

Effects of dietary *Lycium barbarum* polysaccharides on growth performance, digestive enzyme activities, antioxidant status, and immunity of broiler chickens

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ABSTRACT *Lycium barbarum* polysaccharides (LBP) are considered to be the major bioactive components of *L. barbarum* and have been widely used as a well-known traditional Chinese medicine and functional food because of their various biological activities. However, no published research has investigated the use of LBP as a feed additive in broilers. The objective of this study was to evaluate the effects of dietary LBP supplementation on the growth performance, digestive enzyme activities, antioxidant status, and immunity of broiler chickens. A total of 256 one-day-old Arbor Acres male broiler chicks were randomly allotted into 4 groups, with 8 replicates of 8 birds each, and were fed a corn–soybean meal–type basal diet supplemented without (control group) or with 1,000, 2,000, or 4,000 mg/kg LBP for 6 wk. The results showed that compared with the control diet, a significant increase in ADG ($P < 0.05$) during the grower and overall periods was observed in chickens fed the basal diet supplemented with 2,000 mg/kg LBP, whereas supplementation with 1,000 or 2,000 mg/kg LBP decreased feed-to-gain ratio ($P < 0.05$) during the starter period. The

inclusion of LBP in the broiler diets increased overall amylase, lipase, and protease activities ($P < 0.05$). Supplementation with increasing levels of dietary LBP increased the activities of superoxide dismutase and glutathione peroxidase but decreased malondialdehyde content in the serum and liver ($P < 0.05$). Broilers fed with LBP-containing diets exhibited higher serum IgG and IgA concentrations ($P < 0.05$) than the broilers fed with the control diet. Serum tumor necrosis factor α and IL-4 concentrations were significantly elevated in the group fed 2,000 mg/kg LBP compared with the control group ($P < 0.05$). Broilers fed diets supplemented with LBP showed linear ($P < 0.05$) and quadratic ($P < 0.05$) increases in serum IL-6 and interferon gamma concentrations. The results indicated that dietary LBP supplementation can improve growth performance, digestive enzyme activities, antioxidant capacity, and immune function of broilers. In conclusion, LBP may be used as a promising feed additive for broilers, and a supplementation level of 2,000 mg/kg LBP in the broiler diet is recommended.

Key words: *Lycium barbarum* polysaccharides, digestive enzyme, antioxidant status, immunity, broilers

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INTRODUCTION

The use of antibiotic growth promoters in animal feed has led to improved growth performance and feed efficiency in the intensive poultry industry over the last several decades (Agostini et al., 2012). However, possible antibiotic residues and disease resistance have aroused substantial concern regarding the usage of

antibiotics in the animal industry. In the European Union, antibiotic growth promoters have been banned since 2006, and such regulations or bans in poultry diets are similarly expected to impact other countries (Castanon, 2007). Therefore, given the high demand for high-quality poultry products, the development of effective and sustainable special additives that can both increase productive potential and maintain broiler health is imperative (Wu et al., 2019).

Previous studies have demonstrated great potential for plant-extracted natural polysaccharides as an alternative to antibiotic additives (Kong et al., 2007; Qiao et al., 2013; Zhao et al., 2015). Polysaccharides derived from seaweed species such as *Laminaria* have been investigated as an in-feed supplement in piglets

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to promote gastrointestinal function (Gahan et al., 2009; O'Doherty et al., 2010). Dietary supplementation with *Astragalus membranaceus* polysaccharide improved the growth performance of juvenile broilers, potentially due to enhanced digestive enzyme activity and antioxidant capacity (Wu, 2018). In addition, plant polysaccharides have been reported to facilitate various activities such as growth promotion, appetite stimulation, immune enhancement, and antipathogen properties in livestock and poultry production (Chen et al., 2003; Qiu et al., 2007; Gahan et al., 2009; Li et al., 2011). Therefore, plant-extracted natural polysaccharides may be useful as functional feed additives in the poultry industry.

Lycium barbarum, also known as wolfberry, has been widely used in the East Asia for thousands of years as a well-known traditional Chinese herbal medicine to promote health and longevity and as a food supplement (Jin et al., 2013). *L. barbarum* polysaccharides (LBP) are a group of water-soluble glycoconjugates isolated from the aqueous extracts of *L. barbarum* and contain 6 monosaccharides (arabinose, rhamnose, xylose, mannose, galactose, and glucose) (Wang et al., 2009). Many studies have identified various health-promoting activities of LBP, such as antioxidant (Liang et al., 2011; Zhang, et al., 2014a), antistress (Cheng and Kong, 2011), anti-inflammatory (Zhao et al., 2016; Gan et al., 2018), liver protection (Jia et al., 2014), and immunostimulating activities (Zhang et al., 2014b). In mice, high-fat diets supplemented with LBP improved blood lipid metabolism and antioxidant ability (Ming et al., 2009). Ren et al. (2017) reported that supplementing diets with LBP promoted the expression of tumor necrosis factor α (TNF- α) and IL-6 proteins in rat serum. A recent study showed that dietary supplementation of *L. barbarum* extract improved growth and protein deposition in hybrid grouper (*Epinephelus lanceolatus* δ \times *Epinephelus fuscoguttatus* f) (Tan et al., 2019). Although the various biological functions of LBP have received extensive attention, little information is available in the literature on the application of LBP in poultry and animal production, particularly for broilers. Based on the previously reported favorable effects, we speculate that LBP may be an effective feed additive for improving specific aspects of poultry and animal production. Therefore, the objective of the present study was to evaluate the effects of LBP supplementation on growth performance, digestive enzyme activities, antioxidant status, and immune response of broiler chickens.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Foshan University, China. All procedures were conducted in compliance with relevant laws and institutional guidelines. A total of 256 one-day-old Arbor Acres male broiler chicks with similar BW were obtained from a local hatchery. Chicks were individually weighed and randomly allotted into 4 groups with 8 replicates of 8 birds each. The control group was fed a corn-soybean meal basal

Table 1. Composition and nutrient content of experimental diets.

Items	Age (days)	
	Starter	Grower
	(0 to 21 D)	(22 to 42 D)
Ingredient (%)		
Corn	57.20	62.70
Soybean meal (43% CP)	34.70	29.30
Soy oil	2.70	2.80
Fish meal (60.2% CP)	1.50	1.50
Dicalcium phosphate	1.65	1.41
Limestone	1.30	1.30
Salt	0.25	0.21
DL-methionine	0.20	0.20
HCl-lysine	-	0.08
Vitamin-mineral premix ¹	0.50	0.50

¹Supplied per kilogram of diet: vitamin A (*trans*-retinyl acetate), 10,050 IU; vitamin D₃, 2,800 IU; vitamin E (DL- α -tocopheryl acetate), 50 mg; vitamin K₃, 3.5 mg; thiamine, 2.5 mg; riboflavin, 7.5 mg; pantothenic acid, 15.3 mg; pyridoxine, 4.3 mg; vitamin B₁₂ (cyanocobalamin), 0.02 mg; niacin, 35 mg; choline chloride, 1,000 mg; biotin, 0.20 mg; folic acid, 1.2 mg; Mn, 100 mg; Fe, 85 mg; Zn, 60 mg; Cu, 9.6 mg; I, 0.30 mg; Co, 0.20 mg; and Se, 0.20 mg.

diet. Three experimental diets were prepared by adding 1,000, 2,000, and 4,000 mg/kg LBP to the basal diet (supplemented in place of corn) during the starter (day 1 to 21) and grower (day 22 to 42) phases. In the current study, LBP (high-performance liquid chromatography \geq 60%) comprised D-mannose, L-rhamnose, D-glucose, D-galactosamine, and D-xylose, obtained from Xi'an ZeBang Biological Technology Co. Ltd. (Xi'an, China). All diets were formulated to meet the NRC (1994) nutrient requirements. The ingredients and chemical composition of the basal diet are shown in Tables 1 and 2, respectively. All birds were raised in three-layer cages (110 cm \times 60 cm \times 50 cm, 8 birds per cage) in an environmentally controlled room with continuous light and *ad libitum* access to feed and water throughout the 42 D experiment. The room temperature was maintained at 32°C–34°C for the first 3 D and then gradually decreased by 2°C–3°C per week to a final temperature of 22°C. Light was provided for 24 h during the first 1 to 3 D and then reduced to 22 h in the subsequent 4 to 7 D.

Growth Performance

All birds were individually weighed after their arrival from the hatchery. The birds were also weighed on day 21 and 42 after a 12 h fast, and the feed intake of birds in each replicate was recorded to determine ADG and ADFI, and the feed-to-gain ratio was calculated by ADFI/ADG.

Sample Collection

At the end of the experiment (6 wk), one bird with a BW similar to the mean BW of the full replicate cohort was randomly selected and marked. After a 12-h feed withdrawal (water was offered *ad libitum*), blood samples were collected from the wing vein before slaughter using 5-mL vacuum blood tubes; the sample was allowed to clot at 37°C for 2 h and subsequently centrifuged at 3,000 \times g for 10 min at 4°C to obtain serum, which was then stored at -20°C until the analyses of

Table 2. Analyzed composition of experimental diets (%).¹

Analyzed composition (%) ¹	Content of LBP (mg/kg)			
	Control	1,000	2,000	4,000
0 ~ 21 D of age				
ME ² (MJ/kg)	12.92	12.92	12.92	12.92
Crude protein	21.50	21.47	21.49	21.51
Calcium	0.95	0.94	0.96	0.96
Total phosphorus	0.69	0.65	0.68	0.69
Lysine	1.21	1.15	1.16	1.18
Methionine	0.50	0.46	0.47	0.51
22 ~ 42 D of age				
ME (MJ/kg)	13.43	13.43	13.43	13.43
Crude protein	19.60	19.59	19.58	19.57
Calcium	0.88	0.84	0.86	0.87
Total phosphorus	0.64	0.61	0.63	0.65
Lysine	1.09	1.04	1.06	1.08
Methionine	0.44	0.42	0.43	0.43

¹All nutrient levels except ME were analyzed, and values are the means of 2 determinations.

²ME values were calculated using NRC (1994) values.

immunoglobulin (Ig) G, IgM, IgA concentrations and antioxidant enzyme activities. After bleeding, the bird was slaughtered by cervical dislocation, and liver tissue samples were harvested, stored at -80°C , and then used for antioxidant capacity assays. Intestinal digesta was immediately removed by gentle finger stripping of the intestinal segments using the method described by Hashemipour et al. (2013) with some modifications. Briefly, the digesta samples were diluted $10\times$ with ice-cold PBS (pH 7.0) based on sample weight, homogenized for 60 s, and sonicated for 3 cycles of 1 min with 30-s intervals. The samples were then centrifuged at $15,000\times g$ for 30 min at 4°C . The supernatants were divided into aliquots and stored at -80°C for enzymatic assays.

Determination of Digestive Enzyme Activities

Amylase, protease, and lipase activities were determined using ELISA kits (48T, provided by Shanghai Changjin Biotechnology Co., Ltd., China) according to the manufacturer's instructions as described by Gao et al. (2017). Briefly, standards or samples were added in the appropriate Micro-ELISA Strip-plate wells and then incubated with specific antibody followed by a horseradish peroxidase-conjugated antibody specific for the target enzyme. The unbound components were washed away. Tetramethylbenzidine substrate solution was added to each well. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm. The OD was proportional to the activity of the target enzyme, which was calculated by comparing the OD of the samples to the standard curve. One unit of enzyme activity was defined as the amount of enzyme that decreased the absorbance by 0.001 min^{-1} . All assays were performed in triplicate.

Assay of Antioxidant Indices in the Serum and Liver

The liver was isolated on an ice tray and homogenized with cold saline with a weight-to-volume ratio of 1:9, and

then, the homogenate was centrifuged at $9,000\times g$ for 10 min at 4°C . The supernatant fluid was collected for measuring the indices. The activities of total antioxidant capacity, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase and malondialdehyde (MDA) concentrations were determined with commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the method described by Zhang et al. (2009) and Hao et al. (2015). The total antioxidant capacity was measured by the method of ferric-reducing antioxidant power assay. The activity of SOD was determined by measuring the reduction of OD at 450 nm of the reaction solution, catalase by measuring the OD of the reaction solution at 405 nm, GSH-Px by measuring the OD of the reaction solution at 412 nm, and MDA content by measuring OD of the reaction solution at 532 nm with corresponding substrates provided in the assay kits. All the procedures were carried out according to the manufacturers' instructions.

Immunological Measurements

Immunoglobulin levels (IgM, IgG, and IgA) in serum were detected using an ELISA kit purchased from Bogoo Biotechnology Co. Ltd. (Shanghai, China). Serum concentrations of IL-2, IL-4, IL-6, interferon gamma (IFN- γ), and TNF- α were measured using ELISA kits (R&D Systems, Wiesbaden-Nordenstadt, Germany). All measurements were conducted at least in triplicate and according to the manufacturer's instructions.

Statistical Analysis

All data were subjected to ANOVA using the GLM procedure of SPSS 20.0 (SPSS Inc., Chicago, IL). The data were analyzed as a completely randomized design, with the replicate as the experimental unit. Orthogonal polynomial contrasts were used to determine linear and quadratic responses to the increasing level of LBP in diets. Differences were regarded as significant when $P < 0.05$.

RESULTS

Growth Performance

The effects of dietary LBP supplementation on broiler growth performance traits at different phases are shown in Table 3. At 21 and 42 D of age, the inclusion of 2,000 mg/kg LBP increased BW of chickens in a linear and quadratic manner ($P < 0.01$), whereas linearly ($P = 0.014$) and quadratically ($P < 0.001$) decreased the feed-to-gain ratio compared with birds fed the basal diet from 1 to 21 D of age; there were no differences in ADG or ADFI among the groups ($P > 0.05$). From 22 to 42 D of age, broilers fed diets supplemented with 2,000 mg/kg LBP showed linear and quadratic ($P < 0.05$) increases in ADG and ADFI compared with the control group. At 42 D of age, addition of

Table 3. Effects of dietary *Lycium barbarum* polysaccharides on the performance of broiler chickens.¹

Items	LBP levels (mg/kg of feed)				SEM	P-value	
	0	1,000	2,000	4,000		Linear	Quadratic
BW, g							
1 D	43.24	43.23	43.25	43.24	1.587	0.879	0.241
21 D	718.52 ^b	739.31 ^{a,b}	781.13 ^a	722.72 ^b	24.537	0.001	0.006
42 D	2228.41 ^b	2285.52 ^b	2389.71 ^a	2252.33 ^b	68.936	0.002	0.008
ADG, g/bird/D							
1 to 21 D	32.16	33.15	35.14	32.36	0.417	0.273	0.164
22 to 42 D	71.90 ^b	73.63 ^{a,b}	76.60 ^a	72.84 ^{a,b}	0.629	0.039	0.015
1 to 42 D	52.03 ^b	53.39 ^{a,b}	55.87 ^a	52.60 ^{a,b}	0.528	0.101	0.037
ADFI, g/bird/D							
1 to 21 D	54.00	53.10	55.30	52.60	0.684	0.201	0.117
22 to 42 D	137.21 ^b	139.43 ^{a,b}	145.54 ^a	140.00 ^{a,b}	0.975	0.028	0.016
1 to 42 D	95.61	96.27	100.42	96.30	0.862	0.077	0.059
F:G, g/g							
1 to 21 D	1.68 ^a	1.60 ^{b,c}	1.57 ^c	1.63 ^{a,b}	0.013	0.014	<0.001
22 to 42 D	1.91	1.89	1.90	1.92	0.022	0.758	0.219
1 to 42 D	1.84	1.80	1.80	1.83	0.015	0.213	0.148

^{a,b,c} Means within a row with no common superscript letters differ significantly ($P < 0.05$).

Abbreviations: F:G, feed-to-gain ratio; LBP, *Lycium barbarum* polysaccharides.

¹Data are means collected from 8 birds from each treatment (1 bird per replicate).

2,000 mg/kg LBP quadratically ($P = 0.037$) increased ADG and tended ($0.05 < P < 0.10$) toward increased ADFI compared with the control group. The results show that broilers fed with 1,000 to 2,000 mg/kg of LBP exhibited superior feed efficiencies when compared with the control group.

Digestive Enzyme Activities

The effects of dietary LBP on intestinal digestive enzyme activities in broilers at 42 D of age are shown in Table 4. Compared with the control diets, supplementation of LBP in the broiler diets increased amylase, lipase, and protease activities ($P < 0.05$), indicating that LBP can induce the expression of digestive enzymes.

Antioxidant Enzyme Activities

The effects of dietary LBP supplementation on SOD and GSH-Px activities and MDA concentration in the serum and liver of broilers at 42 D of age are shown in Table 5. Broilers fed diets supplemented with LBP increased serum SOD (linear, $P = 0.005$; quadratic, $P = 0.004$) and GSH-Px (linear, $P = 0.003$) activities. However, MDA concentrations of serum were linearly decreased ($P = 0.004$) by the addition of dietary LBP

in feed. Dietary supplementation with LBP linearly and quadratically ($P < 0.01$) increased SOD and GSH-Px activities in the liver. The MDA level in liver was linearly reduced ($P < 0.01$) by the inclusion of LBP.

Serum Concentrations of Antibodies and Cytokines

The effects of dietary LBP on serum concentrations of IgG, IgA, and IgM in broilers are shown in Table 6. LBP supplementation in broiler diets had no effect on serum concentrations of IgM ($P > 0.05$) at any dose, but the serum IgG and IgA concentrations were increased linearly and quadratically ($P < 0.05$) with LBP supplementation. Broilers fed with 2,000 mg/kg of LBP exhibited the highest IgG and IgA levels of all the groups.

The effects of dietary LBP supplementation on serum concentrations of IL-2, IL-4, IL-6, TNF- α , and IFN- γ in broilers are shown in Table 7. Broiler chicks fed 2,000 and 4,000 mg/kg supplement of LBP had higher (linear, $P = 0.006$; quadratic, $P = 0.009$) serum levels of IL-2 than those of control group. Serum TNF- α and IL-4 concentrations in the group fed with 2,000 mg/kg of LBP were significantly elevated compared with the control group ($P < 0.05$). Broilers fed diets supplemented with LBP showed linear ($P < 0.05$) and quadratic

Table 4. Effect of dietary *Lycium barbarum* polysaccharides on digestive enzyme activities of broiler chickens at 42 D of age.¹

Items	LBP levels (mg/kg of feed)				SEM	P-value	
	0	1,000	2,000	4,000		Linear	Quadratic
Amylase (U/mL)	1045.32 ^c	1237.98 ^b	1792.45 ^a	1211.32 ^b	35.781	0.003	0.009
Lipase (U/L)	135.63 ^c	215.51 ^b	254.92 ^a	220.18 ^{a,b}	11.225	0.008	0.016
Protease (U/mL)	146.83 ^c	177.36 ^b	235.57 ^a	183.71 ^b	10.496	0.011	0.037

^{a,b,c} Means within a row with no common superscript letters differ significantly ($P < 0.05$).

Abbreviation: LBP, *Lycium barbarum* polysaccharides.

¹Data are means collected from 8 birds from each treatment (1 bird per replicate).

Table 5. Effects of dietary *Lycium barbarum* polysaccharides on superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) concentration in the serum and liver of broilers at 42 D of age.¹

Items	LBP levels (mg/kg of feed)				SEM	P-value	
	0	1,000	2,000	4,000		Linear	Quadratic
Serum							
SOD (U/mL)	136 ^c	154 ^{ab}	163 ^a	171 ^a	4.352	0.005	0.004
GSH-Px (U/mL)	165 ^b	176 ^a	179 ^a	185 ^a	2.894	0.003	0.137
MDA (nmol/mL)	7.37 ^a	6.62 ^b	5.97 ^b	6.01 ^b	0.273	0.004	0.029
Liver							
SOD (U/mL)	279 ^b	308 ^a	325 ^a	329 ^a	2.315	0.001	0.001
GSH-Px (U/mL)	3.08 ^b	4.35 ^a	4.86 ^a	4.69 ^a	0.139	0.002	0.001
MDA (nmol/mL)	5.12 ^a	4.47 ^b	4.11 ^b	4.05 ^b	0.148	0.004	0.217

^{a,b,c}Means within a row with no common superscript letters differ significantly ($P < 0.05$).

Abbreviation: LBP, *Lycium barbarum* polysaccharides; TNF- α , tumor necrosis factor α .

¹Data are means collected from 8 birds from each treatment (1 bird per replicate).

($P < 0.05$) increases in serum IL-6 concentration. Serum IFN- γ concentrations were also increased in a linear ($P < 0.001$) and quadratic ($P < 0.001$) manner with LBP supplementation.

DISCUSSION

Dietary supplementation with Chinese herbal polysaccharides, such as *Atractylodes macrocephala* Koidz polysaccharides, *Aloe vera* polysaccharide, and *A. membranaceus* polysaccharide, has been used extensively for improving growth performance, immunity, or antioxidant activity to prevent infectious diseases in broiler chickens and pigs (Chen et al., 2003; Li et al., 2011; Qiao et al., 2013). *L. barbarum* is a Chinese traditional medicine with a potent ability to enhance immunity and antioxidation. The present study aimed to evaluate the effects of dietary LBP supplementation on growth performance, immunity, and antioxidant activity in broiler chickens. Supplementation of 2,000 mg/kg LBP in broiler diets improved BW (day 21 or 42), ADG in the grower and overall periods, and ADFI in the grower period. To the best of our knowledge, no available data have evaluated the impact of LBP inclusion in broiler diets on growth performance. However, Tan et al. (2019) reported that dietary supplementation of *L. barbarum* extract could improve body weight in hybrid groupers (*E. lanceolatus* $\delta \times E. fuscoguttatus$ ♀). Another study showed that LBP could inhibit polymorphonuclear neutrophil accumulation and intracellular adhesion molecule-1 expression and mitigate changes in TNF- α levels, nuclear

factor- κ B activation, intestinal permeability, and histology in a rat model. This indicates that LBP can enhance nutrient absorption in the intestine (Ren et al., 2017), which may represent one potential mechanism underlying the beneficial effects of LBP on broiler growth performance. In addition, broiler weight decreased at the highest dietary concentration, and the feed conversion ratio improved when birds received 4,000 mg/kg LBP. The discrepancy can be attributed to the addition method, processing technology and level of phytogetic feed additive in the basal diet, diet composition, and feeding management.

In the current study, the activities of protease, amylase, and lipase in the small intestinal contents of broilers were increased with the addition of LBP. These results suggest that LBP supplementation induced the expression of digestive enzymes. Little is known regarding the underlying mechanisms by which dietary supplementation of LBP modulates digestive enzymes, especially in the small intestine. The present study provides similar results to those reported by other researchers (Jang et al., 2007; Hashemipour et al., 2013). Another study reported that polysaccharides from *A. membranaceus* improved the activities of intestinal digestive enzymes (amylase, lipase, and protease) in broilers (Wu, 2018). However, further research is needed to elucidate this mechanism.

The antioxidant-promoting abilities of LBP were demonstrated in this study by measuring various antioxidant parameters in serum and livers, such as SOD and GSH-Px. SOD and GSH-Px can scavenge reactive oxygen species and thus function as antioxidants.

Table 6. Effects of dietary *Lycium barbarum* polysaccharides on serum concentrations of IgG, IgA, and IgM in broiler chickens at 42 D of age.¹

Items	LBP levels (mg/kg of feed)				SEM	P-value	
	0	1,000	2,000	4,000		Linear	Quadratic
IgA (mg/mL)	0.384 ^b	0.481 ^a	0.489 ^a	0.475 ^a	0.008	0.002	0.016
IgG (mg/mL)	0.749 ^b	0.798 ^a	0.889 ^a	0.794 ^a	0.003	0.004	0.029
IgM (mg/mL)	2.102	2.283	2.314	2.356	0.005	0.113	0.247

^{a,b}Means within a row with no common superscript letters differ significantly ($P < 0.05$).

Abbreviations: Ig, immunoglobulin; LBP, *Lycium barbarum* polysaccharides

¹Data are means collected from 8 birds from each treatment (1 bird per replicate).

Table 7. Effects of dietary *Lycium barbarum* polysaccharides on serum concentrations of IL-2, IL-4, IL-6, IFN- γ , and TNF- α in broiler chickens at 42 D of age.¹

Items	LBP levels (mg/kg of feed)				SEM	P-value	
	0	1,000	2,000	4,000		Linear	Quadratic
IL-2 ($\mu\text{g/mL}$)	175.331 ^b	181.743 ^b	197.378 ^a	196.402 ^a	3.046	0.006	0.009
IL-4 (ng/mL)	81.272 ^c	83.629 ^{b,c}	89.337 ^a	84.792 ^{a,b}	1.853	0.004	0.003
IL-6 (pg/mL)	20.593 ^c	25.196 ^{a,b}	27.571 ^a	23.893 ^b	0.176	0.003	0.017
TNF- α (pg/mL)	34.759 ^b	36.784 ^{a,b}	39.832 ^a	36.971 ^{a,b}	0.287	0.007	0.129
IFN- γ (pg/mL)	61.481 ^c	63.726 ^b	77.365 ^a	76.952 ^a	2.591	<0.001	<0.001

^{a,b,c} Means within a row with no common superscript letters differ significantly ($P < 0.05$).

Abbreviations: IFN- γ , interferon gamma; LBP, *Lycium barbarum* polysaccharides; TNF- α , tumor necrosis factor α .

¹Data are means collected from 8 birds from each treatment (1 bird per replicate).

Superoxide is first degraded into hydrogen peroxide by SOD and subsequently converted into water by a series of enzymes including GSH-Px (Wang et al., 2008). MDA is one of the most frequently used indicators of lipid peroxidation. Increased MDA results from cellular membrane damage initially caused by the increased formation of radicals (Papadimitriou and Loumbourdis, 2002). In the current study, LBP supplementation in broiler diets increased SOD and GSH-Px activities and decreased MDA concentrations in serum and liver, indicating that dietary LBP supplementation enhanced the activity of antioxidant enzymes. Other researchers showed that dietary LBP administration improved SOD and GSH-Px activities and reduced MDA contents in the blood, lung, kidney, and liver of rats (Li, 2007; Ming et al., 2009). Antioxidant effects have been reported for some plants that contain flavonoids, phenolic compounds, ascorbic acid, and tocopherol (Andallu and Varadacharyulu, 2003; Wang et al., 2008). Phytochemical data showed that LBP are rich in flavonoids, phenolic compounds, ascorbic acid, and tocopherol (Peng and Tian, 2001), and it is possible that the observed antioxidant effect is related to these components.

Serum immunoglobulins are important indicators of an animal's immune status (Wang et al., 2017). Plant-extracted natural polysaccharide supplementation was found to improve host immune functions (Li et al., 2011, 2018). Few reports have investigated the effects of LBP on broiler immune responses. In the current experiment, dietary LBP supplementation promoted the humoral immune response leading to an increase in serum IgA and IgG concentrations. Increased immunoglobulin concentrations stimulate complement components to enhance specific immune mechanisms in birds and thereby protecting them against infections. Furthermore, the immune response is controlled by a complex interplay among various cytokines. LBP have been reported to possess immunomodulating activities through the activation of macrophages or lymphocytes to generate nitric oxide and promote cytokine secretion (e.g., TNF- α , IL-1 β , IL-2, IL-6, or IL-12) (Zhang et al., 2014b; Ren et al., 2017; Qian, 2019). In this study, we also observed that serum concentrations of TNF- α ,

IL-2, IL-4, and IL-6 increased linearly and quadratically with increasing levels of LBP in diets, indicating that dietary supplementation with LBP can activate immune functions leading to enhanced antibody production. In addition, IFN- γ is an important indicator cytokine that initiates cell-mediated immune responses (Park et al., 2008) and has broad biological activities against viruses and tumor cells. In the present study, increased serum IFN- γ concentrations were observed in broilers given increasing levels of dietary LBP. Similar findings were reported by Qiao et al. (2013), who demonstrated that dietary supplementation with *A. vera* polysaccharide increased IFN- γ production in serum of weaned piglets. Furthermore, some others had also reported that dandelion root extract (major bioactive components are polysaccharide and flavone) and *Astragalus* polysaccharide supplementation in the diet increased IFN- γ level in serum of pigs (Yuan et al., 2006; Zhao et al., 2019). Therefore, these results implies that dietary supplementation with certain kinds of Chinese herbs could improve the immune function of weaned pigs.

CONCLUSION

To the best of our knowledge, this study provides the first evidence that LBP can serve as an effective and beneficial feed additive for improving the growth performance in broilers, and this effect may be partially attributed to improved digestive enzyme activities, enhanced immune functions, and the antioxidant capacity induced by LBP. An inclusion level of 2,000 mg/kg LBP in broiler diets is recommended.

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REFERENCES

- Agostini, P. S., D. Solà-Oriol, M. Nofrarias, A. C. Barroeta, J. Gasa, and E. G. Manzanilla. 2012. Role of in-feed clove supplementation on growth performance, intestinal microbiology, and morphology in broiler chicken. *Livest. Sci.* 147:113–118.
- Andallu, B., and N. C. Varadacharyulu. 2003. Antioxidant role of mulberry (*Morus indica* L. cv. *Anantha*) leaves in streptozotocin-diabetic rats. *Clin. Chim. Acta.* 338:3–10.
- Castanon, J. I. R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci.* 86:2466–2471.
- 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.
- Cheng, D., and H. Kong. 2011. The effect of *Lycium barbarum* polysaccharide on alcohol-induced oxidative stress in rats. *Molecules.* 16:2542–2550.
- Gahan, D. A., M. B. Lynch, J. J. Callan, J. T. O'Sullivan, and J. V. O'Doherty. 2009. Performance of weanling piglets offered low-, medium- or high-lactose diets supplemented with a seaweed extract from *Laminaria* spp. *Animal.* 3:24–31.
- Gan, F., Q. Liu, Y. Liu, D. Huang, C. Pan, S. Song, and K. Huang. 2018. *Lycium barbarum* polysaccharides improve CCl₄-induced liver fibrosis, inflammatory response and TLRs/NF- κ B signaling pathway expression in wistar rats. *Life Sci.* 192:205–212.
- Gao, Y., J. He, Z. He, Z. Li, B. Zhao, Y. Mu, J. Y. Lee, and Z. Chu. 2017. Effects of fulvic acid on growth performance and intestinal health of juvenile loach *Paramisgurnus dabryanus* (Sauvage). *Fish Shellfish Immunol.* 62:47–56.
- Hao, R., Q. Li, J. Zhao, H. Li, W. Wang, and J. Gao. 2015. Effects of grape seed procyanidins on growth performance, immune function and antioxidant capacity in weaned piglets. *Livest. Sci.* 178:237–242.
- Hashemipour, H., H. Kermanshahi, A. Golian, and T. Veldkamp. 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poult. Sci.* 92:2059–2069.
- Jang, I. S., Y. H. Ko, S. Y. Kang, and C. Y. Lee. 2007. Effect of a commercial essential oil on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Anim. Feed Sci. Technol.* 134:304–315.
- Jia, X., F. Xing, H. Jie, L. F. Man, E. C. Liang, Y. P. Ching, A. Xu, R. C. C. Chang, K. F. So, and G. L. Tipoe. 2014. *Lycium barbarum* polysaccharides therapeutically improve hepatic functions in non-alcoholic steatohepatitis rats and cellular steatosis model. *Sci. Rep.* 4:5587.
- Jin, Mingliang, Qingsheng Huang, Shang, Peng, Zhao, and Ke. 2013. Biological activities and potential health benefit effects of polysaccharides isolated from *Lycium barbarum* L. *Int. J. Biol. Macromol.* 54:16–23.
- Kong, X. F., G. Y. Wu, Y. P. Liao, Z. P. Hou, H. J. Liu, F. G. Yin, T. J. Li, R. L. Huang, Y. M. Zhang, and D. Deng. 2007. Effects of Chinese herbal ultra-fine powder as a dietary additive on growth performance, serum metabolites and intestinal health in early-weaned piglets. *Livest. Sci.* 108:272–275.
- Li, X. M. 2007. Protective effect of *Lycium barbarum* polysaccharides on streptozotocin-induced oxidative stress in rats. *Int. J. Biol. Macromol.* 40:461–465.
- Li, L. L., F. G. Yin, B. Zhang, H. Z. Peng, F. N. Li, N. S. Zhu, D. X. Hou, Y. L. Yin, J. J. Luo, Z. R. Tang, and G. Liu. 2011. Dietary supplementation with *Atractylodes Macrophala Koidz* polysaccharides ameliorate metabolic status and improve immune function in early-weaned pigs. *Livest. Sci.* 142:33–41.
- Li, Y., X. Lei, Z. Yin, W. Guo, S. Wu, and X. Yang. 2018. Transgenerational effects of paternal dietary *Astragalus* polysaccharides on spleen immunity of broilers. *Int. J. Biol. Macromol.* 115:90–97.
- Liang, B., M. Jin, and H. Liu. 2011. Water-soluble polysaccharide from dried *Lycium barbarum* fruits: Isolation, structural features and antioxidant activity. *Carbohydr. Polym.* 83:1947–1951.
- Ming, M., L. Guanhua, Y. Zhanhai, C. Guang, and Z. Xuan. 2009. Effect of the *Lycium barbarum* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. *Food Chem.* 113:872–877.
- National Research Council. 1994. *Nutrient Requirements of Poultry.* 9th ed. Natl. Acad. Sci, Washington, DC.
- O'Doherty, J. V., S. Dillon, and S. Figat. 2010. The effect of lactose inclusion and seaweed extract derived from *Laminaria* spp. on performance, digestibility of diet components and microbial populations in newly weaned pigs. *Anim. Feed Sci. Technol.* 157:173–180.
- Papadimitriou, E., and N. S. Loumbourdis. 2002. Exposure of the frog *Rana ridibunda* to copper: impact on two biomarkers, lipid peroxidation, and glutathione. *Bull. Environ. Contam. Toxicol.* 69:885–891.
- Park, S. S., H. S. Lillehoj, P. C. Allen, P. D. Woon, F. C. Steve, D. A. Bautista, and E. P. Lillehoje. 2008. Immunopathology and cytokine responses in broiler chickens coinfecting with *Eimeria maxima* and *Clostridium perfringens* with the use of an animal model of necrotic enteritis. *Avian Dis.* 52:14–22.
- Peng, X., and G. Tian. 2001. Structural characterization of the glycan part of glycoconjugate LbGp2 from *Lycium barbarum* L. *Carbohydr. Res.* 331:95–99.
- Qian, L. 2019. Modulation of cytokine level and sperm quality of mice by *Lycium barbarum* polysaccharides. *Int. J. Biol. Macromol.* 126:475–477.
- Qiu, Y., Y. L. Hu, B. A. Cui, H. Y. Zhang, X. F. Kong, D. Y. Wang, and Y. G. Wang. 2007. Immunopotentiating effects of four Chinese herbal polysaccharides administered at vaccination in chickens. *Poult. Sci.* 86:2530–2535.
- Qiao, J., H. H. Li, C. J. Zheng, Z. Y. Feng, and W. Wang. 2013. Dietary supplementation with Aloe vera polysaccharide enhances the growth performance and immune function of weaned piglets. *J. Anim. Feed Sci.* 22:329–334.
- Ren, L., J. Li, Y. Xiao, Y. Zhang, J. Fan, B. Zhang, L. Wang, and X. Shen. 2017. Polysaccharide from *Lycium barbarum* L. leaves enhances absorption of endogenous calcium, and elevates cecal calcium transport protein levels and serum cytokine levels in rats. *J. Funct. Foods.* 33:227–234.
- Tan, X., Z. Z. Sun, and C. X. Ye. 2019. Dietary *Lycium barbarum* extract administration improved growth, meat quality and lipid metabolism in hybrid grouper (*Epinephelus lanceolatus* δ \times *E. fuscoguttatus* ♀) fed high lipid diets. *Aquaculture.* 504:190–198.
- Wang, L., X. L. Piao, S. W. Kim, X. S. Piao, Y. B. Shen, and H. S. Lee. 2008. Effects of *Forsythia suspensa* extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. *Poult. Sci.* 87:1287–1294.
- Wang, C. C., S. C. Chang, and B. H. Chen. 2009. Chromatographic determination of polysaccharides in *Lycium barbarum* Linnaeus. *Food Chem.* 116:595–603.
- Wang, W., J. Chen, Z. Hang, W. Li, S. Ding, Y. Wang, S. Dan, and A. Li. 2017. Effects of microencapsulated *Lactobacillus plantarum* and fructooligosaccharide on growth performance, blood immune parameters, and intestinal morphology in weaned piglets. *Food Agr. Immunol.* 1:84–94.
- Wu, S. 2018. Effect of dietary *Astragalus membranaceus* polysaccharide on the growth performance and immunity of juvenile broilers. *Poult. Sci.* 97:3489–3493.
- Wu, S., T. Li, H. Niu, Y. Zhu, Y. Liu, Y. Duan, Q. Sun, and X. Yang. 2019. Effects of glucose oxidase on growth performance, gut function, and cecal microbiota of broiler chickens. *Poult. Sci.* 98:828–841.
- Yuan, S., X. Piao, D. Li, S. Kim, H. Lee, and P. Guo. 2006. Effects of dietary *Astragalus* polysaccharide on growth performance and immune function in weaned pigs. *Anim. Sci.* 82:501–507.
- Zhang, G. F., Z. B. Yang, Y. Wang, W. R. Yang, S. Z. Jiang, and G. S. Gai. 2009. Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poult. Sci.* 88:2159–2166.

- Zhang, M., F. Wang, R. Liu, X. Tang, Q. Zhang, and Z. Zhang. 2014a. Effects of superfine grinding on physicochemical and antioxidant properties of *Lycium barbarum* polysaccharides. *LWT - Food Sci. Technol.* 58:594–601.
- Zhang, X., Y. Li, J. Cheng, G. Liu, C. Qi, W. Zhou, and Y. Zhang. 2014b. Immune activities comparison of polysaccharide and polysaccharide-protein complex from *Lycium barbarum* L. *Int. J. Biol. Macromol.* 65:441–445.
- Zhao, X., L. Li, Q. Luo, M. Ye, G. Luo, and Z. Kuang. 2015. Effects of mulberry (*Morus alba* L.) leaf polysaccharides on growth performance, diarrhea, blood parameters, and gut microbiota of early-weanling pigs. *Livest. Sci.* 177:88–94.
- Zhao, Q., J. Li, J. Yan, S. Liu, Y. Guo, D. Chen, and Q. Luo. 2016. *Lycium barbarum* polysaccharides ameliorates renal injury and inflammatory reaction in alloxan-induced diabetic nephropathy rabbits. *Life Sci.* 157:82–90.
- Zhao, J., G. Zhang, X. Zhou, W. Dong, Q. Wang, C. Xiao, and S. Zhang. 2019. Effect of *Dandelion root* extract on growth performance, immune function and bacterial community in weaned pigs. *Food Agr. Immunol.* 30:95–111.