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Effects of dietary Immunodate supplementation on growth performance, digestive enzyme activity, immune responses, antioxidant status, and gut microbiota in Asian seabass (*Lates calcarifer*)

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Abstract

Functional feed additives may improve fish health and reduce antibiotic reliance in aquaculture. This study evaluated the effects of graded dietary inclusion levels of an immunostimulant supplement, Immunodate, on growth performance, feed utilization, digestive enzyme activities, hematological parameters, antioxidant status, and gut microbiota in Asian seabass (*Lates calcarifer*). A 60-day feeding trial was conducted using five dietary treatments containing 0% (control), 1%, 3%, 5%, and 7% Immunodate. Dietary supplementation appeared to numerically improve growth efficiency without significant differences among treatments; however, the 1% treatment showed the numerically highest weight gain (87.94 ± 5.60 g) and lowest feed conversion ratio (FCR) (1.349 ± 0.032). The 1% and 3% groups exhibited higher lymphocyte counts and lower heterophil values and lymphocyte/heterophil ratios compared to the control ($p < 0.05$). Antioxidant capacity was also significantly modulated ($p < 0.05$); catalase (CAT) and glutathione peroxidase (GPX) activities peaked in the 1% group, whereas superoxide dismutase (SOD) and total antioxidant capacity (TAC) reached their highest levels in the 7% and 5% groups, respectively. Regarding gut microbiota, Immunodate supplementation significantly influenced microbial populations ($p < 0.05$), with the 1% inclusion level yielding the highest counts mannitol egg yolk polymyxin agar (MYPA) ($2.55 \pm 0.02 \times 10^5$ CFU/g), Man, Rogosa and Sharpe (MRS) ($2.57 \pm 0.02 \times 10^5$ CFU/g), and tryptic soy agar (TSA) ($73.11 \pm 12.16 \times 10^8$ CFU/g). Overall, dietary inclusion of Immunodate at 1% appeared most effective in enhancing growth efficiency, stimulating beneficial gut microbiota, and reinforcing the immune and antioxidant systems of *L. calcarifer*.

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Introduction

Functional feed additives have become increasingly important in modern aquaculture as effective strategies for enhancing growth performance, immune competence, and disease resistance while reducing reliance on antibiotics (Rodrigues *et al.*, 2020; Machuca *et al.*, 2022). Among these additives, β-glucans are widely recognized as potent immunomodulators capable of activating innate immune responses in fish through interactions with pattern recognition receptors such as Dectin-1 (Brown and Gordon, 2003; Adams *et al.*, 2008). Numerous studies have demonstrated the beneficial effects of dietary β-glucans on fish immunity and survival. For example, β-glucan supplementation has been reported to increase antibody titers and improve disease resistance in rainbow trout, carp, and sea bass (Sahoo and Mukherjee, 2001; Siwicki *et al.*, 2004; Kumari and Sahoo, 2006; Bonaldo *et al.*, 2007). In addition, growth-promoting and physiological benefits of β-glucans have been reported in several cultured fish species, including salmonids and freshwater carp (Misra *et al.*, 2006; Dawood *et al.*, 2015). However, despite plenty works carried out on effect of β-glucans on fish immunity, the optimal dosage of this immuno-stimulator in fish is remained to be detected. Asian seabass (*Lates calcarifer*) is one of the most economically important marine fish species in the Indo-Pacific region due to its rapid growth rate, high market demand, and adaptability to diverse culture conditions (Schipp *et al*., 2007; Glencross, 2013). Despite these advantages, the fish is sensitive to environmental and nutritional stressors, which may negatively affect growth performance and health. Therefore, the use of functional feeds and immunostimulator strategies has become increasingly important for improving the sustainability of its culture (Faulk and Holt, 2009). Immunodate is an immunostimulant supplement enriched with β-glucan and other bioactive compounds such as essential amino acids, selenium, and zinc, designed to enhance growth and immunity in fish and shrimp (Rangin Zist Parvar Shahrekord Knowledge based Company). There is no data regarding its effect on growth performance and immuno-physiological variables in this fish species. Therefore, the present study aimed to evaluate the effects of graded dietary inclusion levels of Immunodate on growth performance, feed utilization, and digestive enzyme activities in Asian seabass.

Materials and Methods

Experimental design

Fish

Juvenile Asian seabass (*L. calcarifer*) with initial average weight presented in Table 1 were obtained from Makran Agriculture and Industry Company fish farm and were adapted to new condition for two weeks. Fish were kept in 1000-l fiberglass tanks, with 15 fish randomly stocked in each tank, and were fed commercial feed (Faradaneh Aquafeed Manufacturing Company) at 2.5% body weight.

Table 1. Initial body weight (g) and total length (cm) of fish in each dietary treatment.

Treatment	Number of tank	Average initial weight (g)	Average initial length (cm)
1%	6, 7, 14	35.03 ± 6.03	14.02 ± 1.74
3%	4, 10, 15	37.16 ± 5.17	14.24 ± 1.60
5%	11, 12, 13	34.25 ± 7.30	13.64 ± 2.12
7%	2, 5, 9	37.57 ± 5.78	14.06 ± 1.92

Water quality

Water quality parameters including dissolved oxygen, temperature, salinity, and pH were monitored daily using WTW water quality meters (Germany), and the values were maintained at > 6 mg/l, 31-33ºC, 38-41ppt, and 7.7-8.1, respectively.

Feeding experiments

Body weight and total length of all fish were individually measured. Statistical analysis confirmed that there were no significant differences among treatments at the start of the trial, indicating uniform initial conditions among the experimental groups (Steel *et al.*, 1997). The experiment was conducted using a completely randomized design consisting of five dietary treatments containing 0% (control), 1%, 3%, 5%, and 7% Immunodate, each with three replicates. The feeding trial lasted for 60 days. Fish were hand-fed three times daily (at 8 am, 11 am and 5 pm) at a feeding rate of 2.5% of body biomass, and the feeding ratio was adjusted every 15 days based on the biomass of each tank. A commercial basal diet formulated for Asian seabass, produced by Faradaneh Co. (Iran) (protein 44-46%, lipid 14-16%, carbohydrate, fiber 2-3%, ash 8-12%, moisture 6-8%, and phosphorus 1.2-1.4%) was used as the base feed. The Immunodate supplement, in powder form, was incorporated

into the basal diet at the designated inclusion levels. For this purpose, the supplement was first mixed with a small amount of starch paste and then evenly sprayed onto the feed pellets. The prepared pellets were air-dried at room temperature and stored at 4°C until use. Fish in the control group received the basal diet without Immunodate supplementation. Throughout the experimental period, all tanks were continuously aerated. Tank biomass was measured every 15 days to adjust the feeding rate.

Growth performance

At the end of the experiment, all fish were individually weighed and measured. Growth performance and feed utilization were evaluated by calculating weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), and lipid efficiency ratio (LER) using following equations (NRC, 2011). Initial body weight and total length of fish in each dietary treatment were given in Table 1.

WG = (final body weight (FW) (g) − initial body weight (IW) (g)

SGR = (ln FW − ln (IW) × 100/experimental days

PER = Weight gain (g) / Protein intakt (g)

LER = Weight gain (g) / Lipid intake (g)

FCR = total feed consumed (g)/WG (g)

Digestive enzymes activity

Three fish from each tank (nine fish per treatment) were randomly sampled for digestive enzyme analysis. Intestinal tissues were dissected, homogenized in cold buffer, and centrifuged at 4°C. The supernatant was collected and used for enzymatic assays. The activities of lipase and amylase were determined using commercial colorimetric assay kits according to the manufacturer's instructions. Enzyme activities were determined spectrophotometrically using a UV–visible spectrophotometer and expressed as IU/ml following standard enzymatic procedures (Bergmeyer, 1974).

Hematological indices

Blood samples were collected from caudal vein randomly from three fish in each tank (nine fish per treatment). A portion of each sample was used to prepare blood smears for differential leukocyte counts. The smears were air-dried, fixed with methanol, and stained with Giemsa stain. Differential leukocyte counts were performed under a light microscope to identify and count leukocyte types, including lymphocytes and heterophils. The heterophil-to-lymphocyte (H/L) ratio was calculated as an indicator of physiological stress and immune status following standard hematological procedures (Davis *et al.*, 2008).

Liver antioxidant activity

Liver tissues were collected from three fish randomly from each tank, and samples were immediately excised, rinsed with cold physiological saline, frozen in liquid nitrogen, and stored at −80°C until analysis. Prior to biochemical assays, liver tissues were homogenized in cold phosphate buffer and centrifuged at 4°C, and the resulting supernatant was used for biochemical measurements. The activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), as well as total antioxidant capacity (TAC), were determined using commercial assay kits (KPG company) according to the manufacturer's instructions. Enzyme activities were measured spectrophotometrically using a UV–visible spectrophotometer. Protein concentration in the tissue homogenates was determined using the Bradford method (Bradford, 1976), and antioxidant enzyme activities were expressed relative to protein content.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). The normality of data was also checked. Differences among treatments were analyzed using one-way analysis of variance (ANOVA), and when significant differences were detected, Tukey's HSD test was used to compare treatment means. Statistical analyses were performed using SPSS version 26, and differences were considered statistically significant at $p < 0.05$.

Results

The initial biometric characteristics of the experimental fish were similar among treatments. The mean initial weight ranged from 34.25 to

37.57 g, while the mean initial length varied between 13.64 and 14.24 cm across treatments. Results of growth and nutritional performances were given in Table 2. Dietary Immunodate supplementation did not significantly affect growth performance or feed utilization indices of Asian seabass among the experimental treatments ($p > 0.05$). Weight gain per fish ranged from 74.23 ± 4.10 g in the control group to 87.94 ± 5.60 g in the 1% Immunodate group. Although fish fed the 1% Immunodate diet showed numerically higher weight gain and feed intake, no significant differences were observed among treatments. Also, dietary Immunodate supplementation did not significantly affect the specific growth rate (SGR) of Asian seabass among the experimental groups ($p > 0.05$). The highest numerical SGR was recorded in the 1% treatment (2.09 ± 0.04), while the remaining groups showed comparable values. Similarly, FCR, protein efficiency ratio (PER), and lipid efficiency ratio (LER) were not significantly different among groups ($p > 0.05$).

Table 2. Growth performance and feed utilization indices of Asian seabass fed different levels of Immunodate for 8 weeks. WG = weight gain, SGR= specific growth rate, PER= protein efficiency rate, LER= lipid efficiency rate, FCR= feed conversion rate.

Treatment	WG/fish (g)	SGR	Feed/fish (g)	FCR	PER	LER
Control	74.23 ± 4.10 [a]	1.82 ± 0.10 [a]	113.4 ± 3.78 [a]	1.53 ± 0.08 [a]	1.45 ± 0.07 [a]	4.36 ± 0.22 [a]
1%	87.94 ± 5.60 [a]	2.09 ± 0.04 [a]	118.1 ± 4.73 [a]	1.35 ± 0.03 [a]	1.65 ± 0.04 [a]	4.95 ± 0.12 [a]
3%	78.87 ± 9.02 [a]	1.83 ± 0.17 [a]	115.9 ± 6.09 [a]	1.48 ± 0.11 [a]	1.51 ± 0.12 [a]	4.53 ± 0.33 [a]
5%	74.89 ± 5.47 [a]	1.93 ± 0.12 [a]	109.94 ± 2.29 [a]	1.47 ± 0.08 [a]	1.51 ± 0.09 [a]	4.54 ± 0.25 [a]
7%	75.45 ± 11.37 [a]	1.88 ± 0.16 [a]	113.57 ± 7.91 [a]	1.52 ± 0.12 [a]	1.47 ± 0.13 [a]	4.42 ± 0.37 [a]

Digestive enzymes

The activities of digestive enzymes were not significantly influenced by dietary Immunodate supplementation ($p > 0.05$). Lipase activity ranged from 7.89 ± 1.62 IU/ml in the 1% group to 10.57 ± 2.47 IU/ml in the 7% group. Although the highest lipase activity was observed in fish fed the 7% Immunodate diet, this increase was not statistically significant compared to the control group.

Amylase activity showed a numerical increase in all Immunodate-supplemented groups compared to the control. The highest amylase activity was recorded in the 7% group (8.71 ± 1.87 IU/ml), followed by the 1% and 5% groups. However, no significant differences were detected among treatments ($p > 0.05$) (Table 3).

Table 3. Digestive enzyme activities in intestine tissues of Asian seabass fed different levels of Immunodate.

Treatment	Lipase (IU/ml)	Amylase (IU/ml)
Control	8.71 ± 2.19 [a]	4.14 ± 1.41 [a]
1%	7.89 ± 1.62 [a]	7.00 ± 2.32 [a]
3%	8.64 ± 1.35 [a]	6.90 ± 1.18 [a]
5%	8.56 ± 2.10 [a]	7.00 ± 2.16 [a]
7%	10.57 ± 2.47 [a]	8.71 ± 1.87 [a]

Hematological values

Results of hematological values were presented in Table 4. Lymphocyte value was significantly higher in the 1% and 3% treatments compared to other treatments and the control. In contrast, heterophile percentage showed a significant decrease in the 1% and 3% treatments, while the control and higher inclusion levels (5% and 7%) exhibited comparatively greater values. Accordingly, the H/L ratio was significantly reduced in the 1% and 3% groups showing an improvement in haematological status under these dietary treatments.

Table 4. Haematological parameters of Asian seabass fed different levels of Immunodate.

Treatment	Lymphocyte (%)	Heterophile (%)	H/L ratio
Control	69.50 ± 1.41^{c}	30.00 ± 0.19^{a}	0.45 ± 0.16^{a}
1%	75.75 ± 1.61^{a}	24.00 ± 0.77^{c}	0.33 ± 0.14^{c}
3%	74.88 ± 1.38^{a}	25.00 ± 0.40^{c}	0.34 ± 0.09^{c}
5%	70.50 ± 1.59^{b}	28.63 ± 0.70^{b}	0.42 ± 0.15^{b}
7%	69.88 ± 0.91^{c}	29.50 ± 0.66^{a}	0.43 ± 0.08^{b}

Antioxidant activity

The analysis of hepatic antioxidant indices revealed that dietary Immunodate supplementation exerted a dose-dependent effect on the antioxidant status of the fish (Table 5). A significant improvement in CAT and GPX activities was observed in fish fed with the 1% inclusion level, which marked the peak for these specific parameters. Conversely, the SOD activity demonstrated an upward trend with increasing inclusion levels, reaching its maximum in the 7% group, which was significantly higher than all other treatments. Regarding the total antioxidant capacity (TAC), the 5% supplementation level appeared to be the most effective, as it induced a significant elevation compared to other dietary groups.

Table 5. Antioxidant enzyme activities in liver tissues of Asian seabass fed different levels of Immunodate. CAT= (U/mg protein), GPX= (U/mg protein), SOD= (U/mg protein), TAC= (µM/mg protein).

Treatment	CAT (U/mg protein)	GPX (U/mg protein)	SOD (U/mg protein)	TAC (µM/mg protein)
Control	4.04 ± 1.04^{c}	37.49 ± 4.40^{c}	46.94 ± 3.97^{c}	1.38 ± 0.04^{c}
1%	7.50 ± 1.42^{a}	74.53 ± 3.38^{a}	45.31 ± 4.53^{c}	1.53± 0.07^{b}
3%	5.52 ± 2.32^{b}	49.03 ± 5.99^{b}	45.83 ± 2.13^{c}	1.61± 0.01^{b}
5%	5.35 ± 2.36^{b}	53.23 ± 3.79^{b}	52.90 ± 1.56^{b}	1.86 ± 0.06^{a}
7%	3.64 ± 0.64^{c}	30.17 ± 2.85^{d}	65.92 ± 1.88^{a}	1.40 ± 0.05^{c}

Intestinal microbiota composition based on culture-dependent analysis

Intestinal microbial counts were presented in Table 6. Dietary treatments significantly influenced MYP, MRS, and TSA counts ($p < 0.05$). The highest MYP counts were observed in the 1% treatment ($2.55 \pm 0.02 \times 10^5$ CFU/g), whereas the control group showed the lowest values ($1.21 \pm 0.02 \times 10^5$ CFU/g). MRS counts followed a similar trend, with the 1% ($2.57 \pm 0.02 \times 10^5$ CFU/g) and 3% ($2.19 \pm 0.34 \times 10^5$ CFU/g) treatments exhibiting the highest levels, significantly exceeding the control group ($0.26 \pm 0.01 \times 10^5$ CFU/g). Regarding TSA counts, the 1% group reached the highest level ($73.11 \pm 12.16 \times 10^8$ CFU/g), while the control group recorded the lowest count ($36.33 \pm 4.47 \times 10^8$ CFU/g). Overall, dietary supplementation improved intestinal microbial populations, with the 1% inclusion level providing the most pronounced enhancement across all parameters.

Table 6. Intestinal microbial counts of Asian seabass fed different levels of Immunodate.

Treatment	*Bacillus* spp. (MYP) ($\times 10^5$ CFU/g)	Lactic acid bacteria (MRS) ($\times 10^5$ CFU/g)	Total viable bacteria (TSA) ($\times 10^8$ CFU/g)
Control	1.21 ± 0.02^{c}	0.26 ± 0.01^{c}	36.33 ± 4.47^{a}
1%	2.55 ± 0.02^{a}	2.57 ± 0.02^{a}	73.11 ± 12.16^{c}
3%	2.27 ± 0.33^{ab}	2.19 ± 0.34^{a}	53.11 ± 10.44^{b}
5%	1.89 ± 0.06^{bc}	1.40 ± 0.31^{b}	51.89 ± 12.75^{b}
7%	1.91 ± 0.05^{bc}	1.83 ± 0.07^{ab}	54.67 ± 11.37^{b}

Discussion

In the present research work, dietary supplementation with Immunodate produced numerical improvements in several growth and nutritional indices of Asian seabass, with the inclusion of 1% exhibiting the most favourable response. However, these improvements were not statistically significant among treatments. Similar findings have been reported in previous studies. For instance, Bagni *et al.* (2005) showed that dietary β-glucan supplementation did not significantly affect the performance of European seabass, although it improved fish immune responses. Similarly, Sealey *et al.* (2008) showed no significant effect of different dietary β-glucan concentrations on fish growth. These findings support the view that β-glucan functions primarily as an immunomodulator rather than a direct growth promoter (Ai *et al.*, 2007; Meena *et al.*, 2013). The activity of digestive enzymes is an important indicator of digestive efficiency and nutrient utilization in fish. In our present study, lipase and amylase activities varied slightly among treatments but did not differ significantly. This lack of significant variation may be in part due to the attribution to the adequate nutritional composition of the basal diet and favourable rearing conditions, which likely maintained digestive performance at an optimal concentration regardless of dietary supplementation.

In contrast, Immunodate supplementation significantly influenced the intestinal microbiota of Asian seabass. Fish fed the diet containing 1% Immunodate exhibited significantly higher populations of beneficial bacteria, including lactic acid bacteria and *Bacillus* spp., compared to the control group. This shift toward a more favourable gut microbial community suggests a prebiotic effect of Immunodate. Date seed powder, one of the components of Immunodate, contains bioactive compounds and fermentable substrates that can support the proliferation of beneficial intestinal microorganisms. Similar improvements in intestinal healthy fish have been reported by Dawood *et al.* (2020) in Nile tilapia fed fermented date seed.

Dietary Immunodate also significantly affected heamatological variables, particularly at the 5% and 7% inclusion concentrations. These treatments resulted in a significant increase in lymphocyte percentage and a corresponding reduction in the H/L ratio. Because the H/L ratio is widely recognized as an indicator of physiological stress, its reduction suggests lower stress levels and improved immune status (Davis *et al.*, 2008). An increase in lymphocyte value further exhibited stimulation of the innate immune responses through pattern-recognition receptor pathways (Brown and Gordon, 2003; Adams *et al.*, 2008), a mechanism that has been associated with enhanced resistance to bacterial infections in fish (Siwicki *et al.*, 2004; Bonaldo *et al.*, 2007).

The antioxidant defence system also responded positively to Immunodate supplementation in Asian seabass. Fish receiving Immunodate had significantly higher activities of antioxidant enzymes, including CAT, GPX, SOD, and TAC, compared to the control fish. These findings are consistent with previously reported data demonstrating that β-glucans enhance antioxidant defences by reducing reactive oxygen species production and improving cellular integrity (Dawood *et al.*, 2017; Han *et al.*, 2020; Rodrigues *et al.*, 2020).

The antioxidant response, however, differed among individual enzymes. CAT and GPX activities were not dose-dependent, as their activities declined at higher Immunodate inclusion levels. In contrast, SOD activity continued to increase up to the 7% supplementation level, while TAC reached its highest value at 5% Immunodate. These results exhibite that the antioxidant effect of Immunodate may be in part enzyme-specific, with moderate supplementation levels (1–5%) generally providing the greatest improvement of the overall antioxidant activity.

Overall, these present findings of this study exhibit that although Immunodate supplementation insignificantly improved growth performance or digestive enzyme activity of Asian seabass, it positively influenced gut microbiota, hematological parameters, and antioxidant status in Asian seabass. Inclusion of 1% in the fish diet was the most effective in enhancing beneficial intestinal microbiota, whereas inclusion of higher concentrations (5–7%) induced greater enhancements in hematological and antioxidant responses. These findings suggest that Immunodate can enhance the physiological condition and immune competence of Asian seabass, making it as a promising functional feed additive. Nevertheless, further research work, including pathogen challenge trials, is needed to

confirm its protective efficacy under commercial aquaculture conditions.

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Conflict of Interest

The authors do not have any potential conflict of interest to declare.

References

Adams, A and Thompson, KD (2008). β-glucans as immunostimulants in fish. Aquac. Res., 39: 1041-1050.

Ai, Q; Mai, K; Zhang, L; Tan, B; Zhang, W; Xu, W and Li, H (2007). Effects of dietary β−1,3 glucan on innate immune response of large yellow croaker (*Pseudosciaena crocea*). Fish Shellfish Immunol., 22: 394-402.

Bagni, M; Romano, N; Finoia, MG; Abelli, L; Scapigliati, G; Tiscar, PG; Sarti, M and Marino, G (2005). Short and long term effects of a dietary yeast β−glucan (Macrogard) and alginic acid on immune response in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immunol., 18: 311-325.

Bergmeyer, HU (1974). Methods of enzymatic analysis. 1st Edn., New York, USA, Academic Press. PP: 1-1200.

Bonaldo, A; Thompson, KD; Manfrin, A and Adams, A (2007). The influence of dietary β−glucans on the immune response and disease resistance of sea bass (*Dicentrarchus labrax*). Aquaculture, 273: 110-119.

Bradford, MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem., 72: 248-254.

Brown, GD and Gordon, S (2003). Fungal β−glucans and mammalian immunity. Immunity, 19: 311-315.

Davis, AK; Maney, DL and Maerz, JC (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol., 22: 760-772.

Dawood, MAO; Koshio, S; Ishikawa, M and Yokoyama, S (2015). Interaction effects of dietary supplementation of heat killed *Lactobacillus plantarum* and β−glucan on growth performance, immune response and disease resistance of juvenile red sea bream (*Pagrus major*). Fish Shellfish Immunol., 45: 33-42.

Dawood, MAO; Koshio, S and Esteban, MA (2017). Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac., 10: 950-974.

Dawood, MAO; Koshio, S and Esteban, MA (2018). Dietary supplementation of β−glucan improves growth performance, immune responses, and disease resistance in fish. Aquaculture, 495: 674-681.

El Boshy, ME; El Ashram, AM; Abdelhamid, FM and Gadalla, HA (2010). Immunomodulatory effect of dietary β−glucan on Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol., 29: 241-246.

Hadiuzzaman, M; Rahman, MM and Hossain, MS (2022). Immunostimulatory effects of β−glucan in aquaculture species: a review. Aquac. Rep., 23: 101043.

Han, B; Long, WQ; He, JY; Liu, YJ; Tian, LX and Yang, HJ (2020). Effects of dietary β−glucan on growth performance, antioxidant capacity and immune responses of fish: a review. Fish Physiol. Biochem., 46: 1753-1768.

Hoseinifar, SH; Sun, YZ; Wang, A and Zhou, Z (2016). Probiotics as means of diseases control in aquaculture, a review of current knowledge and future perspectives. Front. Microbiol., 7: 242.

Ji, SC; Takaoka, O; Jeong, GS; Lee, SW; Ishimaru, K; Seoka, M and Takii, K (2019). Dietary medicinal herbs improve growth and intestinal health in fish. Aquac. Nutr., 25: 114-124.

Kumari, J and Sahoo, PK (2006). Dietary β−1,3 glucan influences immune responses and disease resistance in Asian catfish (*Clarias batrachus*). Fish Shellfish Immunol., 20: 305-314.

Lin, S; Pan, Y; Luo, L and Luo, L (2011). Effects of dietary β−glucan on immune response and digestive enzyme activities of fish. Aquac. Res., 42: 1459-1467.

Lushchak, VI (2011). Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol., 101: 13-30.

Machuca, M; Esteban, MA and Cuesta, A (2022). Dietary immunostimulants in aquaculture: current knowledge and future perspectives. Aquac. Int., 30: 1201-1220.

Meena, DK; Das, P; Kumar, S; Mandal, SC; Prusty, AK; Singh, SK; Akhtar, MS; Behera, BK; Kumar, K; Pal, AK and Mukherjee, SC (2013). Beta glucan: an ideal immunostimulant in aquaculture (a review). Fish Physiol. Biochem., 39: 431-457.

Misra, CK; Das, BK; Mukherjee, SC and Pattnaik, P (2006). Effect of multiple injections of β−glucan on non specific immune response and disease resistance in *Labeo rohita*. Fish Shellfish Immunol., 20: 305-319.

National Research Council (NRC) (2011). Nutrient requirements of fish and shrimp. 1st Edn., Washington DC, USA, National Academies Press. PP: 1-376.

Rodrigues, MV; Zanuzzo, FS; Koch, JFA; de Oliveira, CAF; Sima, P and Vetvicka, V (2020). Development of fish immunity and the role of β−glucan in

immune responses. Fish Shellfish Immunol., 103: 144-154.

Sahoo, PK and Mukherjee, SC (2001). Effect of dietary $\beta -1,3$ glucan on immune responses and disease resistance of healthy and aflatoxin B1 induced immunocompromised rohu (*Labeo rohita*). Fish Shellfish Immunol., 11: 683-695.

Sealey, WM; Barrows, FT; Hang, A; Johansen, KA; Overturf, K; LaPatra, SE and Hardy, RW (2008). Evaluation of the ability of partially defatted distillers dried grains with solubles to replace fish meal in rainbow trout diets. Aquaculture, 283: 95-101.

Siwicki, AK; Anderson, DP and Rumsey, GL (2004). Dietary intake of immunostimulants by rainbow trout affects non specific immunity and protection against bacterial diseases. Fish Shellfish Immunol., 16: 279-292.

Steel, RGD; Torrie, JH and Dickey, DA (1997). Principles and procedures of statistics: a biometrical approach. 3rd Edn., New York, USA, McGraw Hill. PP: 1-666.