Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2019 / v.21 / n.2 / 001-010

http://dx.doi.org/10.1590/1806-9061-2018-0868

Original Article

Effect of Dietary Curcumin on the Antioxidant Status of Laying Hens under High-Temperature Conditions

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■Keywords

Antioxidant; curcumin; heat stress; laying hen; oxidative damage.

Submitted: 26/October/2018
Approved: 04/February/2019

ABSTRACT

Heat stress induces oxidative stress, and reduces body antioxidant metabolite levels, which can affect poultry production performance. Dietary antioxidants protect birds against the adverse effects of heat stress. The effects of increasing concentrations of dietary curcumin on the antioxidant parameters of layers maintained under high-temperature conditions for nine weeks were evaluated. Roman laying hens (n = 336, 22 weeks old, 1420 g BW) were divided into three treatment groups. The first group served as a thermoneutral control (kept at 25 ± 1 °C). The second group was exposed to high temperatures (32 ± 1 °C, 6 h/d), given a basal diet. The third group was further divided into five treatment groups (100, 150, 200, 250, 300 mg/kg Curcumin) fed a basal diet (treatments H1, H2, H3, H4, H5) under high temperatures conditions (32 ± 1 °C, 6 hours/day). As a result of this study, total superoxide dismutase activity was significantly higher in H2 and H3 groups, and total antioxidant capacity was higher in H2, H3, and H5 groups. Catalase and glutathione peroxidase activity was significantly higher in the H3 group. Malondialdehyde concentration was lowered in curcumin supplemented hens compared with control groups hens. Laying hens in all curcumin treatment groups had slightly higher activities of CAT, SOD, GSH-Px, and T-AOC in the liver, heart, and lungs, compared with heat stressed control group. It was concluded that dietary curcumin given to laying hens under heat stress may enhance their antioxidant status, and alleviate the detrimental effects of stressful environmental conditions.

INTRODUCTION

In hot climatic regions of the world, heat stress (HS) causes oxidative stress in poultry (Lin *et al.,* 2006; Habibi *et al.,* 2014). Oxidative stress causes tissue damage and impair disease resistance, resulting in performance losses (Dhanalakshmi *et al.,* 2007; Rahmani *et al.,* 2017; Nawab *et al.,* 2018). In southern China, in particular, high environmental temperatures disturbs poultry thermoregulation due to long duration of summer. Thermogenic mechanisms depend on fast metabolism in birds (Guo *et al.,* 2007). However, fast metabolism rates increase the oxygen demand in the 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 increases the production of free radicals (Reis *et al.,* 2013), which in turn increases the activities of circulating enzymes, disturbing normal body functions (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 generation of free radicals (Dinkova-Kostova & Talalay 2008; Wu *et al.,* 2016). Several

synthetic antioxidants have been banned due to their liver-carcinogenicity properties (Rahmani *et al.,* 2017). On the other hand, several studies have reported that plant substances included in animal feeds provide the beneficial effects, including antioxidant action, activation of immune responses, as well as stimulation of appetite and improvement of endogenous digestive enzyme secretion (Ledoux, 2009; Toghyani *et al.*, 2011; Nawab *et al.*, 2018). Recently, curcumin, a yellow pigment of turmeric, has been considered a potential natural antioxidant feed additives (Wang *et al.,* 2015; Ramos *et al.,* 2017). Turmeric (*Curcuma longa* L) belongs to ginger family and is found in the southern and southeastern Asia (Nouzarian *et al.,* 2011).

Curcumin is the main compound present in of turmericplant (Nouzarian *et al.,* 2011; Wang *et al.,* 2015; Arslan *et al.*, 2017), and has been shown to have a wide range of therapeutic and pharmacological properties, including antioxidant, anti-inflammatory, free-radical scavenging, lipid peroxidation inhibition, antimicrobial, antiviral, antiprotozoal, and antitumor activities. In addition, turmeric may act boost the immune response (Cleary & McFeeters, 2006; Singh *et al.,* 2010; Zhang *et al.,* 2014; Wang *et al.,* 2015; Pulido-Moran *et al.,* 2016; Amalraj *et al.,* 2017). Those curcumin biological properties make it a potential antioxidant feed additive for poultry. Therefore, the aim of our research was to evaluate the effects of increasing levels of dietary curcumin on antioxidant status of laying hens maintained under high environmental temperature conditions to determine if the harmful effects of heat stress could be alleviated.

MATERIALS AND METHODS

Birds, housing, experimental design, and diets

A total of 336 day-old Roman layers were purchased from Guangzhou poultry industry in Guangzhou, China. Birds were transported to the Department of Animal Sciences, Agricultural College, Guangdong Ocean University, where they were housed in battery cages (2 hens/cage), equipped with hopper feeder and nipple or cup drinker. Hens were provided with water and feed *ad libitum* throughout the experimental period.

Hens were evaluated from 22 (1420 g body weight) to 31 weeks of age (1940 g body weight). Hens were kept in an environmentally-controlled room (with controlled temperature, humidity, and light conditions until 22 weeks of age. In week 22, hens were divided into the following treatment groups: thermoneutral control group (TC; n=84), heat stress control group (HC; n=84) and heat stress treatment groups (HT; n= 84) named as H1, H2, H3, H4, H5. The thermoneutral environment was characterized by comfort temperatures of 25 ± 1 °C and 45-55% relative air humidity (RH). Heat stress was characterized by submitting birds to 32 ± 1 °C for 6 hours/day, between 10:00 and 16:00 h and 55-65% RH for nine weeks. Hens in the TC group were fed a basal diet formulated to supply layer nutritional requirements according to the NRC, (1994) (Table 2). The hens in the heat stress control group were fed the same basal diet as the TC hens, with no curcumin addition, and heat stress treatment group were given basal diet supplemented with curcumin at 100, 150, 200, 250, 300 mg/kg of feed, corresponding to treatments H1, H2, H3, H4, H5, respectively. The experimental treatments are described in Table 1.

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

Ingredients	Percent (%)	Nutrients (Analyzed composition, %)[1]	Content
Corn CP 8%	62.8	ME /(MJ/kg) [1]	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[1]	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 1212µg; 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 (N2SeO3.5H2O) 0.2 mg; I(KI) 0.5mg.

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

Dietary curcumin was obtained from Agricultural Vegetable Limited Company in Xi'an, China. Curcumin was composed of 77% curcumin, 18% dimethoxy curcumin, and 5% bisdemethoxy curcumin (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 at the required amounts to obtain the HS1, HS2, HS3, HS4, and HS5 diets.

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 + 100mg/kg curcumin
Treatment 2	H2	Basal diet + 150mg/kg curcumin
Treatment 3	H3	Basal diet + 200mg/kg curcumin
Treatment 4	H4	Basal diet + 250mg/kg curcumin
Treatment 5	H5	Basal diet + 300mg/kg curcumin

Blood sampling and determination of antioxidant enzyme activities

Blood samples were taken from the wing vein of three randomly-selected hens fasted overnight per replicate during the 3rd, 6th and 9th week 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 the 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 enzymatic activities, and stored at -20 °C. The tissue samples were prepared in PBS (phosphate buffered saline) buffer, and centrifuged at 2,500 g for 10 min at 4 °C. The assays were conducted according to the procedures described by Wang *et al.* (2015). Serum catalase (CAT) activity was assessed by the method described by Sippy *et al.* (2003) (ELISA kit: QuantiChrom, 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 nitroblue tetrazolium reduction reaction in extracts of the collected tissue samples(Sun *et al.* 1988). Serum glutathione peroxidase (Gpx) was measured 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 & Brooks (1979). Serum total antioxidant capacity (TAC) was measured by using a Randox total antioxidant status kit (Randox Laboratories Ltd, Crumlin, UK). Serum malondialdehyde (MDA) levels, as an indicator of lipid peroxidation, were determined by using thiobarbituric acid reactive substances (TBARS) produced during oxidative stress (Ohkawa *et al.* 1979) (ELISA Kit: QuantiChromTM, Bioassay Systems, USA, Catalog No. DTBA-100), according to Ohkawa *et al.* (1979). The assays were conducted according to the manufacturers' protocols.

Statistical analysis

Statistical analysis was carried out using (SPSS Statistical Software, 1968). Data were submitted to one-way analysis of variance, and means compared by Duncan' significant difference test (Steel *et al.,* 1997). All data are expressed as means ± standard error (SEM). Results were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Several stress factors cause the generation of reactive oxygen species (ROS), such as superoxide (O_2) and hydrogen peroxide (H_2O_2), which 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, that ultimately lead to cell death (figure 1)(Maheshwari & Dubey, 2009; Srivastava & Dubey, 2011; Zeng *et al.,* 2014). Antioxidant metabolites (SOD, CAT, T-AOC, and GSH-Px) play the role of defense mediators in animal bodies (Daneshyar, 2012). Decreased or increased concentrations of antioxidant metabolites and free radicles (ROS) have detrimental effects on body tissues, which in turn, result in the manifestation of diseases (Wang *et al.,* 2015). MDA is one of main products of lipid peroxidation, and can be monitored by determining MDA concentrations in serum and tissue samples (Wang *et al.,* 2015).

Curcumin was shown to present excellent antioxidant and anti-inflammatory activities (Yarru *et al.,* 2009; Nouzarian *et al.,* 2011; Rahmani *et al.,* 2017). Curcumin is a main antioxidant element of turmeric plants (Cousins *et al.,* 2007; Wang *et al.,* 2015). It has

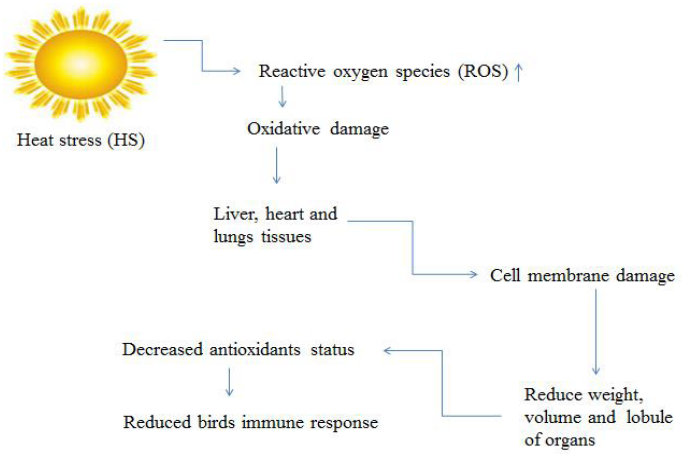

Figure 1 – Effect of heat stress on poultry health.

the specific ability to scavenge free radicals (including superoxide anions and hydroxyl radicals) and hinder lipid peroxidation (Yarru *et al.,* 2009; Wang *et al.,* 2015).

Studies have 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, positive effects of curcumin on the antioxidant and oxidant status of serum and tissues samples of laying hens submitted to heat stress for nine weeks were detected (Figure 2). Serum SOD activity was significantly (p<0.05) higher in hens maintained in TC compared with HS hens fed no curcumin in all evaluated weeks. SOD activity was significantly (p<0.05) increased in heat-stressed groups fed curcumin at 150 and 200 mg/kg (HT150 and HT200) in the 3rd and 9th week of the experiment, respectively, compared with the TC and HC group, which were fed only the basal diet. In the 6th week of the experiment, heat-stressed hens supplemented with all curcumin levels (HT100, HT150, HT200, HT250, HT300 mg/kg) had increased serum SOD activity, but results were not statistically significant (p>0.05). These results were in accordance with Daneshyar (2012).

Table 3 presents antioxidant metabolite activities determined in the serum of the experimental hens. Serum T-AOC activity was significantly (p<0.05) higher in curcumin-fed hens in a dose-dependent manner compared with the heat stressed control group (not supplemented with curcumin) during weeks 3, 6, and 9 of the experiment, as previously discussed by Daneshyar (2012). Serum T-AOC activity was significantly (p<0.05) enhanced in groups HT200, HT150, and HT250 during the 3rd, 6th, and 9th week of the experiment, respectively, compared with the HC group, fed only the basal diet. Serum CAT activity was significantly (p<0.05) increased in the serum samples of hens in heat-stressed curcumin supplemented groups HT150 and HT200 during week 3 and HT200 during week 9 of the experiment when compared with the heat stress (HC) control group, which is in accordance with Wang *et al.* (2015). On the other hand, in 6 week of the experiment, heat stress curcumin supplemented groups (HT100, HT150, HT200, HT250 and HT300 mg/kg) showed significantly (p<0.05) higher serum CAT activity compared with the HC group, given only the basal diet.

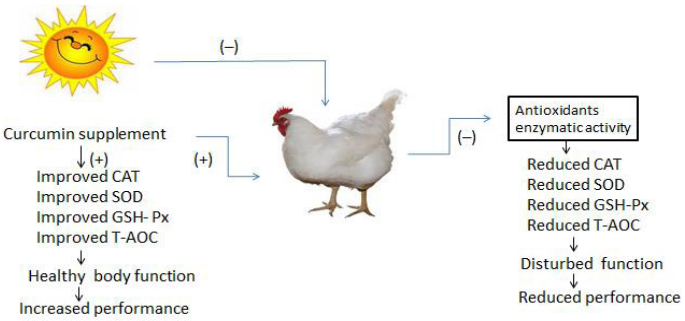

Figure 2 – Effect of dietary curcumin on poultry performance.

CAT; Catalase, SOD; Superoxide dismutase, GSH-Px; glutathione peroxidase, T-AOC; Total antioxidant capacity.

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[1]	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).

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

Furthermore, GSH-Px activity was also significantly (p<0.05) higher in the serum samples of curcumin-fed HT200 group in the 3rd and 6th weeks of the experiment, whereas in the 9th week, curcumin supplementation at 200 and 250 mg/kg (groups HT200 and HT250) increased GSH-Px activity compared with the non-supplemented HC and TC groups. In addition, despite not statistically different (p>0.05), serum MDA levels were lower in all heat-stress groups fed the diets supplemented with curcumin in weeks 3, 6, and 9 of the experiment compared with the heat-stressed group fed the basal diet. Similar findings were reported by Cousins *et al.* (2007) and Daneshyar (2012).

Wang *et al.* (2015) indicated that dietary curcumin counteracts the process of lipid peroxidation and reduces the production of reactive free radicals which, in response, increase the concentrations of antioxidant metabolites in the poultry body. In the present experiment, hens in all curcumin-supplemented groups (H1, H2, H3, H4, H5) had slightly higher activities of CAT, SOD, GSH-Px, and T-AOC in the liver, heart, and lung tissues compared with the-heat stressed control group (Wang *et al.,* 2015).

Table 4 shows the results of antioxidant metabolites determined in the liver of the experimental birds. SOD activity was significantly (p<0.05) increased in the 3rd week (treatmentsH2 and H5), 6th week (treatmentsH2 and H3), and 9th week (treatment H4) of the experiment compared with HC group fed only the basal diet. CAT activity was significantly (p<0.05) increased in liver in the 3rd week (treatment H4), 6th week (treatment H3 and H4), and 9th week (treatment H4) of the experiment compared with the HC group fed only the basal diet. T-AOC activity was also significantly (p<0.05) increased in the 9th week (treatment H3) of the experiment compared with the HC group fed only the basal diet; however, in the 3rd and 6th weeks of the experiment, the curcumin-supplemented diet fed to heat-stressed hens had no significant (p>0.05) effect on T-AOC activity. GSH-Px activity was also significantly (p<0.05) increased in the liver of heat-stressed hens fed curcumin-supplemented diets in the 3rd week (treatment H3), 6th week (treatment H4), and 9th week (treatment H4) of the experiment compared with the HC group fed only the basal diet.

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[1]	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±1.9	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).

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

Antioxidant metabolite activities assayed in the heart are shown in Table 5. SOD activity was significantly (p<0.05) increased in heat-stressed hens fed curcumin-supplemented diets in the 6th week (treatments H2 and H3) and 9th week (treatmentsH2 and H3) compared with the HC group fed only the basal diet, but no statistical differences (p>0.05) were detected in the 3rd week. CAT activity was significantly (p<0.05) increased in heat-stressed hens fed curcumin-supplemented diets in the 3rd week (treatment H3), 6th week (treatments H1 and H5), and 9th week (treatment H5) of the experiment compared with the HC group fed only the basal diet. Heart T-AOC activity was also significantly (p<0.05)

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[1]	Times (Week)	TC	HC	H1	H2	H3	H4	H5
SOD (U/mL)	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
	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 (U/mL)	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}
	6	11.57±0.2	9.81±0.66	12.93±0.1	10.52±0.7	10.76±0.37	11.24±0.39	12.21±0.7
	9	9.99±0.82ab	6.30±0.36b	8.88±0.71ab	9.96±0.78ab	10.43±3.42ab	8.87±0.73ab	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±1.43	3.36±1.68	3.42±0.82	3.55±2.22	3.15±2.87	3.08±1.29
GSH-Px (U/mol)	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}
	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 (nmol/mL)	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}
	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 ($p>0.05$).

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

increased in the 3rd week (treatments H3 and H4) and 6th week (treatments H1 and H2) of the experiment compared with the HC group fed only the basal diet, but in the 9th week, curcumin supplementation did not significantly ($p>0.05$) affect T-AOC activity. Moreover, heart GSH-Px activity significantly ($p<0.05$) increased in the 3rd week (treatments H3 and H4), 6th week (treatmentsH3 and H5), and 9th week (treatment H3) of the experiment compared with the HC group given only basal diet.

Table 6 presents the activities of antioxidant metabolites determined in the lungs of the experimental layers. Lung SOD activity was significantly ($p<0.05$) increased in the 3rd week (treatment H2) and 9th week (treatments H2 and H3) of the experiment in heat-stressed hens fed curcumin-supplemented

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[1]	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±0.78
	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±1.63
	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±1.29^{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±4.52
	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±1.15
	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±1.15

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 ($p>0.05$).

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

diets compared with the HC group fed only the basal diet, but no significant (p>0.05) differences were determined in the 6th week. CAT activity was also significantly (p<0.05) increased in the lungs of hens submitted to heat stress and fed curcumin in the 3rd week (treatmentsH2, H4 and H5), 6th week (treatments H2, H4 and H5), and 9th week (treatments H2, H3 and H4) of the experiment compared with the HC group fed only the basal diet. LungT-AOC activity of heat-stressed hens fed diets supplemented with curcumin was significantly (p<0.05) increased in the 3rd week (treatments H2 and H3) and 9th week (treatmentsH3, H4, and H5) of the experiment compared with the HC group fed only the basal diet, but was not significantly (p>0.05) different in the 6th week. Furthermore, lung GSH-Px activity was also significantly (p<0.05) increased in heat-stressed hens fed curcumin in the 3rd week (treatmentsH3 and H5), 6th week (treatment H1), and 9th week (treatment H3 and H4) of the experiment compared with the HC group given only the basal diet.

The concentration of MDA in the liver, heart, and lungs was decreased in all heat-stressed groups fed graded curcumin levels (H1, H2, H3, H4 and H5) compared with those submitted to heat stress or maintained at comfort temperature and fed only the basal diet (but not significantly), suggesting that dietary curcumin is capable of alleviating the deleterious effects of heat-stress, acting as an antioxidant (Wang *et al.,* 2015). The significantly higher concentrations of MDA in the heat-stress control (HC) hens observed in our study was in accordance with reports from studies with heat-stressed broiler chickens (Zhang *et al.,* 2009; Ledoux, 2009; Habibi *et al.,* 2014; Wang *et al.,*2015) and laying hens (Akbarian *et al.,* 2011). Interestingly, the significantly higher activities of CAT, SOD, T-AOC, and GSH-Px obtained in the heat-stressed hens fed diets supplemented with curcumin (200 and 250 mg/kg) suggest that curcumin may provide a protective mechanism against oxidative stress and lipid peroxidation (Wang *et al.,* 2015). This indicates 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). The mechanism that describes how dietary curcumin can reduce the negative effects of heat stress may explain that stressful environmental conditions stimulate the secretion of corticosteroids, which can be counteracted by dietary curcumin supplementation.

Taken together, the results of our study suggested that laying hens fed dietary curcumin at 200 and 250 mg/kg had better heat tolerance compared with the control groups, which is reflected by higher activities of CAT, SOD, T-AOC, and GSH-Px, as well as lower MDA concentrations in the serum and tissue samples compared with the heat stress control group, which may help to protect the cells and tissues from lipid peroxidation. The results of present study also indicated that curcumin improves the antioxidant metabolites of birds, and can be a suitable feed additive as an alternative to synthetic antioxidants in the poultry diets which may enhance the bird's immunity against stressful environmental conditions.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

Special thanks to Revista Brasileira de Ciência Avícola for giving an opportunity to share knowledge on worldwide heat stress issues and strategies to overcome this problem. The author wishes to thank beloved parents (Rana Nawab Ahmad and Mrs. R. Sultana), grandparents (Saith Bachal Din), uncle (Rana Maqbool Ahmad), brother (Rana Kashif Nawab and Dr. Rana Yasir Nawab) and dearest all teachers for continued support and excellent mentorship. This work was supported by Science and Technology Planning Project of Guangdong Province, China (2010 B090400376).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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

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