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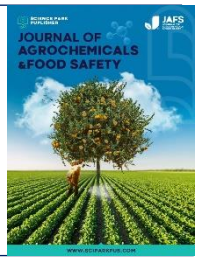

Dietary curcumin influences broiler immunity under high temperature

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Abstract

This study examines the efficacy of curcumin, a bioactive component found in turmeric, in mitigating the negative impacts of heat stress on broiler chicks. 120 unsexed Ross 308 chicks were allocated into four groups: a control group receiving a basal diet and three experimental groups receiving basal diets supplemented with 100, 200, and 400 mg/kg of curcumin, respectively. The trial included 35 days, during which all birds experienced 6 hours of daily heat stress at 35±1°C.

The results demonstrated that curcumin administration markedly enhanced live body weight (LBW) at 35 days of age with respect to the control. Moreover, white blood cell (WBC) counts, lymphocyte and monocyte levels, and the heterophil/lymphocyte (H/L) ratio were markedly elevated in the curcumin-treated groups. Total serum protein and globulin concentrations were raised, although albumin levels remained unchanged. Curcumin supplementation significantly enhanced immunoglobulin levels (IgG, IgA, IgM) and several immunological measures, including lysosomal activity, bactericidal activity, lymphocyte transformation tests, phagocytic index, and phagocyte activity.

Moreover, the groups treated with curcumin demonstrated heightened serum interleukin-2 (IL-2) concentrations, increased blood glucose levels, and diminished corticosterone levels. The bacterial count, encompassing *E. coli* and *Proteus*, was markedly reduced in curcumin-treated groups.

In conclusion, dietary curcumin supplementation effectively enhances the immune response and mitigates the adverse effects of heat stress in broiler chickens, suggesting its potential as a natural antioxidant in poultry diets.

Keywords: Bacteria; Chicken; Growth; Hematology; Immunoglobulin

1. Introduction

Heat stress (HS) remains one of the main concerns in the poultry industry worldwide, mainly in the tropics, due to the high temperatures throughout the year. Broilers are homeothermic animals that are primarily affected by heat stress, leading to decreased growth performance, decreased immune response, and an imbalance of the oxidant/antioxidant mechanism [1-2]. The immunosuppressive effect of HS has been reported, demonstrating that the neuroendocrine system plays a significant role in birds' proper physiological functioning and homeostasis during HS. During the early stages of the temperature challenge, the sympathoadrenal medullary axis activates and regulates homeostasis. The adrenal medulla experiences a hormonal rise in epinephrine, norepinephrine, and glucocorticoids due to the impulse transmitted by sympathetic nerves in response to elevated temperature. As a result, muscle and liver glycogen levels drop while respiratory rates rise [3]. The activation of the hypothalamic-pituitary-adrenal axis intensifies with prolonged

stress exposure. The immunosuppressive effect of HS has been documented, indicating that the neuroendocrine system is crucial for the physiological functioning and homeostasis of birds during HS. In the initial phases of the temperature challenge, the sympathoadrenal medullary axis is activated to regulate homeostasis. The adrenal medulla undergoes an increase in catecholamines (epinephrine and norepinephrine) and glucocorticoids as a result of impulses from sympathetic nerves in reaction to elevated temperature. Consequently, there is a reduction in muscle and liver glycogen levels, accompanied by a rise in respiratory rates [3]. In response to stress, the pituitary gland secretes adrenocorticotropic hormone (ACTH), which is induced by corticotropin-releasing hormone (CRH). ACTH stimulates corticosteroid release and synthesis in the adrenal glands [4]. Corticosterone increases are frequently followed by increases in heterophiles, increasing the heterophile-lymphocyte ratio [5]. Poultry exposed to high temperatures shows a drop in immune system components [6]. Additionally, it was noted that poultry under heat stress showed increased levels of serum inflammatory cytokines like tumor necrosis factor-α, interleukin-1, and interleukin-6 [7-8].

Therefore, alternatives for improving broiler immunity and other strategies to alleviate the adverse effects of HS are needed in the poultry industry. In this sense, supplementing poultry rations with bioactive compounds, such as curcumin, can increase antioxidant activity, contributing to decreased symptoms of HS. Curcumin is the most critical bioactive component originating from turmeric, extracted from the rhizome of *Curcuma longa L*., belonging to the Zingiberaceae family [9]. A potent antioxidant and various other pharmacological effects are attributed to curcumin, including antibacterial, antiviral, lipid-lowering, antidiabetic, and anti-inflammatory properties [9]. The unique biological properties of turmeric have sparked extensive research into its health benefits, particularly focusing on curcumin, its active component [10]. The existing literature regarding the impact of curcumin supplementation on the immunity of heat-stressed challenge chickens is notably sparse. The current study aimed to assess the effects of varying dietary concentrations of curcumin, a natural antioxidant, on the immune responses of challenge chickens subjected to HS.

2. Materials and methods

The experiment was carried out at the Animal and Poultry Research Centre (El-Bostan Farm), part of the Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Egypt. All procedures in the current investigation were authorized by the local Ethics Committee on Animal Use (DUFA-2024-16).

2.1. Bird management

From day one to 35 days of age, chicks were kept in galvanized wire cages with standard dimensions of 65×65×45 cm in a semi-open house with two exhaust fans to maintain normal ventilation. Over the experiment period, food and water were given freely. All experimental chicks were brooded at 33 °C upon arrival and gradually decreased from 30 to 28 °C during the second week. Consistent heat augmentation was executed by utilising digital heaters to keep the optimal temperature. Throughout the trial, the relative humidity was about 60±5%. Chicks were subjected to a standard lighting schedule according to commercial broiler strain guidelines, 23 hours of light/1h darkness during the first week and 20 h light / 2h darkness from the second week until the end of the experiment. A systematic immunisation initiative against Newcastle disease and Gumboro disease was implemented. Thus, the chicks were vaccinated against Gumboro at the age of 12 days through drinking water. The Hatchner B, Lasota, and Colon 30 vaccines were administered at 7, 18, and 28 days of age, respectively.

2.2. Diet and experimental design

For this experiment, 120-7-day unsexed Ross 308 chicks were randomly divided into four treatment groups with five replications of six chickens per replication. The 1st group was fed a basal diet (control group), the 2nd, 3rd, and 4th groups were fed basal diets supplied with different levels of curcumin (100, 200, and 400 mg/kg diet), respectively (Table 1). The experiment lasted for 35 days. From 14 to 35 days of age, all experimental birds were exposed to 6 hours per day (10:00 to 16:00) of continuous heat stress at 35±1^{o}C and 24±2^{o}C for the remaining time and relative humidity 60±5%.

2.3. Temperature-humidity index (THI)

In order to investigate the effects of both humidity and temperature acting together. The equation was updated by [11] to determine the temperature-humidity value. Results of the THI were categorised as follows: no HS if ≤27.8 was achieved, moderate HS if 27.8≤28.9 was obtained, severe HS if more than 28.9 was acquired.

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Table 1. Composition and calculated analysis of the basal diet.

Ingredients (%)	Starter period (7-21 day)	Grower period (22-35 day)
Yellow Corn	54.00	59.00
Soybean Meal (46%)	27.00	21.20
Full fat soya,	5.00	7.00
Gluten (60%)	8.00	7.00
Soya oil	1.50	1.30
Monocalcium Phosphate	1.65	1.65
Lime stone	1.75	1.75
L-lysine	0.25	0.25
DL –methionine	0.20	0.20
Salt (Na Cl)	0.35	0.35
Premix *	0.30	0.30
Total	100	100
Calculated analysis		
Crude Protein %	22.9	21.4
ME (kcal/kg)	3042	3103
Crude Fiber, %	2.70	2.70
Ether extract, %	4.10	4.45
Calcium, %	1.01	1.01
Phosphorus available%	0.50	0.51
Methionine %	0.66	0.61
Lysine %	1.33	1.25
Methionine+Cystine %	1.05	0.98

*: Each kg of vitamin and mineral mixture contains: 12 M IU vitamin A; 5 M IU D3; 80000 mg E; 4000 K mg; 4000 mg B1; 9000 mg B2; 4000 mg B6; 20 mg B12; 15000 mg pantothenic acid; 60000 mg Nicotinic acid; 2000 mg Folic acid; 150 mg Biotin; 400000 mg Choline Chloride; 15000 mg Copper sulphate; 1000 mg calcium Iodide; 40000 mg ferrous sulphate; 100000 mg Manganese oxide; 100000 mg Zinc oxide and 300 mg Selenium selenite. Calculated according to requirements of poultry (National Research Council, 1994).

2.4. Sample collection

By the 35th day of the experiment, five chicks were blood sampled for each treatment. Roughly 3 millilitres of blood were drawn from the brachial vein and placed into vacutainer tubes, which were either pre- or post-treated with K3-EDTA (1 mg/mL). To separate the sera, they were spun at 4,000 rpm for 15 minutes at 4°C. After that, they were placed in a freezer to wait for additional examination. Two parts were taken from the second set of non-coagulated blood samples. The first part was examined soon after collection to determine the white blood cell count and differential, and the second part was spun at 4000 rpm for 15 minutes. After separating the clear plasma, it was placed in a deep freezer and kept at -20°C until biochemical analysis could be carried out. Using commercially available kits, all blood biochemical variables were calorimetrically evaluated. Following this, in accordance with Islamic protocols for collecting cecal content samples, one chicken from each replication was slain, choosing it based on its proximity to the group's average body weight. Afterwards, the samples were examined for different species of *Lactobacillus* (CFU x 10^3) and *total bacterial count* (TBC, CFU x 10^6). The procedures described in [12] were adapted to use different types of agar, and the quantities of *Escherichia coli* and *Proteus* were measured as colony-forming units (CFU x 10^3) and CFU x 10^3, respectively.

2.5. White blood cell count and differential analysis

White blood cell counts (WBCs $10^3/mm^3$) were calculated according to [13]. The percentage of lymphocytes, heterophils, and monocytes was calculated to record the blood WBC differential count [13]. The quantity of heterophils (H) divided by the number of lymphocytes (L) yielded the heterophils to lymphocytes ratio (H/L).

2.6. Protein profile, glucose, and corticosterone analysis

The serum total protein (g/dl) was measured using a spectrophotometer (Beckman DU-530, Germany) and specialised kits provided by Sentinel CH Milano, Italy, in accordance with the protocols outlined by Armstrong and Carr [14]. Specific kits from CH Milano, Italy, were used to test serum albumin (g/dl), in accordance with the methodology outlined by Doumas et al. [15]. Since fibrinogen usually makes up a small fraction, the serum globulin level (g/dl) was calculated as the difference between total protein and albumin [16]. Another metric was the albumin-to-globulin ratio. The Trinder [17] method was employed to ascertain the concentration of glucose in the serum. According to Dehnhard et al. [18], the levels of plasma corticosterone were determined.

2.7. Immune indices

Determining various types of globulins, specifically α-, β-, and γ-globulin, was conducted following the methodology outlined by Bossuyt et al. [19]. Serum immunoglobulin levels (IgG, IgA, and IgM) were assessed using a commercial ELISA method [20]. The lymphocyte transformation test (LTT) was conducted in accordance with the methodology outlined by Balhaa et al. [21]. The bactericidal activity (BA) against the Aeromonas hydrophila strain was assessed following the method established by Rainger and Rowley [22]. Lysozyme activity (LA) was quantified according to Engstad et al. [23]. The phagocytic activity (PA) and phagocytic index (PI) were determined following the methodology outlined by Kawahara et al. [24].

2.8. Serum cytokine analysis

The contents of interleukin-2 (IL-2), IL-6, IL-10, and interferon-gamma (IFN-γ) were measured using chicken ELISA Kits (R&D Systems, Minneapolis, MN, USA).

2.9. Statistical analysis

The model expressed in equation 1 was used for analysis in a one-way analysis of variance (ANOVA) with the GLM technique [25]

$$Yij = \mu + Ti + eij \quad (1)$$

Where Yij is an observation, μ is the overall mean, Ti is the effect of treatment (i = 1,2, …, .4), and eij is the experimental random error. Prior to statistical analysis, the percentage data of the attributes that were analysed underwent square root or arc sine transformation, and the bacterial counts were converted using a Log transformation. The difference among means was determined using Student-Newman-Keuls. Means differences at $p \leq 0.05$ were tested.

3. Results and discussion

3.1. Growth performance

Meat-type chickens subjected to chronic heat stress (CHS) had their live body weight (LBW) affected by various dietary doses of curcumin, as shown in Table 2. The LBW on the seventh day of age exhibited no significant variation across the experimental groups. However, heat-stressed meat-type chickens fed a baseline diet supplemented with varying amounts of curcumin had a significant increase in LBW at 35 days ($P \leq 0.002$) of age with respect to the control. Generally, the LBW of birds subjected to varying curcumin dosages was markedly greater with respect to the control group at 35 days of age. The current study's findings revealed that varying levels of curcumin enhanced the LBW of broiler chicks exposed to CHS with no significant difference between different levels of curcumin by 18.9, 17.2 and 23.0% for (100, 200, and 400 mg curcumin/kg diets), respectively with respect to the control at 35 days of age. The birds fed varying doses of curcumin consumed 1.62, 2.04,2.98 % more feed and grew 20.61, 18.63, and 25% higher with respect to the control. Furthermore, for the entire experimental duration, the percentages of improvement of Feed Conversion Ratio (FCR) were 15.54, 13.99, and 17.62 % for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control group. The observed enhancement in FCR can be linked to the increase in BWG noted during this experiment. In the current study, a progressive enhancement in performance with an elevated amount of curcumin under HS conditions. The observed improvement may result from essential oil compounds in curcumin exhibiting significant antimicrobial

activity, which inhibits bacterial growth. This action supports intestinal flora performance, enhancing digestion and energy utilization, ultimately contributing to improved growth.

Furthermore, curcumin supplementation enhances the production of bile acids and the activities of lipase, amylase, and proteases, which are crucial for metabolism and promote expedited digestion [26]. The small intestine's structure is crucial for nutrient digestion and absorption [27]. Furthermore, the inclusion of phenolic compounds, such as curcumin, has the potential to reduce gut inflammation, enhance nutrient digestibility, and improve metabolic activity [28-30]. Additionally, these substances have the potential to alter the gastrointestinal system's architecture and functioning [31].

Table 2. Effect of different dietary levels of curcumin supplementation on growth performance of broiler chickens.

Parameter	curcumin levels				SEM	p-value
	0	100mg	200mg	400mg		
Initial BW,g	144.2	145.3	146.0	146.0	1.982	0.911
Final BW, g	1745.0^{b}	2076.0^{a}	2045.0^{a}	2147.0^{a}	29.37	0.0001
BWG,g	1600.8^{b}	1930.7^{a}	1899.1^{a}	2001.0^{a}	30.12	0.0001
FI,g/day	3090.0^{b}	3140.0^{a}	3153.0^{a}	3182.0^{a}	29.22	0.046
FCR	1.93^{a}	1.63^{b}	1.66^{b}	1.59^{c}	0.029	0.0001

^{a,b} Means± SEM in the same column, followed by different superscripts, are significantly different at $p \leq 0.05$. BW: Body weight: FI; Feed intake: FCR; Feed Conversion Ratio: SEM; Standard error of mean.

3.2. Leukocyte count and differential analysis

The results from Table 3 showed the white blood cells (WBCs), lymphocytes (L), H/L ratio, and monocyte values of chicks as affected by varying dietary levels of curcumin under CHS. Data from Table 3 illustrated that WBCs for broilers fed control diet supplied with 400 mg curcumin/kg diet were significantly increased with respect to those for groups fed basal diet with 100 mg curcumin/kg diet and control. In addition, results demonstrated that birds fed basal diets supplied with varying doses of curcumin significantly improved lymphocytes (%), monocytes (%), and H/L ratios with respect to the control. Moreover, these previously mentioned parameters for treated groups (curcumin groups) did not represent any statistical change between each other.

A wide variety of metabolites and other components within an animal's body can be evaluated by blood analysis, significantly contributing to understanding its physiological, nutritional, and pathological condition [32]. The findings of this investigation align with those of Lee et al. [33], which indicated an elevated percentage of lymphocytes in the spleen cells of broiler chickens following exposure to turmeric powder. Given the antioxidant [34] and immunomodulatory activities of turmeric [35-36], the elevated lymphocyte proportion appeared to enhance the immune system of birds in our investigation. The latter conclusion was substantiated by the observation that turmeric enhanced the level of globulin, a basis for the synthesis of immunoglobulin, in birds in the present investigation. This present finding aligns with the earlier work by Hosseini-Vashan et al. [37], which involved administering turmeric rhizome powder to chickens subjected to HS. The H/L ratio is frequently utilized as a marker of stress and infections in chickens [38-40]. Turmeric appeared advantageous in mitigating stress and infections in broiler chicks.

3.3. Protein profile

Table 4 illustrates the impact of varying dietary amounts of curcumin supplementation on the protein profile of broiler chicks subjected to HS conditions. The findings indicated a significant increase in the serum total protein of meat-type chickens receiving curcumin supplementation under CHS with respect to the control. Treatment groups given 100, 200, and 400 mg curcumin/kg diets differed from the control group by 16.6%, 22.3%, and 15.5%, respectively, as compared to the control mean. The results demonstrated a non-significant impact of curcumin supplementation on albumin level (g/dl) at 35 days of age. Curcumin treatments significantly elevated the concentration of serum globulin (g/dl) in heat-stressed meat-type

chickens with respect to the control at 35 days of age. The percentage increases were 43.04%, 53.6%, and 39.07% for diets containing 100 mg, 200 mg, and 400 mg of curcumin per kg, respectively, with respect to the control. The results demonstrated that A/G ratio values showed no statistically significant variations among the experimental groups. The findings suggest a notable improvement in total protein and globulin concentrations in heat-stressed broilers administered a basic diet augmented with differing levels of curcumin. This aligns with the observations of Muhammad and Al-Hassani [41], indicated that varying concentrations of turmeric root powder led to a substantial elevation in protein profile in broilers subjected to HS. The reduced A/G ratio observed in the current investigation may signify an enhancement in the immune competence of broilers administered decocted turmeric. Sugiharto et al. [38] proposed that a diminished A/G ratio is associated with enhanced immune competence and disease resistance in meat-type chickens.

3.4. Immunological status

Table 5 presents data on serum α–globulin and β–globulin concentrations (µg/dl). Serum γ–globulin levels in heat-stressed broiler chickens receiving a control diet supplemented with 100 mg or 200 mg of curcumin per kg significantly exceeded those of the control group. Conversely, α-globulin and β-globulin exhibited no significant variation among the experimental groups. The serum immunoglobulin (Ig) fractions (IgG, IgM, and IgA) of meat-type chickens at the end of the experiment, influenced by varying dietary levels of curcumin, are presented in Table 5.

Table 3. Effect of different levels of dietary curcumin supplementation on hematological parameters of broiler chicks under heat stress conditions.

Dietary supplementations	Leukocytic components				
	WBC's (10^3/mm^3)	Heterophils (%)	Lymphocyte (%)	H/L	Monocyte (%)
Control	18.71[b]	30.21	30.71[b]	0.983[a]	14.03[b]
100 mg Curcumin	19.21[b]	32.91	42.12[a]	0.782[b]	15.60[a]
200 mg Curcumin	21.10 [ab]	30.81	45.71[a]	0.674[b]	16.10[a]
400 mg Curcumin	23.30[a]	33.22	47.71[a]	0.696[b]	16.30[a]
SEM	0.712	5.790	1.21	0.001	0.301
P value	0.006	0.067	0.003	0.207	0.002

[a,b] Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean;. WBC's = White blood cell.

Table 4. Effect of different levels of dietary curcumin supplementation on protein profile of broiler chicks under heat stress conditions.

Dietary supplementations	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	4.51[b]	3	1.5[b]	1.99
100 mg Curcumin	5.26[a]	3.1	2.16[a]	1.44
200 mg Curcumin	5.52[a]	3.2	2.32[a]	1.38
400 mg Curcumin	5.21[a]	3.11	2.10[a]	1.48
SEM	0.236	0.125	0.189	0.054
P value	0.006	0.518	0.020	0.074

[a,b] Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean.

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Serum levels of IgG, IgA, and IgM (mg/dl) were significantly elevated (P≤0.009) in response to varying dietary curcumin levels with respect to the control. Results indicated curcumin levels significantly affected serum IgM and IgA concentration (P≤0.006). The groups administered curcumin exhibited elevated serum levels of IgG by (16.6, 24.8, and 16.7), IgM by (30.9, 36.4, and 37.9), and IgA by (41.5, 49.4, and 56.7) in comparison to the control group.

Data for lysosome activity (LA, %), bactericide activity (BA, %), lymphocyte transformation test (LTT, %), phagocytic index (PI, %), and phagocyte activity (PA, %) of broiler chicks at 35 days of age are shown in Table 6.

The analysis of variance indicates that LA % for birds receiving varying dietary levels of curcumin was significantly elevated (P≤0.003) with respect to the control. The serum LA% exhibited significant increases of 69.3%, 51.2%, and 46.8% for diets containing 100, 200, and 400 mg of curcumin/kg, respectively, with respect to the control at 35 days of age. BA in groups fed a control diet with varying dietary levels of curcumin was significantly elevated (P≤0.005) with respect to the control at 35 days of age. The serum BA% exhibited significant increases of 26.6%, 34.5%, and 29.9% for diets containing 100, 200, and 400 mg of curcumin per kg, respectively, with respect to the control at 35 days of age. Furthermore, LTT % for chickens subjected to HS and fed a basal diet with varying levels of curcumin showed a significant increase with respect to the control. The serum LTT% exhibited significant increases of 27.1%, 25.7%, and 31.7% for diets containing 100, 200, and 400 mg of curcumin/kg, respectively, with respect to the control at 35 days of age. These results indicated that treated broiler chicks with dietary curcumin significantly increased (P≤0.004) in their PA% with respect to the control. Thus, the percentages of significant increase in serum PA% were 20.4, 16.6, and 13.4 % for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age. It can be seen that the meat-type chicken's PI concentration was significantly affected by curcumin levels at 35 days of age with respect to the control. Thus, the percentages of a significant increase in serum PI% were 52.3, 71.9, and 85.9% for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age. Moreover, these previously mentioned parameters for treated groups (curcumin groups) did not represent any statistical change between each other.

Table 5. Effect of different levels of dietary curcumin supplementation on serum globulin fractions and immunoglobulin broiler chicks under heat stress conditions.

Dietary supplementations	α–globulin (µg/dl)	β –globulin (µg/dl)	γ –globulin (µg/dl)	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)
Control	1.30	1.07	0.523[b]	41.51[b]	18.41[b]	15.06[b]
100 mg Curcumin	1.13	1.05	1.52[a]	48.41[a]	24.11[a]	21.30[a]
200 mg Curcumin	1.14	1.11	1.73[a]	51.80[a]	25.10[a]	22.51[a]
400 mg Curcumin	1.24	1.07	1.42[ab]	48.40[a]	25.40[a]	23.61[a]
SEM	0.031	0.056	0.201	1.050	1.070	0.980
P value	0.062	0.909	0.001	0.009	0.006	0.002

[a,b] Means in the same column followed by different letters are significantly different at (p ≤ 0.05); SEM, Standard error of mean; IgG, Immuno globulin G; IgM, Immuno globulin M; IgA, Immuno globulin A.

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Table 6. Effect of different levels of dietary curcumin supplementation on immunological status of broiler chicks under heat stress conditions.

Dietary Supplementations	Lysosome activity (LA, %)	Bactriocide activity (BA, %)	Lymphocyte transformation test (LTT, %)	Phagocyte activity (PA, %)	Phagocytic index (PI, %)
Control	8.21[b]	25.50[b]	19.40[b]	14.64[b]	1.07[b]
100 mg Curcumin	13.90[a]	32.30[a]	24.65[a]	17.63[a]	1.63[a]
200 mg Curcumin	12.41[a]	34.30[a]	24.38[a]	17.06[a]	1.84[a]
400 mg Curcumin	12.06[a]	33.12[a]	25.54[a]	16.60[a]	1.99[a]
SEM	1.041	0.880	0.610	0.291	0.125
P value	0.003	0.005	0.001	0.004	0.002

[a,b] Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error.

Table 7 displays the serum IL-2, IL-6, IL-10, and INF levels of meat-type chickens at 35 days of age that were altered by varied amounts of curcumin in their diet. Serum IL-2 concentration for broilers exposed to HS and fed a basal diet with varying levels of curcumin significantly increased with respect to the control ($P \leq 0.0001$). Thus, the percentages of a significant increase in serum IL-2 were 94.1, 2.09, and 22.7% for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age. Also, results indicated that curcumin levels significantly influenced serum IL-6 concentration ($P \leq 0.002$). The percentages of significant increase in serum IL-6 were 12.5%, 17.2%, and 16.7% for the 100, 200, and 400 mg curcumin/kg diets, respectively, with respect to the control at 35 days of age. Additionally, the findings indicated that curcumin levels significantly influenced serum IL-10 ($P \leq 0.006$). The percentages of significant increase in serum IL-10 were 11.3%, 21.5%, and 31.6% for diets containing 100, 200, and 400 mg curcumin/kg, respectively, with respect to the control at 35 days of age. Results indicated that curcumin levels significantly affected serum INF ($P \leq 0.006$) with respect to the control. The percentages of significant increase in serum INF were 40.5%, 37.2%, and 43.3% for diets containing 100 mg, 200 mg, and 400 mg of curcumin per kg, respectively, with respect to the control at 35 days of age.

Heat stress induces oxidative stress in broilers, resulting in cellular damage and compromised immune function. Curcumin is a potent antioxidant, effectively neutralizing free radicals and diminishing oxidative stress. This protective effect can improve overall immune function, including producing IL-2, IL-6, IL-10, and INF.

Srivastava et al. [42] showed that curcumin supplementation strongly affects innate and adaptive immunity, which is in line with our results. This modulation occurs through the functional alteration of various immune cells, including neutrophils, macrophages, monocytes, natural killer cells (NK cells), dendritic cells (DCs), T cells, and B cells. Curcumin interacts with multiple cell types, including macrophages, dendritic cells, B cells, T cells, and natural killer cells, to alter the body's defense mechanisms [43].

3.5. Blood glucose and corticosterone

Table 8 illustrates the impact of varying dietary curcumin supplementation levels on broiler chicks' blood glucose and corticosterone levels subjected to HS. The findings indicated that glucose levels were significantly elevated ($P \leq 0.001$) in the curcumin-treated groups with respect to the control. The plasma glucose levels exhibited significant increases of 15.8%, 12.6%, and 22.4% for the 100, 200, and 400 mg curcumin/kg diets, respectively, with respect to the control at 35 days of age. Heat stress conditions elevate the metabolic demands of the body. Curcumin may reduce stress by lowering corticosterone levels, which could subsequently affect glucose metabolism and increase glucose availability to satisfy heightened energy demands. The results in Table 8 indicate the variations in corticosterone hormone concentration throughout the treatment

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period. The findings revealed a significant reduction in the mean levels of plasma corticosterone hormone (P≤0.001) in heat-stressed broiler chickens with respect to the control. The percentages of significant decrease in serum corticosterone were 36.95%, 44.67%, and 26.51% for the 100, 200, and 400 mg curcumin/kg diets, respectively, compared to the control group at 35 days of age. Curcumin possesses anti-inflammatory and antioxidant properties that may mitigate the physiological stress response. Curcumin may affect the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the stress response by reducing oxidative stress and inflammation. Curcumin can decrease corticosterone secretion by modulating this axis.

3.6. Bacterial count

In Table 9, birds fed diets supplemented with curcumin had a significantly lower percentage of total bacterial count than that of the control. The total bacterial count of the treated groups were (P=0.004) 33.01, 65.60, and 37.73% for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age. Birds fed the basal diet supplemented with different amounts of curcumin had significantly increased (P=0.002) *Lactobacillus sp*. Thus, the percentages of significant increase in *Lactobacillus sp*. were 76.5, 59.13, and 11.3% for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age. *Lactobacillus* species are beneficial probiotics that promote gastrointestinal health, augment nutritional assimilation, and strengthen the immune system. The increase in Lactobacillus sp. indicates that curcumin promotes a favorable gut environment. The curcumin-treated groups showed a substantial decrease (P=0.001) in E. coli and Proteus with respect to the control, as shown in Table 9.

Table 7. Effect of different dietary levels of curcumin supplementation on cytokines of broiler chicks under heat stress conditions.

Dietary supplementations	IL-2 (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)	INF (pg/mL)
Control	6.54[b]	15.42[b]	12.53[c]	4.87[b]
100 mg Curcumin	12.69[a]	17.35[a]	13.94[b]	6.84[a]
200 mg Curcumin	13.73[a]	18.06[a]	15.22[ab]	6.68[a]
400 mg Curcumin	14.57[a]	18.00[a]	16.46[a]	6.98[a]
SEM	0.501	0.246	0.575	0.212
P value	0.0001	0.0002	0.006	0.0004

[a,b] Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); Interleukin -2 (IL-2), IL-6, IL-10 and interferone-gamma (IFN-γ); SEM, Standard error of mean.

Table 8. Effect of different dietary levels of curcumin supplementation on blood glucose and corticosterone of broiler chicks under heat stress conditions.

Dietary Supplementations	Glucose (mg/dl)	Corticosterone (pg/mg)
Control	155.6[b]	4.79[a]
100 mg Curcumin	180.3[a]	3.02[b]
200 mg Curcumin	175.3[a]	2.65[b]
400 mg Curcumin	190.6[a]	3.52[b]
SEM	5.560	0.190
P value	0.001	0.0003

[a,b] Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean.

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Thus, the percentages of significant diminish in *E. coli* were 40.20, 45.22, and 46.73% for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age, concerning the values of *E. coli*. While the percentages of significant diminish in *Proteus* were 59.55, 49.34, and % for (100, 200, and 400 mg curcumin/kg diets), respectively, with respect to the control at 35 days of age, concerning the values of *Proteus.* This suggests that curcumin effectively inhibits this pathogenic bacterium, and reducing pathogenic bacteria like *E. coli* and *Proteus* is crucial for preventing infections and improving overall health and growth performance in broilers.

Table 9. Effect of different dietary levels of curcumin supplementation on bacterial count of broiler chicks under heat stress conditions.

Dietary Supplementations	TBC x 10^3	Lactobacillus x 10^3	E. coli x 10^3	Proteus x 10^3
Control	18.87^{a}	1.15^{c}	1.99^{a}	0.989^{a}
100 mg Curcumin	12.64^{b}	2.03^{b}	1.19^{b}	0.400^{b}
200 mg Curcumin	12.38^{b}	1.83^{b}	1.09^{b}	0.501^{b}
400 mg Curcumin	11.75^{b}	2.43^{a}	1.06^{b}	0.314^{b}
SEM	0.714	0.235	0.133	0.071
P value	0.004	0.002	0.001	0.001

^{a,b,c} Means in the same column followed by different letters are significantly different at ($p \leq 0.05$); SEM, Standard error of mean.

4. Conclusion

Based on the results of improved body weight gain and feed utilization, it can be inferred that broiler chicks grown under CHS perform better when fed curcumin, particularly at a dosage of 100 mg/kg diet. Indicative of an enhanced immunological condition, this enhancement was also noted in the chicks' blood biochemical indicators.

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