ANIMAL MODELS

Dietary taurine supplementation attenuates lipopolysaccharide-induced inflammatory responses and oxidative stress of broiler chickens at an early age

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Abstract

This study was conducted to investigate the effect of taurine as a prophylactic treatment on antioxidant function and inflammatory responses of broilers challenged with lipopolysaccharide (LPS). A total of 256 one-day-old male Arbor Acres broiler chicks were randomly assigned to four treatments with eight replicates of eight birds (eight birds per cage). Four treatment groups were designated as follows: 1) in the **CON group**, broilers fed a basal diet; 2) in the **LPS group**, LPS-challenged broilers fed a basal diet; 3) in the **LPS + T1 group**, LPS-challenged broilers fed a basal diet supplemented with 5.0 g/kg taurine; and 4) in the **LPS + T2 group**, LPS-challenged broilers fed a basal diet supplemented with 7.5 g/kg taurine. The LPS-challenged broilers were intraperitoneally injected with 1 mg/kg body weight (BW) of LPS at 16, 18, and 20 d of age, whereas the CON group received an injection of sterile saline. The results showed that broilers injected with LPS exhibited decreased ($P < 0.05$) the average daily gain (ADG) and the 21-d BW ($P < 0.05$), while taurine supplementation alleviated the negative effects of LPS. Additionally, the LPS-induced increases ($P < 0.05$) in serum alanine transaminase and aspartate transaminase activities were reversed by taurine supplementation. The taurines could alleviate the hepatic oxidative stress, with the presence of lower content of malondialdehyde ($P < 0.05$), higher content of glutathione ($P < 0.05$), and an increased glutathione peroxidase (GSH-Px) activity ($P < 0.05$). The concentrations of interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) in the liver were measured by ELISA kits, and the result showed that dietary taurine supplementation prevented these cytokines increases in the liver of LPS-induced broilers. Taurine reduced the genes expression of IL-1 β , TNF- α , IL-6, cyclooxygenase-2, and inducible nitric oxide synthase, whereas it boosted the expression levels of antioxidant-related genes (nuclear factor erythroid 2-related factor 2, heme oxygenase-1, glutamate-cysteine ligase catalytic subunit, and GSH-Px) in the liver of LPS-induced broilers. In conclusion, dietary taurine supplementation in broilers mitigated LPS-induced defects in ADG, oxidative stress, and inflammatory responses.

Key words: broiler, immunological stress, lipopolysaccharide, liver injury, oxidative damage, taurine

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
ALT	alanine transaminase
AST	aspartate transaminase
BW	body weight
CAT	catalase
COX-2	cyclooxygenase-2
G:F	gain:feed
GCLC	glutamate-cysteine ligase catalytic subunit
GCLM	glutamate-cysteine ligase modifier subunit
GSH	glutathione
GSH-Px	glutathione peroxidase
HO-1	heme oxygenase-1
IL-10	interleukin-10
IL-1 β	interleukin-1 β
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
LPS	lipopolysaccharide
MDA	malondialdehyde
mRNA	messenger ribonucleic acid
MyD88	myeloid differentiation factor 88
NF- κ B	nuclear factor-kappa B;
Nrf2	nuclear factor erythroid 2-related factor 2
PBS	phosphate-buffered saline
ROS	reactive oxygen species
SOD	superoxide dismutase
T-AOC	total antioxidant capacity
TLR4	toll-like receptor 4
TNF- α	tumor necrosis factor- α

Introduction

The endotoxin lipopolysaccharide (LPS) is one of the major constituents of the outer coats in Gram-negative bacteria. Orally or abdominally injected LPS can increase the accumulation of proinflammatory mediators, leading to the production of cytokines and oxidative stress, which, in turn, causes hepatic inflammation (Hou et al., 2013; Wang et al., 2018). The LPS can trigger inflammatory responses in diverse species by activating signal pathways and promoting gene expressions, such as chickens (Chen et al., 2018), mouse (Zhang et al., 2020), and pig (Xia et al., 2018), which are characterized by reduced feed intake, decreased growth performance, and increased release of reactive oxygen species (ROS) and proinflammatory cytokine production (Takahashi et al., 2008; Munyaka et al., 2013; Gadde et al., 2017). It has been extensively used as a model to mimic bacterial infection and immunological stress in laboratory animals. The mechanisms of LPS-induced inflammatory responses have been widely explored. Toll-like receptor 4 (TLR4) serves as a signaling receptor for LPS, which leads to an activation of myeloid differentiation factor 88 (MyD88). The triggering of MyD88 pathways activates the nuclear factor-kappa B (NF- κ B) signaling cascade, which promotes the biosynthesis of proinflammatory cytokines (Liu et al., 2017a). Moreover, inflammation and oxidative stress are tightly intertwined, because the overproduction of proinflammatory cytokines can generate ROS, ultimately inflicting oxidative injury in multiple organ systems (Godbout et al., 2005; Faheem et al., 2015). In the modern poultry industry, intensive production and feeding conditions increase

the risks of exposure to pathogens and stressors. In recent years, the damage caused by immunological and oxidative stress in livestock production has gained considerable attention (Panda et al., 2009; Remus et al., 2014; Salami et al., 2015; Tan et al., 2015; Yang et al., 2019). Immunological and oxidative stress can change the partitioning of nutrients, decrease the growth potential of animals, induce various diseases, and even result in death (Eugeni et al., 1992; Min et al., 2018). Therefore, it is of great importance to understand the LPS-induced immune reaction, which would be helpful to alleviate the harmful consequences of stress and maintain the growth performance of broilers.

Taurine, as a nontoxic endogenous antioxidant, has become an attractive candidate for attenuating various toxins and drug-induced liver disease (Joydeep et al., 2011; Das et al., 2012). Taurine is an amino acid with antioxidant (Cassol et al., 2010), lipid-lowering (De Carvalho et al., 2018), anti-inflammatory, and immunomodulatory functions (Janusz and Ewa, 2014). Lu et al. (2019a) revealed that supplementation with taurine significantly decreased the level of malondialdehyde (MDA) in breast meat of heat-stressed broilers. Growing evidence shows that taurine appears to act as nonspecific radical oxygen species scavenger and a regulator of antioxidant defense system to reduce the occurrence of oxidative stress (De Luca et al., 2015; Coutinho et al., 2017). In addition, taurine can attenuate immune and oxidative damage via downregulating the components of the NF- κ B signaling pathway and its downstream inflammatory target genes (Liu et al., 2017b; Abd-Elhakim et al., 2020; Jangra et al., 2020). Taurine can react with hypochlorous acid to produce taurine monochloramine in vivo, which, in turn, reduces or blocks the production of proinflammatory mediators (Schuller-Levis and Park, 2003, 2004). Inhibition of the release of proinflammatory cytokines by taurine in vitro (Nakajima et al., 2010) has also been reported. However, whether taurine exerts beneficial effects to regulate the broilers' immunosuppressive status due to stress still remains unclear.

Based on the findings above, we used a typical immune stress model by intraperitoneal administration of LPS to investigate the effects of taurine on growth performance, hepatic antioxidant function, and inflammatory responses in LPS-challenged broiler chickens at an early age. Our results will provide useful insights for taurine to alleviate the adverse effects of production stress in broiler chickens.

Materials and Methods

All experimental procedures were conducted according to the guidelines on the Ethical Treatment of Experimental Animals set by the Ministry of Science and Technology in China. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Animals, diets, and experimental design

LPS from *Escherichia coli* serotype O55:B5 was purchased from Sigma-Aldrich Chemical Co. (#L2880; St. Louis, MO, USA). The LPS was dissolved in 0.86% (w/v) sterile saline solution to prepare 1 mg/mL LPS solution. Taurine (purity > 98.5%) was purchased from Jiangyin Huachang Food Biotechnology Co. Ltd. (Jiangyin, Zhejiang, China). A total of 256 one-day-old male Arbor Acres broiler chicks (Hewei Company, Xuancheng, Anhui, China) were randomly distributed into four treatment groups with eight replicates of eight birds. At 16, 18, and 20 d, the LPS-challenged groups were intraperitoneally injected with a dose of 1 mg/kg body weight (BW) LPS solution, whereas the CON

group received an equivalent volume sterile saline injection. All birds were allocated into four treatments: 1) broilers fed a basal diet (CON); 2) LPS-challenged broilers fed a basal diet (LPS); 3) LPS-challenged broilers fed a basal diet and supplemented with 5.0 g/kg taurine (LPS + T1); and 4) LPS-challenged broilers fed a basal diet and supplemented with 7.5 g/kg taurine (LPS + T2). The dosage and its administration routine of LPS were referred to available findings (Chen et al., 2018; Jiang et al., 2019). The supplemental taurine levels were determined according to previous studies (Lu et al., 2019b; Xu et al., 2020).

Housing

During the trial period, birds had free access to feed and water in three-layer cages (120 × 60 × 50 cm; 0.09 m² per chick) with 23:1 (L:D) per day. Meanwhile, the brooding temperature was maintained at 32 to 35 °C for the first 5 d and then gradually decreased by 0.5 °C every day until decreased to 26 °C and then kept constant. In addition, all birds were vaccinated according to a routine immunization program. The basal diet was formulated in accordance with the National Research Council (1994) nutrient requirements of the broiler, and its ingredient composition and nutrient level are listed in Table 1.

Growth performance measurement

On days 1, 15, and 21 of the experiment, the BW and the total feed consumption of broilers were recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and gain to feed ratio (G:F) before (from 1 to 15 d of age) and after the LPS challenge (from 16 to 21 d of age). The G:F = ADG:ADFI.

Sample collection

At 21 d of age, one bird from each replicate was selected for slaughter, and blood samples were collected in a non-anticoagulant sterile blood vessel from the jugular vein. After centrifugation at 4,000 × *g* for 15 min at 4 °C, the top serum in the tube was collected and stored at –80 °C for analysis. After blood sampling, the birds were euthanized by cervical dislocation immediately. The liver tissues were immediately removed, thoroughly washed with phosphate-buffered saline (PBS), stored in liquid nitrogen, and then preserved at –80 °C for further analysis.

Analysis of serum aminotransferase activities

The serum was sampled to measure aspartate transaminase (AST, #C010-1-1) and alanine transaminase (ALT, #C010-2-2) activities. The kits were purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China), and all experimental procedures were performed according to the manufacturer's instructions.

Assay of antioxidant enzymes

One gram of liver tissue preserved at –80 °C was homogenized by Ultra-Turrax homogenizer (Tekmar Co, Cincinnati, OH, USA) with 9 mL of 0.9% ice-cold sodium chloride buffer and then centrifuged at 4,000 × *g* for 10 min at 4 °C, and the supernatant was collected and analyzed quickly (Ge et al., 2019). The total antioxidant capacity (T-AOC, #A015-2-3), superoxide dismutase (SOD, #A003-2-4), glutathione peroxidase (GSH-Px, #A002-1-2), and catalase (CAT, #A007-1-3) activities, as well as glutathione (GSH, #A001-2-2) and MDA (#A007-3-2) contents, were all measured using commercial assay kits (Nanjing Jiancheng Institute of Bioengineering) according to the manufacturer's instructions.

Determination of inflammatory cytokines

Approximately, 0.5 g of liver tissue was homogenized with PBS (PH 7.4) for 30 s, using Ultra-Turrax homogenizer. After that, the homogenization was centrifuged at 3,000 × *g* for 20 min at 4 °C, and the supernatant was collected in tubes, which were immediately stored at –20 °C for the subsequent analysis. The concentrations of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10) in the liver samples were measured by ELISA using chicken-specific quantification kits purchased from Shanghai Yili Biological Technology Co, Ltd (Shanghai, China).

Quantification of messenger ribonucleic acid by real-time PCR

Total RNA from the liver sample (about 50 to 100 mg) was extracted by the addition of 1 mL of TRIzol-reagent (#R601-03; TaKaRa Biotechnology, Dalian, Liaoning, China) according to the manufacturer's instructions. The concentration and purity of the total RNA were assessed using a spectrophotometer (NanoDrop

Table 1. Ingredients and nutrient composition of broiler diets on fed basis

Ingredients	Contents, %	Calculated nutrient levels ^a	Contents, %
Corn	56.58	Apparent metabolizable energy, MJ/kg	12.52
Soybean meal	29.76	Crude protein	21.60
Corn gluten meal	4.96	Lysine	1.18
Soybean oil	3.97	Methionine	0.52
Limestone	1.19	Calcium	1.08
Dicalcium phosphate	1.49	Total phosphorus	0.70
Alanine ¹	0.54	Analyzed composition	
Microcrystalline cellulose ²	0.21	Crude protein	21.33
Salt	0.30	Crude fat	4.81
Premix ³	1	Calcium	1.00
Total	100.00	Total phosphorus	0.66

¹Alanine, the purity was 99%, was used to balance the nitrogen in all diets.

²Microcrystalline cellulose, the purity was 99%, was used to balance the different percentages of supplemental taurine.

³The premix provided per kilogram of diet: retinyl acetate for vitamin A 10,000 IU, cholecalciferol for vitamin D₃ 3,000 IU, DL-α-tocopheryl acetate for vitamin E 30 IU, menadione sodium bisulphate 1.3 mg, thiamin 2.2 mg, riboflavin 8.0 mg, nicotinamide 40 mg, choline chloride 600 mg, calcium pantothenate 10 mg, pyridoxine HCl 4 mg, biotin 0.04 mg, folic acid 1 mg, vitamin B₁₂ (cobalamin) 0.013 mg, Zn 60 mg, Fe 80 mg, Cu 8.0 mg, Mn 110 mg, I 1.1 mg, and Se 0.3 mg.

⁴Calculated value.

Technologies, Wilmington, DE, USA). Subsequently, the RNA was reverse transcribed to cDNA using the PrimeScript RT reagent Kit (Perfect Real Time, SYBR PrimeScriP TaKaRa, China, #3894A) according to the manufacturer's instructions. The cDNA samples were amplified by real-time quantitative polymerase chain reaction with SYBR Premix Ex Taq reagents (Takara Biotechnology). The real-time PCR cycling conditions were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 30 s (Lu et al., 2019a). The messenger ribonucleic acid (mRNA) expression of target genes relative to beta-actin (β -actin) was calculated using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All of the primer sequences are listed in Table 2 and synthesized by Sangon Biotech Co. Ltd. (Shanghai, China).

Statistical analysis

All data were preliminarily processed using Excel 2010. The statistical analyses were performed using statistical software SPSS 22.0 (SPSS Inc, Chicago, IL, USA) with replicates ($n = 8$) as an experimental unit, and the results were presented as means $\pm$ SEM. Data were normally distributed (Shapiro-Wilk test) and tested for homogeneity of variance (Levene's test). Statistical analyses were carried out using one-way analysis of variance followed by the Duncan's multiple test. Differences were regarded as significant at $P < 0.05$.

Results

Growth performance

Before the LPS challenge (1 to 15 d of age), there were no dietary effects on bird growth performance ($P > 0.05$; Table 3). Compared with the CON group, during the LPS challenge (16

to 21 d of age) significantly decreased the ADG ($P < 0.001$) of broilers (Table 3), while the ADG of LPS-challenged broilers was increased ($P < 0.001$) by taurine administration.

Activities of serum AST and ALT

The serum activities of AST and ALT are shown in Figure 1. Compared with the CON group, the serum activities of ALT and AST were significantly increased ($P < 0.05$) in the LPS group, whereas dietary inclusion with taurine decreased the serum activities of ALT and AST ($P < 0.05$).

Hepatic redox status

As shown in Table 4, compared with the non-challenged broilers, LPS injection decreased the activities of GSH-Px, SOD, and the content of GSH, but increased the content of MDA. Dietary taurine supplementation significantly increased ($P < 0.05$) the GSH-Px activity and decreased ($P < 0.05$) the concentration of MDA concentration in the liver of broilers in comparison with the LPS-induced group ($P < 0.05$). Also, broilers in the LPS + T1 group had a higher ($P < 0.05$) content of GSH than the LPS-induced group. In addition, there were no changes in the level of T-AOC and the activity of CAT among all the groups ($P > 0.05$).

Hepatic inflammatory cytokine concentrations

As indicated in Table 5, after injected with LPS, the hepatic concentrations of IL-1 β , IL-6, and TNF- α were significantly increased ($P < 0.05$) in the LPS group compared with the CON group. In contrast, taurine supplementation alleviated the inflammatory responses in LPS-challenged broilers, as evidenced by the decreases in IL-1 β , IL-6, and TNF- α concentrations in the liver of LPS-challenged broilers after receiving taurine ($P < 0.05$).

Table 2. Primer sequences used for quantitative real-time PCR

Gene name		Primers sequence (5'-3')	Accession number
Nrf2	Forward	GAGCCCATGGCCTTTCTCTAT	NM_001007858.1
	Reverse	CACAGAGGCCCTGACTCAAA	
HO-1	Forward	AAACTTCGCAGCCACACAAC	NM_205344.1
	Reverse	GACCAGCTTGAAGTCGTGGA	
GSH-Px	Forward	GACCAACCCGCAGTACATCA	NM_001277853.1
	Reverse	GAGGTGCGGGCTTTCTCTTA	
SOD1	Forward	CCGGCTTGTCTGATGGAGAT	NM_205064.1
	Reverse	TGCATCTTTTGGTCCACCGT	
GCLC	Forward	TGCGGTCTTGCACAAATGG	XM_419910.3
	Reverse	TGCTGTGCGATGAATTCCT	
GCLM	Forward	CCAGAACGTCAAAGCACACG	NM_001007953.1
	Reverse	TCCTCCCATCCCCAGAAAT	
IL-1 β	Forward	GTACCGAGTACAACCCCTGC	NM_204524.1
	Reverse	AGCAACGGGACGGTAATGAA	
TNF- α	Forward	CCCCTACCCTGTCCACAA	NM204267
	Reverse	TGAGTACTGCGGAGGGTTCAT	
IL-6	Forward	CAGCTGCAGGACGAGATGTGCAA	AJ309540
	Reverse	GCACAGGACTCGACGTTCTGCT	
IL-10	Forward	GGAGCTGAGGGTGAAGTTTGA	NM_001004414.2
	Reverse	GACACAGACTGGCAGCCAAA	
TLR4	Forward	TTCAGAACGGACTCTTGAGTGG	AY064697
	Reverse	CAACCGAATAGTGGTGACGTTG	
COX-2	Forward	TGTCCTTTCACTGCTTTCCAT	NM_001167718.1
	Reverse	TTCCATTGCTGTGTTTGAGGT	
iNOS	Forward	CTCAATGGTCAAGAAGAAGCCT	U46504
	Reverse	CTTGTCATCTCTTGCTCTGTA	
β -Actin	Forward	TGCTGTGTTCCCATCTATCG	NM_205518.1
	Reverse	TTGGTGACAATACCGTGTTC	

Table 3. Effects of taurine supplementation on the growth performance of LPS-challenged broilers¹

	Treatment ²				
Item	CON	LPS	LPS + T1	LPS + T2	P-value
1 to 15 d (before the LPS challenge)					
1 d BW, g	43.61 ± 0.11	43.73 ± 0.10	43.66 ± 0.07	43.81 ± 0.07	0.415
15 d BW, g	524.08 ± 2.99	523.66 ± 7.97	564.64 ± 20.84	541.33 ± 15.30	0.135
ADG, g/d	30.03 ± 0.19	30.00 ± 0.50	32.56 ± 1.30	31.10 ± 0.96	0.136
ADFI, g/d	47.51 ± 0.61	47.23 ± 0.39	48.95 ± 2.16	47.49 ± 0.44	0.717
G:F, g/g	0.63 ± 0.01	0.64 ± 0.01	0.67 ± 0.02	0.65 ± 0.01	0.283
16 to 21 d (during the LPS challenge)					
21 d BW, g	743.58 ± 11.93 ^{ab}	720.16 ± 9.35 ^a	782.64 ± 21.09 ^b	763.08 ± 14.65 ^{ab}	0.038
ADG, g/d	43.90 ± 1.01 ^a	39.30 ± 0.38 ^b	43.60 ± 0.99 ^a	44.35 ± 0.52 ^a	<0.001
ADFI, g/d	69.15 ± 1.52	65.48 ± 0.80	68.95 ± 1.37	68.61 ± 0.97	0.127
G:F, g/g	0.64 ± 0.01 ^{ab}	0.60 ± 0.01 ^b	0.63 ± 0.02 ^{ab}	0.65 ± 0.01 ^a	0.055

¹Data are means ± SEM, n = 8.²CON, control basal diet; LPS, LPS challenged broilers fed CON; LPS+T1, LPS challenged broilers fed CON supplemented with 5 g/kg taurine; LPS+T2, LPS challenged broilers fed CON supplemented with 7.5 g/kg taurine.^{a,b}Means within the same row with no common superscript differ significantly ($P < 0.05$). **Figure 1.** Effects of taurine supplementation on the activities of AST(a) and ALT(b) in the serum of LPS-challenged broilers. The results are expressed as the mean ± SEM, n = 8. Histograms labeled with different superscript letters are significantly different ($P < 0.05$).

Hepatic antioxidant gene expression

The mRNA expression of antioxidant genes in the liver of broilers are shown in Table 6. Compared with the CON birds, the LPS injection declined ($P < 0.05$) the mRNA expression levels of nuclear factor erythroid 2-related factor 2 (*Nrf2*), heme oxygenase-1 (*HO-1*), *SOD1*, *GSH-Px*, and glutamate-cysteine ligase catalytic subunit (*GCLC*) in the liver of broilers. However, except for *SOD1*, taurine supplementation reversed these genes expression ($P < 0.05$). No changes were observed in glutamate-cysteine ligase modifier subunit (*GCLM*) mRNA among the four groups ($P > 0.05$).

Hepatic inflammatory gene expression

The data of mRNA expression of inflammatory genes in the liver of broilers are shown in Table 7. Compared with CON broilers, LPS injection increased the mRNA expression levels of *TLR4*, *IL-1β*, *TNF-α*, cyclooxygenase-2 (*COX-2*), and inducible nitric oxide synthase (*iNOS*) in the liver of broilers ($P < 0.05$). In contrast, taurine treatment decreased these genes expression ($P < 0.05$).

Discussion

Broilers under an immunosuppressive status exhibit reduced feed intake, weight gain, and feed conversion ratio

(He et al., 2007), which causes a loss to poultry production. Accumulating evidence have demonstrated that dietary taurine supplementation could improve the growth performance of broiler chickens (Tufft and Jensen, 1992; Lee et al., 2004; Lv et al., 2017; Faraji et al., 2019), but little is known about the effect of taurine on broilers growth performance under immune stress. In this study, we found that an LPS challenge induced growth retardation of broilers, as evidenced by reduced ADG. Similarly, numerous previous studies have also shown that LPS injection damages compromised growth performance of broilers (Li et al., 2015; Wang et al., 2015; Zheng et al., 2016). The reason is mainly due to the LPS injection increased the synthesis of various inflammatory cytokines, which leads to the diversion of available nutrients show a tendency to support immune-related processes instead of growth and development (Yang et al., 2011; Tan et al., 2014). Also, this study found that supplementation with taurine could alleviate the inhibition of the ADG of the LPS-challenged broilers, indicating that taurine may exert a protective effect on the growth as evidenced by an increase in ADG of broilers under immunological stress.

Moreover, many studies have shown that LPS administration causes liver injury and dysfunction in broilers (Morris et al., 2014; Qu et al., 2019; Jangra et al., 2020; Mei et al., 2020). The activities of AST and ALT in the serum are usually used clinically

Table 4. Effects of taurine supplementation on liver oxidative status of LPS-challenged broilers¹

Item	Treatment ²				P-value
	CON	LPS	LPS + T1	LPS + T2	
T-AOC, units/mg protein	0.90 ± 0.02	0.81 ± 0.03	0.90 ± 0.05	0.82 ± 0.05	0.197
SOD, units/mg protein	372.87 ± 9.73 ^a	339.68 ± 9.11 ^b	350.01 ± 8.62 ^{ab}	366.23 ± 12.18 ^{ab}	0.101
GSH-Px, units/mg protein	58.25 ± 1.82 ^a	51.30 ± 1.46 ^b	56.15 ± 1.21 ^a	57.82 ± 1.92 ^a	0.021
GSH, nmol/mg protein	2.86 ± 0.12 ^a	2.11 ± 0.24 ^b	2.67 ± 0.10 ^a	2.40 ± 0.12 ^{ab}	0.03
CAT, units/mg protein	8.07 ± 0.17	7.54 ± 0.17	7.90 ± 0.11	7.85 ± 0.38	0.441
MDA, nmol/mg protein	1.02 ± 0.07 ^b	1.33 ± 0.07 ^a	1.02 ± 0.06 ^b	1.08 ± 0.09 ^b	0.021

¹Data are means ± SEM, n = 8.²CON, control basal diet; LPS, LPS challenged broilers fed CON; LPS+T1, LPS challenged broilers fed CON supplemented with 5 g/kg taurine; LPS+T2, LPS challenged broilers fed CON supplemented with 7.5 g/kg taurine.^{a-b}Means within the same row with no common superscript differ significantly (P < 0.05).**Table 5.** Effects of taurine supplementation on liver cytokines concentrations of LPS-challenged broilers¹

Item	Treatment ²				P-value
	CON	LPS	LPS + T1	LPS + T2	
IL-1β, ng/g protein	148.79 ± 10.04 ^d	317.68 ± 22.13 ^a	260.07 ± 19.96 ^b	175.77 ± 15.36 ^c	<0.01
IL-6, ng/g protein	12.15 ± 1.27 ^c	26.48 ± 1.06 ^a	19.21 ± 1.09 ^b	14.91 ± 1.37 ^c	<0.01
TNF-α, ng/g protein	75.18 ± 3.59 ^c	132.32 ± 7.33 ^a	100.19 ± 5.33 ^b	102.64 ± 3.71 ^b	<0.01
IL-10, ng/g protein	39.48 ± 3.37	40.00 ± 2.73	39.19 ± 2.57	42.51 ± 2.81	0.842

¹Data are means ± SEM, n = 8.²CON, control basal diet; LPS, LPS challenged broilers fed CON; LPS+T1, LPS challenged broilers fed CON supplemented with 5 g/kg taurine; LPS+T2, LPS challenged broilers fed CON supplemented with 7.5 g/kg taurine.^{a-d}Means within the same row with no common superscript differ significantly (P < 0.05).**Table 6.** Effects of taurine supplementation on antioxidant gene expression in the liver of LPS-challenged broilers¹

Item ³	Treatment ²				P-value
	CON	LPS	LPS + T1	LPS + T2	
Nrf2	1.00 ± 0.06 ^a	0.70 ± 0.04 ^b	0.93 ± 0.06 ^a	0.90 ± 0.05 ^a	0.003
HO-1	1.00 ± 0.10 ^a	0.71 ± 0.07 ^b	0.99 ± 0.09 ^a	1.03 ± 0.09 ^a	0.060
SOD1	1.00 ± 0.04 ^a	0.71 ± 0.04 ^b	0.92 ± 0.11 ^{ab}	0.83 ± 0.08 ^{ab}	0.061
GSH-Px	1.00 ± 0.06 ^a	0.74 ± 0.05 ^b	0.91 ± 0.03 ^a	0.93 ± 0.05 ^a	0.004
GCLC	1.00 ± 0.13 ^a	0.63 ± 0.04 ^b	1.22 ± 0.15 ^a	1.23 ± 0.08 ^a	0.001
GCLM	1.00 ± 0.14	1.02 ± 0.10	1.12 ± 0.06	1.08 ± 0.12	0.869

¹Data are means ± SEM, n = 8.²CON, control basal diet; LPS, LPS challenged broilers fed CON; LPS+T1, LPS challenged broilers fed CON supplemented with 5 g/kg taurine; LPS+T2, LPS challenged broilers fed CON supplemented with 7.5 g/kg taurine.³Expressed in arbitrary units. The expression of each target gene for the CON group was assigned a value of 1 and normalized against beta-actin.^{a-b}Means within the same row with no common superscript differ significantly (P < 0.05).

as specific indicators reflecting the liver injury (Senior, 2012). Our results showed that the serum activities of ALT and AST in the CON broilers were similar to the taurine groups but higher in the LPS group. Consistent with our results, Zhang et al. (2020) reported that LPS caused significant increases in the activities of AST and ALT. Reduced AST and ALT activities in serum suggested that LPS-induced hepatic injury was ameliorated by taurine administration. These findings together indicate that taurine could protect the hepatic integrity of broilers. This protective effect may be attributed to its ability to stabilize biomembranes (You and Chang, 1998; Adedara et al., 2017) and scavenge reactive oxygen derivatives (Marcinkiewicz et al., 2000).

Antioxidants in birds play an important role in controlling the negative effects of oxidative stress and further prevent immunopathological damage to host tissues (Costantini and Møller, 2009). The GSH is the main endogenous nonenzymatic

antioxidant and helps to prevent lipid peroxidation through a reaction catalyzed by GSH-Px (Fang et al., 2002). When the activity of GSH-Px was attenuated, excessive free radicals produced in the organism attack polyunsaturated fatty acids and cause lipid peroxidation to form lipid peroxide, such as MDA. In the present study, the administration of LPS resulted in reduced GSH, GSH-Px, and SOD activities, but increased MDA level, indicating that an oxidative stress is present in the liver of LPS-injected broilers. In contrast, we noted that dietary taurine supplementation alleviated the oxidative stress in LPS-challenged broilers, as evidenced by reduced MDA content, increased activity of GSH-Px, and increased content of GSH in the liver of LPS-challenged broilers after receiving taurine. Numerous studies have confirmed that taurine supplementation significantly improved GSH-Px activity, decreased MDA content, and then inhibited lipid peroxidation

Table 7. Effects of taurine supplementation on inflammatory gene expression in the liver of LPS-challenged broilers¹

Item ³	Treatment ²				P-value
	CON	LPS	LPS + T1	LPS + T2	
TLR4	1.00 ± 0.15 ^b	1.40 ± 0.10 ^a	0.90 ± 0.07 ^b	1.05 ± 0.11 ^b	0.021
IL-1β	1.00 ± 0.13 ^b	2.09 ± 0.24 ^a	1.31 ± 0.12 ^b	1.48 ± 0.17 ^b	0.001
TNF-α	1.00 ± 0.16 ^b	1.56 ± 0.10 ^a	1.01 ± 0.17 ^b	1.13 ± 0.12 ^b	0.030
IL-6	1.00 ± 0.15 ^b	1.99 ± 0.42 ^a	1.16 ± 0.20 ^b	1.31 ± 0.19 ^{ab}	0.062
COX-2	1.00 ± 0.25 ^b	1.74 ± 0.12 ^a	1.12 ± 0.07 ^b	1.20 ± 0.11 ^b	0.010
iNOS	1.00 ± 0.17 ^b	1.57 ± 0.23 ^a	0.99 ± 0.11 ^b	1.02 ± 0.06 ^b	0.033
IL-10	1.00 ± 0.20	0.84 ± 0.06	0.92 ± 0.12	0.96 ± 0.23	0.923

¹Data are means ± SEM, n = 8.²CON, control basal diet; LPS, LPS challenged broilers fed CON; LPS+T1, LPS challenged broilers fed CON supplemented with 5 g/kg taurine; LPS+T2, LPS challenged broilers fed CON supplemented with 7.5 g/kg taurine.³Expressed in arbitrary units. The expression of each target gene for the CON group was assigned a value of 1 and normalized against beta-actin.^{a,b}Means within the same row with no common superscript differ significantly (P < 0.05).

(Winiarska et al., 2009; Yang et al., 2010). Similarly, Xiao et al. (2018) showed that taurine reversed LPS-induced oxidative damage. These findings indicate that taurine improving hepatic antioxidant function may be due to the enhanced level of GSH and the increased activity of GSH-Px (Tabassum et al., 2006). We examined the expression levels of genes associated with hepatic redox status. The Nrf2 is a key regulator of cellular defenses against oxidative stress. In response to stress, Nrf2 is activated and translocates to the nucleus (Ma, 2013), resulting in the induction of many antioxidant genes including HO-1 (Hou et al., 2020). In our study, broilers injected with LPS leads to a sharp decline in the expression of genes, including *Nrf2*, *HO-1*, *SOD1*, *GSH-Px*, and *GCLC*, while taurine administration protects against LPS-induced liver injury by upregulating the mRNA levels of *Nrf2* and its downstream *HO-1*, *GCLC*, and *GSH-Px*. Alterations of *GCLC* gene expression in the liver of broilers, which partly, verify the capacity of taurine to enhance the synthesis of GSH and to replenish GSH supplies after LPS-induced depletion. Similarly, it has been reported that taurine could alleviate oxidative impairment caused by hydrogen peroxide via activating the Nrf2 pathway (Jang et al., 2009). Sun et al. (2018) observed that in human neuroblastoma cells, taurine also activated Nrf2 to protect against corticosterone-induced cell death. Therefore, we inferred that taurine improved the antioxidant properties of broilers by regulating the Nrf2 signaling pathway, increasing the activity of GSH-Px, enhancing the synthesis of GSH, and preventing lipid peroxidation.

Besides oxidative stress, substantial evidence have reported that the LPS stimulation could induce classical microglial activation through the activation of neutrophils and macrophages to produce a large number of proinflammatory cytokines that include TNF-α, IL-1β, and IFN-γ, which, in turn, releases free radicals and causes cell and tissue damage (Kobayashi et al., 2013; Zheng et al., 2016). TLR4 serves as a signaling receptor for LPS and is a transmembrane signal transporter that activates intracellular NF-κB (Lu et al., 2008). The NF-κB signaling pathway plays a crucial role in regulating inflammation and cell death (Sun et al., 2015). This molecule played a pivotal role in the development of inflammation by synthesizing and releasing proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and inducible proinflammatory enzymes (COX-2 and iNOS; Okorji et al., 2016), which, in turn, releases free radicals and causes cell and tissue damage.

Proinflammatory cytokines have crucial influences on systemic immune and inflammatory responses. Production of these proinflammatory cytokines results in a wide range of diseases, such as dyslipidemia (Chen et al., 2019), febrile (Liu et al., 2019), diarrhea (Wang et al., 2014), infection (Withanage et al., 2005), inflammation and oxidation (Li et al., 2014), enteric diseases (Swaggerty et al., 2016), immune dysfunction, further tissue damage, or even death (Barton, 2008). The result showed that LPS injection increased the concentrations of IL-1β, IL-6, and TNF-α, and these increases were inhibited by taurine supplementation. From the molecular point of view, the mRNA expression of *IL-1β*, *IL-6*, *TNF-α*, *COX-2*, and *iNOS* was significantly increased in the liver of LPS-challenged broilers, which was consistent with enhanced mRNA expression of TLR4. Our results were in accordance with the report of Mei et al. (2020) in which LPS increased the mRNA expression of NF-κB, *IL-1β*, *IL-6*, *IFN-γ*, and *iNOS* in the liver of broilers. Similar results were observed by Li et al. (2015) and Liu et al. (2015). Ali et al. (2020) reported that LPS treatment significantly increased the levels of cytokines (TNF-α, IL-1β, and IL-6) and enhanced NF-κB phosphorylation in mice. These results suggest that LPS induced change in cytokine patterns possibly by the activation of TLR4 and the regulation of NF-κB (Liu et al., 2015). Notably, in this study, we observed that taurine treatment decreased the mRNA expression of TLR4 in the liver of LPS-treated broilers along with an accompanying decrease in the production of *IL-1β*, *IL-6*, *TNF-α*, *COX-2*, and *iNOS*. In agreement with our findings, Lin et al. (2015) showed that taurine attenuates hepatic inflammation in chronic alcohol-fed rats through the inhibition of TLR4 signaling to reduce the mRNA expression of *iNOS*, *TNF-α*, *IL-6*, and *IL-1β*. Marcinkiewicz and Kontny (2014) found that taurine inhibited the production of proinflammatory cytokines to provide anti-inflammatory effects and protected cells from the cytotoxic effects of inflammation. These results suggest that a possible mechanism by which taurine mitigates hepatic inflammation is associated with the regulation of the TLR4 and NF-κB pathway. The HO-1 plays an important role in oxidative stress and inflammation (Wagener et al., 2003), which can reduce the synthesis of proinflammatory heme proteins such as *COX-2* and *iNOS* (Ryter et al., 2002). Furthermore, Lee et al. (2008) demonstrated that the production of proinflammatory cytokines was associated with HO-1 deficiency in the HO-1 knockout mice model. Taken together, both our findings and

aforementioned investigations show that taurine alleviates the anti-inflammatory process through the regulation of the TLR-4/NF- κ B pathway and promotes the mRNA expression of HO-1 to reduce the large-scale production of proinflammatory cytokines after the LPS challenge.

In conclusion, the present study demonstrates that dietary taurine supplementation improves ADG, enhances hepatic antioxidant status, and ameliorates inflammation in the liver of broiler chickens under immunological stress.

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships and that there was no conflict of interest.

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