Effect of curcumin on laying performance, egg quality, endocrine hormones, and immune activity in heat-stressed hens

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ABSTRACT This study was conducted to evaluate the effect of curcumin on laying performance, egg quality, biochemical indicators, hormone levels, and immune activity in hens under heat stress. Hy-Line brown hens (280-day-old) were fed with 0, 100, 150, and 200 mg/kg of curcumin during a 42-D experiment. Compared with the control treatment, supplementation with 150 mg/kg of curcumin improved laying performance and egg quality by significantly increasing egg production, eggshell thickness, eggshell strength ($P < 0.01$), and albumen height ($P < 0.05$) while decreasing the feed-to-egg ratio. Antioxidant activity was improved by significantly increasing the activity of superoxide dismutase and glutathione peroxidase but decreasing malondialdehyde levels in serum ($P < 0.05$) and significantly increasing the levels of follicle-stimulating hormone, luteinizing hormone, estradiol, IgG, IgA, and complement C_3 activity in serum ($P < 0.05$). These results indicated that supplemental 150 mg/kg curcumin can improve productive performance, antioxidant enzyme activity, and immune function in laying hens under the heat stress conditions applied in the present study.

Key words: curcumin, heat stress, laying hen, laying performance, hormone level

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INTRODUCTION

Among all livestock, poultry is the most sensitive to heat stress owing to its low ability to dissipate body heat. Therefore, high ambient temperature adversely affects poultry production and health (Lara and Rostagno, 2013). Studies have shown that the optimal ambient temperature for laying hens is approximately 20°C to 25°C (Tumova and Gous, 2012). When the temperature exceeds 30°C, signs of heat stress appear (Yardibi and Turkay, 2014). Research indicates that a marked decrease in egg production and feed intake occurs in laying hens subjected to chronic heat stress over a 5-wk period (Mashaly et al., 2004). Moreover, exposure of chickens to extreme heat stress induced a decrease in serum immunoglobulin (**Ig**) IgG and IgM levels (Sangoh et al., 2013). The issue has become a great point of interest in animal agriculture, particularly owing to public awareness and concerns. The importance of animal responses to environmental challenges applies to all species. However, poultry seems to be particularly sensitive to temperature-associated environmental challenges, especially heat stress. It has been suggested that modern poultry genotypes produce more body heat than earlier strains owing to their higher metabolic activity (Deeb and Cahaner, 2002; Settar et al., 1999). Some nutritional strategies focus on alleviating the negative effects of heat stress through supplementation with medicinal herbs (natural extracts with antioxidant potential), micronutrients (vitamins and minerals), and so on. The main aim is to satisfy the special needs of animals, and this has proven advantageous during heat stress (Lin et al., 2006).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1, 6-diene-3,5-dione; diferuloylmethane), extracted from turmeric roots, is a major bioactive polyphenol that has been used widely as a spice, food additive, and herbal medicine in Asia. Numerous studies have demonstrated that curcumin possesses potent biological activity, including antioxidant and anti-inflammatory activity (Sharma et al., 2005). It is known to eliminate oxygen free radicals, inhibit lipid peroxidation, and protect cellular macromolecules such as DNA from oxidative stress (Srinivasan et al., 2006). Epidemiological, clinical, and experimental studies have demonstrated that curcumin is a pharmacologically safe agent. However, in the past few decades, curcumin research has focused on the antioxidant, anti-inflammatory, lipid-lowering, cancer chemopreventive, and potential chemotherapeutic properties of the compound

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(Esatbeyoglu et al., 2012). Research on curcumin in animal husbandry is still in its infancy. It has been established that curcumin ameliorates heat stress by modulating hepatic nuclear transcription factors and heat shock protein 70 in heat-stressed quails (Sahin et al., 2012; Zhang et al., 2015). In addition, turmeric powder improves immunity in laying hens by increasing total Ig and IgG titers after sheep red blood cell injections (Arshami et al., 2013).

Curcumin has become a potential herb-derived additive with its natural, nonresidual, antioxidant, and anti-inflammatory properties. However, information about curcumin as a growth promoter and natural antioxidant in laying hens is scarce. Thus, the purpose of this study was to determine the effect of dietary curcumin as a phytogenic additive on production performance, serum biochemical metabolites, endocrine hormones, and immune activity in laying hens under heat stress.

MATERIALS AND METHODS

Preparation of Curcumin

The curcumin used in the present study was provided by the Kehu Bio-technology Research Center (Guangzhou, China). The purity detection of the same curcumin production was determined by Dr. Zhang's lab (Zhang et al., 2015). The content of curcumin was 98% in this study.

Ethics Statement

This experimental protocol was approved by the Ethical Committee and developed under the supervision of the Institutional Animal Care and Use Committee of Nanjing Agricultural University, Nanjing, China.

General

A total of 240 Hy-Line brown hens (280-day-old) were randomly assigned to 4 experimental diets, with 4 replicates of 15 birds each. The amounts of curcumin supplied with the basal diet were as follows: 0 mg/kg, 100 mg/kg, 150 mg/kg, and 200 mg/kg. A small amount of the basal diet was first mixed with the appropriate amount of curcumin to make a small batch, and the remaining basal diet was added to obtain a homogeneous mixture. All hens were provided with free access to clean water and feed and indoor ventilation and lighting, and their environment was regularly cleaned and disinfected. Diets were formulated following the nutrient requirement recommendations of the NRC (1994) (Table 1). The photoperiod was set at 16 h of light and 8 h of darkness (16L:8D) throughout the study. The house temperature and humidity were measured daily at 9:00 am, 13:00 pm, 17:00 pm, and 21:00 pm The ambient temperature transformation was at 34 ± 2°C for 8 h/D (9:00 am–5:00 pm), followed by 22°C to 28°C for 16 h/D. The house was maintained at a relative humidity of 50 to 65%. The feeding experiment was performed for 42 D after a 10-day adaptation period. The experiment was conducted on July 12, 2018, at the Mechanized Chicken Farm in Panchu, Nanjing, People's Republic of China.

Table 1. Ingredients and nutrient composition of the experimental basal diet (as fed).

Ingredients (%)	Content	Analyzed nutrient	Content
Corn	62.00	ME (MJ/kg)[2]	11.20
Soybean meal	22.00	CP	17.00
Wheat bran	3.00	EE	6.32
Limestone	8.00	Lys	0.78
Premix[1]	3.00	Met + Cys	0.68
NaCl	0.40	Met	0.54
Calcium hydrophosphate	1.60	Ca	3.52
Total	100.00	P	0.48

Abbreviations: Cys, cysteine; CP, crude protein; EE, ether extract; Lys, lysine; ME, metabolic energy; Met, methionine.

[1]The premix provides the following per kg diet: vitamin A, 7000 IU; vitamin D_3, 2500 IU; vitamin E, 36 mg; vitamin K, 32 mg; vitamin B_{12}, 0.025 mg; vitamin B_2, 5.6 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.025 mg; nicotinic acid, 38 mg; folic acid, 1.1 mg; calcium pantothenate, 10 mg; biotin, 0.16 mg; Cu, 10 mg; Fe, 80 mg; Mn, 100 mg; Zn, 60 mg; I, 0.55 mg; and Se, 0.12 mg.

[2]Values are deterministic values except ME.

Laying Performance Measurement

The laying rate, egg weight, and feed intake of each group were recorded daily to calculate egg production (laying hens/total hens × 100%), average egg weight, feed conversion ratio (total feed intake/total egg mass), and average daily feed intake. At the end of the experiment, 8 hens were randomly selected from each replicate and were slaughtered. The serum was centrifuged from the blood (3,000 × *g*, 10 min) and stored at −20°C for later examination of serum biochemical parameters, enzyme activity, and immune function.

Egg Quality Assay

Twenty eggs from each group were collected on the 42nd day of the feeding experiment and stored at 4°C until analysis (<24 h). Shape index, eggshell thickness, shell strength, albumen height, Haugh units, yolk color, and weight were determined (Kirunda et al., 2001) using an egg quality tester (DET6000, NABEL Co., Ltd., Kyoto, Japan).

Serum Biochemical Parameters

The levels of total protein, albumin (**ALB**), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose (**GLU**), cholesterol (**CHO**), triglyceride (**TG**), aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferase were analyzed and determined by an automatic biochemical analyzer (NVAS6805, NOVATECH, Jinan, China). The kits were purchased from Shandong Nuoan Aideer Bioengineering Co., Ltd., Jinan, China.

Enzyme Activity

The activity levels of serum glutathione peroxidase (**GSH-Px**), superoxide dismutase (**SOD**), malondialdehyde (**MDA**), and catalase (**CAT**) were determined by using the corresponding kits as per the manufacturer's instructions. All kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Hormone Determination

Levels of corticosterone (**COR**), estradiol ($\mathbf{E_2}$), follicle-stimulating hormone (**FSH**), luteinizing hormone (**LH**), serum Ig IgG, IgA, ad IgM, complement C_3, complement C_4, and IL-6 were determined by ELISA. The assay kit was purchased from Aoqing Biological Co., Ltd. (Nanjing, China), and the reagent preparation and procedure were carried out as per the instructions. The main instruments were an incubator, a constant-temperature water bath, and a shaker.

Statistical Analysis

Data were analyzed using one-way analysis of variance using SPSS 20.0 (SPSS Inc., Chicago, IL) Differences were considered significant at $P < 0.05$ and extremely significant at $P < 0.01$. Data are expressed as mean ± SE.

RESULTS

Effects of Curcumin on Laying Performance

As shown in Table 2, compared with the control treatment, the 3 increasing concentrations of supplemental curcumin improved egg production by 8.67% ($P < 0.05$), 11.58% ($P < 0.01$), and 1.56% ($P > 0.05$), respectively. The feed conversion ratios decreased by 9.50% ($P < 0.01$), 10.74% ($P < 0.01$), and 2.07% ($P > 0.05$), respectively. Other indicators showed no significant difference ($P > 0.05$).

Effects of Curcumin on Egg Quality

As shown in Table 3, eggs from laying hens fed with a diet supplemented with 100, 150, or 200 mg/kg of curcumin had significantly ($P < 0.01$) increased eggshell thickness and eggshell strength (kg/cm^2) than the control group. The eggshell strength greatly improved by 22.22, 23.22, and 26.74%, respectively, and the eggshell thickness improved by 61.49, 76.40, and 90.06%, respectively, with curcumin supplementation. Whereas Albumen height was numerically increased ($P > 0.05$) in the dietary groups supplemented with 150 or 200 mg/kg of curcumin. In addition, a diet supplemented with 100 mg/kg of curcumin significantly increased albumen height by 21.12% ($P < 0.05$). In addition, no significant differences were found in shape index, yolk color, yolk weight, or Haugh units ($P > 0.05$).

Effects of Curcumin on Serum Biochemical Parameters

As shown in Table 4, supplementation with curcumin significantly increased the level of serum alkaline phosphatase and decreased the level of serum ALB. The serum ALB level in hens was significantly decreased by 16.35% with diets supplemented with 150 mg/kg of curcumin ($P < 0.05$); this level in the other trial groups was not significantly different from that in the control group. Although GLU and low-density lipoprotein cholesterol levels were numerically increased ($P > 0.05$), TG and CHO levels were numerically decreased ($P > 0.05$).

Effect of Curcumin on Serum Antioxidant Indices

As shown in Table 5, compared with the control group, the activity of GSH-Px and CAT was significantly increased by 49.06 and 30.60% in hens fed with a diet supplemented with 150 or 200 mg/kg of curcumin, respectively, and the MDA content was numerically decreased ($P > 0.05$).

Effect of Curcumin on Reproductive Hormones

As shown in Table 6, the COR level in laying hens fed with a diet supplemented with 100, 150, or 200 mg/kg of curcumin was significantly ($P < 0.05$) less than that of the control group as follows: decrease by 17.89, 28.68, and 38.18%, respectively. Increase in dietary supplementation with 150 or 200 mg/kg of curcumin significantly improved the levels of serum E_2 by 17.98% ($P < 0.05$) and 31.81% ($P < 0.05$), respectively. Compared with the other 3 groups, the levels of serum FSH significantly

Table 2. Effects of curcumin on production performance in laying hens.

Item	Control	100 mg/kg	150 mg/kg	200 mg/kg
Egg production (%)	74.74 ± 0.02^{A,a}	81.22 ± 0.03^{A,B,b}	83.40 ± 0.05^{B}	75.90 ± 0.02^{A,a}
AEV (g)	58.91 ± 2.11	57.49 ± 1.07	55.42 ± 1.04	56.78 ± 1.48
ADFI (g)	106.55 ± 5.89	102.29 ± 1.44	103.98 ± 2.48	102.24 ± 1.25
Feed conversion ratio	2.42 ± 0.07^{B}	2.19 ± 0.08^{A}	2.16 ± 0.09^{A}	2.37 ± 0.03^{B}

Abbreviations: ADFI, average daily feed intake; AEV, average egg weight.
^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).
^{A,B}Different capital superscripts within a row indicate a highly significant difference ($P < 0.01$).
Values are mean ± SE of 15 hens. Data with different superscript letters are significantly different ($P < 0.05$).

Table 3. Effects of curcumin on egg quality in laying hens.

Item	Control	100 mg/kg	150 mg/kg	200 mg/kg
Shape index	1.31 ± 0.04	1.28 ± 0.03	1.28 ± 0.04	1.30 ± 0.08
Eggshell thickness (mm)	0.288 ± 0.02^{B}	0.352 ± 0.04^{A}	0.355 ± 0.02^{A}	0.365 ± 0.02^{A}
Eggshell strength (kgf/m^2)	1.61 ± 0.32^{C}	2.60 ± 0.56^{B}	2.84 ± 0.53^{A,B}	3.06 ± 0.55^{A}
Albumen height (mm)	7.14 ± 2.29^{b}	8.72 ± 1.42^{a}	7.98 ± 0.61^{a,b}	7.91 ± 2.11^{a,b}
Yolk color	3.12 ± 0.53	3.00 ± 0.47	3.09 ± 0.50	3.21 ± 0.68
Yolk weight(g)	14.50 ± 1.30	14.17 ± 1.21	14.78 ± 1.12	14.19 ± 1.11
Haugh unit	82.16 ± 9.12	91.93 ± 5.53	89.83 ± 3.19	87.54 ± 5.02

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).
^{A,B}Different capital superscripts within a row indicate a highly significant difference($P < 0.01$).
Values are mean ± SE of 20 eggs.

increased in hens supplied with 100 mg/kg of curcumin by 4.78% ($P < 0.05$), whereas the level of LH was numerically decreased ($P > 0.05$).

Effects of Curcumin on Immune Parameters

As shown in Table 7, activity levels of IgA, IgG, IgM, C_3, C_4, and IL-6 were measured. Compared with the control, increasing supplementation with curcumin increased the activity of IgA, IgG, IgM, C_3, and C_4 and decreased the activity of IL-6. Compared with the control group, the activity levels of IgA, IgG, IgM, and C3 from the laying hens fed with a diet supplemented with 150 mg/kg of curcumin significantly improved by 54.92% ($P < 0.01$), 23.95% ($P < 0.01$), 67.87% ($P < 0.01$), and 22.79% ($P < 0.01$), respectively.

DISCUSSION

It is now known that curcumin can modulate multiple signaling pathways in either a direct or an indirect manner. This polyphenol has been shown to possess pleiotropic activities in animal models of many human diseases (Gupta et al., 2012). Therefore, we speculate that the beneficial effect of curcumin is mechanistically related to this effect on signaling. Based on our experiment, a diet supplemented with curcumin improved the laying performance, egg quality, and physical condition of hens, especially at a concentration of 150 mg/kg of curcumin, which had a significant effect on egg production, eggshell strength, immune activity, antioxidant activity, and hormone levels.

Effects of Curcumin on Production Performance

Heat stress resulted in decreased laying performance and shell quality and poor egg quality by reducing food intake and through the direct effect of heat load (Lin et al., 2004). In another study, high environmental temperature was reported to decrease the egg production, egg quality, and feed intake of laying hens (Mashaly et al., 2004). The decrease in egg production was most likely due to the decrease in feed consumption, reducing the available nutrients for egg production. Daniel and Balnave (1981) indicated that feed intake is reduced before subsequent loss in egg production. It has been reported that exposure to high temperature decreases plasma protein concentrations (Zhou et al., 1998) and plasma calcium concentrations (Mahmoud et al., 1996), both of which are required for egg formation. In the treatment group supplemented with curcumin, the egg production, egg-to-egg ratio, and mortality were significantly lower than those of the control group. The reduction in egg quality is related to factors such as reduced feed intake, increased drinking water, and decreased blood calcium levels. The results of this test showed that the addition of curcumin to the diet

Table 4. Effects of curcumin on parameters of serum in laying hens.

Item	Control	100 mg/kg	150 mg/kg	200 mg/kg
AST (U/L)	295.67 ± 23.75	331.00 ± 31.99	306.60 ± 31.52	295.00 ± 138.49
ALT (U/L)	5.67 ± 3.21	5.33 ± 2.52	7.33 ± 4.16	7.33 ± 2.08
TP (g/L)	49.33 ± 4.30	45.83 ± 3.76	51.80 ± 8.38	50.50 ± 3.32
ALB (g/L)	25.50 ± 1.91^{B}	22.17 ± 1.33^{A}	21.33 ± 0.82^{A}	21.75 ± 0.96^{A}
ALP (U/L)	284.3 ± 132.96^{B,b}	513.17 ± 307.11^{A,a}	496.83 ± 151.98^{A,a}	509.75 ± 166.29^{A,a}
TG (mmol/L)	9.86 ± 1.53	7.42 ± 2.71	7.79 ± 2.81	8.13 ± 3.04
CHO (mmol/L)	2.82 ± 0.19	2.43 ± 0.58	2.58 ± 0.79	2.48 ± 0.81
GLU (mmol/L)	9.09 ± 1.99	9.05 ± 1.45	10.14 ± 1.71	10.21 ± 1.58
HDL-C (mmol/L)	0.62 ± 0.17	0.52 ± 0.17	0.98 ± 0.70	0.78 ± 0.45
LDL-C (mmol/L)	0.09 ± 0.04	0.23 ± 0.37	0.13 ± 0.12	0.10 ± 0.05

Abbreviations: ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHO, cholesterol; HDL-C, high-density lipoprotein cholesterol; GLU, glucose; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; TP, total protein.
^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).
^{A,B}Different capital superscripts within a row indicate a highly significant difference ($P < 0.01$).
Values are mean ± SE of 8 hens.

Table 5. Effects of curcumin on antioxidative capability of laying hens.

Item	Control	100 mg/kg	150 mg/kg	200 mg/kg
SOD (U/ml)	13.40 ± 1.08	13.68 ± 0.54	13.94 ± 0.70	13.28 ± 0.84
GSH-Px (U)	4.81 ± 1.40^{a}	6.38 ± 1.70^{a,b}	5.39 ± 1.28^{a,b}	7.17 ± 1.45^{b}
CAT (U/ml)	6.47 ± 0.49^{a}	6.23 ± 0.97^{a}	8.45 ± 1.67^{b}	7.05 ± 1.55^{a,b}
MDA (nmol/ml)	7.84 ± 1.42	6.92 ± 1.60	6.04 ± 1.79	7.26 ± 1.24

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).

^{A,B}Different capital superscripts within a row indicate a highly significant difference ($P < 0.01$).

Values are mean ± SE of 8 hens.

increased eggshell thickness, eggshell strength, and egg yolk weight. The improvement of eggshell quality is mainly due to the increased feed intake of laying hens, which frees calcium in the serum combined with plasma proteins or other components so that there is enough Ca^{2+} in the blood to participate in the formation of eggshells.

Biochemical blood parameters usually reflect the health of an animal. These parameters are vital indicators of the nutritional and physiological status of birds and mammals (Alagawany and Elhack, 2015). Heat stress causes oxidation of excess unsaturated fatty acids in the cell membrane, producing excess lipid peroxides, which convert excess fat into TG, resulting in elevated levels of TG in serum (Freeman and Crapo, 1982). Heat stress hinders CHO metabolism and excretion pathways, causing the serum CHO concentration to increase. In the present study, the addition of curcumin to the diet of laying hens had little influence on the physiology of birds. No changes in serum total protein, ALB, or GLU concentrations were observed during the whole experiment period in either of the experimental treatment groups compared with the control group.

Effects of Curcumin on Antioxidation

The effect of high ambient temperature as an inducer of oxidative stress has been acknowledged (Lin et al., 2006). The enzymatic antioxidant indicators, including SOD, GSH-Px, and CAT, represents the first line of the body's antioxidant defense; modification of the activity of these enzymes can alter the balance between the production of reactive oxygen species and the antioxidant system (Akbarian et al., 2016), which is decreased at high temperatures. In vitro studies have shown that curcumin inhibits nitric oxide and reactive oxygen species production in macrophages (Joe and Lokesh, 1994; Sreejayan and Rao, 1997); MDA is a product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage. Studies have shown that curcumin has a strong antioxidant effect, and this vigorous antioxidant activity is derived from its structure. The phenolic structure of curcumin can capture free radicals and form strong, stable anthraquinones (Kaneko and Baba, 1999). In the present study, we observed that curcumin significantly improved antioxidant activity by increasing the activity levels of SOD and GSH-Px and decreasing the level of MDA. It has been suggested that curcumin may exert its protective effects by scavenging free radicals and stimulating antioxidant enzymes such as SOD, CAT, and GSH-Px (Manju et al., 2012; Reddy and Lokesh, 1992).

Effects of Curcumin on Hormone Levels

Reproductive organ development, follicular development, maturation, and ovulation in chickens are mainly regulated by the hypothalamus–pituitary–gonad axis (Akazome et al., 2002). Gonadotropins, for example, FSH and LH, play a particularly important role in the course of follicular development and ovulation. Furthermore, E_2 has a feedback effect on the hypothalamus and pituitary to promote follicular development (Tarumi et al., 2014). Studies have shown that heat stress can increase serum hormone COR levels in laying hens while reducing FSH, LH, and E_2 levels (Dobson and Smith, 1995). In this study, adding 150 mg/kg curcumin to the diet can reduce serum COR concentration and increase FSH and LH concentrations in heat-stressed laying hens. The reduction in serum COR concentration in heat-stressed

Table 6. Effects of curcumin on hormone levels of laying hens.

Item	Control	100 mg/kg	150 mg/kg	200 mg/kg
COR (μg/L)	227.69 ± 21.15^{C}	186.96 ± 18.6^{B,b}	162.38 ± 16.83^{A,B,a}	140.76 ± 5.31^{A,a}
E_2 (μg/L)	359.09 ± 68.1^{A,a}	382.52 ± 38.04^{A,B,a,b}	423.64 ± 48.63^{A,B,b,c}	473.34 ± 46.85^{B,c}
FSH (μg/L)	3.56 ± 0.56^{A}	4.69 ± 0.77^{B}	3.74 ± 0.51^{A}	3.09 ± 0.2^{A}
LH	13.43 ± 2.37	16.57 ± 2.39	14.64 ± 1.22	13.33 ± 1.92

Abbreviations: COR, corticosterone; E2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

$^{a–c}$Means within a row with different superscripts differ significantly ($P < 0.05$).

$^{A–C}$Different capital superscripts within a row indicate a highly significant difference ($P < 0.01$).

Values are mean ± SE of 8 hens.

Table 7. Effects of curcumin on immune parameters in laying hens.

Item	Control	100 mg/kg	150 mg/kg	200 mg/kg
IgA (μg/mL)	157.15 ± 13.69^{A}	218.08 ± 23.38^{B,a}	243.46 ± 12.96^{B,C,b}	274.07 ± 34.46^{C,c}
IgG (μg/mL)	482.76 ± 47.3^{Aa}	548.96 ± 36.95^{A,B,b}	598.36 ± 42.14^{B}	594.77 ± 48.19^{B}
IgM (μg/mL)	3.61 ± 0.34^{A}	5.32 ± 0.26^{B}	6.06 ± 0.27^{C}	6.25 ± 0.83^{C}
C_3 (μg/mL)	1,119.69 ± 77.74^{A}	1,248.58 ± 61.53^{B}	1,374 ± 103.29^{C}	1,606.3 ± 75.93^{D}
C_4 (μg/mL)	602.2 ± 61.7^{A}	663.94 ± 57.3^{A}	686.12 ± 76.83^{A}	836.78 ± 67.8^{B}
IL-6 (ng/L)	22.22 ± 1.77^{B}	17.24 ± 1.83^{A}	16.46 ± 1.53^{A}	15.53 ± 1^{A}

$^{a–c}$Means within a row with different superscripts differ significantly ($P < 0.05$).
$^{A–D}$Different capital superscripts within a row indicate a highly significant difference ($P < 0.01$).
Values are mean ± SE of 8 hens.

laying hens by curcumin supplementation may be related to its effect on serum inflammatory response. The anti-inflammatory effects of curcumin are mediated through its ability to block nuclear factor-κB activation in response to various inflammatory stimuli (Singh and Aggarwal, 1995) and to suppress the expression of proinflammatory cytokines, including tumor necrosis factor-α, IL-1β, IL-6, and IL-8 (Abe et al., 1999; Jagetia and Aggarwal, 2007). Inflammatory factors such as IL-1, IL-6, and tumor necrosis factor-α have been reported to increase the secretory activity of the hypothalamic–pituitary–adrenal axis (Chikanza and Grossman, 1996), and this study found that curcumin can reduce serum IL-6 concentrations in heat-stressed laying hens. Therefore, the effect of curcumin on the decline in egg production rates under high-temperature conditions may be that it reduces the concentration of IL-6, thereby increasing the secretion of FSH and LH.

Effects of Curcumin on Immune Activity

The main Ig in poultry are IgA, IgG, and IgM, and the contents of the 3 Ig in serum can represent the overall level of serum Ig in laying hens. Studies have shown that heat stress reduces the level of Ig in the blood of animals (Abe et al., 1999), whereas curcumin has the effect of improving immunity. Furthermore, curcumin supplementation in a diet for rabbits (2, 4, and 6 g/kg) significantly increased serum levels of IgG and IgM, suggesting that curcumin can also improve immune function (Alagawany et al., 2016). The results of this study showed that different levels of curcumin in the diet could increase the level of Ig in the serum of laying hens to different degrees in summer. According to Zhu et al., 2014, curcumin has properties that can improve immunity and protein metabolism, as well as protective effects on cells, via enzymatic and nonenzymatic mechanisms (Zhu et al., 2014). Therefore, based on the increase in serum globulins, we suggest that curcumin exerts beneficial effects on the host immune responses, which could be associated with the presence of carotenoid compounds that can contribute to modulating the immune response by inducing lymphocyte proliferation and increasing antibody production (Chew and Park, 2004; Rajput et al., 2013).

This indicates that curcumin has a certain regulatory effect on the humoral immunity of laying hens in a high-temperature environment and can enhance the body's immunity by increasing serum immunoglobulin levels, which is consistent with the aforementioned studies on the effects of curcumin on animal immune function. These positive effects of using turmeric might be due to its anti-inflammatory, antioxidant, and antibacterial activity.

CONCLUSION

The present study demonstrated that dietary supplementation with curcumin in heat-stressed Hy-Line brown hens increased the activity of antioxidant enzymes and improved immune function. Hence, the improved immune function and antioxidative effect of enzymes have contributed to the improvement of laying performance and egg quality. The present experimental results indicated that curcumin can serve the important function of alleviating heat stress. Thus, curcumin could be used as a natural and safe probiotic for improving laying performance and egg quality in heat-stressed hens.

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