Protective effects of curcumin on laying hens fed soybean meal with heat-induced protein oxidation

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ABSTRACT

This study was performed to investigate the effects of curcumin supplementation on intestinal barrier function, serum biochemical index, amino acid profile, and mRNA expression of nutrient transporters in laying hens fed soybean meal with heat-induced protein oxidation. A total of 288 40-week-old Hy-Line Brown laying hens were allocated into one of three treatments with eight replicates of twelve birds each for a 42-day feeding trial. The birds were fed a soybean meal-corn basal diet and a heated soybean meal (HSBM)-corn basal diet supplemented with or without 150 mg/kg curcumin. HSBM increased circulating diamine oxidase activity and D-lactate levels, and curcumin reduced D-lactate concentrations in birds fed HSBM (p < .05). Birds fed HSBM exhibited a decreased mRNA abundance of occludin and claudin 3 in the intestinal mucosa, and downregulation of claudin 3 expression was reversed by curcumin (p < .05). HSBM decreased total protein and albumin levels in the serum, both of these levels being increased with curcumin (p < .05). The administration of curcumin reversed HSBM-induced downregulation of amino acid concentrations in the serum, liver and yolk to control levels (p < .05). Moreover, HSBM decreased mRNA expression levels of amino acid transporters, glucose transporters, and peptide transporters in the intestinal mucosa, and curcumin almost completely reversed these effects, restoring expression levels of these genes to the control values (p < .05). The results of this study indicate that curcumin administration can alleviate the intestinal barrier injury and compromised nutrient absorption induced by HSBM in laying hens.

HIGHLIGHTS

• Protein oxidation of heated soybean meal (HSBM) impaired the intestinal barrier function of laying hens.
• Curcumin treatment attenuated the intestinal barrier injury induced by HSBM.
• Curcumin treatment improved the reduced nutrient absorption and transport of hens fed HSBM.

Introduction

Protein oxidation is defined as the covalent modification of protein induced by a reaction with reactive oxygen species and/or secondary byproducts of oxidatively modified chemical compounds under oxidative stress (Shacter 2000). Previous studies have identified that heat treatment can induce protein oxidation in vitro, resulting in adverse structural changes in proteins (Wu et al. 2009, 2011; Ye et al. 2013), and administration of oxidised protein can, in turn, generate excessive reactive oxygen species and disrupt the redox state in rodent models (Tang, Wu, Le, Shi 2012; Wu, Le, Wang, et al. 2012). Additionally, oxidation of proteins can result in accelerated apoptosis of intestinal epithelial cells and thus cause mucosal injury and inflammation (Xie et al. 2014; Ge et al. 2020). For broiler chickens, feeding heat-induced oxidised soy protein isolates can disrupt antioxidant defence (Zhang et al. 2017), impair digestive function (Chen et al. 2015; Zhang et al. 2016), and compromise the immune system in the gut (Wu et al. 2014). Protein oxidation can also induce amino acid degradation and oxidative protein aggregation, which can alter the physical and chemical protease recognition sites, thereby reducing the proteolysis susceptibility and digestibility of proteins (Chen et al. 2013). Soybean meal is one of the most important protein feeds for livestock, especially poultry. However, continuous
Curcumin, a natural flavonoid compound with multiple biological properties, is primarily extracted from the rhizome of Curcuma longa. It has been widely reported that curcumin possesses antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, antidiabetic, anticancer, hepatoprotective, and neuroprotective functions (Moghadamtousi et al. 2014; Dei Cas and Ghidoni 2018; Salehi et al. 2019). In previous studies, a protein oxidation model of soybean meal was established in vitro by applying heat treatment (Lu et al. 2017), and a subsequent in vivo experiment performed on broilers showed that heated soybean meal (HSBM) can impair growth performance, disrupt oxidative balance, and compromise digestive function (Lu et al. 2019).

Curcumin administration was observed to reduce the cell apoptosis rate, attenuate inflammation, and prevent the disruption of tight junction proteins, indicating the protective effects of curcumin on the intestinal barrier (Loganes et al. 2017; Wang et al. 2017). In weaning piglets, curcumin has been demonstrated to promote growth performance, improve intestinal integrity and alleviate inflammatory conditions (Gan et al. 2019; 2019). An in vivo study on mice with functional gastrointestinal disorders demonstrated that curcumin administration at a dose of 200 mg/kg can partially alleviate the delayed state of gastric emptying and intestinal propulsion rates, which may be related to the absorption of nutrients (Yu et al. 2017). In broilers, supplementation with curcumin or turmeric has been demonstrated to improve growth performance and carcass characteristics and enhance the antioxidant status of individuals (Attia et al. 2017; Zhang et al. 2019). For laying hens subjected to heat stress, curcumin supplementation at a dose of 150 mg/kg can improve productive performance, antioxidant capacity and immune function, as indicated by the decreased levels of antioxidant enzymes and immunoglobulins in the serum (Liu et al. 2020). However, few studies have investigated the protective effects of curcumin in laying hens fed HSBM, especially concerning the intestinal barrier and absorptive function. According to the biological characteristics of curcumin, we hypothesised that curcumin may be effective in counteracting the harmful impacts of HSBM in laying hens. Therefore, this study was conducted to investigate the effects of dietary curcumin supplementation on intestinal barrier function, serum biochemical parameters, amino acid profile, and expression of intestinal nutrient transporter genes in laying hens fed HSBM.

Materials and methods

Experimental design

This experiment was performed according to the animal experiment guidelines set by the Animal Care and Use Committee of Nanjing Agricultural University. A total of 288 40-week-old Hy-Line Brown laying hens purchased from a commercial farm were allocated into one of three treatments, with each treatment being composed of 8 replicates of 12 birds each. After a preliminary experiment over one week, the birds were fed a soybean meal-corn basal diet and an HSBM-corn basal diet supplemented or not supplemented with 150 mg/kg curcumin (the purity of curcumin was 98%; Cohoo Biotechnology R&D Centre, Guangzhou, P.R. China). HSBM was prepared as described previously (Wu et al. 2014; Lu et al. 2017, 2019). In brief, fresh soybean meal purchased from Yihai Grain and Oil Industry Co., Ltd. (Jiangsu, P.R. China) was heated at 100°C for 8 h for HSBM, and oxidised protein was successfully produced under this condition. The analysed levels of protein carbonyl and free sulfhydryl, two sensitive indices reflecting the degree of protein oxidation, were 7.76 nmol/mg protein and 8.42 nmol/mg protein in fresh soybean meal and 11.2 nmol/mg protein and 7.43 nmol/mg protein in HSBM, respectively. The dry matter in the fresh soybean meal and HSBM were calibrated to an equivalent level during the feed manufacturing process. The supplemental level of curcumin was selected according to the findings of a previous study (Zhang et al. 2019) and the official regulation set by Ministry of Agriculture and Rural Affairs of China (2019), which has stated that the recommended amount of curcumin is 50–150 mg/kg in the mixed feed of broilers, while the maximum limit is 150 mg/kg (based on the mixed feed with a dry matter content of 88%). The ingredient composition and nutrient level of the diets are presented in Table 1. The experiment lasted for six weeks. For each replicate, birds (twelve birds/replicate) were reared in four adjacent stainless-steel cages (40×40×35 cm, three birds per cage) with plastic floors, and mash feed and water were provided for ad libitum consumption. The bird house was electrically
flushed with chilled phosphate-buffered saline (pH 7.40). Next, the mucosa was collected carefully by a sterile glass microscope slide, rapidly frozen in liquid nitrogen, and stored at −80°C. The right lobe of the liver was excised, washed with phosphate-buffered saline, dried with filter paper, and then stored at −20°C for subsequent analysis. In addition, three eggs in each replicate were selected, and the mixed yolks were prepared and stored at −20°C until subsequent analysis.

Serum D-lactate level and diamine oxidase activity measurement

The serum D-lactate (D-LA) content and diamine oxidase (DAO) activity were quantified using commercial colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China) in strict accordance with the manufacturer’s protocols.

Serum biochemical parameters determination

The contents of glucose, total protein, albumin, and uric acid in the serum were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China) according to the methods described by the manufacturer.

Amino acid analysis

The determination of amino acid concentration was performed according to the method of Yin et al. (2009). After hydrolysis and dilution with hydrochloric acid and filtration through a semipermeable membrane, the amino acid contents in the samples (liver and yolk) were measured using an automatic amino acid analyser (Hitachi L-8080, Hitachi, Tokyo, Japan). The measurement of amino acids in the serum after treatment with sulfosalicylic acid was similar to the procedures described above.

RNA extraction and mRNA quantification

A sample of 50.0–60.0 mg of tissue (intestinal mucosa) was homogenised in 1.00 mL of TRIzol Reagent (TaKaRa Biotechnology) for the extraction of total RNA, the concentration of which was determined by measuring the optical density at OD260/280 readings using a NanoDrop ND-1000 UV spectrophotometer (NanoDrop Technologies). Next, RNA samples were diluted to a concentration of 0.500 g/L, and complementary DNA was synthesised from 1 μg of total RNA with a PrimeScript™ RT reagent kit (TaKaRa Biotechnology). The reaction was performed at 15 min at 37°C followed by 5 s at 85°C. The primer sequences of the target and reference genes (zonula occludens 1 (ZO1), occludin (OCLN), claudin 1 (CLDN1), claudin 2 (CLDN2), claudin 3 (CLDN3), B0 amino acid transporter (B0,AT), L-type amino acid transporter 1 (LAT1), alanine-serine-cysteine-threonine transporter 1 (ASCT1), excitatory amino acid transporter 3 (EAAT3), b0,+ amino acid transporter (b0,+AT), y+L amino acid transporter 2 (y+LAT2), cationic amino acid transporter 1 (CAT1), cationic amino acid transporter 2 (CAT2), oligopeptide transporter 1 (PepT1), glucose transporter 2 (GLUT2), sodium-dependent glucose transporter 1 (SGLT1), and β-actin) are presented in Table 2. Each of the complementary DNA products was diluted at a

### Table 1. Composition and nutrient level of the basal diet (g/kg, as fed basis unless otherwise stated).

| Items             | CON   | HSBM  |
|-------------------|-------|-------|
| **Ingredients**   |       |       |
| Maize             | 620   | 620   |
| Soybean meal      | 240   | —     |
| Heated soybean meal | —  | 240   |
| Soybean oil       | 10.0  | 10.0  |
| Limestone         | 80.0  | 80.0  |
| Premix*           | 50.0  | 50.0  |
| **Calculation nutrient levels** |       |       |
| Apparent metabolisable energy (MJ/kg) | 11.2  | 11.2  |
| Crude protein     | 160   | 160   |
| Calcium           | 35.8  | 35.8  |
| Total phosphorus  | 6.17  | 6.17  |
| Available phosphorus | 3.69  | 3.69  |
| Lysine            | 7.92  | 7.92  |
| Methionine        | 3.53  | 3.53  |
| Total sulphur amino acids | 6.33  | 6.33  |

*Premix provided per kilogram of diet: transretinyl acetate, 10,000 U; cholecalciferol, 3000 U; all-rac-α-tocopherol, 30.0 U; menadione, 1.00 mg; thiamine, 1.00 mg; riboflavin, 6.00 mg; nicotinamide, 40.0 mg; choline chloride, 350 mg; calcium pantothenate, 10.0 mg; pyridoxine HCl, 3.00 mg; biotin, 0.100 mg; folic acid, 0.300 mg; cobalamine, 0.010 mg; Cu (copper sulfate), 8.00 mg; Fe (ferrous sulfate), 80.0 mg; Mn (manganese sulfate), 100 mg; I (calcium iodate), 1.00 mg; Se (sodium selenite), 0.300 mg; calcium, 6.25 g; phosphorus, 3.00 g; methionine, 1.00 g.

and automatically controlled at 18–25°C and 40–60% humidity in a 16-h light: 8-h dark cycle.

Sample collection

At the end of the feeding experiment (42 days), one bird per replicate was randomly selected (eight birds per treatment, and twenty-four birds in total) for sampling. Blood samples were collected from a wing vein puncture and centrifuged at 4000 g for 15 min at 4°C to separate the serum for further analysis. After that step, the birds were euthanized by cervical dislocation and necropsied immediately. Approximately 20-cm sections of jejunal and ileal tracts were open longitudinally and resected immediately. Approximately 20-cm sections of jejunal and ileal tracts were open longitudinally and resected immediately.
ratio of 1.9 (wt/vol), and quantitative real-time PCR was subsequently performed on an ABI 7300 Real-Time PCR System (Applied Biosystems) using a SYBR® Premix Ex Taq™ Kit (TaKaRa Biotechnology). The PCR procedure consisted of a first step of 95 °C for 30 s and 40 cycles at 95 °C for 5 s followed by an annealing step at 60 °C for 30 s and a final melting stage of 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, and 60 °C for 15 s. The expression abundance of target genes was normalised to that of β-actin and subsequently calculated according to the 2^−ΔΔCT method (Livak and Schmittgen 2001).

**Statistical analysis**

Data were analysed by one-way analysis of variance (ANOVA) using SPSS statistical software (ver. 19.0 for Windows, SPSS Inc., Chicago, USA). The mathematical model was $X_{ij} = \mu + x_i + \epsilon_{ij}$, where $X_{ij}$ = observed value in each experimental unit, $\mu$ = overall mean, $x_i = effect of each treatment, and $\epsilon_{ij}$ = experimental random error. Every individual bird from each replicate for sampling was used as the experimental unit. Differences among the treatments were examined using Tukey’s multiple-range tests. $p$-values $< .05$ were considered to indicate significant differences. Data are presented as the means and their pooled standard errors.

**Results**

**Intestinal barrier function**

Compared with the control group (Table 3), serum DAO activity was increased from 8.51 U/L to 13.2 U/L by dietary HSBM administration ($p < .001$), but curcumin supplementation did not affect circulating DAO activity in laying hens fed HSBM ($p > .05$). Dietary supplementation with HSBM increased the serum D-LA concentration, the level of which was restored to a control value when curcumin was supplemented ($p < .001$).

As shown in Table 4, the mRNA expression abundance of OCLN in the jejunal mucosa ($p = .016$) and CLDN3 in both jejunal ($p = .007$) and ileal ($p = .025$) mucosa of laying hens fed HSBM was decreased with that in the control group. Curcumin supplementation increased the mRNA expression levels of CLDN3 in the jejunal mucosa ($p = .007$) and upregulated OCLN in the ileal mucosa ($p = .026$) in comparison with the HSBM group. However, curcumin treatment did not alter the mRNA abundance of ZO1, CLDN1 or CLDN2 in the intestinal mucosa of laying hens ($p > .05$).

| Table 2. Sequences for real-time PCR primers. |
|-----------------------------------------------|
| Items | Gene bank ID | Primer sequence, sense/antisense |
|-------|--------------|---------------------------------|
| ZO1   | XM_015278975.2 | TGTAAGACCAACGCAAAGGTGTCGAAAGTGT |
| 5'-3' |
| OCLN  | NM_205128.1 | CTGGAATGGCTCCCTCTGTTTGA |
| 5'-3' |
| CLDN1 | NM_001013611.2 | GGAGATCAGTGAAGGTGTTGTCAG |
| 5'-3' |
| CLDN2 | NM_001277622.1 | CCTGCACTACCTGTGGGCTCAG |
| 5'-3' |
| CLDN3 | NM_204202.1 | GCTGAACTCACTCTTGGGCTACG |
| 5'-3' |
| LAT1  | NM_001030579.2 | AGACCATAGTGGCACTGTCGAG |
| 5'-3' |
| ASCT1 | XM_001232899.5 | CACACTATGGGCGCATGCT |
| 5'-3' |
| EAAT3 | XM_419056.6 | GGCTTTGTTTGTGGTTGAGAA |
| 5'-3' |
| B0AT  | XM_015278975.2 | TGTAGCCACAGCAAGAGGTG |
| 5'-3' |
| LAT2  | XM_015292306.2 | CAHAGAGAAGAAGAAGAAGAAGAAG |
| 5'-3' |
| ASCT1 | NM_001199133.1 | ATGGAATGGCTAAGAAGGCTAAG |
| 5'-3' |
| EAAT3 | XM_424930.6 | TCGTCTGTTGAGTCCACTG |
| 5'-3' |
| B0AT  | XM_015292306.2 | AGCAATGACGGATGCAAGAAGTATAGT |
| 5'-3' |
| LAT2  | XM_015292306.2 | CAGTAGTAATCTGTGTTGAGA |
| 5'-3' |
| CAT2  | NM_001199133.1 | GCAAGGAAGAAGAAGAAGAAGAAGAAG |
| 5'-3' |
| PepT1 | NM_204365.1 | GCCGCGAGAGAAGAAGAAGAAGAAG |
| 5'-3' |
| GLUT2 | NM_207178.1 | CTCATCGTGCTAGTCCACT |
| 5'-3' |
| SGLT1 | NM_001293240.1 | GCCATCGAGGACGCAGGTA |
| 5'-3' |
| β-actin | NM_205151.8 | TGGTGCTGCTGCTATCAG |
| 5'-3' |

| Table 3. Effects of dietary curcumin supplementation on serum diamine oxidase activity and D-lactate concentration of laying hens fed heated soybean meal. |
|-----------------------------------------------|
| Items | CON (U/L) | HSBM (U/L) | HSBM + Cur (U/L) | SEM | p-Value |
|-------|--------|----------|----------------|-----|--------|
| DAO   | 8.51   | 13.2a    | 13.0a          | 0.604 | < .001 |
| D-LA  | 0.714b | 1.28a    | 0.882b         | 0.066 | < .001 |

CON: laying hens fed a soybean meal-com basal diet; HSBM: laying hens fed a heated soybean meal-com basal diet; HSBM + Cur: laying hens fed a heated soybean meal-com basal diet supplemented with 150 mg/kg curcumin; DAO: diamine oxidase; D-LA: D-lactate.

SEM: standard errors of mean.

Mean values within a row with different superscripts letters are significantly different at $p < .05$. 

[ZO1: zonula occludens 1; OCLN: occludin; CLDN1: claudin 1; CLDN2: claudin 2; CLDN3: claudin 3; B0AT: b0, amino acid transporter; LAT1: L-type amino acid transporter 1; ASCT1: alanine-serine-cysteine-threonine transporter 1; EAAT3: excitatory amino acid transporter 3; β0, amino acid transporter; y LAT2: y’L amino acid transporter 2; CAT1: cationic amino acid transporter 1; CAT2: cationic amino acid transporter 2; PepT1: oligopeptide transporter 1; GLUT2: glucose transporter 2; SGLT1: sodium-dependent glucose transporter 1.]

\[X_{ij} = \mu + x_i + \epsilon_{ij}\]
Table 4. Effects of dietary curcumin supplementation on mRNA expression of genes related to intestinal barrier of laying hens fed heated soybean meal.

| Items | CON | HSBM | HSBM + Cur | SEM | p-Value |
|-------|-----|------|------------|-----|---------|
| Jejunum | ZO1 | 1.00 | 0.962 | 1.05 | 0.057 | 0.875 |
| | OCLN1 | 1.00 | 0.632b | 0.651b | 0.061 | 0.016 |
| | CLDN1 | 1.00 | 0.969 | 0.939 | 0.075 | 0.948 |
| | CLDN2 | 1.00 | 0.909 | 0.859 | 0.071 | 0.738 |
| | CLDN3 | 1.00 | 0.674b | 1.01 | 0.053 | 0.007 |
| Ileum | ZO1 | 1.00 | 0.944 | 1.08 | 0.067 | 0.726 |
| | OCLN1 | 1.00 | 0.707b | 1.10b | 0.065 | 0.026 |
| | CLDN1 | 1.00 | 1.05 | 0.956 | 0.093 | 0.930 |
| | CLDN2 | 1.00 | 0.847 | 0.914 | 0.064 | 0.645 |
| | CLDN3 | 1.00 | 0.648b | 0.864b | 0.056 | 0.025 |

CON: laying hens fed a heated soybean meal-corn basal diet; HSBM: laying hens fed a heated soybean meal-corn basal diet supplemented with 150 mg/kg curcumin; ZO1: zona occludens 1; OCLN1: occludin; CLDN1: claudin 1; CLDN2: claudin 2; CLDN3: claudin 3.

1SEM: standard errors of mean.

a,bMean values within a row with different superscripts letters are significantly different at p < .05.

Amino acid profile in the serum, liver and yolk

Compared with the control group, elevated concentrations of valine (Table 6, p = .003), methionine (p = .047), isoleucine (p = .003), and leucine (p = .002) in the serum were observed in the HSBM-treated laying hens, and these indices were normalised to control levels by the administration of curcumin in the diet. Additionally, the birds fed HSBM supplemented with curcumin exhibited a higher serum histidine concentration (p = .012) than did birds fed the control treatment.

The laying hens fed HSBM diets exhibited lower contents of glutamic acid (Table 7, p = .029), glycine (p = .036), alanine (p = .034), tyrosine (p = .023), and arginine (p = .042) in the liver compared with those receiving a control diet, and hepatic tyrosine (p = .023) and arginine (p = .042) levels in hens receiving HSBM were increased with curcumin administration. HSBM supplementation decreased the contents of serine (Table 8, p = .010), methionine (p = .009), isoleucine (p = .037), tyrosine (p = .005), arginine (p = .035), and proline (p = .029) in the yolk compared with the control treatment, and supplementation with curcumin increased the levels of serine (p = .010), methionine (p = .009), and tyrosine (p = .005), which reached the control values.

Table 5. Effects of dietary curcumin supplementation on serum biochemical parameters of laying hens fed heated soybean meal.

| Items | CON | HSBM | HSBM + Cur | SEM | p-Value |
|-------|-----|------|------------|-----|---------|
| Glucose (mmol/L) | 15.9a | 16.3a | 13.2b | 0.422 | .001 |
| Total protein (g/L) | 55.0 | 47.7b | 51.8a,b | 1.57 | .013 |
| Albumin (g/L) | 21.2a | 18.0b | 20.1a | 0.549 | .002 |
| Uric acid (μmol/L) | 196 | 237 | 201 | 8.22 | .077 |

CON: laying hens fed a heated soybean meal-corn basal diet; HSBM: laying hens fed a heated soybean meal-corn basal diet supplemented with 150 mg/kg curcumin.

1SEM: standard errors of mean.
a,bMean values within a row with different superscripts letters are significantly different at p < .05.

content of glucose (p = .001) in the serum were observed after curcumin treatment. The content of uric acid in the HSBM group was numerically higher than that in the other two groups, although it did not reach a significant level (p > .05).

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Table 6. Effects of dietary curcumin supplementation on contents of amino acids in the serum of laying hens fed heated soybean meal (mg/L).

| Items | CON | HSBM | HSBM + Cur | SEM | p-Value |
|-------|-----|------|------------|-----|---------|
| Aspartic acid | 3.29 | 4.01 | 5.17 | 0.380 | .140 |
| Threonine | 26.2 | 38.1 | 32.9 | 2.24 | .081 |
| Serine | 46.8 | 44.8 | 35.4 | 2.12 | .111 |
| Glutamic acid | 26.9 | 29.2 | 28.3 | 1.30 | .837 |
| Glycine | 23.5 | 24.6 | 26.9 | 0.940 | .283 |
| Alanine | 28.3 | 30.5 | 35.1 | 1.29 | .124 |
| Valine | 15.4b | 21.5a | 13.9b | 1.14 | .003 |
| Methionine | 9.62b | 12.0a | 9.68b | 0.487 | .048 |
| Isoleucine | 8.69b | 11.3a | 7.77b | 0.505 | .003 |
| Leucine | 20.5b | 23.9a | 18.6b | 0.729 | .002 |
| Tyrosine | 18.5 | 25.4 | 21.1 | 1.62 | .214 |
| Phenylalanine | 21.4 | 27.3 | 26.2 | 1.21 | .109 |
| Lysine | 33.8 | 48.6 | 42.5 | 2.74 | .072 |
| Histidine | 12.2b | 14.3a,b | 15.4a | 0.475 | .012 |
| Arginine | 47.9 | 54.2 | 51.8 | 2.28 | .559 |
| Proline | 17.5 | 19.1 | 16.6 | 0.651 | .287 |

CON: laying hens fed a heated soybean meal-corn basal diet; HSBM: laying hens fed a heated soybean meal-corn basal diet supplemented with 150 mg/kg curcumin.

1SEM: standard errors of mean.
a,bMean values within a row with different superscripts letters are significantly different at p < .05.

Table 7. Effects of dietary curcumin supplementation on contents of amino acids in the liver of laying hens fed heated soybean meal (mg/g).

| Items | CON | HSBM | HSBM + Cur | SEM | p-Value |
|-------|-----|------|------------|-----|---------|
| Aspartic acid | 14.9 | 14.0 | 14.6 | 0.315 | .157 |
| Threonine | 7.49 | 7.02 | 7.45 | 0.184 | .195 |
| Serine | 7.91 | 7.44 | 8.49 | 0.335 | .258 |
| Glutamic acid | 20.7a | 19.3b | 20.3ab | 0.337 | .029 |
| Glycine | 7.72b | 7.20a | 7.26 | 0.144 | .036 |
| Alanine | 9.10b | 8.53a | 8.82b | 0.140 | .034 |
| Valine | 8.22 | 7.75 | 8.13 | 0.185 | .207 |
| Methionine | 3.37 | 3.07 | 3.34 | 0.126 | .211 |
| Isoleucine | 6.26 | 5.85 | 6.28 | 0.171 | .191 |
| Leucine | 13.5 | 12.7 | 13.3 | 0.312 | .170 |
| Tyrosine | 5.78a | 5.28b | 5.76b | 0.133 | .023 |
| Phenylalanine | 7.21 | 6.83 | 7.13 | 0.154 | .208 |
| Lysine | 12.1 | 11.4 | 11.9 | 0.258 | .177 |
| Histidine | 3.94 | 3.71 | 3.81 | 0.085 | .173 |
| Arginine | 9.96a | 9.22b | 9.84a | 0.192 | .042 |
| Proline | 6.55 | 6.14 | 6.39 | 0.135 | .126 |

CON: laying hens fed a heated soybean meal-corn basal diet; HSBM: laying hens fed a heated soybean meal-corn basal diet supplemented with 150 mg/kg curcumin.

1SEM: standard errors of mean.
a,bMean values within a row with different superscripts letters are significantly different at p < .05.
Table 8. Effects of dietary curcumin supplementation on contents of amino acids in the egg yolk of laying hens fed heated soybean meal (mg/g).

| Items  | CON  | HSBM  | HSBM + Cur | SEM^1 | p-Value |
|--------|------|-------|------------|-------|---------|
| Aspartic acid | 14.1 | 13.0 | 13.6 | 0.401 | .204 |
| Threonine | 7.69 | 7.04 | 7.43 | 0.240 | .201 |
| Serine | 12.6^a | 10.9^b | 12.1^a | 0.368 | .010 |
| Glutamic acid | 18.3 | 17.2 | 17.6 | 0.517 | .231 |
| Glycine | 4.65 | 4.68 | 4.71 | 0.223 | .981 |
| Alanine | 7.78 | 7.26 | 7.44 | 0.233 | .330 |
| Valine | 8.06 | 7.40 | 7.79 | 0.238 | .203 |
| Methionine | 3.38^a | 3.01^b | 3.53^a | 0.108 | .009 |
| Isoleucine | 6.82^a | 6.09^b | 6.54^ab | 0.185 | .037 |
| Leucine | 12.9 | 11.9 | 12.4 | 0.373 | .227 |
| Tyrosine | 6.31^a | 5.67^b | 6.17^a | 0.124 | .005 |
| Phenylalanine | 6.37 | 5.92 | 6.23 | 0.174 | .248 |
| Lysine | 11.3 | 10.4 | 10.8 | 0.336 | .218 |
| Histidine | 3.51 | 3.25 | 3.31 | 0.121 | .332 |
| Arginine | 10.1^a | 9.08^b | 9.58^ab | 0.247 | .035 |
| Proline | 6.17^a | 5.49^b | 5.96^ab | 0.154 | .029 |

CON: laying hens fed a soybean meal-corn basal diet; HSBM: laying hens fed a heated soybean meal-corn basal diet; HSBM + Cur: laying hens fed a heated soybean meal-corn basal diet supplemented with 150 mg/kg curcumin. ^1SEM: standard errors of mean. ^a,bMean values within a row with different superscripts letters are significantly different at p < .05.

Table 9. Effects of dietary curcumin supplementation on mRNA expression of nutrient transporters in the intestinal mucosa of laying hens fed heated soybean meal.

| Items  | HSBM  | HSBM + Cur | SEM^1 | p-Value |
|--------|-------|------------|-------|---------|
| Jejunum | | | | |
| B^4AT | 1.00 | 0.972 | 1.04 | 0.077 | .942 |
| LAT1 | 1.00 | 1.04 | 1.06 | 0.097 | .968 |
| ASC1 | 1.00 | 0.992 | 1.01 | 0.086 | .996 |
| EAAT3 | 1.00^a | 0.732^b | 0.734^b | 0.045 | .011 |
| B^4-LAT | 1.00^a | 0.942 | 0.976 | 0.070 | .951 |
| y^1LAT2 | 1.00^a,b | 0.638^b | 1.13^a | 0.079 | .023 |
| CAT1 | 1.00^a | 0.736^b | 1.06^a | 0.044 | .002 |
| CAT2 | 1.00 | 0.902 | 0.972 | 0.085 | .897 |
| PepT1 | 1.00^a | 0.483^c | 0.739^b | 0.057 | <.001 |
| GLUT2 | 1.00^a | 0.730^b | 0.680^b | 0.049 | .008 |
| SGLT1 | 1.00 | 0.853 | 1.25 | 0.073 | .078 |

CON: laying hens fed a soybean meal-corn basal diet; HSBM: laying hens fed a heated soybean meal-corn basal diet; HSBM + Cur: laying hens fed a heated soybean meal-corn basal diet supplemented with 150 mg/kg curcumin; B^4AT: B^4 amino acid transporter; LAT1: L-type amino acid transporter; ASC1: alanine-serine-cysteine-threonine transporter 1; EAAT3: excitatory amino acid transporter 3; B^4-LAT: B^4 amino acid transporter; y^1LAT2: y^1 amino acid transporter 2; CAT1: cationic amino acid transporter 1; CAT2: cationic amino acid transporter 2; PepT1: oligopeptide transporter 1; GLUT2: glucose transporter 2; SGLT1: sodium-dependent glucose transporter 1. ^1SEM: standard errors of mean. ^a,bMean values within a row with different superscripts letters are significantly different at p < .05.

**Expression of genes responsible for nutrient transport**

HSBM supplementation decreased the mRNA expression levels of EAAT3 (Table 9, p = .011), CAT1 (p = .002), PepT1 (p < .001), and GLUT2 (p = .008) in the jejunal mucosa and B^4AT (p < .001), LAT1 (p = .001), and y^1LAT2 (p = .009) in the ileal mucosa compared with the control group. In contrast, compared with HSBM treatment, curcumin administration increased the mRNA expression levels of y^1LAT2 (p = .023), CAT1 (p = .002), and PepT1 (p < .001) in the jejunal mucosa, as well as B^4AT (p < .001), LAT1 (p = .001), y^1LAT2 (p = .009), and CAT1 (p = .002) in the ileal mucosa, and decreased ileal mucosal SGLT1 (p = .008) gene abundance. Aside from the mRNA expression levels of the previously mentioned genes, there was no difference in the gene abundance of other measured intestinal transporters among the three groups (p > .05).

**Discussion**

In recent years, protein oxidation in the soybean meal has become a serious problem (Hellwig 2019; Duque-Estrada et al. 2020), and some measures must be taken to mitigate its side effects to promote the productive performance of animals. Due to the multifunctional characteristics of curcumin, we investigated whether curcumin supplementation could alleviate the effects of protein oxidation in soybean meal in laying hens. Owing to the excessive free radicals produced during the process of protein oxidation, harmful consequences, such as oxidative stress, can be observed in the intestines of individuals fed diets with oxidised proteins (Assimakopoulos et al. 2006). A previous study reported that oxidised wheat gluten can result in intestinal morphological damage and oxidative stress, contributing to diarrhoea and impaired growth performance in broilers (Liao et al. 2018). When the intestinal mucosa is injured and its permeability increases, the D-LA produced by gastrointestinal bacteria enters the circulating system (Vella and Farrugia 1998), and DAO, a degradative enzyme primarily detected in the intestinal mucosa, is released from the mucosal lamina propria into the peripheral circulation (Wolvekamp and de Bruin 1994). Thus, circulating D-LA concentration and DAO activity can be used to quantitatively assess the maturity and integrity of the intestinal mucosa (Sun et al. 2001; Moriyama et al. 2006). Meanwhile, tight junctions (TJs) between epithelial cells limit ion diffusion and luminal antigen migration from the apical to the basolateral membrane, which is important for maintaining the normal
biological function of the intestinal barrier and epithelial cells (Schulze and Fromm 2009). The results of this study showed that HSBM administration decreased the mRNA expression abundance of intestinal mucosal OCLN and CLDN3, both of which participate in the assembly and disassembly of TJs and are involved in intestinal barrier function (Hartsock and Nelson 2008; Rao 2009; Dorfel and Huber 2012). Taken together, these gene expression results were consistent with increased levels of D-LA content and DAO activity in the serum, suggesting that HSBM may have an adverse effect on intestinal barrier function, which is probably due to the accumulation of oxidative products and microbial changes induced by oxidised proteins (Ge et al. 2020). Regarding the effects of curcumin on the intestinal barrier, the present study demonstrated that curcumin administration decreased serum D-LA levels and increased mucosal mRNA abundance of OCLN and CLDN3 compared to the HSBM group, which indicated the mitigating effects of curcumin on intestinal integrity. In a trial investigating the effects of curcumin on ducks fed corn contaminated with ochratoxin A, researchers observed that curcumin can effectively prevent abnormal histopathological and ultrastructural changes in the intestine and simultaneously improve antioxidant capacity and upregulate the expression of TJs (Ruan et al. 2019). Moreover, oxidised protein can also lead to the production of excessive reactive oxygen species and subsequently induce an imbalance of the redox state in organisms (Tang et al. 2012), while curcumin is an ideal antioxidant according to accumulating published data (Pinlaor et al. 2009; Tapia et al. 2013; Fazal et al. 2015). Thus, curcumin might alleviate oxidative stress and exert protective effects on intestinal mucosal barrier function.

Prior to being utilised by animals, macromolecular nutrients, such as sugars and proteins, need to be degraded into small molecular nutrients for further absorption, and these small and soluble molecules are transported by intestinal nutrient carriers, which are typically divided into amino acid transporters, peptide transporters, and glucose transporters according to their different functions (Kaminski and Wong 2018). A previous study showed that different ingredients and nutritional levels of the diet would result in different types and quantities of nutrient transporters in the small intestine (Gilbert et al. 2010). In the current experiment, HSBM administration decreased jejunal mucosal expression level of GLUT2, which is responsible for the transport of carbohydrates (Mueckler and Thorens 2013), implying that HSBM has negative effects on carbohydrate absorption in laying hens. Curcumin has been confirmed to attenuate abnormalities in glucose metabolism under conditions of nutritional oversupply (He et al. 2012), and the current trial demonstrated that curcumin decreased serum glucose content along with the corresponding downregulated expression of mucosal glucose transporters, which may indicate the hypoglycaemic effect of curcumin. With regard to the transport of small peptides and amino acids, the birds fed diets containing HSBM exhibited reduced mRNA expression levels of nutrient transporters in the intestinal mucosa, including EAAT3, CAT1, B0AT, LAT1, y+LAT2, and PepT1, and curcumin supplementation normalised most of them to the control values, indicating that curcumin could reverse the alteration in the expression pattern of amino acid and peptide transporters in the laying hens fed HSBM. Osmanyan et al. (2018) demonstrated that the decreased mRNA expression of several amino acid and peptide transporters could be observed in broilers fed diets with lower levels of amino acids and proteins, which was consistent with our results, since protein oxidation can lead to a high level of carbonyl groups, formation of aggregates, and oxidation of specific amino acid side chains (Santé-Lhoutellier et al. 2008), eventually resulting in lower amino acid quality, protein solubility, and digestibility of proteins (Wu et al. 2014; Chen et al. 2015; Zhang et al. 2016). Meanwhile, changes in the expression and transcription of nutrient transporters may lead to metabolic disorders of nutrients (e.g. amino acids, peptides, and carbohydrates) in animals (Chen et al. 2002; Paris and Wong 2013; Su et al. 2014; Miska and Fetterer 2017). In keeping with the reduced abundance of intestinal amino acid transporters, the current study showed that birds fed diets with HSBM exhibited decreased contents of amino acids in the liver and yolk. Yang et al. (2017) reported that oxidised tyrosine products induce negative changes in several metabolic systems that involve a variety of amino acids and other functional molecules, and this alteration of metabolism may contribute to the lower deposition of amino acids in the liver and egg. Additionally, the reduced contents of serum total protein and albumin in the HSBM treatment implied that oxidised protein products would cause liver injury and decrease hepatic protein synthesis ability (Li et al. 2014), which might be associated with the higher proportion of underused amino acids in the serum. With regard to curcumin, Kołodziejczyk et al. (2011) demonstrated that curcumin could reduce oxidative and nitrative damage to blood platelets and plasma components, including proteins and lipids,
which may facilitate the digestion and absorption of nutrients. This study demonstrated that adding curcumin to the diet could normalise the contents of several amino acids, suggesting a positive influence of curcumin on the digestion and absorption of amino acids in domestic laying hens. Simultaneously, the restored protein synthesis capacity mediated by curcumin supplementation (Singh et al. 2014), as supported by the increased contents of total protein and albumin in the serum, could account for the improved deposition of amino acids to a certain extent. Furthermore, curcumin was determined to be efficacious in alleviating HSBM-induced intestinal barrier damage in laying hens, which may also provide an explanation for the improved transport and absorption of nutrients in curcumin-treated birds (De Santis et al. 2015).

Conclusions

Taken together, the results of this 6-week experiment indicated that oxidised HSBM protein could induce the impairment of intestinal barrier function and nutrient absorption and downregulate the expression of intestinal transporters in laying hens, and the dietary administration of 150 mg/kg curcumin could alleviate these negative effects.

Ethics approval

This experimental method was approved by the animal experiment guidelines established by the Animal Care and Use Committee of Nanjing Agricultural University.

Disclosure statement

The authors declare that there are no potential conflicts of interest with respect to the authorship and publication of this article.

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