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Effect of dietary curcumin on the antioxidant status of laying hens under high- temperature condition

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ABSTRACT

Heat stress induces oxidative stress, and reduces antioxidant defenses of birds, which may affect poultry-production performance. Dietary antioxidants may protect against heat stress. We evaluated the effect of increasing concentrations of dietary curcumin on antioxidant parameters of hens under high-temperature conditions for nine weeks. Roman laying hens (n = 336, 22 weeks old, 1420 g weight) were divided into three treatment groups. The first group served as a thermo-neutral control (kept at 25 ± 1 °C). The second group was exposed to high temperatures (32 ± 1 °C, 6 h/day), and fed a basal diet. The third group was further divided into five groups, and all were exposed to high temperatures (32 ± 1 °C, 6 h/day) and provided a basal diet supplemented with 100, 150, 200, 250, 300 mg/kg curcumin (H1, H2, H3, H4, H5). All treatments included four replicates of 12 hens. Total superoxide dismutase (SOD) activity was significantly higher in H2 and H3 groups, and total antioxidant capacity (T-AOC) was higher in H2, H3, and H5 groups. Catalase (CAT) and glutathione peroxidase (GSH-Px) activities were significantly higher in the H3 group. Malondialdehyde concentrations were lower in curcumin supplemented hens compared to control groups hens. Hens in all curcumin treatment groups had slightly (but non-significantly) higher activities of CAT, SOD, GSH-Px, and T-AOC in liver, heart, and lung tissues, compared to heat stressed control group. It is concluded that dietary curcumin given to laying hens under heat stress may enhance their antioxidant status, and ameliorate stressful environmental conditions.

1. Introduction

In hot climatic regions of the world, heat stress (HS) causes oxidative stress in poultry birds (Lin et al., 2006; Habibi et al., 2014). Oxidative stress is associated with diseases and tissue damage, and may result in a loss of production performance (Dhanalakshmi et al., 2007; Rahmani et al., 2017). Especially in the southern part of China, high temperatures disturb the process of thermoregulation due to the long duration of summer. Thermogenic mechanisms depend on increased flow of thyroid hormones and a fast metabolism in birds (Guo et al., 2007). However, a fast metabolism increases the need for oxygen in body tissues (Rahmani et al., 2017). Stressful conditions create an imbalance between oxygen demand and supply, thereby resulting in hypoxemia (Hassanzadeh et al., 2014). Hypoxia may increase the production of free radicles (Reis et al., 2013), which in turn increase the activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in damaged liver tissues (Arab et al., 2006; Rahmani et al., 2017).

Antioxidants protect cells from the effects of lipid peroxidation. Lipid peroxidation is an indicator of cellular injury due to the generation of free radicals (Dinkova-Kostova and Paul, 2008; Wu et al., 2016). Several synthetic antioxidants have been banned due to their liver-carcinogenicity properties (Rahmani et al., 2017). Several studies have reported that plant substances in animal nutrition provide the beneficial effects including the activation of immune responses and antioxidant action, stimulation of appetite and improvement of endogenous digestive enzyme secretion (Ledoux, 2009; Toghyani et al., 2011).

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Table 1
Diets fed to laying hens in this study.

Treatments	Abbreviations	Diet
Thermo-neutral control	TC	Basal diet
Heat control	HC	Basal diet
Treatment 1	H1	Basal diet +100 mg/kg curcumin
Treatment 2	H2	Basal diet +150 mg/kg curcumin
Treatment 3	H3	Basal diet +200 mg/kg curcumin
Treatment 4	H4	Basal diet +250 mg/kg curcumin
Treatment 5	H5	Basal diet +300 mg/kg curcumin

Table 2
Ingredients and chemical composition of the experimental basal diet.

Ingredients	Percent (%)	Nutrients (Analyzed composition, %)[a]	Content
Corn CP 8%	62.8	ME/(MJ/kg) [a]	11.42
Soybean meal CP 44%	20.0	CP, %	18.17
Wheat bran 12%	2.0	Ca, %	3.7
Fish meal CP 62%	4.5	TP, %	0.58
Limestone %	9.0	Met, %	0.41
$CaHPO_{4\%}$	1.0	Cys, %	0.29
NaCl %	0.2	Lys, %	0.94
Premix[a]	0.5		

Notes: 1 The premix provided, per kg of diet: Vitamin A 9000 IU; Vitamin D 2500 IU; Vitamin E 20 IU; Vitamin B 1212 μg; Vitamin K 2.4 mg; Mn100 mg; Zn 60 mg; Fe 25 mg, Cu 5 mg; Co 0.1 mg (Mn, Zn, Fe, Cu, Co were provided in the form of sulfates); Se ($N_2SeO_3.5H_2O$) 0.2 mg; I(KI) 0.5 mg.

[a] Calculated values were according to NRC (1994) values for feedstuffs.

Recently, curcumin, a yellow pigment of turmeric, has been considered as a potential natural antioxidant (Wang et al., 2015; Ramos et al., 2017). Turmeric (Curcuma longa L) belongs to the ginger family and is found in southern and southeastern Asia (Nouzarian et al., 2011).

Curcumin is a main ingredient of turmeric (Nouzarian et al., 2011; Wang et al., 2015; Arslan et al., 2017) and has a wide range of therapeutic and pharmacological characteristics including the property of antioxidant, free radical scavenging, inhibition of lipid peroxidation, anti-inflammatory, antimicrobial, antiviral, antiprotozoal, and anti-tumor characteristics, and in addition, turmeric may act as an immune booster (Cleary and McFeeters, 2006; Singh et al., 2010; Zhang et al., 2014; Wang et al., 2015; Pulido-Moran et al., 2016; Amalraj et al., 2017). The above mention biological properties of curcumin make it potential antioxidant feed additive in poultry nutrition. Curcumin supplementation restores the balance of antioxidants and free radicles in the poultry under harsh environmental conditions. Therefore, the aim of our research was to evaluate the effects of increasing levels of dietary curcumin on antioxidant status of laying hens under high temperature conditions which might reduce the harmful effect of heat stress in the poultry farming.

2. Materials and methods

2.1. Laying hens, housing, experimental design, and diets

A total of 336 Roman egg-laying hens were purchased from the Guangzhou poultry industry, China. The experiment was started when hens were 22 weeks old (1420 g weight), and terminated when hens were 31 weeks old (1940 g weight). Hens were kept in an environment-controlled room (with controlled temperature, humidity, and light conditions). Hens were divided into three groups. The first group consisted of a thermo-neutral control (TC) group. The second group consisted of a high temperature control (HC) group. The third group was further divided into five treatment sub-groups (H1–H5, Table 2), with four replicates of 12 hens each. The hens in the first group (thermo-neutral control group) were kept at comfort temperatures of 25 ± 1 °C and 45–55% relative humidity (RH), and fed with a basal diet (Table 1). The hens in the high temperature control and curcumin treatment groups (high temperature groups) were kept at temperatures of 32 ± 1 °C and 55–65% RH for nine weeks for 6 h/day, from 10:00 to 16:00. Dietary curcumin was obtained from the Agricultural Vegetable Limited Company in Xi'an, China. Curcumin is composed of 77% curcumin, 18% demethoxycurcumin, and 5% bisdemethoxycurcumin (Rahmani et al., 2017). The purity of the curcumin used in this study was 95%. Curcumin was first added to a small amount of basal diet and then thoroughly mixed with 100 kg feed. The chickens in H1, H2, H3, H4, H5 treatment groups were given a basal diet supplemented with curcumin at 100, 150, 200, 250, 200 mg/kg of feed, respectively, while birds in thermo-neutral and heat control (TC and HC) groups were fed a basal diet without curcumin supplementation. Chickens were provided with water and feed *ad libitum* throughout the experimental period.

2.2. Data collection

2.2.1. Blood sampling and determination of antioxidant enzyme activities

Blood samples were taken from the wing vein of three randomly selected overnight-fasting hens per replicate per group in the morning of weeks 6 and 9 of the experiment. Each sample was collected in two tubes (one with and one without EDTA as an anticoagulant). Blood samples were kept at room temperature for 45 min, and then serum was obtained by centrifugation at 700*g* for 10 min. Serum was stored in 2 mL plastic vials at −20 °C for further analysis. Liver, lung, and heart tissue samples were also collected for measurement of antioxidant enzyme activities, and stored at −20 °C. The tissue samples were prepared in PBS (phosphate buffered saline) buffer, and centrifuged at 2500 g for 10 min at 4 °C. The assays were conducted according to the procedure described by Wang et al., 2015. Serum catalase (CAT) activity was assessed by using the method described by Wang et al. (2015) (ELISA kit: Quanti-Chrom, BioAssay Systems, USA, Catalog No.ECAT-100). Superoxide dismutase (SOD) activity was measured using the xanthine and xanthine oxidase method (ELISA kit: Cayman Chemical Company, USA, Catalog No. 706002), which measures the inhibition of the nitro blue tetrazolium reduction reaction by using the extracts from the collected tissue sample (Sun et al., 1988). Serum glutathione peroxidase (Gpx) was measured by using H_2O_2 and a specific dye containing an electron donor that results in a pink color during the peroxide reaction (ELISA Kit: QuantiChrom, Bioassay Systems, USA, Catalog No. DPOD-100), following Kokkinakis and Brooks (1979). Serum total antioxidant capacity (TAC) was measured by using a Randox total antioxidant status kit (Randox Laboratories Ltd, Crumlin, UK). The serum concentrations of MDA (lipid peroxidation), were assessed by using theobarbituric acid reactive substances (TBARS) produced during oxidative stress (Ohkawa et al., 1979) (ELISA Kit: QuantiChromTM, Bioassay Systems, USA, Catalog No. DTBA-100), following to Ohkawa et al. (1979). The assays were conducted according to the manufacturers' protocols.

2.2.2. Statistical analysis

Statistical analysis was carried out using SPSS statistical software. Data from all the groups of laying hens were compared using one-way ANOVA followed by Duncan significant difference test (Steel et al., 1997). All data are expressed as means ± standard error (SEM). Results were considered statistically significant at $P < 0.05$.

3. Results and discussion

Various stress factors, such as superoxide (O2) and hydrogen peroxide (H2O2) can cause the generation of reactive oxygen species (ROS), and lead to oxidative stress (Zeng et al., 2014). Oxidative stress can be described as an imbalance between pro-oxidant and antioxidant metabolites (Daneshyar, 2012; Ismail et al., 2013). Elevated levels of ROS can overwhelm cellular homeostasis by initiating lipid peroxidation, oxidation of proteins, and inhibition of enzymes, which may

Table 3
Serum antioxidant metabolites of control hens (TC; no heat stress, no curcumin), heat stressed hens not supplemented with dietary curcumin (HC) and heat stressed hens supplemented with different concentrations of dietary curcumin (H1, H2, H3, H4, H5, for concentrations see Table 1) for nine weeks. Data are shown as mean ± SEM.

Parameters[a]	Time (Week)	TC	HC	H1	H2	H3	H4	H5
SOD (U/mL)	3	43.8 ± 2.83^{a}	40.60 ± 3.32^{b}	42.36 ± 3.00^{ab}	43.44 ± 2.81^{a}	41.12 ± 2.56^{ab}	40.70 ± 2.59^{b}	43.03 ± 2.55^{ab}
	6	43.2 ± 3.25^{a}	35.31 ± 5.76^{b}	39.01 ± 4.00^{ab}	41.84 ± 4.13^{ab}	41.78 ± 2.16^{ab}	38.06 ± 4.63^{ab}	38.40 ± 4.26^{ab}
	9	60.8 ± 4.95^{a}	51.11 ± 3.56^{b}	61.31 ± 5.38^{a}	63.91 ± 3.30^{a}	63.32 ± 5.07^{a}	58.31 ± 5.47^{ab}	57.47 ± 4.05^{ab}
CAT (U/mL)	3	7.03 ± 0.23^{ab}	5.30 ± 0.50^{b}	7.76 ± 0.29^{ab}	10.01 ± 0.56^{a}	8.79 ± 0.40^{a}	7.67 ± 0.67^{ab}	5.97 ± 0.34^{ab}
	6	7.57 ± 0.42^{b}	4.81 ± 0.66^{a}	6.93 ± 0.31^{b}	6.52 ± 0.27^{b}	6.76 ± 0.37^{b}	6.24 ± 0.39^{b}	6.21 ± 0.37^{b}
	9	10.1 ± 0.82^{ab}	6.30 ± 0.36^{c}	8.48 ± 0.71^{b}	9.96 ± 0.78^{ab}	12.43 ± 3.42^{a}	9.87 ± 0.73^{ab}	9.37 ± 0.95^{b}
T-AOC (U/mL)	3	10.5 ± 4.17^{a}	5.59 ± 2.50^{b}	6.16 ± 4.43^{b}	9.57 ± 2.49^{ab}	5.94 ± 2.74^{b}	6.25 ± 1.04^{b}	9.06 ± 1.78^{ab}
	6	4.89 ± 1.53	3.00 ± 1.23	4.77 ± 2.96	4.93 ± 1.66	4.32 ± 1.83	4.23 ± 2.31	4.23 ± 1.63
	9	4.54 ± 1.87	2.07 ± 1.43	4.36 ± 1.68	3.42 ± 0.82	3.55 ± 2.22	5.15 ± 2.87	4.08 ± 1.29
GSH-Px (U/mol)	3	262.2 ± 34.81^{ab}	233.29 ± 12.56^{c}	239.58 ± 19.11^{c}	240.92 ± 12.14^{c}	287.71 ± 33.26^{a}	275.52 ± 16.15^{a}	248.98 ± 18.45^{bc}
	6	324.2 ± 12.39^{a}	297.7 ± 88.11^{a}	313.7 ± 89.66^{a}	288.5 ± 76.83^{a}	551.0 ± 148.25^{b}	328.5 ± 48.96^{a}	288.5 ± 59.99^{a}
	9	321.22 ± 12.39^{ab}	277.66 ± 48.15^{a}	283.25 ± 87.23^{ab}	278.20 ± 46.43^{a}	451.50 ± 98.33^{b}	329.50 ± 78.66^{ab}	278.50 ± 59.99^{a}
MDA (nmol/mL)	3	15.6 ± 5.53^{b}	18.58 ± 4.24^{a}	17.38 ± 4.15^{ab}	17.75 ± 5.17^{ab}	17.42 ± 7.66^{ab}	16.84 ± 7.73^{ab}	16.38 ± 4.52^{ab}
	6	13.00 ± 1.33^{b}	15.77 ± 0.19^{a}	13.56 ± 1.83^{ab}	13.99 ± 0.58^{ab}	13.67 ± 1.15^{ab}	13.11 ± 1.35^{ab}	13.67 ± 1.15^{ab}
	9	14.0 ± 1.53^{b}	16.22 ± 1.07^{a}	15.00 ± 0.88^{ab}	15.67 ± 0.58^{ab}	15.78 ± 1.02^{ab}	14.83 ± 0.88^{ab}	15.67 ± 1.15^{ab}

Note: Numbers with different lowercase letters are significantly different from each other ($P < 0.05$). Numbers not followed by different lowercase letters are not significantly different from each other ($P > 0.05$).

[a] SOD = superoxide dismutase; CAT = catalase; T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

ultimately result in cell death (Maheshwari and Dubey, 2009; Srivastava and Dubey, 2011; Zeng et al., 2014). The antioxidant metabolites (SOD, CAT, T-AOC, and GSH-Px) play the role of the first line antioxidant defense in animal bodies (Daneshyar, 2012). Decreased or increased concentrations of antioxidant metabolites and free radicles (ROS) may have detrimental effects on body tissues, and result in the manifestation of diseases (Wang et al., 2015). MDA is one of main products of lipid peroxidation, and can be monitored by examining MDA concentrations in serum and tissue samples (Wang et al., 2015). Curcumin has excellent antioxidant and anti-inflammatory activities (Yarru et al., 2009; Nouzarian et al., 2011; Rahmani et al., 2017). Curcumin is the main antioxidant element of turmeric plants (Cousins et al., 2007; Wang et al., 2015). It has the specific ability to scavenge free radicles (including superoxide anions and hydroxyl radicals) and hinder lipid peroxidation (Yarru et al., 2009; Wang et al., 2015).

A study has reported that curcumin given to rats had inhibited lipid peroxidation in liver microsomes and erythrocyte membranes (Chattopadhyay et al., 2004; Wang et al., 2015). In our experiment, we have found the positive effects of Curcumin on serum and tissues samples of laying hens during 9 weeks of experimental periods. The findings of the present study demonstrate that the activity of T-AOC was significantly ($P < 0.05$) higher in serum samples of curcumin-treated heat stressed groups as compared to untreated heat stressed control groups during weeks 3, 6, and 9 of the experiment which was similar with (Daneshyar, 2012). The available data also indicated that CAT activity was significantly ($P < 0.05$) higher in serum samples of hens in the heat-stressed curcumin supplemented group at weeks 3, 6, and 9 of the experiment, when compared with the heat stress (HC) control groups which was in accordance with Wang et al., 2015. Furthermore, GSH-Px and SOD activity were also significantly ($P < 0.05$) higher in serum samples of curcumin treatment groups during weeks 3, 6, and 9 of the experiment as compared to the untreated high temperature (HC) control group. These results were in accordance with (Daneshyar, 2012). The concentrations of MDA were significantly ($P < 0.05$) lower in serum

Table 4
Assay of antioxidant metabolites in liver tissue of control hens (TC; no heat stress, no curcumin), heat stressed hens not supplemented with dietary curcumin (HC) and heat stressed hens supplemented with different concentrations of dietary curcumin (H1, H2, H3, H4, H5, for concentrations see Table 1) for nine weeks.

Parameters[a]	Time (Week)	TC	HC	H1	H2	H3	H4	H5
SOD (U/mL)	3	143.88 ± 22.83	140.60 ± 32.32	142.36 ± 32.00	143.44 ± 22.81	141.12 ± 22.56	140.70 ± 22.59	143.03 ± 23.55
	6	143.20 ± 33.25	135.31 ± 53.76	139.01 ± 43.00	141.84 ± 43.13	141.78 ± 23.16	138.06 ± 42.63	138.40 ± 42.26
	9	160.81 ± 42.95	151.11 ± 33.56	151.31 ± 25.38	153.91 ± 33.30	153.32 ± 53.07	158.31 ± 53.47	157.47 ± 42.05
CAT (U/mL)	3	19.32 ± 1.51^{a}	10.64 ± 5.74^{b}	13.66 ± 6.53^{ab}	14.75 ± 1.32^{ab}	16.74 ± 0.99^{ab}	19.02 ± 0.52^{a}	11.49 ± 0.87^{b}
	6	26.66 ± 0.17^{a}	15.41 ± 4.20^{bc}	20.18 ± 3.76^{b}	18.29 ± 3.38^{b}	18.88 ± 5.94^{b}	20.64 ± 2.40^{b}	14.21 ± 0.98^{c}
	9	21.61 ± 4.92	18.20 ± 5.36	21.48 ± 3.26	18.84 ± 4.85	18.26 ± 6.15	22.17 ± 5.77	20.30 ± 4.45
T-AOC (U/mL)	3	8.54 ± 1.17	7.01 ± 1.50	7.16 ± 1.43	7.57 ± 1.09	6.65 ± 1.74	7.25 ± 2.04	7.56 ± 1.23
	6	7.29 ± 1.53^{a}	4.25 ± 1.33^{b}	5.77 ± 2.56^{ab}	5.55 ± 1.36^{ab}	6.32 ± 1.83^{ab}	5.53 ± 1.31^{ab}	4.26 ± 1.63^{b}
	9	6.67 ± 1.7	5.58 ± 1.43	5.70 ± 0.68	6.02 ± 0.2	7.55 ± 3.2	5.85 ± 2.87	5.08 ± 1.9
GSH-Px (U/mol)	3	162.29 ± 34.81	133.29 ± 12.56	139.58 ± 19.11	140.92 ± 12.14	187.71 ± 43.26	175.52 ± 16.15	158.98 ± 18.45
	6	194.62 ± 12.39	197.75 ± 88.11	153.75 ± 49.66	188.50 ± 76.83	151.00 ± 48.25	228.50 ± 48.96	188.50 ± 59.99
	9	191.22 ± 12.39	177.66 ± 48.15^{a}	183.25 ± 67.23	178.20 ± 46.43	151.50 ± 58.33	229.50 ± 68.66^{a}	178.50 ± 59.99
MDA (nmol/mL)	3	3.67 ± 0.53^{b}	4.78 ± 0.24^{a}	3.98 ± 0.05^{ab}	3.75 ± 5.17^{ab}	3.42 ± 7.66^{ab}	4.04 ± 1.73^{ab}	4.38 ± 0.42^{ab}
	6	4.00 ± 1.33^{b}	6.07 ± 1.19^{a}	4.90 ± 1.33^{ab}	5.49 ± 0.56^{ab}	4.67 ± 2.11^{ab}	7.11 ± 0.75^{a}	5.67 ± 1.45^{ab}
	9	6.55 ± 4.53^{b}	8.22 ± 2.07^{a}	8.01 ± 2.88^{a}	7.37 ± 1.78^{ab}	7.78 ± 1.08^{ab}	6.83 ± 0.88^{ab}	9.67 ± 1.45^{a}

Note: Numbers with different lowercase letters are significantly different from each other ($P < 0.05$). Numbers not followed by different lowercase letters are not significantly different from each other ($P > 0.05$).

[a] SOD = superoxide dismutase; CAT = catalase; T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

Table 5
Assay of antioxidant metabolites in heart tissues of control hens (TC; no heat stress, no curcumin), heat stressed hens not supplemented with dietary curcumin (HC) and heat stressed hens supplemented with different concentrations of dietary curcumin (H1, H2, H3, H4, H5, for concentrations see Table 1) for nine weeks.

Parameters[a]	Times (Week)	TC	HC	H	H2	H3	H4	H5
SOD	3	243.88 ± 2.83	240.60 ± 3.32	242.36 ± 3.00	243.44 ± 2.81	241.12 ± 2.56	240.70 ± 2.59	243.03 ± 2.55
/(U/mL)	6	243.20 ± 3.25	235.31 ± 5.76^{b}	239.01 ± 4.00	241.84 ± 4.13	241.78 ± 2.16	238.06 ± 4.63	238.40 ± 4.26
	9	260.81 ± 4.95	251.11 ± 3.56	261.31 ± 5.38	263.91 ± 3.30	263.32 ± 5.07	258.31 ± 5.47	257.47 ± 4.05
CAT	3	13.03 ± 0.23^{a}	4.00 ± 1.04^{bc}	2.76 ± 0.29^{c}	6.01 ± 0.56^{bc}	13.79 ± 0.40^{a}	5.67 ± 0.67^{bc}	7.97 ± 0.34^{b}
/(U/mL)	6	11.57 ± 0.2	9.81 ± 0.6 6	12.93 ± 0.1	10.52 ± 0.7	10.76 ± 0.37	11.24 ± 0.39	12.21 ± 0.7
	9	9.99 ± 0.82 ab	6.30 ± 0.36 b	8.88 ± 0.71 ab	9.96 ± 0.78 ab	10.43 ± 3.42 ab	8.87 ± 0.73 ab	12.37 ± 0.95a
T-AOC/(U/mL)	3	4.54 ± 4.1^{b}	3.59 ± 2.5^{b}	4.16 ± 4.4^{b}	5.57 ± 2.4^{b}	8.94 ± 2.4^{a}	9.25 ± 1.4^{a}	8.06 ± 1.7^{a}
	6	12.89 ± 1.53^{a}	5.00 ± 1.23 b	12.77 ± 2.96^{a}	12.93 ± 1.66^{a}	12.32 ± 1.83^{a}	9.23 ± 2.31^{ab}	9.23 ± 1.63^{ab}
	9	3.54 ± 1.87	3.07 ± 1.4 3	3.36 ± 1.68	3.42 ± 0.82	3.55 ± 2.22	3.15 ± 2.87	3.08 ± 1.29
GSH-Px	3	262.29 ± 34.81^{ab}	233.29 ± 12.56^{c}	239.58 ± 19.11^{c}	240.92 ± 12.14^{c}	287.71 ± 33.26^{a}	275.52 ± 16.15^{a}	248.98 ± 18.45^{bc}
/(U/mol)	6	324.62 ± 12.39^{a}	297.75 ± 88.11^{a}	313.75 ± 89.66^{a}	288.50 ± 76.83^{a}	551.00 ± 148.25^{b}	328.50 ± 48.96^{a}	288.50 ± 59.99^{a}
	9	321.22 ± 12.39^{ab}	277.66 ± 48.15^{a}	283.25 ± 87.23^{ab}	278.20 ± 46.43^{a}	451.50 ± 98.33^{b}	329.50 ± 78.66^{ab}	278.50 ± 59.99^{a}
MDA	3	5.67 ± 5.53^{b}	10.58 ± 4.24^{a}	7.38 ± 4.15^{ab}	8.75 ± 5.17^{ab}	8.42 ± 7.66^{ab}	6.84 ± 7.73^{ab}	9.38 ± 4.52^{a}
/(nmol/mL)	6	5.00 ± 1.3	5.77 ± 0.19	5.56 ± 1.3	5.99 ± 0.8	5.67 ± 1.5	5.11 ± 1.5	5.67 ± 1.5
	9	9.08 ± 1.53	10.22 ± 1.07	8.00 ± 0.88	8.67 ± 0.58	10.78 ± 1.02	14.83 ± 0.88	10.67 ± 1.15

Note: Numbers with different lowercase letters are significantly different from each other ($P < 0.05$). Numbers not followed by different lowercase letters are not significantly different from each other ($P > 0.05$).
[a] SOD = superoxide dismutase; CAT = catalase; T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

Table 6
Assay of antioxidant metabolites in lung tissues of control hens (TC; no heat stress, no curcumin), heat stressed hens not supplemented with dietary curcumin (HC) and heat stressed hens supplemented with different concentrations of dietary curcumin (H1, H2, H3, H4, H5, for concentrations see Table 1) for nine weeks.

Parameters[a]	Time (Week)	TC	HC	H1	H2	H3	H4	H5
SOD (U/mL)	3	86.24 ± 2.32^{a}	80.20 ± 3.32^{b}	84.36 ± 3.22^{ab}	86.44 ± 2.22^{a}	82.12 ± 2.23^{ab}	80.70 ± 2.32^{b}	85.03 ± 2.55^{ab}
	6	88.20 ± 3.25^{a}	70.31 ± 5.76^{b}	78.01 ± 4.00^{ab}	82.84 ± 4.13^{ab}	82.78 ± 2.16^{ab}	76.06 ± 4.63^{ab}	76.40 ± 4.26^{ab}
	9	100.81 ± 4.95^{a}	81.11 ± 3.56^{b}	91.31 ± 5.38^{a}	93.91 ± 3.30^{a}	93.32 ± 5.07^{a}	88.31 ± 5.47^{ab}	87.47 ± 4.05^{ab}
CAT (U/mL)	3	18.03 ± 0.23^{a}	8.01 ± 1.4 c	7.74 ± 1.20^{c}	16.01 ± 0.56^{ab}	12.79 ± 0.40^{bc}	16.67 ± 0.67^{ab}	16.97 ± 0.34^{ab}
	6	21.57 ± 0.42^{a}	4.85 ± 2.6 c	8.83 ± 3.31^{c}	22.52 ± 0.27^{a}	13.76 ± 0.37^{b}	19.24 ± 0.39^{a}	20.21 ± 0.37^{a}
	9	19.19 ± 0.82^{a}	8.30 ± 0.36^{b}	16.48 ± 0.71^{ab}	29.96 ± 0.78^{a}	22.43 ± 3.42^{a}	23.87 ± 0.73^{a}	16.37 ± 0.95^{ab}
T-AOC (U/mL)	3	2.54 ± 1.17	1.59 ± 0.50	1.16 ± 0.43	2.57 ± 2.49	2.94 ± 0.74	1.25 ± 1.04	1.06 ± 0.7 8
	6	1.89 ± 1.53	1.00 ± 1.23	1.77 ± 2.96	1.93 ± 1.66	1.32 ± 1.83	1.23 ± 2.31	1.23 ± 1.6 3
	9	6.54 ± 1.87^{ab}	4.07 ± 1.43^{b}	4.36 ± 1.68^{b}	5.42 ± 0.82^{b}	8.55 ± 2.22^{a}	9.15 ± 2.87^{a}	8.45 ± 1.2 9^{a}
GSH-Px (U/mol)	3	242.29 ± 34.81	233.29 ± 12.56	239.58 ± 19.11	241.92 ± 12.14	247.71 ± 33.26	245.11 ± 16.15	248.98 ± 18.45
	6	304.62 ± 12.39	297.75 ± 88.11	313.75 ± 89.66	208.50 ± 76.83	211.00 ± 148.25	218.50 ± 48.96	208.50 ± 59.99
	9	321.22 ± 12.39	297.16 ± 48.15	303.15 ± 87.13	308.20 ± 46.13	311.50 ± 92.33	309.50 ± 38.66	298.50 ± 49.99
MDA (nmol/mL)	3	6.67 ± 5.53	7.58 ± 4.24	7.38 ± 4.15	7.75 ± 5.17	6.42 ± 7.66	6.84 ± 7.73	6.38 ± 4.5 2
	6	5.00 ± 1.33	4.77 ± 0.19	4.56 ± 1.83	4.99 ± 0.58	4.67 ± 1.15	4.11 ± 1.35	4.67 ± 1.1 5
	9	5.08 ± 1.53	5.22 ± 1.07	5.00 ± 0.88	4.67 ± 0.58	4.78 ± 1.02	4.83 ± 0.88	5.67 ± 1.1 5

Note: Numbers with different lowercase letters are significantly different from each other ($P < 0.05$). Numbers not followed by different lowercase letters are not significantly different from each other ($P > 0.05$).
[a] SOD = superoxide dismutase; CAT = catalase; T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase; MDA = malondialdehyde.

samples of heat-treatment groups supplemented with curcumin during weeks 3, 6, and 9 of the experiment as compared to the high temperature control group. Similar findings were reported by Cousins et al. (2007) & Daneshyar (2012).

It has indicated that dietary curcumin counteracts the process of lipid peroxidation and reduces the generation of reactive free radicals (ROS) which in, turn, enhance the antioxidant metabolites in the poultry. While hens in all curcumin treatment groups (H1, H2, H3, H4, H5) had slightly higher activities of CAT, SOD, GSH-Px, and T-AOC in liver, heart, and lung tissues compared to the heat stressed control group, the difference was not significant ($P > 0.05$) (Wang et al., 2015). Serum and tissue antioxidant metabolites and MDA concentrations are presented in Tables 3–6, respectively. The lower concentration of MDA in hens in treatment group H3 (exposed to high temperatures and supplemented with 200 mg/kg curcumin) as compared to high temperature and comfort temperature control groups (not supplemented with curcumin), suggested that dietary curcumin has the capability to ameliorate the deleterious effects of heat-stress as an antioxidants (Wang et al., 2015). The significantly higher concentrations of MDA in heat-stress control (HC) hens observed in our study is in accordance with data from heat-stressed broiler chickens (Zhang et al., 2009; Ledoux, 2009; Habibi et al., 2014; Wang et al., 2015) and egg laying chickens (Akbarian et al., 2011). Significantly, the activities of CAT, SOD, T-AOC, and GSH-Px were higher in hens fed curcumin supplemented diets, and this can be interpreted as a protective mechanism against oxidative stress and lipid peroxidation (Wang et al., 2015). This might indicate the potential of dietary curcumin to initiate the biosynthesis of antioxidant enzymes, as well as to reduce heat-stress induced oxidative damage (Yarru et al.,

2009). Mechanism that describes how dietary curcumin can reduce the negative effects of heat stress might explain that stressful environmental conditions stimulate the secretion of corticosteroids, which can be counteracted by dietary supplementation of curcumin.

To summarize, the results of our current study suggested that laying hens that were fed dietary curcumin had better heat tolerance than the control groups, which is reflected by higher activities of CAT, SOD, T-AOC, and GSH-Px, as well as lower MDA concentrations in serum and tissue samples as compared to heat stress control group, which may help to protect cells from lipid peroxidation. Our results also indicate that curcumin can improve the antioxidant defense system of birds, and can be suitable as an alternative to synthetic antioxidants in poultry diets.

Ethics approval and consent to participate

According to rules and regulation of Guangdong Ocean University Animal Care and use Committee (Guangdong Province, China).

Declaration of competing interest

We all authors agree that there is no conflict of interest with any organization regarding the material discussed in the manuscript.

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