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Dietary incorporation of biological curcumin nanoparticles improved growth performance, ileal architecture, antioxidative status, serum lipid profile, and humoral immune response of heat-stressed broiler chickens

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ABSTRACT

Heat stress greatly impairs poultry productivity, underscoring the urgent need for effective strategies to mitigate these adverse effects and improve overall poultry health. This study assessed the impact of dietary curcumin nanoparticles (CurNPs) on blood metabolites, immunity, redox status, ileal histomorphometry, and growth of broilers subjected to heat stress. A total of 400 one-day-old Ross-308 broiler chicks were randomly distributed into five groups, each consisting of eight replicates with ten birds per replicate. The chicks were fed a basal diet containing CurNPs at concentrations of 0, 100, 200, 300, or 400 mg/kg feed, designated as 0CurNPs, 100CurNPs, 200CurNPs, 300CurNPs, and 400CurNPs, respectively. Dietary CurNPs supplementation linearly ($P > 0.001$) improved weight gain, feed conversion ratio and European production efficiency index, while feed intake decreased linearly ($P > 0.001$) with increasing CurNPs supplementation. Carcass traits and serum renal and hepatic function biomarkers remained unaffected by the treatment. Serum cholesterol and LDL levels exhibited linear and quadratic ($P > 0.05$) reduction in all treated groups, although triglycerides and VLDL levels reduced linearly ($P > 0.05$) only in the 300CurNPs group. The inclusion of CurNPs resulted in a linear and quadratic increase ($P > 0.05$) in ileal villi height and a linear elevation ($P > 0.05$) in the villi height-to-crypt depth ratio. The redox status was improved with CurNPs supplementation, as serum MDA levels showed a linear decrease ($P > 0.05$) in the 300CurNPs and 400CurNPs groups, while SOD levels increased linearly and quadratically ($P > 0.05$) across all treated groups. Furthermore, dietary CurNPs exhibited linear ($P > 0.001$) increases in serum levels of IgM, IgG, and IgA, though antibody titres against NDV and AIV were unaffected. In conclusion, CurNPs proved to be an effective growth promoter, enhancing growth, intestinal architecture, redox status, and humoral immunity in heat-stressed broilers.

Introduction

Poultry production under intensive commercial conditions faces numerous challenges, particularly environmental stress from high temperatures, leading to increased mortality and economic losses (Abdel-Moneim et al., 2021). Heat stress triggers contagious diseases, and hinders growth, digestive function, and immune responses (Elbaz et al., 2022a). These issues have led to the use of antibiotic growth promoters (AGPs) to improve productivity and immunity, however, the overuse of AGPs has encountered increasing opposition and has been prohibited in the European Union. Similarly, the United States is moving toward enacting comparable regulations. The prevalent and often indiscriminate use of AGP in poultry not only raises concerns about animal welfare but also poses significant risks to human health by contributing to the emergence of antibiotic-resistant bacteria (Abdel-Moneim et al., 2020b; 2020c).

This growing resistance necessitates the development and adoption of safer and more sustainable alternatives for promoting growth and preventing diseases in poultry. Numerous investigations have thoroughly explored diverse natural feed additives and intestinal modulators

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as AGP alternatives, such as essential oils (Elbaz et al., 2022b), prebiotics (Abd El-Hack et al., 2021; Shehata et al., 2022; Yang et al., 2023), exogenous enzymes (Saleh et al., 2021), probiotics (Abd El-Hack et al., 2020; Elbaz et al., 2023), and phytogenics (Ashour et al., 2021; Dosoky et al., 2021; Mesalam et al., 2021; Abd El-Hack et al., 2023). Among these, phytogenic feed additives and their derivatives are particularly noteworthy because of their wide range of bioactivities, including immune-modulatory, antioxidant, antimicrobial, and anti-inflammatory effects (Johannah et al., 2018; Abd El-Hack et al., 2019). Phytogenics can act as a potent alternative to AGPs by promoting intestinal health and combating microbial threats (Prasad et al., 2014).

Turmeric, a common culinary spice, contains the bioactive compound curcumin, which has garnered considerable attention for its therapeutic attributes. Curcumin exhibits several health benefits, such as antioxidant, antimicrobial, anti-inflammatory, and gastroprotective activities (Prasad et al., 2014; Zhang et al., 2015; Gera et al., 2017). As reported by Platel and Srinivasan (2000) and Chattopadhyay et al. (2004), the incorporation of curcumin in animal diets enhanced the digestibility and metabolism of nutrients by activating the digestive enzymes and stimulating the secretion of bile acids. Furthermore, curcumin has the potential to reduce blood cholesterol concentrations, ameliorate liver functionality, and improve intestinal architecture (Seo et al., 2008). Nevertheless, curcumin's practical application is hindered by its inadequate bioavailability, attributed to low absorption rates, rapid metabolic processes, and quick systemic elimination (Geevarghese et al., 2023).

Innovative formulations such as phospholipid complexes, liposomes, adjuvants, micelles, and nanoparticles have been developed to overcome these limitations. Among these strategies, nanoencapsulation stands out as a particularly effective method (Siddiqui et al., 2022). This approach significantly enhances curcumin's bioavailability and efficacy, maximizing its impact on the health and performance of broilers (Abdel-Moneim et al., 2022a; Siddiqui et al., 2022). Transforming curcumin into nanoparticles effectively overcomes its inherent bioavailability limitations, such as poor solubility and limited absorption (Abdel-Moneim et al., 2022b). Compared to traditional curcumin supplements, CurNPs demonstrate significantly enhanced bioavailability by exhibiting prolonged circulation within the body, improved tissue penetration, and resistance to metabolic degradationThese properties make CurNPs a promising innovation for addressing bioavailability issues and advancing poultry nutrition and health management (Geevarghese et al., 2023). Consequently, this study aimed to evaluate the impacts of the dietary addition of CurNPs on growth performance, carcass characteristics, antioxidative status, serum biochemical indices, and immunity of heat-stressed broilers.

Material and methods

Ethics statement

The experiment was carried out at the research farm of the Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority (EAEA). All procedures followed the national experimental animal care guidelines and were approved by the Institutional Ethical Committee of EAEA.

Curcumin nanoparticles

Curcumin nanoparticles (CurNPs) were biosynthesized by *Bacillus subtilis* LA4 using the same procedure reported by Reda et al. (2020) at the Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University. In brief, the biosynthesis process involved mixing 20 mL of cell-free bacterial supernatant with 30 mL of 0.27 mM sterilized curcumin solution in 250 mL conical flasks. The mixture was incubated at 30 °C with shaking at 160 rpm for 72 h to allow nanoparticle formation.

Birds and experimental design

A total of four hundred one-day-old Ross-308 broiler chicks were evenly distributed into five experimental groups, each consisting of eight replicates/ ten birds. The basal diets were formulated following the strain recommendations by Aviagen (2018), as specified in Table 1. These birds were fed a basal diet supplemented with 0, 100, 200, 300, or 400 mg of CurNPs/kg feed (0CurNPs, 100CurNPs, 200CurNPs, 300CurNPs, and 400CurNPs, respectively). The CurNPs were directly incorporated into the feed during diet preparation to ensure even distribution. The birds were housed in floor cages measuring 100 cm length × 70 cm width × 60 cm height. The chicks were kept at 33 °C for the first three days of age (DOA), after which the temperature was gradually reduced to 31 °C for another 48 h. Subsequently, the birds were kept at the natural summer ambient temperature, averaging 30.9 °C with 59.4% relative humidity during the experiment. The temperature-humidity index (THI) was calculated following Tao and Xin (2003) equation: $THI = 0.85t_{db} + 0.15t_{wb}$, where t_{wb} and t_{db} are wet and dry bulb temperatures, respectively, as shown in Fig. 1. The lighting program was maintained at 23L:1D for the first 7 days, followed by 18L:6D for the rest of the experimental period. The chicks had unrestricted access to feed and water and were maintained under the same environmental and management conditions (Abdel-Moneim et al., 2020a).

Growth and carcass traits

At 10, 24 and 35 DOA, body weight gain (BWG) and feed intake (FI) were recorded on a pen basis, and the European production efficiency index (EPEI), and feed conversion ratio (FCR) were calculated

Table 1
Ingredients and nutrient levels of the basal diet at different growth phases.

Ingredients, g.kg^{-1}	Starter (d 0-10)	Grower (d 11-24)	Finisher (d 25-35)
Corn	528.60	575.80	617.60
Soybean meal, 48%	391	339.8	301.0
Plant oil	37.2	44.10	44.10
Dicalcium phosphate.	18.2	16.30	14.00
Limestone	10.0	9.30	9.00
Salt	4.20	3.20	3.20
DL-Methionine	3.50	3.20	2.80
L-Lysin HCL	2.00	1.90	1.90
L-Threonine	1.30	1.10	1.10
Choline chloride 60%	0.90	0.90	0.90
Sodium bicarbonate	0.10	1.40	1.40
Premix Blank	3.00	3.00	3.00
Total	1000	1000	1000
Calculated values, %			
Crude protein	23.37	21.23	19.66
Metabolizable energy, Kcal. kg^{-1}	2977	3078	3130
Crude fat	6.04	6.87	7.00
Crude fiber	3.90	3.65	3.47
Calcium	0.93	0.85	0.77
Available phosphorus	0.49	0.44	0.40
Potassium	1.05	0.95	0.90
Sodium	0.18	0.14	0.14
Chloride	0.29	0.23	0.23
Dig. Lysine	1.35	1.21	1.12
Dig. Methionine and Cysteine	1.04	0.96	0.88
Analyzed values, %			
Crude protein	23.62	21.46	19.78
Crude fat	6.11	6.93	7.10
Crude fiber	4.38	3.92	3.84

Premix Per 1 Kg: 1400 IU Vitamin A, 3000 IU Vitamin D3, 50 mg Vitamin E, 4 mg Vitamin K, 3 mg Vitamin B6, 6 mg Vitamin B12, 60 mg Niacin, 20 mg Pantothenic acid, 0.20 mg folic acid, 150 mg Choline, 48 mg Ca, 3.18 mg P, 100 mg Mn, 50 mg Fe, 80 mg Zn, 10 mg Cu, 0.25 mg Co, 1.5 mg Iodine. Curcumin nanoparticles were incorporated into broilers diets at levels of 0, 100, 200, 300, and 400 mg.kg^{-1}.

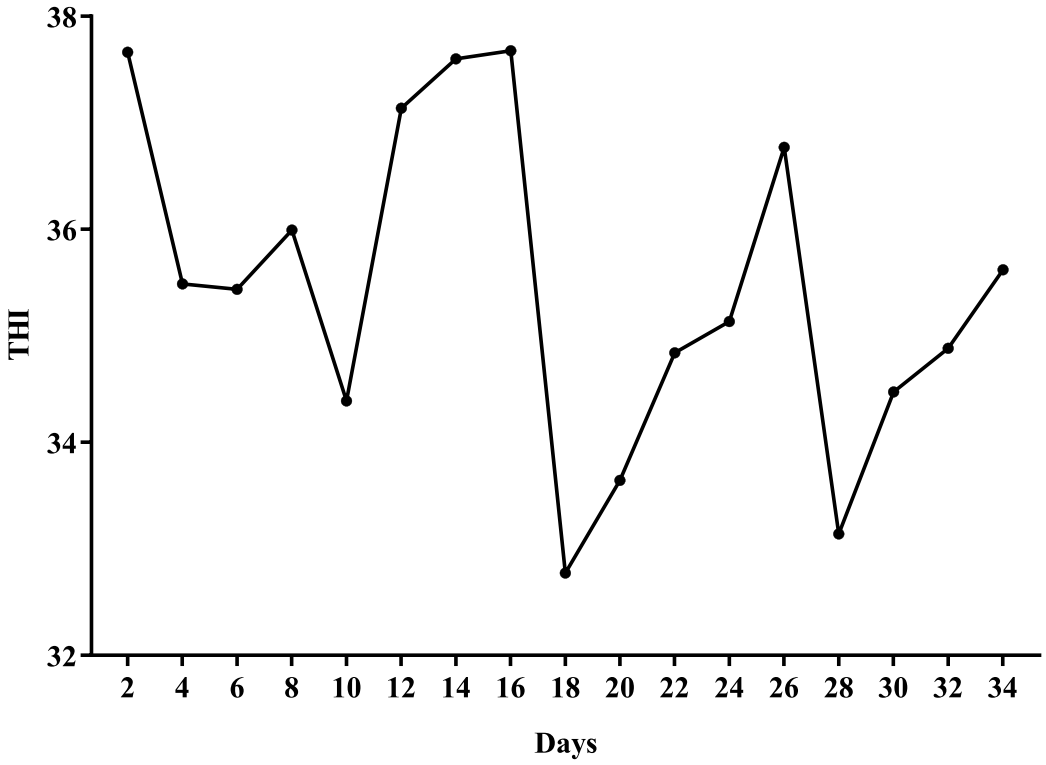

Fig. 1. Average temperature-humidity index (THI) during the experimental period.

(Abdel-Moneim et al., 2022b). On 35 DOA, eight chicks per group, representative of the group's average weight, were randomly chosen for carcass evaluation to ensure unbiased sampling. The selected birds were fasted for 12 h before being individually weighed, slaughtered, de-feathered, and eviscerated. The eviscerated carcass, body parts (breast, thigh, drumstick, and wings), breast and leg meat, and lymphoid organs (spleen, thymus, and bursa of Fabricius) were weighed. All measurements were taken using a calibrated digital scale to ensure accuracy and expressed as a percentage of live BW (Abo Ghanima et al., 2020).

Ileal histomorphometry

Samples from the mid-ileum were collected after immediately slaughtering under standardized conditions to ensure consistency. The collected samples were fixed in a 10% formalin-saline solution, dehydrated, cleared, embedded in paraffin wax, and sectioned. By means of a rotary microtome, sections of 4-5 μm thickness were attained and stained with hematoxylin and eosin to be observed with light microscopy for ileal morphometric evaluation. Measurements of villus height and crypt depth were taken, using image analysis software (ImageJ, Bethesda, MD, USA), and their ratio was calculated. A total of eight well-oriented villi and villus-associated crypts from eight intestinal cross-sections were selected. The mean values were used for further calculations (Abdelhady et al., 2021).

Serum biochemical indices

Prior to slaughter, non-heparinized sterile tubes were used for collecting blood samples from the wing vein. Stress-reducing measures, including gentle handling and minimizing handling time, were applied to reduce distress during sampling. The collected blood samples were centrifuged for 15 min at 3400 × *g* at 4 °C to separate the serum. Sera samples were then stored in aliquots at −20 °C to prevent repeated freeze-thaw cycles and preserve sample integrity until biochemical analyses were performed. Serum levels of metabolic indicators (glucose, albumin, total protein, creatinine, uric acid, aspartate aminotransferase (AST), and alanine aminotransferase (ALT)), and lipid profile (triglycerides (TG), cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL)), were measured using a spectrophotometer (Shimadzu UV 1601) with commercial kits from Stanbio Laboratory (Boerne, Texas, USA). The concentration of serum superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH) were conducted using commercial kits from Spinreact Co. (Girona, Spain). Antibody titers against NDV and AIV and serum IgA, IgM, and IgG concentrations were determined using ELISA quantitation kits (Cat# FT275003, FT274012, CSB-E11232Ch, CSB-E16200C, CSB-EQ027259CH, respectively) from Indical Bioscience GmbH (Leipzig, Germany) and Cusabio (Houston, Texas, USA).

Statistical analysis

The collected data were analyzed using a One-way ANOVA for a completely randomized design, utilizing the SPSS software (ver. 19.0, SPSS Inc., IL, USA). Data were subjected to analysis after conducting the homogeneity of variance and normality tests. The replication pen was designated as the experimental unit for growth performance parameters, while the individual birds served as the experimental units for the other measurements. Orthogonal polynomial contrast analysis was applied to assess the linear and quadratic effects of increasing dietary CurNPs inclusion levels on each measurement. Differences among the means were evaluated using Tukey's multiple-range test at $P > 0.05$.

Results

Growth performance

Table 2 illustrates the impact of CurNPs incorporation on the growth performance of heat-stressed broilers. BWG, EPEI and FCR were linearly ($P > 0.001$) improved with CurNPs supplementation throughout the experimental periods, while FI was reduced linearly ($P > 0.001$). The 400CurNPs group exhibited the highest BWG and EPEI, alongside the

Table 2
Effect of dietary curcumin nanoparticles on growth performance of broiler chickens exposed to heat stress from 1 to 35 d of age.

Parameter[1]	Curcumin nanoparticles level, mg.kg^{-1}					SEM[2]	*P-values*		
	0	100	200	300	400		ANOVA	L	Q
Starter (1-10 d)									
BWG, g.bird.period^{-1}	274.2^{c}	277.5^{b}	281.1^{a}	283.7^{a}	283.9^{a}	0.665	<0.001	<0.001	0.071
FI, g.bird.period^{-1}	315.0^{a}	308.4^{b}	308.3^{b}	303.1^{b}	304.0^{b}	1.003	<0.001	<0.001	0.182
FCR, g feed.g gain^{-1}	1.149^{a}	1.112^{b}	1.097^{bc}	1.069^{c}	1.071^{c}	0.006	<0.001	<0.001	0.095
EPEI	263.2^{c}	280.9^{b}	290.5^{ab}	301.3^{a}	300.6^{a}	2.456	<0.001	<0.001	0.014
Grower (11-24 d)									
BWG, g.bird.period^{-1}	765.3^{b}	776.0^{a}	780.5^{a}	783.0^{a}	782.4^{a}	1.549	<0.001	<0.001	0.032
FI, g.bird.period^{-1}	1187.2^{a}	1179.3^{ab}	1176.4^{bc}	1170.6^{bc}	1168.5^{c}	1.683	0.022	<0.001	0.484
FCR, g feed.g gain^{-1}	1.551^{a}	1.520^{b}	1.507^{b}	1.496^{b}	1.494^{b}	0.005	0.001	<0.001	0.111
EPEI	276.5^{a}	292.0^{b}	299.6^{ab}	303.8^{a}	303.8^{a}	1.879	<0.001	<0.001	0.004
Finisher (25-35 d)									
BWG, g.bird.period^{-1}	902.5^{b}	913.1^{b}	940.6^{a}	931.5^{a}	943.2^{a}	2.706	<0.001	<0.001	0.027
FI, g.bird.period^{-1}	1760.1^{a}	1753.8^{ab}	1752.2^{b}	1744.2^{c}	1744.9^{c}	1.254	<0.001	<0.001	0.374
FCR, g feed.g gain^{-1}	1.950^{a}	1.921^{b}	1.863^{c}	1.873^{c}	1.851^{c}	0.007	<0.001	<0.001	0.036
EPEI	276.4^{c}	290.2^{b}	307.6^{a}	305.6^{a}	310.9^{a}	2.064	<0.001	<0.001	0.001
Overall (1-35 d)									
BWG, g.bird.period^{-1}	1942.0^{c}	1966.7^{b}	2002.3^{a}	1998.2^{a}	2009.5^{a}	4.626	<0.001	<0.001	0.021
FI, g.bird.period^{-1}	3262.4^{a}	3241.6^{ab}	3237.0^{bc}	3217.8^{c}	3217.4^{c}	3.868	<0.001	<0.001	0.346
FCR, g feed.g gain^{-1}	1.680^{a}	1.649^{b}	1.617^{c}	1.611^{c}	1.602^{c}	0.006	<0.001	<0.001	0.056
EPEI	272.0^{c}	287.7^{b}	299.2^{a}	303.6^{a}	305.1^{a}	2.086	<0.001	<0.001	0.003

Means in the same row with different superscripts are significantly different, n = eight pens/group, [1]BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio, EPEI = European production efficiency index, L = linear, Q = quadratic [2]SEM = standard error of means. Values with different superscript letters are statistically different ($P < 0.05$).

lowest FCR and FI, with no statistical differences when compared to 200CurNPs and 300CurNPs groups, indicating a plateau effect at higher doses.

Carcass traits

Carcass characteristics, including eviscerated carcass, body parts (breast, thigh, and drumstick), and breast and leg meat, were not altered by the dietary incorporation of CurNPs (Table 3). However, a linear ($P > 0.05$) decrease in the relative weight of the wing was observed as CurNPs concentration increased in the diet.

Serum metabolites

As shown in Table 4, serum concentrations of renal and hepatic function biomarkers were not influenced by dietary incorporation of CurNPs except for a linear ($P > 0.05$) elevation in globulin and total protein levels. The 300CurNPs and 400CurNPs groups exhibited the highest levels of these parameters compared to the untreated birds.

Lipid profile

The impact of dietary CurNPs incorporation on the serum lipid profile of heat-stressed broilers is presented in Table 5. The results revealed that CurNPs exhibit hypocholesterolemic effect, evidenced by a quadratic and linear ($P > 0.05$) reduction in LDL and TC, along with a linear ($P > 0.05$) decrease in TG and VLDL as the dietary inclusion level of CurNPs increased. Birds in the 300CurNPs group showed the lowest values of the aforementioned parameters compared to the unsupplemented group. Additionally, all treated birds exhibited higher ($P > 0.05$) HDL concentrations compared to the untreated ones.

Ileal histomorphometry

As depicted in Figs. 2 and 3, VH increased linearly and quadratically ($P > 0.01$) with increased dietary levels of CurNPs. Additionally, the VH/CD ratio showed a linear ($P > 0.01$) increase in all treated groups. The highest VH and VH/CD ratio were observed in the 300CurNPs, 400CurNPs, and 200CurNPs, respectively. Conversely, the ileal CD was not altered by the dietary inclusion of CurNPs.

Antioxidant status

Fig. 4 illustrates the impact of dietary CurNPs inclusion on the redox status of broilers. Serum concentrations of MDA decreased linearly ($P > 0.05$), while SOD levels elevated both linearly and quadratically ($P > 0.05$) as the CurNPs levels in the diet increased. However, serum GSH concentrations remained unchanged across all experimental groups regardless of dietary CurNPs supplementation.

Table 3
Effect of dietary curcumin nanoparticles on carcass traits of broiler chickens exposed to heat stress at 35 d of age.

Parameter	Curcumin nanoparticles level, mg.kg^{-1}					SEM[1]	*P-values*		
	0	100	200	300	400		ANOVA	L	Q
Eviscerated carcass, %	69.76	70.14	72.10	73.43	71.55	0.561	0.228	0.086	0.265
Breast, %	27.46	27.37	27.15	27.58	27.44	0.056	0.163	0.668	0.221
Thigh, %	13.36	13.46	13.48	12.72	13.44	0.199	0.752	0.695	0.803
Drumstick, %	9.738	9.826	9.616	9.786	9.708	0.046	0.689	0.769	0.905
Wing, %	7.163^{a}	6.906^{a}	6.202^{b}	6.852^{a}	6.245^{b}	0.108	0.004	0.027	0.348
Breast meat, %	23.07	23.37	23.12	23.04	23.36	0.112	0.824	0.763	0.836
Leg meat, %	17.02	17.18	17.39	16.52	17.06	0.169	0.616	0.642	0.820
Total meat, %	40.09	40.55	40.51	39.57	40.42	0.205	0.551	0.828	0.942

Means in the same row with different superscripts are significantly different, n = eight birds/group, [1]SEM = standard error of means, L = linear, Q = quadratic. Values with different superscript letters are statistically different ($P < 0.05$).

Table 4
Effect of dietary curcumin nanoparticles on serum hepatic and renal function biomarkers of broiler chickens exposed to heat stress at 35 d of age.

Parameter	Curcumin nanoparticles level, mg.kg^{-1}					SEM[1]	*P-values*		
	0	100	200	300	400		ANOVA	L	Q
Total protein, g.dl^{-1}	3.90^{c}	4.01^{bc}	4.09^{abc}	4.38^{a}	4.23^{b}	0.053	0.020	0.003	0.414
Albumin (A), g.dl^{-1}	2.12	2.11	2.22	2.35	2.18	0.044	0.456	0.254	0.447
Globulin (G), g.dl^{-1}	1.78^{b}	1.91^{ab}	1.87^{ab}	2.03^{a}	2.05^{a}	0.036	0.071	0.008	0.953
A/G ratio	1.19	1.11	1.19	1.17	1.07	0.033	0.730	0.463	0.601
AST, IU.L^{-1}	148.9	149.0	146.9	136.1	138.9	2.098	0.146	0.057	0.840
ALT, IU.L^{-1}	25.46	25.89	26.62	20.52	24.12	1.112	0.464	0.321	0.959
Glucose, mg.dl^{-1}	154.3	160.0	157.0	161.5	160.4	1.550	0.608	0.236	0.638
Uric acid, mg.dl^{-1}	4.62	4.91	4.48	4.81	4.44	0.263	0.980	0.822	0.814
Creatinine, mg.dl^{-1}	0.692	0.734	0.730	0.732	0.734	0.027	0.988	0.691	0.762

Means in the same row with different superscripts are significantly different, n = eight birds/group, [1]SEM = standard error of means, L = linear, Q = quadratic, AST = aspartate aminotransferase, ALT = alanine aminotransferase. Values with different superscript letters are statistically different ($P < 0.05$).

Table 5
Effect of dietary curcumin nanoparticles on serum lipid profile of broiler chickens exposed to heat stress at 35 d of age.

Parameter	Curcumin nanoparticles level, mg.kg^{-1}					SEM[1]	*P-values*		
	0	100	200	300	400		ANOVA	L	Q
Total cholesterol, mg.dl^{-1}	208.4^{a}	190.9^{b}	191.0^{b}	178.6^{b}	184.3^{b}	2.621	0.001	>0.001	0.035
Triglycerides, mg.dl^{-1}	200.3^{a}	193.3^{ab}	197.7^{a}	185.9^{b}	193.2^{ab}	1.578	0.031	0.034	0.282
HDL, mg.dl^{-1}	39.74^{c}	49.58^{b}	48.80^{b}	58.82^{a}	51.97^{ab}	1.664	0.002	0.001	0.036
LDL, mg.dl^{-1}	128.6^{a}	102.7^{b}	102.6^{b}	82.54^{c}	93.70^{bc}	3.920	>0.001	>0.001	0.023
VLDL, mg.dl^{-1}	40.06^{a}	38.65^{ab}	39.54^{a}	37.19^{b}	38.64^{ab}	0.316	0.031	0.034	0.282

Means in the same row with different superscripts are significantly different, n = eight birds/group, [1]SEM = standard error of means, L = linear, Q = quadratic, HDL = high-density lipoprotein cholesterol, LDL = low-density lipoprotein cholesterol, VLDL = very low-density lipoprotein cholesterol. Values with different superscript letters are statistically different ($P < 0.05$).

Immune response

Results presented in Table 6 reveal the impacts of dietary incorporation of CurNPs on humoral immunity and lymphoid organs' relative weight. Increasing the dietary inclusion level of CurNPs resulted in a linear ($P > 0.01$) increase in serum levels of IgG, IgA, IgM, and total immunoglobulin. The highest levels were observed in the 300CurNPs and 400CurNPs groups compared to the other groups. However, antibody titres against NDV and AIV, as well as the relative weights of the spleen, thymus and bursa of Fabricius, were not affected by CurNPs treatment.

Discussion

The application of phytogenics and their bioactive compounds in poultry diets has gained popularity as an alternative to AGPs. These natural additives demonstrated their function in enhancing gut health by reducing the presence of harmful bacteria, boosting performance, and improving digestibility through stimulating digestive enzyme secretion, bile production, and intestinal architecture. Recently, nanoform of these bioactives have gained attention as a potential strategy to improve poultry performance and health. Nanotechnology techniques enhance the bioavailability of curcumin, leading to better absorption, which in turn supports poultry growth and overall health (Siddiqui et al., 2022). Our results revealed that incorporating CurNPs in the diets of heat-stressed broilers increased their BWG and EPEI by 3.16% and 11.26%, respectively, while reducing FCR and FI 4.17% and 1.18%, respectively. The same results were reported by (Reda et al., 2020) who noticed significant increase in BWG and decrease in FCR and FI of Japanese quails treated with CurNPs up to 0.5 g.kg^{-1} diet. Rahmani et al. (2018) also observed that the treatment of 200 ppm CurNPs improved WG and FCR with no effect on FI. Moreover, Zhang et al. (2015) highlighted curcumin's beneficial role in mitigating heat stress-related growth retardation in poultry. The growth-promoting activity of CurNPs may be attributed to their ability to stimulate digestive enzymes, enhance bile secretion, improve nutrient absorption, and boost thyroid gland function (Durrani et al., 2006; Niamsa and Sittiwet, 2009). Furthermore, curcumin's potent antioxidant, antimicrobial, anti-inflammatory, probiotic-like, and gastroprotective properties (Prasad et al., 2014; Zhang et al., 2015; Gera et al., 2017) might contribute to optimize feed utilization, allowing broilers to achieve better growth performance with reduced feed intake.

The findings of the current study on carcass characteristics of broilers are consistent with previous investigations. Reda et al. (2020) reported that feeding quails with CurNPs-supplemented diets up to 0.5 g.kg^{-1} did not affect their carcass traits. Wang et al. (2015) found that adding turmeric extract at 100 to 300 mg.kg^{-1} did not affect dressing percentage. Additionally, it was noted by Ahmed et al. (2018) that neither the gizzard weight nor the dressing percentage was significantly influenced by dietary turmeric treatments. While these studies observed minimal changes in carcass traits, they noted significant improvements in growth performance, suggesting CurNPs' impact on carcass traits may be limited, possibly due to nutrient allocation prioritizing metabolic and immune functions rather than carcass yield. On the other hand, Durrani et al. (2006) found increased thigh and breast weights and a higher dressing percentage in broilers fed a diet containing 5 g.kg^{-1} *Curcuma longa* powder.

The hepatoprotective impact of CurNPs against the harmful effect of heat stress on broilers is well-observed in the present study. Dietary CurNPs reduced the activity of AST and ALT and increased serum globulin and total protein levels. The positive impact of CurNPs on liver health is likely due to their broad-spectrum antioxidant capabilities. It has been reported that curcumin can neutralize and inhibit the formation of reactive nitrogen and oxygen species (Kim et al., 2013; Liang et al., 2024; Shang et al., 2024). Additionally, it has been reported that curcumin treatment, under stress conditions, down-regulated the expression of heat shock protein 70, activated the Nrf2-mediated phase II detoxifying enzyme systems, and enhanced the activity of several detoxification-related enzymes, including glutathione S-transferase, manganese superoxide dismutase (MnSOD), and glutathione peroxidase to counteract oxidative stress (Liu et al., 2023; Mesalam et al., 2023). Additionally, curcumin upregulated the mitochondrial MnSOD gene and

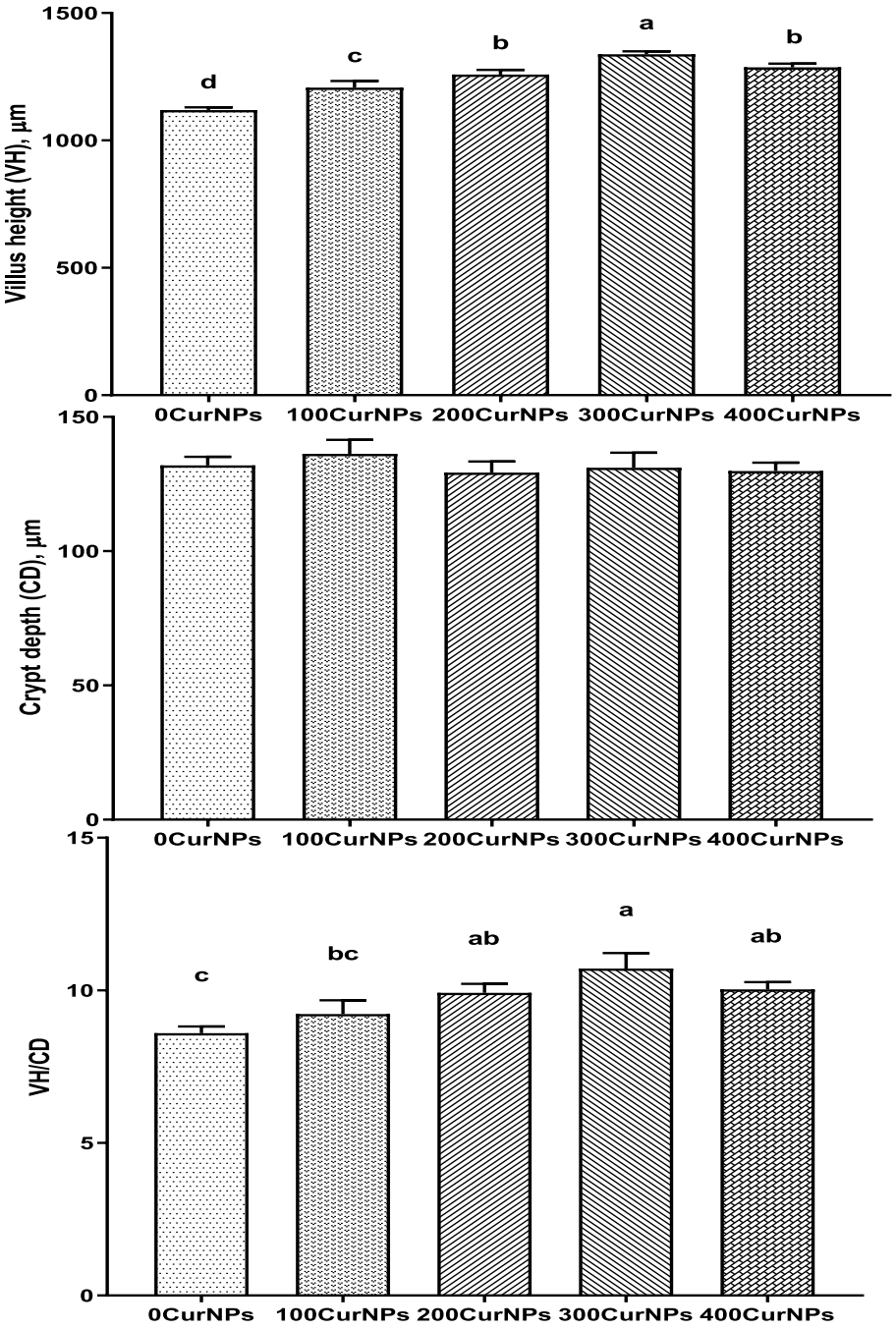

Fig. 2. Effect of dietary curcumin nanoparticles on ileal villus height (VH), crypt depth (CD), and VH/CD ratio of broiler chickens at 35 d of age. 0CurNPs, 100CurNPs, 200CurNPs, 300CurNPs, and 400CurNPs = 0, 100, 200, 300, and 400 mg.kg^{-1} curcumin nanoparticles, respectively. Data are presented as the mean values with their standard errors, n = eight birds/group. Values with different superscript letters are statistically different ($P < 0.05$). P values for the linear and quadratic contrast of VH, CD, and VH/CD are ($P < 0.01$), ($P = 0.498$) and ($P < 0.01$), and ($P < 0.01$), ($P = 0.891$) and ($P = 0.062$), respectively.

alleviated hepatic mitochondrial dysfunction in heat-stressed broilers (Zhang et al., 2018; Nawab et al., 2019).

In the present study, the dietary inclusion of CurNPs significantly reduced serum TC and TG levels. Our findings demonstrated the hypocholesterolemic effect of CurNPs and improved lipid profile of broilers, consistent with previous research demonstrating curcumin's ability to lower LDL and TG while improving liver function (Seo et al., 2008; Gandhi et al., 2011). Numerous studies support curcumin's cholesterol-lowering effects (Rajput et al., 2013; Xie et al., 2018; Pornanek and Phoemchalard, 2019) and its role in promoting fat digestion (De Beer et al., 2008). However, results across studies have not been entirely consistent. For instance, Nouzarian et al. (2011) reported reduced triglycerides with no changes in total cholesterol or LDL:HDL ratio, while Emadi and Kermanshahi (2007) observed reduced LDL and increased HDL with unchanged triglycerides. On the other hand, the study of Mehala and Moorthy (2008) found no significant changes in triglycerides, total cholesterol, or LDL. These discrepancies are likely due to variations in curcumin dosages across different studies. The cholesterol-lowering effects of CurNPs might be attributed to their ability to mobilize cholesterol from extrahepatic tissues for catabolism in the liver, enhance its conversion to bile acids by activating cholesterol-7-α-hydroxylase, and reduce intestinal cholesterol absorption (Badran et al., 2020). Additionally, curcumin may regulate lipid metabolism by inhibiting key liver enzymes such as acetyl-CoA carboxylase,

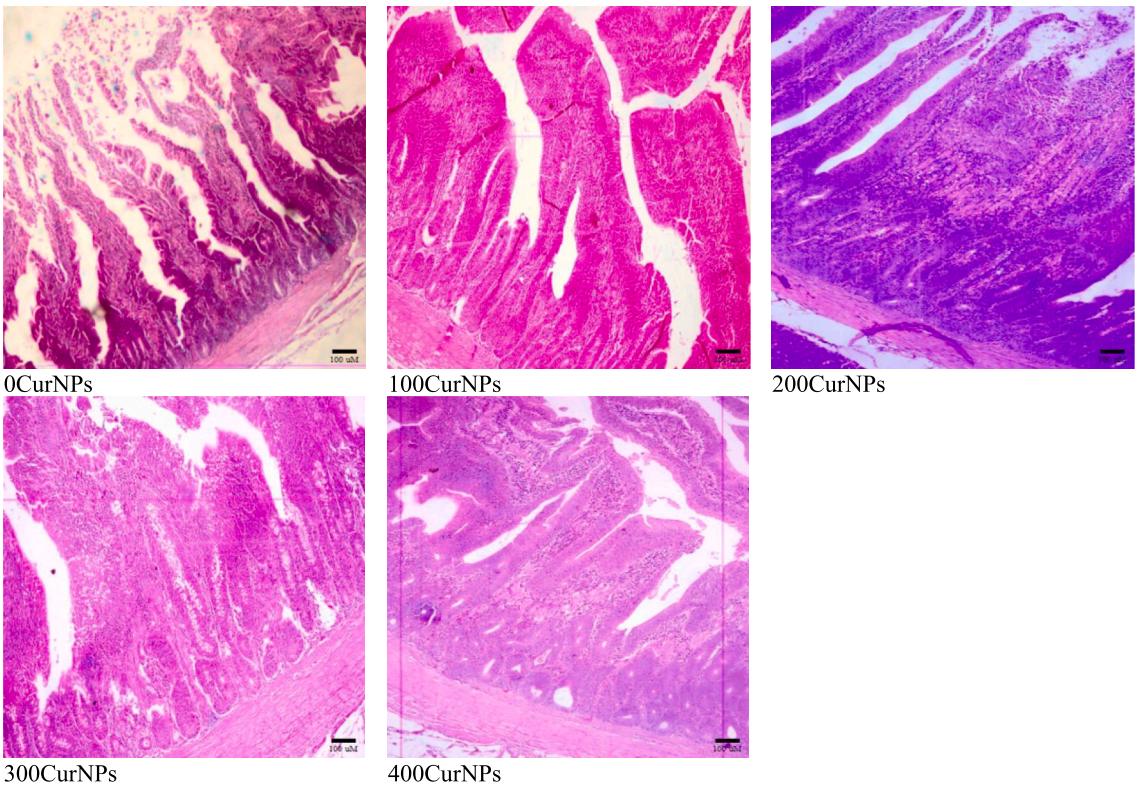

Fig. 3. Effect of dietary curcumin nanoparticles on ileal architecture of broiler chickens at 35 d of age (H&E, x100, $n = 8$). 0CurNPs, 100CurNPs, 200CurNPs, 300CurNPs, and 400CurNPs = 0, 100, 200, 300, and 400 mg.kg^{-1} curcumin nanoparticles, respectively.

the rate-limiting enzyme in fatty acid synthesis, and HMG-CoA reductase, a critical enzyme in cholesterol synthesis, ultimately reducing fatty acid esterification and cholesterol synthesis (Nouzarian et al., 2011; Pornanek and Phoemchalard, 2019).

The incorporation of CurNPs significantly improves intestinal histomorphometry in heat-stressed broilers. Improved intestinal histomorphometry is closely linked to enhanced nutrient absorption and overall gut health, facilitating better growth performance and resilience under stressful conditions. Studies have demonstrated that dietary supplementation with CurNPs, curcumin or turmeric powder increases VH and villi width, resulting in better nutrient uptake and gut function (Rajput et al., 2013; Rahmani et al., 2018; Kpomasse et al., 2023). The enhanced intestinal morphology can be attributed to CurNPs' antioxidant properties, which reduce oxidative stress and inflammation in the gut (Prasad et al., 2014). Curcumin's ability to modulate gut health is linked to its action on intestinal cells, where it helps to maintain tight junction integrity, reduce intestinal permeability, and promote the growth of beneficial gut bacteria (Geevarghese et al., 2023). Additionally, CurNPs upregulate the expression of genes associated with intestinal health and the antioxidant defense system, further supporting their beneficial role in maintaining the intestinal structure during heat stress (Sahin et al., 2012; He et al., 2015). By preserving the integrity of the intestinal lining and enhancing nutrient absorption, CurNPs play a critical role in improving the overall performance and health of heat-stressed broilers. These effects not only enhance growth performance but also provide a protective mechanism against the detrimental effects of heat stress on gut health.

Curcumin nanoparticles have been documented as a powerful antioxidant with significant potential to counter the harmful effects of heat stress in broilers by boosting their antioxidant defense mechanisms and shielding them from oxidative damage. Unlike conventional curcumin, CurNPs offer superior bioavailability and solubility, which allows for better cellular absorption and heightened antioxidant efficacy (Siddiqui et al., 2022). Our findings showed a decrease in serum concentrations of MDA and an increase in SOD in the CurNP-treated groups. These results are consistent with Reda et al. (2020) and Rahmani et al. (2018), who reported elevated SOD and GSH activities alongside reduced MDA in quails and broilers fed with CurNPs.

The primary mechanism through which CurNPs combat oxidative stress is by scavenging free radicals, neutralizing them before they can cause cellular damage. CurNPs donate electrons or hydrogen atoms to stabilize ROS, thereby halting the chain reactions that lead to oxidative stress (Lushchak, 2014; Abdel-Moneim et al., 2020). Moreover, curcumin has been shown to reduce oxidative stress by modulating hepatic nuclear transcription factors and decreasing lipid peroxidation in the muscles and serum of quails (Sahin et al., 2012). CurNPs also amplify the activities of endogenous antioxidant enzymes like SOD, CAT, and GPx, which are vital for maintaining cellular redox balance and detoxifying ROS, thus curbing oxidative damage. CurNPs have been found to upregulate the expression and activity of these enzymes, further strengthening the broilers' innate antioxidant defenses (Geevarghese et al., 2023). This enhanced enzyme action helps protect against lipid peroxidation, protein oxidation, and DNA damage, which are commonly associated with heat stress in poultry. Another key function of CurNPs lies in their modulation of oxidative stress-related signaling pathways, particularly the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway. Nrf2 is essential for cellular defense against oxidative damage, and CurNPs are believed to activate this pathway, promoting the expression of various antioxidant genes, including those that code for SOD, CAT, and GPx (He et al., 2015). In addition to these antioxidant effects, CurNPs help maintain mitochondrial function in heat-stressed broilers by minimizing ROS production and improving mitochondrial membrane integrity, which ensures efficient energy metabolism and limits oxidative harm to the cells (Ghosh et al., 2015).

The immunomodulatory effects of curcumin are notably enhanced when delivered as nanoparticles in heat-stressed broilers. This approach improves curcumin's bioavailability and efficacy, enabling better absorption and more effective interaction with immune cells, which in turn

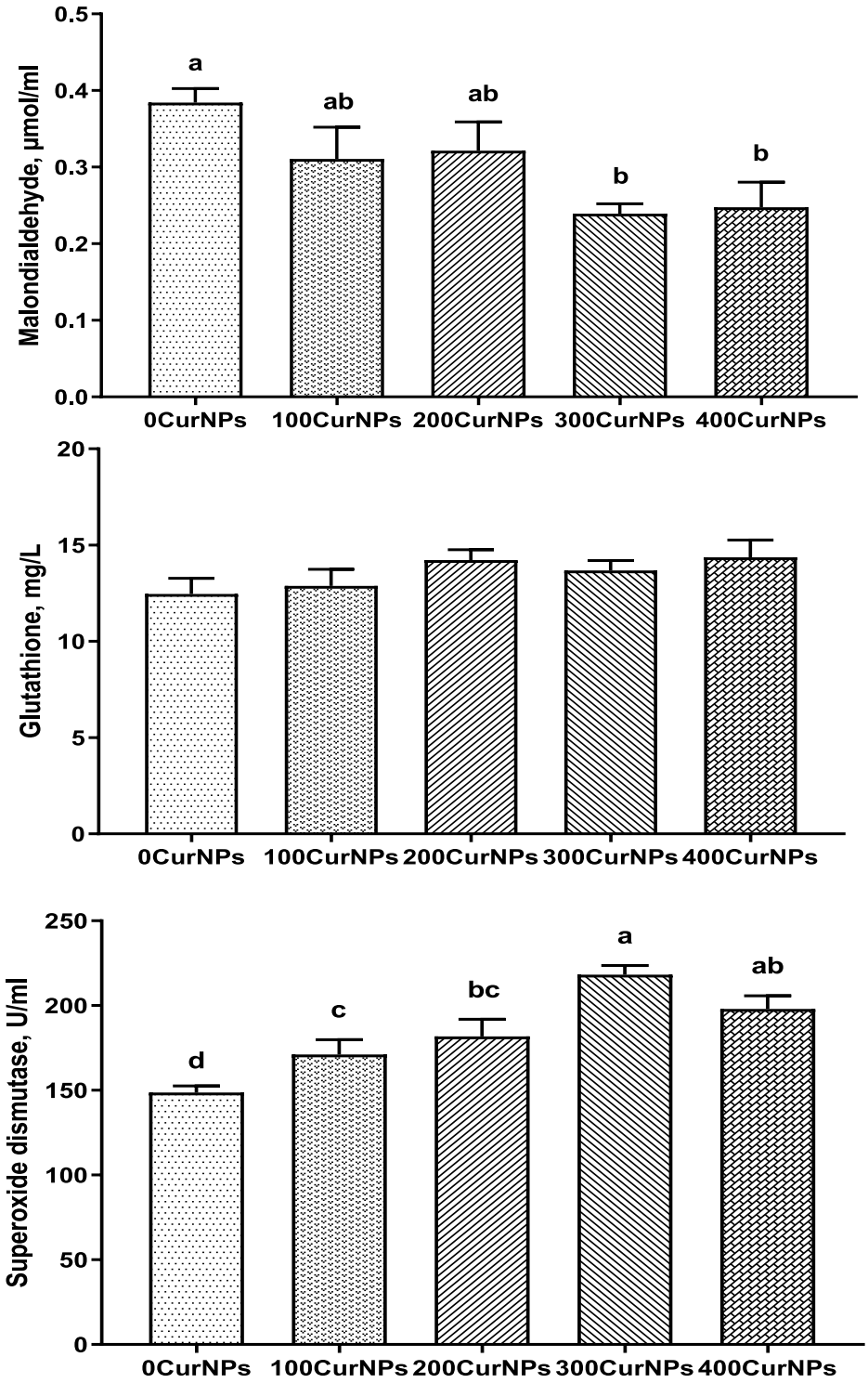

Fig. 4. Effect of dietary curcumin nanoparticles on serum MDA, GSH, and SOD levels of broiler chickens at 35 d of age. 0CurNPs, 100CurNPs, 200CurNPs, 300CurNPs, and 400CurNPs = 0, 100, 200, 300, and 400 mg.kg^{-1} curcumin nanoparticles, respectively. Data are presented as the mean values with their standard errors, n = eight birds/group. Values with different superscript letters are statistically different ($P < 0.05$). P values for the linear and quadratic contrast of MDA, GSH, and SOD are ($P = 0.002$), ($P = 0.065$) and ($P < 0.01$), and ($P = 0.542$), ($P = 0.628$) and ($P = 0.047$), respectively.

boosts immune function and regulates inflammatory responses. Heat stress often weakens the immune system in broilers by elevating inflammatory cytokines like IL-6, TNF-α, and IL-1β, which worsen oxidative stress. CurNPs counteract this by modulating the immune response. In our study, dietary inclusion of CurNPs increased serum immunoglobulin levels, though antibody titers against NDV and AIVwere not significantly affected. Consistent with these findings, Reda et al. (2020) and Badran et al. (2020) reported that CurNPs elevated immunoglobulin concentrations in quails and broilers, while Emadi and Kermanshahi (2007) found similar effects with turmeric supplementation.

The stimulating ability of CurNPs to the production of immunoglobulins, which are essential for humoral immunity, might be attributed to their ability to modulate pathways like PI3K/Akt, which are involved in cell survival and immune function (Sahin et al., 2012; Ghosh

Table 6
Effect of dietary curcumin nanoparticles on humoral immune response and relative weight of lymphoid organs of broiler chickens at 35 d of age.

Parameter	Curcumin nanoparticles level, mg.kg^{-1}					SEM[1]	*P-values*		
	0	100	200	300	400		ANOVA	L	Q
IgM, μg.ml^{-1}	206^{c}	260^{b}	261^{b}	307^{a}	295^{ab}	0.089	>0.001	>0.001	0.094
IgG, μg.ml^{-1}	372^{c}	385^{bc}	384^{bc}	419^{a}	407^{ab}	0.054	0.026	0.004	0.779
IgA, μg.ml^{-1}	199^{c}	206^{bc}	205^{bc}	224^{a}	218^{ab}	0.029	0.029	0.008	0.811
Total Igs, μg.ml^{-1}	778^{c}	850^{bc}	849^{bc}	949^{a}	920^{ab}	0.166	0.002	>0.001	0.331
NDV titre	4.88	5.79	5.60	5.94	5.83	0.147	0.149	0.051	0.206
AIV titre	3.17	3.47	3.60	4.13	3.58	0.116	0.114	0.063	0.164
Spleen, %	0.124	0.112	0.122	0.118	0.114	0.002	0.514	0.434	0.924
Thymus, %	0.360	0.378	0.356	0.382	0.352	0.005	0.280	0.746	0.280
Bursa of Fabricius, %	0.178	0.176	0.174	0.208	0.156	0.009	0.513	0.854	0.411

Means in the same row with different superscripts are significantly different, n = eight birds/group, [1]SEM = standard error of means, L = linear, Q = quadratic, IgM = immunoglobulin M, IgG = immunoglobulin G, IgA = immunoglobulin A, NDV = Newcastle disease virus, AIV = Avian influenza virus (H_9N_1). Values with different superscript letters are statistically different ($P < 0.05$).

et al., 2015). Another mechanism behind CurNPs' immunomodulatory impact is their ability to downregulate pro-inflammatory cytokines. CurNPs suppress IL-1β, IL-6, and TNF-α, which are typically high during heat stress, thereby restoring immune balance and preventing excessive inflammation. This anti-inflammatory effect is mediated through the inhibition of NF-κB signaling pathways, crucial for regulating inflammation and immune responses (Geevarghese et al., 2023). Additionally, CurNPs enhance levels of anti-inflammatory cytokines like IL-10, which help maintain immune homeostasis and reduce tissue damage. Beyond their anti-inflammatory role, CurNPs also boost immune cell activity like T cells, macrophages, and natural killer, which are impaired by heat stress. CurNPs have been shown to improve the proliferation and activation of these immune cells, helping broilers combat pathogens more effectively under heat stress (Rahmani et al., 2018; Reda et al., 2020). CurNPs also support gut health, which is closely linked to immune function. Heat stress often increases intestinal permeability, raising the risk of infection. CurNPs improve gut barrier function by reducing oxidative damage and promoting the growth of beneficial microbiota, ultimately improving nutrient absorption and strengthening the immune system (Prasad et al., 2014).

This study demonstrates that the dietary incorporation of CurNPs effectively enhances the growth performance and overall health of heat-stressed broiler chickens. CurNPs exhibit potent hypocholesterolemic, antioxidant and immunomodulatory impacts, which contribute to enhanced well-being. Furthermore, CurNPs supplementation positively impacts intestinal histomorphometry, suggesting improved nutrient absorption and gut health. Conclusively, the supplementation of CurNPs presents a promising approach to counteract the detrimental effects of heat stress on broiler health and productivity. Nevertheless, the economic feasibility of CurNPs production is a critical factor that must be considered to ensure their practical application in commercial poultry farming.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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