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Some immune Responses of Raw Fiber (Vitacel) on Giant Sturgeon (*Huso Huso*)

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ABSTRACT-The present study examined the effects of Vitacel raw fiber on great sturgeon (Huso huso) immunocompetent cell population size including lymphocyte and neutrophil populations and lysozyme activity after oral administration of the feed and Vitacel at 1.3% of the feed for 90 days. Generally, a significant increase was seen in the neutrophil count of fish receiving Vitacel as compared to the control group 90 days post treatment ($P<0.05$). Also, lysozyme activity in fish that received Vitacel was significantly higher than the control group 90 days post treatment ($P<0.05$). The results suggest that Vitacel may effectively promote immune responses in great sturgeon (Huso huso).

Keywords: Huso huso, Lysozyme, Raw fiber, Vitacel

INTRODUCTION

With worldwide fish production and intensive cultivation systems, fish are subjected to many diseases leading to great losses and decreases in fish production. A number of approaches have been applied in an attempt to address this problem including sanitary prophylaxis, disinfection, chemotherapy with particular emphasis on the use of antibiotics, and in recent years, vaccination against specific diseases (1). An alternative approach has been the application of various compounds to boost or stimulate the innate immune system of cultured fish. These compounds, known as immunostimulants, include bacteria and bacterial products, complex carbohydrates, nutritional factors, animal extracts, cytokines, lectins, plant extracts and synthetic drugs such as levamisole (2).

Phytogenics can exert multiple effects on organisms, including the improvement of feed palatability, efficiency and digestion, reduction of nitrogen excretion and improvement of gut flora and health status (3, 4). Meanwhile, other properties of herbs, such as antioxidant and antiviral properties, or their effects on the immune system cannot be ignored. One such example is Vitacel R 200 (raw fiber) which is a

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pure substance with at least 70% crude fiber (5). Previous studies were conducted at the University of Mexico to examine the effect of crude fiber on the increase of intestinal villi. Much of the finest fibers stimulate the circulation of blood in the intestinal villi, which improves the digestibility of nutrients (5). Besides mammals, no data is available on the effect of Vitacel on aquatic animals, particularly fish .
Therefore, the aim of this study was to evaluate the effects of Vitacel on a number of immune variables of the giant sturgeon (*Huso huso*).

MATERIALS AND METHODS

Fish

Juvenile sturgeon (*Huso huso*) weighing 100-110 g from a Caspian Sea fish farm were used for the experiments. The fish were acclimated to new conditions provided in 12 polypropylene tanks (300 l) with the flow rate set at 0.5 litre s^{-1}, water temperature 22-25^{o}C and dissolved oxygen 4-6 ppm under a natural photoperiod (10L : 14D) for 90 days. Adaptation to these tanks was performed for 10 days with a commercially pelleted diet from BioMar products used for rainbow trout 1.5% of the body weight per day.

Feeding

Raw fibre (Vitacel®) received from JRS (Germany) was used in this study. Two experimental diets containing 0 (control) or 13 g kg-1 feed were prepared from a commercial pellet diet (Chineh, Iran).

Experimental design

Fish were randomly divided into 2 groups with 47 in each group in three replicates. In treatment groups, fish were fed 1.3 % Vitacel per kg food according to the manufacturer's recommended inclusion level for a period of 90 days. Fish were fed at the same fixed rate of 1.5% body weight thrice daily at 0700, 1200 and 1800 h. Fish in each pond were group-weighed biweekly and the amounts of feed given were readjusted accordingly. A control group was included without any treatment.

Blood Sample Collection and Lysozyme Assay

Fish blood samples were collected by caudal vein puncture in heparinised syringes after the fish were anesthetized with clove oil at 50 ul/L on 15 and 90 days post-trial. Blood samples were centrifuged at 7000×g for 30 minutes and separated sera were used for lysozyme assay described by Ellis (1990) with some modifications. Briefly, aliquots (1.75 ml) of *Micrococcus lysodeikticus* suspension (0.375 mg/L of 0.05 sodium phosphate buffer, pH 6.2) was mixed with 250 µl of each sample and the optical density was measured after 15 and 180 seconds by a spectrophotometer at 670 nm. PBS was used as the blank and results were expressed in amounts of lysozyme (µg) per one milliliter of serum sample. During collection time, blood smears were obtained, air dried, fixed in 96% ethanol for 30 min then stained by Giemsa staining for 30 min. The stained smears were then examined for lecoucyte differential counts under a compound light microscope (6, 7).

Statistical Analysis

The results were subjected to independent-samples t-tests to compare different treatments using SPSS 15. Correlation coefficients were significant at P<0.05.

RESULTS

Lysozyme Content

The results of lysozyme contents are shown in table 2. After 15 days, lyzosyme levels in fish fed with Vitacel were insignificantly higher than the control (P<0.05) (table 2). When the level of lysozyme was measured 90 days post-treatment, its content in fish fed withVitacel significantly increased compared to the control (P<0.05) (table 2).

Differential Count

Lymphocyte and neutrophil profiles of the experimental fish following oral administration of Vitacel are shown in Table 1. Lymphocyte counts in the Vitacel group was significantly lower than the control group on day 90 post-treatment (P<0.05). Also, neutrophil counts in the Vitacel group significantly increased at the end of the experiment as compared to the control group (P<0.05).

Table 1. Leucocyte profiles of *Huso huso* following oral administration Vitacel at 22°C. (Mean ± S.D).

Time post –treatment (day)		
Trial	**15**	**90**
Lymphocyte (%)		
A	**34.33^{b}**	**24.33^{b}**
B	**53.67^{a}**	**42.00^{a}**
Neutrophil (%)		
A	**32.67^{a}**	**46.67^{a}**
B	**22.00^{b}**	**32.00^{b}**
Monocyte (%)		
A	**1.00^{a}**	**0.80^{a}**
B	**1.00^{a}**	**1.00^{a}**
Eosinophil (%)		
A	**22.33^{a}**	**27.66^{a}**
B	**24.33^{a}**	**25.66^{a}**

A: Vitacel group, B: control group, * Values are significantly different (P<0.05).

DISCUSSION

No mortality occurred during the 90-day test of the effect of Vitacel on certain immunological juvenile sturgeon weighing approximately 100 g in the treatment groups. The results of this study showed the improvement of the sturgeon immune system by Vitacel substance. Lysozyme, a strong antibacterial enzyme of the innate immune system has been studied in different organs or tissues of various fish species in continuous contact with microorganisms (8). These data showed that Vitacel changed the level of lysozyme in the serum of juvenile *Huso huso*. This result is supported by the changing appearance of neutrophils and lymphocytes in fish fed with Vitacel as an immunostimulant substance. In addition, the increased weight of fish fed with Vitacel is indicative of increased levels of the plasma growth hormone (9) which has positive effects on the excretion of lysozyme contents in the fish serum. The differences in the levels of lysozyme in fish fed with Vitacel compared to the control group may not be due to differences in the functional organization of fish, but the proportion of cells in particular organs involved in the neutralization of the Vitacel.

CONCLUSION

The comparison of these findings on the concentration of serum lysozyme in fish has shown that the level of lysozyme is dependent on various factors. The results of this study showed that *H. huso* juvenile reacts to Vitacel by increasing lysozyme levels in the serum.

Table 2. The level of lysozyme in fish sera of *Huso huso* following oral administration Vitacel at 22 °C. (Mean ± S.D) (P<0.05).

Time post –treatment (day)		
Trial	**15**	**90**
0 g kg^{-1} Vitacel R	**7.5 ± 0.01^{b}**	**4.2 ± 0.01^{b}**
13 g kg^{-1} Vitacel R	**19.8 ± 0.02^{a}**	**15 ±0.01^{a}**

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اثر فیبر خام (ویتاسل) بر جمعیت سلولهای ایمنی و میزان لیزوزیم فیل ماهی

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چکیده- به منظور بررسی تاثیر ویتاسل بر میزان لنفوسیت و نوتروفیل و فعالیت لیزوزیم فیل ماهیان 100 گرمی، آزمایشی به مدت 90 روز انجام گرفت. فیبر خام (ویتاسل) به میزان 1/3درصد به جیره غذایی فیل ماهیان اضافه گردید. در پایان دوره آزمایش، میزان سلولهای ایمنی (لنفوسیت و نوتروفیل) و فعالیت لیزوزیم در مقایسه با گروه کنترل مورد تجزیه و تحلیل قرار گرفتند. نتایج بدست آمده نشان داد که افزودن ویتاسل به مدت90روز در جیره غذایی فیل ماهیان سبب افزایش معنی دار میزان نوتروفیل نسبت به گروه کنترل شده است ($p<0/05$). فعالیت لیزوزیم نیز پس از افزودن ویتاسل به مدت90روز در جیره غذایی فیل ماهیان به طور معنی داری بیشتر از فعالیت لیزوزیم نسبت به گروه شاهد بود ($p <0/05$).

واژه های کلیدی: **ویتاسل، فیل ماهی، لیزوزیم،فیبر خام**

* به ترتیب استادیار، استاد، مربی و مربی

** مکاتبه کننده