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Effects of Polysaccharides-Rich Extract from *Gracilaria lemaneiformis* on Growth Performance, Antioxidant Capacity, Immune Function, and Meat Quality in Broiler Chickens

Shengwang Jiang[1], Chaoyun Yang[1], Yintao Xiao[2], Saizhen Zheng[2], Qian Jiang[2], and Jiashun Chen[2]

[1]College of Animal Science, Xichang University, Xichang, Sichuan 615013, P.R. China
[2]College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128, P.R.China

This study investigated the effects of dietary supplementation with *Gracilaria lemaneiformis* polysaccharides (GLPs) on the growth performance, antioxidant capacity, immune function, and meat quality of broiler chickens. A total of 320 one-day-old Arbor Acres broiler chicks were individually weighed and randomly assigned to four groups of eight replicate cages (10 broilers per cage). Birds were fed a basal diet supplemented with 0 (control), 1,000, 2,000, or 4,000 mg/kg GLPs. Compared to that of the control group, dietary supplementation with 2,000 mg/kg GLPs linearly increased the average daily weight gain during days 0-42 ($P < 0.05$) and linearly decreased the feed to gain ratio during days 1–21 and 22–42 ($P < 0.05$). Broilers fed GLP-supplemented diets showed linear ($P < 0.05$) and quadratic ($P < 0.05$) increases in serum superoxide dismutase ($P < 0.05$), glutathione peroxidase, and catalase activities in the liver, whereas GLP supplementation decreased serum and liver malondialdehyde concentrations ($P < 0.05$). A linear increase in serum catalase activity was observed following supplementation with 2,000 or 4,000 mg/kg GLPs ($P < 0.05$). Broilers fed GLP-supplemented diets showed linear ($P < 0.05$) and quadratic ($P < 0.05$) increases in serum immunoglobulin (Ig) A, IgG, interleukin (IL)-6, IL-1β, IL-10, and interferon-γ concentrations ($P < 0.05$), and a trend towards linear improvement in IL-4 levels ($P = 0.089$). Dietary GLP supplementation increased the *Lactobacillus* spp. population compared to that of the control group ($P < 0.05$) and 2,000 and 4,000 mg/kg of GLPs nearly decreased the population of *E. coli* in the cecum ($P = 0.056$). Therefore, dietary GLP supplementation may improve broiler growth performance by altering antioxidant capacity, immune function, and the gut microbiota composition. Considering the effects of different doses of GLP on the above parameters, 2,000 mg/kg of GLPs was identified as the best dose.

Key words: antioxidant capacity, broilers, *Gracilaria lemaneiformis* polysaccharides, growth performance, immune function

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Introduction

In recent years, challenges in the poultry industry have become increasingly apparent. Long-term growth in an enclosed, high-density, and stressful environment results in a low state of broiler immunity, making them susceptible to infectious diseases (Dong et al., 2021). Although antibiotic growth promoters (AGPs) are widely used in broiler diets to improve growth performance and health (Baurhoo et al., 2009), concerns associated with the development of antibiotic-resistant bacteria and food safety have limited their use in animal feed (brega et al., 2008). AGPs were banned in poultry feed in the European Union and in other countries in 2006. Additionally, the Chinese government banned AGP use in animal feed in July 2020.

Plant extracts, polysaccharides, essential oils, and polyphenols increase nutrient absorption, and improve immune responses and antioxidant activities in broilers (Hashemipour et al., 2013; Ao and Kim, 2020; Zhang et al., 2021). *Gracilaria lemaneiformis* is a red marine macroalga that is widely distributed along various Chinese coasts. It has also been used in traditional Chinese medicine. The primary constituents of dried *G. lemaneiformis* extract are carbohydrates, amino acids, minerals, vitamins, and polysaccharides (Lu et al., 2022). Polysaccharides are one of the major functional components of *G. lemaneiformis* and mainly comprise 3,6-anhydro-L-galactose and D-galactose (Long et al., 2021a). *G. lemaneiformis* polysaccharides (GLPs) possess various biological activities, including hypoglycemic (Sun et al., 2018),

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Correspondence: Dr. Jiashun Chen, College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128, P.R. China. (Email: jschen@hunau.edu.cn)

antioxidant (Wu et al., 2017), antiviral (Fan et al., 2012), anti-inflammatory (Gong et al., 2021), gut microbiota-modulating (Zhang et al., 2020), and immunomodulatory (Ren et al., 2017) activities. Xuan et al. (2013) report that dietary supplementation with *G. lemaneiformis* enhances the growth performance and health of juvenile black sea bream. Yu et al. (2016) have demonstrated that supplementation with a moderate GLP concentration improves growth performance and resistance to salinity stress, increases the length and integrity of intestinal microvilli, and enhances the immunity of juvenile Pacific white shrimp (*Litopenaeus vannamei*). To the best of our knowledge, no study has reported the effects of GLPs in broiler chickens. GLPs may exert antioxidant activity, stimulate the immune system, and improve broiler growth performance. Therefore, this study investigated the effects of dietary GLP supplementation on the growth performance, immunity, antioxidant activity, cecal microflora, and meat quality of broiler chickens.

Materials and Methods

GLP Preparation

G. lemaneiformis was collected from Nan'ao Island, Guangdong, China. GLP powder was purchased from Shanxi Sinuote Biotechnology Co., Ltd. (Xi'an, China). The GLP powder was incubated overnight in a methanol/dichloromethane/water (4:2:1; v/v/v) solution at a ratio of 10:1 (v/w, mL/g) with shaking to remove low molecular weight compounds. The polysaccharide content isolated from *G. lemaneiformis* was ≥ 50%, as determined using the phenol-sulfuric acid method (Zhang et al., 2020).

Birds, experimental design, and management

All experimental procedures were approved by the Biomedical Research Ethics Committee of Hunan Agricultural University (No. 20220039). A total of 320 1-day-old male Arbor Acres broiler chicks were purchased from a commercial hatchery (Changsha, China). All broilers were individually weighed and randomly assigned to four groups with eight replicate cages (10 broilers per cage), according to their initial body weight. Birds in the four groups were fed a basal diet supplemented with 0 (control group), 1,000, 2,000, or 4,000 mg/kg GLPs. According to the recommendations of the National Research Council (NRC, 1994), a basal diet was formulated to meet or exceed the nutrient requirements of broilers during the starter (1–21 days) and grower (22–42 days) phases (Table 1). During the trial period, all birds were housed in temperature-controlled rooms with steel cages (120 × 60 × 50 cm), feeders, and nipple drinkers. The temperature was maintained at 34 °C during the first week and was reduced by 2 °C–3 °C each week until a final temperature of 24 °C was obtained. Continuous lighting was provided throughout the experiments. All birds had *ad libitum* access to food and water.

Growth performance

On days 21 and 42, all birds were weighed after a 12-h fast, and feed intake was recorded simultaneously. These values were used to determine the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR: feed intake/body weight gain). In addition, mortality was recorded throughout the experiment.

Sample collection

At the end of the experiment, one bird was randomly selected from each cage and euthanized by cutting the jugular vein. Blood samples were collected (5 mL) in 10 mL anticoagulant-free vacutainer tubes and immediately centrifuged at 3,000 ×*g* for 10 min at 4 °C to obtain serum. Serum samples were stored at −20 °C until further analysis. After blood collection, liver tissue samples were harvested, washed in normal saline, and stored in liquid nitrogen for the assessment of antioxidant enzymes. In addition, pectoralis major muscle samples were collected and stored at 4 °C for the assessment of meat color, pH, and drip loss.

Assessment of antioxidant enzymes in the serum and liver

Liver homogenates were prepared by homogenizing liquid nitrogen-frozen tissues with an ice-cold homogenate medium (physiological saline solution) at a ratio of 1:9 (liver, wt/vol) until no tissue particles were visible (approximately 35 s) using

Table 1. **Composition and nutrient content of experimental diets**

Items	Age (days)	
	Starter (0 to 21 days)	Grower (22 to 42 days)
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
Total	100	100
Analytical composition[2]		
ME, kcal/kg[3]	3087	3212
Crude protein,%	21.49	19.58
Calcium,%	1.04	0.95
Total phosphorus,%	0.67	0.65
Lysine,%	1.21	1.08
Methionine,%	0.51	0.43

[1]Supplied per kilogram of diet: vitamin A (*trans*-retinyl acetate), 10,050 IU; vitamin D_3,2,800 IU; vitamin E (DL-α-tocopheryl acetate), 50 mg; vitamin K_3, 3.5 mg; thiamine, 2.5 mg; riboflavin, 7.5 mg; pantothenic acid, 15.3 mg; pyridoxine, 4.3 mg; vitamin B_{12}(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.

[2]All nutrient levels except metabolizable energy were analyzed and values are the mean of two determinations.

[3]ME = metabolizable energy, ME values have been calculated using NRC (1994) values.

a motor-driven homogenizer (JXFSTPRP-24, Shanghai Jingxin Industrial Development Co., Ltd., Shanghai, China). After centrifugation at 4,500×*g* for 15 min, the homogenate supernatant was then separated, aliquoted, and stored at -80 °C for antioxidant enzyme analysis.

The activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), and the concentration of malondialdehyde (MDA) in the serum and liver were measured using commercially available colorimetric diagnostic kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Assessment of serum immunoglobulins and inflammatory factors

Serum levels of immunoglobulin A (IgA), IgM, IgG, interleukin (IL)-1β, IL-2, IL-4, IL-10, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) were determined using enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All samples were assessed in triplicate.

Assessment of meat quality

Meat quality was assessed in eight birds selected from each group (one bird per replicate). A portable pH meter (H199163, Hanna Instruments, Woonsocket, RI, USA) was used to determine the pH of breast muscle samples 45 min and 24 h after the birds were sacrificed. The 3nh NR200 Precision Colorimeter (Nanshan District, Shenzhen, China) was used to determine the trichromatic coordinates (L* = lightness; a* = redness; b* = yellowness) of breast muscle samples at 45 min and 24 h after the birds were sacrificed. A method described by Hiscock et al. (2022) was used to determine the cooking loss of the pectoralis major muscle after 24 h of storage of harvested samples at 4 °C. The 48 h drip loss of the pectoralis major muscle was determined using the method described by Wu et al. (2020). The water-holding capacity (WHC) of breast meat was determined using the method described by Kauffman et al. (1986).

Assessment of cecal microflora

At the end of the experiment, cecal digesta samples were collected from eight birds from each treatment group. Briefly, 1 g of fresh digesta was collected from the middle portion of the cecum, stored in bottles with a CO_2 current, and transported to the laboratory to estimate bacterial counts, as described by Long et al. (2021b). Digesta samples were suspended in 99 mL sterile 0.9% saline and homogenized in a stomacher for 5 min. Each homogenate was diluted 10-fold (10% w/v) in sterile ice-cold normal saline. Diluted samples (0.1 mL) were inoculated onto selective agar for bacterial enumeration. *E. coli* was incubated on MacConkey agar at 37 °C for 24 h. *Lactobacillus* spp. were incubated on Luria-Bertani agar and Briggs liver agar in an anaerobic incubator at 37 °C for 48 h. Agars were purchased from Beijing Ruizekang Technology Co., Ltd. (Beijing, China).

Statistical analysis

Data have been analyzed with analysis of variance using the general linear model procedure in SAS (SAS Institute Inc., Cary, NC, USA). The linear and quadratic effects of different GLP concentrations were assessed using orthogonal polynomials. Cages were used as experimental units to analyze performance data. For analysis of other data, each broiler per replicate was treated as an experimental unit. Data are expressed as the mean ± standard error of the mean (SEM). A P-value < 0.05 indicated statistical significance.

Results

Effects of GLPs on Growth Performance in Broilers

Data regarding the effects of dietary supplementation with 2,000 mg/kg GLPs on the growth performance of broilers during different phases are presented in Table 2. During days 1–21, GLP supplementation decreased the FCR in broilers ($P < 0.05$). During days 22–42, GLP supplementation increased ADG and decreased FCR ($P < 0.05$). Compared to that of the control diet, supplementation with GLPs linearly increased ($P < 0.05$) ADG over the entire study period (days 1–42).

Effects of GLPs on antioxidant capacity in broilers

Data on the effects of dietary GLP supplementation on the levels of antioxidant enzymes in the serum and liver on day 42 are presented in Table 3. Serum SOD activity was higher in broilers fed a diet supplemented with 2,000 mg/kg GLPs than in those fed the control diet (linear, $P = 0.028$; quadratic, $P = 0.013$). Supplementation with 1,000–4,000 mg/kg GLPs linearly increased CAT activity ($P = 0.024$), but decreased MDA concentration (linear, $P = 0.005$; quadratic, $P = 0.043$) in the serum. In addition, dietary supplementation with 2,000 and 4,000 mg/kg GLPs increased the activity of GSH-Px (linear, $P = 0.024$; quadratic, $P = 0.089$) and CAT (linear, $P = 0.019$; quadratic, $P = 0.038$), but significantly decreased the MDA concentration (linear, $P = 0.035$; quadratic, $P = 0.011$) in the liver.

Effects of GLPs on serum immunoglobulins and inflammatory factors in broilers

The effects of dietary GLP supplementation on serum immunoglobulins and inflammatory factor levels in broilers are presented in Table 4. The levels of serum IgA (linear, $P = 0.026$; quadratic, $P = 0.011$) and IgG (linear, $P = 0.015$; quadratic, $P = 0.031$) were higher in broilers fed diets supplemented with 2,000 or 4,000 mg/kg GLPs than in those fed the control diet. The levels of serum IL-6 (linear, $P = 0.021$; quadratic, $P = 0.036$), IL-1β (linear, $P = 0.014$; quadratic, $P = 0.028$), and IL-10 (linear, $P = 0.029$; quadratic, $P = 0.012$) were significantly increased in broilers fed GLP supplemented diets. In addition, dietary supplementation with 2,000 and 4,000 mg/kg GLPs increased the levels of serum TNF-α (linear, $P = 0.042$; quadratic, $P = 0.033$) and IFN-γ (linear, $P = 0.034$; quadratic, $P = 0.046$).

Effects of GLPs on meat quality of broilers

Descriptive statistics for meat quality traits are presented in Table 5. Dietary GLP supplementation exerted neither linear nor quadratic effects ($P > 0.05$) on breast meat quality parameters (meat pH, color, WHC, drip loss, and cooking loss).

Effects of GLPs on the cecal microflora of broilers

The composition of broiler chicken cecal microflora is shown

Table 2. **Effects of dietary Gracilaria lemaneiformis polysaccharides (GLPs) on the growth performance of broiler chickens from 0 to 42 days (d) of age.**

Items	GLP level (mg/kg)				SEM	*P*-value	
	0	1000	2000	4000		Linear	Quadratic
0 to 21 d							
ADFI (g/d/bird)	44.23	46.31	47.64	45.25	0.342	0.175	0.214
ADG (g/d/bird)	29.46	31.25	33.43	30.54	0.133	0.353	0.156
FCR (feed/gain, g/g)	1.50^{a}	1.48^{ab}	1.43^{b}	1.48^{ab}	0.015	0.036	0.015
22 to 42 d							
ADFI (g/d/bird)	129.32	132.44	137.52	130.28	1.638	0.215	0.342
ADG (g/d/bird)	75.74^{b}	80.53^{ab}	85.45^{a}	79.92^{ab}	0.973	0.029	0.041
FCR (feed/gain, g/g)	1.71^{a}	1.64^{a,b}	1.61^{b,c}	1.65^{a,b}	0.012	0.036	0.103
0 to 42 d							
ADFI (g/d/bird)	86.25	89.35	92.55	89.70	0.852	0.087	0.289
ADG (g/d/bird)	53.05^{b}	55.85^{a,b}	59.41^{a}	54.70^{a,b}	0.369	0.045	0.113
FCR (feed/gain, g/g)	1.59	1.56	1.52	1.56	0.011	0.098	0.127

^{a,b,c} Means within rows with different superscript letters differ significantly ($P < 0.05$), whereas values with no letters or the same superscript letters are not significantly different ($P > 0.05$).
ADG, average daily gain; ADFI, average daily feed intake; FCR, feed-to-gain ratio; SEM, standard error of the mean.

Table 3. **Effect of dietary Gracilaria lemaneiformis polysaccharides (GLPs) on antioxidant activities of broiler chickens at 42 days of age.**

Items	GLP level (mg/kg)				SEM	*P*-value	
	0	1000	2000	4000		Linear	Quadratic
Serum							
SOD,U/mL	136.78^{bc}	143.52^{b}	169.55^{a}	153.42^{ab}	1.745	0.028	0.013
GSH-Px,U/mL	204.21	216.44	228.36	221.39	2.214	0.102	0.239
CAT, U/mL	4.78^{b}	6.32^{a}	8.32^{a}	7.43^{a}	0.258	0.024	0.378
MDA, nmol/mL	6.39^{a}	4.27^{b}	3.66^{b}	4.89^{b}	0.322	0.005	0.043
Liver							
SOD, U/mg	319.32	321.41	332.63	335.79	1.874	0.127	0.316
GSH-Px, U/mg	4.59^{bc}	5.89^{ab}	6.88^{a}	6.59^{a}	0.017	0.024	0.089
CAT, U/mg	4.41^{b}	5.53^{ab}	6.37^{a}	7.11^{a}	0.014	0.019	0.038
MDA, nmol/mg	5.45^{a}	3.74^{b}	2.57^{b}	3.48^{b}	0.163	0.035	0.011

^{a,b,c} Means within rows with different superscript letters differ significantly ($P < 0.05$), whereas values with no letters or the same superscript letters are not significantly different ($P > 0.05$).
SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde; SEM, standard error of the mean.

in Figure 1. The abundance of *Lactobacillus* spp. was higher in broilers fed diets supplemented with 1,000–4,000 mg/kg GLPs than in those fed the control diet ($P < 0.05$). However, broilers fed diets supplemented with 2,000 and 4,000 mg/kg GLPs showed a trend towards a reduction in the abundance of *E. coli* compared to broilers fed the control diet ($P = 0.056$).

Discussion

Dietary supplementation with seaweed or seaweed extracts improves the growth performance and feed efficiency of pigs (Gahan et al., 2009; Walsh et al., 2013), fish (Xuan et al., 2019), and chickens (Abudabos et al., 2013). To the best of our knowledge, no previous study has reported the effects of dietary GLPs on broiler chickens. In this study, dietary supplementation with 2,000 mg/kg GLPs improved ADG and decreased FCR in broilers. Nhlane et al. (2020) report that dietary supplementation with 2.5% green seaweed improves feed intake and overall body weight gain in indigenous chickens. In addition, Yu et al. (2016) suggest that dietary supplementation with 2%–3% *G. lemaneiformis* increases the final body weight, overall weight gain, and specific growth ratio of juvenile Pacific white shrimp (*Litopenaeus vannamei*). However, the effects of dietary supplementa-

Table 4. **Effect of dietary Gracilaria lemaneiformis polysaccharides (GLPs) on serum immunoglobulins and inflammatory factors of broiler chickens at 42 days of age.**

Items	GLP level (mg/kg)				SEM	*P*-value	
	0	1000	2000	4000		Linear	Quadratic
IgA, mg/mL	4.35^{c}	6.24^{b}	7.56^{a}	7.01^{ab}	0.126	0.026	0.011
IgM, mg/mL	1.33	1.38	1.40	1.42	0.081	0.472	0.693
IgG, mg/mL	2.21^{bc}	2.57^{b}	3.65^{a}	3.29^{a}	0.132	0.015	0.031
IL-6, pg/mL	101.65^{b}	125.03^{a}	137.42^{a}	136.11^{a}	8.485	0.021	0.036
IL-1β, pg/mL	203.34^{b}	223.62^{a}	238.73^{a}	237.55^{a}	12.436	0.014	0.028
IL-4, ng/mL	74.22	78.68	82.36	81.03	4.598	0.089	0.525
IL-10, ng/mL	25.68^{b}	32.97^{a}	36.31^{a}	37.27^{a}	3.467	0.029	0.012
TNF-α, pg/mL	31.63^{b}	40.54^{ab}	47.94^{a}	46.32^{a}	4.831	0.042	0.033
IFN-γ, pg/mL	62.35^{b}	66.37^{b}	88.68^{a}	85.79^{a}	2.356	0.034	0.046

^{a,b,c} Means within rows with different superscript letters differ significantly ($P < 0.05$), whereas values with no letters or the same superscript letters are not significantly different ($P > 0.05$).
IgA, immunoglobulin (Ig)A; IgM, immunoglobulin (Ig)M; IgG, immunoglobulin (Ig)G; IL-1β, interleukin (IL)-1β; IL-6, interleukin (IL)-6; IL-4, interleukin (IL)-4; IL-10, interleukin (IL)-10; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; SEM, standard error of the mean.

Table 5. **Effect of dietary *Gracilaria lemaneiformis* polysaccharides (GLPs) on the meat quality of breast muscle in broiler chickens at 42 days of age.**

Items	GLP level (mg/kg)				SEM	*P*-value	
	0	1000	2000	4000		Linear	Quadratic
$pH_{45\ min}$	5.84	5.86	5.89	5.88	0.022	0.241	0.438
$pH_{24\ h}$	6.04	6.02	6.01	6.05	0.034	0.304	0.763
Lightness(L^*)$_{45\ min}$	52.34	51.97	52.78	53.14	0.621	0.592	0.378
Redness(a *)$_{45\ min}$	11.84	11.76	11.82	12.05	0.452	0.613	0.794
Yellowness(b *)$_{45\ min}$	7.47	7.61	7.71	7.66	0.385	0.296	0.582
Lightness(L^*)$_{24\ h}$	51.23	50.86	51.39	52.21	0.619	0.241	0.336
Redness(a *)$_{24\ h}$	10.65	10.34	10.76	11.45	0.832	0.462	0.812
Yellowness(b *)$_{24\ h}$	6.94	7.14	7.32	7.21	0.521	0.684	0.302
Drip loss, %	3.42	3.21	3.33	3.37	0.243	0.693	0.316
Cooking loss, %	27.65	28.12	29.45	28.62	1.263	0.417	0.758
WHC,%	88.57	88.63	88.25	87.79	1.94	0.641	0.293

^{a,b,c} Means within rows with different superscript letters differ significantly ($P < 0.05$), whereas values with no letters or the same superscript letters are not significantly different ($P > 0.05$).
WHC, water-holding capacity; SEM, standard error of the mean.

tion with marine seaweed on the growth performance of poultry remain controversial. Abudabos et al. (2013) have demonstrated that dietary supplementation with 30 g/kg *Ulva lactuca* does not affect feed intake or body weight gain in broiler chickens. El-Deek and Brikaa (2009) reported that the inclusion of 3% seaweed in the diets of ducks does not significantly affect their growth performance. These conflicting results may be attributed to differences in diets, animal species, feeding environments, and growth stages. In addition, GLPs consist of agarose and these oligosaccharides act as potential prebiotics with several health-promoting properties, including modulation of the composition of gut microbes (Lu et al., 2022), anti-oxidation (Tang et al., 2021), and anti-inflammatory effects (Liu et al., 2016), which may be responsible for stimulating broiler growth. However, the beneficial effects of natural GLPs on growth performance and the underlying mechanisms warrant further investigation.

Excessive reactive oxygen species (ROS) may disrupt metabolism in animals, damage cell structures, and accelerate oxidation, thereby causing various diseases (Hou et al., 2022). SOD, GSH-Px, and CAT are the primary parameters used to evaluate antioxidant levels in organisms. SOD participates in oxygen metabolism, scavenging, and the reduction of superoxide to water and molecular oxygen (Hao et al., 2015). GSH-Px reduces lipid hydroperoxides to alcohols and free hydrogen peroxide to water (Cheng et al., 2020). MDA levels indicate the degree of organic lipid peroxidation and are associated with cell damage (Cheng et al., 2019). GLPs increase the activity of total antioxidant capacity, GSH-Px, CAT, and SOD, and decrease the concentra-

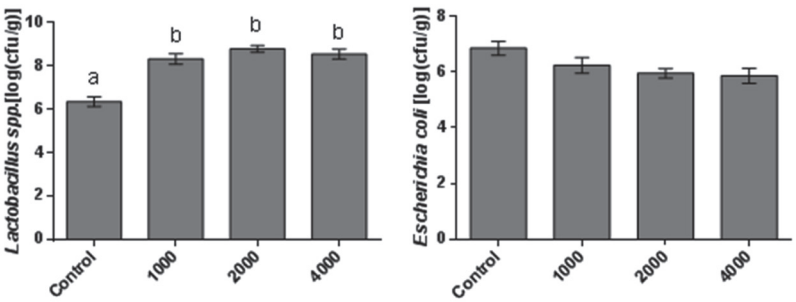

Fig. 1. **Effect of dietary *Gracilaria lemaneiformis* polysaccharides on cecal microbial populations in broilers.** Bacterial number is expressed as log10 colony forming units per gram wet digesta. Each bar represents the mean for eight birds per treatment ± standard error (SE). [a-b] Bars with different letters differ significantly ($P < 0.05$).

tions of MDA and intracellular ROS in HepG2 cells (Long et al., 2022). Wen et al. (2017) report that GLPs enhance the activity of SOD, GSH-Px, and CAT and reduce MDA levels in the serum and liver of mice, exhibiting excellent antioxidant activity. Tang et al. (2021) report that modified GLPs exhibit better free radical-scavenging ability. The current study revealed that dietary supplementation with 2,000 and 4,000 mg/kg GLPs linearly and/or quadratically increased SOD, CAT, and GSH-Px activity and reduced the concentration of MDA in the serum and liver of broilers. Similarly, Liu et al. (2020a) report that dietary supplementation with algae-derived polysaccharides increases SOD activity in the liver and CAT activity in the serum, and reduces MDA levels in the serum and liver of broilers. The findings of this study suggest that polysaccharides derived from marine organisms, such as GLPs, may be used as natural antioxidants in broilers and that their antioxidant activity is correlated with growth performance.

Serum immunoglobulins (IgA, IgM, and IgG) are important components of the humoral immune system and play critical roles in immune responses and gut epithelial protection against pathogens (Chen et al., 2019). Natural polysaccharides derived from seaweeds also promote host immune responses (Liu et al., 2020b). To the best of our knowledge, this study is the first to demonstrate that dietary supplementation with 2,000 and 4,000 mg/kg GLPs increased serum IgA and IgG levels in broilers. Zou et al. (2021) report that dietary supplementation with *Enteromorpha*-derived polysaccharides increases IgA and IgG serum concentrations in weaned piglets. Excessive immunoglobulins stimulate complement components to enhance specific immune mechanisms in birds and protects them against infection (Long et al., 2020). Furthermore, inflammatory cytokines play essential roles in systemic and local immune responses (Han et al., 2021). Both *in vitro* and *in vivo* studies have demonstrated that GLPs regulate immune activity by activating macrophages or lymphocytes to promote the secretion of cytokines (e.g., TNF-α, IL-6, IL-1β, IL-4, and IL-10) (Liu et al., 2016; Han et al., 2020). In this study, broilers fed diets with 2,000 and 4,000 mg/kg GLPs had high serum concentrations of IL-6, IL-1β, TNF-α, IFN-γ, and IL-10. Similar findings are reported by Zou et al. (2021), who demonstrate that pigs fed diets containing *Enteromorpha*-derived polysaccharides enhance humoral immune response by increasing serum IL-6 and TNF-α concentrations. These results suggest that the inclusion of seaweed extracts in diets enhances humoral immune responses in both livestock and poultry.

The appearance, texture, juiciness, flavor, WHC, and nutritional value of meat are key parameters in meat production. Color, pH, cooking loss, drip loss, and shear strength are the main indicators of meat quality (Choi and Kim, 2009). In this study, dietary GLP supplementation had no effect on meat quality parameters. These results are consistent with those reported by Nhlane et al. (2020), who demonstrate that dietary supplementation with seaweed do not adversely affect the pH or color of breast meat in Boschveld hens. It is noteworthy that the pH of meat (5.8–6.1) reported in this study falls within the normal pH range of poultry meat (5.7–6.1) proposed by Werner et al. (2008). Furthermore, dietary GLPs did not affect the cooking loss, drip loss, or WHC of meat, indicating that the inclusion of GLPs in the diet did not interfere with the normal oxidative stability of broiler meat. Oxidative stress is a biological phenomenon that adversely affects meat quality by increasing the rate at which meat pH declines, consequently reducing WHC (Wang et al., 2016). The results of this study are consistent with those of a previous study, which reported that WHC, drip loss, and cooking loss are not altered in broilers fed seaweed-supplemented diets (Matshogo et al., 2020). Future studies should investigate the physicochemical characteristics of chickens fed seaweed-based diets.

Diarrhea caused by *E. coli* is one of the most common poultry diseases and leads to high morbidity and mortality (Liang et al., 2021). *Lactobacillus* is a beneficial probiotic strain that balances the gut microbiota, maintains the mucus layer, inhibits pathogenic bacteria, such as *E. coli*, and boosts immunity (Wang et al., 2017; Long et al., 2021b). Dietary supplementation with

seaweed or seaweed extracts modifies selected populations of gut microbes (e.g., *Lactobacillus* and *E. coli*) in pigs (Mcdonnell et al., 2010; Sweeney et al., 2011). In this study, dietary supplementation with GLPs increases the abundance of *Lactobacillus* and nearly reduces the abundance of *E. coli*, which is consistent with the results of previous studies by O'Doherty et al. (2010), Huang et al. (2019), and Lu et al. (2022). Diets containing complex carbohydrates contribute to the growth of polysaccharide bacteria, such as *Lactobacillus* and some *Lachnospiraceae* (Flint et al., 2012; Fava et al., 2019). It is possible that GLPs are used as substrates for degradation by *Lactobacillus* (Huang et al., 2019). In addition, sulfated polysaccharides derived from *G. lemaneiformis* alleviate diarrheal symptoms by altering the composition of *E. coli* in mice (Liu et al., 2019). Intragastric treatment with sulfated polysaccharides derived from *G. Lemaneiformis* increases acetic acid, propionic acid, and butyric acid levels, and decreases fecal pH in mice (Han et al., 2021). Therefore, polysaccharides may be more readily used by bacteria, such as *Lactobacillus,* to produce short-chain fatty acids, which in turn rapidly decrease the gut pH. This reduction subsequently suppresses the growth of pH-sensitive *E. coli*, providing energy for intestinal cells and protecting the intestinal barrier (Knudsen et al., 2012; Clavijo and Flórez, 2018).

This study revealed that dietary GLP supplementation improved ADG and decreased the FCR in broiler chickens. In addition, dietary GLP supplementation enhanced SOD, GSH-Px, and CAT activity; decreased MDA levels in the serum and liver; increased IgA, IgG, IL-6, TNF-α, IFN-γ, IL-1β, and IL-10 levels in the serum; and modulated the composition of the cecal microbiota. Altogether, this study suggested that GLPs might be used as potential additives to improve the health and growth performance of broilers, and a concentration of 2,000 mg/kg was considered the best dose for GLP inclusion in broiler diets.

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Authors' Contributions

Shengwang Jiang, Chaoyun Yang, and Jiashun Chen designed the study; Yintao Xiao and Qian Jiang acquired the data and performed experiments; Yintao Xiao and Saizhen Zheng conducted the animal experiments; Jiashun Chen and Shengwang Jiang performed data analysis; Shengwang Jiang, Chaoyun Yang, and Jiashun Chen wrote the manuscript. All authors have read and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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