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Research Article

Protective Effects of Sanyar Prebiotic on Immunity and Tissue Changes of Common Carp (*Cyprinus carpio*) Exposed to $CuSO_4$ Stress

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Prebiotics, as feed additives, can activate the antioxidant and immune systems of fish and significantly improve the survival of aquaculture animals in stressful conditions. The exposure of fish to copper sulfate leads to oxidative stress, suppression of the immune system, and destruction of vital body tissues. This study aimed to investigate the protective effects of Sanyar prebiotic on the immunity and tissue changes of common carp (*Cyprinus carpio*) exposed to $CuSO_4$ stress. Two hundred common carp fish with an average weight of 12.33 ± 0.93 g were stocked in four treatments (triplicates) for 60 days. The experimental treatments contained Sanyar commercial prebiotics in 100 g of food, which included *P*1 (1 ml prebiotic liquid), *P*2 (0.1 g prebiotic powder), *P*3 (0.5 ml liquid and 0.05 g powder), and treatment C (control treatment without any additives). At the end of the experimental period, the fish were exposed to acute toxicity of Cu (0.1 m/l) for 96 hours. Gill, kidney, and liver tissue samples were collected and evaluated after 96 hours. According to the results obtained, there were significant differences in specific growth rate (SGR) and final weight ($p < 0.05$). The lowest feed conversion ratio (FCR) was observed in the Sanyar treatments (1.56 ± 0.37), which had a significant difference from the control treatment (1.85 ± 0.35) ($p < 0.05$). The results showed that immunity indices such as lysozyme, cortisol, and ACH50 increased in the experimental treatments compared to the control treatment ($p < 0.05$). The highest and lowest lysozyme activities were observed in *P*2 (16.35 ± 0.57) and control treatments (13.15 ± 1.00), respectively ($p < 0.05$). Generally, no significant difference was observed between growth and nutrition indices in Sanyar treatments ($p < 0.05$). Symptoms of kidney, liver, and gill tissue damage were reported from mild (hyperemia and infiltration of inflammatory cells) to severe (hemorrhage and necrosis). The lowest severity of tissue damage was observed in the treatments fed with Sanyar food supplement. In general, the results of this study showed that the addition of Sanyar prebiotic to the diet of common carp improves the growth indicators and immune response and can also be beneficial in increasing fish resistance to copper toxicity.

1. Introduction

Common carp (Cyprinus carpio) is one of the most important fish cultivated in the world today and belongs to the carp family (Cyprinidae) [1]. This fish has an important role in increasing the rate of aquaculture production worldwide as one of the most valuable cultivated species [2, 3]. Nowadays, strengthening the immune system of aquatic animals against environmental stress is one of the important goals of the aquatic industry, and the use of immune stimulants in the food diet leads to the growth and strengthening of the health of aquatic animals [4]. It has been proven that the use of new food supplements such as prebiotics, probiotics, and synbiotics is very effective on growth, food efficiency, and production of digestive enzymes [5, 6].

A prebiotic refers to a food component that cannot be digested and exerts a positive influence on the host by specifically promoting the proliferation and/or functionality of a particular bacterium or a restricted group of bacteria within the colon [7]. It has been reported that prebiotics as functional compounds in the aquaculture industry improve growth, feed efficiency, intestinal microbiota, digestive enzyme activity, intestinal morphology, immune status, disease resistance, intermediary metabolism, and stress responses [8].

Sanyar prebiotic is derived from *Saccharomyces cerevisiae* yeast. Sanyar contains cell wall carbohydrates mannan-oligosaccharide and beta-glucan, which mannan-oligosaccharide as a prebiotic and beta-glucan as a strong antioxidant are effective in strengthening the immune system of aquatic animals. On the other hand, Sanyar is a very nutritious substance that contains essential amino acids for growth, B vitamins, and growth stimulants including peptides and nucleotides and is also rich in minerals, especially chromium, phosphorus, and selenium. The types of prebiotics used in the aquaculture industry include beta-glucans, mannan-oligosaccharides, lactose, fructo-oligosaccharides, and inulin, whose effects have been proven in aquatic animals [9–11]. However, there is not enough scientific information about the effect of using Sanyar resources on aquatic animals such as common carp. Especially, the protective effects of Sanyar prebiotic against metals stress in fish have not been investigated.

Copper is an essential trace element that plays a crucial role in the proper development and metabolic processes of living organisms. Nevertheless, exceeding the prescribed limit of copper can pose significant risks as it can form persistent metallic compounds that have the potential to accumulate in water and disrupt the delicate balance of the biological system [12]. The rise in environmental pollution can also have adverse effects on aquaculture operations. The toxicity of copper has been observed to induce harmful effects on fish species [13]. This is because fish are one of the aquatic organisms that are able to accumulate heavy metals in their tissue [12]. Based on the importance of the type and quality of food on aquatic production and the role of fish immunity, the possibility of increasing the resistance of fish exposed to copper sulfate was investigated after feeding with Sanyar prebiotic diet. Various studies have been carried out related to the resistance of fish to food supplements including various types of prebiotics [14–18]. Several studies have been conducted on the role of dietary supplements in enhancing resistance against various stresses, such as toxins and diseases [19, 20]. Farhangi et al. [17] investigated the protective and histopathological effects of garlic extract (*Allium sativum*) on rainbow trout (*Oncorhynchus mykiss*) exposed to acute copper toxicity. Komar [21] conducted a study to examine the beneficial and protective effects of a prebiotic, microbial levan, on common carp (*Cyprinus carpio*) fry during experimental exposure to fipronil. Mokhbatly et al. [22] investigated the protective effects of *Spirulina platensis* and β-glucan (BG) supplementation in African catfish (*Clarias gariepinus*) exposed to chlorpyrifos. Hajirezaee et al. [23] reported the protective effects of vitamin C in common carp (*Cyprinus carpio*) exposed to titanium nanoparticles. However, this study represents the first investigation into the effects of Sanyar prebiotic on aquatic animals. Additionally, this study aimed to determine the effectiveness of the Sanyar prebiotic on the growth and resistance of common carp exposed to copper.

2. Materials and Methods

2.1. Experiment Design. Two hundred common carp juveniles (12.33 ± 0.93 g) were purchased from the local farm in Golestan province (Voshmgir Dam) and transported to the fisheries laboratory in Gonbad Kavous University. The fish were kept in on 1500-L tank for two weeks to acclimatize with the conditions laboratory [24]. Then, fish with similar weights were selected and distributed in 12 tanks (35-L volume) at a density of 15 fish per tank (4 treatments with 3 replications). The tanks were continuously aerated, and 30% of water was exchanged daily to maintain water quality [25]. The fish were reared for 60 days under a daily feeding rate of 3% of the biomass, divided into three times (7:00, 12:00, and 17:00). The biomass in the tank was recorded biweekly to adjust the feed amounts. Unconsumed feed and fish feces were siphoned daily.

Sanyar prebiotic product (liquid and powder) was purchased from Sepehr Javan Kavoshgar Company (registered number 37481, Dezful, Iran) (Table 1) for the resistance of fish against stress caused by copper sulfate. Sanyar prebiotic in powder and liquid form was used in 100 g of carp fish diet (SFC code from Faradane company which contained 38% protein, 4% lipid, 3% fiber, 7% ash, 5% moisture, and 1% phosphorus) based on the dose suggested by the manufacturer. Generally, four treatments were used in this experiment, which include *P*1 (1 ml prebiotic liquid), *P*2 (0.1 g prebiotic powder), *P*3 (0.5 ml liquid and 0.05 g powder), and treatment C (control treatment without no additives).

2.2. Growth Indices. Bioassay of fish was carried out in 3-time intervals (initial, middle, and final) after anesthesia with $200\,\mathrm{mg{\cdot}l^{-1}}$ clove powder [26]. Half an hour after feeding, the uneaten feed was siphoned, then dried (60°C for 24 h), and weighed for evaluation of feed efficiency. The weight of the fish was measured using a digital scale with an accuracy of 0.01 g in each tank. Parameters including weight gain rate (WGR, %), specific growth rate (SGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), protein efficiency ratio (PER), and survival percentage (SP) were measured to investigate the performance of experimental diets containing Ajwain supplements on fish growth based on the following formulas [27]:

Table 1: Analysis of Sanyar prebiotic compounds.

Test type	Results
Dry matter (%)	95
Total protein (%)	50
Total carbohydrate (%)	40
Mannan-oligosaccharide (%)	10
Beta-glucan (%)	15
Nucleotide (%)	5
Vitamin B1 (mg/100 g)	12
Vitamin B2 (mg/100 g)	20
Vitamin B3 (mg/100 g)	75
Vitamin B5 (mg/100 g)	60
Escherichia coli (CFU/g)	Negative
Salmonella (in 25 g of sample)	Negative

$$\begin{aligned}
\mathrm{WGR}(\%) &= 100 \times \frac{(\text{final weight}-\text{initial weight})}{\text{initial weight}},\\
\mathrm{SGR}\left(\frac{\%}{\text{day}}\right) &= \frac{(\mathrm{Ln\ WF} - \mathrm{Ln\ WI})}{\mathrm{t}} \times \frac{100}{\text{days}},\\
\mathrm{FCR} &= \frac{F}{(\mathrm{Wf\text{-}Wi})}, \text{ where F and } (\mathrm{Wf\text{-}Wi}) \text{ are feed consumption and body weight gain},\\
\mathrm{FCE}\% &= \left[\frac{g\,(\text{live weight gain})}{g\,(\text{dry feed eaten})}\right] \times 100,\\
\mathrm{PER} &= \frac{\text{weight gain}\,(g)}{\text{protein intake}\,(g)},\\
\mathrm{SP} &= \left(\frac{\text{final number of fish}}{\text{initial number of fish}}\right) \times 100.
\end{aligned} \tag{1}$$

2.3. Pathological Studies under Stress Conditions with Copper Sulfate. The fish were exposed to a concentration of 0.1 mg of copper sulfate for 96 h at the end of the experiment period (60 days period) to determine the level of resistance [28]. The studied concentration is obtained based on the minimum effective concentration (LOEC = lowest observed effect concentration) equal to LC10, 96 h of copper sulfate, and it is a concentration without any losses [29]. After the toxicity test and examining the lesions caused by copper toxicity on fish tissues, 3 fish samples were randomly caught from each treatment and placed in a 10% formalin solution. Then, the samples were transferred to the pathobiology laboratory, and tissue sections were prepared. Histological and lesion studies were performed using identification keys and light microscopy [30]. After determining the volume of water per volumetric unit, copper sulfate was weighed and added to the water to determine the concentration of the poison. The behavior of fish exposed to copper poison was recorded during the experimental stages. The experiment conditions including temperature and pH were constant for all the samples during the test period. Also, feeding was stopped at this stage.

2.4. Mucus Immunity Assay. After 96 h of stress with copper sulfate, the immunity indices of carp fish fed with Sanyar prebiotic were investigated by direct mucus collection based on the method of Guardiola et al. [31]. Seven fish were randomly caught from each replication, and mucus removal was carried out by massaging the surface of the fish in less than 2 minutes. Briefly, skin mucus was obtained by gently rubbing the dorsolateral surface of fish in a front-to-caudal direction, transferred to sterile tubes, and centrifuged for 10 minutes at 3000 rpm at a temperature of 4°C by a centrifuge machine [32]. Then, the samples were stored in 2 ml microtubes and kept at −80°C for the mucus immunity test. Mucus protease activity was evaluated based on the azocasein hydrolysis method described by Palaksha et al. [33]. Lysozyme enzyme was measured by turbidity method using a spectrophotometer [34]. Protease enzyme was measured using the method of Walter [35]. Complement 50 (ACH50) was measured using the method of Oriol Sunyer and Trot [36] based on the analysis of rabbit red blood cells, and alkaline phosphatase enzyme activity, glucose, and total protein were measured by a commercial kit of Pars Azmoun using a spectrophotometer [37]. Total immunoglobulin (IgM) was measured by the method of Siwicki and Anderson [38]. The cortisol level of the skin mucus was measured by the competitive ELISA method (ELISA) using a commercial kit (IBL Co., Gesellschaft für Immunchemie und Immunbiologie).

2.5. Data Analysis. The normality of the data was determined by the Shapiro–Wilk test. Significant differences between treatments were considered by one-way analysis of variance (one-way ANOVA). Duncan test was used at a significant level of 95% ($p < 0.05$) to compare means (mean ± SD). Statistical analyses were performed by SPSS 21 and Excel 2019 software.

3. Result

3.1. Effects of Sanyar Prebiotic on Growth and Nutrition Factors. The growth and nutrition factors of common carp, fed with different levels of Sanyar prebiotic, are presented in Table 2. The initial weight in all treatments was about 12.33 ± 0.93 g, and no significant difference was observed between the treatments ($p > 0.05$). At the conclusion of the 60-day rearing period, the treatments supplemented with various levels of Sanyar prebiotic demonstrated significantly higher final weight, weight gain, and weight gain percentage compared to the control group without prebiotic treatment. The specific growth rate (SGR) in the treatment groups that received prebiotics exhibited a significant increase compared to the control group (1.00 ± 0.17) ($p < 0.05$). Feed conversion ratio (FCR) was the lowest among the treatments fed with Sanyar compared to the control treatment, so the lowest and highest values were observed in the *P*2 treatments (1.49 ± 0.31) and the control group (1.85 ± 0.35), respectively. The feed conversion ratio (FCR) decreased in the treatments fed with Sanyar, feed conversion efficiency (FCE), had a significant increase ($p < 0.05$). Protein conversion efficiency (PER) of the treatments fed with different levels of Sanyar prebiotic had a significant increase compared to the control treatment ($p < 0.05$).

3.2. Protective Effects of Prebiotics on Immunity and Mucus Stress Indices. The results of the protective effects of Sanyar prebiotic on the mucus immunity of common carp exposed to copper sulfate stress are shown in Table 3. Lysozyme activity showed a significant difference between the experimental and control treatments ($p < 0.05$), with the highest value observed in the *P*2 treatment (16.35 ± 0.57 U/ml/min) and the lowest in the control treatment (13.15 ± 1.00 U/ml/min). A significant difference in the concentration of ACH50 was found between the different Sanyar prebiotic treatments and the control treatment without additives ($p < 0.05$), with the lowest concentration observed in the control treatment (132.58 ± 3.12). Mucus protein levels also exhibited a significant difference between the experimental and control treatments, with the highest amount observed in the *P*2 treatment. Mucus protease levels were significantly different in fish fed with Sanyar prebiotics and exposed to copper sulfate stress, with the highest observed value. The highest was observed in the *P*2 treatment and the lowest in the control treatment ($p < 0.05$) (Table 3).

The results of the protective effects of Sanyar prebiotic on mucus stress indices in common carp exposed to copper sulfate stress are shown in Figure 1. Cortisol concentration in the control and *P*3 treatments had a significant increase compared to the *P*1 and *P*2 treatments ($p < 0.05$). Glucose levels of fish mucus in the control and *P*3 treatments were significantly increased compared to other treatments ($p < 0.05$). Alkaline phosphatase had a statistically significant increase in the control treatment compared to other experimental treatments (Figure 1).

3.3. The Results of Histological Studies. After exposing the fish fed with Sanyar prebiotic to copper sulfate for 96 hours, the highest amount of lesions was observed in the gill tissue and the least amount of lesions was observed in the liver and kidney tissues. However, the severity of lesions observed in the treatments fed with Sanyar was higher than in the control treatment. Complications such as hyperemia and bleeding in the gills and kidneys were more obvious (Table 4). The main lesions of the gill tissue included hyperemia of the secondary lamellae, edema, detachment of the covering epithelium of the secondary lamellae, hyperplasia at the base of the secondary lamellae, and necrosis of the covering cells (Figure 2). Mild levels of renal duct destruction, hyperemia, bleeding, and the accumulation of melanoma macrophages were observed in the kidney tissue (Figure 3). However, the liver tissue showed minimal damage, indicating that the severity of the lesions was considerably lower in the treatments where Sanyar was administered. Notably, liver lesions such as hyperemia and the accumulation of melanoma macrophages were only observed in the experimental group (Figure 4). It is worth mentioning that no deaths occurred throughout the duration of the experiment (Figure 4). No casualties occurred during the experiment.

4. Discussion

Resilience and strengthening of aquatic animals against environmental stress is one of the important objectives of the aquaculture industry, which will be implemented by adding immune stimulants to the diet. This study was conducted based on the positive results obtained from various probiotic and prebiotic food supplements in the past and investigating the effects of a new prebiotic compound (Sanyar). Based on this, Jafarian et al. [39] reported that adding 0.1 g of Sanyar prebiotic powder to the diet of common carp causes a significant increase in the final biomass weight, final density, and protein yield ratio and a decrease in the feed conversion ratio. In this study, Sanyar prebiotic caused a significant increase in growth and nutrition factors ($p < 0.05$), so the changes in weight gain in the treatments fed with the ration containing Sanyar were more than the control treatment. However, no significant difference was observed in the treatments containing Sanyar powder and liquid ($p > 0.05$). Although

Table 2: Mean ± SD of growth parameters of common carp fed with diets containing Sanyar after 60 days.

Growth parameters	Treatments			
	*P*0	*P*1	*P*2	*P*3
Initial weight (g)	12.62 ± 1.02	12.43 ± 0.99	12.32 ± 0.86	11.97 ± 0.88
Final weight (g)	20.69 ± 1.71^{b}	22.32 ± 2.13^{a}	22.51 ± 2.09^{a}	21.94 ± 1.84^{a}
Body weight (%)	64.51 ± 14.07^{b}	80.40 ± 19.82^{a}	83.39 ± 19.51^{a}	83.98 ± 18.05^{a}
Feed intake (g)	14.44 ± 0.23	14.78 ± 0.34	14.63 ± 0.12	14.78 ± 0.22
SGR (%/day)	0.82 ± 0.14^{b}	0.97 ± 0.18^{a}	1.00 ± 0.17^{a}	1.00 ± 0.16^{a}
FCR	1.85 ± 0.35^{a}	1.56 ± 0.37^{b}	1.49 ± 0.31^{b}	1.53 ± 0.28^{b}
FCE	55.93 ± 10.94^{b}	66.98 ± 14.87^{a}	69.64 ± 14.75^{a}	67.44 ± 12.36^{a}
PER	1.47 ± 0.28^{b}	1.76 ± 0.39^{a}	1.83 ± 0.38^{a}	1.77 ± 0.32^{a}
SP	100	100	100	100

The lowercase letters indicate significant differences between treatments ($P < 0.05$).

Table 3: Mean ± SD of skin mucus immune parameters of common carp fed with diets containing Sanyar after 60 days.

Skin mucus immune parameters	Treatments			
	*P*0 (control)	*P*1	*P*2	*P*3
Lysozyme (U/ml//min)	13.15 ± 1.00^{c}	15.51 ± 0.31^{ab}	16.35 ± 0.57^{a}	14.49 ± 0.25^{b}
ACH50 (%)	132.58 ± 3.12^{b}	138.47 ± 2.40^{a}	142.13 ± 0.95^{a}	139.40 ± 2.09^{a}
TP (g/dl)	56.28 ± 0.86^{d}	64.54 ± 1.24^{b}	67.75 ± 1.56^{a}	60.32 ± 1/47^{c}
Protease (U/mg)	21.52 ± 1.34^{c}	25.06 ± 1.05^{b}	28.87 ± 0.59^{a}	23.29 ± 0/65^{bc}

The lowercase letters indicate significant differences between treatments ($P < 0.05$).

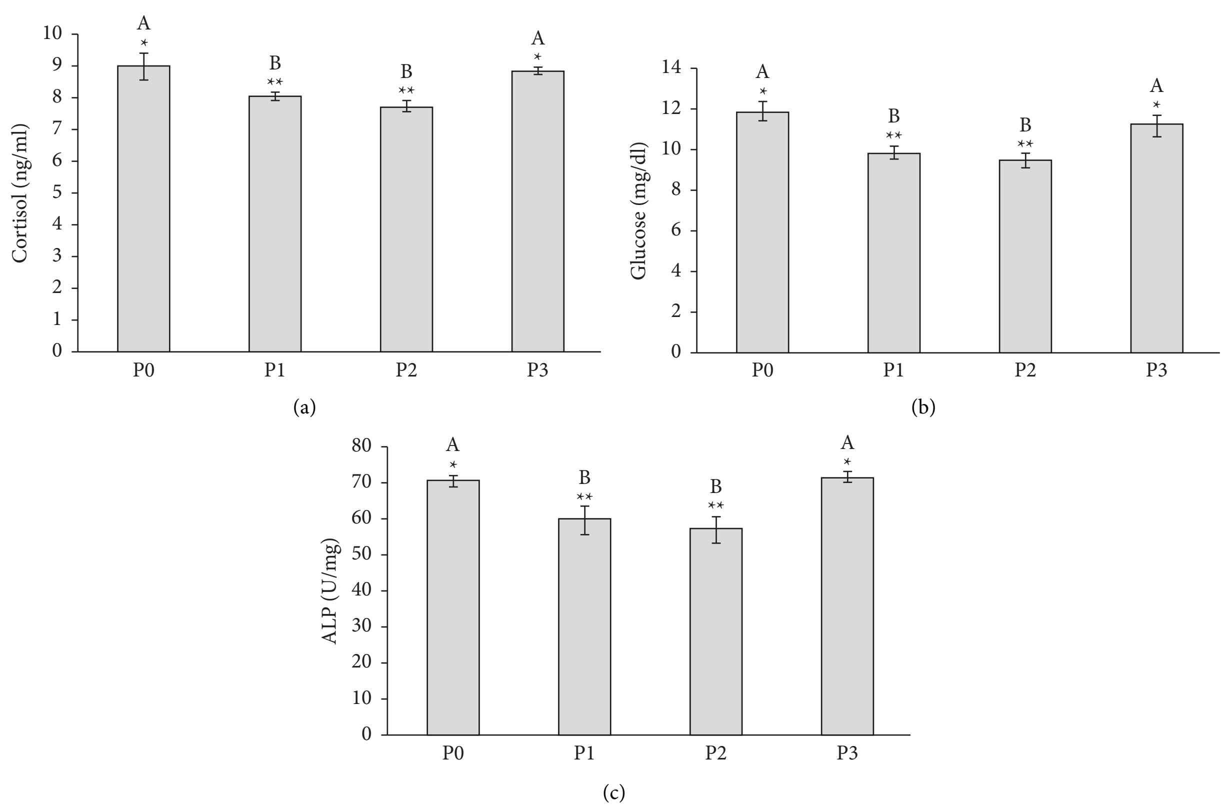

Figure 1: Mean ± SD of stress parameters of common carp fed with diets containing Sanyar after 60 days. Diverse letters show significant differences ($p < 0.05$) in different groups in the Duncan test. Significant differences ($p < 0.05$) between the means obtained are marked by asterisks.

Sanyar prebiotic had a good effect on the growth and nutrition of fish, no difference was observed in the type of combination with food or in addition to water.

Prebiotics are beneficial food compounds that selectively stimulate the growth and activity of a number of bacteria in the digestive system of the host, which are known as growth

Table 4: The common lesions of the fish exposed to the concentration of copper after 96 h.

A: gill

Hyperplasia	Epithelial cells necrosis of gill	Expansion of secondary lamella	Hyperemia	Hemorrhage	Edema	Concentrations (mg/l)
+++	++	++	++	++	++	*P*0
+++	++	++	++	++	++	*P*1
++	++	++	++	++	++	*P*2
++	++	++	++	++	++	*P*3

B: kidney

Inflammatory cells infiltration	Hyperemia	Hemorrhage	Necrosis of tubule	Concentrations (mg/l)
++	+	+	+	*P*0
++	+	−	+	*P*1
+	−	−	−	*P*2
+	−	−	−	*P*3

C: liver

Inflammatory cells infiltration	Hyperemia	Hemorrhage	Necrosis	Concentrations (mg/l)
++	+	−	−	*P*0
++	+	−	−	*P*1
+	−	−	−	*P*2
+	−	−	−	*P*3

(−): No lesions; (+): lesions <20%; (++): lesions> 20–60%; (+++): lesions> 60%.

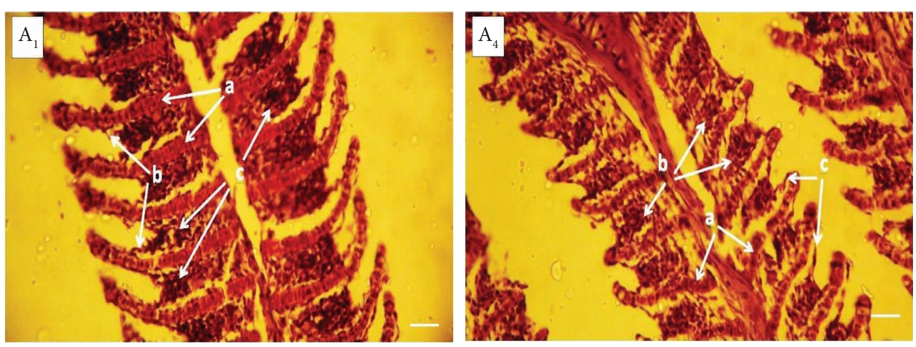

Figure 2: Tissue section prepared from fish gill exposed to copper sulfate (H&E, bar = 25 μm, ×400). A1: control treatment fed with basal diet and arrowheads: hyperemia of secondary lamellae (a), edema and detachment of covering epithelium in secondary lamellae (b), and basal hyperplasia in secondary lamellae (c). A4: *P*3 treatment fed with Sanyar supplement and arrowheads: hyperemia of secondary lamellae (a), edema and detachment of covering epithelium in secondary lamellae (b), and basal hyperplasia in secondary lamellae (c).

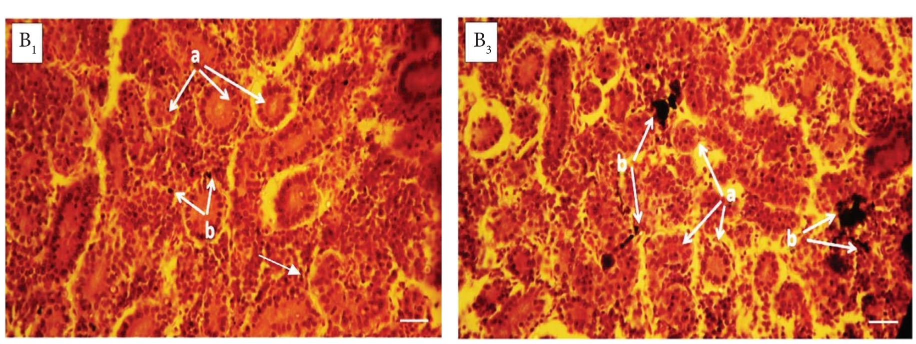

Figure 3: Tissue section prepared from fish kidney exposed to copper sulfate (H&E, bar = 40 μm, ×400). B1: control treatment fed basal diet and arrowheads: normal renal tubules (a) and accumulation of melanoma macrophages (b). B3: *P*2 treatment fed with Sanyar supplement and arrowheads: normal renal tubules (a), accumulation of melanoma macrophages (b), and destruction of renal tubules (c).

stimulants and immune stimulants in aquatic animals [40]. Types of prebiotics used in the aquaculture industry include beta-glucans, mannan-oligosaccharides, lactose, fructo-oligosaccharides, and inulin, which have been proven effective in aquatic animals. Numerous studies have been published on this topic, indicating that the inclusion of

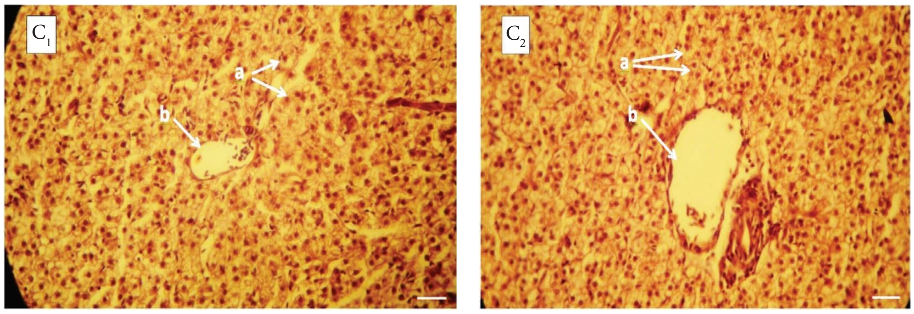

Figure 4: Tissue section prepared from fish liver exposed to copper sulfate (H&E, bar = 40 μm, ×400). C1: control group fed basal diet and arrowheads: normal hepatocytes (a) and central vein (b). C2: *P*2 treatment fed with Sanyar supplement and arrowheads: normal hepatocytes (a) and central vein (b).

Immunogen® as a dietary supplement, specifically at a level of 1.5%, can have a beneficial impact on growth parameters and immune responses in shabout (*Tor grypus*) fish [41]. The combined effects of thyme essential oil (TEO) and prebiotic (Immunogen®) on growth performance and innate immunity of rainbow salmon, *Oncorhynchus mykiss*, showed an increase in the performance and well-being of rainbow salmon in the treatment 2% TEO + 0.2% Immunogen [42]. Prebiotic compounds improve intestinal microbial flora, facilitate digestion and absorption, and increase the value of food through the production of enzymes and specific metabolic substances [43, 44]. In this research, the Sanyar prebiotic facilitates the absorption of nutrients and weight growth in fishes through an indirect effect on the microbial flora of the digestive system. According to the study of Laice et al. [6], adding 0.2 mg/l of synbiotic to water in the biofloc system (*Bacillus toyi*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, mannan-oligosaccharides, lysine, methionine, choline, vitamin C, vitamin E, and dextrose) caused an increase in the final weight, final length, weight gain, and specific growth rate in biofloc + synbiotic treatment compared to the control treatment. In the past years, functional additives such as various prebiotics have been introduced as good candidates to reduce the health effects and disease resistance caused by this replacement by strengthening the fish immune system [45].

The results of stress and immunity indices of carp fish exposed to copper sulfate indicate an increase in fish resistance due to prebiotic function (Table 3). Cortisol and glucose levels increased in the mucus of the control treatment at the end of the fish feeding period and exposure to copper sulfate, while the cortisol level in fish fed with Sanyar prebiotic decreased and had a significant difference with the control treatment. Exposure of fish to copper sulfate as a toxic substance and nervous system stimulant causes stress in fish. The results showed that there was less stress in the treatments fed with a ration containing Sanyar, and a significant difference was observed compared to the control treatment. Also, this result shows the effect of the Sanyar prebiotic in reducing stress and increasing the level of immunity in common carp fish. Several studies have shown that the increase of toxic elements in water causes direct effects on fish organs, severe reduction of oxygen due to stress, and increased oxygen consumption by fish [46]. The level of alkaline phosphatase as a stress factor in the treatment containing Sanyar prebiotic showed a significant decrease compared to the control treatment, and the lowest level was observed in the *P*2 treatment. These differences show the effect of these food supplements on reducing the stress level in fish. Alkaline phosphatase activity (ALP) is a polyfunctional enzyme involved in membrane transport activities [47, 48]. The increase in the level of these enzymes is probably related to cytolysis and the leakage of enzymes in the immune system, which indicates tissue damage in organs such as the liver and kidney [49]. In our study, the use of the Sanyar prebiotic increased the activity of lysozyme and complement 50 (ACH50) in common carp. However, the highest amount was obtained in the *P*2 treatment (0.1 g per kg of food). The results of this study are consistent with the findings of Zarei Nozari et al. [50] and Razavi Amri et al. [51]. In this regard, immune factors were significantly increased in treatments containing Sanyar commercial prebiotics (0.2 g powder and 1 ml liquid) [39]. The functional results of improving the immune response of common carp in Matala Har are consistent with the findings of Hosseinifar et al. [11] and Abasubong et al. [52]. In research, food supplements with 0.5% of galactomannan and oligosaccharide (GMOS diet) or 0.02% of a combination of essential oils (PHYTO diet) increased fish serum lysozyme after *Vibrio anguillarum* infection, so the combined use of both additives can be useful to combat infection in European sea bass [45]. The increase in the activity of lysozyme enzyme and serum complement protein can indicate the strengthening of the fish immune system, which is probably due to the effectiveness of the active ingredients in Sanyar. The serum complement protein is a strong nonspecific defense mechanism to protect fish against a variety of invasive organisms. Immune stimuli increase the power of xenophagy and increase the production of lysozyme [53]. Numerous studies have highlighted the enhanced resistance of aquatic animals when fed with prebiotics, exhibiting increased resilience to various biotic and abiotic stresses. In the context of immunological indicators, a study on rainbow trout (*Oncorhynchus mykiss*) infected with *Aeromonas hydrophila* demonstrated the protective effects of a prebiotic on these indicators. Specifically, the challenge group exhibited higher levels of serum cortisol and glucose. However, fish that were under challenge and fed a diet supplemented with Immunogen showed lower levels of cortisol and glucose compared to the challenge group [54].

The study on gilthead sea bream (*Sparus aurata*) investigated the effects of mannan-oligosaccharide and synbiotic supplementation on growth performance and immune response both before and after thermal stress at 34°C. Following thermal stress, the experimental diets led to a significant increase in monocyte counts, lysozyme activity, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the fish. Notably, the fish fed with a biomin-supplemented diet exhibited the lowest concentration of cortisol. It is important to note that "synbiotic" refers to a combination of prebiotics and probiotics working together synergistically to promote beneficial effects on the host health [55].

Histopathology is a suitable method to observe the toxic effects of chemicals in the aquatic environment. This method provides valuable insights into the extent of tissue damage resulting from the intensity and duration of exposure to toxic substances. Additionally, it helps assess the tissue capacity for adaptation and tolerance when exposed to such harmful agents [56]. Chemical pollutants stimulate various organs such as gills, kidneys, and liver, and these tissues are suitable evidence to investigate the effects of external pollutants. The gill tissue has less protection than other tissues and is directly adjacent to water. Therefore, this organ is more sensitive and can be the first defense barrier of the fish body against chemical pollutants. In this study, the destruction of gill tissue of fish exposed to copper sulfate stress included lesions such as hyperemia, bleeding, hyperplasia of the apex in the gill filaments, edema, and expansion of secondary lamellae in all treatments. However, these lesions were different and less in fish fed with Sanyar. Major lesions in liver and kidney tissues included cellular inflammation and hyperemia. Several studies have also reported the effects of pollutants and environmental stress on gill tissue [57, 58]. However, the investigated damages (hyperplasia, hypertrophy, hyperemia, and edema) were more in the control group than in the Sanyar-treated fish, and fewer damages were observed in the Sanyar-treated fish. This finding indicates the protective effect of Sanyar on fish organs exposed to copper toxicity. Similar to our results, several other studies evaluated the positive performance of prebiotics in exposure to toxins. Mokhbatly et al. [22] investigated the protective effects of *Spirulina platensis* and β-glucan (BG) supplementation in African catfish (*Clarias gariepinus*) exposed to chlorpyrifos. The obtained results showed that the fish exposed to the poison had lower growth and nutritional factors than the control treatment. Also, the blood factors of the fish fed with the used supplements had a better condition. The study conducted by Gupta [59] demonstrates the ameliorative and protective effects of microbial levan, a prebiotic, on common carp (*Cyprinus carpio*) fry exposed to fipronil experimentally. The findings indicate that the inclusion of microbial levan at a concentration of 0.75% effectively mitigates the stress induced by fipronil, owing to its potent nutraceutical properties. One of the methods of removing heavy metals from the environment is the use of microorganisms [60]; therefore, strengthening bacteria with nutrients such as prebiotics can help to increase the population of beneficial bacteria in the digestive system of fish. According to Zoghi et al. (2014), many microbes have the ability to remove heavy metals, and some of them are lactic acid bacteria (LAB). Lactic acid produced by these bacteria could suppress the growth of pathogenic microbes by making the environment more acidic. Sanyar commercial prebiotic plays a vital role in creating an optimal environment that promotes the growth of beneficial bacteria while suppressing the proliferation of pathogenic bacteria. This unique mechanism strengthens the fish's immune system, empowering it to effectively combat and mitigate the adverse effects of copper sulfate contamination. By enhancing the fish's natural defense mechanisms, Sanyar prebiotic contributes to a healthier and more resilient aquatic ecosystem.

5. Conclusion

The results of this study showed that the use of Sanyar prebiotic can improve the feed and growth of fish, reduce stress, and increase immunity in fish exposed to environmental factors. Based on the results, 1 mg/kg Sanyar prebiotic is recommended for common carp feed supplementation.

Data Availability

The data presented in this study are available from the corresponding author upon responsible request.

Disclosure

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

Conflicts of Interest

The authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript: Gonbad Kavous University.

Authors' Contributions

RR collected field data and carried out experiments. MF designed the study and analyzed the data and histopathology. HA, HJ, and MA collected field data, gave input, and proofread the final manuscript. All authors have participated in conception and design, or analysis and interpretation of the data; drafting the article or revising it critically for important intellectual content; and approval of the final version.

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