

## Effect of dietary A-Max supplementation as a prebiotic on growth performance and hemato- immunological parameters of great sturgeon (*Huso huso* Linnaeus, 1758) juveniles

Akrami R.<sup>1\*</sup>, Chitsaz H.<sup>1</sup>, Lakzaei F.<sup>2</sup>

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### Abstract

An 8-week feeding trial was conducted to evaluate the effects of dietary commercial prebiotic A-Max, a mixture of mannan oligosaccharides, fructo oligosaccharides and  $\beta$  glucan, on growth and hemato-immunological parameters of great sturgeon (*Huso huso*) juveniles. After acclimation, fish (initial weight of  $28.79 \pm 0.85$  g) were placed into 12 tanks (30 fish per tank). Fish were fed a basal diet (containing 44% protein and 20% lipid) supplemented with 0 (control), 0.5, 1.0 and 1.5 g kg<sup>-1</sup> A-Max in a totally randomized design in triplicate groups. At the end of the trial, growth factors and haemato-immunological parameters were assessed. Fish fed 1.5 g kg<sup>-1</sup> prebiotic mixture displayed higher growth performances and feed efficiency compared to the control group ( $p > 0.05$ ). There were no significant differences in survival rate among all treatment groups ( $p > 0.05$ ). The results showed that RBC, WBC, lymphocyte, neutrophil, haematocrit and total protein were significantly affected by 1.5 g kg<sup>-1</sup> dietary prebiotic mixture ( $p < 0.05$ ). An elevation of monocyte and haemoglobin ( $p > 0.05$ ) was found in the fish fed diet containing 1.0 g kg<sup>-1</sup> prebiotic. In addition fish fed the diet with 1.5 g kg<sup>-1</sup> prebiotic mixture showed a significant increase in lysozyme activity, respiratory burst activity, serum total immunoglobulin (Ig) and alternative complement activity (ACH50) ( $p < 0.05$ ) compared with those fed the diets supplemented with other levels of prebiotics. The results showed that the addition of 1.5 g kg<sup>-1</sup> prebiotic mixture to the diet of great sturgeon juveniles improving growth performance, some haematological parameters and immune response and seemed to be an effective immunostimulant.

**Keywords:** Prebiotic mixture, Growth, Hematology, Immune response, Great sturgeon (*Huso huso*)

1-Department of Fisheries, Azadshahr Branch, Islamic Azad University, Azadshahr, Iran.

2 Iranian Fisheries Sciences Research Institute, Tehran, Iran

\*Corresponding author's E-mail: akrami.aqua@gmail.com

## Introduction

Growth enhancement and disease resistance of aquaculture organisms are two of the most important concerns (Li *et al.*, 2005b). The use of dietary additives in fish farms is one of the methods commonly used to improve weight gain, feed efficiency, and/or disease resistance in cultured fish. Therefore, several kinds of additives for aquafeed that are used to improve the performance of fish have been studied (Cho and Lee, 2012). Recently, immunostimulants such as prebiotics have shown promise as preventive and environmentally friendly alternatives to antibiotics in aquaculture (Sang *et al.*, 2009). Prebiotics are non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth and/or activity of health-promoting bacteria in the intestinal tract (Gibson, 2004). A-Max is a commercial prebiotic mixture of mannan oligosaccharides, fructo oligosaccharides and immunostimulating compound such as  $\beta$ -glucan. The mentioned elements are derived from the cell wall of yeast (*Saccharomyces cerevisiae*). Mannan oligosaccharides (MOS) and fructo-oligosaccharides (FOS) are two frequently used prebiotics with unique chemical structures that may improve the gut health and ecosystem of the host in different ways (Ye *et al.*, 2011).  $\beta$ -glucans are polysaccharides extracted from yeast cell walls. Their immunostimulating function is linked to the presence of species receptors at the surface of macrophages and other phagocytic cells of fish (Verlhac

Trichet, 2010). Intake of prebiotics could significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota (Gibson and Roberfroid, 1995). The introduction of prebiotics in sturgeon nutrition could be an interesting alternative to improve feed efficiency and sturgeon health. Sturgeons are valuable species, which are currently highly endangered (Safarpour Amlashi *et al.*, 2011). Sturgeon culture has seen considerable progress in recent years because artificial culture up to marketable size is important to reduce pressure on natural populations of sturgeon in the Caspian Sea (Pourkazemi, 1997). Great sturgeon, *Huso huso*, is an important aquaculture species in Russia, Eastern Europe, Japan and Iran. This species is good in aquaculture activities (Mohseni *et al.*, 2006). Bans and restrictions on antibiotics as feed additives in fish culture in many countries have resulted in the increase in studies on alternative dietary supplements such as probiotics and prebiotics to enhance the health and production of cultured fish (Hoseinifar *et al.*, 2011a). However, despite recent advances on the administration of prebiotics on other species, the information on the efficacy of dietary prebiotic in the culture of sturgeon fish has been limited (Akrami *et al.*, 2009; Hoseinifar *et al.*, 2011a; Ta'ati *et al.*, 2011; Razeghi Mansour *et al.*, 2012; Akrami *et al.*, 2013). Hence, this study aimed to assess the effect of a prebiotic mixture on the growth performance and haemato-immunological parameters of great sturgeon (*H. huso*) juvenile.

## Materials and methods

### *Prebiotic mixture (A-Max)*

A-Max is a commercial prebiotic mixture of mannan oligosaccharides (14.62%), fructo oligosaccharides (8.06%) and immunostimulating compound such as  $\beta$ -glucan (15.58%). The mentioned elements are derived from the cell wall of yeast (*S. cerevisiae*).

### *Diet preparation*

To prepare the diets, a commercial pelleted diet (containing 44% protein, 20% lipid, 7% ash and 22.71 MJ kg<sup>-1</sup> GE) was crushed, mixed with the appropriate prebiotic mixture (A-Max) concentration and water, and made again into the pellets, which were allowed to dry for 18 h at 45 °C by air circulation and stored at 4 °C until use. The control diet was prepared by adding only water (Cerezuela *et al.*, 2008; Akrami *et al.*, 2013). The dietary prebiotic mixture (A-Max) was supplemented at levels of 0 (control), 0.5, 1.0 and 1.5 g kg<sup>-1</sup> dry food for the four experimental groups. The control group received no prebiotic mixture supplement. The approximate chemical composition of formulated diet was determined according to standard methodology (AOAC, 2005).

### *Feeding and culture system*

Great sturgeon Juveniles were obtained from Shahid Marjani Sturgeon Hatchery Center (Gorgan, Iran) and stocked in the experimental fiberglass tanks (2 m × 2 m × 0.5 m) for 2 weeks before the beginning of the

experimental regime, in order to condition the fish to the laboratory system and handling procedures, and then, 360 fish at a mean weight of 28.79±0.85 g were randomly allocated to 12 tanks, with 30 fish in each tank, and three replicates per experimental group. The tanks were connected to a continuous circulating system. Continuous aeration was provided to each tank through air stones connected to a central air compressor. During the experimental period, water temperature, dissolved oxygen and pH were 25.5±1.5 °C, 4.86±0.28 mg L<sup>-1</sup> and 7.9±0.2, respectively. During the trial, the fish were hand-fed at a rate of 2-5% of the body weight per day (Razeghi Mansour *et al.*, 2012), spread across 3 feeding times (08:00, 14:00 and 20:00 h). The daily weight of feed consumed by the fish in each tank was recorded at the end of each day. Dead fish were recorded, and if a fish died during the day it was assumed that it had not consumed feed that day. The feeding trial was carried out for 8 weeks.

### *Growth performance*

In order to analyze the growth indices all of fish from each tank were weighed every 2 weeks, at least 12 h after the last feeding. The fish were weighed by a digital scale (to the nearest 0.01 g) after they had been anesthetized. Based on the results of the biometry, the daily ration of the fish in the supplemented groups and in the control was determined. At the end of the feeding trial, weight gain (WG%), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate were

calculated according to the following formulae:

Weight gain (WG g) = final weight of fish - initial weight of fish (Tacon, 1990).

Specific growth rate (SGR %/day) =  $100 \times [\ln \text{ final weight of fish} - \ln \text{ initial weight of fish}] / \text{days of feeding}$  (Hevroy *et al.*, 2005).

Feed conversion ratio (FCR) = dry feed fed (g) / wet weight gain (g) (Hevroy *et al.*, 2005).

Survival rate % =  $100 \times [\text{initial number of fish} - \text{final number of fish}] / \text{initial number of fish}$  (Ai *et al.*, 2006).

#### *Blood sample collection*

At the end of the experiment, 6 fish were sampled randomly from each tank and were anaesthetized with clove solution and about 2 mL of blood was drawn from the caudal vein, using a non-heparinized syringe, after they were starved for 24 h. Then, blood samples were introduced to both heparinized and non-heparinized tubes in order to perform haematological and immunological studies, respectively. For serum isolation, blood samples into non-heparinized tubes were centrifuged at 3000 rpm for 10 min (using a Heraeus Labofuge 400) and the sera were removed with a disposable transfer pipette. The latter was stored in the freezer at -20 °C for analysis of total protein, glucose and Immunological parameters test (Ibrahim *et al.*, 2010).

#### *Hemato-biochemical assays*

In order to study the haematological parameters, the blood samples were suspended in heparinized tube in order to blood cell investigations. The erythrocyte (RBC) and leukocyte (WBC) counts were determined using a Neubauer haemocytometer (Blaxhall and Daisley, 1973). Hemoglobin levels (Hb) were obtained by the cyano-

methemoglobin spectrophotometry method (Dorafshan *et al.*, 2008). Haematocrit was measured using the standard microhematocrit method and reported as percentages. To estimate the differential leukocyte counts (lymphocytes, monocyte and neutrophils) blood smears were prepared, air-dried, fixed in methanol, and stained using May–Giemsa solution (Blaxhall and Daisley, 1973). Total protein and glucose levels were determined by the Biuret and glucose oxidase methods, respectively (Asadi *et al.*, 2009).

#### *Immunological assays*

##### *Lysozyme activity*

Lysozyme level was determined by turbidometric assay according to the method of Ellis (1990). With slight modifications. Aliquots (1.75 mL<sup>-1</sup>) of *Micrococcus lysodeikticus* suspension (Sigma) (0.375 mg mL<sup>-1</sup>, 0.05 M PBS, pH 6.2) were mixed with 250 µL<sup>-1</sup> of each sample and the optical density was measured after 15 and 180 s by spectrophotometer (Biophotometer Eppendorf) at 670 nm. PBS was used as the blank and results were expressed in amounts of lysozyme (µg) per 1 mg of sample calibrated using a standard

curve determined with hens egg white lysozyme (Sigma) in sterile sodium phosphate buffer.

#### *Respiratory burst activity*

The generation of intracellular superoxide radicals by sole phagocytes was determined by the reduction of nitro-blue tetra-zolium (NBT) according to the technique described by Secombes (1990) and Boesen *et al.* (2001). Phagocyte monolayers were washed with L<sup>-15</sup> medium and HBSS (Hank's Balanced Salt Solution) to remove any trace of antibiotics. Then, 100 µL NBT, dissolved at 1mg mL<sup>-1</sup> in HBSS, were added to the wells and the phagocytes incubated at 22 °C for 30 min. After incubation, the medium was removed and the culture was washed twice with isotonic PBS, fixed with 100 µL/well of 100% methanol for 3 min, and washed twice with 70% methanol; then, the cells were air dried. Formazan was solubilized in 120 µL of KOH (2 M) plus 120 µL of dimethyl sulfoxide (DMSO) and the absorbance was read spectrophotometrically (Hitachi) at 620 nm using KOH/DMSO as a blank.

#### *Serum total immunoglobulin (Ig)*

Serum total immunoglobulin (Ig) levels were determined according to the method described by Siwicki and Anderson (1993). Briefly, serum total protein content was measured using a microprotein determination method (C-690; Sigma), prior to and after precipitating down the immunoglobulin molecules, using a 12% solution of polyethylene glycol (Sigma). The

difference in protein content represents the Ig content.

#### *Alternative complement activity (ACH50)*

Alternative complement activity was assayed according to the procedure of Yano (1992). By using rabbit red blood cells (RaRBC). The volume of serum yielding 50% haemolysis was determined and used to calculate the complement activity of the sample (value of ACH50 is in units per mL).

#### *Statistical analysis*

The normality and homogeneity of data were explored by examining the residual plots. The data were subjected to one-way analysis of variance (ANOVA), and if significant ( $p < 0.05$ ) differences were found, Duncan's multiple range test was used to rank the groups using SPSS (version 15).

## **Results**

The effects of the different levels of dietary prebiotic mixture (A-Max, from USA) on the growth performance and feed utilization of great sturgeon juvenile are shown in Table 1. At the end of the trial, there were no significant differences in growth and feeding parameters such as weight gain, SGR and FCR between juveniles fed control and prebiotic mixture supplementation diets ( $p > 0.05$ ). No mortality was observed during the experiment ( $p > 0.05$ ).

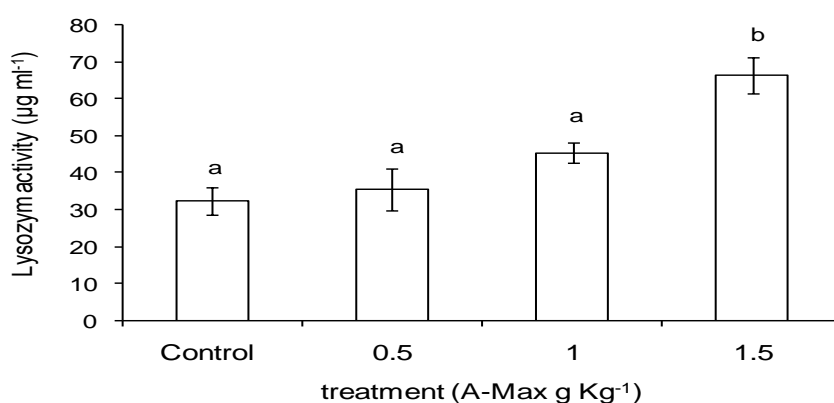
**Table 1: Growth performance and feed utilization of great sturgeon juvenile fed with diets containing different levels of prebiotic (A-Max) for 60 days.**

Levels of prebiotic (g kg <sup>-1</sup> )	Control	0.5	1	1.5
Parameters				
Weight gain %	612.7±41.8	628.4±64.9	637.23±56.6	644.2±42.4
SGR (%/day)	3.41±0.11	3.47±0.23	3.54±0.19	3.57±0.16
FCR	0.86±0.04	0.85±0.07	0.83±0.05	0.81±0.02

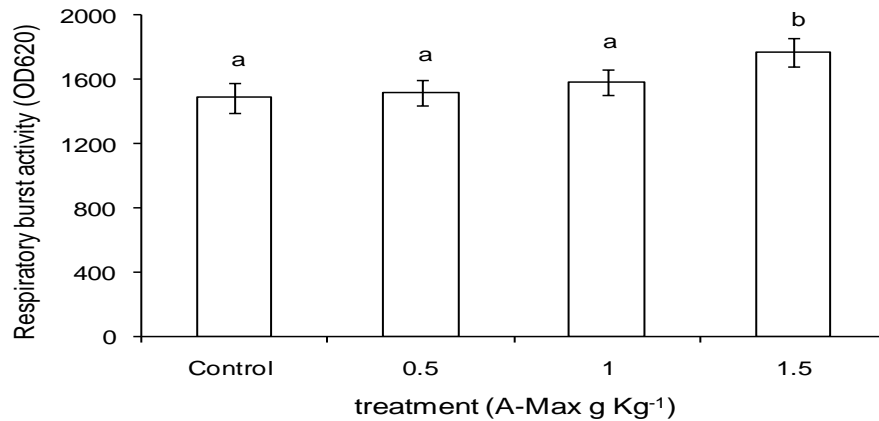
Data expressed as mean ± SD, (n=30). Values in the same row sharing the same superscript letter are not significantly different ( $p>0.05$ ).

The effects of the different levels of dietary prebiotic mixture (A-Max) on the innate immune responses of great sturgeon juvenile are shown in Figs. 1–4. All innate immune responses measured (i.e. lysozyme activity, respiratory burst activity, total immunoglobulin and alternative complement activity) were significantly higher ( $p<0.05$ ) in fish fed the diet with 1.5 g kg<sup>-1</sup> prebiotic compared to the control group. The lysozyme activity (66.3±4.8 µg mL<sup>-1</sup>) and respiratory burst activity (1768±87.9 OD620) of serum were significantly affected by 1.5 g kg<sup>-1</sup> dietary prebiotic mixture ( $p<0.05$ ). There were no significant

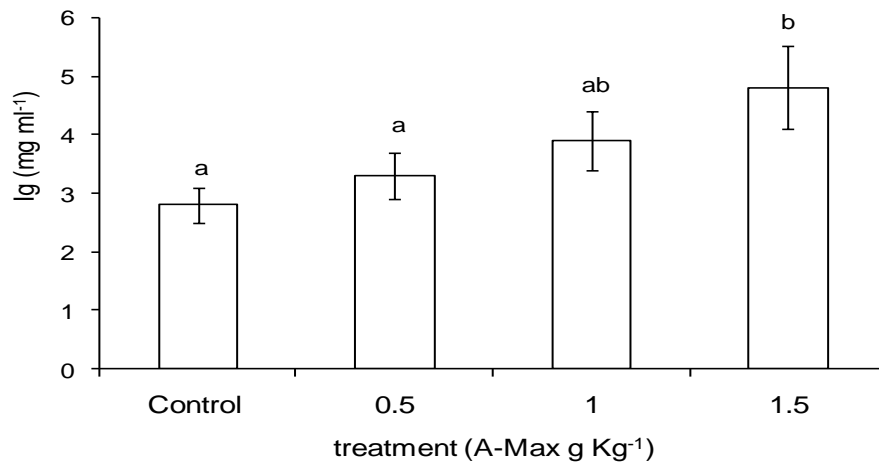
difference between the 0.5 and 1 g kg<sup>-1</sup> prebiotic mixture group and the control group ( $p>0.05$ ) (Figs. 1 and 2). Significant differences in total immunoglobulin and alternative complement activity (ACH50) levels were observed between the treatments ( $p<0.05$ ). Fish fed 1.5 g kg<sup>-1</sup> prebiotic mixture played significantly elevated total immunoglobulin (4.8±0.7mg mL<sup>-1</sup>) and ACH50 (85.3±12.6 U mL<sup>-1</sup>) compared to the control group ( $p<0.05$ ). There were no significant difference between the 0.5 g kg<sup>-1</sup> prebiotic mixture and the control group ( $p>0.05$ ) (Figs. 3 and 4).



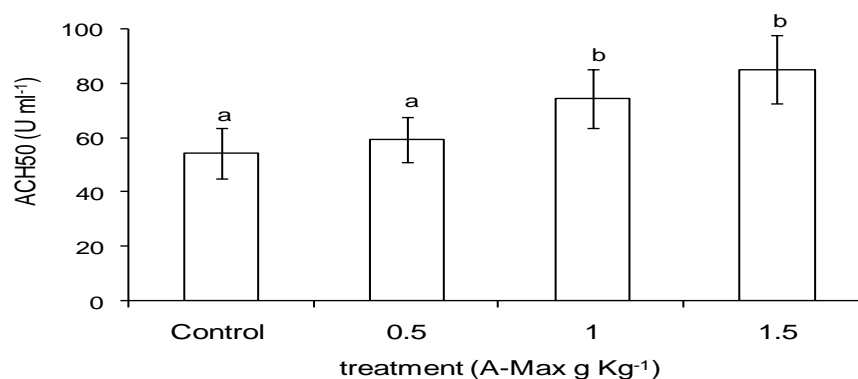
**Figure 1: Serum lysozyme activity of great sturgeon juvenile fed with diets containing different levels of prebiotic (A-Max) for 60 days. Data represent the mean±SD. Bars assigned with different superscripts are significantly different ( $p<0.05$ ).**



**Figure 2:** Respiratory burst activity (OD620) of great sturgeon juvenile fed with diets containing different levels of prebiotic (A-Max) for 60 days. Data represent the mean $\pm$ SD. Bars assigned with different superscripts are significantly different ( $p<0.05$ ).



**Figure 3:** Serum total immunoglobulin (Ig) levels of great sturgeon juvenile fed with diets containing different levels of prebiotic (A-Max) for 60 days. Data represent the mean $\pm$ SD. Bars assigned with different superscripts are significantly different ( $p<0.05$ ).



**Figure 4:** Serum alternative complement activity (ACH50) of great sturgeon juvenile fed with diets containing different levels of prebiotic (A-Max) for 60 days. Data represent the mean $\pm$ SD. Bars assigned with different superscripts are significantly different ( $p<0.05$ ).

Hematological and biochemical parameters of great sturgeon juvenile fed on different levels of dietary

prebiotic mixture (A-Max) are shown in table 2. The number of erythrocyte (RBC), leucocytes (WBC),

lymphocytes, neutrophils and hematocrit were significantly ( $p < 0.05$ ) higher in the groups that were fed prebiotic mixture diet at  $1.5 \text{ g kg}^{-1}$  feed (Table 2). An elevation of hemoglobin level and monocyte count was found following feeding with  $1.5 \text{ g kg}^{-1}$  prebiotic diet although they were not significantly different ( $p > 0.05$ ).

Prebiotic mixture added diets affected the blood glucose level, being relatively lower in all treated groups compared with the control (Table 2). The lowest glucose content and highest protein content were found in the fish fed the diet with  $1.5 \text{ g kg}^{-1}$  prebiotic mixture compared to the control group ( $p < 0.05$ ).

**Table 2: Hematological and blood serum biochemical parameters of great sturgeon juvenile fed with prebiotic A-Max added diet at different levels for 60 days.**

Levels of prebiotic (g kg <sup>-1</sup> )	Control	0.5	1	1.5
<b>Parameters</b>				
RBC (10 <sup>6</sup> mL <sup>-1</sup> )	0.8±0.07 <sup>c</sup>	0.82±0.13 <sup>c</sup>	1.04±0.1 <sup>b</sup>	1.56±0.02 <sup>a</sup>
WBC (10 <sup>3</sup> mL <sup>-1</sup> )	18.6±0.95 <sup>c</sup>	19.2±0.95 <sup>c</sup>	20.83±0.89 <sup>b</sup>	22.76±0.21 <sup>a</sup>
Lymphocytes (%)	74.33±3.78 <sup>b</sup>	71.51±2.15 <sup>b</sup>	73.66±1.52 <sup>b</sup>	79.47±1.94 <sup>a</sup>
Monocytes (%)	3.9±0.14	3.92±0.17	4.12±0.15	4.18±0.12
Neutrophils (%)	15.32±1.21 <sup>b</sup>	16.47±1.72 <sup>ab</sup>	17.35±1.52 <sup>ab</sup>	19.68±0.57 <sup>a</sup>
Hemoglobin (g dL <sup>-1</sup> )	8.06±0.08	8.03 ± 0.07	9.51 ± 0.51	9.91 ± 0.26
Hematocrit (%)	26.16±1.6 <sup>b</sup>	26.31 ± 3.21 <sup>b</sup>	30.16 ± 4.1 <sup>b</sup>	35.76 ± 0.81 <sup>a</sup>
Glucose (mg l <sup>-1</sup> )	76±10.14 <sup>a</sup>	70±8.5 <sup>ab</sup>	61.66±2 <sup>bc</sup>	56±1 <sup>c</sup>
Total protein (mg l <sup>-1</sup> )	1.8±0.4 <sup>a</sup>	1.8±0.3 <sup>a</sup>	2.1±0.5 <sup>b</sup>	2.3±0.4 <sup>b</sup>

Data expressed as mean±SD, (n=6). Data assigned with different superscripts indicate significant differences ( $p < 0.05$ ).

## Discussion

To our knowledge, this is the first study to investigate the effects of A-Max, a mixture of prebiotics on great sturgeon (*H. huso*) juvenile. The result of present study showed that there were no significant differences in growth related parameters such as weight gain, SGR and FCR between great sturgeon juveniles fed control and prebiotic supplemented diets ( $p > 0.05$ ). Similar to our results, several other studies have revealed that growth parameters have remained unaffected with prebiotic

applications in fish (Pryor *et al.*, 2003; Genç *et al.*, 2006; Genç *et al.*, 2007; Welker *et al.*, 2007; Sado *et al.*, 2008; Akrami *et al.*, 2010; Dimitroglou *et al.*, 2010; Peterson *et al.*, 2010; Ai *et al.*, 2011; Hoseinifar *et al.*, 2011b; Peterson *et al.*, 2012; Razeghi Mansour *et al.*, 2012). The absence of positive significant effects on growth may be attributed to the inability of intestinal microbiota to ferment excessive prebiotic levels and the subsequent accumulation of indigestible material in the intestine which may cause irritation



to the gut (Hoseinifar *et al.*, 2011b; Soleimani *et al.*, 2012). Also Ye *et al.* (2011) explained that considerable variation in growth, feed utilization and health benefits with the dietary use of prebiotics and/or probiotics is likely dependent on fish species, feeding duration, fish nutritional/physiological status, ambient culturing conditions and supplementation level as well as the type of pre- and probiotics. Growth enhancement as a result of prebiotic administration has been reported in several previous studies on a variety of fish species fed dietary prebiotics (Li and Gatlin, 2004; Li *et al.*, 2005a; Mahious *et al.*, 2006; Staykov *et al.*, 2007; Torrecillas *et al.*, 2007; Yilmaz *et al.*, 2007; Grisdale-Helland *et al.*, 2008; Gultepe *et al.*, 2010; Ye *et al.*, 2011; Gultepe *et al.*, 2012; Soleimani *et al.*, 2012; Akrami *et al.*, 2013). Unlike this study, Akrami *et al.* (2015) found that there was a significant improvement in the weight gain and feed conversion ratio in rainbow trout treated with 1.5 g kg<sup>-1</sup> A-Max.

At the end of the feeding trial, haematological parameters such as erythrocyte (RBC), leucocytes (WBC), lymphocytes, neutrophils and haematocrit were improved by the inclusion of 1.5 g kg<sup>-1</sup> dietary prebiotic. Similar to these results, Andrews *et al.* (2009) observed a significant improvement in WBC, RBC and Hb, in rohu (*Labeo rohita*) fed on the MOS supplemented diet in comparison with those fed on the control diet. On the contrary, Welker *et al.* (2007) reported that RBC, WBC, Hct, Hb and plasma protein levels were not affected in

Channel catfish (*Ictalurus punctatus*) fed 0.2% mannan oligosaccharide. Sado *et al.* (2008) and Gultepe *et al.* (2012) showed that dietary MOS had no significant effect on hematological parameters of Nile tilapia (*Oreochromis niloticus*) and Gilthead Seabream (*Sparus auratus*) respectively. Also Hoseinifar *et al.* (2011b) and Razeghi Mansour *et al.* (2012) explained that supplementation with fructooligosaccharide and mannan oligosaccharide had no effects on hematological parameters of giant sturgeon (*H. huso*) juvenile. Stress responses by the fish as a result of daily feeding on  $\beta$ -glucan may increase RBC, WBC, Hct and Hb of the blood. Ebrahimi *et al.* (2012) also reported increased WBC counts in common carp (*Cyprinus carpio*) after feeding on Immunogen prebiotic which is a mixture of mannan oligosaccharide and  $\beta$ -glucans. The observed increases in the leucocyte and total protein appear to be signs of enhanced health status of the prebiotic-fed fish.

In the present study, the lowest glucose content and highest protein content were found in the fish fed diet 1.5 g kg<sup>-1</sup> prebiotic mixture compared to the control group ( $p < 0.05$ ). Andrews *et al.* (2009) showed a significant improvement in serum protein in *L. rohita* fed on the MOS supplemented diet in comparison with those fed on the control diet. On the contrary, Welker *et al.* (2007) and Sado *et al.* (2008) reported that plasma protein levels was not affected in Channel catfish and tilapia fed mannan oligosaccharide (MOS), respectively. Also, Hoseinifar

*et al.* (2011a) observed that glucose and total protein levels were not affected in beluga fed FOS. Subsequently, Akrami *et al.* (2015) studied the effects of dietary intake of prebiotic mixture (A-Max) in rainbow trout, and they observed that this supplement cannot significantly affect serum total protein, albumin, and glucose levels.

Stimulation of the immune response of fish through dietary supplements is of high interest for commercial aquaculture (Soleimani *et al.*, 2012). The innate immune system is very important in this regard because aquatic animals are continually vulnerable to numerous opportunistic pathogens and this part of immune response provides the first line of defense for the host (Magnadóttir, 2006). The result of the present research showed that lysozyme activity increased significantly in the group treated with 1.5 g kg<sup>-1</sup> prebiotic ( $p < 0.05$ ). Similar to these results, Soleimani *et al.* (2012) observed a significant improvement in serum lysozyme activity and serum alternative complement activity (ACH50) in Caspian roach (*Rutilus rutilus*) fry fed on the FOS-supplemented diet in comparison with those fed on the control diet. In another research, serum lysozyme activity enhanced significantly in stellate sturgeon (*A. stellatus*) juveniles fed on the diet supplemented with 1% fructooligosaccharide compared with other groups (Akrami *et al.*, 2013). Similarly, application of FOS as prebiotic was found to enhance the lysozyme activity of Nile tilapia (*O. niloticus*) (He *et al.*, 2003), Red drum

(*Sciaenops ocellatus*) (Zhou *et al.*, 2010) and Japanese flounder (*Paralichthys olivaceus*) (Ye *et al.*, 2011). In contrast to these results, no significant effects were observed on serum lysozyme activity of Atlantic salmon (*Salmo salar*) and Siberian sturgeon (*Acipenser baerii*) fed on FOS and arabinoxylan-oligosaccharides compared with the control diet, respectively (Grisdale-Helland *et al.*, 2008; Geraylou *et al.*, 2012). This contradictory result may be attributable to the low dosage (Soleimani *et al.*, 2012), degree of polymerization (Geraylou *et al.*, 2012), different duration of prebiotic administration, life stage and/or different fish species (Geraylou *et al.*, 2012; Soleimani *et al.*, 2012).

In the current study, respiratory burst activity, total immunoglobulin and alternative complement activity increased significantly in the great sturgeon juveniles with 1.5 g kg<sup>-1</sup> prebiotic compared with control group ( $p < 0.05$ ). Similarly, Geraylou *et al.* (2012) and Akrami *et al.* (2013) showed a significant improvement in alternative complement activity (ACH50) in Siberian sturgeon and stellate sturgeon fed on the arabinoxylan-oligosaccharides and MOS supplemented diet in comparison with those fed on the control diet, respectively. Additionally, Soleimani *et al.* (2012) observed a significant improvement in serum total immunoglobulin (Ig) levels in Caspian roach (*R. rutilus*) fry fed on the FOS-supplemented diet. On the contrary, no significant differences in respiratory

burst activity were observed for the Siberian sturgeon (*A. baerii*) fed arabinoxylan-oligosaccharides (Geraylou *et al.*, 2012) and stellate sturgeon (*A. stellatus*) fed with FOS (Akrami *et al.*, 2013).

In conclusion, the result indicated that prebiotic mixture (A-Max) at the level of 1.5 g kg<sup>-1</sup> in the diet of great sturgeon juvenile improved growth performance, immune response and some blood parameters of great sturgeon juvenile and it is appropriate for supplementation in the diet of cultured great sturgeon juveniles.

#### References

- Ai, Q., Mai, K., Tan, B., Xu, W., Duan, Q., Ma, H. and Zhang, L., 2006.** Replacement of fish meal by meat and bone meal in diets for large Yellow croaker (*Pseudosciaena crocea*). *Aquaculture*, 260, 255-263.
- Ai, Q., Xu, H., Mai, K., Xu, W., Wang, J. and Zhang, W., 2011.** Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. *Aquaculture*, 317, 155-161.
- Akrami, R., Hajimoradloo, A., Matinfar, A. and Abedian Kinari, A., 2009.** Effect of dietary prebiotic inulin on growth performance, intestinal microflora, body composition and hematological parameters of juvenile beluga, *Huso huso* (Linnaeus, 1758). *Journal of World Aquaculture Society*, 40, 771-779.
- Akrami, R., Karimabadi, A., Mohammadzadeh, H. and Ahmadifar, E., 2010.** Effect of dietary mannan oligosaccharide on growth performance, survival, body composition and salinity stress resistance in Kutum (*Rutilus frisii kutum*) fry stage. *Journal of Marine Science and Technology Research*, 8, 47-57.
- Akrami, R., Iri, Y., Khoshbavar Rostami, H. and Razeghi Mansour, M., 2013.** Effect of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival, lactobacillus bacterial population and hemato-immunological parameters of stellate sturgeon (*Acipenser stellatus*) juvenile. *Fish & Shellfish Immunology*, 35, 1235-1239.
- Akrami, R., Razeghi Mansour, M., Chitsaz, H., Ghasempour Alamdar, O. and Amani Denji, K., 2015.** Prebiotic (A-Max) and growth of juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Aquaculture*, 27, 61-71.
- Andrews, S. R., Sahu, N. P., Pal, A. K. and Kumar, S., 2009.** Haematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella. *Aquaculture Research*, 41, 61-69.
- AOAC, (Association of Official Analytical Chemists) 2005.** Official methods of analysis. 18th ed. MD.,

- Gaithersburg, USA: Association of Official Analytical Chemists.
- Asadi, F., Hallajian, A., Asadian, P., Shahriari, A. and Pourkabir, M., 2009.** Serum lipid, free fatty acid, and proteins in juvenile sturgeons: *Acipenser persicus* and *Acipenser stellatus*. *Comparative Clinical Pathology*, 18, 287-289.
- Blaxhall, P.C. and Daisley, K.W., 1973.** Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.
- Boesen, H.T., Larsen, M.H., Larsen, L.H. and Ellis, A.E., 2001.** In vitro interactions between rainbow trout (*Oncorhynchus mykiss*) macrophages and *Vibrio anguillarum* serogroup O2a. *Fish & Shellfish Immunology*, 11, 415-431.
- Cerezuela, R., Cuesta, A., Meseguer, J. and Esteban, M.A., 2008.** Effects of inulin on gilthead seabream (*Sparus aurata* L.) innate immune parameters. *Fish & Shellfish Immunology*, 24, 663-668.
- Cho, S.H. and Lee, S.M., 2012.** Onion Powder in the Diet of the Olive Flounder, *Paralichthys olivaceus*: Effects on the Growth, Body Composition, and Lysozyme Activity. *Journal of World Aquaculture Society*, 43, 30-38.
- Dimitroglou, A., Merrifield, D.L., Spring, P., Sweetman, J., Moate, R. and Davies, S.J., 2010.** Effects of mannan oligosaccharide (MOS) supplementation on growth performance, feed utilisation, intestinal histology and gut microbiota of gilthead sea bream (*Sparus aurata*). *Aquaculture*, 300, 182-188.
- Dorafshan, S., Kalbassi, M.R., Pourkazemi, M., Mojazi Amiri, B. and Soltan Karimi, S., 2008.** Effects of triploidy on the Caspian salmon (*Salmo trutta caspius*) haematology. *Fish Physiology and Biochemistry*, 34, 195-200.
- Ebrahimi, Gh., Ouraji, H., Khalesi, M.K., Sudagar, M., Barari, A., Zarei Dangesaraki, M. and Jani Khalili, K.H., 2012.** Effects of a prebiotic, Immunogen<sup>®</sup>, on feed utilization, body composition, immunity and resistance to *Aeromonas hydrophila* infection in the common carp *Cyprinus carpio* (Linnaeus) fingerlings. *Journal of Animal Physiology and Animal Nutrition*, 96, 591-599.
- Ellis, A.E., 1990.** Lysozyme assays. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Kaattari, S.L. and Rowley, A.F., editors. *Techniques in fish immunology*. Poland: SOS Publications. pp.101-103.
- Genç, M.A., Yilmaz, E. and Genç, E., 2006.** Yeme eklenen mannan-oligosakkarit'in karabalıkların (*Clarias gariepinus* (Burchell, 1822)) gelişimine, barsak ve karaciğer histolojisine etkileri. *Journal of Fish Aquatic Science*, 23, 37-41.
- Genç, M.A., Yilmaz, E., Genç, E. and Aktas, M., 2007.** Effect of dietary mannanoligosaccharid on growth, body composition and intestine and liver histology of the hybrid tilapia (*Oreochromis niloticus* × *O.aureus*).

- Israeal Journal of Aquaculture-Bamidgeh*, 59, 10–16.
- Geraylou, Z., Souffreau, C., Rurangwa, E., D'Hondt, S., Callewaert, L., Courtin, C.M., Delcour, J.A., Buyse, J. and Ollevier, F., 2012.** Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (*Acipenser baerii*) performance, immune responses and gastrointestinal microbial community. *Fish & Shellfish Immunology*, 32, 718–724.
- Gibson, G.R., 2004.** Fibre and effects on probiotics (the prebiotic concept). *Clinical Nutrition Supplements*, 1, 25–31.
- Gibson, G.R. and Roberfroid, M.B., 1995.** Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*, 125, 1401–1412.
- Gridale-Helland, B., Helland, S.J. and Gatlin, D.M., 2008.** The effect of dietary supplementation with mannanoligosacchare, fructooligosaccharide or galactooligosaccharide on the growth Atlantic salmon (*Salmo salar*). *Aquaculture*, 283, 163–167.
- Gultepe, N., Salnur, S., Hossu, B. and Hisar, O., 2010.** Dietary supplementation with mannanoligosaccharides (MOS) from Bio-Mos enhances growth parameters and digestive capacity of gilthead sea bream (*Sparus aurata*). *Aquaculture Nutrition*, 17, 482–487
- Gultepe, N., Hisar, O., Salnur, S., Hossu, B., Tansel Tanrikul, T. and Aydm, S., 2012.** Preliminary assessment of dietary mannanoligosaccharides on growth performance and health status of gilthead seabream *Sparus auratus*. *Journal of Aquatic Animal Health*, 24, 37–42.
- He, S., Xu, G., Wu, Y., Weng, H. and Xie, H., 2003.** Effects of IMO and FOS on the growth performance and non-specific immunity in hybrid tilapia. *Chinese Feed*, 23, 14–15 (In Chinese).
- Hevroy, E.M., Espe, M., Waagbo, R., Sandness, K., Rund, M. and Hemer, G.I., 2005.** Nutrition utilization in Atlantic salmon (*Salmo salar*) fed increased level of fish protein hydrolysate during a period of fast growth. *Aquaculture Nutrition*, 11, 301–313
- Hoseinifar, S.H., Mirvaghefi, A., Merrifield, D.L., Mojazi Amiri, B., Yelghi, S., Darvish, and Bastami, K., 2011a.** The study of some haematological and serum biochemical parameters of juvenile beluga (*Huso huso*) fed oligofructose. *Fish Physiology and Biochemistry*, 37, 91–96
- Hoseinifar, S.H., Mirvaghefi, A., Mojazi Amiri, B., Rostami, H.K. and Merrifield, D.L., 2011b.** The effects of oligofructose on growth performance, survival and autochthonous intestinal microbiota of beluga (*Huso huso*) juveniles. *Aquaculture Nutrition*, 17, 498–504
- Ibrahim, M.D., Fathi, M., Mesalhy, S. and Abdel-Aty, A.M., 2010.** Effect of dietary supplementation of inulin and vitamin C on the growth,

- hematology, innate immunity, and resistance of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 29, 241–246
- Li, P. and Gatlin, D.M., 2004.** Dietary brewers yeast and the prebiotic Grobiotic AE influence growth performance, immune responses and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. *Aquaculture*, 231, 445–456
- Li, P., Burr, G.S., Goff, J., Whiteman, K.W., Davis, K.B., Vega, R.R., Neill, W.H. and Gatlin, D.M., 2005a.** A preliminary study on the effects of dietary supplementation of brewers yeast and nucleotides, singularly or in combination, on juvenile red drum *Sciaenops ocellatus*. *Aquaculture Research*, 36, 1120–1127
- Li, P., Delbert, M. and Gatlin III, D.M., 2005b.** Evaluation of the prebiotic GroBioticTMAE and brewers yeast as dietary supplements for sub-adult hybrid striped bass *Morone chrysops* times *M. saxatilis* challenged in situ with *Mycobacterium marinum*. *Aquaculture*, 248, 197–205
- Magnadóttir, B., 2006.** Innate immunity of fish (overview). *Fish & Shellfish Immunology*, 20, 137–51.
- Mahious, A.S., Gatesoupe, F.J., Hervi, M., Metailler, R. and Ollevier, F., 2006.** Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima*. *Aquaculture International*, 14, 219–229
- Mohseni, M., Pourkazemi, M., Bahmani, M., Falahatkar, B., Pourali, H.R. and Salehpour, M., 2006.** Effects of feeding rate and frequency on growth performance of yearling great sturgeon, *Huso huso*. *Journal of Applied Ichthyology*, 22, 278–82
- Peterson, B.C., Bramble, T.C. and Manning, B.B., 2010.** Effects of Bio-Mos® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. *Journal of World Aquaculture Society*, 41, 149–155
- Peterson, B.C., Booth, N.J., Barrows, F.T. and Manning, B.B., 2012.** Improved survival in channel catfish fed mannanoligosaccharides in an extruded diet. *Journal of Animal Science*, 2, 57–61
- Pourkazemi, M., 1997.** Reviewing the status of the Caspian Sea sturgeon and how to protect its reserves. *Iranian Journal of Fisheries Science*, 6, 13–22.
- Pryor, G.S., Royes, J.B., Chapman, F.A. and Miles, R.D., 2003.** Mannan oligosaccharides in fish nutrition: Effects of dietary supplementation on growth and gastrointestinal villi structure in gulf of Mexico sturgeon. *North American Journal of Aquaculture*, 65, 106–111
- Razeghi Mansour, M., Akrami, R., Ghobadi, S. H., Amani Denji, K., Ezatrahimi, N. and Gharaei, A., 2012.** Effect of dietary mannan oligosaccharide (MOS) on growth performance, survival, body composition, and some

- hematological parameters in giant sturgeon juvenile (*Huso huso* Linnaeus, 1754). *Fish Physiology and Biochemistry*, 38, 829–835
- Sado, R.J., Bicudo, A.J.D.A. and Cyrno, J.P.E., 2008.** Feeding dietary mannan oligosaccharid to juvenile nile tilapia (*Oreochromis niloticus*), has no effect on hematological parameters and showed decreased feed consumption. *Journal of World Aquaculture Society*, 39, 821–826
- Safarpour Amlashi, A., Falahatkar, B., Sattari, M. and Tolouei Gilani, M.H., 2011.** Effect of dietary vitamin E on growth, muscle composition, hematological and immunological parameters of sub-yearling beluga *Huso huso* L. *Fish & Shellfish Immunology*, 30, 807–814
- Sang, H.M., Trung Ky, L. and Fotedar, R., 2009.** Dietary supplementation of mannan oligosaccharide improves the immune responses and survival of marron, *Cherax tenuimanus* (Smith, 1912) when challenged with different stressors. *Fish & Shellfish Immunology*, 27, 341–348
- Secombes, C.J., 1990.** Isolation of salmonid macrophages and analysis of their killing activity. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., van Muiswinkel, W.B., (Eds.), *Techniques in Fish Immunology*. SOS Publication, FairHaven, NJ. pp. 137–154
- Siwicki, A.K. and Anderson, D.P., 1993.** Nonspecific defense mechanisms assay in fish: II. potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. *Fish Disease Diagnosis and Prevention Methods*. Olsztyn, Poland. pp. 105–112.
- Soleimani, N., Hoseinifar, S.H., Merrifield, D.L., Barati, M. and Hassan Abadi, Z., 2012.** Dietary supplementation of fructooligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach (*Rutilus rutilus*) fry. *Fish & Shellfish Immunology*, 32, 316–321
- Staykov, Y., Spring, P., Denev, S. and Sweetman, J., 2007.** Effect of mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture International*, 15, 153–161
- Ta'ati, R., Soltani, M., Bahmani, M. and Zamini, A.A., 2011.** Growth performance, carcass composition and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster. *Iranian Journal of Fisheries Science*, 10, 324–335
- Tacon, A.G.J., 1990.** Standard methods for the nutrition and feeding of farned fish and shrimp. Argent Laboratories Press, Redmond. USA. 424P.
- Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Robaina, L., Real, F., Sweetman, J., Tort, L. and Izquierdo, M.S., 2007.** Immune stimulation and improved infection

- resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. *Fish & Shellfish Immunology*, 23, 969–981
- Verlhac Trichet, V., 2010.** Nutrition and immunity: an update. *Aquaculture Research*, 41, 356–372
- Welker, T.L., Lim, C., Yildirim-Aksoy, M., Shelby, R. and Klesius, P.H., 2007.** Immune response and resistance to stress and *Edwardsiella ictaluri*, fed diets containing commercial whole-cell yeast or yeast subcomponents. *Journal of World Aquaculture Society*, 38, 24–35
- Yano, T., 1992.** Assay of hemolytic complement activity. In: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Hattari, S., Rowley, A.F., editors. *Techniques in Fish Immunology*. New Jersey: SOS Publications. pp. 131–141
- Ye, J.D., Wang, K., Li, F.D. and Sun, Y.Z., 2011.** Single or combined effects of fructo- and mannan oligosaccharide supplements and *Bacillus clausii* on the growth, feed utilization, body composition, digestive enzyme activity, innate immune response and lipid metabolism of the Japanese flounder *Paralichthys olivaceus*. *Aquaculture Nutrition*, 17, 902–911
- Yilmaz, E., Gence, M.A. and Gence, E., 2007.** Effect of dietary mannan oligosaccharides on growth, body composition, intestine and liver histology of rainbow trout (*Oncorhynchus mykiss*). *Israel Journal of Aquaculture- Bamidgeh*, 59, 182–188.
- Zhou, Q.C., Alejandro Buentello, J. and Gatlin III, D.M., 2010.** Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). *Aquaculture*, 309, 253–257.