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)

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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

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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