

Effects of Dietary Supplementation with Glycyrrhiza Polysaccharides on Growth Performance, Histomorphology, Immunity, and Oxidative Stress in Largemouth Bass (*Micropterus salmoides*)

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Abstract

To investigate the effects of dietary supplementation with Glycyrrhiza polysaccharide (GCP) on the growth performance of largemouth bass (*Micropterus salmoides*), 450 fish with an initial body weight of 11.46 ± 0.51 g were randomly divided into five groups, with three replicates per group. Throughout an 8-week nutritional trial, fish were assigned to one of five experimental groups and administered diets supplemented with 0 (control group), 500, 1000, 1500, or 2000 mg/kg GCP. The experimental results showed that the addition of GCP to the basal diet significantly increased the FBW, WGR, and SGR of largemouth bass, while the FCR was significantly reduced. Compared with the control group, crude protein content in fish muscle was significantly increased in the 1000 mg/kg GCP group, while crude lipid content was significantly decreased in both the 1000 and 1500 mg/kg GCP groups. The administration of 1000–1500 mg/kg significantly boosted the activities of antioxidant enzymes (T-SOD, CAT, GSH) in the liver and intestine, and significantly decreased the MDA content compared to the control group. The activities of α -amylase and trypsin were significantly increased at 1500 mg/kg but significantly decreased at 2000 mg/kg. As GCP supplementation increased, the expression levels of *nrf2*, *gclc*, *gclm*, and *nqo1* peaked at 1500 mg/kg of GCP. In contrast, the expression of *keap1* was significantly reduced at 1000 mg/kg and 1500 mg/kg but significantly increased at 2000 mg/kg. These results suggest that GCP may enhance antioxidant enzyme activity by regulating the Nrf2-Keap1 pathway. Following 96 h of waterborne cadmium (Cd) exposure, T-SOD activity was significantly elevated at the 1500 mg/kg group, which exhibited a robust antioxidant capacity at 96 h. Analysis of inflammatory gene expression revealed that the relative expression levels of the *il-1 β* , *il-6*, and *tnf- α* genes were significantly downregulated in the 1500 mg/kg GCP group, while the expression of the anti-inflammatory gene *il-10* showed an opposite trend. Collectively, our findings indicated that the optimal supplementation level of GCP in largemouth bass diets was 1000–1500 mg/kg.

1. Introduction

The largemouth bass (*Micropterus salmoides*) is a high-quality freshwater commercial fish widely cultured worldwide. Endowed with characteristics including rapid growth, tender flesh and abundant nutritional value, this species occupies a prominent position in China's freshwater aquaculture industry (Zhang et al., 2020). With the industrialized fish culture models, largemouth bass faces many challenges, such as deteriorating farming environments (heavy metal pollution, water quality fluctuations, etc.) (Thai et al., 2021), frequent stress responses (Lee et al., 2023) and increasing pressure on disease prevention and control (Hu et al., 2020). These factors not only lead to reduced growth performance and lower feed utilization efficiency but also cause oxidative damage and immune dysfunction in the fish, ultimately affecting their health status and farming profitability (Chen et al., 2023a). Therefore, exploring safe, efficient, and environmentally friendly feed additives to enhance the growth, antioxidant capacity, immune function, and stress resistance of largemouth bass has become a prominent research area in the current aquaculture field.

Natural plant polysaccharides, as an important class of bioactive substances, have attracted widespread attention in aquatic feed applications due to their various biological effects such as regulating animal growth, enhancing antioxidant and immune functions, and improving intestinal health (Song et al., 2021). Licorice (*Glycyrrhiza uralensis*), a plant used in both traditional medicine and cuisine, contains licorice polysaccharides (*Glycyrrhiza polysaccharides*, GCP) as a key component. GCP demonstrates a range of beneficial biological activities, including antioxidant, immunomodulatory, and anti-inflammatory effects (Du et al., 2017). Existing studies have shown that plant polysaccharides can positively impact fish through multiple pathways. In terms of growth performance, polysaccharides can enhance nutrient digestion and absorption, improve feed-utilization efficiency, and thereby accelerate fish growth. Astragalus polysaccharides can notably enhance the weight gain and specific growth rates of crucian carp (*Carassius auratus*) while lowering the feed conversion ratio. (Li et al., 2023b). Atractylodes macrocephala polysaccharide and yam polysaccharide can effectively enhance the anti-apoptotic capacity of grass carp (*Ctenopharyngodon idella*) hepatocytes (Sun et al., 2023), and prevent *Aeromonas hydrophila* infection (Tian et al., 2025). Polysaccharides may boost the antioxidant defense in fish and shield them from oxidative stress by elevating the activity of enzymes like superoxide dismutase (SOD) and catalase (CAT) (Dai et al., 2018), elevating the content of non-enzymatic antioxidants like reduced glutathione (GSH) (Jia et al., 2018), and reducing the production of lipid peroxidation products such as malondialdehyde (MDA) (Ahmadifar et al., 2024). For intestinal health, polysaccharides can improve intestinal tissue morphology, promote intestinal villi development, enhance digestive enzyme activity, regulate the balance of intestinal microbiota, and boost the digestive absorption and immune barrier functions of the intestine (Liu et al., 2022). Furthermore, polysaccharides can influence the health status of organisms by regulating serum biochemical indicators and modulate the physiological functions of fish at the molecular level through their effects on gene expression in the Nrf2-Keap1 signaling pathway (Yu et al., 2023b).

However, comprehensive investigations into the application of GCP in largemouth bass aquaculture are currently limited. It remains unclear how GCP influences the synergistic influences on growth efficiency, antioxidant status, tissue's well-being, and immune function in largemouth bass reared under complex conditions, such as heavy metal stress. However, studies have shown that licorice polysaccharides may alleviate body damage by binding to metal ions (Yang et al., 2024). Cadmium (Cd), a prevalent heavy metal pollutant, is frequently discharged into aquatic environments (Hu et al., 2020; Lacave et al., 2020), posing significant threats to aquatic life. Fish are highly sensitive to Cd toxicity (Renieri et al., 2017). Exposure to high or low concentrations of Cd can lead to the overproduction of reactive oxygen species (ROS) in organisms. This, in turn, induces oxidative stress and disrupts the antioxidant system balance (Chen et al., 2024). This also leads to tissue damage in organs such as the intestines and liver, impairing normal physiological functions in fish (Guardiola et al., 2013; Yu et al., 2021; Zheng et al., 2016). In this context, studying the effects of GCP on largemouth bass under Cd exposure is crucial for elucidating its regulatory mechanisms and assessing its potential for future applications.

Our study systematically investigated the effects of varying dietary supplementation levels of GCP on the growth performance, serum biochemical parameters, intestinal and liver histomorphology, digestive

enzyme activities, antioxidant capacity, and associated gene expression in largemouth bass. The research aimed to elucidate the efficacy and underlying mechanisms of GCP in largemouth bass, ascertain its optimal supplementation level, and provide theoretical foundations and data support for the scientific application of GCP in largemouth bass aquaculture.

2. Materials and methods

2.1 Diet and experimental design

The experimental fish were obtained from a commercial fish farm (Jiyuan, Henan Province) with an average body weight of 11.46 ± 0.51 g and body length of approximately 10.25 ± 0.26 cm. A total of 450 healthy largemouth bass were chosen for the study and randomly allocated into five groups, with three replicates in each group. The control group was fed a basal diet, while the other groups were fed diets supplemented with 500, 1000, 1500, and 2000 mg/kg of GCP, respectively. The feed ingredients were thoroughly mixed and pelleted using a 2.5mm pellet mill (Shanghai Taiyi Machinery Co., Ltd.). The formulated feed was subsequently dried under indoor conditions and stored in sealed containers. The composition of the feed formula is shown in Table 1. The GCP used in this experiment was produced by Lansly Technology Co., Ltd. (Henan, China) with a purity of 98%. It is primarily composed of four monosaccharides: rhamnose, arabinose, galactose, and glucose. Before starting the formal trials, the test fish were acclimated for two weeks in the indoor recirculating aquaculture system. The specific culture conditions were maintained as follows: water temperature $25.0 \pm 0.5^{\circ}\text{C}$, pH 7.5 ± 0.5 , dissolved oxygen > 5.5 mg/L, nitrite < 0.5 mg/L. The fish were fed at 8:00 AM and 4:00 PM until visibly satiated, and one-third of the water in each aquarium was replaced daily with dechlorinated tap water.

Table 1
The basic formulation and nutritional levels of the experimental diet (% dry matter).

Ingredients	Content	Nutritional level ³	
Fish meal ¹	40.00	Crude protein(%)	49.31
Chicken meal	20.00	Crude lipid(%)	12.46
Soybean meal	18.00	Ash (%)	5.05
Yeast hydrolysate	5.00	Moisture (%)	8.85
Wheat flour	9.50		
Premix ²	1.00		
Mixed oil	4.00		
Choline chloride	0.50		
Calcium dihydrogen phosphate	1.00		
Cellulose	1.00		
Total	100		
Note: 1. Fish meal, crude protein, crude lipid and ash were 65.76%, 13.13% and 5.92%, respectively.			
2. The premix, purchased from Charoen Pokphand Group, (per kilogram of diet) provided: vitamin K 30.0 mg, Ca 300.0 mg, Mg 10.0 mg, Zn 3.0 mg, Fe 22.0 mg, Cu 1.7 mg, I 0.29 mg, Se 0.02 mg, vitamin A 8000 IU, vitamin C 230.0 mg, vitamin D 800 IU, vitamin E 90.0 mg, vitamin K 8.0 mg, thiamin 10.0 mg, nicotinic acid 100.0 mg, pantothenic acid 50.0 mg, vitamin B6 15.0 mg, vitamin B12 10.0 mg, inositol 150.0 mg.			
3. The nutritional levels are all measured values.			

2.2 Sample Collection

A 24-hour fast was enforced before the experiment concluded; Nine fish from each group were randomly selected and anesthetized using 100 mg/L MS-222, and rinsed with distilled water thereafter. Blood was drawn from the caudal vein and spun at 3500 rpm (4°C) for 10 minutes to prepare for serum biochemical analysis. The fish were subsequently put on ice, and scissors were used to open their abdominal cavities to gather liver and intestinal tissue samples, along with dorsal muscle samples for standard muscle nutrient composition analysis. All procedures were performed using sterile instruments. Collected liver and intestinal specimens were treated as follows: part of the samples was preserved for histopathological examination, while the rest stored at -80°C for subsequent assays of gene expression and antioxidant activity.

2.3 Growth performance

Parameters for evaluating growth performance included WGR, SGR, FI, FCR, CF, HSI, VSI and SR (weight gain rate, specific growth rate, feed intake, feed conversion ratio, condition factor, hepatosomatic index, viscerosomatic index and survival rate). The specific calculation formulas are as follows:

$$\text{WGR (\%)} = 100 \times (\text{Wt} - \text{W0}) / \text{W0}$$

$$\text{SGR (\%/d)} = 100 \times (\ln \text{Wt} - \ln \text{W0}) / t$$

$$\text{FI (g/fish/d)} = \text{W}_{\text{total}} / [(\text{Nt} + \text{N0}) / 2] / T$$

$$\text{FCR} = \text{Wf} / (\text{Wt} - \text{W0})$$

$$\text{CF (g/cm}^3\text{)} = \text{Wt} \times 100 / \text{L}^3$$

$$\text{HSI (\%)} = 100 \times \text{Wl} / \text{Wt}$$

$$\text{VSI (\%)} = 100 \times \text{Wv} / \text{Wt}$$

$$\text{SR (\%)} = 100 \times \text{Nt} / \text{N0}$$

Where W0 is the initial body weight; Wt is the final body weight; W_{total} is the total feed consumption; Wf is the total food intake; N0 is the initial number of fish; Nt is the final number of fish; L is the body length; Wl is the liver weight; Wv is the visceral mass weight.

2.4 Determination of feed nutritional levels and muscle nutritional components

Moisture, crude protein, crude fat and crude ash contents of experimental feed and largemouth bass muscle samples were assayed according to GB 5009.3–2016, GB 5009.5–2016, GB 5009.6–2016 and GB 5009.4–2016, respectively.

2.5 Analysis of serum biochemical parameters

A fully automatic biochemical analyzer (Au-5800, Beckman, USA) was used to determine serum biochemical parameters. Assayed parameters comprised aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), globulin (GLB), total bile acid (TBA), total protein (TP), urea (UREA), triglyceride (TG), total cholesterol (CHO), glucose (GLU), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

2.6 H&E staining observation

Liver and intestinal tissues were fixed in 10% paraformaldehyde for 24 h, dehydrated in graded ethanol solutions, double-rinsed with xylene, and finally embedded in paraffin for histopathological assessment. The trimmed paraffin blocks were sectioned (5µm) using a microtome. Finally, the tissue samples were stained with hematoxylin and eosin (H&E) and examined under an optical microscope (Nikon ECLIPSE E100).

2.7 Analysis of Antioxidant indicators and ATP content

The levels of malondialdehyde (MDA, A003-1), glutathione (GSH, A006-2-1), total superoxide dismutase (T-SOD, A001-2), catalase (CAT, A007-1-1) activity, and adenosine triphosphate (ATP, A095-1-1) in liver tissues and intestines were determined using the assay kits from Nanjing Jiancheng Bioengineering Institute, strictly following the manufacturer's protocols.

2.8 Analysis of intestinal digestive enzyme activity

The activities of pepsin (G1210W), trypsin (G1209W), and α -amylase (G0510W) were measured using the kits from Suzhou Grace Biotechnology Co., Ltd.

2.9 Real-time quantitative PCR analysis

Total RNA was isolated using Trizol reagent (Servicebio, China), and its concentration was quantified with a NanoDrop spectrophotometer (Thermo Fisher ND2000, USA). All samples had A260/A280 ratios between 1.8 and 2.0 (optimal range), confirming their suitability for subsequent experiments. cDNA was then synthesized using a reverse transcription kit (B639251, Sangon Biotech) according to the manufacturer's protocol. qPCR detection was performed on a CFX Connect real-time PCR system (Bio-Rad, USA). The cycling conditions included an initial denaturation at 95 °C for 3 minutes, followed by 40 cycles of 5 seconds at 95 °C and 30 seconds of extension at 60 °C. The Ct values for each gene were normalized using the Ct value of β -actin from the same RNA sample. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method and β -actin as the internal reference gene. All primer sequences for the genes used are shown in Table 2.

Table 2
Primer sequences for qRT-PCR

Genes	Forward primer sequences	Reward primer sequences	Accession number
<i>nrf2</i>	AACGCAAGATGGAGAACATAGT	ACATGCTGAAGACCTCCAGGT	XM_038720536.1
<i>keap1</i>	ATGCGGTTGGTGGATCACAT	CAGCAACACCCACACCAATG	XM_038713667.1
<i>gclc</i>	TCTTTGTCGTCCTGCTCACC	AGCGTTTCTCTTCTGTGCGA	XM_038737635.1
<i>gclm</i>	AAGGGCTACCTTGTCAGTGC	GATTTGGCGGCATCCGTTAC	XM_038715955.1
<i>hmox1</i>	ACATGCTAGATAAGCATGCAACC	ACGGTGAAGTGCATGATGGG	XM_038694281.1
<i>gst</i>	TATCTCGACCTGCACTCCCA	CTTTCCGAACTCCTCGCTGT	XM_038703864.1
<i>nqo1</i>	GGTGAAGGAGCAGCAAGAGT	CCCGTCTGGTTAGGCACTTT	XM_038701508.1
<i>claudin</i>	GAACAACCGGAGCAGAAGGA	CAACAAACAGGGCACTTCCG	XM_038718401.1
<i>occludin</i>	GCTCAGACTTCTTGCCGGAT	ATGGGGCCTCTGTAGCTGTA	XM_038718311.1
<i>zo-1</i>	CAACAGCATCTTGCCACCAC	CGTAGCTCCACAGGCTTCAA	XM_038701018.1
<i>hsp70</i>	TCGTCACCAAGCTGAGTTCC	GCTGTGGCTCTCCAGATGTT	XM_038695197.1
<i>c3</i>	GTGGAGGCCGAGTTGAGAAA	CAACACCTTTTGCCGGAGTG	XM_038714041.1
<i>c4</i>	AACATCGTTGCTGACTCGGT	CGTAGCCTGTTGTCCAGGTT	XM_038711701.1
<i>il-1β</i>	TGCCTGAACATACACCCAGAATC	TGTGTTGGAATGGTTTACCGAC	XM_038733429.1
<i>il-10</i>	CAGAGAGCCTCTGCGTCCATTA	GCAGTGCTGAAGAAAGACAGCA	XM_038696252.1
<i>il-6</i>	GTCCCTGACCCCAACATCTCC	CGATCTGCCAGGTTCTCCTGC	XM_038732985.1
<i>tnf-α</i>	ACCAGCCCTCGACAGAAATG	GGGGAAGCAGAGCAGTCTTT	XM_038723994.1
<i>β-actin</i>	ATTGCCGACAGGATGCAGAA	CTGCTGGAAGGTGGACAGAG	XM_038695351.1

2.10 Cd exposure experiment

After the feeding trial, the remaining largemouth bass were subjected to a 96-hour Cd challenge. Cd chloride monohydrate ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$, 99.95% purity; CAS No.: 35,658-65-2) was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). The Cd concentration was set at 9.804 mg/L (Duan et al., 2025). No mortality was observed in largemouth bass during the Cd exposure period. At 24, 48, 72, and 96h of exposure, three fish were randomly taken from every group for liver tissue collection. The samples were used for antioxidant index detection and inflammatory gene expression analysis.

2.11 Statistical analysis

Statistical analyses were displayed as “Mean ± SEM”. The data from the experiments were statistically analyzed using IBM SPSS Statistics version 27.0.1, applying one-way ANOVA and Tukey's post hoc test, and the graphs were generated with GraphPad Prism version 9.5.0. $P < 0.05$ indicates statistical significance between groups.

3. Results

3.1 Effects of GCP on the growth performance of largemouth bass

As indicated in Table 3, the FBW, WGR, and SGR of largemouth bass in the experimental groups were notably higher than those in the control group at the end of the 8-week feeding trial ($P < 0.05$). Notably, the highest values for these growth indicators were achieved with 1500 mg/kg of dietary supplementation. No significant differences ($P > 0.05$) were observed in FI, CF, HSI, VSI, or SR between the GCP groups and the control group. The FCR in the 1000 mg/kg, 1500 mg/kg, and 2000 mg/kg groups was significantly lower than that in the control group ($P < 0.05$), with the lowest value observed at 1500 mg/kg.

Table 3

The effects of adding different levels of GCP to feed on the growth performance of largemouth bass.

Item	Treatment group				
	Control	500mg/kg	1000mg/kg	1500mg/kg	2000mg/kg
IBW (g)	11.46 ± 0.96	11.51 ± 0.78	11.44 ± 0.83	11.43 ± 0.55	11.54 ± 0.63
FBW (g)	47.73 ± 0.81 ^a	50.13 ± 0.9 ^b	55.77 ± 0.29 ^c	58.77 ± 1.16 ^d	54.43 ± 1.02 ^c
WGR (%)	331.63 ± 9.9 ^a	349.61 ± 3.59 ^b	407.59 ± 2.93 ^c	433.25 ± 7.53 ^d	391.69 ± 3.96 ^e
SGR (% /d)	2.62 ± 0.03 ^a	2.71 ± 0.03 ^b	2.90 ± 0.01 ^c	2.99 ± 0.04 ^d	2.86 ± 0.03 ^c
FI (g /fish /d)	1.28 ± 0.07	1.22 ± 0.11	1.19 ± 0.05	1.21 ± 0.05	1.27 ± 0.07
FCR	1.78 ± 0.06 ^a	1.72 ± 0.04 ^a	1.53 ± 0.03 ^b	1.49 ± 0.04 ^b	1.55 ± 0.02 ^b
CF (g/cm ³)	1.52 ± 0.19	1.6 ± 0.22	1.65 ± 0.01	1.69 ± 0.07	1.56 ± 0.1
HSI (%)	1.08 ± 0.31	1.28 ± 0.19	1.26 ± 0.57	1.16 ± 0.05	1.19 ± 0.13
VSI (%)	7.31 ± 0.42	6.53 ± 0.27	6.21 ± 0.33	6.78 ± 0.49	7.06 ± 0.21
SR (%)	96.00 ± 2.00	93.00 ± 3.00	97.00 ± 3.00	96.00 ± 2.00	92.00 ± 2.00
Abbreviation: IBW, Initial body weight; FBW, Final body weight; WGR, weight gain rate; SGR, specific growth rate; FI, feed intake; FCR, Feed conversion ratio; CF, condition factor; HSI, hepatosomatic index; VSI, viscerasomatic index; SR, Survival rate.					
Note: In the same row, data with different superscript letters show significant difference ($P < 0.05$).					

3.2 Effects of GCP on muscle composition of largemouth bass

The nutritional profile of largemouth bass muscle was impacted by GCP, notably through changes in protein and fat metabolism. As depicted in Table 4, the 1000 mg/kg group significantly promoted muscle protein deposition. The 2000 mg/kg GCP group showed significant adverse effects compared with the control group, notably reduced moisture and ash content ($P < 0.05$).

Table 4

The effect of adding different levels of GCP to feed on the muscle composition of largemouth bass.

Items	Control	500mg/kg	1000mg/kg	1500mg/kg	2000mg/kg
Moisture (%)	72.45 ± 0.48 ^a	72.14 ± 0.31 ^a	71.44 ± 0.37 ^b	73.86 ± 0.97 ^{ac}	71.26 ± 0.97 ^b
Crude lipid (%)	6.11 ± 0.01 ^a	6.17 ± 0.17 ^a	5.24 ± 0.3 ^b	4.98 ± 0.09 ^b	7.44 ± 0.29 ^c
Crude protein (%)	17.28 ± 0.43 ^a	17.18 ± 0.37 ^a	18.33 ± 0.85 ^b	17.43 ± 0.52 ^a	17.81 ± 0.23 ^c
Ash (%)	4.15 ± 0.58 ^a	4.62 ± 0.15 ^b	5.08 ± 0.54 ^c	3.77 ± 0.62 ^d	3.49 ± 0.71 ^d
Note: In the same row, data with different superscript letters show significant difference ($P < 0.05$).					

3.3 Effects of GCP on serum biochemical indices of largemouth bass

As illustrated in Table 5, the supplementation of feed with 1500 mg/kg GCP, in comparison to the control group, resulted in the significant elevation in the levels of TP, HDL-C, LDL-C, ALB, TBA, and CHO ($P < 0.05$). The activities of ALP, ALT, and AST in the GCP experimental group were significantly lower than those in the control group ($P < 0.05$). As the GCP content increased, the urea content progressively decreased and was significantly lower than that of the control group ($P < 0.05$). GLU and GLB did not show significant variation among the groups ($P > 0.05$).

Table 5

The effects of adding different levels of GCP to feed on the serum biochemical indices of largemouth bass.

Item	Treatment group				
	Control	500mg/kg	1000mg/kg	1500mg/kg	2000mg/kg
TP (g/L)	12.8 ± 0.53 ^a	14.83 ± 0.31 ^b	21.63 ± 0.64 ^c	22.4 ± 0.85 ^{cd}	23.1 ± 0.79 ^d
TG (mmol/L)	1.77 ± 0.81 ^a	1.3 ± 0.48 ^a	2.93 ± 0.44 ^b	1.79 ± 0.21 ^a	1.96 ± 0.36 ^{ab}
ALP (U/L)	26.13 ± 0.81 ^a	15.2 ± 1.71 ^{bc}	13.93 ± 1.45 ^c	14.13 ± 1.03 ^c	16.9 ± 1.65 ^b
ALT (U/L)	34.27 ± 1.48 ^a	28.67 ± 3.06 ^b	24.33 ± 3.21 ^{bc}	20 ± 2.65 ^c	24.33 ± 4.04 ^{bc}
AST (U/L)	152.33 ± 4.93 ^a	123 ± 6.24 ^b	131.17 ± 2.57 ^b	114.33 ± 5.86 ^c	127.67 ± 7.51 ^b
AST/ALT	4.45 ± 0.27 ^a	4.31 ± 0.27 ^a	5.45 ± 0.68 ^{ab}	6.32 ± 1.2 ^b	4.71 ± 0.81 ^a
HDL-C (mmol/L)	0.47 ± 0.07 ^a	0.65 ± 0.28 ^{ab}	0.62 ± 0.13 ^{ab}	0.88 ± 0.25 ^b	0.91 ± 0.15 ^b
LDL-C (mmol/L)	0.39 ± 0.17 ^a	0.33 ± 0.06 ^a	0.52 ± 0.02 ^{ab}	0.92 ± 0.18 ^c	0.74 ± 0.22 ^{bc}
GLU (mmol/L)	5.98 ± 0.78	5.68 ± 0.61	5.89 ± 0.94	6.97 ± 0.36	6.35 ± 0.76
UREA (mmol/L)	2.57 ± 0.76 ^a	2.37 ± 0.21 ^a	1.97 ± 0.12 ^a	1.33 ± 0.15 ^b	1.03 ± 0.06 ^b
ALB (g/L)	2.67 ± 0.51 ^a	3.83 ± 0.29 ^b	5.07 ± 0.31 ^c	4.6 ± 0.72 ^{bc}	4.47 ± 0.29 ^{bc}
GLB (g/L)	15.13 ± 1.25	16.03 ± 1.36	15.67 ± 1.66	17.73 ± 0.47	15.3 ± 1.51
ALB/GLB	0.18 ± 0.04 ^a	0.24 ± 0.01 ^b	0.33 ± 0.03 ^c	0.26 ± 0.03 ^b	0.29 ± 0.05 ^{bc}

Abbreviation: TP, total protein; TG, triglycerides; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLU, glucose; UR, urea; ALB, albumin; GLB, globulin; CHO, total cholesterol; TBA, total bile acid.

Note: In the same row, data with different superscript letters show significant difference ($P < 0.05$).

Item	Treatment group				
	Control	500mg/kg	1000mg/kg	1500mg/kg	2000mg/kg
CHO (mmol/L)	2.42 ± 0.74 ^a	2.98 ± 1.75 ^{ab}	4.61 ± 0.16 ^{ab}	5.01 ± 0.98 ^b	4.87 ± 1.29 ^b
TBA (μmol/L)	1.5 ± 0.1 ^a	2.23 ± 0.76 ^b	2.57 ± 0.21 ^{bc}	3.27 ± 0.32 ^c	2.31 ± 0.21 ^b
Abbreviation: TP, total protein; TG, triglycerides; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLU, glucose; UR, urea; ALB, albumin; GLB, globulin; CHO, total cholesterol; TBA, total bile acid. Note: In the same row, data with different superscript letters show significant difference ($P < 0.05$).					

3.4 Effects of GCP on H&E staining of liver and intestine in largemouth bass

High supplementation level of GCP exceeding 1500 mg/kg may lead to multi-organ dysfunction in largemouth bass. Within the control group, the liver tissue showed a normal liver lobule structure and neatly arranged hepatocytes (Fig. 1A, a). When GCP was supplemented at appropriate level (GCP ≤ 1500 mg/kg), no significant liver tissue damage was observed (Fig. 1A, b, c and d). However, at a GCP supplementation level of 2000 mg/kg, the hepatocyte arrangement became disordered and exhibited vacuolization (Fig. 1A, e). In the control group (Fig. 1B, a), the intestinal villi exhibited an intact, well-organized. Following GCP supplementation, the high-level group exhibited villus atrophy along with epithelial cell shedding (Fig. 1B, e). In contrast, the appropriate-level groups (Fig. 1B, b, c, and d) remained well-preserved villus morphology, with an intact structure, regular shape, and an absence of discernible damage.

3.5 Effects of GCP on antioxidant activities and ATP content in liver and intestine of largemouth bass

After supplementation of different level of GCP, the antioxidant activities of the liver and intestine exhibited similar trends. As shown in Fig. 2, GCP supplementation (500–2000 mg/kg) significantly boosted the antioxidant capacity of the liver and intestine, leading to increased T-SOD, CAT, and GSH activities and concurrently decreased MDA contents compared to the control group. Overall, the optimal activation of the antioxidant system was observed at level of 1000–1500 mg/kg coincided with enhanced ATP synthesis capacity, suggesting potential optimization of cellular energy metabolism. However, at a level of 2000 mg/kg supplementation, although the T-SOD (Fig. 2a) and CAT (Fig. 2b) activities were still superior to those of the control group, they showed significant decreases compared with the low and medium GCP levels.

3.6 Effect of GCP on digestive enzyme activities in largemouth bass

Figure 3 shows that the activities of α -amylase, trypsin, and pepsin all initially increased and then decreased with the increasing GCP supplement. All GCP groups exhibited significantly higher enzyme activities compared to the control group ($P < 0.05$). The highest activities of α -amylase and trypsin were observed in the 1500mg/kg group.

3.7 Effects of GCP on the gene expression levels of Nrf2-Keap1 pathway in the liver of largemouth bass

Figure 4 reveals that qPCR analysis found a significant increase in *nrf2* gene expression in the 1500 mg/kg group compared to the control group (Fig. 4a), along with a notable decrease in *keap1* gene expression, which was significantly downregulated, exhibiting an opposite trend to *nrf2*. With increasing GCP levels, the relative expression levels of Nrf2-Keap1 pathway downstream genes associated with antioxidation (*gclc*, *gclm*, and *nqo1*) peaked at 1500 mg/kg, while *hmx1* and *gst* genes reached their peak at 1000 mg/kg.

3.8 Effects of GCP on the expression levels of *claudin*, *occludin*, *zo-1*, *hsp70*, *c3*, and *c4* genes in the intestine of largemouth bass

As illustrated in Fig. 5, the expression levels of *claudin*, *occludin*, and *zo-1* genes were the lowest in the control group compared to the GCP-supplemented groups. As GCP supplementation increased, the relative expression levels of the three genes demonstrated a general upward trend. Compared with the control group, the expression level of *hsp70* was significantly higher at 1500 mg/kg ($P < 0.05$). The expression levels of *c3* and *c4* genes increased with the rising level of GCP, and the 2000 mg/kg group exhibited significantly higher expression by comparison with the control group ($P < 0.05$).

3.9 Effects of GCP on antioxidant enzyme activities and ATP content in liver tissue of largemouth bass after acute Cd exposure

At the conclusion of the 8-week dietary experiment, the leftover largemouth bass from each group were subjected to waterborne Cd exposure. Samples were collected at designated time points to determine liver antioxidant enzyme activities. T-SOD activity was consistently low in the control group, whereas it was generally higher in the groups that received GCP supplementation (Fig. 6a). At 72 and 96 hours, GCP supplementation at levels of 1000mg/kg and 1500mg/kg significantly decreased MDA content compared to the control group ($P < 0.05$). Following Cd exposure, the control group, the activities of GSH and CAT were somewhat inhibited. At the supplementation level of 2000 mg/kg, CAT activity was significantly reduced at 24 and 48 hours compared to the control group while ATP content was elevated at all time points.

3.10 Effects of GCP on the expression levels of inflammatory genes in liver tissues of largemouth bass following acute Cd exposure

As presented in Fig. 7, Supplementing the diet with 1500 mg/kg GCP significantly upregulated *il-10* gene expression while downregulating the expression levels of *il-1 β* , *il-6*, and *tnf- α* compared with the control group ($P < 0.05$). When GCP was supplemented at 2000 mg/kg, the levels of *il-6* and *tnf- α* gene

expression sharply increased in the liver, suggesting that a high level of GCP supplementation may disrupt immune homeostasis.

4. Discussion

This study investigated the regulatory effects of GCP on largemouth bass from multiple dimensions, including growth performance, antioxidant capacity, and gene expression of the Nrf2-Keap1 pathway. Through a 96-hour Cd exposure experiment, the protective effects of GCP on the liver of fish were preliminarily assessed. Existing studies have confirmed that Chinese herbal extracts can enhance immune and antioxidant functions in fish and promote growth (Hou et al., 2022). Plant polysaccharides, which are non-cytotoxic and have low side effects, are highly promising as high-quality feed additives and antibiotic alternatives in aquaculture (Kan et al., 2023; Yang et al., 2024). Among them, GCP has been demonstrated to alleviate TCDD-induced liver damage in Jian carp (*Cyprinus carpio* var. jian) (Du et al., 2024), and Codonopsis polysaccharides can also improve the growth performance of *Procambarus clarkii* (Liu et al., 2020). This study takes largemouth bass as the research subject, further enriching the application scenarios of plant polysaccharides in aquaculture. Meanwhile, through gene expression analysis of the Nrf2-Keap1 pathway, it provides a theoretical basis for elucidating the molecular mechanism by which GCP regulates the antioxidant capacity of largemouth bass and alleviates heavy metal stress.

Growth performance is the most direct indicator of aquaculture proficiency and serves as the primary determinant of farming efficiency (Li et al., 2025; Zhang et al., 2021). Studies have shown that adding 0.6g/kg of *Lycium barbarum* polysaccharides to feed can markedly promote the growth of largemouth bass, and alleviate the damage caused by high-carbohydrate diets (Qi et al., 2025). Feeding zebrafish with feed supplemented with wheat bran polysaccharides can significantly improve their growth rate and decrease the FCR (Chen et al., 2022). Our study revealed that FBW, WGR, and SGR were optimal in the 1500 mg/kg GCP group; conversely, a reduction in these parameters was observed at the 2000 mg/kg supplementation level. It is speculated that this phenomenon may be due to high levels of polysaccharides inhibiting the growth of largemouth bass (Liu et al., 2022; Ren et al., 2020), or due to a dose-effect relationship, where the beneficial effects of polysaccharides diminish beyond a certain supplementation threshold (Pathinathan et al., 2024). Muscle composition is a key parameter for evaluating fish meat quality and nutritional value. Moisture, crude fat, crude protein, and ash levels have a direct impact on the texture, taste, and nutritional value of fish meat (Lei et al., 2023). Dietary supplementation with mannoooligosaccharides enhances the nutritional value of striped jack (*Trachinotus ovatus*) muscle by boosting its crude protein levels while lowering its crude fat levels (Hoang et al., 2023). In this study, compared with the control group, the 1000 mg/kg group showed significantly higher crude protein and ash content, while the 1500 mg/kg group exhibited significantly lower crude fat content. Previous studies have shown that low-dose *Ganoderma lucidum* polysaccharides can promote fat catabolism and reduce lipid accumulation (Jia et al., 2023), which is consistent with our finding. Based on this, to determine whether GCP exert such effects in largemouth bass, we measured their serum biochemical indicators. Elevated serum levels of ALT and AST can be indicative of liver disease or

damage, thereby serving as important biomarkers for the assessment and monitoring aquatic animal health (Sheikhzadeh et al., 2011). Serum AST and ALT activity observed in the 2000 mg/kg group indicate that high-dose GCP supplementation has the potential to induce liver damage in largemouth bass. TP, GLB, and ALB serve as valuable indicators for assessing the metabolic status of fish protein. An increase in the levels of these indicators can promote protein deposition in the body and enhance the fish's immune system (Liu et al., 2024). Throughout our investigation, the GCP experimental group presented substantially higher levels of TP, GLB, and ALB, along with an elevated A/G ratio, compared to the control group. This discovery highlights the potential of appropriate GCP supplementation to mitigate lipid metabolism disorders, enhance immunity, and improve growth performance in fish (Yu et al., 2023a).

The liver and intestine are key organs for digestion and metabolism in fish, and their structural integrity is important for ensuring the health of the fish (Chen et al., 2023b; Xu et al., 2023). Emerging evidences have demonstrated that polysaccharides exert hepatoprotective effects via the gut-liver axis (Li et al., 2020; Wang et al., 2021). Hawthorn polysaccharides can alleviate oxidative stress-induced cellular damage in mouse livers and maintain normal liver tissue morphology (Wang et al., 2025). In our study, no significant liver damage was observed in largemouth bass when GCP was supplemented within the range of 500 to 1500 mg/kg. However, the 2000 mg/kg group displayed liver abnormalities, including pronounced cytoplasmic vacuolation. This may be attributed to the metabolic burden imposed on the liver by excessive GCP, which disrupts intracellular substance metabolism and subsequently results in abnormal lipid metabolism, ultimately leading to vacuole formation (Chen et al., 2025; Miao et al., 2022). Studies by Song et al. indicate that silymarin improves intestinal tissue morphology and facilitate villus development in juvenile largemouth bass (Song et al., 2025). Our study demonstrated that GCP supplementation level at 500–1500 mg/kg promoted well-structured intestinal villi. This effect is likely due to GCP's role in enhancing the proliferation and differentiation of intestinal epithelial cells, thereby preserving villus structural integrity and enhancing intestinal digestion and absorption (Wang et al., 2024). T-SOD, CAT and GSH play a critical role in the elimination of reactive ROS and the prevention of oxidative damage in the body (Huang et al., 2022). MDA, which is formed during lipid peroxidation, serves as a marker for levels of oxidative damage, whereas ATP is involved in cellular energy metabolism (Zhang et al., 2022). The findings of our study indicate that GCP dietary supplementation significantly increased antioxidant enzyme activities and GSH content, while reducing MDA levels and boosting ATP levels in the liver and intestines of largemouth bass. Similar results were observed when guar gum was added to the diet (Zhao et al., 2024). During carbohydrate digestion, α -amylase hydrolyzes starch into simple sugars, whereas trypsin and pepsin break down large protein molecules into small peptides and amino acids (Ma et al., 2023). Fermented wheat bran polysaccharides considerably augment the actions of trypsin and pepsin in the intestines of common carp, thereby improving the fish's digestive capacity (Wang et al., 2020). In our study, the supplementation of GCP to the feed significantly increased the activity of α -amylase, trypsin, and pepsin in the intestines. This effect was particularly notable at GCP supplementation levels of 1000 to 1500 mg/kg, where a substantial boost in enzyme activity was detected. These observations are in agreement with past studies that demonstrate polysaccharides' role in increasing digestive enzyme activity in fish (Cholaraj and Venkatachalam, 2023).

The Nrf2-Keap1 pathway plays a vital role in the antioxidant defense system within the body. Operating as a transcription factor, *nrf2* translocated into the nucleus upon activation to manage the expression of different downstream targets, such as antioxidant enzymes (*gclc*, *gclm*, *hmo1*, *gst*, and *nqo1*) and detoxification enzymes. *keap1* functions as a negative regulator of *nrf2* by binding to it and facilitating its degradation (Jadeja et al., 2016; Luo et al., 2021). Previous studies have demonstrated that emodin supplementation upregulates *nrf2* expression, downregulates *keap1* expression, and improve the liver health in juvenile largemouth bass (Zhao et al., 2025). GCP primarily achieves upstream regulation of the Nrf2-Keap1 pathway by scavenging excess intracellular reactive ROS and inhibiting Keap1-mediated ubiquitination degradation of *nrf2* (Gao et al., 2025), thereby promoting nuclear translocation of *nrf2* to initiate transcription of downstream antioxidant genes (Fei et al., 2022). In our study, dietary supplementation with GCP significantly upregulated the expression of *nrf2* and downregulated *keap1* in the liver of largemouth bass, while simultaneously upregulating the expression of downstream antioxidant genes. These findings are consistent with previous studies on polysaccharide-mediated regulation of the Nrf2-Keap1 signaling pathway in fish (Song et al., 2025). *Claudin*, *occludin*, and *zo-1* are core molecules that maintain tight junctions in intestinal epithelial cells and the maintenance of the intestinal barrier function, and their expression levels are closely associated with intestinal barrier integrity (Li et al., 2023a). During our study, dietary supplementation of 1000–1500 mg/kg GCP significantly elevated the intestinal expression of *claudin* and *occludin* genes in largemouth bass. When the GCP supplementation was raised to 2000 mg/kg, it resulted in a significant upregulation of the *zo-1*. These findings demonstrate that GCP may improve the intestinal barrier function in largemouth bass through the regulation of tight junction-related gene expression, which consequently promotes nutrient absorption and utilization efficiency. As a stress-inducible protein, *hsp70* exhibits elevated expression in response to cellular injury or stress, and performs a key protective and reparative function in cells (Yang et al., 2015). The 1000mg/kg GCP group demonstrated a significant upregulation of *hsp70* expression. In contrast 2000mg/kg GCP group displayed a reduction in *hsp70* expression compared to the 1000 mg/kg group; however, this expression level remained elevated by comparison with the control group, thereby contributing to the maintenance of normal cellular physiological functions. *C3* and *c4* serve critical in the immune defense of fish, participating in immune responses and immune regulation. Previous studies have shown that consuming *Coriolus versicolor* polysaccharides via the oral route significantly elevates serum *c3* and *c4* levels in crucian carp (Wu et al., 2013). Dietary supplementation with *Hericium erinaceus* polysaccharides (Gou et al., 2018), *Ficus carica* polysaccharides (Yang et al., 2015) and soybean isoflavones (Zhou et al., 2015) enhance complement levels in fish, thereby strengthening the innate immune barrier and reducing complement-mediated tissue inflammation. These results align with the present study, in which GCP supplementation led to a significant increase in the expression of intestinal *c3* and *c4*. It is speculated that GCP may activate intestinal immune cells, promote the transcription and expression of complement genes, thereby enhancing the intestinal immune response capacity in largemouth bass and improving their resistance to pathogens.

Exposure to Cd, a highly toxic heavy metal, results in the overproduction of ROS in fish, which consequently induces oxidative stress. This process compromises antioxidant enzyme activity, exacerbates lipid peroxidation, and disrupts cellular energy metabolism (Liu et al., 2018). Dietary GCP

supplementation enhanced the antioxidant capacity and energy metabolism in the liver of largemouth bass following acute Cd exposure. This finding aligns with earlier research on the antioxidant defense properties of polysaccharides against heavy metal stress (Jia et al., 2023). Polysaccharides can enhance the body's intrinsic antioxidant defense, facilitate the synthesis and secretion of antioxidant enzymes, and bolster the capacity for reactive ROS scavenging (Li et al., 2019). Dietary supplementation with seaweed polysaccharides enhanced the activities of T-SOD and CAT and increased GSH content in the liver of common carp (*Cyprinus carpio* L.) exposed to copper, while markedly reducing MDA levels (Liu et al., 2015). Liu et al. reported that polysaccharides from *Ganoderma lucidum* attenuated mitochondrial injury in the carp liver caused by carbon tetrachloride; they also enhanced ATP content and facilitated the preservation of energy metabolism balance (Liu et al., 2017). This study demonstrates that GCP enhances the activities of T-SOD, GSH, CAT, as well as ATP content, in the liver of largemouth bass exposed to exposure. Dietary GCP supplementation significantly lowered MDA levels compared to the control group, with the lowest levels seen after 96 hours of Cd exposure. Earlier research has indicated that exposure to Cd triggers oxidative stress responses and causes immune system dysfunction in fish (Guo et al., 2017). *Il-1 β* , a core cytokine mediating innate immunity and inflammation, is essential for maintaining tissue homeostasis in fish. Under physiological conditions, *il-1 β* triggers appropriate inflammatory responses to eliminate foreign substances and facilitate tissue repair, thereby playing an essential part in the maintenance of homeostasis (Safari et al., 2022). Conversely, *il-10* is a cytokine with anti-inflammatory properties that works to reduce excessive inflammatory reactions (Gou et al., 2018). A study by Xu et al. revealed that dietary chitosan supplementation markedly decreased the amounts of pro-inflammatory cytokines in hybrid sturgeon juveniles (*Acipenser baerii* × *Acipenser schrenckii*) following *Aeromonas hydrophila* infection (Xu et al., 2023). *Tnf- α* is a key inflammatory mediator known to induce oxidative stress (Zhang et al., 2023) and modulate imbalance within the cytokine network (Hong et al., 2013). *Il-6*, an inflammatory cytokine, triggers the production of various acute-phase proteins and influences the growth and specialization of inflammatory cells (Liu et al., 2021). Our study found a notable rise in *il-6* and *tnf- α* expression in the group given 2000 mg/kg of GCP. This phenomenon may be attributed to the excessive GCP supplementation exerting a stimulatory effect on the liver, thereby leading to an upregulation in the production of pro-inflammatory cytokines. The underlying mechanisms remain to be fully elucidated in future.

5. Conclusion

In conclusion, this study demonstrates that dietary supplementation with 1000-1500 mg/kg GCP can effectively enhance the growth performance of largemouth bass, with the promoting effect diminishing beyond this dosage. The optimal GCP dose activates the hepatic Nrf2-Keap1 signaling pathway, upregulates antioxidant gene expression, alleviates oxidative stress, and improves intestinal immunity through immune cell activation and enhanced complement gene expression, thereby boosting the fish's stress resistance and disease resistance capabilities. Based on these findings, in aquaculture applications, this dosage can serve as a routine feed additive for healthy juvenile largemouth bass, effectively balancing growth promotion and stress resistance while alleviating hepatointestinal damage

in fish exposed to Cd-contaminated water. Future studies should validate its practical farming stability, investigate synergistic effects with other plant-derived additives, elucidate the regulatory mechanisms of gut microbiota, and assess the safety of metabolic residues.

Declarations

CRedit authorship contribution statement

Yunping Duan: Initial Draft Writing, Editing and Reviewing, Managing Projects, Processing Data. Xihong Sun: Visualization, Validation. Yuhe Bai: Methodology, Investigation, Data curation. Weichuan Zhang: Project administration. Yong Huang: Writing - review & editing.

Ethics statement

The experimental procedures of this study were approved by the Animal Management and Use Committee of Henan University of Science and Technology (HAUST-024-F1218009).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

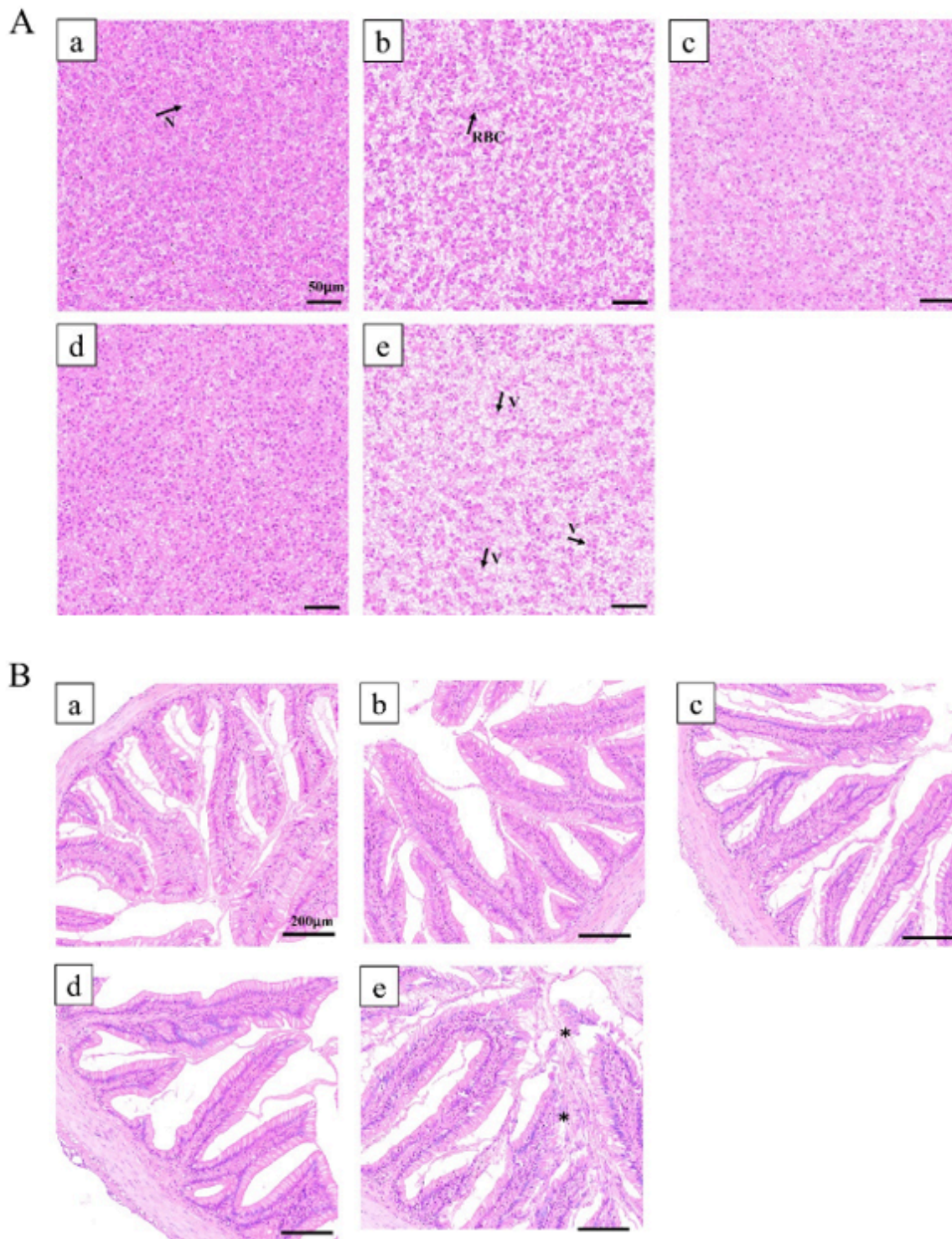


Figure 1

The effects of adding different levels of GCP to feed on the liver tissue and intestinal morphology of largemouth bass.

Note: The scale bar for liver tissue HE is 50 micrometers, and the scale bar for intestine is 200 micrometers. (a) Control group. (b) 500mg/kg group. (c) 1000mg/kg group. (d) 1500mg/kg group. (e)

2000mg/kg group. N represents the nucleus, RBC stands for red blood cells, V denotes the vacuolization phenomenon of cells, and * indicates the shedding of intestinal epithelial cells.

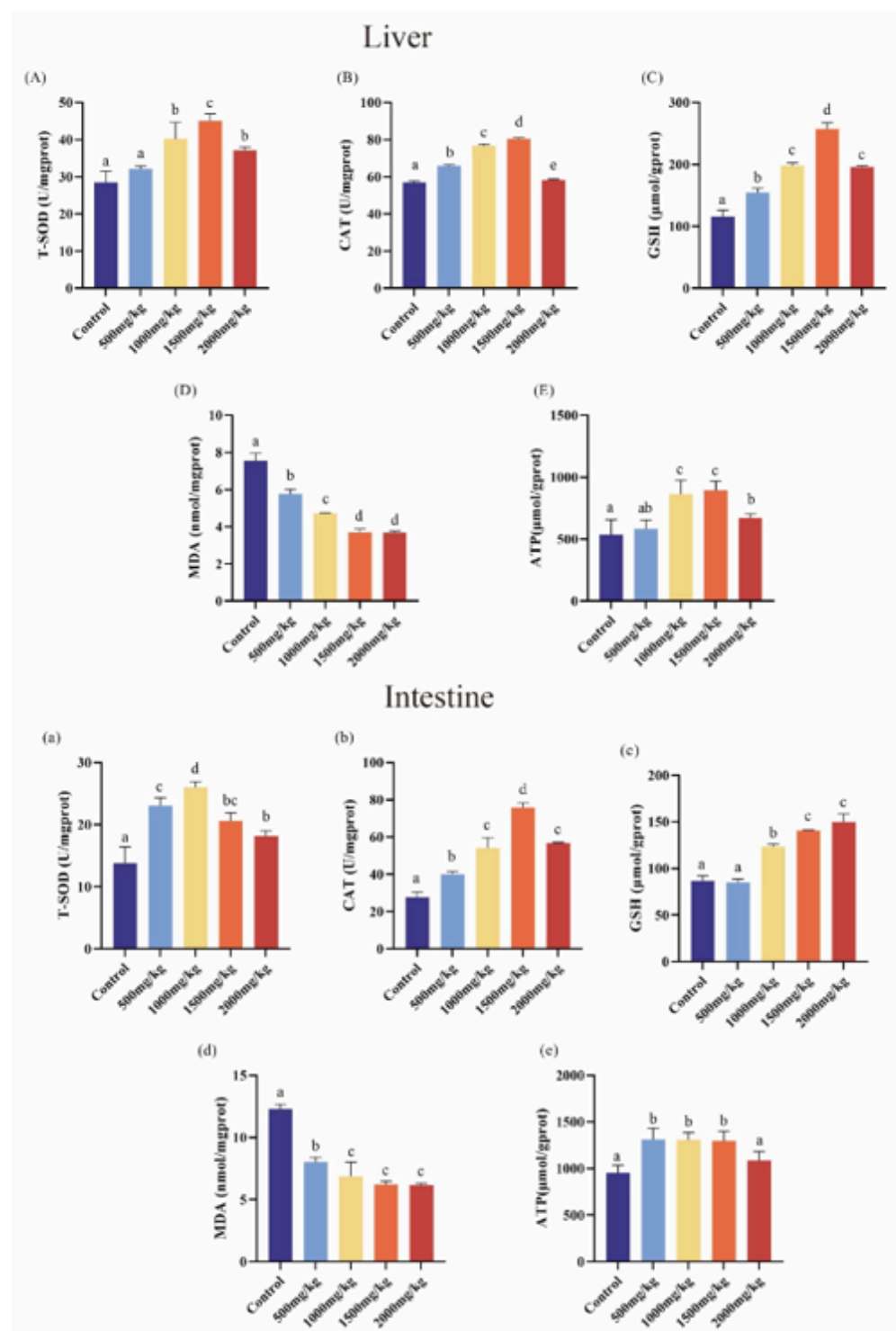


Figure 2

The effects of adding different levels of GCP to feed on the antioxidant activity and ATP content in largemouth bass.

Note: (A, a) T-SOD, total superoxide dismutase; (B, b) CAT, catalase; (C, c) GSH, glutathione; (D, d) MDA, malondialdehyde;(E, e) ATP, adenosine triphosphate. n=6. Different letters indicate statistically significant differences among the experimental groups ($P < 0.05$).

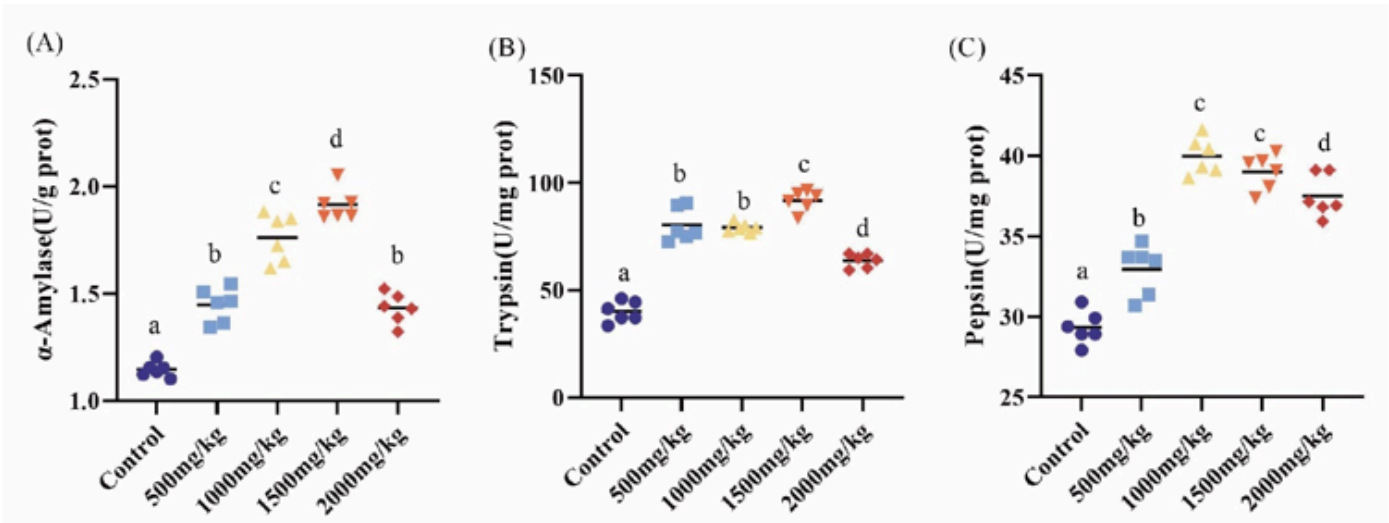


Figure 3

The effects of adding different levels of GCP to feed on the digestive enzyme activity in the intestine of largemouth bass.

Note: Different letters indicate statistically significant differences among the experimental groups ($P < 0.05$).

Liver

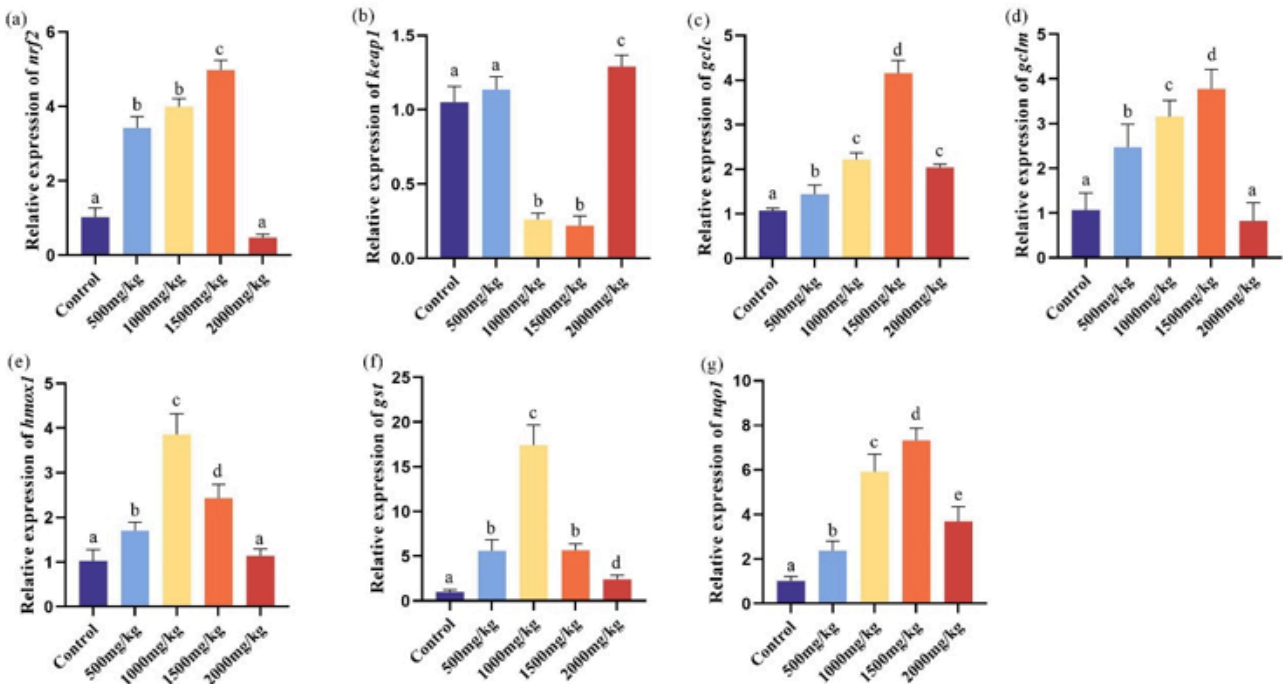


Figure 4

The impact of adding different levels of GCP to feed on the Nrf2-Keap1 signaling pathway genes in largemouth bass.

Note: (a) *nrf2*, nuclear factor erythroid 2-related factor 2; (b) *keap1*, kelch-like ECH-associated protein 1; (c) *gclc*, glutamate-cysteine ligase catalytic subunit; (d) *gclm*, glutamate-cysteine ligase modifier subunit; (e) *hmox1*, heme oxygenase 1; (f) *gst*, glutathione S-transferase; (g) *nqo1*, NAD (P) H quinone dehydrogenase 1. n=6. Different letters indicated statistically significant differences between the experimental groups ($P < 0.05$).

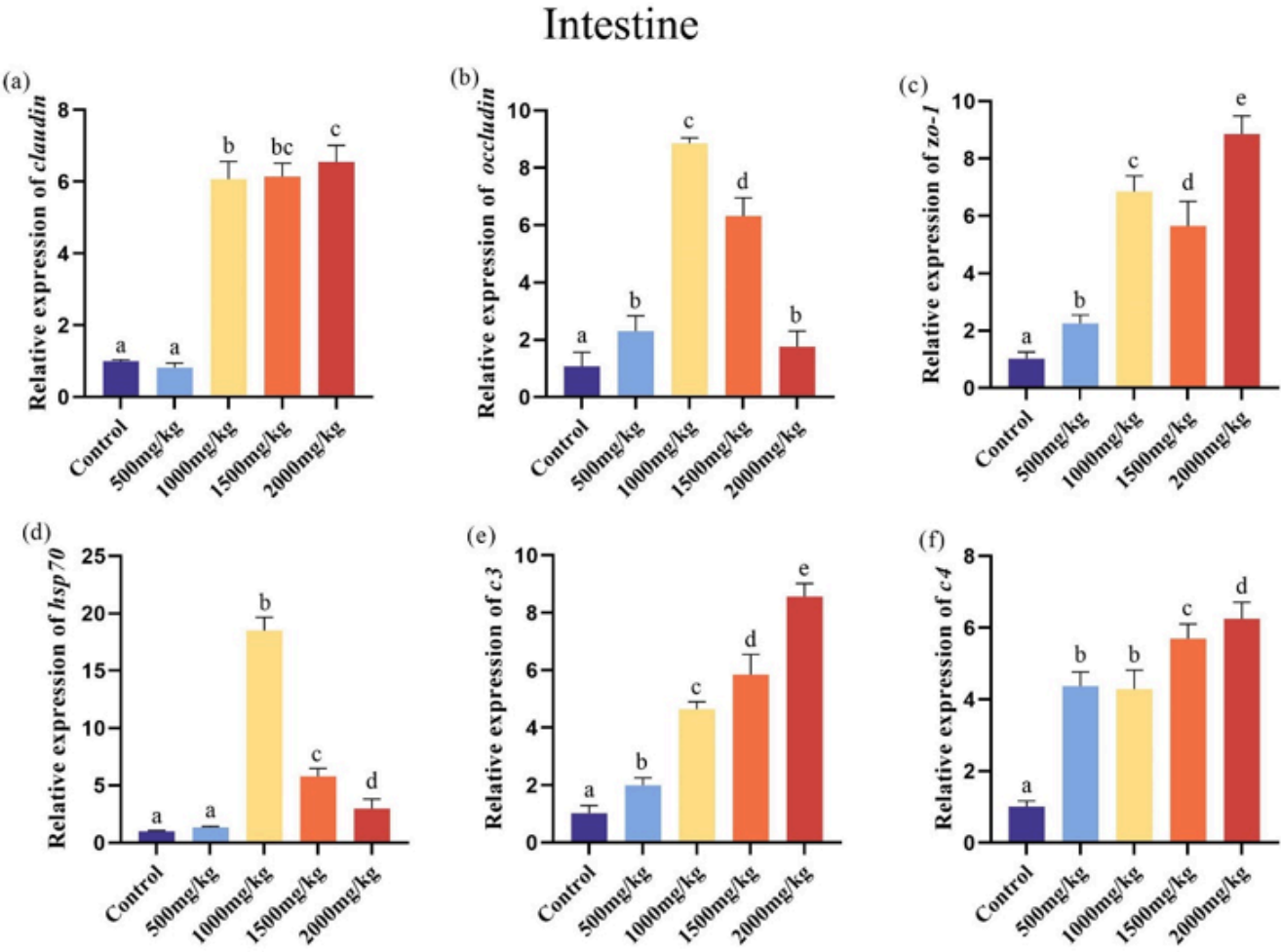


Figure 5

The effects of adding different levels of GCP to the feed on the *claudin*, *occludin*, *zo-1*, *hsp70*, *c3*, and *c4* genes in largemouth bass.

Note: (a) *claudin*; (b) *occludin*; (c) *zo-1*, zonula occludens 1; (d) *hsp70*, heat shock protein 70; (e) *c3*, complement component 3; (f) *c4*, complement component 4; n=6. Different letters indicated statistically significant differences between the experimental groups ($P < 0.05$).

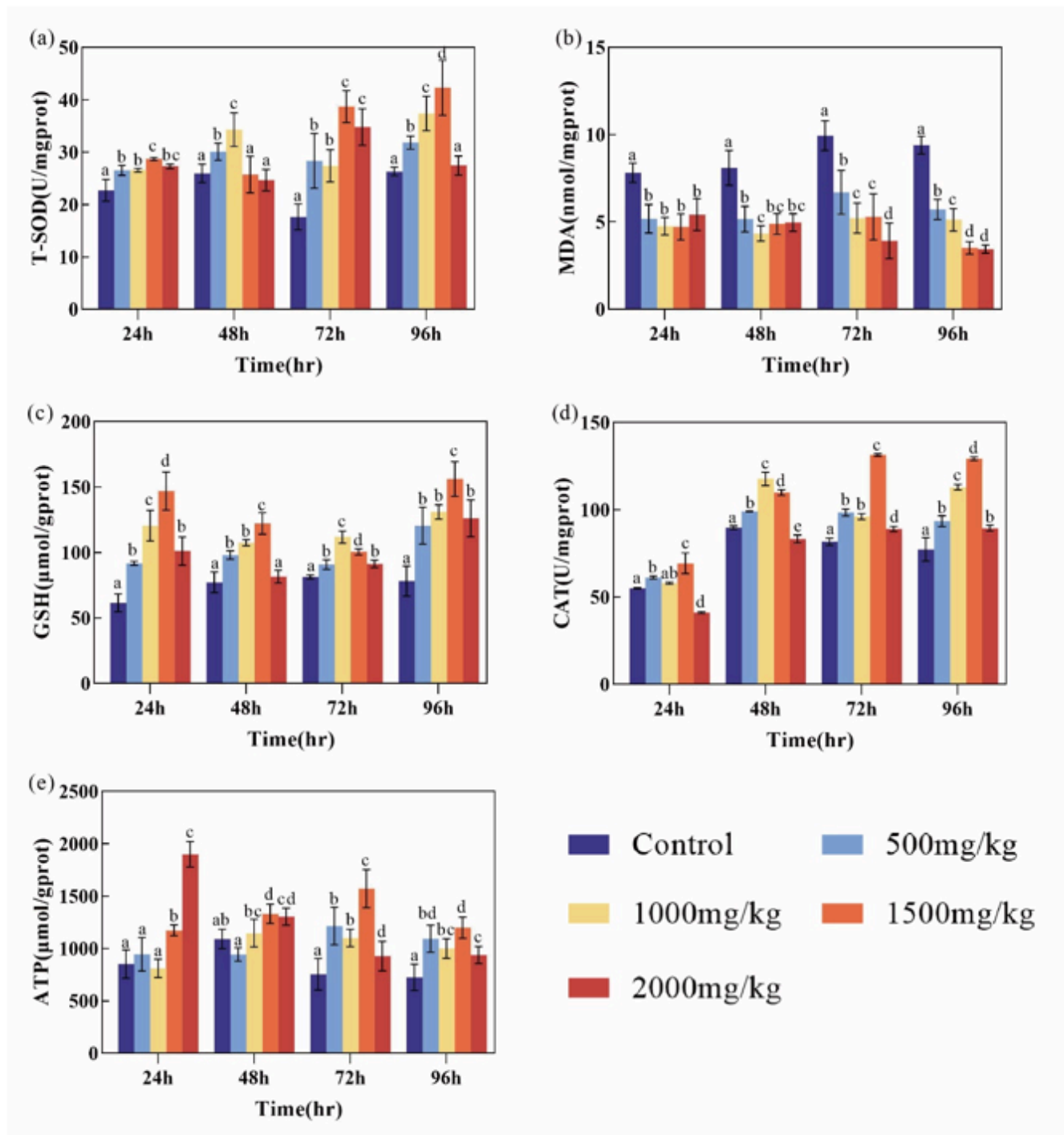


Figure 6

The effects of GCP supplementation in the diet on the antioxidant activity and ATP content in the liver tissue of largemouth bass after acute Cd exposure.

Note: Different letters indicate statistically significant differences ($P < 0.05$) between experimental groups compared to the control group alone.

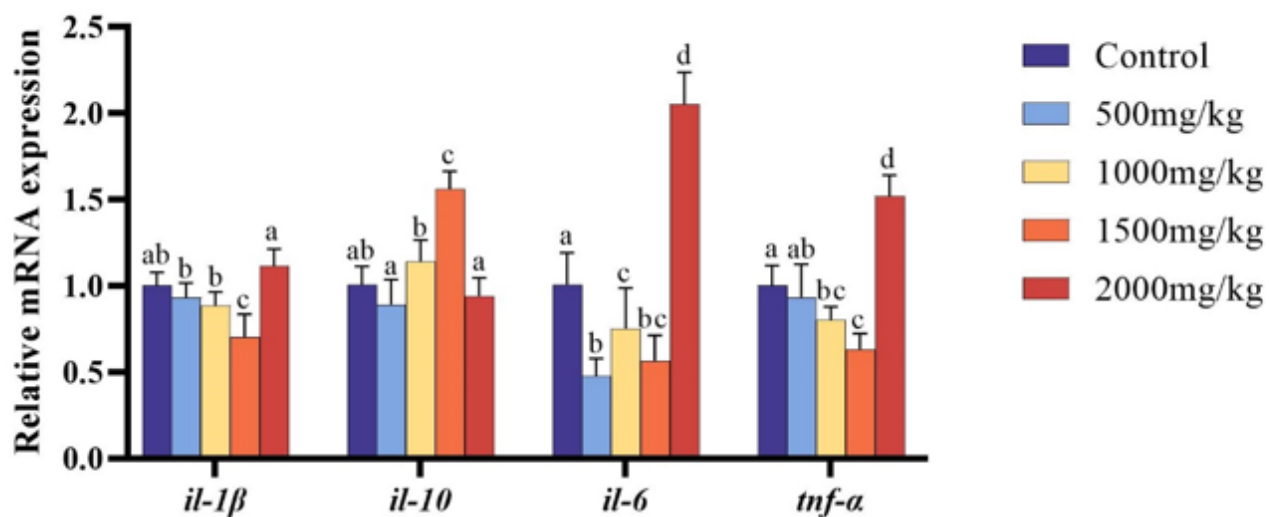


Figure 7

The effect of GCP supplementation in the diet on the expression of inflammatory genes in the liver tissue of largemouth bass after acute cadmium exposure.

Note: *il-1β*, interleukin-1β; *il-10*, interleukin-10; *il-6*, interleukin-6; *tnf-α*, tumor necrosis factor-α. n=6. Different letters indicated statistically significant differences between the experimental groups ($P < 0.05$).