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Hilyses®, fermented *Saccharomyces cerevisiae*, enhances the growth performance and skin non-specific immune parameters in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Effects of Hilyses®, fermented *Saccharomyces cerevisiae* (*S. cerevisiae*), on growth, body composition and skin mucus immune components in rainbow trout were quantified. Ninety rainbow trout (105 ± 5 g) were randomly assigned to 2 groups in triplicates and fed dietary Hilyses® (5 g kg^{-1}) or control diet without Hilyses® for 50 days. Results of this study demonstrated that growth performance increased significantly by the dietary yeast supplement; however body composition was not affected in treatment group. At the 45th and 50th day of feeding trial, results of mucus samples demonstrated that yeast supplementation in treatment group significantly promoted enzyme activities, namely lysozyme, protease, alkaline phosphatase and esterase compared to control group. Significant increases were also observed in hemagglutination and antibacterial activity against *Yersinia ruckeri* in fish fed treatment diet. The present study suggests that fermented *S. cerevisiae* may effectively promote the growth performance and skin non-specific immune parameters in rainbow trout.

1. Introduction

In the intensive fish culture, suitable conditions for high numbers of pathogenic microorganisms are provided. Fish skin epithelium is the most important defense sites for protection against pathogens. Firstly, it prevents pathogen adherence by being continuously produced and sloughed off. Secondly, it comprises a number of immune components such as lysozyme, protease, immunoglobulins, complement proteins, C-reactive protein, lectins, proteolytic enzymes and various other antibacterial proteins and peptides [1–4].

High fish mortality, resulting in serious economic loss, is associated with the stress and increased infection rate caused by intensive rearing conditions. Many studies have looked into the modulation of the immune system in fish in order to prevent disease outbreaks and the possibility of altering nutrition to favor normal growth and enhance fish health [5,6]. Immunostimulants are biological and synthetic compounds that enhance non-specific defense mechanism in fish and they confer protection under adverse conditions. In contrast with numerous data on improved blood immune system with immunostimulants, there is little information regarding their effects on mucosal immunity [4,7,8].

Baker's yeast (*Saccharomyces cerevisiae*) (*S. cerevisiae*) is a natural product from the baker's industry that contains various immunostimulating compounds such as β-glucan, nucleic acids, mannan oligosaccharides and chitin. Hilyses® yeast is an additive obtained from fermentation of specific strains of *S. cerevisiae*, consists of peptide, free amino acid, glutamate, glutamine and nucleotides. Previously *S. cerevisiae* has been proved to enhance the blood immune responses and growth in fish species [9–11,11–15]. There is no published information concerning the effects of dietary *S. cerevisiae* on innate immune parameters in the epidermal mucus in fish species. The objective of this study was to determine the effect of Hilyses® yeast dietary supplementation on growth, body chemical composition and cutaneous mucosal immune response in rainbow trout.

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2. Material and methods

2.1. Fish

Ninety specimens (105 ± 5 g weight) of rainbow trout (*Oncorhynchus mykiss*) obtained from a fish farm in Karaj, Iran were kept in running (flow rate 0.5 lit s^{-1}) river water in 6 polypropylene tanks (300 l) at 15 ± 1 °C with a natural photoperiod (10L:14D). Fish were allowed to acclimatize for 14 days before the experiment and fed with pelleted diet (Chineh, Iran). The chemical composition of the commercial feed was 36% protein, 14% crude lipid, 4% crude fiber, 10% crude ash and 1% phosphorous.

2.2. Feeding

Fermented *S. cerevisiae* (Hilyses®) received from ICC (Brazil) was used in this study. Two experimental diets containing 0 (control) or 5 g kg^{-1} feed of Hilyses® were prepared from a commercial pellet diet. Fish were divided randomly into two groups (45 fish/group) with 15 fish in each tank and each resulting group was fed one of the two different diets at a total daily rate of 1.5% body weight for 50 days.

2.3. Fish performance

At the end of feeding period, 5 fish from each individual tank were starved for 24 h, then weighted and factors such as weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) were calculated as following:

Weight gain = final weight (g) − initial weight (g);

$$SGR = 100/(\ln W_2 - W_1)/T$$

where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period; FCR = dry feed intake (g)/weight gain (g).

2.4. Whole body chemical analysis

After calculating the growth parameters, fish were used for chemical carcass analysis. Dry matter was calculated by gravimetric analysis following oven drying at 55 °C for 48 h. Protein levels were calculated from the determination of total nitrogen by Kjeldhal digestion. Crude fat content was determined gravimetrically following extraction of the lipids according to the Soxhlet method (AOAC 1998) [16]. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550 °C for 12 h.

2.5. Mucus collection

Five fish were randomly sampled from each tank at day 45 and 50. After being kept 24 h without feeding, the fish were anaesthetized with clove oil (50 μl L^{-1}) and the skin mucus was collected by gentle scraping with enough care to avoid contamination with blood. The mucus was then homogenized with 4 volumes of Tris-buffered saline (TBS, 50 mM Tris–HCl, pH 8.0, 150 mM NaCl) and centrifuged at 1700 g for 30 min at 4 °C. The supernatant was frozen overnight at −20 °C and then transferred to −80 °C for longer term storage. Following the lyophilization (Alpha-1-2LD, Germany), 300 mg of the lyophilized skin mucus was added to 1 mL TBS buffer, and centrifuged at 1700 *g* for 30 min at 4 °C to obtain the supernatant. Protein concentrations of the resulting supernatants were determined by the dye binding method [17] to calculate the specific enzyme activities.

2.6. Analysis of enzyme activities in mucus samples

Alkaline phosphatase activity was determined through incubation of mucus supernatants with 4 mM para-nitrophenyl phosphate (Sigma) in 100 mM ammonium bicarbonate buffer containing 1 mM magnesium chloride, pH 7.8 at 30 °C following the method given in Palaksha et al. (2008) [18]. One unit of activity was defined as the amount of enzyme required to release 1 μmol of para-nitrophenyl product in 1 min.

Lysozyme activity was determined using a turbidometric assay [15]. 25 μl mucus supernatants were incubated in a 96 well plate with 75 μl of lyophilized *Micrococcus lysodeikticus* cells (Sigma, 75 μg ml^{-1}) in triplicate. A unit of lysozyme activity was defined as the amount of enzyme causing a decrease in absorbance of 0.001 per min (450 nm) and expressed as U mg^{-1} of mucus sample.

Protease activities were determined using the azocasein hydolysis assay described by Palaksha et al. (2008) [18]. The azocasein hydrolysis assay involved incubation of mucus supernatants with 100 mM ammonium bicarbonate buffer containing 0.7% azocasein, pH 7.8 for 19 h on a shaker at 30 °C, precipitation with 4.6% trichloroacetic acid followed by a 10 min centrifugation at 10,600 *g*. Equal volumes of the resultant supernatant was added to the microplate well containing 0.5 M NaOH and the increase in the OD values was measured at 405 nm.

Esterase activity was determined through incubation of mucus supernatants with 0.4 mM para-nitrophenyl myristate in 100 mM ammonium bicarbonate buffer containing 0.5% Triton X-100, pH 7.8 at 30 °C following the method given in Palaksha et al. (2008) [18]. The absorbance was measured continuously for 2 h at 405 nm by ELISA reader. The activity was defined as the amount of enzyme required to release 1 μmol of para-nitrophenyl product in 1 min.

2.7. Bactericidal activity

The bactericidal activity of fish mucus was determined by minimal inhibitory concentration (MIC) method. *Yersinia ruckeri* (BCCM5/LMG3279) used in this assay was inoculated with tryptic soy broth (Merck, Germany) and grown for 16 h at 25 °C. Fifty microliter of mucus supernatant (equivalent to 30 μg protein) was serially diluted for 8 times in tryptic soy broth. Equal volume of bacterial suspension was added to all the wells and after adding 50 μl of tryptic soy broth to all the wells, incubation overnight at room temperature was performed. The last well with visible bacterial inhibition was considered as the minimal inhibitory concentration in each sample.

2.8. Hemagglutination assay

Two milliliters of citrated chicken blood was suspended in 10 ml TBS and centrifuged at 2500 *g* for 10 min. The supernatant was aspirated without disturbing the blood cells. 10 ml TBS was added and mixed by inverting. Following the centrifugation and washing two more times, the supernatant was aspirated and 2.5% chicken red blood cell (C-RBC) suspension in TBS was prepared. In round bottomed microtiter plate, 50 μl of mucus supernatant (equivalent to 30 μg protein) was serially diluted in TBS buffer. Equal volumes of 2.5% C-RBC was added to all the wells and kept for 1 h at room temperature. The reciprocal of the highest dilution that gave agglutination was taken as the hemagglutinating titer.