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Effects of fucoidan on growth performance, immunity, antioxidant ability, digestive enzyme activity, and hepatic morphology in juvenile common carp (*Cyprinus carpio*)

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Fucoidan with its excellent biological activities such as growth promotion, antioxidant and strong immunity, is widely used in animal production. The present study was conducted to investigate the influences of feeding fucoidan on growth performance, biochemical indices, immunity, the antibacterial ability of plasma, the digestive enzyme activity of the intestine, antioxidant capacity, and the histological structure of liver in juvenile common carp. Five experimental diets added with 0 (Diet 1), 500 (Diet 2), 1,000 (Diet 3), 1,500 (Diet 4), and 2,000 (Diet 5) mg/kg fucoidan were fed to triplicate groups of 30 fish (35.83 ± 0.24 g) respectively for 8 weeks. The results showed that fish fed diets with a fucoidan supplementation of 1,666.67–1,757 mg/kg might have the best growth performance ($p < 0.05$). The levels of plasma total protein (TP) and albumin (ALB) in Diet 3, Diet 4, and Diet 5 were higher than those in Diet 1 and Diet 2 ($p < 0.05$). Moreover, the contents of plasma C3, LYZ, and IgM; the antibacterial ability of serum; and the activity of SOD, CAT, POD, and GPX in the liver, and ACP, AKP, LPS, AMS, and TRY in the intestine significantly improved; the contents of LPO and MDA in the liver were notably decreased in diets with fucoidan supplement ($p < 0.05$). Furthermore, the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and the contents of total bilirubin (TB) and glucose (Glu) in Diet 5 were the highest among the groups. Meanwhile, proinflammatory factors (plasma IL-6 and IL-1β) had a higher expression, but anti-inflammatory factors (plasma IL-1) had a lower expression in Diet 5 ($p > 0.05$). It indicated that a higher dose (2,000 mg/kg) of fucoidan may induce inflammation and metabolic disorders. Interestingly, histological results of liver also indicated that dietary fucoidan intake in certain amounts (500–1,500 mg/kg) could ameliorate hepatic morphology, but the high dosage (2,000 mg/kg) probably damaged the liver. To the best of our knowledge, this is the first study on the application of fucoidan as a functional additive to juvenile common carp. The results of the present study can be used to guide the application of fucoidan in healthy aquaculture and can further reveal the effect and mechanism of fucoidan on the nutritional physiology of aquatic animals.

KEYWORDS

fucoidan, common carp, growth, immunity, antioxidant

Introduction

Feed supplements are usually used in the aquaculture industry to improve fish growth rate and production. Fucoidan is a type of natural active polysaccharide mainly extracted from marine brown algae (Pomin, 2015) containing sufficient L-fucose and sulfate eater groups (Li et al., 2008). Fucoidan has been found to be effective at growth promotion (Dawood et al., 2018; Fazio et al., 2019) and gastrointestinal function regulation (Cui et al., 2020; Liu et al., 2020), and to have anti-inflammatory (Kirindage et al., 2022; Wang et al., 2022), anti-oxidation (Sony et al., 2019; Abdel-Daim et al., 2020; Mahgoub et al., 2020), anti-tumor (Han et al., 2015a; Han et al., 2015b; Jin et al., 2022), anti-bacterial (Yu et al., 2015; Zhu et al., 2021), and immunomodulatory (Wang et al., 2019; Ikeda-Ohtsubo et al., 2020) properties. Recently, fucoidan has attracted the attention of aquatic animal researchers. However, an extreme limitation is that fucoidan research on aquatic animals has mainly focused on a few species, such as *Penaeus monodon* (Chotigeat et al., 2004; Sivagnanavelmurugan et al., 2014), *Marsupenaeus japonicus* (Traifalgar et al., 2010), *Pelteobagrus fulvidraco* (Yang et al., 2014), *Litopenaeus vannamei* (Sinurat et al., 2016), *Labeo rohita* (Mir et al., 2017; Adnan et al., 2018), *Pagrus major* (Sony et al., 2019), *Carassius auratus* (Cui et al., 2020), and *Oreochromis niloticus* (Abdel et al., 2021), and it has been found to improve the growth rate and stimulate the immune system. Therefore, fucoidan is considered as a promising feed additive to enhance the growth and immunity in aquatic animals.

As a freshwater economic fish, common carp (*Cyprinus carpio*), is one of the key aquaculture species in the world. Common carp production provides an economical, tasty, hyperproteic, healthy food supplement for people around the world. Hence, researchers have focused on improving the growth (Mohammadian et al., 2021; Khorshidi et al., 2022; Zhang et al., 2022), disease resistance (Ahmadifar et al., 2022; Ghafarifarsani et al., 2022) and stress resistance (Banaee et al., 2022; Dawood et al., 2022; Xue et al., 2022b) of common carp. As mentioned earlier, fucoidan as feed additive has remarkable effects on improving growth and immunity, but its application in fish feed research is limited. To the best of our knowledge, there is no available information about the effect of fucoidan on common carp.

Therefore, the present study was conducted to determine the dietary fucoidan effects on growth performance, biochemical parameters, specific and non-specific immunity, antibacterial ability, antioxidant capacity, digestive enzyme activity, and the histological structure of the liver in juvenile common carp, and the results of the study help to determine the optimal amount of fucoidan that could be effectively used in carp culture.

Materials and methods

Diet formulation and preparation

The basal diet was based on soybean meal as carbohydrate sources and fish meal as protein sources. Five isonitrogenous (38%) and isolipidic (6.6%) experimental diets (Table 1) were formulated and supplemented with 0 (Diet 1), 500 (Diet 2), 1,000 (Diet 3), 1,500 (Diet 4), and 2,000 (Diet 5) mg/kg fucoidan (purity ≥98%, Xi ‘an Risen Biotechnology Co., LTD., China). The selected dosages of fucoidan were based on the findings of earlier studies (Sony et al., 2019; Cui et al., 2020). All feed ingredients were crushed and sifted by a 60-mesh sieve, weighed, and mixed by a stepwise expansion method to make pellet feed with a diameter of 1 mm, which was dried naturally outside in a drafty place that is not exposed to direct sunlight and then stored at −20°C until feeding.

Experimental fish and design

Common carp juveniles were purchased from an aquafarm (Chongqing, China) and acclimated for 14 days in a cement breeding pond (6 m × 6 m × 0.8 m) feeding with the commercial feed (Tongwei Co. LTD., China) twice a day. After acclimation to the trial conditions, 450 healthy fish (35.83 ± 0.24 g) were randomly assigned to 15 cement breeding ponds (2.1 m × 1.2 m × 0.8 m), each with 30 fish species. The feeding trial was conducted outdoors for 8 weeks in the aquaculture base of Southwest University (Chongqing, China). Fish in triplicate groups were fed the respective experimental diets to apparent satiation twice daily at 8:00 and 17:00. Meanwhile, the feed intake and mortality of fish in each pond were recorded. During the trial, the dissolved oxygen was ≥6.5 mg/L and the water temperature ranged from 25 to 28°C. The total ammonia nitrogen and nitrites remained< 0.05 mg/L, and the pH was kept at 7.4–8.2. The natural photoperiod was applied.

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TABLE 1 Ingredients and proximate nutrient compositions of experimental diets (dry).

Items	Dietary fucoidan supplementations (mg/kg)				
	Diet 1 (0)	Diet 2 (500)	Diet 3 (1,000)	Diet 4 (1,500)	Diet 5 (2,000)
Ingredients (%)					
Fish meal	26	26	26	26	26
Bean pulp	35	35	35	35	35
Rapeseed meal	11	11	11	11	11
Bran	8	8	8	8	8
Flour	12	11.5	11	10.5	10
Soybean oil	4	4	4	4	4
$C_a(H_2PO_4)_2$	1	1	1	1	1
Choline chloride	1	1	1	1	1
Binder	0.50	0.50	0.50	0.50	0.50
1.5% premix	1.50	1.50	1.50	1.50	1.50
Nutrient content (%)					
Crude protein	38.05	38.10	37.81	38.13	37.96
Crude lipid	6.59	6.60	6.65	6.64	6.61
Moisture	9.12	9.14	9.01	9.15	9.06
Ash	16.71	16.59	16.64	16.38	16.50
Met + Cys	1.20	1.21	1.19	1.23	1.21
Lys	2.43	2.41	2.39	2.42	2.43
Thr	1.53	1.55	1.54	1.52	1.51

The compound premix provides vitamins and minerals per kg of feed as below: V_C = 200 mg, V_A = 30,000 IU, V_E = 600 mg, V_{D3} = 25,000 IU, V_{B1} = 50 mg, V_{B2} = 60 mg, V_K = 100 mg, niacin = 100 mg, V_{B12} = 0.2 mg, calcium pantothenate = 120 mg, folic acid = 20 mg, biotin = 7 mg, inositol = 250 mg, $CuSO_4{\cdot}5H_2O$ = 7.20 g, $MnSO_4{\cdot}H_2O$ = 5.16 g, $FeSO_4{\cdot}7H_2O$ = 15.56 g, Na_2SeO_3 = 2.10 g, KI = 6.58 g.

Sampling

At the end of the trial, carps were starved for 24 h, weighed and dissected, and then counted to calculate growth index. Twelve fish were caught randomly from each pond, and anesthetized by applying eugenol for sampling. Among them, six fish were selected for blood collection purposes; their body length and weight (body, viscera, and liver) were then recorded for the purpose of computing the somatic indices. The blood of three fish was drawn from their caudal vein using sterile disposable syringes (2 ml), rinsed with heparin sodium solution. After the centrifugation (3,500 rpm, 15 min, 4°C) of the sample, the plasma was separated and stored at −80°C for biochemical index detection. Non-heparinized blood of 3 fish was centrifuged (3,500 rpm, 15 min, 4°C) to obtain the serum for antibacterial activity test. Three fish from each pond were sacrificed to obtain liver and intestine collected in an ultralow-temperature refrigerator for analysis of enzyme activity. Moreover, three hepatic tissues from each pond were placed in 2-ml centrifuge tubes containing Bouin's solution for micromorphological analysis. After 24 h of fixation, the samples were washed and stored using 70% ethanol solution until the preparation of tissue section.

Determination of plasma biochemical parameters

The assay of plasma biochemical indices in this study comprised alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), total bilirubin (TB), urea nitrogen (UN), creatinine (Cr), glucose (Glu), triglycerides (TG), and total cholesterol (TC), which were all measured by using a fully automated biochemical analyzer (Chemray 240, Shenzhen, China).

Immune, antioxidant, and digestive enzyme indices assay

The content of interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-10 (IL-10), complement 3 (C3), lysozyme (LYZ), and immunoglobulin M (IgM) in the plasma; the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), lipid peroxide (LPO), and malonaldehyde (MDA) in the liver; and the activities of acid phosphatase (ACP), alkaline phosphatase (AKP), lipase (LPS), amylase (AMS), and trypsin (TRY) in the intestine were determined by utilizing the commercial kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The above kits were carried out in accordance with the manufacturer's specifications.

Antimicrobial activity of serum *in vitro*

The serum from non-heparinized blood was used for antibacterial activity test *in vitro*. For this purpose, *Aeromonas hydrophila* (BBAh1) stored in the Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education) (Li et al., 2019) was grown in tryptic soy broth for 24 h at 30°C. Bacterial cells were centrifuged and suspended in sterile PBS and adjusted to an optical density of 0.5 at 546 nm. The diluted bacterial suspension was mixed with serum homogenates for incubation at 37°C for 1 h. After incubation, the number of viable bacterial cells was counted (Eslami et al., 2022).

Liver histological processing

Hepatic samples were fixed in Bouin's solution. Then, the fish tissues were placed in plastic cassettes and processed (gradual dehydration in 70%–100% alcohol, clearing in xylene, and paraffin wax embedding). Five-micron-thick sections were cut on a microtome and then stained with hematoxylin and eosin (HE). Slides were examined with a light microscope (Nikon DXM1200).

Calculations and statistical methods

The growth performance parameters were calculated as follows (Geetanjali et al., 2020; Jayant et al., 2021):

Initial body weight (IW, g);
Final body weight (FW, g);
Final body length (FL, cm);

$$\text{Weight gain}(WG, \%) = (FW - IW) \times 100/IW;$$

$$\text{Specific growth rate}(SGR, \%/\text{day}) = (\text{Ln } FW - \text{Ln } IW) \times 100/\text{day};$$

$$\text{Feed conversion ratio}(FCR) = \text{total diet fed}/\text{total wet weight gain};$$

$$\text{Hepatosomatic index}(HI, \%) = \text{liver weight} \times 100/\text{body weight};$$

$$\text{Viscera-somatic index}(VSI, \%) = \text{visceral weight} \times 100/\text{body weight};$$

$$\text{Condition factor}(CF) = \text{final weight} \times 100/(\text{final body length});$$

$$\text{Survival rate}(SR, \%) = \text{final quantity} \times 100/\text{initial quantity}.$$

All of the data were expressed as the mean ± SEM. The data were analyzed by one-way analysis of variance and Tukey's multiple range test using SPSS 19.0 (IBM, Chicago, USA). Differences were considered significant at $p< 0.05$. The optimal dietary requirement of fucoidan for juvenile carp was calculated by using broken line regression analysis (Robbins et al., 2006).

Results

Growth performance, feed utilization, and somatic indices

The growth performance, feed utilization, and somatic indices of juvenile common carp fed the experimental diets containing different levels of fucoidan were presented in Table 2. At the end of the 8-week culturing trial, Diet 3, Diet 4, and Diet 5 were higher in FW, WG, and SGR than Diet 1 and Diet 2 ($p< 0.05$). Moreover, FCR in Diet 3 and Diet 4 was significantly lower than in the other diets ($p< 0.05$). VSI in Diet 3 and Diet 4 was significantly higher than in Diet 1, and fish fed diet 1,500 had a higher VSI than fish fed diet 500. Nevertheless, there were nearly no significant changes in HI, CF, and SR among all the treatments ($p > 0.05$). Furthermore, the regression analysis between fucoidan supplementation and WG showed that the optimal dietary content of fucoidan was 1,757 mg/kg (Figure 1), and the regression analysis between fucoidan content and SGR showed that the optimal dietary supplementation of fucoidan was 1,666.67 mg/kg for juvenile common carp (Figure 2). Meanwhile, the survival rate of common carp fed with dietary fucoidan remained unaffected compared to the control group, which was 100% in all diets.

Plasma biochemical parameters

The effects of dietary fucoidan on plasma biochemical parameters of juvenile common carp are presented in Table 3. The highest levels of plasma ALT, AST, TB, and Glu were found in Diet 5, which were statistically higher than other diets ($p< 0.05$). Additionally, plasma TP and ALB in Diet 3, Diet 4, and Diet 5 were higher than that in Diet 1 and Diet 2 ($p< 0.05$), whereas nearly no statistically significant differences were observed in UN, Cr, TG, and TC for any of the treatment ($p > 0.05$).

Immune parameters and antibacterial activity of serum

Table 4 shows that dietary supplementation with fucoidan containing different levels in common carp impacted the immune parameters in plasma. The values of proinflammatory cytokines IL-6 and IL-1β showed a decreasing trend in Diet 2, Diet 3, and Diet 4, and an increasing trend in Diet 5 ($p< 0.05$). Conversely, the values of anti-inflammatory cytokine IL-10 showed an increasing trend in Diet 2 and Diet 3, and a decreasing trend in Diet 4 and Diet 5 ($p< 0.05$). Furthermore, compared with Diet 1, dietary addition of fucoidan significantly improved the C3, LYZ, and IgM content

TABLE 2 Effect of dietary fucoidan supplementations on growth performance, feed utilization, and somatic indices of juvenile *Cyprinus carpio* (N = 18).

Items	Dietary fucoidan supplementations (mg/kg)				
	Diet 1 (0)	Diet 2 (500)	Diet 3 (1,000)	Diet 4 (1,500)	Diet 5 (2,000)
Initial body weight (IW, g)	36.10 ± 0.46	35.27 ± 0.57	35.68 ± 0.75	35.90 ± 0.52	36.20 ± 0.43
Final body weight (FW, g)	129.16 ± 4.51^{a}	148.33 ± 6.13^{b}	170.00 ± 3.89^{c}	182.50 ± 7.50^{c}	178.33 ± 6.72^{c}
Weight gain (WG, %)	259.49 ± 15.52^{a}	322.41 ± 20.16^{b}	378.69 ± 14.48^{c}	409.53 ± 22.34^{c}	393.61 ± 19.58^{c}
Specific growth rate (SGR, %/day)	2.26 ± 0.08^{a}	2.55 ± 0.08^{b}	2.78 ± 0.05^{c}	2.88 ± 0.08^{c}	2.83 ± 0.07^{c}
Feed conversion ratio (FCR)	1.21 ± 0.01^{de}	1.18 ± 0.01^{ce}	1.11 ± 0.01^{ab}	1.07 ± 0.01^{a}	1.14 ± 0.01^{bc}
Hepatosomatic index (HI, %)	2.43 ± 0.19^{ab}	2.56 ± 0.18^{ab}	2.81 ± 0.23^{b}	2.16 ± 0.18^{a}	2.58 ± 0.20^{ab}
Viscera–somatic index (VSI, %)	9.01 ± 0.36^{a}	9.76 ± 0.38^{ab}	9.94 ± 0.45^{ac}	11.19 ± 0.72^{c}	10.80 ± 0.38^{bc}
Condition factor (CF)	2.75 ± 0.06^{ab}	2.67 ± 0.06^{a}	2.87 ± 0.05^{b}	2.71 ± 0.05^{ab}	2.83 ± 0.04^{ab}
Survival rate (SR, %)	100.00	100.00	100.00	100.00	100.00

Data expressed as mean ± SEM.
Different letters in the same line indicate that the indexes are significant differences ($p < 0.05$).

($p < 0.05$). Moreover, the highest value of C3 and LYZ appeared in Diet 3, and the highest value of IgM appeared in Diet 4. Quite noticeably, the number of *A. hydrophila* colonies notably diminished ($p < 0.05$) in the fish serum feeding Diet 3 (cfu = 226 ± 21), Diet 4 (cfu = 301 ± 12), and Diet 5 (cfu = 309 ± 18) compared to Diet 1 (cfu = 362 ± 17) and Diet 2 (cfu = 343 ± 12) (Figure 3).

Antioxidative parameters in liver

As displayed in Table 5, antioxidative parameters in the liver were also affected significantly by dietary fucoidan supplementation. Distinctly, the activity of enzymes in antioxidant systems SOD (in Diet 3 and Diet 4), CAT (in Diet 2 to Diet 5), and POD and GPX (in Diet 3 to Diet 5) was significantly increased by feeding fucoidan ($p <$ 0.05). Meanwhile, the content of the peroxidation product LPO (in Diet 3 to Diet 5) and MDA (in Diet 2 to Diet 5) was dramatically decreased by the supplementation of fucoidan ($p < 0.05$).

Digestive enzyme activity in the intestine

The digestive enzyme activity in the intestine was also affected by feeding fucoidan (Table 6). Obviously, all the digestive enzyme activity in Diet 3, Diet 4, and Diet 5 was significantly elevated compared to Diet 1 ($p < 0.05$). Notably, the activity of LPS in Diet 5 was the highest ($p < 0.05$), and the activity of AMY and TRY in Diet 3 and Diet 4 was higher than the other Diets ($p < 0.05$).

Histological observation of liver

The effect of dietary supplementation of fucoidan on the hepatic morphology of juvenile common carp is presented in Figure 4. Compared to Diet 1 and Diet 5, the hepatic tissues of fish feeding Diet 2, Diet 3, and Diet 4 were obviously more intact: hepatocytes were arranged neatly, with a clear cellular boundary and the nucleus mainly in the cell center, and slight vacuolization was observed. In Diet 5, hepatocytes were arranged irregularly, with a blurred cellular

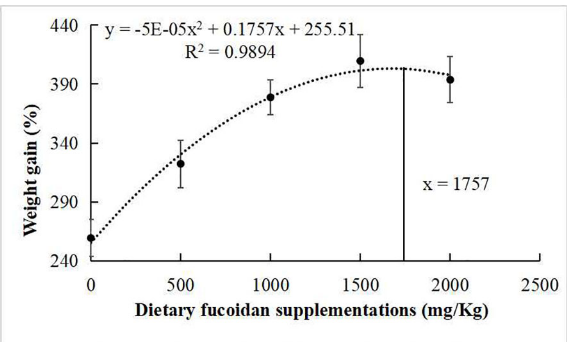


FIGURE 1
Broken line regression analysis between dietary fucoidan supplementation and weight gain of juvenile *Cyprinus carpio*.

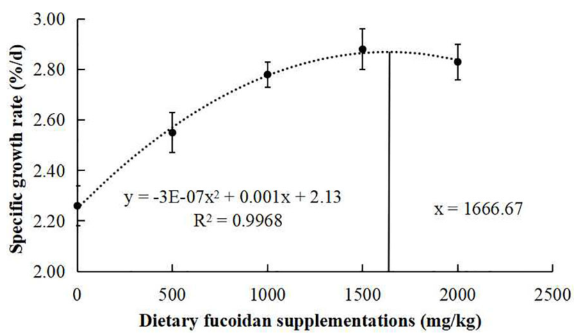


FIGURE 2
Broken line regression analysis between dietary fucoidan content and specific growth rate of juvenile *Cyprinus carpio*.

TABLE 3 Effect of dietary fucoidan supplementations on plasma biochemical indexes of juvenile *Cyprinus carpio* (N = 9).

Items	Dietary fucoidan supplementations (mg/kg)				
	Diet 1 (0)	Diet 2 (500)	Diet 3 (1,000)	Diet 4 (1,500)	Diet 5 (2,000)
Alanine aminotransferase (ALT, U/L)	30.00 ± 0.57^{a}	31.66 ± 3.71^{a}	36.00 ± 1.15^{a}	50.00 ± 1.73^{a}	264.33 ± 32.83^{b}
Aspartate aminotransferase (AST, U/L)	223.66 ± 15.30^{bc}	228.66 ± 1.76^{c}	195.66 ± 10.58^{a}	199.66 ± 2.02^{ab}	294.66 ± 1.20^{d}
Total protein (TP, g/L)	27.00 ± 1.00^{a}	27.00 ± 0.57^{a}	31.33 ± 1.33^{b}	31.33 ± 0.33^{b}	29.66 ± 1.76^{ab}
Albumin (ALB, g/L)	11.33 ± 0.33^{a}	11.33 ± 0.33^{a}	13.66 ± 0.33^{b}	13.66 ± 0.33^{b}	13.33 ± 0.88^{b}
Total bilirubin (TB, μmol/L)	1.56 ± 0.26^{a}	1.50 ± 0.25^{a}	1.43 ± 0.24^{a}	1.66 ± 0.13^{a}	1.90 ± 0.20^{b}
Urea nitrogen (UN, μmol/L)	33.13 ± 0.49^{a}	33.26 ± 0.20^{a}	33.23 ± 0.74^{a}	31.76 ± 0.21^{a}	32.53 ± 0.72^{a}
Creatinine (Cr, μmol/L)	29.66 ± 3.17^{ab}	31.33 ± 1.76^{b}	24.66 ± 1.45^{ab}	20.66 ± 1.20^{a}	27.66 ± 5.69^{ab}
Glucose (Glu, μmol/L)	4.61 ± 0.33^{a}	5.96 ± 0.99^{a}	5.65 ± 0.41^{a}	4.25 ± 0.28^{a}	7.89 ± 0.67^{b}
Triglyceride (TG, μmol/L)	1.95 ± 0.77^{a}	1.52 ± 0.39^{a}	1.43 ± 0.30^{a}	1.99 ± 0.37^{a}	1.16 ± 0.18^{a}
Total cholesterol (TC, μmol/L)	6.43 ± 0.56^{ab}	6.70 ± 0.15a^{b}	6.90 ± 0.80^{b}	6.33 ± 0.31^{ab}	5.20 ± 0.26^{a}

Data expressed as mean ± SEM.
Different letters in the same line indicate that the indexes are significant differences ($p< 0.05$).

boundary and the nucleus located at the cellular periphery (or even disappeared), cellular swelling, severe vacuolization, and even melanin macrophage and lymphocyte infiltration.

Discussion

Fucoidan as a functional supplement with various benefits, such as improved growth rate, feed utilization, immunity, and antioxidant capacity, was used in aquatic animals (Pomin, 2015; Yu et al., 2015). In the current study, the effect of fucoidan, a type of sulfated polysaccharide extracted from brown algae, as a feed additive in the soy protein-based diet for juvenile common carp has been evaluated.

The effect of fucoidan on growth

The growth rate (FW, WG, and SGR) of juvenile common carp supplemented with fucoidan were enhanced compared with the control group in the present study. Similarly, fucoidan additive in the diets of rohu (*L. rohita*) (Mir et al., 2017; Adnan et al., 2018), red sea bream (*P. major*) (Sony et al., 2019), gible carp (*C. auratus*) (Cui et al., 2020), and Nile tilapia (*O. niloticus*) (Abdel et al., 2021) improved growth performance. Notably, the regression analysis between dietary fucoidan supplementation and SGR or WG showed that the optimal dietary content of fucoidan was 1,666.67 mg/kg or 1,757 mg/kg for the growth of juvenile common carp in this study. However, the best recipes of fucoidan in *P. major* (0.4%) (Sony et al., 2019), *C. auratus* (30 g/kg) (Cui et al., 2020), and *L. rohita* (2%) (Mir et al., 2017) are very different. Therefore, a study on the appropriate amount of fucoidan added to different fishes was very important and necessary.

The effect of fucoidan on immunoregulation

In the present study, dietary fucoidan could significantly improve the immunity of juvenile common carp by downregulating the level of proinflammation factors (IL-6 and IL-1β) and enhancing the specific (IgM) and non-specific (lysozyme and C3) immunity. Analogously, immune cells' activation and the

TABLE 4 Effect of dietary fucoidan supplementations on plasma immune parameters of juvenile *Cyprinus carpio* (N = 9).

Items	Dietary fucoidan supplementations (mg/kg)				
	Diet 1 (0)	Diet 2 (500)	Diet 3 (1,000)	Diet 4 (1,500)	Diet 5 (2,000)
Interleukin-6 (IL-6, ng/L)	24.06 ± 0.61^{d}	20.89 ± 0.51^{c}	16.73 ± 0.42^{b}	13.29 ± 0.48^{a}	27.66 ± 0.38^{e}
Interleukin-1β (IL-1β, ng/L)	76.78 ± 1.60^{c}	64.60 ± 1.51^{b}	40.08 ± 1.2^{a}	62.79 ± 1.61^{b}	119.69 ± 1.55^{d}
Interleukin-10 (IL-10, ng/L)	487.65 ± 6.72^{c}	566.16 ± 5.58^{d}	587.49 ± 4.90^{e}	381.85 ± 6.26^{b}	335.674 ± 6.16^{a}
Complement 3 (C3, μg/ml)	315.84 ± 5.10^{a}	334.02 ± 5.57^{b}	1082.69 ± 5.59^{e}	856.14 ± 5.85^{d}	792.31 ± 6.53^{c}
Lysozyme (LYZ, μg/ml)	34.22 ± 3.74^{a}	40.58 ± 3.22^{ab}	66.07 ± 3.83^{c}	46.26 ± 3.23^{b}	55.05 ± 3.16^{b}
Immunoglobulin M (IgM, μg/ml)	844.99 ± 6.92^{a}	848.34 ± 7.12^{a}	872.26 ± 5.92^{b}	896.91 ± 6.29^{c}	891.81 ± 5.66^{bc}

Data expressed as mean ± SEM.
Different letters in the same line indicate that the indexes are significant differences ($p< 0.05$).

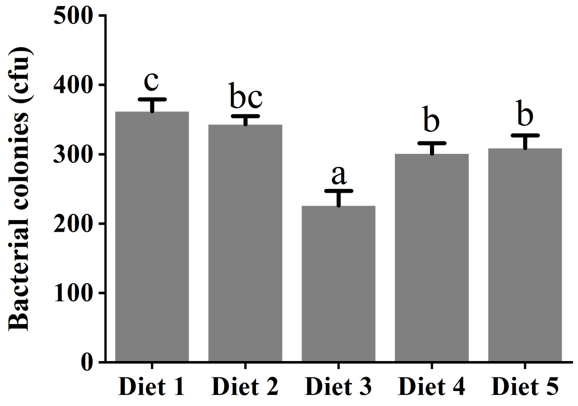


FIGURE 3
Challenge with *Aeromonas hydrophila* in serum of juvenile *Cyprinus carpio* fed dietary fucoidan. Different superscript letters indicate marked differences among the groups ($p < 0.05$). Diet 1, Diet 2, Diet 3, Diet 4, and Diet 5 refer to fish fed diets with varying levels of fucoidan (0, 500, 1,000, 1,500, and 2,000 mg/kg).

high expression of IgM, IgG, and IgA in plasma were observed in mice with dietary fucoidan (Makoto et al., 2019). The results of cytokines in this study showed that IL-6 and IL-1b decreased in Diets 2, 3, and 4, and increased in Diet 5. On the other hand, IL-10 increased in Diets 2 and 3, but decreased in Diets 4 and 5. Therefore, we could speculate that fucoidan might regulate the level of inflammation and have a proinflammatory effect at a high dose, which means that the dosage of fucoidan added in the feed should not be that high; otherwise, it will have adverse effects. Obviously, fucoidan could regulate the expression of inflammatory cytokines by activating inflammation-related signaling pathways, such as the NF-kB and eNOS signaling pathways (Wang and Chen, 2015; Caroline et al., 2019). Moreover, the survival rate of fry of *L. rohita* infected with *A. hydrophila* was significantly improved, and the content of lysozyme in plasma was also markedly increased (Adnan et al., 2018). Interestingly, we found that plasma lysozyme activity was negatively correlated with serum antimicrobial capacity in this study, which means that lysozyme activity in plasma is an important index to measure the antibacterial ability of the body (Kord et al., 2021).

The effect of fucoidan on antioxidant activity

The antioxidant system consisted of enzymes (SOD, CAT, POD, and GPX) and non-enzyme (glutathione and vitamins E and C) components, which jointly regulated the antioxidant level and maintained homeostasis (Sony et al., 2019; Abdel-Daim et al., 2020). In addition, the lipid peroxidation product LPO and its decomposition product MDA could directly reflect the rate and degree of lipid peroxidation in tissues (Mahgoub et al., 2020). In this regard, we evaluated the antioxidant capacity of the liver tissue in juvenile common carp fed with dietary fucoidan. The results showed that feeding with fucoidan significantly increased the activities of SOD, CAT, POD and GPX, and decreased the levels of LPO and MDA in common carp. Similarly, *P. fulvidraco* fed with dietary fucoidan could increase SOD and CAT activities and decrease MDA content (Yang et al., 2014). It was found that the antioxidant activity of fucoidan usually played a role through molecular pathways, such as MAPK (Kim et al., 2011; 2012), Keap1-Nrf2-ARE (Zhang et al., 2012), PI3K-Akt (Han et al., 2015a; 2015b), and the TLR-NFkB signaling pathway (Asanka et al., 2019).

The effect of fucoidan on hepatic function

The liver function could be regulated by using proper feed supplements in aquafeeds (Hemre et al., 2002), which would be reflected in antioxidant levels (Mohammadi et al., 2021), inflammation index (Yang et al., 2021), and the histological structure of the liver (Cui et al., 2020). ALT and AST levels in liver were much higher than those in plasma, but liver cell necrosis could lead to ALT and AST levels that are two times higher than plasma. Therefore, plasma ALT and AST could serve as indices to evaluate the degree of hepatic damage (Josekutty et al., 2013). Previous studies have documented that the activities of plasma ALT and AST restored the normal level in mice fed the diet containing 100 mg/(kg·day) fucoidan after BCG vaccine and lipopolysaccharide-induced immunological liver injury; it suggested that fucoidan could significantly reduce the necrosis of hepatocytes and protect the liver

TABLE 5 Effect of dietary fucoidan supplementations on liver antioxidant activities and oxidative stress of juvenile *Cyprinus carpio* (N = 9).

Items	Dietary fucoidan supplementations (mg/kg)				
	Diet 1 (0)	Diet 2 (500)	Diet 3 (1,000)	Diet 4 (1,500)	Diet 5 (2,000)
Superoxide dismutase (SOD, U/L)	44.70 ± 2.96^a	48.99 ± 2.51^a	61.75 ± 3.65^b	64.37 ± 3.16^b	50.77 ± 2.45^a
Catalase (CAT, U/L)	29.40 ± 1.91^a	34.70 ± 1.38^b	34.20 ± 1.10^b	36.20 ± 1.41^b	37.6 ± 1.46^b
Peroxidase (POD, U/L)	45.91 ± 2.34^a	45.40 ± 2.14^a	54.33 ± 2.16^b	57.68 ± 1.54^b	53.77 ± 1.62^b
Glutathione peroxidase (GPX, U/L)	63.50 ± 1.54^a	67.90 ± 1.52^a	101.00 ± 1.65^c	104.00 ± 1.61^c	74.80 ± 1.33^b
Lipid peroxide (LPO, U/L)	13.90 ± 0.72^c	13.30 ± 0.72^c	9.90 ± 0.65^b	6.99 ± 0.71^a	10.70 ± 0.40^b
Malonaldehyde (MDA, U/L)	16.30 ± 0.48^d	14.00 ± 0.54^b	7.19 ± 0.36^c	5.32 ± 0.43^a	13.60 ± 0.23^b

Data expressed as mean ± SEM.
Different letters in the same line indicate that the indexes are significant differences ($p < 0.05$).

TABLE 6 Effect of dietary fucoidan supplementations on digestive enzyme activity in the anterior intestine of juvenile *Cyprinus carpio* (N = 9).

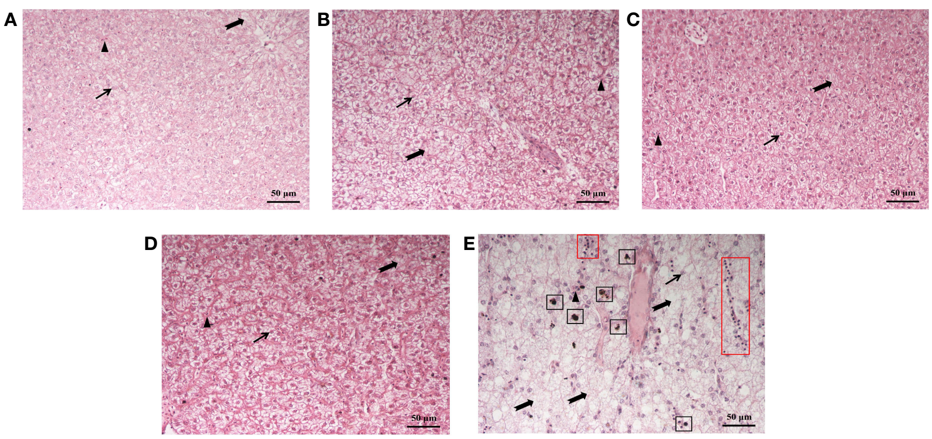
Items	Dietary fucoidan supplementations (mg/kg)				
	Diet 1 (0)	Diet 2 (500)	Diet 3 (1,000)	Diet 4 (1,500)	Diet 5 (2,000)
Acid phosphatase (ACP, U/mgprot)	0.39 ± 0.01[a]	0.39 ± 0.01[a]	0.45 ± 0.02[b]	0.45 ± 0.01[b]	0.45 ± 0.01[b]
Alkaline phosphatase (AKP, U/mgprot)	0.60 ± 0.02[a]	0.69 ± 0.03[b]	0.71 ± 0.02[b]	0.71 ± 0.03[b]	0.69 ± 0.02[b]
Lipase (LPS, U/mgprot)	0.07 ± 0.01[a]	0.05 ± 0.01[a]	0.12 ± 0.01[b]	0.15 ± 0.01[b]	0.21 ± 0.02[c]
Amylase (AMS, U/mgprot)	0.73 ± 0.01[a]	0.82 ± 0.02[b]	0.90 ± 0.02[c]	0.89 ± 0.03[c]	0.88 ± 0.02[bc]
Trypsin (TRY, U/mgprot)	216.30 ± 15.01[a]	197.43 ± 14.43[a]	361.86 ± 18.47[c]	382.60 ± 13.27[c]	283.48 ± 15.58[b]

Data expressed as mean ± SEM.
Different letters in the same line indicate that the indexes are significant differences ($p < 0.05$).

(Xiao et al., 2017). Opposite results in the present study showed that the activity of plasma ALT and AST, and the content of TB and Glu were significantly increased in Diet 5 compared to other diets. It indicated that a higher dose (2,000 mg/kg) of fucoidan may induce liver injury and metabolic disorders. Moreover, hepatic micromorphology can be used to assess the healthy status of the liver. In our study, histological observation of the liver showed that the diet with 500–1,500 mg/kg fucoidan could ameliorate the morphology of hepatic tissue to a certain extent, such as reducing vacuolation, inflammatory response, and improving blood circulation in the liver. These findings clearly show that fucoidan may protect the liver, whose mechanism may be associated with the suppression of hepatocyte apoptosis, intrahepatic inflammation, and antioxidant activity (Ahmad et al., 2022; Xue et al., 2022a), whereas a high dosage of fucoidan, particularly at 2,000 mg/kg, led to the increase of plasma ALT and AST levels, as well as significant pathological changes in liver tissue. Thus, it indicated that the addition of low dosage of fucoidan could ameliorate the hepatic morphology of juvenile common carp, whereas the high dosage probably caused further damage to the liver.

The effect of fucoidan on digestive enzyme activity

ACP was one of the signature enzymes of lysosomes, and its activity could represent the intensity of intracellular digestion in the intestine (Maritza et al., 2012; Zacarias et al., 2013). AKP could directly participate in the transfer of phosphate groups and play a key regulatory role in metabolism. Furthermore, AKP was also an important immunoreactive enzyme, and its activity was often used as a key indicator to diagnose foreign pathogens and environmental pollution (Jean, 2020; Singh and Lin, 2021). In the present study, the activity of ACP, AKP LPS, AMS, and TRY in the intestine was notably enhanced in groups feeding fucoidan, which indicated that fucoidan could effectively improve the digestive ability of the intestine of juvenile common carp. Similar results were observed in *C. auratus* feeding fucoidan (Cui et al., 2020). Of course, the intestinal digestive function was closely related to intestinal microbial composition (Wu et al., 2019; Amenyogbe et al., 2022; Chang et al., 2023). Thus, future studies are needed to explore the effect of fucoidan on intestinal microorganisms of common carp.



Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the Committee of Laboratory Animal Experimentation at Southwest University.

Author contributions

FL and HS conceived the ideas and designed the methodology. YL, DH, and CR caught and dissected fish individuals. FL and CZ collected data. FL and GL analyzed data. FL and HS led the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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