Dietary curcumin enhances intestinal antioxidant capacity in ducklings via altering gene expression of antioxidant and key detoxification enzymes

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ABSTRACT The study investigated the effects of dietary curcumin supplementation on tissue distribution of curcumin and its metabolites, intestinal antioxidant capacity, and expression of detoxification-related genes in ducks. A total of 720 one-day-old male Cherry Valley Pekin ducklings (initial BW 58.6 ± 0.1 g) were randomly assigned to 4 dietary groups each with 6 replicates of 30 ducks using a single factorial arrangement design. Ducks in the control group were fed a basal diet and the remainder were fed the basal diet supplemented with 200, 400, or 800 mg/kg curcumin. The experiment lasted for 21 D. Curcumin was present at 13.12 to 16.18 mg/g in the cecal digesta, 75.50 to 575.40 μg/g in jejunal mucosa, 35.10 to 73.65 μg/g in liver, and 7.02 to 7.88 μg/mL in plasma. The jejunal and hepatic contents of curcumin increased significantly ($P < 0.05$) in response to supplementation with 400 and 800 mg/kg of curcumin respectively, compared with 200 mg curcumin/kg group. There was a linear ($P < 0.001$) effect of dietary curcumin on relative abundance of *SOD1*, *GPX1*, *CAT*, *HO-1*, and *Nrf2* transcripts, and a quadratic ($P < 0.001$) increase in the activities of GSH-Px and T-AOC in jejunal mucosa. The expression of *CYP1A4*, *CYP2D17* increased and *CYP1B1*, *CYP2A6* decreased linearly ($P < 0.001$) with dietary curcumin concentrations. In addition, dietary curcumin increased gene expression of *GST*, *MRP6,* and *ABCB1* in jejunal mucosa. In conclusion, dietary supplementation with 200 to 800 mg/kg curcumin enhanced the accumulation of curcumin and its metabolites in jejunum as well as increasing the antioxidant capacity and detoxification potential, which play major roles in the protection of duck intestines against damage.

Key words: curcumin, ducks, tissue distribution, intestinal antioxidation, cytochrome P450

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INTRODUCTION

Reactive oxygen species (**ROS**) are inevitably formed due to metabolic processes, but their levels are increased by the various stressful conditions that exist on poultry farms and oxidative damage may occur when their levels exceed the capacity of the antioxidant defense system to eliminate them (Fellenberg and Speisky, 2006; Estévez, 2015). Among the various organs in poultry, the intestine is the major site of nutrient absorption and it is the first organ exposed to oxidative damage (Gu et al., 2012; Tang et al., 2018). In poultry, previous studies indicate that the antioxidant status of the intestines reflects the capacity to utilize nutrients and the productivity (Ma et al., 2014; Liao et al., 2018). Thus, during the first phase of avian species, adding natural antioxidants is recommended to strengthen the antioxidant status of the intestine, which provides protection and support of rapid growth (Bai et al., 2018; Zhang et al., 2018).

Curcumin, one of the attractive coloring materials and spice components widely used in Indian and Southeast Asian food, is a polyphenolic carotenoid isolated from the rhizome of turmeric (*Curcuma longa*) that has antioxidant, anti-inflammatory, antimicrobial, antiviral, and antifungal properties (Hussain et al., 2017). It has been recommended as a natural dietary supplement for poultry products (Rajput et al., 2013b). Diets supplemented with curcumin increased growth performance through improving intestinal structure and feed efficiency in chickens (Rajput et al., 2013a). Curcumin

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increased antibody production and alleviated the negative effects of heat stress, avian influenza, coccidia, and mycotoxins in poultry (Zhang et al., 2015; Umar et al., 2016; Galli et al., 2018; Ruan et al., 2019; Wang et al., 2018).

Curcumin is a lipophilic natural antioxidant that has a variety of pharmacological activities, closely related to its excellent antioxidant properties. Previous studies indicated that curcumin is a potent inducer of heme oxygenase-1 (**HO-1**) through the transcription factor (nuclear factor erythroid 2-related factor 2, **Nrf2**) binding to the antioxidant-responsive element (**ARE**) and initiating the transcription of genes encoding detoxifying enzymes and cytoprotective proteins (Balogun et al., 2003). Phase I and phase II detoxifying enzymes are mainly found in the liver and intestines, and are important for resisting xenobiotics and eliminating endogenous substances through redox or hydrolysis reactions (Xu et al., 2005). Cytochrome P450 (**CYP**) enzymes metabolize various exogenous and endogenous compounds, which is reported to be a potent inhibitor of CYP1A1, CYP1A2, and CYP2B1 activities in rat liver microsomes (Thapliyal and Maru, 2001). Shamsi et al. (2017) revealed that curcumin is a strong inhibitor of human CYP2C9 and CYP3A4. Zhang et al. (2018) demonstrated that dietary curcumin supplementation significantly ameliorated the heat-stressed broilers' overproduction of ROS through activating hepatic Nrf2-mediated phase II antioxidant enzyme systems. Despite the previous studies indicating the likely importance of curcumin for poultry nutrition, the impact on intestinal antioxidant status and expression of detoxification-related genes are unknown. Similarly, the deposition of curcumin and its metabolites in different tissues and its effect on ducklings' performance have not yet been investigated. The main objectives of this study, therefore, were to determine the effects of added curcumin on tissue distribution, performance, intestinal antioxidant capacity, and expression of detoxification-related genes in young ducks.

MATERIALS AND METHODS

Birds, Diets, and Management

The study was approved by the Animal Care and Use Committee of the Institute of Animal Science, Guangdong Academy of Agriculture Sciences and performed in accordance with animal welfare and ethics. A total of 720 male 1-day-old Cherry Valley Pekin ducklings from Guiliu Poultry Co., Ltd (Foshan, China) with initial BW of 58.6 ± 0.1 g were randomly allotted to 4 treatments each with 6 replicates of 30 birds per pen. Ducks were reared on plastic wire-floor pens (length 300 cm × width 200 cm), and they were studied for 21 D. The birds were provided with ad libitum access to water and pelleted diets. The light regime was 23 L: 1 D. The temperature of the room was maintained at 32 to 34°C for the first 3 D and then reduced by 2 to 3°C per week to a final temperature of 26°C. The basal diet was a corn-soybean meal-based diet without antibiotics (Table 1), formulated to fulfill the nutritional requirements of starter ducks according to the recommendations of the National Research Council (NRC, 1994). The natural curcumin (purity ≥ 98%, Nanjing Nutri-herb Biotech Co., Ltd, Nanjing, China) was added to the basal diet at the expense of carrier to produce experimental diets containing 0, 200, 400, and 800 mg/kg. The curcumin concentration was analyzed by high-performance liquid chromatography (**HPLC**) according to Pan et al. (1999). The analyzed levels of dietary curcumin were 0, 196, 413, and 822 mg/kg of diet.

Table 1. Formulated and analyzed nutrient composition of the basal diet.

Ingredient	%, as fed basis
Corn	63.80
Soybean meal	32.00
L-Lysine·HCl	0.10
DL-Methionine	0.14
Limestone	1.06
Calcium hydrogen phosphate	1.60
Sodium chloride	0.30
Premix[1]	1.00
Total	100.00
Formulated nutrient composition	
Metabolizable energy, kcal/kg	2819.00
Dry matter, %	88.57
Crude protein, %[2]	20.05
Ether extract, %[2]	2.96
Ca, %	0.90
Phytate P, %	0.66
Available P, %	0.42
Total Lys, %	1.10
Total Met, %	0.45
Total Met+Cys, %	0.80
Total Thr, %	0.80
Total Trp, %	0.24

[1]The premix provided per kilogram diet: vitamin A 10,000 IU; vitamin D_3 3,000 IU; vitamin E 20 mg; vitamin K_3 2.0 mg; vitamin B_1 2.0 mg; vitamin B_2 8.0 mg; vitamin B_6 4.0 mg; vitamin B_{12} 0.02 mg; chloride choline 500 mg; nicotinic acid 50 mg; D-pantothenic acid 20 mg; folic acid 1.0 mg; biotin 0.2 mg; Fe 60 mg; Cu 10 mg; Mn 80 mg; Zn 60 mg; I 0.2 mg; Se 0.3 mg; Co 0.25 mg.
[2]Measured values. Other nutrient compositions are calculated values.

Tissue Sampling and Storage

At 21 D of age, after 12 h of feed deprivation, 12 ducks per treatment (2 per replicate) with BW close to the replicate average were selected. Individual blood samples were collected from the wing vein; plasma samples were separated by centrifugation of blood at 1,200 × *g* for 10 min at 4°C and stored at −20°C for analysis. The birds were then stunned, and slaughtered quickly after blood collection; the samples of liver and the midpoints of the jejunum were harvested and rinsed quickly with phosphate-buffered saline (**PBS**); jejunal mucosa was scraped from the midpoints of the jejunum; and cecal digesta was collected, snap-frozen in liquid nitrogen, and stored at −80°C.

Table 2. Characteristic fragment ions of curcumin and key metabolites and their optimized MS/MS conditions.

Analyte	Scan Q1	Scan Q3	Declustering potential (ev)	Collision energy (ev)
Curcumin	366.8	217.0	−56	−17
	366.8	148.9	−56	−24
DHC	368.8	149.1	−58	−24
	368.8	218.9	−58	−18
THC	370.9	234.8	−83	−24
	370.9	192.9	−83	−30

DHC, dihydrocurcumin; THC, tetrahydrocurcumin.

Growth Performance

Feed intake was recorded weekly on a per replicate basis. All birds were weighed on day 21. The average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**; feed: gain, g: g) were calculated from 1 to 21 D of age. Feed conversion was calculated and corrected for mortality.

Distribution of Curcumin and Curcumin Metabolites in Tissues

Curcumin and curcumin metabolites were extracted from plasma and tissues according to the guidelines, as previously described (Pan et al., 1999). The composition of extracts of tissues and plasma samples, and curcumin, dihydrocurcumin (**DHC**), and tetrahydrocurcumin (**THC**) standards were analyzed by HPLC performed on a Shimadzu HPLC system using the following reverse-phase conditions: Agilent XDB C18 column (1.8 μm, 4.6 × 50 mm); constant flow rate of 0.5 mL min; the gradients were mixtures of water (solvent A) and acetonitrile; 60 to 15% A over 0 to 8 min, 15% A for 8 to 9.5 min, and re-equilibration with 60% A for 3.5 min. The HPLC system was connected to a tandem triple quadrupole MS analyzer (API 4000, AB SCIEX, Foster City, CA).

The compounds were quantified in multiple reaction monitoring (**MRM**) mode using optimized MS/MS conditions, which are listed in Table 2. The quantitative ion pairs were 366.8/217.0 for curcumin, 368.8/149.1 for DHC, and 370.9/234.8 for THC. The MS/MS conditions were optimized using the 3 standards. The MS conditions were as follows: source, Turbo IonSpray; ion polarity, negative; IonSpray voltage, –4,500 V; source temperature, 550°C; gas, nitrogen; curtain gas, 25 psi; nebulizing gas (GS1), 55 psi; collision gas (GS2), 55 psi; scan type, MRM; Q1 resolution: unit; Q3 resolution: unit. The Analyst 1.5.2 software (AB SCIEX, Foster City, CA) was used to control the instrument and to acquire and process all of the MS data.

Assay of Intestinal Antioxidant Indices

Intestinal mucosa was homogenized in ice-cold PBS and then centrifuged at 10,000 × g at 4°C for 10 min, and the supernatant was stored at –80°C. The activities of total superoxide dismutase (**T-SOD**), glutathione peroxidase (**GSH-Px**), total antioxidant capacity (**T-AOC**), the contents of reduced glutathione (**GSH**), malondialdehyde (**MDA**), and 8-hydroxy-2′-deoxyguanosine (**8-OHdG**) were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The protein content of supernatants was determined using Coomassie Brilliant Blue G250 (Sigma, St. Louis, MO) with bovine serum albumin standards.

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction

Total RNA was isolated from the frozen jejunum mucosal samples using Trizol reagent (Invitrogen, Carlsbad, CA), and dissolved in RNase-free water. The concentration of extracted RNA was qualified by the NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and the integrity of RNA was verified by denaturing polyacrylamide gel electrophoresis. The cDNA was synthesized using a PrimeScript RT reagent kit with gDNA Eraser (Takara Biotechnology, Dalian, China), and was stored at –20°C. The cDNA was diluted with nuclease-free water (1: 9) directly before quantitative real-time polymerase chain reaction (**qPCR**) analysis. The primers (Table 3) were designed based on duck gene sequences using Premier 5.0 software and synthesized by Sangon Biotech (Shanghai, China).

The qPCRs were performed using an iTaq Universal SYBERGreen Supermix (Takara) in a CFX96 Real Time PCR Detection System (Bio-Rad, Hercules, CA). The protocol of qPCR was as follows: denaturation at 95°C for 3 min followed by 39 cycles at 95°C for 15 s, 30 s at the annealing temperature (T_A), and 72°C for 15 s. Specificity of PCR products was evaluated by the analysis of melting curves. All measurements were carried out in triplicate, each in a volume of 20 μL, and the average values were obtained. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative transcript abundance of intestinal genes (Livak and Schmittgen, 2001). The expression of β-actin was selected as an internal control to normalize the expression of targeted genes.

Table 3. GenBank accession number, sequences of forward and reverse primers used for real-time PCR and expected fragments sizes.

Transcripts	Accession number	Primer sequence (5′-3′)	Size (bp)
SOD1	XM_013097859.1	F: CCTGTGGTGTCATCGGAATA R: TTGAACGAGGAAGAGCAAGTA	116
GPX1	KU048803.1	F: CAGTACATCATCTGGTCGCC R: CCTGGATCTTGATGGTTTCG	127
CAT	KU048802.1	F: CTGTTGAGGAAGCAGGAAGG R: GAAAGACCAGGATGGGTAGTTG	101
HO-1	KU048806.1	F: CCCATGCCTACACTCGCTAT R: GCCTCCTCCAAGACTCGTTT	217
Nrf2	NM_001310777.1	F: GTTGAATCATCTGCCTGTGG R: TAAGCTAGGTGGTCGAGTGC	171
CYP1A4	XM_005023163.3	F: AGCACATCAGGGACATCACA R: TACATGAGGCACCAGGACAG	172
CYP1B1	XM_005029378.2	F: CGATATCTTTGGTGCAGGGC R: CGTAGATGAAGGCCTCCAGA	185
CYP2A6	KX687985.1	F: CTGCAGAGAATGGCATGAAG R: CCTGCAAGACTGCAAGGAA	294
CYP2C9	XM_005031519.3	F: GGTTTGTGTTGTTTGCCTGC R: ACTGGCCCATACTCTTTGCT	168
CYP2D17	XM_005012209.3	F: TCTTCACCACTCTCCTGCAG R: GCCTAGACCTGCTGCATTTC	195
CYP3A8	XM_021272860.1	F: GGCCAAACCCAGATGAGTTC R: GCTTGAGAGGGACCTGAGTT	207
GST	KU048805.1	F: TGCTGTGCTTTCTGGGTTTC R: AGGCCCGAAATACCAGGAAA	190
MRP6	XM_013098438.2	F: GCAGACACCCATTGGAAACT R: GCCTTTGGTGTAGCCACAAT	150
ABCB1	HG917092.1	F: TGGCCTGGTTTGATGATCCT R: GCCCAGCCAACATCTTCATT	243
β-actin	EF667345.1	F: GCTATGTCGCCCTGGATTT R: GGATGCCACAGGACTCCATAC	160

SOD1, superoxide dismutase 1; *GPX1*, glutathione peroxidase 1; *CAT*, catalase; *HO-1*, heme oxygenase 1; *Nrf2*, nuclear factor erythroid 2-like 2; *CYP1A4*, cytochrome P450 1A4-like; *CYP1B1*, cytochrome P450 1B1; *CYP2A6*, cytochrome P450 2A6; *CYP2C9*, cytochrome P450 2C9; *CYP2D17*, cytochrome P450 2D17; *CYP3A8*, cytochrome P450 3A8-like; *GST*, glutathione S-transferase; *MRP6*, multidrug resistance-associated protein 6; *ABCB1*, ATP-binding cassette subfamily B member 1.

Statistical Analysis

Replicate (n = 6) was taken as the experimental unit. Except where noted otherwise, 2 sampled birds per replicate were used. The effects of dietary curcumin were examined using 1-way analysis of variance (**ANOVA**) procedure of SAS software (SAS Institute, Gary, NC). When significant effects of diet were demonstrated, orthogonal polynomial contrasts were examined. Statistical significance was satisfied when $P < 0.05$.

RESULTS

Tissue Distribution of Curcumin and Curcumin Metabolites

The distributions of curcumin, DHC, and THC in duck tissues are shown in Figures 1–3. There were no differences ($P > 0.05$) among treatments in concentrations of curcumin, DHC, and THC in cecal contents or plasma. Compared with the 200 mg curcumin/kg group, however, using 400 and 800 mg curcumin/kg diet led to higher ($P < 0.05$) jejunal mucosa and liver curcumin concentrations, respectively (Figure 1). The DHC concentration in jejunum of 400 mg curcumin/kg group was also higher than 200 curcumin/kg group, whereas the 800 mg curcumin/kg group resulted in the highest DHC concentration in liver ($P < 0.05$) (Figure 2). Furthermore, the concentrations of THC in jejunal mucosa and liver increased in a dose-related manner when dietary supplementation with curcumin increased ($P < 0.05$) (Figure 3).

Growth Performance

The performance of the ducks is summarized in Table 4. Compared with controls, ducks fed 200, 400, or 800 mg/kg curcumin had no significant difference ($P > 0.05$) in 21-D BW, ADFI, ADG, or FCR.

Antioxidant Variables and Related Gene Expression in the Jejunal Mucosa

Table 5 shows that the jejunum mucosal activities of GSH-Px and T-AOC increased linearly ($P < 0.001$) and quadratically ($P < 0.001$) as dietary curcumin contents increased; jejunum mucosal content of MDA and 8-OHdG showed a linear decrease ($P < 0.05$). Dietary curcumin levels increased the jejunum mucosal expression of *SOD1*, *GPX1*, catalase (*CAT*), *HO-1,* and *Nrf2* in a linear ($P < 0.001$) manner (Table 6).

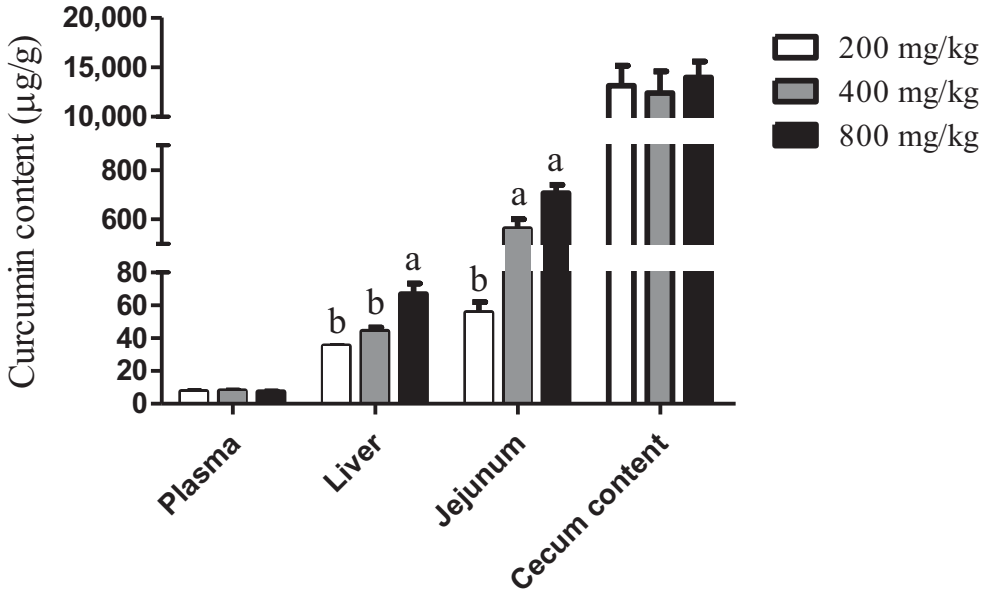

Figure 1. Effect of dietary curcumin on tissues distribution of ducks at 21 D of age. n = 6. Mean values within groups that do not share a common letter (a, b) are significantly different ($P < 0.05$).

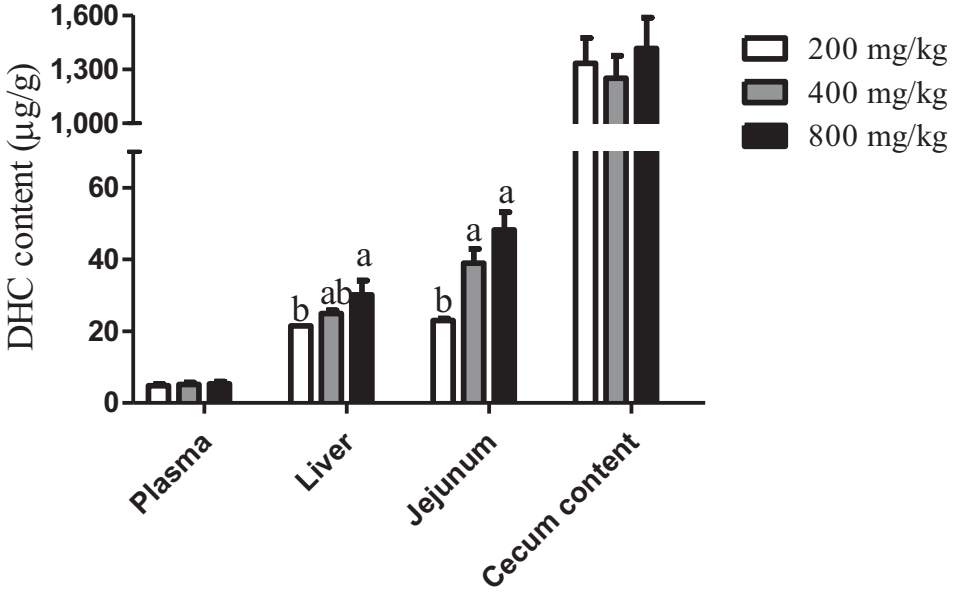

Figure 2. Effect of dietary curcumin on DHC (dihydrocurcumin) tissues distribution of ducks at 21 D of age. n = 6. Mean values within groups that do not share a common letter (a,b) are significantly different ($P < 0.05$).

Jejunum Mucosa Expression of Genes Related to CYP450 and Phase II Enzymes

As shown in Table 7, the relative abundance in jejunal mucosa of *CYP1A4* and *CYP2D17* transcripts increased and that of *CYP1B1* and *CYP2A6* transcripts decreased linearly to curcumin supplementation ($P < 0.001$). Increasing dietary curcumin linearly ($P < 0.001$) and quadratically ($P < 0.05$) increased the relative expression of *ABCB1* in jejunal mucosa, while that of *GST* and *MRP6* increased linearly ($P < 0.001$) with dietary curcumin levels. There were no significant effects of dietary curcumin on jejunum mucosal expression of *CYP2C9* or *CYP3A8* ($P > 0.05$).

DISCUSSION

Effect of Dietary Curcumin Supplementation on Growth Performance and Tissues Distribution of Curcumin and its Metabolites in Ducklings

Curcumin is a lipophilic polyphenolic component that has been used to alleviate or overcome several

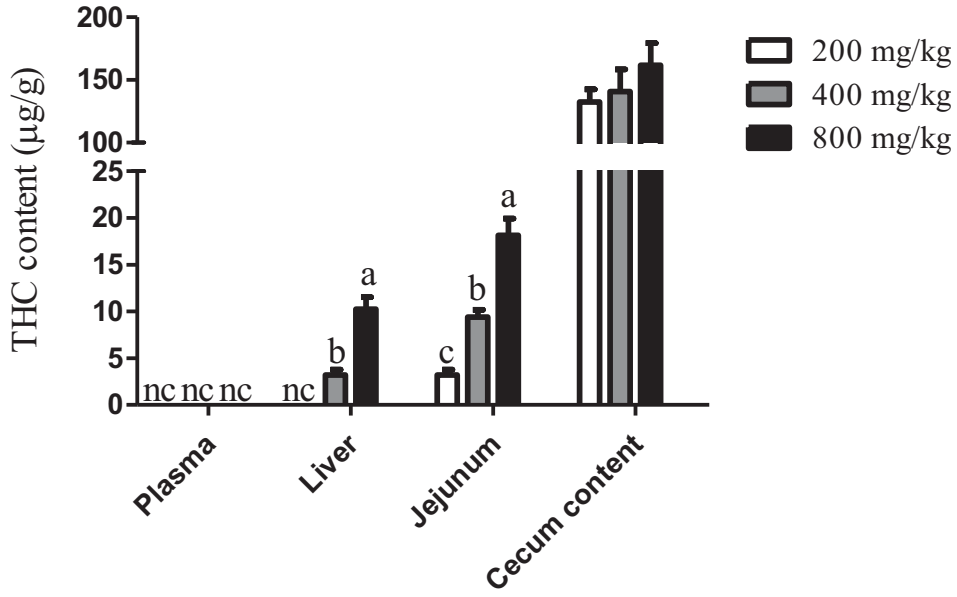

Figure 3. Effect of dietary curcumin on THC (tetrahydrocurcumin) tissues distribution of ducks at 21 D of age. n = 6. Mean values within groups that do not share a common letter (a–c) are significantly different ($P < 0.05$). nc = not detected.

problems in the poultry industry (Umar et al., 2016; Galli et al., 2018; Ruan et al., 2019; Wang et al., 2018; Zhang et al., 2018), but accumulation of curcumin and its metabolites in tissues, which might explain its role, was not previously known. Ravindranath and Chandrasekhara (1980) showed, after oral administration of 400 mg curcumin to rats, that about 60% of the dose was absorbed; only traces (<5 μg/mL) in blood and negligible quantities in liver and kidney (<20 μg/g) were observed from 15 min up to 24 h after administration. Another study (Pan et al., 1999) using mice showed distribution of curcumin in the intestines, spleen, liver, kidneys, and brain, which were 117.04, 26.06, 26.90, 7.51, and 0.41 μg/g, respectively, 1 h after oral administration (0.1 g/kg BW). Similarly, the distribution of curcumin in the intestinal mucosa was 1.4 mg/g, liver (3.67 μg/g), heart (807.6 ng/g), kidneys (206.8 ng/g), plasma (16.1 ng/mL), and urine (2.0 ng/mL) 2 h after oral administration of curcumin (0.34 g/kg BW) to mice (Marczylo et al., 2009). Dietary curcumin (2%) has shown to yield low (1 to 12 nM) curcumin levels in plasma, whereas tissue concentrations of curcumin in colonic mucosa and liver were 0.2 to1.8 μmol/g and 0.1 to 0.9 nmol/g, respectively (Sharma et al., 2001). In addition, the peak concentration of curcumin in plasma was 0.36 μg/mL after intravenous delivery of curcumin (0.01 g/kg) to rats (Yang et al., 2007). In the present experiment, after feeding ducks with 200 to 800 mg/kg curcumin for 21 D, the concentrations of curcumin ranked from highest in the cecal digesta to lowest in plasma. The concentrations of curcumin metabolites DHC and THC in jejunal mucosa and liver were much lower than those of curcumin but also increased in a dose-dependent fashion with increasing dietary curcumin. The above studies revealed that

Table 4. Growth performance of ducks fed curcumin from hatch to 21 D.[1]

Curcumin (mg/kg)	21 D BW (kg)	ADFI (g)	ADG (g)	FCR (feed: gain g: g)
0	1.268	94.47	59.89	1.58
200	1.398	93.99	61.23	1.54
400	1.409	93.03	61.27	1.52
800	1.388	94.42	60.73	1.56
SEM	0.020	0.802	0.868	0.006
P-values				
Curcumin	0.722	0.566	0.508	0.183

[1]Means are based on 30 birds per pen and 6 replicate pens per diet.
BW, body weight; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.

the concentrations in the intestine and liver were higher than kidney, heart, and plasma. The accumulation of nutrients with antioxidant properties in various body tissues can provide protection against oxidative damage, which adversely affects their functions (Xie et al., 2018).

In the present study, dietary supplementation of curcumin (200 to 800 mg/kg) did not affect growth performance. Similar results were obtained in Ross broiler chicks by Nouzarian et al. (2011), who found that inclusion of 3.3, 6.6, or 10 g/kg turmeric powder into diets did not affect ADFI, ADG, and FCR between 1 and 21 D of age. Rajput et al. (2013a) reported that 200 mg/kg curcumin in diets of broilers increased 42-D BW and improved FCR, but did not influence ADFI. Other studies (Gowda et al., 2009; Yarru et al., 2009; Rangsaz and Ahangaran, 2011) reported that supplementing basal diets with curcumin did not improve performance in broilers, but can significantly offset the reduced growth, otherwise induced by aflatoxin B1 (**AFB1**). The addition of 50 or 100 mg/kg curcumin to diets also improved

Table 5. Jejunal antioxidant capacity of ducks fed curcumin from hatch to 21 D.[1]

Curcumin (mg/kg)	GSH (mg/g prot)	T-SOD (U/mg prot)	GSH-Px (U/mg prot)	T-AOC (U/mg prot)	MDA (nmol/mg prot)	8-OHdG (nmol/mg prot)
0	3.69	140.5	45.18	0.59	0.42	10.5
200	4.51	163.4	54.11	0.63	0.35	9.2
400	5.07	166.7	65.25	0.87	0.23	8.1
800	5.47	178.8	57.96	0.72	0.21	7.3
SEM	0.22	2.15	1.548	0.078	0.040	0.180
P-values						
Curcumin	0.024	0.008	<0.001	0.035	0.016	0.028
Linear	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
Quadratic	0.217	0.199	<0.001	<0.001	0.084	0.183

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.
GSH, reduced glutathione; T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; T-AOC, total antioxidant capacity; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

Table 6. Relative expression of genes related to antioxidation in the jejunal mucosa of ducks fed curcumin from hatch to 21 D.[1]

Curcumin (mg/kg)	*SOD1*	*GPX1*	*CAT*	*HO-1*	*Nrf2*
0	1.042	1.016	1.019	1.023	1.022
200	1.259	1.456	1.465	1.449	1.072
400	1.822	2.010	2.172	1.896	1.745
800	2.859	2.209	2.776	2.096	3.010
SEM	0.210	0.199	0.262	0.196	0.205
P-values					
Curcumin	<0.001	0.003	<0.001	0.006	<0.001
Linear	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.391	0.737	0.759	0.656	0.089

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.
SOD1, superoxide dismutase 1; *GPX1*, glutathione peroxidase 1; *CAT*, catalase; *HO-1*, heme oxygenase 1; *Nrf2*, nuclear factor erythroid 2-like 2.

FCR in heat-stressed broilers at 22 to 42 D of age (Zhang et al., 2015). Previous research and our findings indicated that low or high concentrations of curcumin had no effects on growth by birds under experimental control conditions, and the differences between our results and those reported by Rajput et al. (2013a) could be explained by the bird species, experimental period, and/or the housing system employed.

Effect of Dietary Curcumin Supplementation on Antioxidation in Jejunal Mucosa of Ducklings

Numerous studies have revealed that curcumin and its metabolites showed a powerful capacity for protecting tissues from ROS injury (Ak and Gulcin, 2008; Barzegar and Moosavi-Movahedi, 2011). HO-1 is an essential enzyme involved in the conversion of heme into biliverdin and bilirubin, which are the 2 most potential endogenous antioxidants involved in the defense system against ROS (Chiu et al., 2002). The transcription factor Nrf2 is a key regulator in cellular antioxidant and detoxifying response against oxidants (Li and Kong, 2009; Bai et al., 2016). Previous studies have indicated that mice received dietary curcumin (0.2%) for 14 wk had obviously downregulated intestinal mucosal cyclooxygenase-2 expression and decreased levels of DNA adducts, reflecting direct antioxidant effects (Tunstall et al., 2005). Shen et al. (2006) identified curcumin-regulated Nrf2 dependence in mouse small intestine. Wang et al. (2012) concluded that curcumin could protect human intestinal epithelial cells against H_2O_2-induced disruption of tight junctions via the HO-1 pathway.

Very little is known about the effect of curcumin on the intestinal antioxidant status of ducklings. It was found here that the addition of curcumin to the diets linearly increased SOD, GSH-Px, and T-AOC activity and GSH content in jejunal mucosa while linearly decreasing MDA and 8-OHdG contents. These results were consistent with curcumin increasing jejunum mucosal expression of *SOD1*, *GPX1*, *CAT*, *HO-1*, and *Nrf2*. Similar findings have been made in fish (Jiang et al., 2016), where increasing dietary curcumin (0 to 5 g/kg) showed significantly lower MDA and protein carbonyl contents in the intestine; gene expression and activities of glutathione reductase, CAT, T-SOD, GSH-Px, and GST were increased. However, overdosing consumption of curcumin could promote hepatic oxidation through the generation of ROS and proinflammatory cytokines IL-6, and decreased levels of SOD and GST in rats (Qiu et al., 2016). The results obtained in the present study showed that supplementing duck diets with curcumin could enhance the activities of SOD, GSH-Px, and T-AOC through

Table 7. Relative expression of genes related to CYP450 and phase II detoxifying enzymes in the jejunal mucosa of ducks fed curcumin from hatch to 21 D.[1]

Curcumin (mg/kg)	*CYP1A4*	*CYP1B1*	*CYP2A6*	*CYP2C9*	*CYP2D17*	*CYP3A8*	*GST*	*MRP6*	*ABCB1*
0	1.010	1.024	1.020	1.016	1.008	1.035	1.020	1.011	1.010
200	1.458	0.898	1.144	1.135	1.752	1.493	1.565	1.070	0.967
400	2.354	0.731	0.701	1.100	1.619	1.342	2.147	1.622	1.351
800	2.896	0.696	0.484	1.099	2.322	1.303	3.153	1.832	2.665
SEM	0.106	0.080	0.091	0.210	0.145	0.200	0.106	0.072	0.113
P-values									
Curcumin	0.008	0.001	<0.001	0.968	<0.001	0.108	0.004	<0.001	<0.001
Linear	<0.001	<0.001	<0.001		<0.001		< 0.001	<0.001	<0.001
Quadratic	0.456	0.407	<0.001		0.982		0.768	0.217	<0.001

[1]Means are based on 2 birds per pen and 6 replicate pens per diet.

CYP1A4, cytochrome P450 1A4-like; *CYP1B1*, cytochrome P450 1B1; *CYP2A6*, cytochrome P450 2A6; *CYP2C9*, cytochrome P450 2C9; *CYP2D17*, cytochrome P450 2D17; *CYP3A8*, cytochrome P450 3A8-like; *GST*, glutathione S-transferase; *MRP6*, multidrug resistance-associated protein 6; *ABCB1*, ATP-binding cassette subfamily B member 1.

the antioxidant gene expression of *Nrf2*, *HO-1*, *SOD1*, and *GPX1* which plays a pronounced antioxidant role in the protection of duck intestines against damage.

Effect of Dietary Curcumin Supplementation on Gene Expression of CYP450 and Phase II Detoxifying Enzymes in Jejunal Mucosa of Ducklings

Phase I and phase II metabolisms are the 2 pathways for xenobiotic metabolism. Phase I metabolic enzymes are mainly of the CYP450 superfamily and contribute to the metabolism of a large variety of xenobiotics, including drugs, carcinogens, and herbs. The CYP450 enzymes catalyze reactions such as oxidation, reduction, hydroxylation, hydrolysis, etc. in order to increase the polarity of substrate molecules facilitating their excretion. In poultry, nutritional factors can regulate cytochrome and metabolism, and accumulation of substances in the tissues reflects its physiological functions (Yarru et al., 2009; Perez et al., 2016; Kulcsár et al., 2017); there is little information on the effects of curcumin on expression of cytochrome enzymes in birds. At present, the regulation by curcumin on CYP450 is mainly known for human and animal liver; it inhibited the activity of CYP3A in rats and the activities of CYP2D6, CYP1A2, CYP3A4, and CYP2C9 in humans (Appiah-Opong et al., 2007; Kim et al., 2015). Curcumin protected chicks from AFB1-induced liver damage by inhibiting the hepatic activities of CYP1A1, CYP1A2, CYP2A6, and CYP3A4 by preventing bioactivation of AFB1–8,9-exo-epoxide to AFBO. (Yarru et al., 2009; Zhang et al., 2016). Muhammad et al. (2017) found a dose-dependent inhibition by curcumin of hepatic CYP2A6 at the mRNA and protein levels in AFB1-treated broilers; maximal inhibition of liver CYP2A6 enzyme activity was achieved with 450 mg/kg curcumin. It was shown here that dietary curcumin reduced jejunum mucosal expression of *CYP1B1* and *CYP2A6* in ducks, while increasing that of *CYP1A4* and *CYP2D17*. Overall, curcumin selectively targeted expression of CYP450 isoenzymes which would be expected to protect against oxidative damage of xenobiotics.

The phase II metabolic enzyme system mainly includes GST, glutathione reductase, glucuronyl transferase, etc. The related groups of xenobiotics and their metabolites are conjugated with endogenous molecules to facilitate excretion. The ATP-dependent efflux transporters such as ATP-binding cassette subfamily B (ABCB) and multidrug resistance-associated protein (MRP) locate to apical and basal membranes of intestinal epithelial cells and affect the oral bioavailability of exogenous substances and intestinal efflux clearance (Takano et al., 2006). In particular, the expression levels of ABCB1, ABCB2, and ABCB3 in the gut play an important role in intestinal absorption and maintenance of barrier function (Leslie et al., 2005). Studies revealed that curcumin and its metabolite THC were effective multidrug resistance modulators. They impacted on the activities of ABCB1 and MRP1 protein in vivo, which play an important role in regulating the intestinal absorption of xenobiotic compounds (Chearwae et al., 2004). In the present experiment with ducklings, the addition of curcumin to diets significantly increased the transcript abundance of *MRP6* and *ABCB1* in the jejunal mucosa, which would be expected to favor elimination processes and to maintain the balance of intracellular substances.

CONCLUSIONS

Generally, according to our experimental results, dietary supplementation with curcumin enhanced the accumulation of curcumin and its metabolites as well as increasing the antioxidant capacity and detoxification potential, whereas lipid peroxidation decreased in the jejunal mucosa. The accumulation of nutrients with antioxidant properties in various body tissues could provide protection against oxidative damage. Therefore, further studies are required to test whether curcumin could protect the intestine against the oxidative damage induced by various stressful conditions.

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