5: Effect of dietary *curcumin* (CURC) supplementation on the cholesterol profile of laying hens exposed to cyclic heat stress conditions

Items	Thermoneutral (24°C)[1]				Heat stress (35°C)				SEM	*P-value*		
	0	100	200	300	0	100	200	300		HS	CURC	HS × CURC
Total CH, mg/dL	171^{e}	165^{f}	135^{h}	153^{g}	214^{a}	207^{b}	188^{d}	194^{c}	1.17	<0.001	<0.001	<0.001
HDL, mg/dL	58.0^{d}	60.2^{cd}	74.0^{a}	64.1^{b}	51.1^{e}	53.6^{e}	62.5^{bc}	57.7^{d}	0.89	<0.001	<0.001	0.013
LDL, mg/dL	127^{d}	118^{e}	102^{g}	110^{f}	159^{a}	153^{ab}	146^{c}	150^{bc}	1.43	<0.001	<0.001	<0.001
Yolk CH, mg/dL	18.0^{c}	14.4^{d}	11.6^{e}	12.1^{de}	27.5^{a}	26.0^{ab}	24.9^{b}	25.3^{b}	0.52	<0.001	<0.001	0.001
Liver CH, mg/dL	7.6	6.3	4.7	5.2	12.5	10.1	8.6	9.1	0.57	<0.001	<0.001	0.739

[1]Treatment groups: the laying hens were maintained at 24°C as a thermoneutral or exposed to a cyclic heat stress of 35°C (HS), the hens were further divided into four subgroups supplemented with curcumin (CURC) at 0, 100, 200, and 300mg/kg of the diet. Total CH: total cholesterol; HDL: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol. Means within the same raw with different superscripts significantly differ (P<0.05). SEM: standard error of means.

Table 6: Effect of dietary *curcumin* (CURC) supplementation on the immune response of laying hens exposed to cyclic heat stress conditions

Items	Thermoneutral (24°C)[1]				Heat stress (35°C)				SEM	*P-value*		
	0	100	200	300	0	100	200	300		HS	CURC	HS × CURC
TWBC, 10^3/µL	67.8	72.1	100.3	93.1	46.5	49.9	81.3	74.7	1.17	<0.001	<0.001	0.33
H/L ratio	0.37^{d}	0.35^{d}	0.32^{d}	0.36^{d}	0.89^{a}	0.78^{b}	0.65^{c}	0.71^{bc}	0.018	<0.001	<0.001	<0.001
T-lymphocytes SI	3.4^{c}	3.6^{c}	4.9^{a}	4.5^{b}	1.4^{d}	1.4^{d}	1.5^{d}	1.5^{d}	0.07	<0.001	<0.001	<0.001
B-lymphocytes SI	2.1	2.2	3.5	3.3	0.9	1.0	2.2	2.1	0.05	<0.001	<0.001	0.072

[1]Treatment groups: the laying hens were maintained at 24°C as a thermoneutral or exposed to a cyclic heat stress of 35°C (HS), the hens were further divided into four subgroups supplemented with curcumin (CURC) at 0, 100, 200, and 300mg/kg of the diet. TWBC: total white blood cells; T-lymphocytes SI: T-cell lymphocyte stimulation index; B-lymphocytes SI: B-cell lymphocyte stimulation index. Means within the same raw with different superscripts significantly differ (P<0.05). SEM: standard error of means.

Cholesterol profile

Table 5 illustrates the impact of HS, CURC, and their interaction on hens' liver, egg yolk and blood plasma cholesterol profiles. Although plasma HDL cholesterol levels diminished, HS significantly elevated liver, egg yolk, and plasma total cholesterol, as well as plasma LDL cholesterol. Conversely, dietary CURC supplementation reduced (P<0.05) plasma total cholesterol, LDL, liver cholesterol, and egg yolk cholesterol, while simultaneously elevating HDL levels with increasing dietary CURC levels. A notable interaction effect (P<0.05) was detected between HS×CURC treatments in the plasma TCH, LDL, HDL, and yolk cholesterol. Hens administered 200mg/kg CURC exhibited the optimal cholesterol profile under both normal and heat stress conditions.

Immunological parameters

Table 6 illustrates the impact of HS, CURC, and their combinations on the immune-related indicators in laying hens. The findings indicated that HS resulted in significant elevation in the H/L ratio and a significant reduction in TWBCs and T- and B-lymphocyte SI. The immunological measurements showed improvements (P<0.05) when the dietary CURC level rose. Moreover, HS×CURC demonstrated a significant interaction on T-lymphocyte proliferation and H/L ratio. Following CURC administration at 200 mg/kg, the H/L ratio in the heat-stressed hens significantly reduced to its nadir.

DISCUSSION

Comprehending the deteriorative mechanism of HS on avian performance is essential for alleviating the economic downturn in the poultry sector. Numerous studies have demonstrated that heat stress exposure significantly impairs laying hens' productivity and compromises welfare (Nawab et al. 2018; Kim and Lee 2023; Kim et al. 2024; Tesakul et al. 2025). Thermal stress induces detrimental consequences on chickens, such as disruption in egg production, feed consumption, energy availability, nutrient digestibility, and metabolism (Zhang et al. 2017; Ahmed 2025). In accordance with previous research findings, the current study revealed that heat stress dramatically decreased both egg weight and production (He et al. 2018; Abbas et al. 2022; Kim and Lee 2023; Kim et al. 2024; Tesakul et al. 2025). It is assumed that the birds under HS decreased feed intake and conversion efficiency are directly related to the apparent drop in egg production (Mignon-Grasteau et al. 2015; Abd El-Hack et al. 2017a; Abd El-Hack et al. 2017b).

Curcumin has been possibly included into chicken diets to improve their performance under different limiting conditions (Nawab et al. 2019; Liu et al. 2020; Nawab et al. 2020; Hafez et al. 2022; Eleiwa et al. 2023). According to the current study, CURC increased the EP, EW, FI, and FCR by 200mg/kg. These findings support previous studies suggesting that curcumin may enhance chicken layer productivity (Park et al. 2012; Rahardja et al. 2015; Liu et

al. 2020; Li et al. 2024). The advantageous effects of CURC on EP and EW may be ascribed to the enhanced FI of laying hens, which liberates plasma proteins, calcium, and other nutrients essential for egg production (Liu et al. 2020). CURC has been found to enhance nutritional absorption by altering intestinal architecture, namely by increasing villus height and surface area as well as crypt depth (Awad et al. 2008; Guo et al. 2023; Xu et al. 2024; Abdel-Moneim et al. 2025).

Numerous physiological cascades are triggered by heat stress (Moustafa et al. 2021; Al-Otaibi et al. 2022), which might be a factor in the laying hens' worse performance in our study. For example, heat stress (HS) causes lipid peroxidation (Islam et al. 2018), activates the hypothalamic-pituitary-adrenal (HPA) axis (Quinteiro-Filho et al. 2012; Huang et al. 2024) and triggers inflammation (Li et al. 2020). As a result, laying hens exposed to HS exhibit a marked increase in inflammatory cytokines (Table 3) and stress indicators (Table 4). Under heat stress conditions, these physiological changes may cause laying hens to become immunosuppressed (Li et al. 2020; Abbas et al. 2022).

The present investigation indicated that dietary CURC supplementation enhanced the heat-stressed laying hens' physiological indices and productive performance. CURC reduced the elevated levels of stress indicators and inflammatory cytokines in HS-hens. CURC has a significant impact on oxidation and inflammation (Yeung et al. 2019; Chen et al. 2024; Gharib et al. 2025; Jain et al. 2025). The primary activator of TNF-α and interleukin production, nuclear factor kappa B (NF-κB), may be inhibited by CURC (Wang et al. 2009; Jain et al. 2025), or it may suppress the biosynthesis of prostaglandin E_2 (PGE_2), an inflammatory mediator (Koeberle et al. 2009). Also, CURC may inhibit the production of inflammatory cytokines by downregulating intracellular signaling protein kinases (Jurenka 2009). CURC supplementation may indirectly reduce the inflammatory cytokines release by alleviating many metabolic disorders, including insulin resistance, dyslipidemia, and hyperglycemia (Panahi et al. 2016; Chen et al. 2024; Abdel-Moneim et al. 2025). Also, CURC supplementation leads to decreased CORT levels, which may safeguard laying hens against oxidative damage to mitochondrial metabolism, steroidogenesis, and ovarian function under heat stress (Wang et al. 2009; Spiers et al. 2014; Yu et al. 2025). Hens fed with 200mg/kg of CURC had the lowest levels of MDA, indicating reduced systemic oxidative stress under heat stress conditions. Reports indicate that CURC phenolic groups facilitate its ability to neutralize nitric oxides, superoxide ions, and hydroxyl free radicals, safeguarding tissues and organs against lipid peroxidation (Nawab et al. 2019; Zhao et al. 2024; Wu et al. 2024; Udayakumar et al. 2024; Hernández-García et al. 2025).

On the other hand, as shown in Table 5, HS raised the laying hens' levels of dangerous cholesterol. The effects of glucocorticoid hormones produced because of HPA-axis activation in HS-birds may be responsible for the observed findings (Gonzalez-Rivas et al. 2020; Huang et al. 2024). Additionally, HS is involved in many processes that promote the production of cholesterol (Jastrebski et al. 2017). Other studies have revealed that HS causes elevated hepatic triglyceride levels and cholesterol production in avian liver and blood, which aligns with our observations (Lu et al. 2019; Emami et al. 2020; Al-Otaibi et al. 2022; Gharib et al. 2025). Conversely, the current investigation confirmed that CURC supplementation in the laying hens' diets at 200-300mg/kg mitigated the adverse CH profile caused by heat stress exposure. Numerous studies indicated that CURC reduced belly fat and plasma cholesterol levels in broiler and laying hens (Rajput et al. 2013; Muhshi et al. 2024; Abdel-Moneim et al. 2025). The depressive impact of CURC on cholesterol profile may be explained by the hepatic modulation of cholesterol-metabolizing enzymes and fatty acid synthase in liver (Seo et al. 2008; Yang et al. 2024; Gharib et al. 2025). Peschel et al. (2007) revealed that CURC significantly correlates with the expression of hypocholesterolemic and hypolipidemic genes. CURC was shown to reduce plasma LDL cholesterol by inhibiting cholesterol absorption in the gastrointestinal tract or enhancing cholesterol clearance from the bloodstream via overexpression of LDL receptors (Feng et al. 2010). The results underscore the need of CURC supplementation in layer diets, particularly in hot climates, to mitigate cholesterol-related atherosclerosis in egg consumers (Rahbar and Nabipour, 2010).

The present investigation demonstrated a significant reduction in immunological markers in heat-stressed laying hens (Table 6). The TWBC count, T-lymphocyte SI, and B-lymphocyte SI were reduced by 24, 63 and 46%, respectively. At the same time, the H/L ratio was elevated twofold in response to HS exposure. The link between HS and immunosuppression in chickens has been well established, primarily through the stimulation of the HPA axis and the subsequent CORT secretion (Padgett and Glaser 2003; Webster Marketon and Glaser 2008). Moreover, Mehaisen et al. (2017) indicated that exogenous administration of CORT in broiler chickens inhibited lymphocyte activation, reduced TWBC counts, and elevated the H/L ratio. Therefore, it is essential to highlight the positive effect of CURC on the immune stimulation of laying hens in this research. Our findings indicated that dietary CURC supplementation at 200mg/kg significantly elevated hens' TWBC, T-cell, and B-cell stimulation indexes, while reducing the H/L ratio relative to the other CURC groups. CURC is recognized for its immune modulatory properties and ability to enhance T-cell activation (Allegra et al. 2022; Ghoushi et al. 2024; Fouad et al. 2025). Due to curcumin's unique inhibitory effect on activating the CORT-HPA pathway, dietary treatment of 200mg/kg curcumin markedly reduced the H/L ratio in the HS chickens. Furthermore, suppressing leukocyte protein synthesis by heat stress may cause an irreversible reduction of lymphocyte proliferation (Kamel et al. 2017).

Conclusion

The laying hens' physiological state and productivity both improved when CURC was added to their diet. Up to 200mg/kg, the total performance characteristics showed a linear improvement in tandem with CURC increasing in layer diets. Additionally, adding CURC to heat-stressed chickens' diets improved immune responses, decreased the negative CH profile, and reduced stress and inflammatory symptoms. These findings indicate that CURC can serve as an effective dietary supplement to sustain laying hen performance while enhancing physiological, antioxidant,

and immunological responses under heat stress.

DECLARATIONS

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