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Effects of dietary *Radix Rehmanniae Preparata* polysaccharides on the growth performance, immune response and disease resistance of *Luciobarbus capito*

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ABSTRACT

This work explores the effects of dietary *Radix Rehmanniae Preparata* polysaccharide (RRPP) supplementation on the growth performance, nonspecific immune responses, immune- and growth-related gene expression and disease resistance to *Aeromonas hydrophila* in *Luciobarbus capito*. Diets containing five concentrations of 0%, 0.05%, 0.1%, 0.2% and 0.4% RRPP were fed to fish for 60 d. The results indicated that the growth performance significantly increased in the 0.1%, 0.2% and 0.4% RRPP groups compared with that in the control ($P < 0.05$). The activities of serum lysozyme (LAZ), acid phosphatase (ACP), superoxide dismutase (SOD), alkaline phosphatase (AKP) and total protein (TP) were significantly increased in the appropriate RRPP supplemented groups ($P < 0.05$). With respect to immune- and growth-related genes, such as interleukin (*IL*)-*1β*, *IL-8*, tumor-necrosis factor (*TNF*)-*α*, interferon (*IFN*)-γ, growth hormone (*GH*), insulin-like growth factor (*IGF*)-*I* and *IGF-II*, up-regulation were observed in the three organs (kidney, spleen, gut) of the fish fed with RRPP, compared with the control. In contrast, the mRNA expression of *IL-10* and transforming-growth factor (*TGF*)-*β* were downregulated. After challenge with *A. hydrophila*, the final survival rate was significantly higher in fish fed the RRPP supplement than that in the control group ($P < 0.05$). In conclusion, RRPP enhanced the growth performance, immune response and disease resistance of *Luciobarbus capito*, with the greatest effects at 0.2% RRPP.

1. Introduction

Luciobarbus capito belongs to the family cyprinidae, barbinae and *barbus* genus, and the species is native to the Aral Sea of Uzbekistan [1]. In 2003, *Luciobarbus capito* was first introduced by China and subsequently promoted in many cities. As a large economic fish, *Luciobarbus capito* has the characteristics of wide feeding, rapid growth, tender meat and saline-alkali resistance, and it is suitable for saline-alkali water or freshwater aquaculture in China [2]. The expansion in the size of *Luciobarbus capito* farming has led to a high susceptibility of the fish to various disease pathogen. Traditional control methods rely mainly on antibiotics and chemotherapeutics, which cause potential harm to the quality and safety of water products [3]. Therefore, it is urgently necessary to find safe additives to replace antibiotic drugs. The application of Chinese herbal medicine in aquaculture is in line with the criteria for disease prevention in development of pollution-free aquatic products and production of green aquatic products.

Radix Rehmanniae Preparata (RRP) is processed from *Rehmannia glutinosa* Libosch, a comprehensive traditional Chinese herbal medicine that belongs to the family Scrophulariaceae [4]. Pharmacological studies showed that RRP possesses wide actions of nourishing yin, supplementing blood, boosting essence, and filling bone marrow. Different compounds such as polysaccharides, oligosaccharides, glycosides, iridoid glycosides, flavonoid, phenol glycoside ionone, furfurals, and microelements have been isolated from RRP [5,6]. Polysaccharides are important chemical components of RRP, and their bioactivities have been studied, including the effects of anti-anxiety [7], anti-fatigue [8], anti-diabetes [9], alleviation of intestinal mucositis [10], antitumour [11] and immune enhancement [12]. In this study, we investigated the effects of RRPP on the growth and immune function of *Luciobarbus capito* and explored its feasibility as a feed additive in fish diets.

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2. Materials and methods

2.1. Fish and RRPP

In this study, *Luciobarbus capito* were obtained from Suqian Institute of Agricultural Sciences, Jiangsu Academy of Agricultural Sciences, China. The fish were acclimatized to laboratory conditions (pH: 6.5–7.5, water temperature: 25 ± 1 °C, dissolved oxygen: 5.5–6.5 mg/L, and nitrites: 0.01–0.02 mg/L) for two weeks prior to the experiment. All fish were fed with a commercial diet three times per day.

RRPP is a commercial product purchased from Yangling Ciyuan biotech Co., Ltd (Xi-an City, China). The polysaccharide content reached 95.18%, and the material was stored at −20 °C.

2.2. Experimental diets

The commercially formulated diet was selected as the basal diet (control, Con). The diets contained approximately 37.9% crude protein, 8.6% lipid and 13.6% ash. The basal diets were supplemented with different RRPP levels (0.05%, 0.1%, 0.2% and 0.4%). All ingredients were mixed thoroughly into a mixture and pelletized with a flat die pellet mill. The pellets were subsequently air-dried and stored in plastic bags at −20 °C. The formulation and composition of all diets are shown in Table 1.

2.3. Experimental groups and feed management

The experimental design was based on a completely randomized design. A total of 450 fish (46.20 ± 1.02 g) were randomly assigned to five groups: a control group (CK) fed with a basal diet, and four treatment groups (0.05% RRPP group, 0.10% RRPP group, 0.20% RRPP group and 0.40% RRPP group) fed with a basal diet supplemented with 0.05%, 0.1%, 0.2% and 0.4% RRPP respectively. Each group contained 90 fish with three replicates, and each replicate included 30 fish.

The feeding trial was completed in a recirculating aquaculture system which consisting of a series of plastic tanks (100 × 80 cm). The diets were hand-fed to the fish three times a day at 9:00, 13:00 and 17:00. The feeding trial lasted for 60 d at a rate of approximately 3% of body weight and was subsequently adjusted every 15 d. Regular monitoring of water quality was conducted during feeding, and environmental stability was maintained.

2.4. Growth parameters

The body weight and length of each fish were measured at the beginning and end of the feeding trial. The parameters of fish were calculated using the following formulas:

Weight gain rate (WGR, %) = 100 × (final body weight-initial body weight)/initial body weight;

Specific growth rate (SGR, %) = 100 × [(Ln (final body weight) - Ln (initial body weight)]/number of days;

Condition factor (CF, g/cm^3) = 100 × (body weight)/(total length) [3];

Feed conversion ratio (FCR) = feed intake/(final body weight-initial body weight) [13,14].

Table 1
Diet formulation and proximate composition of each group (%).

Ingredients	CK	0.05% RRPP	0.10% RRPP	0.20% RRPP	0.40% RRPP
Fish meal	20	20	20	20	20
Soybean meal	19	19	19	19	19
Rapeseed meal	20	20	20	20	20
Wheat bran	12	12	12	12	12
Wheat middlings	20	20	20	20	20
Soybean oil	2	2	2	2	2
Salt	0.2	0.2	0.2	0.2	0.2
$Ca(H_2PO_4)\cdot H_2O$	1.5	1.5	1.5	1.5	1.5
Premix	2.3	2.3	2.3	2.3	2.3
CMC-Na	3	2.95	2.90	2.80	2.60
RRPP	0	0.05	0.10	0.20	0.40
Nutrient composition (DM basis)/%					
Crude protein	35.15	35.17	34.92	35.10	34.88
Crude lipid	5.44	5.50	5.47	5.51	5.52
Crude ash	8.23	8.33	8.36	8.29	8.32
Moisture	11.16	10.97	11.18	10.98	11.18

2.5. Sample collection

At the end of the feeding trial, six fish from each tank were randomly collected and anaesthetized using MS-222. Blood samples were collected from the caudal vein using a 5-mL syringe and were allowed to clot for 4 h at 4 °C. Serum was obtained by centrifugation (4000 rpm, 10 min, 4 °C) and was stored at −20 °C until analysis. The fish were placed on an ice plate, and the immune organs (kidney, spleen and gut) were quickly separated and stored in liquid nitrogen.

2.6. Serum immune parameters

2.6.1. LAZ activity

Serum LAZ activity was measured by the immunoturbidimetric method with a commercial kit (Nanjing Jiancheng Bioengineering Institute, China). According to the instructions, 20 μL serum was mixed with 200 μL Micrococcus lysodeikticus suspension (0.2 mg/mL), and the absorbency at 530 nm was determined at 0.5 and 2.5 min. One unit of lysozyme activity was defined as the amount of lysozyme that caused a decrease in absorbency of 0.001 min^{-1}.

2.6.2. SOD, ACP and AKP activities

SOD activity in serum was measured using the hydroxylamine method. ACP and AKP activity in the serum were measured via the tingholes method using commercial test kits (Bio-Chain, Inc., USA).

2.6.3. TP concentration

Serum TP concentration was determined via enzymatic procedures using an automatic biochemical analyser (Beckman Coulter AU680).

2.7. RNA isolation and gene expression analysis

At the end of the feeding trial, the gut, kidney and spleen were dissected from twelve fish per group (four fish per tank). Total RNA was extracted from these three tissues using the RNAiso Plus kit Trizol Reagent (TaKaRa). The RNA concentration and quality were assessed by agarose gel (1%) electrophoresis and NanoDrop (A260:280 ratio), respectively. Subsequently, RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit (TaKaRa) following the manufacturer's instructions. The cDNA were stored at −20 °C for later analysis.

The expressions of immune-related genes (*IL-1β*, *IL-8*, *IL-10*, *TNF-α*, *TGF-β*, *IFN-γ*) and growth-related genes (*GH*, *IGF-I*, *IGF-II*) were assessed with real-time quantitative PCR (RT-qPCR). The RT-qPCR primers were designed according to the cloned sequences, which were published in GenBank, and the *18S* gene was used as a housekeeping gene. The primer sequences, thermocycling conditions and GenBank number are shown in Table 2. RT-qPCR was amplified in a Roche LightCycler® 96 real-time PCR System (Roche, CH) using SYBR® Premix Ex Taq (Takara). The total volume of the PCRs was 10 μL and consisted of 5 μL of SYBR® Green I, 1 μL of cDNA, 0.25 μL (each) of forward and reverse primers, and 3.5 μL of RNase-Free ddH_2O. The cycling conditions were applied as follows: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. All samples were run in triplicate. A melt curve analysis was performed after amplification to verify the accuracy of each amplicon. Relative gene expressions were calculated using the

Table 2
Primer sequences and annealing temperatures for polymerase chain reactions for gene expression in *Luciobarbus capito*.

Target gene	Target gene Primer	Annealing temp (°C)	GenBank no.
18S	CCGCTTTGGTGACTCTAGAT CTTGGATGTGGTAGCCGTTTC	59.7 °C	NR_145818
IL-1β	ATGACCCGAACGAGTACGACA CCTGGTTCATTTCCACCCTCT	60.3 °C	MH720264
IL-8	TCCTCGGATTCCTGACCATT 5′TGTGAGTTTGGAGGGAAGAGC	59.2 °C	MH720265
IL-10	GCTTCTACTTGGACACCATTCT CCCGCTTGAGATCCTGAAATA	57.5 °C	MH720266
TNF-α	CGCTCAACAAGTCTCAGAACAATC AAGCCGCCTGAAACGAAAG	60.7 °C	MH720267
TGF-β	CAGAGGAGGAAGAAAGTTTAGAGC GAAGGCAAAGATAAAGCCAGAA	58.5 °C	MH720268
IFN-γ	CATTCGCATGGAGAAAGATAGT ACACGCTTCAGCTCAAACAA	56.7 °C	MH720269
GH	AGCTCCTTCGCATCTCTTTC CAGCTTCTCAGTGATCTGGTT	55.3 °C	MH720270
IGF-I	TATTTCAGCAAACCGACAGGA TTACGGGCGCACAGTACATC	58.9 °C	MH720271
IGF-II	ATCGTGGGATTGTGGAAGAG CTGTAGGGATGTGGCTGAAA	56.4 °C	MH720272

$2^{-\Delta\Delta Ct}$ method [15].

2.8. Challenge test

After feeding trial, 10 fish from each tank (30 fish in each group) were selected for the challenge test with *A. hydrophila*. The median lethal dose (LD_{50}) for *A. hydrophila* was determined by 2×10^7 CFU/mL. The fish were injected intraperitoneally with 0.2 mL phosphate buffer saline (PBS) quickly containing 2×10^7 CFU/mL *A. hydrophila*. The mortality was recorded for 7 d, and the fish survival rate was calculated using the following equation:

Survival rate = [(initial fish no. - dead fish no.)/initial fish no.] × 100% [16].

2.9. Statistical analysis

The results were presented as the means ± S.E. All data were subjected to a one-way analysis of variance (ANOVA) followed by LSD multiple range tests. A *P*-value of < 0.05 indicated a significant difference. All analyses were run using Statistical Product and Service Solutions (SPSS 20.0) software.

3. Results

3.1. Growth performance

The effect of RRPP on the growth performance of *Luciobarbus capito* is shown in Table 3. At the end of the trial, the average final body weight (FW), WGR, SGR and FCR in the 0.1%, 0.2% and 0.4% RRPP supplemented groups were significantly higher than those in the control group and the 0.05% RRPP ($P < 0.05$) group. The highest values were recorded on the treatment group fed with 0.2% RRPP. However, no significant differences were noted in CF among all groups ($P > 0.05$).

3.2. Serum immune parameters

As shown in Table 4, LAZ activity, ACP activity and SOD activity increased in all experimental groups. LAZ activity and ACP activity were significantly increased in the 0.05%, 0.2% and 0.4% RRPP supplemented groups compared with the control group ($P < 0.05$). SOD activities of fish fed 0.1%, 0.2% and 0.4% RRPP were significantly higher than that of the control group ($P < 0.05$). For AKP activity, only the 0.4% RRPP group showed a significant difference compared with the control ($P < 0.05$). Serum TP concentration was significantly higher in the 0.1% and 0.2% RRPP supplemented groups, and the peak values were 53.40 and 54.87(g/L) respectively.

3.3. Expressions of immune- and growth-related genes

The expression profiles of immune-related genes (*IL-1β*, *IL-8*, *IL-10*, *TNF-α*, *TGF-β*, *IFN-γ*) and growth-related genes (*GH*, *IGF-I*, *IGF-II*) were examined in the kidney (Fig. 1. A), spleen (Fig. 1. B) and gut (Fig. 1. C) of *Luciobarbus capito*. As shown in Fig. 1, the mRNA levels of pro-inflammatory immune factors (*IL-1β*, *IL-8*, *TNF-α*, *IFN-γ*) and growth -related genes (*GH*, *IGF-I*, *IGF-II*) were up-regulated in the three organs of the fish fed with RRPP, whereas the two anti-inflammatory cytokines (*IL-10* and *TGF-β*) were down-regulated.

The expression level of *IL-1β* was significantly increased in the three organs of the fish in the 0.05% RRPP supplemented group, but in the same organs, the 0.1% and 0.2% RRPP groups showed significantly reduced expression of *TGF-β*. In the gut, *IL-8* and *TNF-α* transcription significantly increased in the 0.1% and 0.2% RRPP groups, respectively. In the kidney and spleen, the expression of *IL-10* was significantly down-regulated in fish fed the experimental diets, whereas the *IFN-γ* mRNA levels were significantly higher in the 0.2% and 0.4% RRPP groups, respectively. The current study revealed that feeding with 0.2% RRPP could significantly up-regulate growth-related gene (*GH*, *IGF-I*, *IGF-II*) expression in all treatment groups, except for *IGF-I* in the gut.

3.4. Disease resistance

The results of the challenge study revealed that the mortality of *Luciobarbus capito* increased dramatically at 2–5 d after injection of *A. hydrophila*. The survival rate of fish was increased in all groups treated with RRPP. The final survival rates (%) were 23.3, 43.2, 50.0, 63.4 and 56.7 in the 0%, 0.05%, 0.1%, 0.2% and 0.4% RRPP supplemented groups, respectively (Fig. 2).

4. Discussion

Plant polysaccharides are effective ingredients of medicinal plants, which have been studied widely for their antiviral, antibacterial,

Table 3
Effects of RRPP on growth performance and feed utilization of *Luciobarbus capito*.

Group	IW (g)	FW (g)	WGR(%)	SGR(%/d)	CF (g/cm^3)	FCR
CK	46.55 ± 0.90^a	99.88 ± 2.61^a	114.57 ± 5.61^a	1.24 ± 0.04^a	1.13 ± 0.01^a	2.35 ± 0.09^a
0.05% RRPP	46.84 ± 1.18^a	102.03 ± 2.53^a	117.26 ± 5.17^a	1.26 ± 0.04^a	1.14 ± 0.02^a	2.32 ± 0.09^a
0.1% RRPP	45.28 ± 0.99^a	117.57 ± 2.64^b	159.65 ± 5.83^b	1.56 ± 0.03^b	1.17 ± 0.01^a	1.75 ± 0.07^c
0.2% RRPP	46.07 ± 1.01^a	121.23 ± 2.82^b	163.15 ± 6.12^b	1.68 ± 0.04^c	1.17 ± 0.01^a	1.72 ± 0.07^c
0.4% RRPP	46.28 ± 1.01^a	118.84 ± 2.45^b	156.78 ± 5.29^b	1.54 ± 0.03^b	1.17 ± 0.01^a	2.00 ± 0.08^b

Values (mean ± SE, n = 6) with different superscripts in the same column are significantly different ($P < 0.05$).
IW: Average initial body weight; FW: Average final body weight; WGR: Weight gain rate.
SGR: Specific growth rate; CF: Condition factor; FCR: Feed conversion ratio.

Table 4
Effects of RRPP on serum immune parameters of *Luciobarbus capito*.

Group	LAZ (U/ml)	AKP (U/100 mL)	ACP (U/100 mL)	SOD (U/ml)	TP (g/L)
CK	49.82 ± 0.78^{a}	1.14 ± 0.04^{a}	7.63 ± 0.12^{a}	84.57 ± 2.84^{a}	44.65 ± 0.76^{a}
0.05% RRPP	65.62 ± 1.10^{bc}	1.06 ± 0.05^{a}	8.08 ± 0.11^{b}	85.61 ± 2.35^{a}	43.32 ± 0.72^{a}
0.1% RRPP	52.14 ± 0.64^{a}	1.21 ± 0.04^{a}	7.73 ± 0.13^{a}	105.59 ± 3.15^{c}	53.40 ± 0.70^{b}
0.2% RRPP	66.87 ± 0.91^{c}	1.2 ± 0.04^{a}	9.11 ± 0.10^{c}	97.74 ± 2.57^{b}	54.87 ± 0.62^{b}
0.4% RRPP	63.88 ± 0.68^{b}	1.79 ± 0.05^{b}	8.8 ± 0.12^{c}	96.32 ± 3.02^{b}	44.82 ± 0.70^{a}

Values (mean ± SE, n = 6) with different superscripts in the same column are significantly different ($P < 0.05$).

antitumour, and immune enhancement abilities [17]. Many plant polysaccharides are considered as candidates for the development of immunostimulants in aquaculture. In the current study, we confirmed that the diet supplemented with RRPP significantly affected the growth performance, several serum immune parameters, immune- and growth-related gene expression and disease resistance of *Luciobarbus capito*. This study is the first attempt to investigate the effects of RRPP as an immunostimulant on the growth performance, immunological parameters and disease resistance of *Luciobarbus capito*. In this study, dietary supplementation with RRPP at 0.1%, 0.2%, or 0.4% for 60 d significantly increased the growth performance (FW, WGR, SGR and FCR) of *Luciobarbus capito*. Similar to our results, positive effects of dietary herbal polysaccharides on fish growth performance have been consistently reported in white shrimps [18], grass carp [19] and *Oreochromis niloticus* [20].

LAZ is a low molecular alkaline protein widely distributed in the mucus, lymphoid tissue, serum and other bodily fluids of fish [21], which plays an important role in nonspecific immune responses, splits the β-1, 4 glycosidic bonds in the peptidoglycan of bacterial cell walls [22]. Previous studies have reported enhancement of LAZ activity through the administration of herbal polysaccharide-containing substances, such as *Coriolus versicolor* and *Astragalus* polysaccharide fed to *crucian carp* [23] and *Micropterus salmoides* [24], respectively. The current study showed that fish fed a 0.05%, 0.2% or 0.4% RRPP supplemented diet showed significantly enhanced serum LAZ activity.

AKP and ACP are two important hydrolytic enzymes that participate in signal transduction and energy transformation and also regulate nonspecific immunity and nutrient metabolism [25,26]. In this work, we showed that 0.4% RRPP supplementation increased the AKP level significantly, and the ACP level was significantly increased in the 0.2% and 0.4% RRPP-treated groups. SOD, an antioxidant enzyme that resists oxidative damage also plays an important role in defence against invading pathogens and is used to evaluate non-specific immune responses. In the current study, the SOD activity in the three treatment groups (0.1%, 0.2%, and 0.4% RRPP) was significantly higher than that in the control group, and these results indicate that RRPP can increase serum SOD activity. In other studies on plant polysaccharides, it has been found that *Enteromorpha prolifera* polysaccharide increased ACP and SOD activities, whereas AKP activity was unaffected in the coelomic fluid of sea cucumbers [27].

TP plays a significant role in immune response. Increases in the level of total serum protein are thought to be associated with an intensive innate immune response [28]. In the current study, a significantly higher level of serum TP was observed in the 0.1% and 0.2% RRPP groups. In line with our results, TP was increased in grass carp [29] and in *crucian carp* [30].

The inflammatory response is a key element in the innate immune response system and is primarily mediated by cytokines [31]. IL-1β, IL-8, TNF-α and IFN-γ are pro-inflammatory cytokines, the upregulation of which are involved in inflammatory responses, and the level of expression is generally considered to be an indicator of whether an inflammatory response occurs. Anti-inflammatory cytokines IL-10 and TGF-β are regulatory cytokines with pleiotropic roles in the immune system, and they not only inhibit excessive cytokine release but also control inflammatory processes [32]. In the current study, feeding of four doses of RRPP for 60 d up-regulated the expression of *IL-1β*, *IL-8*, *TNF-α* and *IFN-γ* and down-regulated the expression of *IL-10* and *TGF-β* in the kidney, spleen and gut of *Luciobarbus capito*. The level of regulation was significant in the appropriate RRPP-treated group and particular organs. Similarly, dietary administration of *Hericium caput-medusae Pers.* polysaccharide for 14 or 21 d significantly enhanced expression of *IL-1β* and *TNF-α* and reduced expression of *IL-10* genes in

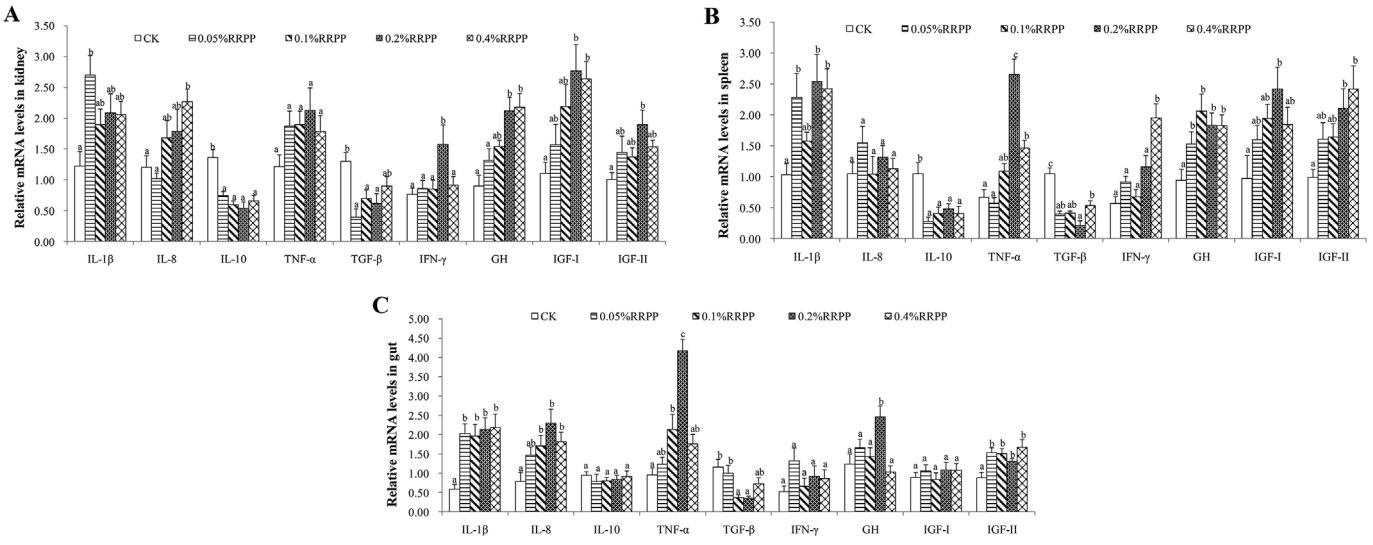

Fig. 1. Relative expression of immune-related genes (*IL-1β*, *IL-8*, *IL-10*, *TNF-α*, *TGF-β*, *IFN-γ*) and growth-related genes (*GH*, *IGF-I*, *IGF-II*) in the kidney (A), spleen (B) and gut (C) of *Luciobarbus capito* fed diets supplemented with graded levels of RRPP for 60 d. Bars represent the mean values ± S.E. of nine samples in each group. Different letters above a bar denote the significant difference between treatments ($P < 0.05$).

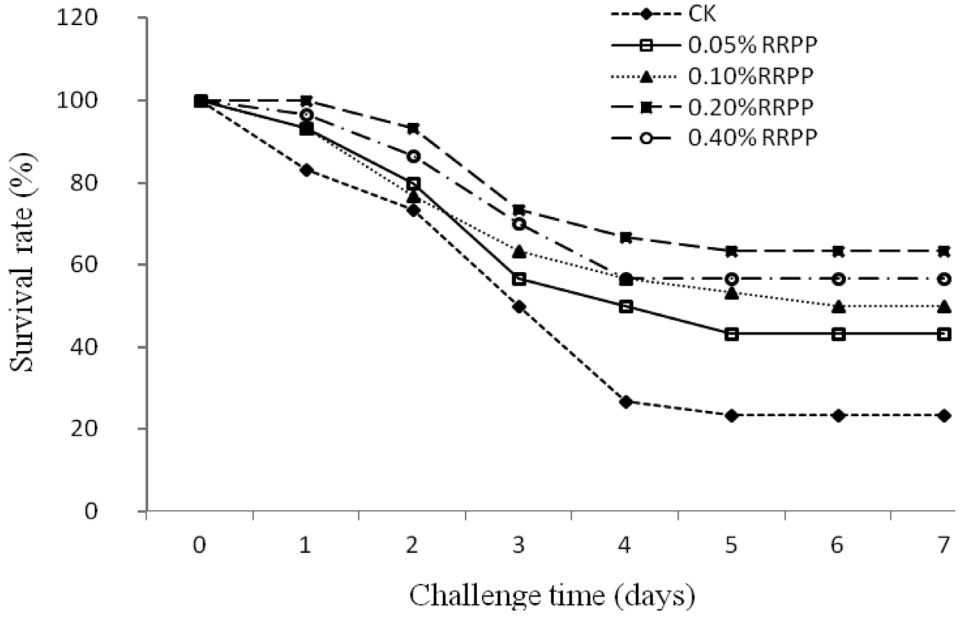

Fig. 2. Survival rate (%) of *Luciobarbus capito* in each group after fish were challenged with *A. hydrophila*. Bars represent the mean values ± S.E, n = 6).

the kidney of grass carp [33]. Injected with *Astragalus polysaccharides* and *chitosan*, in both spleen and head kidney tissues of yellow catfish, mRNA expressions of IFN rose up in the whole immune period [34]. Contrary to our conclusion, dietary supplementation with 0.50–2.00 g kg^{-1} *Lycium barbarum* extract (polysaccharides content, 80.0%) significantly decreased *IL*-8 mRNA levels and significantly increased *IL-10* and *TGF-β1* mRNA levels in the liver of hybrid grouper [35].

The GH-IGF axis plays an integral role in mammalian and fish growth in which GH, IGF-I and IGF-II are important hormones involved in the growth [36]. In bony fish, *GH* and *IGF* mRNA have been detected in numerous organs such as the pituitary gland, brain, liver, eye, gills, heart, kidney, spleen, muscle, pancreatic islets, and gonads [37,38]. In particular, *IGF-I* and *IGF-II* gene are controlled by *GH* in all organs, and the levels of *IGF-I* mRNA positively correlate with body growth rate [39]. In the current study, the growth performance (FW, WGR, SGR) of *Luciobarbus capito* was enhanced in parallel with increased *GH*, *IGF-I* and *IGF-II* gene expression.

Many plant polysaccharides are used as immunostimulants in fish disease control. Administration of marine macroalga polysaccharide enhanced disease resistance against *A. hydrophila* in *Cyprinus carpio* [40]. White shrimps fed a trehalose polysaccharide supplementation diet showed higher survival rate than those fed a basal diet after challenge with *Vibrio alginolyticus* [41]. Dietary supplementation of *Chlorophytum borivilianum* polysaccharide improved disease resistance in Labeo rohita challenged with *Yersinia ruckeri* [42]. Similarly, all fish fed with RRPP showed significantly higher survival rates than those of the controls after *A. hydrophila* challenge, and the best survival rate was observed in the 0.2% RRPP group with a survival rate of 63.4%. Therefore, RRPP enhanced protection against pathogenic bacteria. This result might be due to RRPP elevation of nonspecific immune parameters (LAZ, SOD, AKP, ACP, TP), upregulation of the expression of pro-inflammatory cytokines (*IL-1β*, *IL-8*, *TNF-α*, *IFN-γ*), and down-regulation of the expression of anti-inflammatory cytokines (*IL-10*, *TGF-β*).

In this study, RRPP have a better effect on the growth performance, immunity and disease resistance of *Luciobarbus capito* at the concentration of 0.2%. In addition, we found that 4% of prepared rehmannia root powder can promote growth performance, regulate immune response and prevent pathogenic infection in common carp in previous studies [43]. The polysaccharide extraction rate and content of prepared Rehmannia root were 6.8% [44] and 95.18%, respectively. Therefore, the concentration of *Radix Rehmanniae Preparata* polysaccharides is approximately 0.2%, which is consistent with this research.

5. Conclusions

In conclusion, RRPP enhanced growth performance and innate immune responses, modulated expression of immune- and growth-related genes, and consequently improved disease resistance against *A. hydrophila* in *Luciobarbus capito*. The effects were especially remarkable at concentration of 0.2% RRPP. This study offers support to the hypothesis that RRPP as a plant polysaccharide has the potential for development of a new source of immunostimulants.

Conflicts of interest

The authors declare no conflict of interest.

Ethics statement

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals, and was approved by the Institutional Animal Care and Use Committee of Henan Normal University. The fishes were anaesthetized with diluted MS-222 before been euthanized, and all efforts were made to minimize suffering.

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