Effect of dietary Ergosan and Hilyses on growth performance, hematological variables and immune response in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

This study was conducted to evaluate the efficiency of Ergosan, an algal extract containing alginic acid and Hilyses, a fermented *Saccharomyces cerevisiae*, on growth, immune response and hematological parameters in rainbow trout (*Oncorhynchus mykiss*). Fish with an initial average weight of 70-75 g were fed with Ergosan (5 g kg^{-1}) and Hilyses (10 g kg^{-1}) for a period of 70 days. The Results revealed that dietary supplementation of Ergosan and Hilyses enhanced the growth of all treated fish significantly compared to fish fed with non-supplemented diet. Dietary intake of Ergosan significantly increased the red blood cell, white blood cell and neutrophil levels compared to the Hilyses-fed and control groups. The highest IL-8 level and lysozyme activity were recorded in the Hilyses-fed fish followed by Ergosan. Serum total protein content was enhanced in fishes administrated with Ergosan compared to the control group. The findings of this study suggested the potential of Ergosan and Hilyses to activate growth performance and immunological parameters in rainbow trout.

Keywords: rainbow trout, Ergosan, Hilyses, growth, immune response.

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Introduction

Nowadays, intensive culture of fish species with exposure to various stress factors such as handling, crowding and infection has led to immune depression and outbreaks of infections (El-Boshy, El-Ashram, AbdelHamid & Gadalla 2010). Immunostimulants comprise a group of biological and synthetic compounds that increase the nonspecific cellular and humoral defence mechanisms in fish species. These substances, such as β-glucan, peptidoglycan, chitin, chitosan yeast and vitamin combinations, as well as various products derived from plants and animals are effective in preventing disease (Gopalakannan & Arul 2006).

Baker's yeast (*Saccharomyces cerevisiae*) is a natural product used for the bakers' industry which contains various immunostimulating compounds such as β-glucan, nucleic acids, mannan oligosaccharides and chitin (Tukmechi, Andarani, Manaffar & Sheikhzadeh 2011). Hilyses yeast, an additive obtained from fermentation of specific strains of *S. cerevisiae* consists of nucleotides, glutamine, glutamate, free amino acid and peptide. Effects of *S. cerevisiae* fermentation product on fish growth and immunity were previously reported (Barnes, Durben, Reeves & Sandes 2006; He, Zhou, Liu, Shi, Yao, Ringø & Yoon 2009).

Alginic acid is derived from several genera of brown algae including Macrocystis, Laminaria, Lessonia, Ascophyllum, Alaria, Ecklonia, Eisenia, Neroe-

cystis, Sargassum, Cystoseira and Fucus (Peddie, Zou & Secombes 2002). Ergosan is an algal extract composed of 0.002% unspecified plant extract, 1% alginic acid from Laminaria digitata, and 98.998% algal based carrier. Algines have been used for a range of commercial applications including thickening agents, gelling agents and dispersion stabilizers (Peddie *et al*., 2002). There are some studies in the literature revealing the effects of Ergosan on fish growth, immunity and hematological parameters (Peddie *et al*,. 2002; Montero-Rocha, McIntosh, Sanchez-Merino & Flores 2006; Jalali, Ahmadifar, Sudagar & Takami 2009; Gioacchini, Lombardo, Avella, Olivotto & Carnevali 2010). Therefore, this study aimed to evaluate and compare the efficiency of two immunostimulants, Ergosan and Hilyses, after 70 days administration on some haematological and immunological parameters as well as growth performance of rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Fish and Experimental design

A total number of 99 rainbow trout weighing 70-75 g were obtained from a fish farm in Karaj, and maintained in tanks with continuously aerated free-flowing dechlorinated fresh water at 14±1 ºC. The fish were acclimatized to the laboratory condition for 12 days and fed with commercial pelleted diet (Faradaneh, Fish feed manufature, Iran) at a level of 3% body weight four times daily. The proximate composition of basal diet comprised 38% crude protein, 14% crude lipid, 10% crude ash, and 4% crude fiber. Fsh were randomly distributed in three equal groups. Each group consisted of three replicates of 11 fish For a period of 70 days were fed with diet containing 5 g kg^{-1} of Ergosan according to manufacturer's recommendation (Schering-Plough Aquaculture, UK) and 10 g kg^{-1} of Hilyses (according to manufacturer's recommendation).

Growth performance

At the end of the feeding period, all fish from each individual tank were weighted, and weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) were calculated as follows:

Weight gain = final weight (g) - initial weight (g)

FCR = feed given (g)/weight gain (g)

SGR = $((\ln W_2 - \ln W1)/T)\ 100$

Where W_1, W_2, and T are the initial weight, final weight, and number of days in the feeding trial, respectively.

Blood collection

After 70 days of the feeding period, 10 fish in each tank were anaesthetized with clove oil bath (50 µl l^{-1}), then bled from the caudal peduncle by heparin syringes. The blood was then divided into two aliquots. One was heparinized, and the other was permitted to clot for 30 min at room temperature and then maintained for 5 h at 4°C. The clotted samples were centrifuged for 15 min at 3000 RPM at 4°C in order to collect the serum. The heparinized blood was immediately utilized for the haematological assays.

Haematological parameters

Red blood cells (RBCs) and white blood cells (WBCs) were counted using a Neubauer haemocytomter after dilution with phosphate-buffered-saline (PBS). Differential leukocyte counts namely neutrophil and lymphocyte were determined using blood smears under a light microscope. Hematocrit values (Hct) were determined by placing fresh blood in glass capillary tubes and centrifuging for 5 min in a microhematocrit centrifuge.

Level of interleukin-8 (IL-8) in fish serum was quantified using an IL-8 ELISA kit (Koma Biotech, Seoul, Korea) according to the manufacturer's instructions. Briefly, a rabbit anti-Human IL-8 pre-coated 96-well plate was incubated with 100 µL of standard or sample to each well in duplicate. After incubation for 2 h at room temperature, the plates were washed four times with PBS, 0.01% (v/v) Tween 20. After washing, biotinylated purified anti-Human IL-8 antibody (5 µg mL^{-1}) was added and incubated for 2 h. The plates were washed four times and incubated with 100 µl of the streptavidin-HRP conjugate (1:20 dilutions) for 30 min. After final incubation with 100 µl of color devel-

opment reagent (pink-ONE TMB solution) for 15 min, the reaction was stopped by adding 100 µL of the 2 M sulphuric acid to the wells. The plates were read at 450 nm using a microtiter plate reader. OD readings were converted to pg mL^{-1} using a standard curve and the appropriate dilution factor.

Lysozyme assay

Serum lysozyme activity was estimated by turbidimetric assay as described by Demers & Bayne (1997). *Micrococcus lysodeikticus* (*M. lysodeikticus*) (Sigma) suspended in 0.1 M phosphate citrate buffer, pH 5.8 at a concentration of 750 µg $mL^{-1}$was added to 25 µL of serum samples in 96-well microtiter plates. Immediately after the addition of 150 µL of *M. lysodeikticus*, the optical density was determined. A lysozyme activity unit was calculated as the amount of enzyme producing a decrease in absorbance of 0.001 min^{-1}.

Serum protein level

The protein concentration was determined according to biuret method using a commercial kit (Ziestchem diagnostics Co. Ltd., Tehran, Iran) in accordance with the manufacturer's protocols.

Statistical analysis

All data were presented as means ± SEM of three replicates treatments. Statistical analysis of data was carried out using one-way ANOVA and Tukey multiple range tests using SPSS for windows software, version 17. Differences in treatment means were considered significant at $P<0.05$.

Results

The growth parameters of weight gain, FCR and SGR in fish fed with Ergosan and Hilyses increased compared to the control fish group. No significant difference in growth performance between treatment groups was noted (Table 1).

Higher RBC, WBC and neutrophil counts were found in fish fed Ergosan than in fish fed Hilyses and control diet. No significant differences in Hct and lymphocyte values were shown in different groups (Table 2).

Table 1 Growth performance of rainbow trout fed with immunostimulant supplemented diet for 70 days

Treatments	Growth parameters		
	Weight gain	Feed conversion ratio	Specific growth rate
Control	74.32 ± 8.32^{a}	1.83 ± 0.18^{a}	3.24 ± 0.78^{a}
Ergosan	119.28 ± 4.03^{b}	1.19 ± 0.33^{b}	4.42 ± 0.31^{b}
Hilyses	113.03 ± 7.11^{b}	1.21 ± 0.09^{b}	4.38 ± 0.18^{b}

Means with the same letter in the same column are not significantly different ($P<0.05$).

Higher IL-8 was found in fish fed Hilyses (228.22 ± 23.88) and Ergosan (142.11 ± 0.4) than was observed in the control group. Higher lysozyme activity was shown in the Hilyses group (5.33 ± 1.38) followed by Ergosan group compared with the control group. The protein level of fish that received Ergosan increased compared with control group but no significant differences were found between treatment groups (Table 3).

Discussion

Fish feed supplementation with Ergosan and Hilyses was clearly beneficial, as indicated by the increased growth performance. In this study, all treatment groups showed higher gains in the body weight and SGR than the control group after a period of 70 days feeding. These findings are in accordance with the those on rainbow trout that received fermented *S. cerevisiae* (Barnes *et al*. 2006) and Ergosan (Gioacchini *et al*. 2010). Although the exact mechanism of improved growth in treatment groups is not clear, a number of factors such as better digestibility, immunomodulatory effects, positive effects on digestive enzyme activities, a reduction in pathogenic bacteria and an increase in positive bacteria in the fish gut have been proposed (Heidarieh *et al*. 2012). Hematological parameters of fish blood are useful tools that aid in diagnosis of disease. It can also be used to study immnuopotentiators. Haematological parameters showed significant changes in Ergosan-treated group in comparison with Hilyses-treated and control groups. In this study, elevated levels of RBC, WBC and neutrophil were observed in fish that received Ergosan. Similarly a higher number of lymphocytes were observed in the

Table 2 Hematological changes of rainbow trout fed with immunostimulant supplemented diet for 70 days

Treatments	Hematological parameters				
	RBC	WBC	Hct	Lymphocyte	Neutrophil
	($\times 10^6 mm^3$)	($\times 10^4 mm^3$)	(%)	(% of WBC)	(% of WBC)
Control	9.44±0.68^a	59.80±4.97^a	40.25±1.45^a	92.66±3.20^a	7.33±3.21^a
Ergosan	12.18±0.33^b	109.0±19.82^b	39.33±2.08^a	88.70±4.35^a	11.66±3.05^b
Hilyses	11.18±1.95^{ab}	34.33±0.57^a	34.33±0.57^a	92.00±4.20^a	7.00±3.01^a

Means with the same letter in the same column are not significantly different (P<0.05).

Table 3 Immunological changes of rainbow trout fed with immunostimulant supplemented diet for 70 days

Treatments	Immunological parameters		
	IL-8	Lysozyme	Total protein
	(pg mL^{-1})	(U mL^{-1})	(g dL^{-1})
Control	73.58±3.42^a	1.43±0.42^a	0.41±0.02^a
Ergosan	142.11±0.4^b	2.43±0.29^b	0.54±0.01^b
Hilyses	228.22±23.88^c	5.33±1.38^c	0.42±0.03^{ab}

Means with the same letter in the same column are not significantly different (P<0.05).

blood of fish that received Ergosan-supplemented diets (Jalali *et al*. 2009; Heidarieh, Soltani, Tamimi & Toluei 2011). Total and differential leukocytes counts are important indices in fish as leukocytes are involved in phagocytic and immune responses to pathogens (Tukmechi *et al*. 2011).

Plasma proteins include the humeral elements of the nonspecific immune system such as immunoglobulin (Tukmechi *et al*. 2011). In our study, the serum protein level was found to increase in fish fed with Ergosan. This study also indicated that fish treatment with Hilyses and Ergosan were effective in enhancing serum lysozyme activity. Lysozymes have an important role in innate immunity by lysis of bacterial cell walls, especially gram-positive bacteria and stimulate phagocytosis of bacteria. It is believed that the main sources of serum lysozyme are leukocyte cells, particularly basophils, monocytes and the lower rate from macrophages (Saurabh & Sahoo 2008). A significant increase in neutrophil count was just observed in an Ergosan treatment group. Such increase can cause an enhancement in lysozyme level of serum as seen in this study. However, no changes were found in neutrophil levels of fish administrated with Hilyses. Therefore, other mechanisms might be involved in elevating lysozyme activity in fish treated with Hilyses.

IL-8 is a chemokine produced by cell types of macrophages/monocytes, epithelial cells, neutrophils, fibroblasts, and endothelial cells upon infection or stimulated by cytokines such as IL^{-1} and TNF-α (Jimenez, Coll, Salguero & Tafalla 2006). In this study, significant increase in IL-8 level was shown in fish sera administrated with Hilyses and Ergosan. Intraperitoneal injection of Ergosan (1% alginic acid) at doses > 2.5 mg kg^{-1} augmented the expression of chemokines (IL-8) in rainbow trout leucocytes (Peddie *et al*. 2002). Similarly, in rainbow trout fed on alginic acid, IL-8 gene expression in spleen was significantly higher compared with control group (Gioacchini *et al*. 2010). The effect of various immunostimulants is related to what pattern recognition receptors they bind to and the immune responses of these receptors effect (Bricknell & Dalmo 2005). In this study, it seems that alginic acid and products from fermented S. Service (Hilyses) are legends for different receptors, and may be expected to cause different immune responses in fish (Iwamoto, Kurachi, Nakashima, Kim, Yamaguchi, Oda, Iwamoto & Muramatsu 2005).

In conclusion, these results show that Ergosan (5 g kg^{-1}) and Hilyses (10 g kg^{-1}) are able to enhance some nonspecific immunity and growth status of rainbow trout. Therefore, use of these immunostimulators can benefit the fish farmers via increasing the growth performance and immune system of fish.

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بررسی اثرات جیره حاوی ارگوسان و Hilyses بر روی عملکرد رشد، پارامترهای خونی و پاسخ ایمنی در ماهی قزل آلای رنگین کمان (*Oncorhynchus mykiss*)

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چکیده

در این مطالعه اثرات جیره حاوی ارگوسان و عصاره جلبک حاوی اسید آلژینیک و Hilyses بر روی رشد، سیستم ایمنی و پارامترهای خونی ماهی قزل آلا مورد ارزیابی قرار گرفت. ماهیان با میانگین وزن اولیه ۷۵–۷۰ گرم با جیره حاوی ارگوســان به غلظت ۵ گرم و Hilyses به غلظت ۱۰ گرم در کیلوگرم غذا به مدت ۷۰ روز تغذیه شدند. نتایج حاصله نشان داد که میزان رشد در گروه‌های تغذیه شده با جیره‌های حاوی ارگوسان و Hilyses از گروه شاهد بیشتر است. علاوه بر این تعداد گلبولهای قرمز و سفید و همچنین تعداد نوتروفیل‌ها در گروه تغذیه شده با جیره حاوی ارگوسان از بقیه تیمارها بیشتر بود. بالاترین میزان IL ۸ و فعالیت لیزوزیم ابتدا در گروه تغذیه شده با هیلسیس و سپس در گروه تغذیه شده با ارگوسان ثبت گردید. میزان کل پروتئین سرم در تیمار تغذیه با ارگوسان از گروه کنترل بالاتر بود. این نتایج نشان می‌دهد که ارگوسان و Hilyses قادر به بهبود عملکرد رشد و پارامترهای ایمنی در ماهیان قزل آلا هستند.

واژه‌های کلیدی: قزل الای رنگین کمان، ارگوسان، Hilyses، پارامترهای خونی، پاسخ ایمنی.

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