Fish and Shellfish Immunology 80 (2018) 505–513

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Dietary administration of eryngii mushroom (*Pleurotus eryngii*) powder on haemato-immunological responses, bactericidal activity of skin mucus and growth performance of koi carp fingerlings (*Cyprinus carpio* koi)

Omid Safari*, Mehrdad Sarkheil

Department of Fisheries, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Keywords:
Prebiotic
Pleurotus eryngii
Koi carp (*Cyprinus carpio* koi)
Immune response
Bactericidal activity

ABSTRACT

The aim of present study was to evaluate the effects of edible eryngii mushroom powder, *Pleurotus eryngii* (PE), for 63 days on haematological parameters, the serum immune responses, skin mucus, bactericidal activity, stress resistance, growth performance and digestive enzyme activities of Koi carp fingerlings (*Cyprinus carpio* koi). Fish were divided into five groups and each group was fed with dietary PE with five graded levels (0, 0.5, 1, 1.5 and 2%). The results showed a significant dose-dependent increase of Ht, Hb, MCV and MCH levels in fish fed dietary PE ($P < 0.05$). The highest levels of WBCs, lymphocytes and monocytes were measured in fish fed 1.5% and 2% of dietary PE ($P < 0.05$). The activities of total IG, lysozyme, Alternative haemolytic complement activity in serum of fish fed with 2% of dietary PE for 63 days as well as 5-min air exposure challenge test were significantly higher than other groups ($P < 0.05$). The most bactericidal activity was observed in skin mucus of fish fed with 1.5% of dietary PE against *Streptococcus iniae* ($P < 0.05$). The highest ratio of the lactobacillus count to the total viable count was observed in fish fed 2% of dietary PE. The α-amylase activity of fish fed with dietary PE (1, 1.5 and 2%) were significantly higher than control group ($P < 0.05$). Feeding fish 2% of dietary PE increased the trypsin and lipase activity compared to others groups ($P < 0.05$). The growth performance of fish fed 1.5% of dietary PE improved compared to control group ($P < 0.05$). The results revealed that feeding koi fish with dietary supplementation of PE (1.5 and 2%) improved the selected humoral innate immune responses, bactericidal activity of skin mucus and growth performance of koi fish.

1. Introduction

Globally the ornamental fish sector is growing and their production and trade is a profitable activity in aquaculture industry [1]. The value of global trade of ornamental fish is estimated to be more than USD 15 billion with an annual growth of 8% [2]. The commercial production of koi fish as an ornamental strain of common carp, *Cyprinus carpio*, is emerged in the past few decades [3]. However, increasing use of intensified aquaculture systems has accelerated the outbreaks of disease with huge economic losses [4]. To prevent and control diseases; vaccines, antibiotics and chemotherapeutics commonly have been used in aquaculture industry [5]. The application of antibiotics and chemotherapeutics is harmful in aquaculture because of development of drug resistant microorganisms [6], environmental hazards [7] and human health hazards [8].

During the last decades, several studies have been conducted on the modulation of fish immune system by using immunostimulants as dietary additives in order to maintain fish health and to improve growth performance. An immunostimulant is a natural or chemical compound that stimulate both specific and non-specific immune mechanisms to protect fish against invading pathogens [9,10]. Supplementation of dietary prebiotics with fish diets can be a potential alternative to prevent and control pathogens in aquaculture [8,11]. Nowadays, edible mushrooms as a potential source of polysaccharides namely β-glucan and other bioactive compounds such as oligosaccharides, dietary fibers, glycoproteins, proteins, peptides, amino acids, triterpenoids, alkaloids, alcohols, phenols, polyphenols, vitamins, and minerals [12,13] are considered as natural prebiotics.

Several species of king oyster mushroom (*Pleurotus* spp.) are commercially grown as food and medicine in many countries [14]. *Pleurotus eryngii* is a popular type of edible mushroom due to nutritional value and biological functions and its production has greatly increased during last few decades [15]. Several studies on *P. eryngii* have revealed a number of therapeutic functions such as immunostimulatory [16], antitumor, antioxidative [17], antimicrobial and antiviral activities [15,18]. Moreover, this species contain high levels of carbohydrates

* Corresponding author. Department of Fisheries, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, P.B. 91773, Mashhad, Iran.
E-mail address: omidsafari@um.ac.ir (O. Safari).

https://doi.org/10.1016/j.fsi.2018.06.046
Received 15 April 2018; Received in revised form 14 June 2018; Accepted 26 June 2018
Available online 28 June 2018

(9.6% of the fresh weight), dietary fibers (4.6% of the fresh weight), chitin (0.5% of the fresh weight) [19] and low level of lipid (0.8% of the fresh weight) [20]. The results of a study revealed that the dietary supplementation of *P. eryngii* and *Lactobacillus plantarum* stimulated growth, immunity and disease resistance of the Pangasius catfish, *Pangasius bocourti* [21]. Katya et al. [13] reported that the replacement of fish meal by 6.3% of fermented by-product of mushroom, *Pleurotus ostreatus* (FBPM) in diet of juvenile Amur catfish, *Silurus asotus,* improved growth performance, lysozyme activity and chemiluminescent response of fish. To our knowledge, little information is available about the effects of dietary edible mushroom supplementation in ornamental fish. Furthermore, the commercial production and trade of ornamental fish especially koi is expanding all over the world and more information about improvement of their health and immune system is needed. Therefore, the present study was conducted to evaluate the effects of dietary edible mushroom, *P. eryngii* (PE), on haemato-immunological parameters, bactericidal response of skin mucus, stress resistance, digestive enzyme activities as well as growth performance of koi fish as an ornamental strain of common carp, *Cyprinus carpio*.

2. Materials and methods

2.1. Pleurotus eryngii preparation

Eryngii mushroom, *P. eryngii* (PE) used in this study was obtained from Research Institute of Industrial Biotechnology, ACECR (Khorasan Razavi, Mashhad, Iran). PE samples were dried in an oven at 50 °C for 24 h and then ground into powder before adding them to the diets.

2.2. Experimental diets

A basal diet (340 g kg^{-1}, crude protein; 50 g kg^{-1}, crude lipid; 15.80 MJ kg^{-1}, gross energy) as the control diet was developed by WUFFDA (windows-based user-friendly feed formulation, done again; University of Georgia, Georgia, USA) software (NRC) (Table 1). To prepare the experimental diets, PE at the levels of 0, 5, 10, 15 and 20 g kg^{-1} was employed. PE was replaced with Carboxymethyl cellulose (CMC). Diets were isonitrogenous and isoenergetic. Feed ingredients were converted into a uniform paste by adding water, and then the dough passed through a meat grinder with a diameter of 2 mm. Finally, the wet pellets were dried at 30 °C for 24 h, and stored at 4 °C until use.

Table 1
Composition of the basal diets (g/kg dry matter) fed fingerling of koi fish (7.36 ± 0.056 g).

Ingredients	Eryngii mushroom powder	g kg^{-1} (dry-weight basis)
Fish meal		175
Wheat flour		245
Soybean meal		250
Corn gluten		150
Soybean oil		25
Fish oil		25
Mineral premix		35
Vitamin premix		35
CMC		25
Anti-fungi		15
BHT		15
Vit C		5
Chemical composition		
Dry matter	1000	967.3
Crude protein	297.9	340
Crude fat	39.3	50
Crude fiber	214.1	380
Ash	89.2	680
Cross energy (Mj/Kg)	19.94	15.80

2.3. Experimental design

Fingerling of *Cyprinus carpio* koi (7.36 ± 0.05 g) were obtained from Toos Koi Co. (Khorasan Razavi, Iran). Fish were randomly distributed into 15 glass aquarium (capacity of 150 l) at the density of 15 $tank^{-1}$ with daily water exchange rate of 20% at three replicates for each experimental diet. Prior to onset of the nutritional trail, fish were fed the control diet for two weeks. Fish were fed with experimental diets to appetent satiation three time daily for 63 days. Fish were maintained under photoperiod of 12:12 (light: dark). Water temperature was maintained at 25.5 ± 1.5 °C throughout feeding trail. Dissolved oxygen (6.53 ± 0.21 mg L^{-1}) and pH (7.64 ± 0.14) were measured every week. All experiments on koi fish were done according to animal ethic rights' FUM.

2.4. Evaluation of growth performance

At the end of feeding trial, each fish was individually weighted (± 0.01) on an electronic scale (AND, Japan) and the growth performance parameters including specific growth rate (SGR % day^{-1}), feed conversion ratio (FCR) and condition factor (CF) were calculated using the following equations:

Specific growth rate (SGR; % day^{-1}) = $[(\ln W_f - \ln W_i)/t] \times 100$

Feed conversion ratio (FCR) = $(\text{Feed}_{\text{consumed}}/W_{gain})$

Condition factor (CF) = $W_f/ L_f^{\ 3} \times 100$

Survival rate (%) = $(N_f / N_i) \times 100$

Where W_i, W_f, W_{gain}, L_f, N_f, N_i, t and $\text{Feed}_{\text{consumed}}$ are initial weight, final weight, weight increment (g), final length (cm), final number of fish, initial number of fish, time period (day) and consumed feed (g), respectively.

2.5. Haemato-immunological assays

After 24 h of last feeding time in the 63 t h day, six fish from each glass aquarium were anesthetized by clove powder (500 mg l^{-1}). The blood samples were collected from the caudal vein. Then, an aliquot of blood samples were stored in heparinized tubes to determine haematological parameters including RBCs, WBCs, hematocrit (Ht), hemoglobin (Hb), mean corpuscular hemoglobin volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential WBCs (neutrophils, lymphocytes and monocytes). The reminders of blood samples were introduced to non-heparinized tubes, centrifuged at 1000 × g for 5 min at 4 °C. The collected serum samples were stored immediately at −20 °C until assay of the activities of total immunoglobulin (IG), lysozyme (LYZ) and Alternative haemolytic complement activity (ACH50). All assays were done one by one at three replicates.

RBCs and WBCs counting were performed using a Newbauer hemocytometer by suspension of whole blood in the diluents described by Natt and Herrick [22]. Hematocrit (Ht) was determined using the standard microhematocrit method as described by Brown [23]. For this purpose, blood-filled heparinized microhaematocrit capillary tubes were centrifuged at 7000 × g for 10 min using a microhaematocrit centrifuge and the hematocrit (Ht) values were read directly and reported as packed cell volume percentage. The hemoglobin (Hb) concentration was measured according to cyan-methaemoglobin method explained by Blaxhall and Daisley [24] using a spectrometer at a wavelength of 540 nm. Mean corpuscular hemoglobin volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were calculated using the total RBC count, Hb concentration and Ht [25]. Neutrophil, lymphocyte and monocyte cells were measured using May-Grunwald-Giemsa blood smears [26]. Blood smears were studied by light microcopy in order to make blood cell

counts.

Lysozyme activity in serum was determined according to the method of Kumari et al. [27] based on the lysis of the lysozyme sensitive Gram positive bacterium, *Micrococcus lysodeikticus* (Sigma). The serum sample (15 μL) was placed into wells of a 96-well plate in triplicate. Then, 150 μL of *M. lysodeikticus* suspension prepared in 0.02 M sodium acetate buffer, pH 5.8 (0.02 mg L^{-1}) and added to each wall. An initial absorption value was measured at 450 nm using a spectrophotometry (HACH DR/4000, USA). Second absorption value was measured after holding sample at room temperature for 1 h. The difference between the absorption values was calculated and the lysozyme activity was expressed as μg mL^{-1}. The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 μg mL^{-1} in same buffer were taken as the standard.

The total immunoglobulin (IG) activity of the serum was determined as described by Siwiki and Anderson [28]. Briefly, 100 μL of the serum were mixed with an equal amount of 12% polyethylene glycol and incubated for 2 h under constant agitation at room temperature to precipitate the immunoglobulin. After centrifugation at 3000 × g for 15 min, the supernatant was removed and the remaining protein was determined after it was subtracted from the total serum protein concentration.

Alternative haemolytic complement activity (ACH50) of the serum was assayed based on method described by Yano [29]. Briefly, the diluted serum samples (range, 50–250 μL) were dispensed into test tube and total volume were made up to 250 μL with the barbitone buffer in presence of ethyleneglycol-bis (2-aminoethoxy)-tetraacetic acid (EGTA) and Mg^{2+}. Then, 100 μL New Zealand rabbit red blood cells (RaRBC) was added and incubated at 20 °C for 90 min. At this point, 3.15 ml of NaCl was added to each tube and centrifuged at 1600 × g for 5 min. The absorbance of supernatant was read at 414 nm. The serum volume producing 50% haemolysis (ACH50) was determined and the number of ACH50 units ml^{-1} was calculated for each group.

2.6. Digestive enzymes measurements

At the end of feeding trial, fish were starved for 24 h and three fish from each glass aquarium sampled for enzymatic analysis. The intestines were isolated and rinsed with cold distilled water at 4 °C [30]. The intestine content was extracted and homogenized in phosphate buffered saline (pH 7.5; 30 g/70 ml PBS) using a homogenizer (DI 18 Disperser). The homogenate was then centrifuged at 15000 × g, 4 °C, 15 min and the supernatant stored in liquid nitrogen until analyses. The activity of α-amylase was measured by the 3,5-dinitrosalicylic acid method described by Worthington [31], using 1% starch (diluted in a buffer at pH 6.9, 0.02 M Na_2HPO_4 and 0.006 M NaCl) as substrate at final reading 540 nm. Trypsin activity was measured using N-α-benzoyl-dlarginine-pnitroanilide (BAPNA) as substrate. BAPNA (1 mM in 50 mM Tris-HCl, pH 8.2, 20 mM $CaCl_2$) was incubated with the enzyme extract at 25 °C and absorbance was recorded at final reading 410 nm [32]. Lipase activity was assayed at 25 °C using 0.4 mM 4-nitrophenylmyristate as substrate and absorbance was recorded at final reading 405 nm [33]. All enzyme activities were measured in triplicate by a microplate scanning spectrophotometer (Power Wave HT, BioTek®, USA) and defined as specific activity (U/mg protein/min). All the reagents used in the measuring of enzyme activities were purchased from Sigma-Aldrich.

2.7. Bacteriological analysis

At the end of feeding trial, koi fish (three fish from each glass aquarium) was transported alive to the laboratory, anesthetized with ice, rinsed with benzalkolium chloride (0.1% for 60 min) and then, fish were dissected to remove the intestinal tract. The digestive tracts were homogenized with sodium chloride (0.9 w/v) using a homogenizer (DI 18 Disperser), and the homogenate was centrifuged at 5000 × g, 4 °C for 5 min. One hundred microliter of prepared samples were inoculated in plate count agar (PCA; Merck Co.) and de Man, Rogosa, and Sharpe media (MRS; Merck Co.) at three replicates to determine the total viable counts (TVC) of heterotrophic aerobic bacteria and presumptive lactic acid bacteria (LAB), respectively. The plates were incubated at room temperature (25 °C) for five days and colony forming units (CFU) g^{-1} were calculated from plates containing 30–300 colonies [34].

2.8. Fish mucus parameters

2.8.1. Mucus collection

At the end of feeding trial, skin mucus samples were collected from fish (three fish from each glass aquarium) that starved for 24 h [35]. Briefly, the tested fish were anesthetized using clove powder (500 mg l^{-1}) and then transferred into polyethylene bags that contain NaCl (50 mM; 5 ml g^{-1} fish; Merck, Germany) for about 1 min. Fish were slowly shaken inside the plastic bags to collect skin mucus and then returned to recovery glass aquariums. The obtained mucus samples were immediately transferred to 15 ml sterile centrifuge tubes, centrifuged at 1500 × g, at 4 °C, for 10 min and the supernatants were stored in 2 ml tubes at −80 °C until future assays.

2.8.2. Mucus bactericidal test

The bactericidal activity of fish skin mucus samples were evaluated based on a standardized single disc method [36] against five bacterial strains including *Aeromonas hydrophila* (ATCC 7966), *Streptococcus iniae* (ATCC29178), *Streptococcus faecium* (ATCC 12755), *Micrococcus luteus* (MTCC 3911) and *Yersinia ruckerii* (ATCC 29473).

At first, bacteria were cultured in the nutrient broth medium (Merck, Germany) and incubated at 37 °C for 24 h in a shaking incubator at 200 rpm (JSSI-200C L; JSR, Gongju-City, Korea). Then, the surface of nutrient agar (Merck, Germany) completely inoculated with bacteria (1.5×10^8 CFU ml^{-1}). Paper discs (6 mm diameter) were inoculated with 150 ml of the mucus sample for 20 min to absorb the mucus and placed over solidified agar gel. The plates were incubated at 37 °C for 24 h. Finally, the diameter of the growth inhibition zones was measured by digital caliper (Guilin Guanglu Measuring Instrument, Zone Guilin, China).

2.9. Air exposure challenge

In order to evaluate biological responses of fish fed the experimental diets against air exposure challenge, three fish from each glass aquarium were exposed to air for 5 min on day 63. Then, the activities of total IG, LYZ and ACH50 in blood samples of exposed fish were measured according to methods described above.

2.10. Statistical analysis

All data of this study were presented as Mean ± SD. The statistical analysis were performed using SPSS software (Version, 19, IBM SPSS, Armonk, NY, USA). The percentage data were transformed using the arcsine method. Normality assumption of data were determined using the Kolmogrov-Smirnov test. Differences between the means were analyzed using One-Way analysis of variance (ANOVA). The Duncan multiple range test was also used to compare significant differences among the means at $P < 0.05$.

3. Results

3.1. Haematological assays

The variations in haematological parameters of koi fish fed with different levels of dietary eryngii mushroom (PE) after 63 days are shown in Table 2. The level of RBCs was not significantly affected by dietary PE ($P > 0.5$, Table 2). WBC levels in fish fed 1.5% and 2% of

Table 2
The haematological parameters of koi fish fed diets with different levels of dietary eryngii mushroom powder (PE) (*P. eryngii*) after 63 days (Mean ± SD, n = 3).

Haematological parameters	Dietary Eryngii mushroom powder levels (%)				
	Control	0.5%	1%	1.5%	2%
RBC (× 10^6/μl)	1.64 ± 0.1^a	1.87 ± 0.07^a	1.89 ± 0.8^a	2 ± 0.09^a	2.15 ± 0.11^a
WBC (× 10^3/μl)	33.20 ± 0.2^a	34.45 ± 1.34^{ab}	35.15 ± 1.27^{ab}	36 ± 1.39^b	36.98 ± 1.76^b
Hb (mmol/l)	2.74 ± 0.015^a	3.01 ± 0.11^b	3.05 ± 0.13^b	3.282 ± 0.12^c	3.52 ± 0.15^d
Hematocrit (%)	22.36 ± 0.115^a	24.1 ± 0.13^b	24.17 ± 0.14^b	24.63 ± 0.15^c	25.82 ± 0.12^d
MCV (fl)	148.33 ± 0.305^a	151.28 ± 1.23^b	152 ± 1.33^b	153.71 ± 1.32^c	158.92 ± 1.30^d
MCH (pg)	31.23 ± 0.095^a	32.16 ± 1.11^{ab}	33.25 ± 1.13^b	36.72 ± 1.13^c	38.21 ± 1.14^c
MCHC (mmol/l)	12.1 ± 0.1^a	13.68 ± 1.34^a	13.72 ± 1.33^a	14.09 ± 1.36^a	14.38 ± 1.39^a
Lymphocyte (%)	86.90 ± 0.1^a	87.13 ± 1.30^{ab}	88.27 ± 1.45^{abc}	89.37 ± 1.32^{bc}	90.27 ± 1.43^c
Monocyte (%)	3.46 ± 0.11^a	5.21 ± 1.23^{ab}	5.89 ± 1.56^{ab}	6.17 ± 1.49^b	6.48 ± 1.42^b
Neutrophil (%)	6.12 ± 0.59^a	7.66 ± 2.5^a	5.84 ± 3.01^a	4.64 ± 2.81^a	3.25 ± 2.85^a

Values with different letters within a row are significantly different (ANOVA, $P < 0.05$).

dietary PE were significantly higher than control group ($P < 0.05$). Hb and hematocrit levels increased significantly in treated groups compared to control group ($P < 0.05$) and the levels of these parameters in fish fed 1.5% and 2% of dietary PE were significantly higher than fish fed 0.5% and 1% of dietary PE ($P < 0.05$). The results revealed an elevation of MCV in treated groups compared to control group ($P < 0.05$) and the highest level observed in fish fed 2% of dietary PE ($P < 0.05$). MCH level increased significantly in fish fed 1%, 1.5% and 2% of dietary PE compered control group ($P < 0.05$). There was no significant difference in MCHC level of fish fed experimental diets and control diet ($P > 0.05$). Lymphocyte and monocyte levels increased significantly only in fish fed 1.5% and 2% of dietary PE compared to control group ($P < 0.05$). The results showed that there was no significant difference in neutrophil level among fish fed experimental diets and control diet ($P > 0.05$).

3.2. Immunological assays

The effects of feeding of koi fish with different levels of dietary PE on serum immune parameters are shown in Fig. 1(A–C). The serum total IG and ACH50 activities in the 1.5% and 2% PE fed groups increased significantly compared to control group ($P < 0.5$). The highest total IG and ACH50 activity were observed in 2% PE fed group ($P < 0.05$). All experimental diets except of 0.5% of dietary PE, increased lysozyme activity compared to control diet ($P < 0.05$). Lysozyme activity in fish fed 2% of dietary PE was significantly higher compared to other experimental diets ($P < 0.05$).

The effects of air exposure challenge for 5 min on serum immune parameters in fish fed with different levels of dietary PE for 63 days are shown in Fig. 2 (A–C). The results revealed that all experimental diets except of 0.5% of dietary PE increased post challenge total IG and lysozyme activity compared to control diet ($P < 0.05$). Elevation of post challenge ACH50 activity was observed only in fish fed 1.5% and 2% of dietary PE compared to control group ($P < 0.05$).

3.3. Bacteriological analysis

Fig. 3 shows the results of the LAB count to TVC ratio (%) of intestine extracted from koi fish feeding with dietary PE for 63 day. Fish fed with 1%, 1.5% and 2% of dietary PE showed significantly higher LAB/TVC ratio (%) compared to control group ($P < 0.05$). The highest LAB/TVC ratio (56.43%) was observed in fish fed with 2% of dietary PE ($P < 0.05$).

3.4. Mucus bactericidal activity

Growth inhibitory zone formed by skin mucus of fish fed different levels of dietary PE for 63 days against five bacterial strains including Aeromonas *hydrophila*, *Streptococcus iniae*, *Streptococcus faecium*, *Micrococcus luteus* and *Yersinia ruckerii* in plating test was shown in Fig. 4. The results revealed that all mucus samples obtained from fish fed dietary PE had significant bactericidal effects against all the bacteria strains ($P < 0.05$). The maximum diameter of growth inhibitory zone was formed around all mucus samples in 1% and 1.5% PE fed groups ($P < 0.05$). The highest bactericidal effect was also observed against *Staphyloccus iniaee* in 1.5% PE fed group ($P < 0.05$).

3.5. Growth performance

Table 3 shows the results of growth performance of koi fish fed different levels of dietary PE after 63 days. The results revealed that fish fed with 1.5% of dietary PE had significantly higher final weight, weight gain and SGR % than control group ($P < 0.05$). There were no significant differences in the CF of PE fed groups and control group ($P > 0.05$). FCR of fish fed with dietary PE was significantly lower than control group ($P < 0.05$). The lowest value of FCR was observed in fish fed with 1.5% of dietary PE ($P < 0.05$). Also, feeding test fish with PE-diets improved the survival rate compared to that of fish fed control ($P < 0.05$).

3.6. Digestive enzyme activities

Table 4 shows the effects of different levels of dietary PE on digestive enzyme activities of koi fish after 63 days. Trypsin and lipase activities of fish fed 2% of dietary PE were significantly higher than control group ($P < 0.05$). The α-amylase activity increased significantly in fish fed 1%, 1.5% and 2% of dietary PE compared to control diet ($P < 0.05$). The highest level of α-amylase activity was observed in 2% PE fed group ($P < 0.05$).

4. Discussion

4.1. Haemato-immunological parameters and stress resistance

It is well known that the application of many different immunostimulants can be effective in different fish species [36–38]. The cultivation of organic fish has been considered over last decade and therefore the use of natural immunostimulants are currently receiving considerable attention in aquaculture industry [38]. Mushroom due to containing bioactive compounds are added to diets to improve fish immune system and growth performance [21], [13].

The teleost fish innate immune system has both cellular and humoral components. At the cellular level leukocytes, mainly monocytes, neutrophils, macrophages as well as T and B lymphocytes are the major cell types. Humoral elements participate in the innate immune response including lysozyme, complement (classical and alternative pathways), hemolysin, transferring and C-creative protein [39], [10]. Some researcher reported that extract of mushroom, *P. eryngii*, with the highest

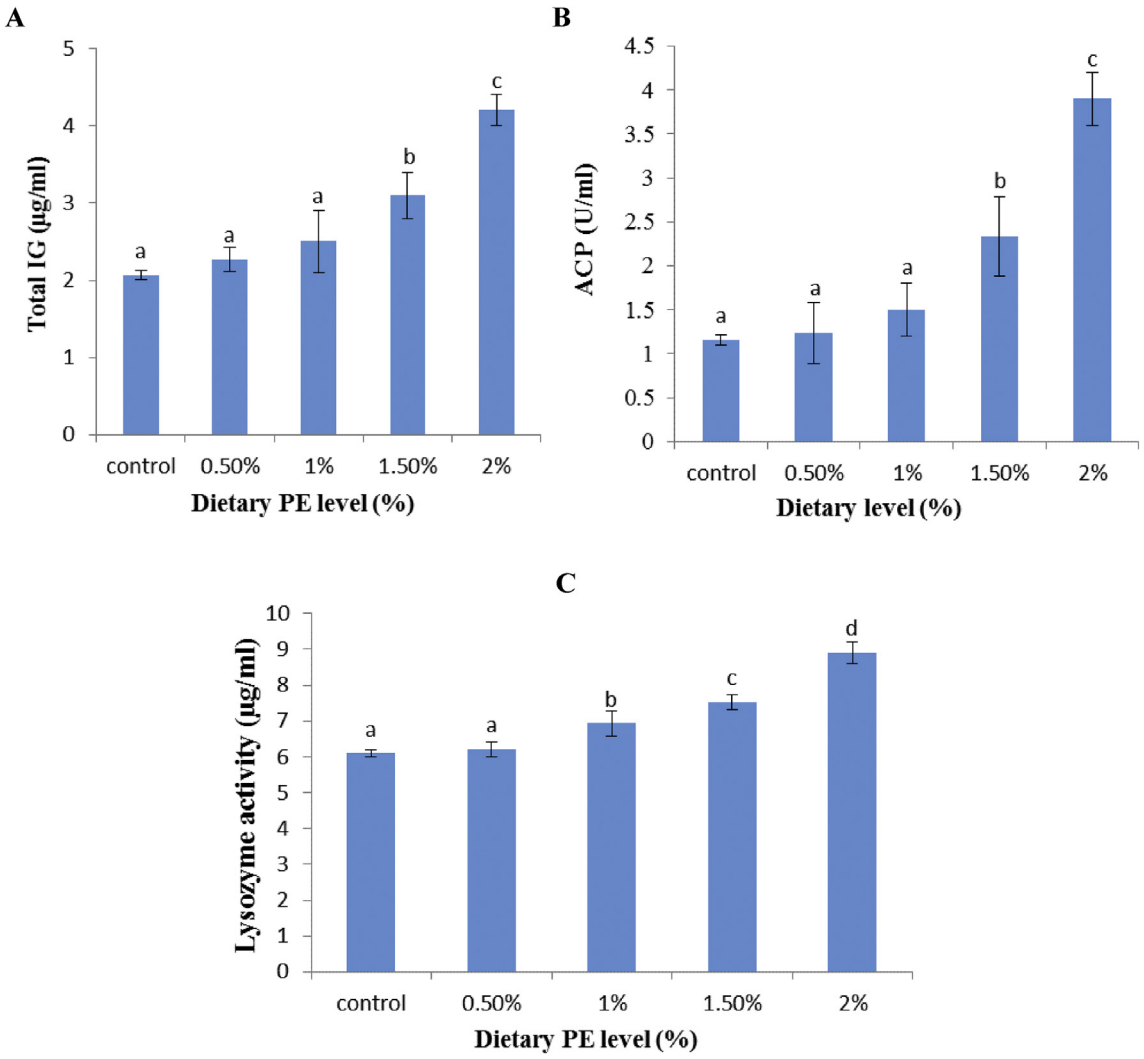

Fig. 1. Total immunoglobulin (µg/ml) (A), Alternative haemolytic complement activity (ACH50) (U/ml) (B) and lysozyme (µg/ml) (C) activities of koi fish fed with different levels of dietary eryngii mushroom powder (PE) (*P. eryngii*) after 63 days (Mean ± SD, $P < 0.05$).

β-glucan (18.94%) content, displayed immune-stimulating activity [1]. Haematological indices are regarded as useful indicators to investigate the potential effects of prebiotics in aquafeeds [11], [40]. The results of present study showed significant dose-dependent increase of hematocrit, Hb, MCV and MCH levels in fish fed dietary PE ($P < 0.05$). WBC counts, lymphocytes and monocytes levels in fish fed 1.5% and 2% of dietary PE increased significantly compared to control group. By contrast, dietary PE did not have significant effects on neutrophils and MCHC levels. Sahoo and Mukherjee [41] reported the increasing WCBs count in *Labeo rohita* fingerlings fed with immunostimulants such as levamisole and ascorbic acid. Haematological parameters including, RBC, WBC counts, and haemoglobin concentration of *Channa striata* fingerlings fed with three commercial prebiotics (β-glucan, galactooligosaccharide and mannan-oligosaccharide) for 16 weeks increased significantly compared to control diet [42]. Ashish Kumar et al. [43] also observed a significant improvement in WBC count in *catla catla* fed on yeast RNA, x-3 fatty acid and b-carotene. Feeding of Asian sea bass, *Lates calcarifer,* fingerlings with various concentrations of *Cissus quadrangularis* plant (stem) and lipopolysaccharide (LPS from bacteria) supplemented diets resulted in increased leucocytes and Hb levels compared to control group [44]. However, the results of a study showed that haematological parameters including WBCs, haemoglobin and lymphocyte did not significantly improve after feeding of beluga, *Huso huso* with dietary fructooligosaccharide [45].

In this study, positive effects of dietary PE on serum total immunoglobulin, lysozyme and Alternative haemolytic complement activities was observed. The highest levels of these parameters were measured in fish fed 2% of dietary PE. Similarly, Khodadadian Zou et al. [46] reported that serum non-specific immune parameters including total IG, lysozyme and ACH_{50} significantly increased in common carp (*Cyprinus carpio*) fingerlings fed with different levels (0.5%, 1% and 2%) of dietary white bottom mushroom (*Agaricus bisporus*) powder for 8 weeks. The results of different studies also revealed that dietary supplementation of mushrooms in different fish species such as *P. eryngii* ($3\,g\,kg^{-1}$) in Pangasius catfish, *Pangasius bocourti* [21], *P. ostreatus* in Amur catfish, *Silurus asotus* [13], mushroom beta-glucan in orange-spotted grouper, *Epinephelus coioides* [47] and *Lentinula edodes* in rainbow trout, *Oncorhynchus mykiss* [48] stimulated serum innate immune responses including lysozyme, respiratory burst and phagocytic activities. Some researcher reported that extract of mushroom, *Pleurotus eryngii*, with the highest β-glucan (18.94%) level among the 16 species of culinary medicinal mushrooms, displayed immune-stimulating activity [12]. It is reported that β-glucan may increase the number of immune system cells such as macrophages by binding to particular cell receptors and directly activating the cells [49]. Furthermore, some immunostimulants such as Lentinan and grifolan extracted from mushroom, *Lentinula edodes* and *Grifola frondosa*, respectively, enhance the functions of macrophages and natural killer

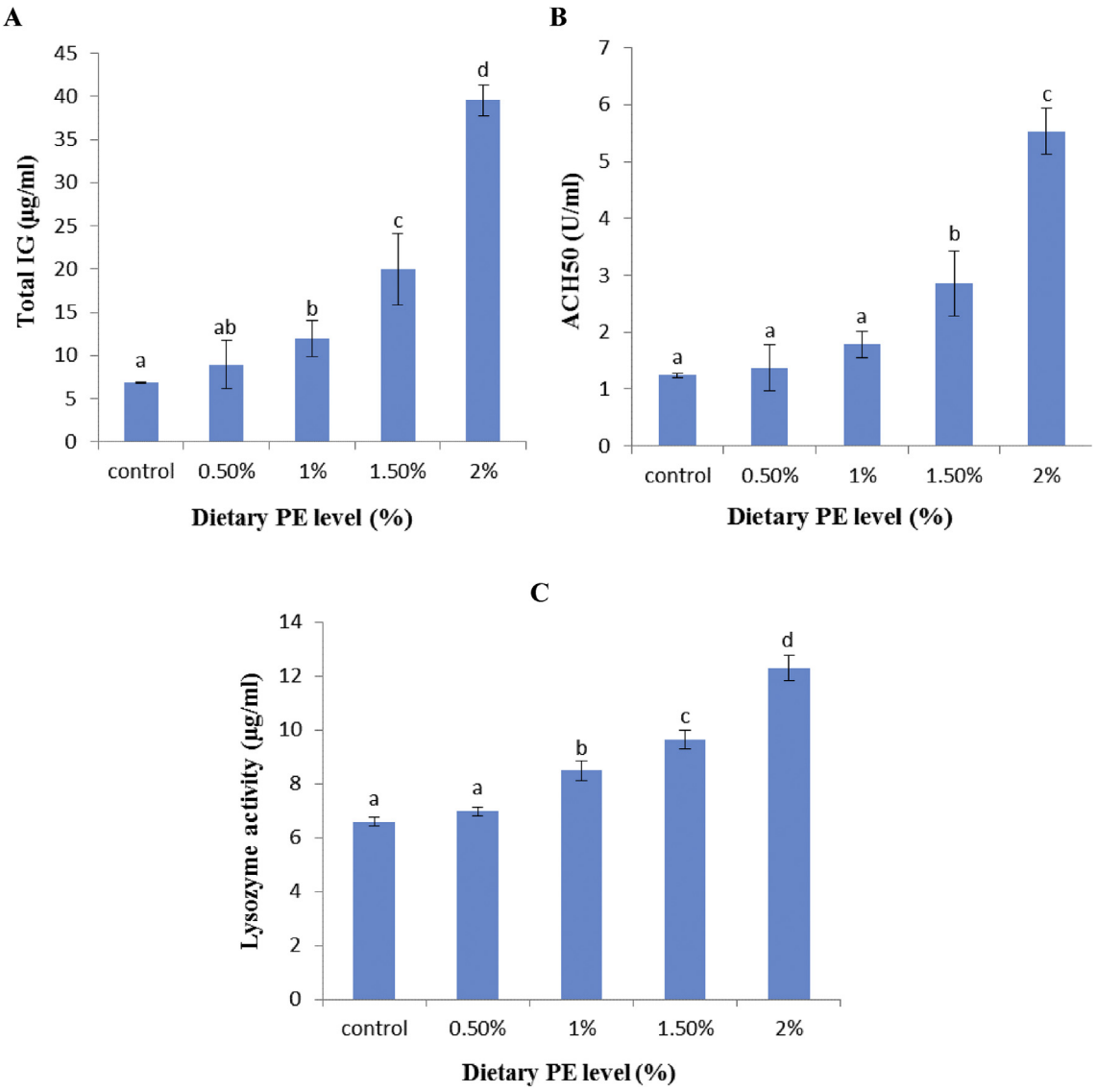

Fig. 2. Total immunoglobulin (µg/ml) (A), Alternative haemolytic complement activity (ACH50) (U/ml) (B) and lysozyme (µg/ml) (C) activities of koi fish fed with different levels of dietary eryngii mushroom powder (PE) (*P. eryngii*) after air exposure challenge (Mean ± SD, $P < 0.05$, n = 3).

cells [50], [51].

Exposure of target animal to different physical, chemical and biological stressors is a way to evaluate the effectiveness of immunostimulants as dietary supplements for a period of post-feeding. Several studies have proved that under acute and short-term stress, the fish immune response pattern is stimulatory, whereas under chronic stress condition, the immune response shows suppressive effects and therefore the chances of an infection may be enhanced [52]. The results of this study showed that the exposure of koi fish to air for 5 min after feeding with experimental diets induced innate immune responses. So that, the post challenge total IG and lysozyme activity increased in fish fed 1%, 1.5%, 2% of dietary PE and elevation of ACH50 activity was observed in 1.5% and 2% treated groups in compared to control.

4.2. Bacteriological analysis

The application of feed additives such as probiotics, prebiotics and synbiotics (probiotics + prebiotics), have been shown the potential to improve gastrointestinal (GI) morphology and microbial balance in aquatic animals [53]. Lactic acid bacteria (LAB) are sometimes abundant in the intestine tract especially in freshwater fish and some strains have been considered for beneficial effects on fish health [54]. The results of present study revealed approximately 2-fold and 2.5-fold increase in LAB/TVC ratio (%) of fish fed with 1%, 1.5% and 2% of dietary PE compared to control group, respectively. Hoseinifar et al. [55] reported the increase in lactic acid bacteria (LAB) level in intestinal microbiota of common carp (*Cyprinus carpio*) larvae fed with low levels of dietary short chain fructooligosacchairde (sc-FOS) for 7 weeks. It is well known that prebiotics mainly through its fermentation by specific beneficial bacteria (such as *Lactobacillus*, and *Bifidobacterium*) alter the composition of intestinal microbiota [56].

4.3. Mucus bactericidal activity

The results of present study showed that skin mucus of fish fed with different levels (0.5%, 1%, 1.5% and 2%) of dietary PE had bactericidal activities against five bacterial strains including *Aeromonas hydrophila, Streptococcus iniae*, *Streptococcus faecium*, *Micrococcus luteus* and *Yersinia ruckerii*. The finding of other researchers have revealed that bactericidal activity of skin mucus increase in rainbow trout, *Oncorhynchus mykiss* and Caspian roach, *Rutilus rutilus caspicus* fed with dietary supplements of *Saccharomyces cerevisiae* and Vit C, respectively [57,58]. A rise in

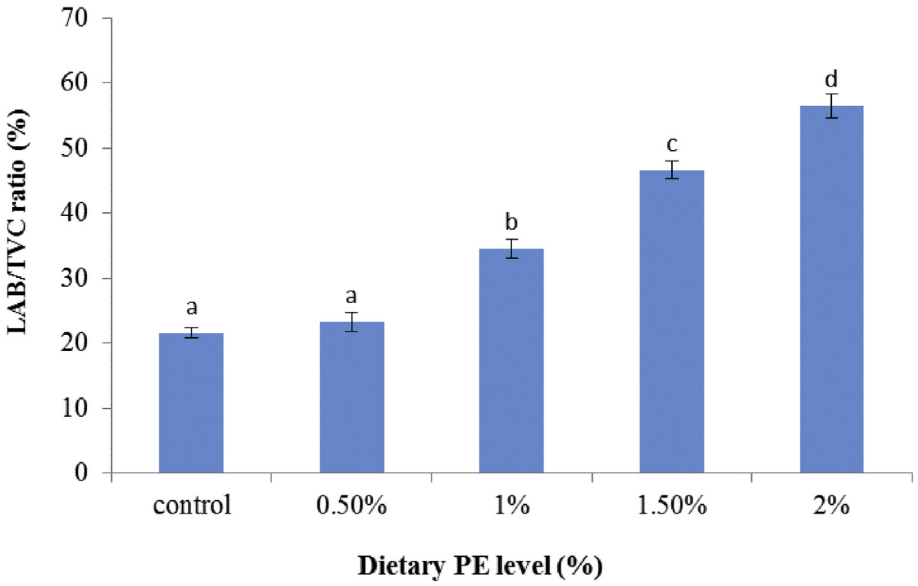

Fig. 3. Lactic acid bacteria (LAB) count (CFU g^{-1}) to total viable heterotrophic aerobic bacteria count (CFU g^{-1}) ratio (%) of intestinal extracted from koi fish fed with different levels of dietary eryngii mushroom powder (PE) (*P. eryngii*) after 63 days (Mean ± SD, P < 0.05).

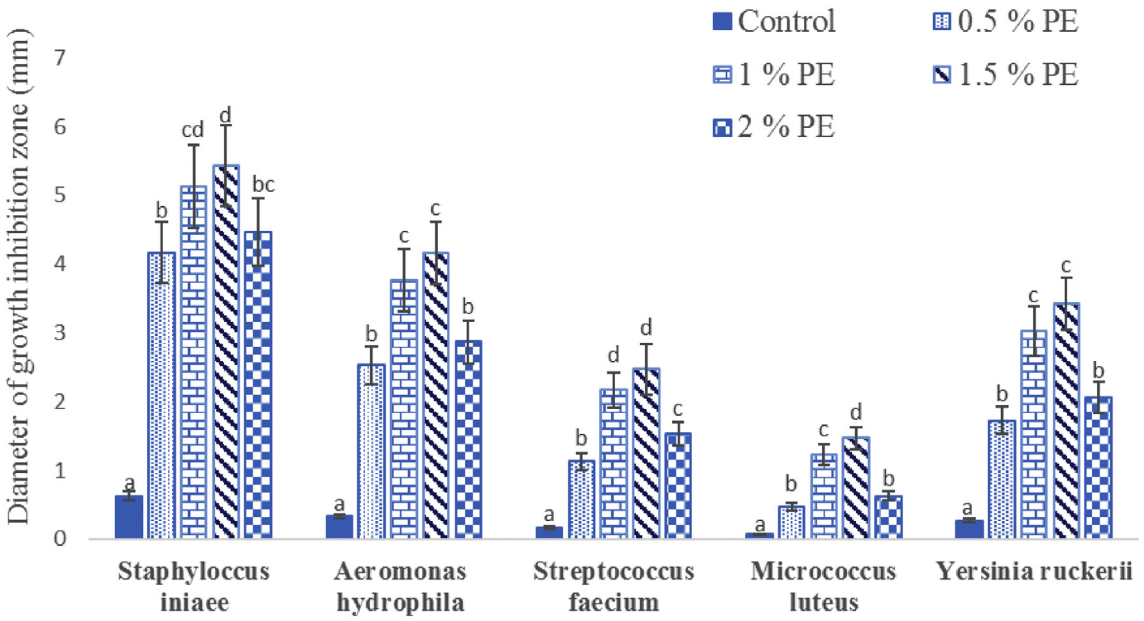

Fig. 4. Diameter of growth inhibition zone (mm) formed by skin mucus of koi fish feeding on the experimental diets against five bacterial strains including *Aeromonas hydrophila, Streptococcus iniae*, *Streptococcus faecium*, *Micrococcus luteus* and *Yersinia ruckerii* (Mean ± SD, P < 0.05).

Table 3
Growth performance and feed utilization of koi fish fed with different levels of dietary eryngii mushroom powder (PE) (*P. eryngii*) after 63 days (Mean ± SD, n = 3).

	Dietary Eryngii mushroom powder levels (%)				
	0 (control)	0.5	1	1.5	2
Initial weight (g)	7.42 ± 0.04^{a}	7.33 ± 0.09^{a}	7.32 ± 0.01^{a}	7.35 ± 0.09^{a}	7.39 ± 0.05^{a}
Final weight (g)	15.33 ± 0.46^{a}	15.61 ± 0.46^{ab}	15.65 ± 0.93^{ab}	17.35 ± 1.39^{b}	16.42 ± 0.73^{ab}
Weight gain (g)	7.91 ± 0.43^{a}	8.28 ± 0.37^{ab}	8.33 ± 0.93^{ab}	10.00 ± 1.30^{b}	9.03 ± 0.68^{ab}
SGR (%BW day^{-1})	1.15 ± 0.04^{a}	1.19 ± 0.02^{a}	1.19 ± 0.08^{a}	1.35 ± 0.10^{b}	1.26 ± 0.06^{ab}
FCR	2.88 ± 0.38^{c}	2.76 ± 0.34^{b}	2.74 ± 0.44^{b}	2.13 ± 0.43^{a}	2.56 ± 0.26^{b}
CF (%)	1.57 ± 0.11^{ab}	1.38 ± 0.19^{a}	1.67 ± 0.09^{b}	1.59 ± 0.13^{ab}	1.55 ± 0.11^{b}
Survival rate (%)	82.22 ± 3.84^{a}	91.10 ± 3.85^{b}	93.33 ± 6.67^{b}	97.77 ± 3.85^{b}	95.55 ± 3.85^{b}

Values with different letters within a row are significantly different (ANOVA, P < 0.05).

Table 4
Digestive enzyme activities of koi fish fed with different levels of dietary eryngii mushroom powder (PE) (*P. eryngii*) after 63 days (Mean ± SD, n = 3).

	Dietary Eryngii mushroom powder levels (%)				
	Control	0.5	1	1.5	2
Trypsin (U/mg protein/min)	0.576 ± 0.032^{a}	0.62 ± 0.21^{a}	1.12 ± 0.31^{ab}	1.25 ± 0.924^{ab}	1.95 ± 0.18^{b}
α-amylase (U/mg protein/min)	1.67 ± 0.025^{a}	1.87 ± 0.15^{a}	2.3 ± 0.11^{b}	3.11 ± 0.125^{c}	4.03 ± 0.21^{d}
Lipase (U/mg protein/min)	0.32 ± 0.02^{a}	0.38 ± 0.01^{a}	0.37 ± 0.02^{ab}	0.41 ± 0.065^{ab}	0.45 ± 0.09^{b}

Values with different letters within a row are significantly different (ANOVA, $P < 0.05$).

antibacterial properties of skin mucus may be due to nitrogenous materials existing in the skin mucus (e.g. lysozyme, proteases, immunoglobulins, mucins, and lectins) [59]. Therefore, bactericidal activity of skin mucus can be used as a potential indicator in determining the function of fish immune system.

4.4. Growth performance and digestive enzymes

The beneficial effects of dietary supplementation of mushroom powder and its derivatives on growth performance of some finfish species such as Amur Catfish [60], Kelp grouper [61], red tilapia [62] and Pangasius catfish [21] have demonstrated in the literature. Similarly, feeding of koi fish with 1.5% of dietary PE resulted in better growth performance (final weight and SGR%) and lower FCR. In contrast, Dobsicova et al. [63] reported no effect of the long-term dietary administration of oyster mushroom (*P. ostreatus*) β-1.3/1.6-D-glucanon on any biometrical parameter (i.e. total length, standard length, body weight, hepatosomatic and spleen somatic index, and condition factor) of Common carp (*C. carpio*). In the present study, also no significant change in condition factor (CF) of koi fish fed with dietary PE was observed compared to control group.

In this study an enhancement of trypsin, α-amylase and lipase activities was observed in koi fish fed with dietary PE at 63 days. A-amylase activity increased in fish fed with all dietary PE except in 0.5% PE fed group. Fish fed with 2% of dietary PE had higher trypsin and Lipase activities. This was in accordance with the finding of other researches [64] who found that protease and α-amylase activities in the intestine and hepatopancreas content of allogynogenetic crucian carp, *Carassius auratus gibelio*, fed xylooligosaccharide significantly increased compared to control diet. In contrast, the finding of Anguiano et al. [65] showed that inclusion of four prebiotics (fructo-oligosaccharide, Bio-MOS, transgalacto-oligosaccharide and GroBiotic-A) in diet of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*) and red drum (*Sciaenops ocellatus*) had no significant effects on trypsin, α-A-amylase and lipase activities at week 8. Guerreiro et al. [66] also found an enhancement of trypsin and lipase activities in White Sea bream (*Diplodus sargus*) fed fructooligosaccharides (scFOS), xylooligosaccharides (XOS) and galactooligosaccharides (GOS), at 15 days, however, by the end of the trial at 12 weeks those differences disappeared. The existing differences in these studies may be attribute to the sharp contrast between physiology and architecture of gastrointestinal tract in carnivores (e.g. hybrid striped bass and red drum) and omnivores/herbivores (e.g. crucian carp) fish [65].

In conclusion, the results of present study revealed that dietary administration of eryngii mushroom powder in diet of koi fish at levels of 1.5% and 2% improved growth performance, stimulated selected humoral innate immune responses and bactericidal activity of skin mucus and modulated intestinal microbiota. Therefore, mushroom can be considered as a natural prebiotic for modulation of fish immune system and prevention of disease outbreaks in aquaculture.

Conflicts of interest

There is no conflict of interest about the present manuscript.

Acknowledgement

The authors are grateful to the Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad for providing facilities and equipment and Toos Koi Company for providing fish for this research work.

References

[1] M.A. Jaleel, M.S. Musthafa, A.J. Ali, M.J. Mohamed, M.S.A. Kumar, V. Natarajan, G. Thiagarajan, Studies on the Growth Performance and Immune Response of Koi Carp Fingerlings (Cyprinus carpio Koi) Fed with Azomite vol. 4, (2015), pp. 160–169 (3).
[2] C. Goswami, V.S. Zade, Analysis of International Trade and Economical & Commercial Scope of Ornamental Fishes, (2015), pp. 36–40.
[3] N.K. Tripathi, K.S. Latimer, T.L. Lewis, V.V. Burnley, Biochemical reference intervals for koi (Cyprinus carpio), Comp. Clin. Pathol. 12 (2003) 160–165, http://dx.doi.org/10.1007/s00580-003-0495-x.
[4] W. Sirimanapong, A. Adams, E.L. Ooi, D.M. Green, D.K. Nguyen, C.L. Browdy, B. Collet, K.D. Thompson, The effects of feeding immunostimulant β-glucan on the immune response of Pangasianodon hypophthalmus, Fish Shellfish Immunol. 45 (2015), http://dx.doi.org/10.1016/j.fsi.2015.04.025.
[5] D. Ramesh, S. Souissi, Effects of potential probiotic *Bacillus subtilis* KADR1 and its subcellular components on immune responses and disease resistance in *Labeo rohita*, Aquacult. Res. 49 (2018) 367–377, http://dx.doi.org/10.1111/are.13467.
[6] W. Fish, Bacterial Fish Pathogens, (2007), http://dx.doi.org/10.1007/978-1-4020-6069-4.
[7] A. Rico, K. Satapornvanit, M.M. Haque, J. Min, P.T. Nguyen, T.C. Telfer, P.J. van den Brink, Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review, Rev. Aquacult. 4 (2012) 75–93, http://dx.doi.org/10.1111/j.1753-5131.2012.01062.x.
[8] O.E. Heuer, H. Kruse, K. Grave, P. Collignon, I. Karunasagar, F.J. Angulo, Human health consequences of use of antimicrobial agents in aquaculture, Clin. Infect. Dis. 49 (2009) 1248–1253, http://dx.doi.org/10.1086/605667.
[9] E. Vallejos-Vidal, F. Reyes-López, M. Teles, S. MacKenzie, The response of fish to immunostimulant diets, Fish Shellfish Immunol. 56 (2016) 34–69, http://dx.doi.org/10.1016/j.fsi.2016.06.028.
[10] E. Mehana, A. Rahmani, S. Aly, Immunostimulants and fish culture: an overview, Annu. Res. Rev. Biol. 5 (2015) 477–489, http://dx.doi.org/10.9734/ARRB/2015/9558.
[11] D.L. Merrifield, A. Dimitroglou, A. Foey, S.J. Davies, R.T.M. Baker, J. Bøgwald, M. Castex, E. Ringø, The current status and future focus of probiotic and prebiotic applications for salmonids, Aquaculture 302 (2010) 1–18, http://dx.doi.org/10.1016/j.aquaculture.2010.02.007.
[12] N. Abdullah, R. Abdulghani, S.M. Ismail, M.H.Z. Abidin, Immune-stimulatory potential of hot water extracts of selected edible mushrooms, Food Agric. Immunol. 28 (2017) 374–387, http://dx.doi.org/10.1080/09540105.2017.1293011.
[13] K. Katya, Y.H. Yun, G. Park, J.Y. Lee, G. Yoo, S.C. Bai, Evaluation of the efficacy of fermented by-product of mushroom, pleurotus ostreatus, as a fish meal replacer in juvenile amur catfish, silurus asotus: effects on growth, serological characteristics and immune responses, Asian-Australasian J. Anim. Sci. 27 (2014) 1478–1486, http://dx.doi.org/10.5713/ajas.2014.14038.
[14] O. J. K, Effectiveness of immunotherapies from oyster mushroom (pleurotus species) in the management of immunocompromised patients, Int. J. Immunol. 3 (2015) 8, http://dx.doi.org/10.11648/j.iji.s.2015030201.12.
[15] Y. Sun, X. Hu, W. Li, Antioxidant, antitumor and immunostimulatory activities of the polypeptide from Pleurotus eryngii mycelium, Int. J. Biol. Macromol. 97 (2017) 323–330, http://dx.doi.org/10.1016/j.ijbiomac.2017.01.043.
[16] H. Chen, Y. Ju, J. Li, M. Yu, Antioxidant activities of polysaccharides from Lentinus edodes and their significance for disease prevention, Int. J. Biol. Macromol. 50 (2012) 214–218, http://dx.doi.org/10.1016/j.ijbiomac.2011.10.027.
[17] SPOOBG_2004_v33n7_1092.pdf, (n.d.).
[18] A. Gregori, M. Švagelf, J. Pohleven, Cultivation techniques and medicinal properties of Pleurotus spp, Food Technol. Biotechnol. 45 (2007) 238–249.
[19] P. Manzi, L. Gambelli, S. Marconi, V. Vivanti, L. Pizzoferrato, Nutrients in edible mushrooms: an inter-species comparative study, Food Chem. 65 (1999) 477–482, http://dx.doi.org/10.1016/S0308-8146(98)00212-X.
[20] S.P. Wasser, A.L. Weis, Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (review), Int. J. Med. Mushrooms 1

(1999) 31–62, http://dx.doi.org/10.1615/IntJMedMushrooms.v1.i1.30.
[21] H. Van Doan, S. Doolgindachbaporn, A. Suksri, Effects of Eryngii mushroom (Pleurotus eryngii) and Lactobacillus plantarum on growth performance, immunity and disease resistance of Pangasius catfish (Pangasius bocourti, Sauvage 1880), Fish Physiol. Biochem. 42 (2016) 1427–1440, http://dx.doi.org/10.1007/s10695-016-0230-6.
[22] J.-K. Wan, W.-L. Chu, Y.-Y. Kok, K.-W. Cheong, Assessing the toxicity of copper oxide nanoparticles and copper sulfate in a tropical Chlorella, J. Appl. Phycol. (2018) 1–13.
[23] Hematology _ Principles and Procedures (Book, (1993) [WorldCat, (n.d.).
[24] P.C. Blaxhall, K.W. Daisley, Routine haematological methods for use with fish blood, J. Fish. Biol. 5 (1973) 771–781, http://dx.doi.org/10.1111/j.1095-8649.1973.tb04510.x.
[25] R.G. Lee, J. Foerster, J. Jukens, F. Paraskevas, J.P. Greer, G. Rodgers, Wintrobe's Clinical Hematology, tenth ed., Lippincott Williams & Wilkins, New York, 1998.
[26] B. Bank, Medical Laboratory, (n.d.).
[27] J. Kumari, P.K. Sahoo, T. Swain, S.K. Sahoo, A.K. Sahu, B.R. Mohanty, Seasonal variation in the innate immune parameters of the Asian catfish Clarias batrachus, Aquaculture 252 (2006) 121–127 https://doi.org/10.1016/j.aquaculture.2005.07.025.
[28] A.K. Siwicki, D.P. Anderson, G.L. Rumsey, Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis, Vet. Immunol. Immunopathol. 41 (1994) 125–139, http://dx.doi.org/10.1016/0165-2427(94)90062-0.
[29] T. Yano, Techniques in fish immunology : immunological and pathological techniques of aquatic invertebrates/[edited by J.S. Stolen. [et al.]. - Version details - Trove, (n.d.). https://trove.nla.gov.au/work/14038786?q&versionId=16595361 (accessed March 21, 2018).
[30] E.K. Sohn, Y.S. Chung, S.A. Johari, T.G. Kim, J.K. Kim, J.H. Lee, Y.H. Lee, S.W. Kang, I.J. Yu, Acute toxicity comparison of single-walled carbon nanotubes in various freshwater organisms, BioMed Res. Int. 2015 (2015), http://dx.doi.org/10.1155/2015/323090.
[31] W.E. Manual, K. Worthington, T.D. Guide, H. To, C. Worthington, A. Deaminase, A. Dehydrogenase, A.A. Oxidase, A.A. Oxidase, A. Aminotransferase, B. Agarase, C. Anhydrase, G. Oxidase, G. Oxidase, G. Decarboxylase, G. Dehydrogenase, G. Kinase, O. Decarboxylase, Worthington Enzyme Manual the Worthington Enzyme Manual Contains Technical Information on Enzymes Including Molecular Weight, (1993).
[32] B.F. Erlanger, N. Kokowsky, W. Cohen, The preparation and properties of two new chromogenic substrates of trypsin, Arch. Biochem. Biophys. 95 (1961) 271–278, http://dx.doi.org/10.1016/0003-9861(61)90145-X.
[33] A. Gawlicka, B. Parent, M.H. Horn, N. Ross, I. Opstad, O.J. Torrissen, Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (Hippoglossus hippoglossus): indication of readiness for first feeding, Aquaculture 184 (2000) 303–314, http://dx.doi.org/10.1016/S0044-8486(99)00322-1.
[34] S.H. Hoseinifar, A. Mirvaghefi, M.A. Amoozegar, D.L. Merrifield, E. Ringø, In vitro selection of a synbiotic and in vivo evaluation on intestinal microbiota, performance and physiological response of rainbow trout (Oncorhynchus mykiss) fingerlings, Aquacult. Nutr. 23 (2017) 111–118, http://dx.doi.org/10.1111/anu.12373.
[35] S. Subramanian, S.L. MacKinnon, N.W. Ross, A comparative study on innate immune parameters in the epidermal mucus of various fish species, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 148 (2007) 256–263, http://dx.doi.org/10.1016/j.cbpb.2007.06.003.
[36] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clin. Pathol. 45 (1966) 493–496 http://www.ncbi.nlm.nih.gov/pubmed/5325707 , Accessed date: 21 March 2018.
[37] D. Rairakhwada, A.K. Pal, Z.P. Bhathena, N.P. Sahu, A. Jha, S.C. Mukherjee, Dietary microbial levan enhances cellular non-specific immunity and survival of common carp (Cyprinus carpio) juveniles, Fish Shellfish Immunol. 22 (2007) 477–486, http://dx.doi.org/10.1016/j.fsi.2006.06.005.
[38] S. Bilen, M. Bulut, A.M. Bilen, Immunostimulant effects of Cotinus coggyria on rainbow trout (Oncorhynchus mykiss), Fish Shellfish Immunol. 30 (2011) 451–455, http://dx.doi.org/10.1016/j.fsi.2010.12.013.
[39] B. Magnadóttir, Innate immunity of fish (overview), Fish Shellfish Immunol. 20 (2006) 137–151, http://dx.doi.org/10.1016/j.fsi.2004.09.006.
[40] P. Fric, Probiotics and prebiotics - renaissance of a therapeutic principle, Cent. Eur. J. Med. 2 (2007) 237–270, http://dx.doi.org/10.2478/s11536-007-0031-5.
[41] P.K. Sahoo, S.C. Mukherjee, Journal of aquaculture in the tropics., Oxford and IBH, http://agris.fao.org/agris-search/search.do?recordID=US201302948721, (1999) , Accessed date: 21 March 2018.
[42] M.B. Munir, R. Hashim, S.A.M. Nor, T.L. Marsh, Effect of dietary prebiotics and probiotics on snakehead (Channa striata) health: haematology and disease resistance parameters against Aeromonas hydrophila, Fish Shellfish Immunol. 75 (2018) 99–108, http://dx.doi.org/10.1016/j.fsi.2018.02.005.
[43] A.K. Jha, A.K. Pal, N.P. Sahu, S. Kumar, S.C. Mukherjee, Haemato-immunological responses to dietary yeast RNA, ω-3 fatty acid and β-carotene in Catla catla juveniles, Fish Shellfish Immunol. 23 (2007) 917–927, http://dx.doi.org/10.1016/j.fsi.2007.01.011.
[44] C. Devakumar, A. Chinnasamy, Dietary administration of natural immunostimulants on growth performance, haematological, biochemical parameters and disease resistance of Asian Sea bass Lates calcarifer (Bloch, 1790), Aquacult. Res. 48 (2017) 1131–1145, http://dx.doi.org/10.1111/are.12955.
[45] S.H. Hoseinifar, A. Mirvaghefi, D.L. Merrifield, B.M. Amiri, S. Yelghi, K.D. Bastami, The study of some haematological and serum biochemical parameters of juvenile beluga (Huso huso) fed oligofructose, Fish Physiol. Biochemist 37 (2011) 91–96, http://dx.doi.org/10.1007/s10695-010-9420-9.
[46] H. Khodadadian Zou, S.H. Hoseinifar, H. Kolangi Miandare, A. Hajimoradloo, Agaricus Bisporus Powder Improved Cutaneous Mucosal and Serum Immune Parameters and Up-regulated Intestinal Cytokines Gene Expression in Common Carp (Cyprinus carpio) Fingerlings, Elsevier Ltd, 2016, http://dx.doi.org/10.1016/j.fsi.2016.09.050.
[47] C.S. Chang, S.L. Huang, S. Chen, S.N. Chen, Innate immune responses and efficacy of using mushroom beta-glucan mixture (MBG) on orange-spotted grouper, Epinephelus coioides, aquaculture, Fish Shellfish Immunol. 35 (2013) 115–125, http://dx.doi.org/10.1016/j.fsi.2013.04.004.
[48] E. Baba, G. Uluköy, C. Öntaş, Effects of feed supplemented with Lentinula edodes mushroom extract on the immune response of rainbow trout, Oncorhynchus mykiss, and disease resistance against Lactococcus garvieae, Aquaculture 448 (2015) 476–482, http://dx.doi.org/10.1016/j.aquaculture.2015.04.031.
[49] a Mueller, J. Raptis, P.J. Rice, J.H. Kalbfleisch, R.D. Stout, H.E. Ensley, W. Browder, D.L. Williams, The influence of glucan polymer structure and solution conformation on binding to (1– > 3)-beta-D-glucan receptors in a human monocyte-like cell line, Glycobiology 10 (2000) 339–346, http://dx.doi.org/10.1093/glycob/10.4.339.
[50] a S. Daba, O.U. Ezeronye, Anticancer effect of polysaccharides isolated from higher basidiomycetes mushrooms, Afr. J. Biotechnol. 2 (2003) 672–678.
[51] L. Hong, W. Weiyu, W. Qin, G. Shuzhen, W. lebin, Antioxidant and immunomodulatory effects of a α-glucan from fruit body of maitake (Grifola frondosa), Food Agric. Immunol. 24 (2013) 409–418, http://dx.doi.org/10.1080/09540105.2012.704901.
[52] L. Tort, Stress and immune modulation in fish, Dev. Comp. Immunol. 35 (2011) 1366–1375, http://dx.doi.org/10.1016/j.dci.2011.07.002.
[53] A. Abid, S.J. Davies, P. Waines, M. Emery, M. Castex, G. Gioacchini, O. Carnevali, R. Bickerdike, J. Romero, D.L. Merrifield, Dietary synbiotic application modulates Atlantic salmon (Salmo salar) intestinal microbial communities and intestinal immunity, Fish Shellfish Immunol. 35 (2013) 1948–1956, http://dx.doi.org/10.1016/j.fsi.2013.09.039.
[54] F.J. Gatesoupe, Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments, J. Mol. Microbiol. Biotechnol. 14 (2007) 107–114, http://dx.doi.org/10.1159/000106089.
[55] S.H. Hoseinifar, H. Eshaghzadeh, H. Vahabzadeh, N. Peykaran Mana, Modulation of growth performances, survival, digestive enzyme activities and intestinal microbiota in common carp (Cyprinus carpio) larvae using short chain fructooligosaccharide, Aquacult. Res. 47 (2016) 3246–3253, http://dx.doi.org/10.1111/are.12777.
[56] M. Roberfroid, G.R. Gibson, L. Hoyles, A.L. McCartney, R. Rastall, I. Rowland, D. Wolvers, B. Watzl, H. Szajewska, B. Stahl, F. Guarner, F. Respondek, K. Whelan, V. Coxam, M.-J. Davicco, L. Léotoing, Y. Wittrant, N.M. Delzenne, P.D. Cani, A.M. Neyrinck, A. Meheust, Prebiotic effects: metabolic and health benefits, Br. J. Nutr. 104 (2010) S1–S63, http://dx.doi.org/10.1017/S0007114510003363.
[57] N. Sheikhzadeh, M. Heidarieh, A. Karimi Pashaki, K. Nofouzi, M. Ahrab Farshbafi, M. Akbari, Hilyses®, fermented Saccharomyces cerevisiae, enhances the growth performance and skin non-specific immune parameters in rainbow trout (Oncorhynchus mykiss), Fish Shellfish Immunol. 32 (2012) 1083–1087, http://dx.doi.org/10.1016/j.fsi.2012.03.003.
[58] Z. Roosta, A. Hajimoradloo, R. Ghorbani, S.H. ossei, Hoseinifar, The effects of dietary vitamin C on mucosal immune responses and growth performance in Caspian roach (Rutilus rutilus caspicus) fry, Fish Physiol. Biochem. 40 (2014) 1601–1607, http://dx.doi.org/10.1007/s10695-014-9951-6.
[59] M. Ángeles Esteban, An overview of the immunological defenses in fish skin, ISRN Immunol 2012 (2012) 1–29, http://dx.doi.org/10.5402/2012/853470.
[60] K. Katya, Y.H. Yun, H. Yun, J.Y. Lee, S.C. Bai, Effects of dietary fermented by-product of mushroom, Pleurotus ostreatus, as an additive on growth, serological characteristics and nonspecific immune responses in juvenile Amur catfish, Silurus asotus, Aquacult. Res. 47 (2016) 1622–1630, http://dx.doi.org/10.1111/are.12623.
[61] R. Harikrishnan, M.C. Kim, J.S. Kim, C. Balasundaram, M.S. Heo, Effect of Coriolus versicolor supplemented diet on innate immune response and disease resistance in kelp grouper Epinephelus bruneus against Listonella anguillarum, Fish Shellfish Immunol. 32 (2012) 339–344, http://dx.doi.org/10.1016/j.fsi.2011.11.026.
[62] A.R.J. Mohd Din, S.A. Razak, V. Sabaratnam, Effect of mushroom supplementation as a prebiotic compound in super worm based diet on growth performance of red tilapia fingerlings, Sains Malays. 41 (2012) 1197–1203.
[63] R. Dobšíková, J. Blahová, I. Mikulíková, H. Modrá, E. Prášková, Z. Svobodová, M. Škorič, J. Jarkovský, A.K. Siwicki, The effect of oyster mushroom β-1.3/1.6-D-glucan and oxytetracycline antibiotic on biometrical, haematological, biochemical, and immunological indices, and histopathological changes in common carp (Cyprinus carpio L.), Fish Shellfish Immunol. 35 (2013) 1813–1823, http://dx.doi.org/10.1016/j.fsi.2013.09.006.
[64] B. Xu, Y. Wang, J. Li, Q. Lin, Effect of prebiotic xylooligosaccharides on growth performances and digestive enzyme activities of allogynogenetic crucian carp (Carassius auratus gibelio), Fish Physiol. Biochemist 35 (2009) 351–357, http://dx.doi.org/10.1007/s10695-008-9248-8.
[65] M. Anguiano, C. Pohlenz, A. Buentello, D.M. Gatlin, The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (Sciaenops ocellatus) and hybrid striped bass (Morone chrysops×Â M. saxatilis), Br. J. Nutr. 109 (2013) 623–629, http://dx.doi.org/10.1017/S0007114512001754.
[66] I. Guerreiro, C.R. Serra, P. Pousão-Ferreira, A. Oliva-Teles, P. Enes, Prebiotics effect on growth performance, hepatic intermediary metabolism, gut microbiota and digestive enzymes of white sea bream (Diplodus sargus), Aquacult. Nutr. 24 (2018) 153–163, http://dx.doi.org/10.1111/anu.12543.