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Research Article

Effects of Dietary Supplementation of PrimaLac, Inulin, and Biomin Imbo on Growth Performance, Antioxidant, and Innate Immune Responses of Common Carp (*Cyprinus carpio*)

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Prebiotics, probiotics, and synbiotics have been successfully used in dietary supplements to achieve aquatic animal health and therefore increase the quality and sustainability of fish production. In the present study, four hundred and twenty common carps, *Cyprinus carpio* (25.37 ± 0.22 g; mean ± SE) were randomly attributed to seven treatments, fed with diets containing PrimaLac (probiotic), inulin (prebiotic), and Biomin Imbo (synbiotic), 1 and 2 g/kg for each supplement. After 60 days of feeding, an increase in final weight (FW), weight gain (WG), specific growth rate (SGR), and survival rate (SR) was recorded in the treatment groups compared to that of the control ($P < 0.05$). The food conversion ratio (FCR) in the treatment groups significantly decreased ($P < 0.05$). The treated groups showed significant improvements in serum immune parameters: lysozyme (LYZ), alternative complement (ACH50), total immunoglobulin (total Ig), and myeloperoxidase (MPO) ($P < 0.05$). Feeding fish with supplemented diets significantly showed enhanced antioxidant status: catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). Malondialdehyde (MDA) activity was significantly lower in fish fed dietary additives ($P < 0.05$). Compared with the control group, enzyme parameters revealed that supplementation could significantly decrease alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) ($P < 0.05$). The biochemical parameters including triglyceride (TRIG), cholesterol (CHO), glucose (GLU), and cortisol (CORT) decreased with dietary supplementation ($P < 0.05$). Total protein (TP) increased in fish fed experimental diets ($P < 0.05$). Fish fed pro, pre, and synbiotic exhibited significantly higher digestive enzymes (amylase, lipase, and protease) ($P < 0.05$). Skin mucus parameters (total Ig, ACH50, LYZ, protease, and ALP) were significantly enhanced in groups fed dietary additives ($P < 0.05$). Thus, the best recommended inclusion level of supplementation is 2 g/kg for inulin and 2 g/kg for PrimaLac and doses of 1 or 2 g/kg for Biomin Imbo. Dietary PrimaLac, inulin, and Biomin Imbo could be recommended as beneficial feed additives to enhance growth performance, innate immune and antioxidant systems, and promoted biochemical parameters and digestion of common carp; also, the effect of dietary synbiotic was superior to that of prebiotic and probiotic.

1. Introduction

The aquaculture industry has become one of the most important food-producing sectors and has had a rapid development rate in recent years due to the growing population (FAO, 2020). Increased infectious disease, especially under intensive culture systems, has resulted in the transfer of pathogenic organisms among countries and is responsible for considerable economic losses in aquaculture [1, 2]. Prohibitions of the utilization of antibiotics as feed additives in some countries have encouraged a variety of beneficial dietary supplements like probiotics, prebiotics, and synbiotics [3, 4].

Probiotics are living microorganisms administered via feed or directly to the rearing water [5]. Probiotics enhance gut microflora and enzymatic contributions to better digestion and absorption of food [6, 7]. Moreover, they elevate antibodies and phagocytic activity against pathogenic microorganisms [8, 9]. A variety of Gram-positive and Gram-negative bacteria have been used as probiotics [10]. Lactobacillus, Streptococcus, and Bifidobacterium are the most popular probiotic strains. PrimaLac is a multistrain commercial probiotic comprised of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium*, and *Bifidobabacterium thermophiles* [11]. Previous studies revealed that PrimaLac supplementation could promote immunity while improving health in chickens and fish [12, 13].

In an attempt to improve the immune system of animals, prebiotics, nondigestible food additives, are used as energy sources for endogenous bacterial species, which produce extracellular digestive enzymes in the colon [14, 15]. Prebiotics improve hematological parameters, immune-related gene expression, and stress resistance and increase fermentation products [15, 16]. Therefore, they promote the suppression of infectious diseases and host growth and well-being [17, 18]. Inulin belongs to a class of carbohydrates that contain fructose polymers, terminal glucose sucrose, and small oligosaccharides [19, 20]. Its derivatives are found in a wide range of plants species. Inulin promotes bifidobacterium and lactobacillus, which improve health, immune responses, and survival rates of many fish species [19, 21, 22].

Synbiotics are a mixture of prebiotics and probiotics in a form of synergism [23]. Synbiotics can have greater consequences compared to the activity of the prebiotic or probiotic alone [24]. Synbiotics can enhance assimilation and utilization of nutrients and enzymatic activities as well as hematological and biochemical parameters [25, 26]. Therefore, they can protect against infectious pathogens [27]. Biomin Imbo is comprised of *Entercoccus faecium* IMB52 (DSM530) as a probiotic and fructooligosaccharide (FOS) as a prebiotic. Studies revealed that Biomin Imbo improves fish growth performance and survival rate by enhancing the activities of digestive enzymes, antioxidant status, and immunity [28–32].

The innate immune system comprised of numerous distinct and interdependent immune components demonstrates the influence of nutritional treatments on the immunological status of fish [33–35]. Supplements can elevate nonspecific immune mechanisms and enhance performance and disease resistance in fish [36]. Also, antioxidants regulate levels of free radicals, prevent cellular damage, and are considered as a health indicator [37]. Little information is available on the comparative effect of probiotics, prebiotics, and synbiotics on the hematological and antioxidant status of common carp (*Cyprinus Carpio*). Common carp is a key aquaculture species cultured in many countries [21]. The aim of this study was to evaluate the effects of PrimaLac, inulin, and Biomin Imbo on the growth, blood biochemical parameters, and humoral and mucosal immune responses in common carp.

2. Material and Methods

2.1. Experimental Animals and Conditions. Four hundred twenty common carp weighing 25.37 ± 0.22 g (mean ± SE) were purchased from a local farm in Karaj, Iran. Fish were acclimated to experimental conditions and fed a commercial diet for two weeks prior to feeding trials. Each treatment included three indoor cylindrical polyethylene tanks with a freshwater volume of 300 L. Water was filtered through the sea star aquarium purification filter (HX-1180F2, China). The experiment started with seven experimental groups, each treatment repeated in triplicates, with a density of 20 fish per tank. All tanks were aerated and mixed continuously using air stones. During the entire experiment, the water temperature was maintained at 22.8 ± 1.2°C, dissolved oxygen at 6.75 ± 0.5 mg L^{-1}, and pH at 7.5 ± 0.45. Daily water exchange was approximately 50%. The light regime was set at 12 h light: 12 h darkness. Fish were fed the experimental diet for 60 days.

2.2. Diet Preparation and Feeding Trial. Basal diet formulation is shown in Table 1, which is used as a control. Seven treatments in triplicates containing as CON (0, control); PLL (1 g/kg PrimaLac), PLH (2 g/kg PrimaLac), INL (1 g/kg inulin), INH (2 g/kg inulin), BIL (1 g/kg Biomin Imbo), and BIH (2 g/kg Biomin Imbo) were prepared. Such concentrations were chosen based on previous studies using these dietary supplementations for other fish species [29, 30, 38, 39]. To prepare each experimental diet, the proper amount of supplementations was dissolved in distilled water and sprayed on diets. All experimental diets were coated with fish oil. Pellets were air-dried at room temperature for 24 h. Then, the diet was stored at -20°C until used. Fish were fed at 3% of body weight twice daily.

2.3. Sample Collection. Blood was sampled using a sterile syringe from the caudal vessels of 24 h starved anesthetized fish (100 mg/l eugenol, CMV [40]). The serum samples were centrifuged at 1600 × g for 10 minutes. The supernatant was collected in a fresh sterile tube and was stored at -20°C to determine biochemical, immune, and antioxidant status. Portions of the entire intestinal tissues were homogenized in sterile saline solution (0.85% NaCl) and centrifuged at 3000 g under 4°C for 10 minutes. Supernatants were collected and stored at -80°C for further analyses [41]. Skin mucus samples were obtained based on Subramanian et al. Fish were placed into polyethylene bags containing 5 mL of

Table 1: Analysis of the commercial feed for *C. carpio* (Faradaneh Co. Shahrekord, Iran).

Analyses	composition
Protein	37%
Lipid	6%
Fiber	6%
Digestible phosphorus	1.25%
Moisture	7%

50 mM NaCl (Merck, Germany). The bags were gently shaken by hand for approximately 1–2 min. Mucus was collected and transferred to 10 mL sterile tubes and centrifuged at 1500 × g (4°C) for 10 minutes; then, samples were stored at -80°C [42].

2.4. Growth Performance. Growth performance, feed utilization, and survival parameters were calculated using the following equations:

$$\begin{aligned}
&\text{Weight gain (WG; g)} = \text{final bodyweight} - \text{initial bodyweight},\\
&\text{Specific Growth Rate (SGR; \%day}^{-1}\text{)}\\
&\quad = [(\ln\ (\text{final bodyweight}) - \ln\ (\text{initial bodyweight}))/\text{trial period}] \times 100,\\
&\text{Feed conversion rate (FCR)} = \text{feed intake (g)/weight gain (g)},\\
&\text{Survival rate (SR; \%)} = (\text{final number of fish/initial number of fish}) \times 100.
\end{aligned} \tag{1}$$

2.5. Immune Parameters. The method described by Ellis [43] was used for the determination of serum lysozyme (LYZ) activity. Briefly, *Micrococcus luteus* was added to hen egg-white lysozyme (Sigma, USA). A reduction in absorbance of 0.001/min at 450 nm was regarded as one unit of lysozyme activity. The alternative complement pathway activity (ACH50) was measured using the method described by [44] based on sheep red blood cell hemolysis. The serum total immunoglobulin (total Ig) activity was analyzed using the microprotein method. The immunoglobulin molecules were precipitated down by a 12% solution of polyethylene glycol. Total Ig levels were presented after subtracting protein content before and after precipitation [44]. Myeloperoxidase (MPO) activity was determined by Quade and Roth [45]. The blood smears were diluted with Hank's balanced salt solution (Ca^{2+}, Mg^{2+}free). Mixtures of 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide were then added. After incubation for two minutes, sulphuric acid was added. Lastly, absorbance was read via a spectrophotometer at 450 nm via a UV-VIS spectrophotometer (Thermo Spectronic, UK). Protease activity in mucus was determined according to Ross et al. [46].

2.6. Antioxidant Status. Superoxide dismutase (SOD) was determined based on converting the superoxide anion to the hydrogen peroxide method [47]. Malondialdehyde (MDA) levels were determined based on thiobarbituric acid reaction at 95°C (Buege, 1978). The glutathione peroxidase (GPx) activity was measured by the oxidation of NADPH to $NADP^+$ with an ELISA reader [48]. Catalase (CAT) was determined by decreased H_2O_2 absorbance [49]. All parameters were measured by Zellbio commercial kits (Zellbio, Veltinerweg, Germany).

2.7. Enzymatic and Soluble Protein Assay. Alanine transaminase (ALT), alkaline phosphatase (ALP), and aspartate transaminase (AST) were determined by commercial kits (Pars Azmun, Co., Tehran, Iran) [35]. The total protein content was measured as described by Bradford [50].

2.8. Biochemical Analysis. Cortisol (CRT) levels were assayed by a commercial kit (Pars Azmun Co., Tehran, Iran) using the manufacturer's instructions based on the radioimmunoassay method [51]. Glucose (GLU) concentrations were measured according to the colorimetric glucose oxidase method [52]. Globulin (GLO) activity measurement was based on subtraction of the albumin value from the total protein value of the same sample. Moreover, albumin (ALB) was measured following the method of Wotton and Freeman [53]. Cholesterol (CHOL) and triglyceride (TRIG) were determined by the enzymatic colorimetric test described by Abell et al. [54] and Cole [55].

2.9. Digestive Enzyme Analysis. Protease activity was measured based on azocasein hydrolysis by the Ross et al. [46] method, by absorbing at 450 nm. The assay of amylase activity was determined according to the substrate 4.6-ethylidene-(G7)-ρ-nitrophenyl-(G1)-α-D-maltoheptaoside to reduce oligosaccharide at 405 nm [56]. Lipase activity was assayed using fatty acid release and triglyceride hydrolysis in an emulsion of olive oil at 580 nm [57].

2.10. Statistical Analysis. The Kolmogorov-Smirnov test was used to check the normality and homogeneity of variance. Data analyses were conducted using SPSS software version 20.00 (SPSS Inc., Chicago, IL, USA) and are represented as the mean ± standard error (SE). Statistical significances were processed via one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests ($P < 0.05$).

3. Results

3.1. Growth Performance. The overall results for fish growth performance are shown in Table 2. In the beginning, no significant difference was observed in the IW between groups. The FW and WG, at the end of the experiment, were positively affected by the experimental treatments ($P < 0.05$). Determination of SGR showed a significant increase in the experimental treatments over the control treatment ($P < 0.05$). However, no statistical variations were recorded in SGR in fish fed supplemented diets ($P < 0.05$). The fish fed supplemented diets had lower FCR values compared to those fed the control diet. Also, SR significantly increased in PLH, INH, BIL, and BIH compared to the control group ($P < 0.05$).

Table 2: Growth parameters of common carp fed different experimental diets.

Parameters	CON	PLL	PLH	INL	INH	BIL	BIH
IW (g)	26.00 ± 0.60	25.51 ± 0.59	25.39 ± 0.84	25.53 ± 0.86	24.58 ± 0.68	24.97 ± 0.46	25.60 ± 0.35
FW (g)	44.83 ± 0.72^{a}	53.68 ± 0.72^{bcd}	55.34 ± 0.79^{cd}	52.00 ± 0.47^{b}	52.83 ± 0.21^{bc}	55.55 ± 0.69^{cd}	56.01 ± 0.58^{d}
WG (g)	18.83 ± 0.12^{a}	28.17 ± 0.67^{bc}	29.95 ± 0.19^{bc}	26.47 ± 1.22^{b}	28.25 ± 0.88^{bc}	30.57 ± 1.04^{c}	30.40 ± 0.22^{c}
SGR (% d^{-1})	0.90 ± 0.01^{a}	1.24 ± 0.03^{b}	1.30 ± 0.03^{b}	1.18 ± 0.06^{b}	1.27 ± 0.05^{b}	1.33 ± 0.046^{b}	1.30 ± 0.005^{b}
FCR	3.22 ± 0.05^{a}	2.16 ± 0.06^{bc}	2.02 ± 0.01^{bc}	2.30 ± 0.10^{b}	2.14 ± 0.06^{bc}	1.98 ± 0.06^{c}	1.99 ± 0.01^{c}
SR (%)	93.33 ± 2.88^{a}	98.33 ± 2.88^{ab}	100.00 ± 0.00^{b}	98.33 ± 1.66^{ab}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}

Data are expressed as the mean ± SE ($n = 3$). Different letters (a–c) in the same row indicate significant differences among the treatments ($P < 0.05$). CON (0, control); PLL (1 g/kg PrimaLac); PLH (2 g/kg PrimaLac); INL (1 g/kg inulin); INH (2 g/kg inulin); BIL (1 g/kg Biomin Imbo); and BIH (2 g/kg Biomin Imbo). IW: initial weight; FW: final weight; WG: weight gain; SGR: specific growth rate; FCR: food conversion ratio; SR: survival rate.

Table 3: Serum immune parameters of common carp fed different experimental diets.

Parameters	CON	PLL	PLH	INL	INH	BIL	BIH
LYZ (U/ml)	09.03 ± 0.19^{a}	12.88 ± 0.23^{b}	16.31 ± 0.29^{cd}	11.23 ± 0.56^{b}	15.22 ± 0.37^{c}	17.59 ± 0.47^{d}	17.75 ± 0.31^{d}
ACH50 (U/ml)	39.98 ± 1.16^{a}	44.35 ± 0.73^{ab}	50.74 ± 1.10^{c}	43.79 ± 1.00^{b}	48.39 ± 1.09^{bc}	50.31 ± 0.68^{c}	50.74 ± 0.69^{c}
Total Ig (mg/ml)	12.14 ± 0.33^{a}	14.87 ± 0.66^{ab}	16.54 ± 0.71^{b}	14.07 ± 0.42^{ab}	15.72 ± 0.36^{b}	20.06 ± 0.76^{c}	20.53 ± 0.83^{c}
MPO (OD at 540 nm)	4.10 ± 0.10^{a}	6.02 ± 0.40^{bc}	6.95 ± 0.07^{cd}	6.68 ± 0.26^{b}	6.74 ± 0.25^{bc}	7.99 ± 0.22^{d}	9.23 ± 0.18^{e}

Data are expressed as the mean ± SE ($n = 6$). Different letters (a–e) in the same row indicate significant differences among the treatments ($P < 0.05$). LYZ: lysozyme; ACH50: alternative complement activity; total Ig: total immunoglobulin; MPO: myeloperoxidase.

3.2. Immune Parameters. The serum immune parameters of common carp fed the dietary supplements are presented in Table 3. Each of the experimental groups displayed significantly higher LYZ compared to that of the control group ($P < 0.05$). Serum ACH50 activity significantly increased in fish fed supplemented diets, except for PLL ($P < 0.05$). PLL ACH50 activity closely resembled that of the control. No significant difference was observed for the total Ig between PLL, INL, and the control group. However, other treatments displayed higher total Ig levels than those of the control ($P < 0.05$). The highest level of total Ig was recorded in the fish fed Biomin Imbo (BIL and BIH) ($P < 0.05$). MPO analyses indicated a significant increase among all treatments compared to the control group ($P < 0.05$). Additionally, BIH had the most MPO levels among the treatments ($P < 0.05$). Mucus ACH50 analyses (Figure 1) indicated a significant increase among all treatments compared to the control group ($P < 0.05$). In addition, the value of LYZ increased in fish groups fed the supplemented diets, except INL, compared to the control group ($P < 0.05$). Mucus protease activities did not influence in PLL and INL groups; however, significant increases were observed in other treatments compared to the control group ($P < 0.05$).

3.3. Antioxidant Status. Results of serum antioxidants are presented in Table 4. Compared to the control group, CAT content was significantly higher in all treated groups ($P < 0.05$). SOD activity in treatment groups was significantly increased over those in the control group, except for INL ($P < 0.05$). Moreover, the highest MDA values were recorded in the control group versus all treatment groups ($P < 0.05$). All experimental groups showed higher GPx levels than the control group ($P < 0.05$), though no significant differences were recorded among them.

3.4. Enzyme Parameters. Results of serum enzyme activities are presented in Table 5. PLH, BIL, and BIH had significantly lower ALT activities than those of the other groups ($P < 0.05$), and the other treatments displayed no significant differences. The AST activity in fish fed supplemented diets was significantly lower than that in the control group, except for INL ($P < 0.05$). Moreover, the ALP activity of the serum in the groups fed with PLH, BIL, and BIH was significantly lower than that in other groups ($P < 0.05$). Mucus ALP activities (Figure 1) had no statistical variations in PLL and INL groups; however, significant increases were observed in other treatments compared to the control group ($P < 0.05$).

3.5. Biochemical Analysis. Biochemical parameters are presented in Table 6. TP content in the fish fed PrimaLac and Biomin Imbo significantly increased compared to the control group ($P < 0.05$), whereas the other treatments displayed no significant differences. Furthermore, there was no significant difference in serum ALB and GLO activities among all treatments. There were no significant differences in serum TRIG levels among INL, PLL, and the control group. Other treatments displayed significantly higher TRIG levels ($P < 0.05$). Except for INL, dietary supplementations significantly increased CHOL in relation to the control treatment ($P < 0.05$); however, there were no significant differences among other treatments. Fish in treatment groups had lower plasma GLU concentrations than the control ($P < 0.05$); although, there were no significant differences among PLL, INL, and the control. In addition, no significant differences were found among the experimental groups regarding plasma CORT; however, the highest value was in the control ($P < 0.05$).

3.6. Digestive Enzyme Analysis. Intestinal enzyme activities of common carp, after 60 days of trial period, are shown in

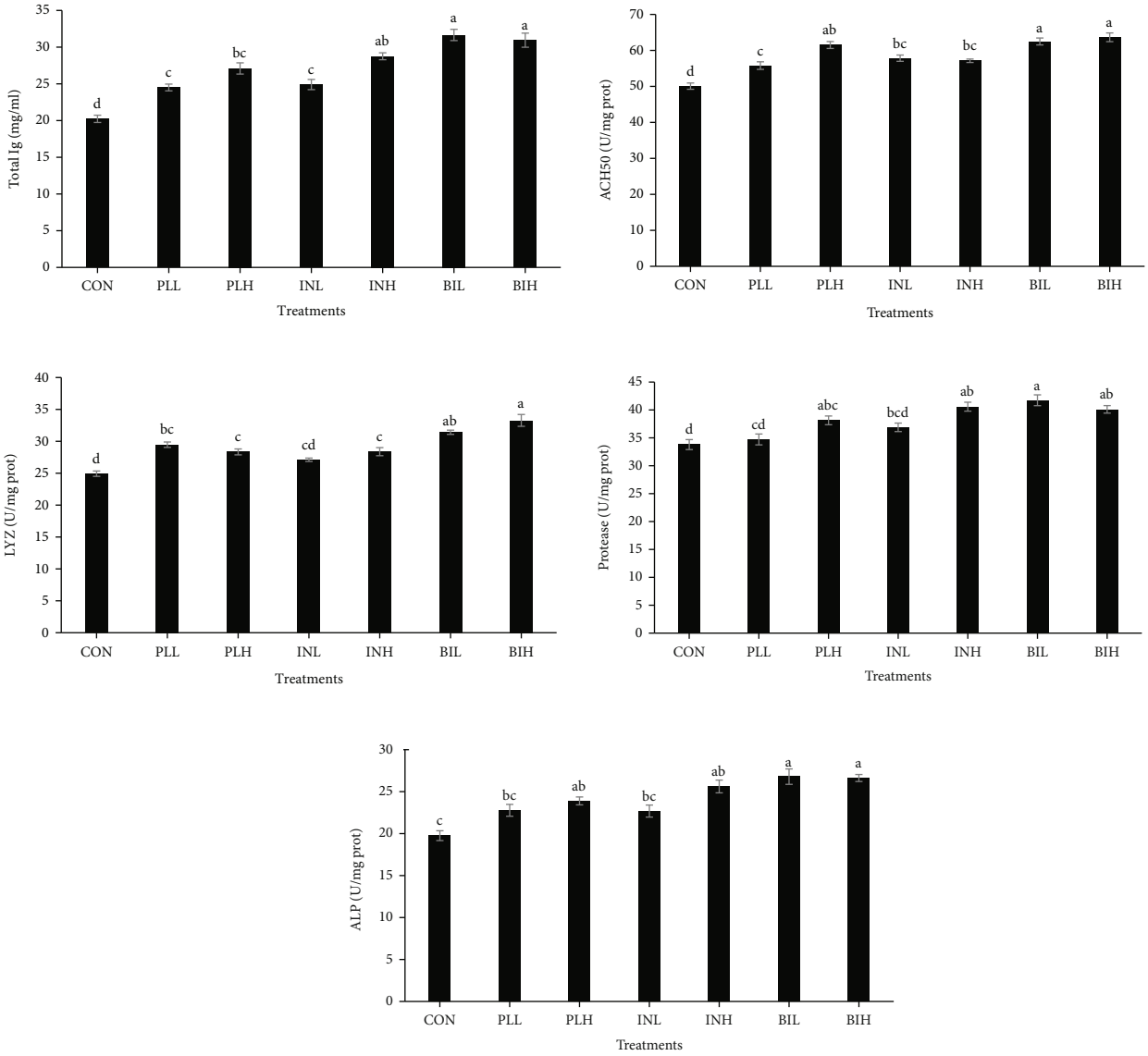

Figure 1: Skin mucus parameters of common carp fed different experimental diets. Values are presented as the mean ± SE ($n = 6$). Different letters (a–d) in each row indicate statistically significant differences ($P < 0.05$). Total Ig: total immunoglobulin; ACH50: alternative complement activity; LYZ: lysozyme; ALP: alkaline phosphatase.

Table 7. The amylase contents of fish fed CON, PLL, and INL diets were significantly lower than those in other groups ($P < 0.05$). All the treated groups showed a significant increase in lipase value compared to the control group ($P < 0.05$), except for INL. Also, significant improvements in protease value were observed in experimental treatments compared to the control group ($P < 0.05$).

4. Discussion

The results revealed that these additives could influence the growth performance, serum immune and biochemical factors, serum and intestinal enzymes, antioxidant capacity, and mucosal immunity, as well as promote significant increases in the innate immune responses of the common carp. The results of the growth indices indicated that FW, WG, and SGR increased in common carp fed dietary supplementations. Fish fed supplemented diets also had lower FCR levels. These results are in agreement with previous studies on beneficial effects of dietary pre-, pro- and synbiotics on growth performance parameters, including, diets supplemented with probiotics for juvenile roach (*Rutilus rutilus)* [58], common carp [59], Nile tilapia (*Oreochromis niloticus)* [60], Asian sea bass, (*Lates calcarifer*) [61], white shrimp [62] synbiotic in Texas cichlid (*Herichthys cyanoguttatus)* [31], and prebiotics in rainbow trout (*Oncorhynchus mykiss)* [22]. Dietary pre- and probiotics and their combination can stimulate fish appetite and intestinal microflora [63]. Increased nutritional efficiency may be associated with improving digestive enzyme activity and degradation of indigestible nutrients, making them available for adsorption [59]. Probiotic supplements not only colonize intestinal epithelial cells but also adhere to the mucosa [9]. A similar finding was obtained by Jin et al. that PrimaLac supplementation

Table 4: Antioxidant parameters of common carp fed different experimental diets.

Parameters	CON	PLL	PLH	INL	INH	BIL	BIH
CAT (U/ml)	15.65 ± 0.30^{a}	20.69 ± 0.75^{b}	20.45 ± 0.94^{b}	21.61 ± 0.76^{b}	23.27 ± 0.70^{bc}	25.20 ± 0.66^{c}	25.30 ± 0.81^{c}
SOD (U/ml)	25.13 ± 0.52^{a}	28.35 ± 0.62^{b}	32.84 ± 0.83^{c}	27.98 ± 0.45^{ab}	31.82 ± 0.47^{c}	33.80 ± 0.70^{c}	32.96 ± 0.74^{c}
MDA (nmol/ml)	2.15 ± 0.08^{c}	1.40 ± 0.04^{ab}	1.28 ± 0.03^{a}	1.57 ± 0.04^{b}	1.29 ± 0.03^{a}	1.24 ± 0.02^{a}	1.23 ± 0.02^{a}
GPx (U/ml)	47.00 ± 1.00^{a}	54.85 ± 0.94^{b}	58.23 ± 0.69^{bc}	56.21 ± 0.56^{bc}	57.81 ± 1.18^{bc}	60.66 ± 1.15^{bc}	59.26 ± 1.26^{bc}

Data are expressed as the mean ± SE ($n = 6$). Different letters (a–c) in the same row indicate significant differences among the treatments ($P < 0.05$). CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde; GPx: glutathione peroxidase.

Table 5: Serum enzyme of common carp fed different experimental diets.

Parameters	CON	PLL	PLH	INL	T4INH	BIL	BIH
ALT (U/ml)	12.21 ± 0.39^{c}	12.66 ± 0.43^{c}	09.03 ± 0.42^{a}	12.48 ± 0.40^{c}	11.22 ± 0.32^{bc}	09.12 ± 0.36^{a}	09.75 ± 0.26^{ab}
AST (U/ml)	14.06 ± 0.29^{c}	11.63 ± 0.26^{b}	11.81 ± 0.24^{b}	13.36 ± 0.24^{c}	10.82 ± 0.17^{ab}	10.20 ± 0.32^{a}	10.01 ± 0.38^{a}
ALP (U/ml)	21.49 ± 0.49^{de}	23.21 ± 0.54^{e}	18.73 ± 0.45^{bc}	21.97 ± 0.71^{de}	20.44 ± 0.44^{cd}	15.78 ± 0.36^{a}	17.67 ± 0.32^{ab}

Data are expressed as the mean ± SE ($n = 6$). Different letters (a–e) in the same row indicate significant differences among the treatments ($P < 0.05$). ALT: alanine aminotransferase; AST: aspartate transaminase; ALP: alkaline phosphatase.

Table 6: Biochemical parameters of common carp fed different experimental diets.

Parameters	CON	PLL	PLH	INL	INH	BIL	BIH
TP (g/dL)	3.27 ± 0.06^{a}	3.79 ± 0.09^{bc}	3.98 ± 0.06^{c}	3.42 ± 0.10^{ab}	3.53 ± 0.16^{abc}	3.73 ± 0.06^{bc}	3.77 ± 0.06^{bc}
ALB (g/dL)	2.34 ± 0.15	2.75 ± 0.39	2.55 ± 0.20	2.37 ± 0.12	2.53 ± 0.17	2.61 ± 0.09	2.76 ± 0.14
GLO (g/dL)	0.93 ± 0.11	1.04 ± 0.39	1.43 ± 0.24	1.04 ± 0.02	1.00 ± 0.01	1.12 ± 0.10	1.01 ± 0.15
TRIG (mg/dL)	167.46 ± 4.36^{b}	153.33 ± 1.93^{ab}	140.80 ± 1.89^{a}	152.99 ± 5.57^{ab}	146.36 ± 4.42^{a}	138.86 ± 2.67^{a}	141.46 ± 2.26^{a}
CHO (mg/dL)	111.02 ± 2.45^{c}	93.61 ± 2.67^{ab}	95.52 ± 2.59^{ab}	102.56 ± 2.32^{bc}	93.82 ± 2.32^{ab}	87.91 ± 2.12^{a}	89.50 ± 1.74^{a}
GLU (mg/dL)	90.75 ± 2.21^{c}	83.61 ± 1.67^{bc}	78.49 ± 1.70^{ab}	83.88 ± 1.77^{bc}	79.73 ± 2.34^{ab}	74.19 ± 1.45^{a}	73.08 ± 1.44^{a}
COR (nmol/L)	62.41 ± 1.43^{c}	52.62 ± 0.97^{b}	50.87 ± 0.79^{ab}	52.60 ± 1.64^{ab}	49.73 ± 1.07^{ab}	46.84 ± 1.17^{a}	48.20 ± 1.06^{ab}

Data are expressed as the mean ± SE ($n = 6$). Different letters (a–c) in the same row indicate significant differences among the treatments ($P < 0.05$). TP: total protein; ALB: albumin; GLO: globulin; TRIG: triglyceride; CHO: cholesterol; GLU: glucose; COR: cortisol.

Table 7: Digestive parameters of common carp fed different experimental diets.

Parameters	CON	PLL	PLH	INL	INH	BIL	BIH
Amylase (U/mg prot)	7.94 ± 0.36^{a}	8.19 ± 0.29^{a}	11.17 ± 0.35^{bc}	7.65 ± 0.35^{a}	9.84 ± 0.30^{b}	11.05 ± 0.37^{bc}	11.67 ± 0.30^{c}
Lipase (U/ mg prot)	3.58 ± 0.15^{a}	4.53 ± 0.21^{bc}	6.50 ± 0.20^{e}	4.42 ± 0.15^{ab}	5.40 ± 0.21^{cd}	6.32 ± 0.22^{de}	6.04 ± 0.15^{de}
Protease (U/ mg prot)	15.77 ± 0.23^{a}	19.11 ± 0.26^{bc}	21.21 ± 0.65^{cd}	18.22 ± 0.33^{b}	20.40 ± 0.49^{bc}	23.37 ± 0.31^{d}	23.07 ± 0.74^{d}

Data are expressed as the mean ± SE ($n = 6$). Different letters (a–d) in the same row indicate significant differences among the treatments ($P < 0.05$).

in poultry diets stabilizes the microflora environment of the avian digestive tract [64]. Moreover, prebiotics improves the intestinal mucosal barrier, enhances the growth of commensal microbiota, and protects against pathogenic microorganisms in the colon epithelium [15, 17]. Inulin is a nondigestible fiber, encouraging lactic acid bacteria, and increasing fermentation products [4, 20]. Likewise, the administration of synbiotics can prevent gastrointestinal disorders, providing additional energy and nutrients to the host. Previous studies revealed that synbiotics can elevate gut epithelial barrier function, villus height, and short-chain fatty acids production, thus utilizing all available nutrients and overall metabolic processes [27, 60]. It appears that the effects of these immunostimulants on growth performance indices could vary and might depend on fish species, the type of pre-and probiotics, and the fermentation process [4, 65].

Different kinds of pre-, pro, and synbiotics have been supplemented in diets for many cultured fish species and have been reported to affect immune parameters [66]. In the present study, dietary supplementation significantly increased the serum and mucus LYZ levels. LYZ is a lysosomal enzyme with bactericidal activity, which prevents pathogen invasion within and outside hemocytes [17, 67]. This improvement may be related to an increase in WBC number expressed in neutrophils, monocytes, and the small number of macrophages [68, 69]. Studies revealed that inulin adheres to lectin-like receptors on leukocytes and

macrophages and then stimulates immune system function [70, 71]. In this regard, previous studies demonstrated increased serum LYZ in white shrimp after feeding with *Bacillus subtilis* [72] and PrimaLac ([13]),rainbow trout fed with inulin ([22]), and red tilapia (*Oreochromis niloticus*) fed with synbiotic Jerusalem artichoke (*Helianthus tuberosus*) and *Lactobacillus rhamnosus* [60]. Additionally, synbiotic *Pediococcus acidilactici* and fructooligosaccharide significantly increased the mucus LYZ level in angelfish (*Pterophyllum scalare*) [73]. Mousavi et al. [74] reported that common carp fed 1% inulin powder had a significantly higher LYZ than the control.

ACH50 is a liver globulin released in response to phagocytic activity and inflammation in conjunction with LYZ [75]. The present results showed that common carp fed with PrimaLac, inulin, and Biomin Imbo had significantly greater serum and mucus ACH50 levels. This enhancement might be due to the effect of supplements on liver function as an ACH50 primary source, also, to an increasing trend of serum LYZ [76, 77]. Different works have reported ACH50 enhancement in Indian major carp (*Labeo rohita*) fed with pre-, pro, and synbiotic [78], in European seabass (*Dicentrarchus labrax*) fed Pdp11 probiotic [10], and Nile tilapia fed *Lactobacillus paracasei* [79].

Ig is a glycoprotein considered an important serum and mucus protein [80]. It is an indicator of innate immunity, producing specific antibody responses [81]. The results here revealed that both serum and mucus total Ig increased in fish fed high levels of Primalac and inulin, as well as fish fed Biomin Imbo diets. In line with other investigations, dietary galactooligosaccharide, *Pediococcus acidilactici* [82], and *Lactobacillus fermentum, ferulic acidin* [83] increased serum and mucus total Ig levels in common carp. Following these results, angelfish fed with a synbiotic enhanced diet had a greater mucus total Ig level [73]. Ig elevation might be due to B lymphocyte elevation and the total protein in treated fish [84].

MPO is a heme-containing enzyme mostly released in fish by neutrophils and utilizing oxidative radicals [85]. MPO is an important enzyme that produces hypochlorous acid to eliminate ingested microbes [86]. All supplemented diets increased serum MPO levels. In agreement with this finding, dietary administration of prebiotic microbial levan increased MPO levels in common carp [87]. MPO activity was also significantly increased in serum and skin of Nile tilapia fed *Bacillus subtilis* and *Bacillus licheniformis* [88].

Fish have a complex system of antioxidant enzymes, reducing oxidative stress through lipid peroxidation and catalyzing the dismutation of superoxide radicals to hydrogen peroxide (H_2O_2) and oxygen (O_2) [89]. Antioxidant capacity following pre-, pro-, and synbiotic administration protects fish from overinflammation, apoptosis, and maintenance of energy homeostasis [90]. CAT and GPx scavenge hydrogen peroxide and water before hydroxyl radicals are produced [91]. MDA level is a suitable indicator of lipid peroxidation [92]. SOD also involves tissue injury following oxidative processes and phagocytosis [93]. Our findings revealed that supplementation elevated the main antioxidant enzymes such as CAT, SOD, and GPx and also decreased MDA. Studies have revealed that pro- and prebiotics can produce antioxidant enzymes and eliminate reactive oxygen species (ROS). Dawood et al. [94] demonstrated that probiotics had protective effects against oxidative stress by increasing SOD and GPx activities in Nile tilapia. Our result is similar to those of Syed et al. who reported that 15-20 g/kg of dietary inulin significantly increased SOD compared to other treatments in Asian seabass (*Lates calcarifer*) [93]. Similar antioxidant potentials were reported in giant prawn (*Macrobrachium rosenbergii*) treated with a probiotic microencapsulated with polysaccharide [95]. Additionally, narrow clawed crayfish (*Astacus leptodactylus*) fed prebiotic galactooligosaccharide exhibited higher LYZ, CAT, and SOD [66]. Devi et al. reported that synbiotic supplementation could significantly elevate the antioxidant capacity in earlier life stages against pathogens [78].

ALT, AST, and ALP are present in hepatocytes, raised in cases of acute liver injury and hepatotoxicity following disease, pollution, and toxicant exposure. ALP catalyzes the hydrolysis of phosphomonoesters at alkaline pH [96]. Mucus ALP has an antimicrobial activity for aquatic pathogens [97]. Despite that, AST is a protein metabolism enzyme in the transamination of amino acids [98]. The results of enzyme parameters in this study indicated that a higher dose of PrimaLac and both doses of Biomin Imbo modulated serum enzyme levels and prevented liver damage in common carp. Additionally, fish fed higher doses of probiotic prebiotic, and both doses of synbiotic showed a significant increase in skin mucus ALP. These results could be correlated to those obtained in white shrimp treated with probiotic *Bacillus* OJ and isomaltooligosaccharides [99]. In addition, Yarahmadi et al. [76] determined that lower levels of ALT and AST in treated and challenged rainbow trout indicated that prebiotic supplementation had a protective effect against *A. hydrophila.* Moreover, Caspian white fish (*Rutilus frisii kutum*) fed with PrimaLac supplemented diet exhibited mucus ALP enhancement [11].

TP level (e.g., proteases, lysozymes, lectins, and globulins) is considered a tool to analyze nonspecific humoral immunity against pathogens, and overall health, and is an indicator of liver function [78, 81, 100]. The present results suggested that PrimaLac and Biomin Imbo supplementation could elevate the serum TP level, which promoted the health and immune status of the fish [86]. However, no significant effect was observed in fish fed prebiotic inulin, and there were no significant differences in serum ALB and GLO among the treated fish. The increase in TP level may be attributed to an increase in serum proteins like agglutinins, lectins, LYZ, and immunoglobulins [101]. Mehrabi et al. [102] reported that a synbiotic Biomin Imbo supplemented diet significantly increased TP and ALB activity but this additive did not affect GLO level. In another study, Grey mullet (*Mugil cephalus*) fed a prebiotic supplemented diet displayed no changes in TP, ALB, and GLO levels [103].

Biochemical analysis revealed that high doses of PrimaLac and inulin, as well as both Biomin Imbo levels, modulated TRIG activity. This may prevent fatty liver pathological changes by modulating the metabolism of lipids and lipoproteins [104]. Dietary supplementations can also

reduce hepatic lipogenesis by the limitation of lipogenic enzymes [105]. Gupta reported that prebiotic decreases the incorporation of acetate into TP involved lower TRIG accumulation [87]. Following these results, dietary supplementation of probiotic *Geotrichum candidum* has been reported to decrease TRIG level in Indian major carp [106].

CHO is a major structural component of biomembranes, the outer layers of plasma lipoproteins, and is a precursor of steroid hormones [107, 108]. In the present study, feeding common carp with immunostimulants decreased serum CHO. Reduction in CHO level prevents fatty liver and hepatobiliary syndrome [109]. Based on previous research, the addition of *Bacillus cereus* in a Pengze crucian carp (*Carassius auratus*) diet [110] and a synbiotic in a Caspian salmon (*Salmo trutta caspius*) diet [111] decreased CHO level. However, no significant difference was observed in grey mullet (*Mugil cephalus*) fed Immunogen prebiotic [103] and rainbow trout fed dietary inulin [22].

GLU is transported to the liver tissue through the blood and stored as glycogen for an energy source. It can be used to evaluate stress conditions [112]. In this study, GLU levels were significantly lower for fish fed the high dose of pre- and probiotic as well as both doses of synbiotic. Similarly, immunogen supplementation could decrease fish GLU in Siberian sturgeon (*Acipenser baeri*) [100]. Dawood et al. reported that *Aspergillus oryzae* as a probiotic additive reduced GLU levels in Nile tilapia [94]. GLU is regulated by hypoglycemic hormone stimulation like insulin, glucagon, and cortisol during stressful conditions, and it elevates the immune status and resistance to stress [113].

CORT is a suitable indicator of the endocrine response to acute stress. CORT levels in the serum of all fish fed with supplementation diets exhibited declining trends. Pre, pro-, and synbiotic supplements might stimulate the hypothalamic-pituitary-interrenal (HPI) axis that controls the secretion of CORT [114]. Similarly, Agrimos® MOS as a prebiotic supplement reduced serum CORT activity in zebrafish (*Danio rerio*) [114]. Additionally, the lowest concentration of CORT was observed in Indian major carp fed with a Biomin Imbo supplemented diet [115]. These findings are in accordance with the previous study conclusion that 2% inulin administration increased the CORT level of rainbow trout [22].

Digestive enzyme evaluation provides insight into feed utilization and the digestive capacity of fish [116]. Pro, pre, and synbiotic supplements in the present study generally resulted in the elevation of digestive enzymes in common carp, which may explain the better growth and feed utilization [117]. The addition of probiotics in a diet can modify the composition of beneficial bacteria in the gut, which in turn can enhance feed efficiency and digestibility by stimulating the secretion of enzymes [118, 119]. Prebiotics and probiotics have also been shown to improve intestine morphology [120] and allow more nutrients and indigestible components to be degraded [121]. They can become established in the mucus and epithelium of the digestive tract, producing extracellular enzymes such as proteases and lipases, as well as vitamins, fatty acids, and essential amino acids [122, 123]. Prebiotics may increase gut microbiota metabolites and provide better hydrolysis substrates [124] which might improve digestion and absorption in many fish species [125]. Likewise, white shrimp treated with *Bacillus* showed higher protease and amylase in the midgut and intestine [126]. On the other hand, dietary PrimaLac as a probiotic improved digestive enzyme in white shrimp postlarvae [13]. Xylooligosaccharide supplements increase protease and amylase activity in crucian carp (*Carassius auratus gibelio*) [127].

The mucosal barrier is the first line of defense and immunity against microbial infections [33]. In this study, an increase in mucus protease levels in PLH, INH, BIL, and BIH was observed. Mucus protease can kill bacteria by cleaving their proteins and elevating innate immunity [82]. Results also showed that common carp fed with pre-, pro-, and synbiotic supplements also had higher total Ig, ALP, ACH50, and LYZ content in their mucus, as mentioned previously. In line with our results, inulin improved mucus LYZ, ALP, total Ig, and protease levels of rainbow trout [22]. Mirghaed et al. [11] reported that PrimaLac increased skin mucus immune parameters in Caspian white fish. Moreover, single or combined administrations of plant extract and synbiotic significantly increased mucus immune parameters of zebrafish [29]. An increase in skin mucus parameters may be partially due to an increase in mucus cell numbers and their stimulation to release contents as a defense response [128]. Probiotics may also enter the blood and migrate from mucosal sites to the systemic lymphoid tissues [129].

5. Conclusion

The present study explored the effects of PrimaLac, inulin, and Biomin Imbo supplementation on growth performance and selected mucosal and serum immune parameters of common carp. Based on our results, the recommended dose of supplementation is 2 g/kg for inulin and 2 g/kg for PrimaLac and doses of 1 or 2 g/kg for Biomin Imbo. Also, results suggest Biomin Imbo as a synbiotic has the potential to be more effective in immune modulation when compared to inulin and PrimaLac. Therefore, their use as feed supplements could be of interest as feed supplements in the aquaculture industry. However, further research is recommended for disease challenges to confirm the results obtained.

Data Availability

The data that support the findings of this study are available upon reasonable request to the corresponding authors.

Ethical Approval

All experiments were accompanied following the protocol approved by the committee of ethics of the faculty of sciences of the University of Tehran (357; 8 November 2000).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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