Effects of *Glycyrrhiza* polysaccharide in diet on growth performance, serum antioxidant capacity, and biochemistry of broilers

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ABSTRACT In the present study, we analyzed the effects of *Glycyrrhiza* polysaccharide (GCP) on growth performance, serum antioxidant capacity, and biochemistry of broilers. A total of 600, one-day-old AA broilers randomly divided into 5 treatment groups with 6 replicate pens of 20 birds per cage received dietary supplementation with GCP (0, 200, 500, 1,000, and 1,500 mg/kg) for 42 d. The supplementation of GCP linearly decreased \( P < 0.05 \) feed conversion rate on day 22 to 42. Dietary supplementation with GCP reduced \( P < 0.05 \) serum total cholesterol on day 21 and 42 and linearly improved \( P < 0.05 \) albumin and high-density lipoprotein cholesterol. Dietary supplementation with 1,000 or 1,500 mg/kg GCP significantly increased \( P < 0.05 \) serum total superoxide dismutase (T-SOD) activity on day 21 and 42 and reduced \( P < 0.05 \) serum malondialdehyde content on 21 d. Dietary supplementation with 1,000 or 1,500 mg/kg GCP significantly improved \( P < 0.05 \) interleukin-1β (IL-1β) and interferon-γ (IFN-γ) expressions in liver on day 21 and 42. At the end of the experiment, we randomly selected 20 broilers from 3 treatment groups (0, 1,000, and 1,500 mg/kg), respectively, to perform an lipopolysaccharide (LPS)-induced acute stress experiment. The 60 broilers were divided into 6 treatment groups with 10 birds per cage. The experiment was designed as a 3!2 factorial arrangement with GCP (0, 1,000, or 1,500 mg/kg) and LPS (injection of saline or 1 mg/kg body weight) levels as treatments. When the grouping was finished, the broilers were immediately intraperitoneally injected with LPS or normal saline. Six hours after challenged, serum antioxidant and liver immunity were analyzed. The results showed that dietary GCP prevented LPS-induced reductions in T-SOD activity and increases in malonaldehyde content \( P < 0.05 \). Also, dietary GCP supplementation mitigated the LPS-induced increase in IL-1β and IFN-γ in the liver. Supplementation with 1,500 mg/kg GCP showed the most optimal effect in broilers. GCP has the potential to be used as feed additive in broilers.

**Key words:** *Glycyrrhiza* polysaccharide, antioxidant, biochemistry

INTRODUCTION

Current intensive poultry breeding practices are plagued with huge losses because of the presence of stressful conditions that reduce growth and immune performance consequently facilitating the spread of diseases. Although antibiotic growth promoters have been used in animal husbandry for long to solve these questions, they can bring adverse effects to consumers (Cheng et al., 2014; Garcia-Migura et al., 2014). Hence, new approaches that are beneficial for the consumers as well as for the animals are highly needed.

Polysaccharides—high-molecular-weight long-chain carbohydrates composed of monosaccharides, such as aldose or ketose, bound together by glycosidic linkages—are widely present in plants, animals, and microorganisms (Ayeka et al., 2016; Huang et al., 2017). Plant polysaccharides are rich in varieties, wide in sources, and easily extracted from plant flower, seeds, roots, stems, and leaves (Ao and Kim, 2020). It is generally considered that plant polysaccharides derived from Chinese herbs have remarkable pharmacological capacities...
including, among others, immunomodulatory, antioxidant, and antitumor functions (Xiong et al., 2019; Chen et al., 2016; Li et al., 2019).

Licorice (*Glycyrrhiza glabra*) is a popular traditional medicinal plant that belongs to the legume family. In traditional Chinese medicine, it invigorates spleen and qi, clears heat and toxic materials, and is widely used in various traditional Chinese medicine prescriptions (Jiang et al., 2020). Based on phytochemical analyses, licorice contains multiple components including triterpene saponins, flavonoids, sugar, and starch (Asl and Hosseinzadeh, 2008; Zhang and Ye, 2009). However, while certain licorice extracts have multiple biological activities (Pastorino et al., 2018), the individual use of them as food additive in the poultry breeding industry is scarcely described (Alagawany et al., 2019). Glycyrrhiza polysaccharide (GCP) is one of the active ingredients of licorice, and its biological activity is closely connected with the spatial structure of alpha-D-pyran polysaccharide (Zeng et al., 2015). Increasing evidence from pharmacological and clinical studies suggests that GCP can exert immunomodulatory, antitumor, wound repair and scar inhibition, antioxidant, and anticough effects (Chen et al., 2013; Nosalova et al., 2013; Wu et al., 2017; Lian et al., 2018). Because of its wide range of biological properties, GCP has gained increased attention and has the potential as food additive in the poultry breeding industry instead of antibiotic growth promoters. In the present study, we analyzed the effect of GCP on growth performance and antioxidant capacity in broilers as well as its effect on antioxidant capacity and liver immunity after lipopolysaccharide (LPS)-induced acute stress. The data obtained in this study might provide a sound base for the potential use of GCP as a food additive in poultry.

**MATERIALS AND METHODS**

All procedures were approved by the Animal Care and Use Committee of Henan University of Science and Technology.

**Experiment 1**

**Birds and Experimental Treatments** Six hundred one-day-old male AA broilers were purchased from a commercial broiler hatchery in Luoyang, China. The birds were examined on arrival for signs of disability and early illness. All healthy chicks with similar average body weight (37 ± 0.5 g) were randomly assigned to 5 experimental diets based on the amount of GCP (Lansela, Luoyang, China) added to the basic diet consisting of corn-soybean meal feed. The dietary treatments with GCP were as follows: 1) control (basal diet), 2) control + 200 mg/kg GCP, 3) control + 500 mg/kg GCP, 4) control + 1,000 mg/kg GCP, and 5) control + 1,500 mg/kg GCP. There were 6 replicates per treatment of 20 birds per cage. The broilers were vaccinated with Newcastle disease vaccine and the infectious bursal vaccine on day 7 and 14 of the experiment, respectively. The entire experiment lasted for 42 d. Broilers were placed in the terrace of a mechanical ventilated room under controlled temperature, humidity, and lighting conditions. They were raised in three-layer ladder cages. Every day, all birds had free access to food and water. Each cage had a plastic drinking trough, and water and feed were administered every morning, noon, and evening. The temperature of the feeding room was kept at 33 ± 1°C in the initial stage (day 1–5) and then shifted to 22 ± 1°C (day 5–42) for the remaining experiment duration. From the beginning to the end of the experiment, the humidity was maintained between 40 and 60%, natural ventilation was maintained, and illumination was maintained for 17 h every day. In general, the housing and care of birds was in line with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (third edition, 2010).

**Preparation of Experimental Diets** To prepare the experimental diets, the required amount of GCP per 1-kg basal diet of each group was weighed out according to the proportion, and GCP was dissolved in 300 mL of distilled water and then evenly sprayed on the basal diet. Then, the prepared mixture was dried and stored in a dry place. Basal diet complied with the required nutrient level for birds as defined by the NRC (1994) (Table 1).

**Growth Performance** On day 0, 21, and 42, body weight and daily feed intake of birds in each cage were recorded to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion rate (FCR) of each group. Chicken mortality was recorded during the experiment for each treatment group.

| Items       | Content         |       |       |
|-------------|-----------------|-------|-------|
| Ingredients |                 |       |       |
| Corn        | 58.36           | 64.22 |       |
| Soybean meal| 31.25           | 27.08 |       |
| Soybean oil | 4.00            | 3.00  |       |
| Corn gluten meal | 2.80 | 2.5       |
| Limestone   | 1.29            | 1.42  |       |
| CaHPO4      | 1.49            | 1.04  |       |
| NaCl        | 0.30            | 0.3   |       |
| L-Lys       | 0.03            | 0.03  |       |
| DL-Met      | 0.23            | 0.16  |       |
| Premix^2    | 0.25            | 0.25  |       |
| Total       | 100.00          | 100.00|       |
| Nutrient levels^2 |       |       |
| ME (MJ/kg)  | 12.65           | 12.67 |       |
| CP          | 21.00           | 19.00 |       |
| Ash         | 6.0             | 8.0   |       |
| Ca          | 9.00            | 1.10  |       |
| TP          | 0.40            | 0.45  |       |
| Met         | 1.10            | 1.04  |       |
| Lys         | 0.52            | 0.90  |       |

^1The premix provided the following per kg of diets: Mn(MnSO4·H2O) 60 mg; Fe(FeSO4·H2O) 60.5 mg; Zn(ZnSO4·7H2O) 88 mg; Cu(CuSO4·5H2O) 8.8 mg; MnO2 (0.7 mg; Se(Na2SeO3) 0.288 mg; VA 11500 IU; VA 3500 IU; VE 90 mg; VK3 3 mg; VB1 3.38 mg; VB2 9.00 mg; VB6 8.96 mg; VB12 0.025 mg; choline chloride 800 mg; calcium panthenate 13 mg; niacin 45 mg; biotin 0.08 mg; folic acid 1.20 mg.

^2Nutrient levels were calculated values.
recorded daily after which performance parameters were corrected for mortality.

**Serum Sample Measurements** Six birds from each replicate were randomly selected from each cage on day 21 and 42, 5 mL of blood was collected through the wing veins in 10 mL heparin tubes and centrifuged at 3,000 g for 15 min at 4°C, and the serum was separated and stored at −20°C until further analysis of serum indicators. The levels of total protein (TP), albumin (ALB), globulin (GLB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by using an automatic clinical chemistry analyzer (HITACHI 912; Hitachi, Tokyo, Japan). Levels of total superoxide dismutase (T-SOD), glutathione (GSH), and malonaldehyde (MDA) in serum were determined using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) via an automated spectrophotometric analyzer (MAPADA, Shanghai, China). All steps were carried out according to the manufacturer’s procedures.

**Real-Time quantitative PCR analysis** After blood collection, the same birds were sacrificed by cervical dislocation and exsanguinated after which the liver was removed to quantify the mRNA concentrations of IL-1β, IL-2, and IFN-γ by quantitative real-time PCR. β-actin was used as a reference gene for normalization. Additional information of the primers used in this study is shown in Table 2. All primers were designed by using chicken sequences in GeneBank and synthesized by a biotechnology company (Sagon Biotech, Shanghai, China). The total RNA of liver tissue samples was extracted with TRIzol according to the manufacturer’s instructions (Solarbio, Beijing, China), and the integrity of the RNA was assessed by visualization on agarose gel. RNA concentration and purity were determined by using a Nanodrop ND-2000 spectrophotometer (Thermo Scientific, Ottawa, ON, Canada). cDNA synthesis was carried out according to the instructions of the kit manufacturer (Takara Biotechnology, Dalian, China). Quantitative real-time PCR was performed on a CFX Connect Real-Time PCR Detection System (Bio-Rad) using a SYBR Green PCR kit (Takara Biotechnology, Dalian, China). Results were calculated using the 2−ΔΔCT method (Livak and Schmittgen, 2001).

**Experiment 2**

At the end of the experiment 1, we concluded that supplementation of 1,000 and 1,500 mg/kg GCP in the feed was effective, although without obvious differences between the 2 groups. Thus, we randomly selected 20 broilers from GCP groups supplemented with 0, 1,000, and 1,500 mg/kg, respectively, to perform an LPS-induced acute stress experiment. The 60 broilers were divided into 6 treatment groups with 10 birds per cage. The experiment was designed as a 3 × 2 factorial arrangement with GCP treatments (supplementation with 0, 1,000, or 1,500 mg/kg) and LPS challenge (injection of saline 9 g/L w/v) or LPS (1 mg/kg body weight). LPS was dissolved in sterile saline to generate a concentration of 500 mg/mL. Broilers received intraperitoneal injection of LPS or equivalent amount of normal saline. All experimental conditions are consistent with the first experiment. Six hours after challenge, the blood was collected, and the birds were killed. The antioxidant capacity of serum and liver gene expression in broilers were determined by the same methods as described for experiment 1.

**Statistical Analysis** All data were recorded in Excel and analyzed using the general linear model procedures of SPSS19.0 software (SPSS Inc., Chicago, IL). Linear and quadratic contrasts were used to assess the effects of increasing dietary concentrations of supplemental GCP by orthogonal polynomials in experiment 1. The serum antioxidant and gene expression levels obtained were analyzed by 3 × 2 factor arrangement in experiment 2, and the main effects were GCP treatment and LPS stimulation. Comparisons between the different groups were performed with Duncan’s multiple comparison test. The variability of the data was expressed as the pooled standard error of the mean, and P < 0.05 was considered statistical significant.

**RESULTS**

**Experiment 1**

**Broiler Growth Performance** The effect of different concentrations of GCP on the growth performance of broilers is shown in Table 3. Although ADG and ADFI enhanced with increasing doses of GCP, it failed to reach statistical significance (P > 0.05) in dietary treatments at all time-points considered. At all time-points considered, supplementation of the diet with GCP resulted in lower ADFI. From day 22 to 42, a linear reduction (P < 0.05) of ADFI was observed.

**Serum Protein and Lipid Levels** The effect of different concentrations of GCP on blood protein and lipid levels is shown in Table 4. On day 21, supplementation of the diet with GCP linearly reduced (P < 0.05) serum TG; however, no differences were observed (P >

| Table 2. Primers for RT-qPCR analysis. |
|---|---|---|---|
| Gene | Accession number | Forward primers sequences (5′-3′) | Reverse primers sequences (5′-3′) | Product size (bp) |
| β-actin | NM 205,518.1 | ACCGCAAATGCTTCTAAACC | ATAAAGCCATGCAATCTCG | 113 |
| IL-1β | NM204524 | CAGGCTCACGGAGGAGGCTT | ACTGTTGTTGCTGCTGAACTCC | 84 |
| IL-2 | GU 119,890.1 | CACACCAACTGAGACCTG | TCTTGCATTCAGTCCGGTGTT | 188 |
| IFN-γ | NM205149 | ACTGACCCGATGTTGTTGATG | TGCCATTAGCAATTGCATCTCG | 288 |

Abbreviations: IL-1β, interleukin 1 beta; IL-2, interleukin 2; IFN-γ, interferon-γ.
0.05) in serum TP, ALB, GLB, total cholesterol, HDL-C, or low-density lipoprotein cholesterol among treatments. On day 42, the supplementation of GCP linearly improved (P, 0.05) ALB and HDL-C, whereas it reduced (P, 0.05) blood TG. Although dietary GCP supplementation also had an effect on the other parameters under study, it failed to reach (P > 0.05) statistical significance among groups.

**Serum AST and ALT** The effect of dietary supplementation with increasing concentrations of GCP on blood AST and ALT is shown in Table 5. On day 21 or 42, a general dose-dependent decrease in serum AST and ALT levels was observed; however, they were not statistically different (P > 0.05) among the treatments.

**Antioxidant Capacity** The effect of GCP on serum oxidative capacity is shown in Table 6. On day 21, compared with the control group, supplementation of diets with 500, 1,000, or 1,500 mg/kg GCP significantly increased GSH (P < 0.05) and T-SOD (P < 0.05) levels, and a significant dose-dependent linearly increase in GSH and T-SOD levels was observed in dietary GCP supplementation birds. No statistical significance was observed when comparing the GSH and T-SOD levels between the birds treated with 1,000 and 1,500 mg/kg GCP, respectively. MDA was lower in diets supplemented with GCP birds than in control diet, although it did not reach statistical significance (P > 0.05). On day 42, significantly higher levels of T-SOD (P < 0.05) paralleled with significantly lower levels of MDA (P < 0.05) were observed in birds treated with 500, 1,000, and 1,500 mg/kg GCP compared with control diet, and they all showed a linear relationship among groups. No statistical significance was observed when comparing the T-SOD and MDA levels between the dietary 1,000 and 1,500 mg/kg GCP supplementation groups, respectively. Compared with the control diet, differences in GSH failed to reach statistically significance in the GCP-supplemented groups (P > 0.05).

**Gene Expression** The effect of GCP on mRNA expression levels of inflammatory markers in the liver is shown in Table 3.
in Figure 1. On day 21, significantly higher levels of IL-1β were observed in birds fed a GCP-supplemented diet than in those fed the control diet (P < 0.05). Compared with control diet, supplementation of diets with 1,000 and 1,500 mg/kg GCP resulted in increased levels of IL-1β; however, no difference was observed between the 2 experimental groups. Although higher levels of IL-2 and IFN-γ were observed in GCP-treated birds than in those fed control diet, this failed to reach statistical significance (P > 0.05). At day 42, supplementation of diets with 1,000 and 1,500 mg/kg GCP resulted in significantly higher levels of IL-2 and IFN-γ mRNA than control diet (P < 0.05). Differences in IL-1β levels observed in the different groups failed to reach statistical significance (P > 0.05).

**Experiment 2**

**Serum Antioxidant Capacity** The data of the serum antioxidant capacity are shown in Table 7. Upon challenge with LPS, supplementation of diets with 1,000 or 1,500 mg/kg GCP significantly increased (P < 0.05) the serum enzyme activity of GSH but failed to significantly (P > 0.05) change the serum T-SOD activity and MDA content compared with the nonsupplemented group. Also, the serum GSH, T-SOD, and MDA changes in the unchallenged groups were identical to those of the challenged groups. In animals fed the control diet, LPS challenge resulted in significantly increased (P < 0.05) MDA content while no significant decrease (P > 0.05) was observed in the serum enzyme activities of GSH and T-SOD compared with the unchallenged group. Similarly, in animals fed diets supplemented with 1,000 or 1,500 mg/kg GCP, LPS challenge resulted in a significantly increased (P < 0.05) MDA content while no significant decrease (P > 0.05) was observed in the serum activities of GSH activity and T-SOD compared with the unchallenged group. This result suggested that supplementation of the diet with GCP can alleviate the LPS-induced decrease in antioxidant capacity. In both the challenged or unchallenged groups, dietary supplementation with 1,500 mg/kg GCP showed the most positive effects.

**Gene Expression** The effect of GCP on LPS-induced changes in the levels of inflammatory markers is shown in Figure 2. When the broilers were challenged with LPS, dietary treatment with 1,000 or 1,500 mg/kg GCP, significantly reductions (P < 0.05) in liver IL-1β and IFN-γ mRNA levels but not (P > 0.05) in IL-2 mRNA expression were observed compared with the non-supplemented group. Obviously, supplementation of the diet with 1,500 mg/kg GCP resulted in lower liver IL-1β and IFN-γ expressions (P < 0.05) in the challenged groups. However, there were no significant differences (P > 0.05) in the liver IL-1β, IL-2, and IFN-γ mRNA expressions among 0, 1,000, and 1,500 mg/kg GCP in the unchallenged groups. When fed the diet supplemented with 0, 1,000, and 1,500 mg/kg GCP, respectively, LPS significantly increased (P < 0.05) liver IL-1β and IFN-γ expressions but not (P > 0.05) IL-2 compared with the unchallenged group.

**DISCUSSION**

In intensive farming, the growth of broilers is, among others, affected by feeding density, temperature, and

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**Table 5.** Effects of dietary Glycyrrhiza polysaccharide (GCP) on blood AST and ALT in broilers.

| Item          | 0          | 200        | 500        | 1,000      | 1,500      | SEM       | P value   |
|---------------|------------|------------|------------|------------|------------|-----------|-----------|
|               | Levels of dietary GCP (mg/kg) |            |            |            |            |           |           |
| Day 21        |            | Linear     | Quadratic  |            |            |           |           |
| AST (IU/L)    | 331.00     | 319.50     | 310.25     | 277.00     | 293.00     | 25.96     | 0.305     | 0.436     |
| ALT (IU/L)    | 17.45      | 16.20      | 13.95      | 10.45      | 12.70      | 2.45      | 0.121     | 0.391     |
| Day 42        |            | Linear     | Quadratic  |            |            |           |           |
| AST (IU/L)    | 384.25     | 345.75     | 332.00     | 299.75     | 312.25     | 73.85     | 0.348     | 0.083     |
| ALT (IU/L)    | 20.05      | 18.55      | 17.55      | 15.30      | 15.30      | 1.70      | 0.114     | 0.625     |

**Table 6.** Effects of dietary GCP on serum oxidative capacity of broilers at day 21 and 42 in the whole experiment.

| Item          | 0          | 200        | 500        | 1,000      | 1,500      | SEM       | P value   |
|---------------|------------|------------|------------|------------|------------|-----------|-----------|
|               | Levels of dietary GCP (mg/kg) |            |            |            |            |           |           |
| Day 21        |            | Linear     | Quadratic  |            |            |           |           |
| GSH (μmol/L)  | 15.18 b    | 15.26 b    | 16.46 b    | 16.79 b    | 16.76 a    | 0.53      | 0.037     | 0.263     |
| T-SOD (U/mL)  | 210.35 b   | 215.81 b   | 227.95 c   | 238.24 a,c | 247.67 a   | 7.37      | <0.001    | 0.623     |
| MDA (nmol/m)  | 4.34       | 4.30       | 4.26       | 4.21       | 4.10       | 0.09      | 0.069     | 0.510     |
| Day 42        |            | Linear     | Quadratic  |            |            |           |           |
| GSH (μmol/L)  | 15.88      | 16.54      | 17.12      | 17.28      | 17.14      | 0.60      | 0.067     | 0.427     |
| T-SOD (U/mL)  | 217.63 b   | 227.33 b   | 245.99 a   | 250.37 a,c | 247.69 a   | 10.09     | 0.021     | 0.170     |
| MDA (nmol/m)  | 4.48 a     | 4.30 b     | 4.22 b,c   | 4.17 b,c   | 4.00 c     | 0.12      | 0.002     | 0.848     |

**Abbreviations:** GCP, Glycyrrhiza polysaccharide; GSH, glutathione; MDA, malonaldehyde; T-SOD, superoxide dismutase.

**SEM:** Means with different superscripts within the same row differ significantly (P < 0.05).
pathogenic microorganisms (Zuowei et al., 2011; Répérant et al., 2012; Liu et al., 2016). A variety of Chinese herbal extracts can promote the growth of poultry (Wallace et al., 2010; Diaz-Sanchez et al., 2015). Experiments exploring the many known biological activities GCP were mainly performed in mice or human cell lines with only a few reports describing the efficacy of GCP in poultry. Hence, we evaluated the effect of dietary supplementation with GCP on the growth performance, serum antioxidant capacity, and biochemistry immunity in broilers.

In experiment 1 of the present study, it was shown that diets supplemented with 200 mg/kg to 1,500 mg/kg GCP improved ADG, ADFI, and FCR to different degrees when considering all time-points. Similar results were described in previous studies on Chinese herbal polysaccharides. For example, Deng et al. (2015) demonstrated that dietary supplementation with polysaccharide from mycelia of Cordyceps sinensis improves the BWG and health of pacific white shrimp. Also, Yang et al. (2019a) reported that both Astragalus polysaccharide and ginseng polysaccharide improves the ADG and FCR and reduced diarrhea rate of piglets. In poultry, polysaccharides were shown to positively influence growth performance (Guo et al., 2004; Abdullahi et al., 2016; Ao and Kim, 2020), but this finding was not confirmed by Chen et al. (2003). Different effects on production performance may be associated with the source of polysaccharide, supplementation dosages, and animal age. Our present study indicated that supplementation of the diet with GCP improved growth performance of broilers, and this finding may be related to GCP reduced FCR. The improvement of growth performance may be related to the improvement of digestibility. It was found that supplementation of the diet with herbal polysaccharides can increase the digestibility of dry matter and nitrogen in broilers (Park and Kim, 2020). Besides, some studies have reported that the activity of digestive enzymes was increased by herbal extracts (Zahran et al., 2014; Long et al., 2020). In addition, herbal extracts may also increase digestibility by changing intestinal flora (Cross et al., 2007; Liu et al., 2018; Hesabi Nameghi et al., 2019). Therefore, additional studies focusing on the effects of GCP on growth performance of chickens are still needed.

The antioxidant system maintains a balance between the production and elimination of free radicals through, among others, the activity of antioxidant enzymes such as GSH, SOD, and CAT (Li et al., 2016b). The degree of lipid peroxidation is a marker of oxidative damage and can be estimated through MDA levels (Zhao et al., 2014). Previous studies confirmed that herbal polysaccharides enhance the antioxidant capacity of animals. For example, Xue et al. (2009) indicated that Achyranthes bidentata polysaccharides decrease serum MDA content and improve the activities of GPx and SOD in rats exposed to streptozotocin-induced oxidative stress. Also, Qiu et al. (2014) reported enhanced antioxidative

| Table 7. Effects of GCP on serum antioxidant capacity of broilers challenged with lipopolysaccharide. |
|---------------------------------------------------------------|
| **Item** | **Saline** | **LPS** | **Saline** | **LPS** | **Saline** | **LPS** | **SEM** | **GCP** | **LPS** | **Interaction** |
| GSH (μmol/L) | 15.61 | 14.06 | 15.74 | 15.50 | 16.16 | 15.68 | 0.65 | 0.128 | 0.092 | 0.381 |
| T-SOD (U/mL) | 205.39<sup>a</sup> | 199.33<sup>a</sup> | 232.88<sup>b</sup> | 221.31<sup>b</sup> | 244.39<sup>b</sup> | 227.33<sup>b</sup> | 6.15 | 0.001 | 0.017 | 0.492 |
| MDA (nmol/mL) | 4.35<sup>a</sup> | 5.57<sup>b</sup> | 4.06<sup>b</sup> | 5.08<sup>b</sup> | 4.02<sup>a</sup> | 5.00<sup>a</sup> | 0.23 | 0.061 | <0.001 | 0.746 |

<sup>a,b</sup>Means with no common superscript within each row are significantly (<i>P</i> &lt; 0.05) different.

Abbreviations: GCP, Glycyrrhiza polysaccharide; GSH, glutathione; LPS, lipopolysaccharide; MDA, malonaldehyde; T-SOD, superoxide dismutase.
capacity of broilers upon injection of *Lycium barbarum* polysaccharide and demonstrated increased levels of GPx and SOD paralleled with reduced levels of MDA. In addition, administration of *Astragalus* polysaccharide to mice with carbon tetrachloride–induced liver injury resulted in an enhanced antioxidant capacity of the liver and increased GSH-PX and SOD levels together with reduced MDA content (Hamid et al., 2017). Also, *Agaricus blazei Murill* polysaccharide significantly increased the activity of SOD and GPx and decreased the content of MDA in chicken serum (Lv et al., 2018). Our current results indicated that GCP improves the antioxidant capacity of broilers by increasing the GSH and T-SOD activities in serum while reducing the MDA content, potentially due to the stimulatory effect of herbal polysaccharides on the production of reactive oxygen species (Li and Li, 1997).

Serum contains a variety of proteins that play an important role in animal physiological and pathological activities (Trefts et al., 2017). The nutritional status of an organism is reflected by its TP levels (Kholif et al., 2014). Meanwhile, GLB and ALB play a role in either stimulating or moderating immune activation depending on the condition of the animal (Laan et al., 1998; Sahloul et al., 2010). In the present study, we analyzed the level of nutrition-related serum lipid metabolism. The levels of total cholesterol, TG, HDL, and LDL are important markers of lipid metabolism of the body and, as such, reflect the nutritional and immune status. The effect of the modulatory effect of herbal extracts on serum protein and lipid levels in broilers was previously described (Akbari and Torki, 2014; Li et al., 2016a; He et al., 2019). In the present study, we showed that dietary supplementation with GCP had beneficial effects on serum protein and lipids levels, especially for the groups supplemented with 1,000 or 1,500 mg/kg. By improving the ability of protein synthesis and metabolic energy, GCP thus promotes the utilization of protein and improves the performance of the organism. We also showed that GCP significantly reduced serum TG levels at day 21 and 42 and HDL-C levels at day 42. These data indicate that GCP might oxidize excessive fat and as such promote fat metabolism providing the energy needed for enhanced protein synthesis and growth of the animals.

The liver plays an important role in regulating metabolism (Guo et al., 2017; Liu et al., 2019b). Therefore, growth performance can be affected by the liver health. The content of aminotransferases ALT and AST—the most sensitive liver enzymes—in serum reflects liver health. In our study, the values of ALT and AST in GCP-supplemented diet are lower than those in control diet. In addition, the liver is an important mediator of the immune response. It resists the invasion of viruses and bacteria, closely controls the secretion of cytokines,
participates in regulation of immunity and inflammatory response, regulates tissue and matrix repair, and is involved in the removal of aging and degenerative blood and tumor cells (Kubes and Jenne, 2018). Hence, in order to further evaluate the health status of the liver, we analyzed the common proinflammatory factors IL-1β, IL-2, and IFN-γ. IL-1β and IL-2 play a role in the activation and regulation of the immune system depending on the pathophysiological condition (Wang et al., 2014). IFN-γ is critical for host defense against a variety of pathogens (Wang et al., 2019). In the present study, we showed that GCP increased the expression of IL-1β, IL-2, and IFN-γ at both day 21 and 42. To a certain level, an increase of those proinflammatory factors has a beneficial effect on the overall immune function of the body. As such, GCP enhances the liver health which, subsequently, improves animal growth performance.

Considering that in experiment 1, dietary supplementation with 1,000 or 1,500 mg/kg GCP had a better effect on liver index, and these concentrations were selected for the next experiment. In the second part of our study, we analyzed the effect of GCP on LPS-induced acute stress. Exposure of an organism to LPS—a gram-negative bacteria-derived endotoxin—initiates an immune and oxidative stress response (Ngkelo et al., 2012; Yücel et al., 2017; Wei et al., 2019). Thus, inhibition of an LPS-induced stress response is considered a key strategy to prevent disease and improve production performance. In the present study, the LPS-induced oxidative stress and inflammatory response were related to increased MDA levels. Treatment of broilers with 1 mg/kg LPS for 6 h resulted in significantly increased MDA serum levels accompanied with reduced GSH and T-SOD levels; however, dietary GCP supplementation alleviated the inhibitory effect of LPS, especially at a concentration of 1,500 mg/kg. The increase of serum antioxidant capacity of broilers may be related to the absorption and metabolism of GCP into the blood circulation. Over the past decade, researchers have tried to investigate natural sources to find new feed additives to prevent and treat oxidative stress in livestock (Abu and Ibrahim, 2018). Our findings provide strong supporting evidence that GCP may be a potential antioxidant.

Liver plays an important role in resisting bacteria and their toxic products, such as LPS (Yi et al., 2014). Upon activation, TLR4—the main recognition receptor of LPS—activates the NF-κB pathway resulting in induction of, among others, the proinflammatory cytokines IL-β, IL-2, IL-6, and TNF-α (Takeuchi et al., 1999; Chi et al., 2015). Meanwhile, LPS-induced oxidative stress equally activates an immune response (Yuan et al., 2016). Previous studies have demonstrated that various herb extracts have the potential to alleviate liver immune stress induced by LPS. For example, Yang et al. (2019c) reported that supplementation with leonurine hydrochloride can reduce the expression of inflammatory factors mediated by TLR4/NF-κB signal pathway, thus alleviating LPS-induced in broilers. Also, berberine resulted in reduced LPS-induced TNF-α and IL-1β levels in the liver (Yang et al., 2019b). Moreover, broilers fed diets supplemented with carvacrol essential oils and challenged by LPS had decreased proinflammatory cytokines, including IL-1β, IL-6, TNF-α, and IL-2 (Liu et al., 2019a). In the present study, we evidenced that GCP supplements prevented an LPS-induced increase in IL-1β and IFN-γ expression levels, especially at a concentration of 1,500 mg/kg, while maintaining the physiological balance suggesting that GCP efficiently reduced the LPS-induced inflammatory response.

**CONCLUSIONS**

In the present study, we reported that dietary supplementation with GCP promoted the growth and development of broilers and improved serum antioxidant and serum biochemistry. In addition, GCP improved the LPS-induced decline in serum antioxidant capacity and liver immune response. Taken together, our results provide a rationale for the application of GCP as a feed additive in broilers.

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**DISCLOSURES**

In this experiment, the authors asserted that they had no conflict of interest.

**REFERENCES**

Abdullahi, A. Y., S. Kallon, X. Yu, Y. Zhang, and G. Li. 2016. Vaccination with Astragalus and ginseng polysaccharides improves immune response of chickens against H5N1 Avian Influenza Virus. Biomed. Res. Int. 2016:1510264.

Abu, H. S. H., and S. A. Ibrahim. 2018. Effect of dietary polyphenol-rich grape seed on growth performance, antioxidant capacity and ileal microflora in broiler chicks. J. Anim. Physiol. Anim. Nutr. 102:268–275.

Akbari, M., and M. Torki. 2014. Effects of dietary chromium picolinate and peppermint essential oil on growth performance and blood biochemical parameters of broiler chicks reared under heat stress conditions. Int. J. Biometeorol. 5:1383–1391.

Alagawany, M., S. S. Elnesr, M. R. Farag, M. E. Abd El-Hack, A. F. Khafaga, A. E. Taha, R. Tiwari, M. I. Yatoo, P. Bhatt, G. Marapan, and K. Dhama. 2019. Use of licorice (Glycyrrhiza glabra) herb as a feed additive in poultry: current Knowledge and Prospects. Animals (Basel) 9:536.

Ao, X., and I. H. Kim. 2020. Effects of Achyranthes bidentata polysaccharides on performance, immunity, antioxidant capacity, and meat quality in Pekin ducks. Poult. Sci. 99:4884–4891.

Asl, M. N., and H. Hosseinzadeh. 2008. Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. Phytother Res. 22:709–724.

Ayeka, P. A., Y. Bian, P. G. Mwitari, X. Chu, Y. Zhang, R. Uzayienega, and E. O. Otachi. 2016. Immunomodulatory and anticancer potential of Gan cao (Glycyrrhiza uralensis Fisch.) polysaccharides by CT-26 colon carcinoma cell growth inhibition and cytokine IL-7 upregulation in vitro. BMC Complement Altern. Med. 16:206.
Kubes, P., and C. Jenne. 2018. Immune responses in the liver. Annu. Rev. Immunol. 36:247–277.

Cheng, G., H. Hao, S. Xie, X. Wang, M. Dai, L. Huang, and Z. Yuan. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front Microbiol. 5:217.

Chen, J., X. Jin, J. Chen, and C. Liu. 2013. Glycyrrhiza polysaccharide induces apoptosis and inhibits proliferation of human hepatocellular carcinoma cells by blocking PI3K/AKT signal pathway. Tumour Biol. 34:1381–1389.

Chen, H. L., D. F. Li, B. Y. Chang, L. M. Gong, J. G. Dai, and G. F. Yi. 2003. Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers. Poult. Sci. 82:364–370.

Chen, J., X. Q. Zhu, L. Yang, Y. Luo, M. Y. Wang, X. T. Liu, K. X. Liang, and X. L. Gu. 2016. Effect of Glycyrrhiza uralensis Fisch polysaccharide on growth performance and immunologic function in mice in Ural City, Xinjiang, Asian Pac. J. Trop. Med. 9:1078–1083.

Chi, X., X. Wei, W. Gao, J. Guan, X. Yu, Y. Wang, X. Li, and J. Cui. 2015. Dexamethasone ameliorates acute lung injury following orthotopic autologous liver transplantation in rats probably by inhibiting Toll-like receptor 4-nuclear factor kappa B signaling. J. Transl Med. 13:190.

Cross, D. E., R. M. McDevitt, K. Hillman, and T. Acamovic. 2007. The role of peroxisome proliferator-activated receptor-alpha in the regulation of hepatic insulin sensitivity. J. Neuroendocrinol. 19:62–72.

Guo, F. C., B. A. Williams, R. P. Kwakkel, H. S. Li, X. P. Li, J. Y. Luo, W. K. Li, and M. W. Versteegen. 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci. 83:175–182.

Deng, B. W. Z., W. Tao, and E. Al. 2015. Effects of polysaccharides from mycelia of cordyceps sinensis on growth performance, immunity and antioxidant indicators of the white shrimp litopenaeus vannamei. Aquac. Nutr. 21:173–179.

Diaz-Sanchez, S., D. D’Souza, D. Biswas, and I. Hanning. 2007. Botanical alternatives to antibiotics for use in organic poultry production. Poult. Sci. 94:1419–1430.

Garcia-Migura, L., R. S. Hendriksen, L. Fraile, and F. M. Aarestrup. 2014. Antimicrobial resistance of zoonotic and commensal bacteria in Europe: the missing link between consumption and resistance in veterinary medicine. Vet. Microbiol. 170:1–9.

Guo, F. C., B. A. Williams, R. P. Kwakkel, H. S. Li, X. P. Li, J. Y. Luo, W. K. Li, and M. W. Versteegen. 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci. 83:175–182.

Gao, R., X. Xu, Y. Lu, and X. Xie. 2017. Physiological oxygen tension reduction increases hepatocyte dedifferentiation in vitro culture. Sci. Rep. 7:5923.

Hamid, M., D. Liu, Y. Ablulrahim, Y. Liu, G. Qian, A. Khan, F. Gan, and K. Huang. 2017. Amelioration of CCl4-induced liver injury in rats by selenizing Astragalus polysaccharides: role of pro-inflammatory cytokines, oxidative stress and hepatic stellate cells. Res. Vet. Sci. 114:202–211.

He, S., S. Li, M. A. Arowolo, Q. Yu, F. Chen, R. Hu, and J. He. 2019. Effect of resveratrol on growth performance, rectal temperature and serum parameters of yellow-feather broilers under heat stress. Avia Poult. Bio Rev. 9:125–141.

Li, X. L., W. L. He, Z. B. Wang, and T. S. Xu. 2016a. Effects of Chinese herbal mixture on performance, egg quality and blood biochemical parameters of laying hens. J. Anim. Physiol. Anim. Nutr. 100:1041–1049.

Li, W. H., L. Wang, H. Y. He, J. Chen, and Y. R. Yu. 2016b. Expression of neutrophil gelatinase-associated lipocalin in low osmolar contrast-induced nephropathy in rats and the effect of N-acetylcysteine. Exp. Ther. Med. 12:3175–3180.

Li, X., L. Ma, and L. Zhang. 2019. Molecular basis for Poria cocos mushroom polysaccharide used as an antitumor drug in China. Prog Mol Biol Transl Sci. 163:263–296.

Li, Z. K., and D. D. Li. 1997. The immunomodulatory effect of achyranthes bidentata polysaccharides. Acta Pharm. Sin. 12:881–887.

Lian, K. X., X. Q. Zhu, J. Chen, G. Liu, and X. L. Gu. 2018. Selencylation modification: enhancement of the antioxidant activity of a Glycyrrhiza uralensis polysaccharide. Glycoconj J. 35:243–253.

Liu, Q. W., J. H. Feng, Z. Chao, Y. Chen, L. M. Wei, F. Wang, R. P. Sun, and M. H. Zhang. 2016. The influences of ambient temperature and crude protein levels on performance and serum biochemical parameters in broilers. J. Anim. Physiol. Anim. Nutr. (Berl). 100:301–308.

Liu, S. D., M. H. Song, W. Yun, J. H. Lee, H. B. Kim, and J. H. Cho. 2019a. Effect of carvacrol essential oils on immune response and inflammation-related genes expression in broilers challenged by lipopolysaccharide. Poult. Sci. 98:2026–2033.

Liu, Z., X. Wang, S. Ou, M. A. Arowolo, D. X. Hou, and J. He. 2018. Effects of Achyranthes bidentata polysaccharides on intestinal morphology, immune response, and gut Microbiome in yellow broiler chickens challenged with Escherichia coli K88. Polymers 10:1233.

Liu, P., R. Yao, H. Shi, Y. Liu, S. Liu, Y. Yang, H. Yang, and S. Li. 2019b. Effects of Cold-inducible RNA-binding protein (CIRP) on liver Glycosylation during acute Cold exposure in C57BL/6 mice. Int. J. Mol. Sci. 20:1470.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408.

Long, L. N., B. J. Kang, Q. Jiang, and J. S. Chen. 2020. Effects of dietary Lycium barbarum polysaccharides on growth performance, digestive enzyme activities, antioxidant status, and immunity of broiler chickens. Poult. Sci. 99:744–751.

Lv, A., M. Ge, X. Hu, W. Liu, G. Li, and R. Zhang. 2018. Effects of Agaricus blazei Murill polysaccharide on Cadmium Poisoning on the MDa5 signaling pathway and antioxidant function of chicken peripheral blood Lymphocytes. Biol. Trace Elem. Res. 181:122–130.

National Research Council (NRC). 1994. Nutrient Requirements of Poultry. 9th ed. rev. National Academy Press, Washington, DC.

NgKoLo, A. K., M. Meja, M. Youdon, I. Adcock, and P. A. Kirkham. 2012. LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and Gi2 dependent PI-3kinase signalling. J. Inflamm. (Lond). 9:1–9.

Noskova, G., D. Fleskova, L. Jurecek, V. Sadlonova, and B. Ray. 2013. Herbal polysaccharides and cough reflex. Respir. Physiol. Neurobiol. 187:47–51.

Park, J. H., and I. H. Kim. 2020. Effects of dietary Achyranthes bidentata polysaccharides and cough growth, digestive enzyme activities, antioxidant status, and immunity of broiler chickens. Poult. Sci. 99:463–470.

Pastorino, G., L. Cornara, S. Sours, F. Rodrigues, and M. Oliveira. 2018. Liquorice (Glycyrrhiza glabra): a phytochemical and pharmaceutical review. Phytother Res. 32:2223–2339.

Qi, S., J. Chen, X. Chen, Q. Fan, C. Zhang, D. Wang, X. Li, X. Chen, X. Chen, C. Liu, Z. Gao, H. Li, and Y. Hu. 2014. Optimization of selenylation conditions for lycium barbarum polysaccharide based on antioxidant activity. Carbohydr. Polym. 103:148–153.

Rêpérant, J. M., M. Dardi, M. Pages, and M. Thomas-Hénaff. 2012. Pathogenicity of Eimeria praecox alone or associated with Eimeria.
acervulina in experimentally infected broiler chickens. Vet. Parasitol. 187:333–336.

Sahloul, T. M., H. M. Emara, F. E. El-Deeb, E. F. Aboueladab, and D. H. El-Bushuty. 2010. Cultivation of mushroom using the environment residues at home and maximizing the utilization of nutrition and health. J. Agric. Biotech. 1:157–166.

Takeuchi, O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda, and S. Akira. 1999. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11:443–451.

Trefts, E., M. Gannon, and D. H. Wasserman. 2017. The liver. Curr. Biol. 27:R1147–R1151.

Wallace, R. J., W. Oleszek, C. Franz, I. Hahn, K. H. Baser, A. Mathe, and K. Teichmann. 2010. Dietary plant bioactives for poultry health and productivity. Br. Poult. Sci. 51:461–487.

Zhang, Q., and M. Ye. 2009. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). J. Chromatogr. A. 1216:1954–1969.

Zhao, F., K. Li, L. Zhao, J. Liu, Q. Suo, J. Zhao, H. Wang, and S. Zhao. 2014. Effect of Nr2 on rat ovarian tissues against atrazine-induced anti-inflammatory response. Int. J. Clin. Exp. Pathol. 7:2780–2789.

Zuowei, S., L. Yan, L. Yuan, H. Jiao, Z. Song, Y. Guo, and H. Lin. 2011. Stocking density affects the growth performance of broilers in a sex-dependent fashion. Poult. Sci. 90:1406–1415.