Growth performance, carcass composition, and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic, Immunoster

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Abstract

Growth performance, carcass composition, and immunophysiological indices in juvenile great sturgeon (*Huso huso*) fed on commercial prebiotic Immunoster (IS) were investigated. After a four-week acclimatization period, 270 great sturgeon juveniles weighing 95.68 ± 10.05 g were randomly distributed into 9 fiberglass tanks in three replicates and kept at a density of 30 fish per tank for a period of 8 weeks at water temperature $20.55 \pm 5.11\,^{\circ}$C and dissolved oxygen 6.73 ± 0.35 mg L^{-1}. IS was added at two levels of 1% and 3% to the basal diet in place of cellulose. At the end of the trial, blood sampling and carcass analysis were conducted. Weight, length, body weight increase (BWI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) were significantly affected by IS 3% compared with IS 1% and the control ($P<0.05$). There was significant difference ($P<0.05$) in crude protein of carcass between the experimental groups. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) showed significantly higher levels in experimental treatments compared with the control ($P<0.05$). Immunoglobulin M (IgM) level and lysozyme activity in IS 1% group were higher than the control group. It can be concluded that IS can enhance growth performance and improve some immunophysiological indices of great sturgeon.

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Introduction

Sturgeons, which their evolutionary history goes back to 100 million years ago are anadromous and potamodromous species of the Northern hemisphere (Bahmani, 1998). 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). The days of culture in sturgeon fish are too long. The results of such studies can lead to prevent different kinds of diseases.

Prebiotic is expressed as a non-digestible food ingredient that profitably affects the host by selectively stimulating the growth and /or activation of one or a limited number of bacteria in the intestine that can enhance host health status (Gibson and Roberfroid, 1995). Prebiotics are carbohydrates, which can be classified into monosaccharides, oligosaccharides and polysaccharides (RingØ et al., 2010).

Mannan oligosaccharides (MOS) are complex carbohydrates derived from yeast cell walls. These materials contain mannose as the primary carbohydrate element. MOS has beneficial effects on the growth of cattle, swine, and avian species (Moran, 2004 cited in Salze et al., 2008). Data about the effect of MOS on sturgeons is very rare.

Immunostimulants can be divided into biological substances and synthetic chemicals which stimulate and enhance lymphocytes' activity, antibody production, protection against pathogens and lysozyme activity (Sakai, 1999).

β-glucans are the most important structural polysaccharides in the cell walls of plants, fungi, algae, yeast and bacteria. They show immunostimulatory properties

and increase survival rate, disease resistance and modulate innate and acquired immunity responses in fish (Dalmo and Bogwald, 2008).

Immunoster™ (IS) is a commercial prebiotic and an immunostimulant derived from the cell wall of a single source of brewers yeast, *Saccharomyces cerevisiae*. This substance contains MOS and β-glucans.

The effects of MOS on the growth performance, hematological parameters and immune responses have been studied in several aquatic species including gulf sturgeon, *Acipenser oxyrinchus* (Pryor et al., 2003), rainbow trout, *Oncorhynchus mykiss* (Staykov et al., 2007), European sea bass, *Dicentrachus labrax* (Torrecillas et al., 2007, 2010), channel catfish, *Ictalurus punctatus* (Welker et al., 2007), cobia, *Rachycentron canadum* (Salze et al., 2008), red drum, *Sciaenops ocellatus* (Burr et al., 2008), Nile tilapia, *Oreochromis niloticus* (Sado et al., 2008), Atlantic salmon, *Salmo salar* (Grisdale-Helland et al., 2008) and rohu, *Labeo rohita* (Andrews et al., 2009).

The objective of the present study is to evaluate the influence of IS on the growth efficiency, carcass composition, hematological and biochemical indices and immune responses of *Huso huso*.

Materials and methods

The study was conducted in Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing Center, Rasht, Iran and Dr. Dadman International Sturgeon Research Institute, Rasht, Iran in 2009. Prior to the feeding trials, fish were fed on the basal diet to apparent satiation four times per

day for a 4-week acclimatization period. Then, 270 great sturgeon juveniles with a mean body weight of 95.68 ± 10.05 g were randomly allocated into 9 fiberglass tanks (2 × 2 × 0.53 m) and kept at a density of 30 fish per tank. The tanks were continuously aerated. Water of the tanks (using filtered water from the Sefidroud River) was replaced every 12 h to prevent accumulation of faeces and uneaten food. During the trial, water temperature, oxygen concentration and pH value were kept at $20.55 ± 5.11°C$, $6.73 ± 0.35$ mg L^{-1} and $7.92 ± 0.09$, respectively. The completely randomized design of this study consisted of three treatments (Control, IS 1% and IS 3%) in triplicates. All groups were fed on their respective diets four times daily (08.00, 14.00, 20.00 and 02.00 h) for a period of 8 weeks. The feeding rate was initially 4% of body weight per day and gradually decreased to 2%.

The ingredients of the experimental diets (based on the formulation of Dr. Dadman International Sturgeon Research Institute) are presented in Table 1. IS was supplied by Awill company, Victoria, Australia. IS was added at two levels of 1% and 3% to the basal diet in place of cellulose, except the control. All dry ingredients were thoroughly mixed for 30 min in a food mixer. Then, liquid ingredients were added to the diets and ingredients were mixed again for 20 min. The mixture was placed into a commercial meat grinder for thorough mixing and extruded through a 4 mm diameter strand and dried in a drier at 30 °C for 24 h. The pellets were packed in sterile bags and sealed and stored at -15 °C for a week. Analysis of the experimental diets is

showed in Table 2. Proximate analysis of the diets was conducted according to the standard methods of AOAC (Association of Official Analytical Chemists) (1995) in the laboratory of veterinary organization, Rasht, Iran. Moisture content was estimated by drying the samples to constant weight at 105 °C in an oven (Memmert, Germany), crude protein content ($N×6.25$) was measured using a Kjeldahl system (Buchi, Switzerland), crude lipid content was determined by a Soxhlet system (Buchi, Switzerland) and ash content was measured by weight after incinerating at 550 °C for 6h in a hotspot furnace (Gallenkamp, England). A bomb calorimeter (Parr, USA) was utilized to measure the energy content. Biometric data was recorded after feeding was ceased for 24h. Fish were individually weighed and measured once every two weeks and ration was adjusted according to new biomass of each tank.

The growth performance of juveniles such as body weight increase (BWI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), hepatosomatic index (HSI) and survival rate were calculated based on the standard formulae: BWI = (final body weight- initial body weight) × 100/ initial body weight, SGR = (ln final weight- ln initial weight) × 100/days, FCR = feed consumption / body weight gain, PER = weight gain / protein intake, CF = (body weight / body length^3) × 100, HSI = (liver weight / body weight) × 100 and survival rate = (final number of fish / initial number of fish) × 100 (Hung et al., 1993 , 1997, Haghighi et al., 2009; Luo et al., 2010).

Table 1: Ingredients of the experimental diets

Ingredients (%)	Control	IS 1%	IS 3%
Kilka fish meal	42	42	42
Meat meal	9	9	9
Soybean meal	19.5	19.5	19.5
Wheat flour	11	11	11
Sunflower oil	9	9	9
Molasses	1.5	1.5	1.5
Lecithin	0.2	0.2	0.2
L-Methionine	0.5	0.5	0.5
L-carnitine	0.1	0.1	0.1
Salt	1.5	1.5	1.5
Vitamin C	0.1	0.1	0.1
Vitamin E	0.1	0.1	0.1
Cellulose	3	2	0
Vitamin premix[*]	1.5	1.5	1.5
Mineral premix[**]	1	1	1
Immunoster	0	1	3

[*] Vitamin premix (g 100 g^{-1} vitamin premix except A, 160000 IU and D$_3$, 40000 IU): E, 4; K$_3$, 0.2; B$_1$, 0.6; B$_2$, 0.8; B$_3$, 1.2; B$_5$, 4; B$_6$, 0.4; B$_9$, 0.2; B$_{12}$, 0.8; H$_2$, 0.02; C, 6; Inositol, 2; BHT (butylated hydroxyl toluene), 2.

[**] Mineral premix (g 100 g^{-1} mineral premix): Fe, 2.6; Zn, 1.25; Se, 0.2; Co, 0.048; Cu, 0.42; Mn, 1.58; I, 0.1; Cholin chloride, 1.2.

Table 2: Proximate composition of experimental diets (Dry matter basis)

Ingredients (%)	Control	IS 1%	IS 3%
Moisture	6.1	5.9	6.2
Crude protein	42	41.3	42.2
Crude lipid	15	15.2	14.8
Fiber	2.4	2.2	2.3
Ash	10.1	10.2	10.1
NFE	30.5	31.1	30.6
Gross energy (MJ kg^{-1})	14.65	14.69	14.62

[*] NFE, nitrogen free extract = 100- (Protein + Lipid + Fiber + Ash)

At the end of the trial, six fish per treatment (two fish per replicate) were randomly selected to carry out the carcass analysis. Proximate analysis of carcass was

performed according to AOAC (1995). Livers were excised and weighed to calculate HSI.At the end of trial, nine fish per treatment (three fish per replicate) were randomly captured and blood samples were collected using a 2–mL syringe from the caudal vein to evaluate immunophysiological indices. The extracted blood was divided in two sets of Eppendorf tubes. One set contained heparin for hematology studies and the other one (non-heparinized) was centrifuged at 3000 rpm for 10 min in order to measure biochemical and immune indices. All sets were stored at $80^{\circ}C$ until analyzed. Before the blood samplings, fish were starved for 24 h. Hematocrit (Hct) values were determined using microhematocrit heparinized capillary tubes. The amount of hemoglobin (Hb) was measured according to the cyanmethemoglobin method. Red blood cell (RBC) and white blood cell (WBC) were counted in a Neubauer hemocytometer. Mean corpuscular volume (MCV= [Hematocrit $\times$ 10 / RBC]), mean corpuscular hemoglobin (MCH= [Hemoglobin $\times$ 10 / RBC]) and mean corpuscular hemoglobin concentration (MCHC= [Hemoglobin $\times$ 100 / Hematocrit]) were calculated. To estimate the differential leucocyte count, blood smears were prepared, air-dried, fixed in methanol and stained using Giemsa (Merck, Germany) (Klontz, 1994; Ameri Mahabadi, 2000; Zorriehzahra et al., 2010). Leucocytes in blood smears were categorized into lymphocytes, neutrophils, eosinophils and monocytes. Total serum protein was evaluated using the biuret

reaction (Doumas et al., 1981; Soltani et al., 2010). Albumin was measured using the bromocresol green binding method (Doumas et al., 1971; Soltani et al., 2010). A digital freezing osmometer (Roebling, Germany) was utilized to assess osmolarity. Ca^{2+} and Mg^{2+} values were determined using colorimetric method using an autoanalyzer (Technicon RA-1000, USA) according to (Kazemi et al., 2006). Na^+ and K^+ concentrations were measured by means of flame photometer (Jenway, England). Immunoglobulin M (IgM) content was estimated according to the method described by Siwicki and Anderson (1993). Lysozyme levels were determined based on the method of Ellis (1990). Levene's test was used to determine the homogeneity of variance. The means of all parameters such as growth performance, carcass composition and immunophysiological indices were subjected to one-way analysis of variance ANOVA, and comparisons among treatment means were made by Tukey as a post-hoc test using SPSS software (version 17). Statistical significance was accepted at the P<0.05 level. All data in the text are presented as mean ± SD.

Results

Growth indices are summarized in Table 3. Fish fed on IS at 1% and 3% had better growth performance during the 8-week feeding trial. Final weight, final length, BWI, SGR, FCR, PER and CF were significantly affected by IS 3% compared to IS 1% and the control (P<0.05). HSI was insignificantly higher in fish fed on IS 3% (P>0.05). Survival rate was 100% in all experimental treatments.

Table 3: Growth performance of *Huso huso* in the 8-week feeding trial (N=90 per treatment for growth indices and N= 6 per treatment for HSI)

Growth index	Control	IS 1%	IS 3%	*P*-value
Initial weight (g)	95.08 ± 10.30^a	96.32 ± 9.82^a	95.65 ± 10.11^a	0.710
Final weight (g)	290.28 ± 58.23^a	306.51 ± 58.18^a	354.17 ± 57.28^b	0.000
Initial length(cm)	30.84 ± 1.09^a	30.96 ± 0.89^a	30.85 ± 1.11^a	0.655
Final length (cm)	42.26 ± 2.57^a	42.67 ± 2.20^a	44.52 ± 2.34^b	0.000
BWI (%)	207.15 ± 13.85^a	219.52 ± 19.78^a	271.08 ± 10.68^b	0.005
SGR (% day^{-1})	1.99 ± 0.87^a	2.06 ± 0.11^a	2.34 ± 0.51^b	0.006
FCR	1.70 ± 0.10^b	1.58 ± 0.11^b	1.30 ± 0.49^a	0.005
PER	1.39 ± 0.08^a	1.52 ± 0.10^a	1.84 ± 0.06^b	0.001
CF	0.38 ± 0.005^a	0.39 ± 0.005^{ab}	0.41 ± 0.010^b	0.021
HSI (%)	3.59 ± 0.37^a	3.38 ± 0.35^a	3.66 ± 0.38^a	0.404

Means in the same row with different superscripts are significantly different (P<0.05)

Carcass proximate composition is presented in Table 4. There was significant difference in crude protein among the experimental groups (P<0.05). Crude protein of fish fed on IS at 1% and 3% was significantly (P<0.05) higher than the fish fed on the control diet. Lipid, moisture and ash content of the whole body were not significant (P>0.05). Table 5 shows the levels of hematological indices of great sturgeon juveniles at the end of the trial. MCV and MCH were significantly higher in IS-fed groups compared to the control diet-fed group (P<0.05). There was an insignificant increase in Hct, WBC and neutrophil in fish fed on IS at 1% and 3% (P>0.05). Significant differences (P<0.05) were observed in lymphocyte, eosinophil and monocyte among the treatments.

Table 4: Carcass proximate composition of *Huso huso* in the 8-week feeding trial (N=6 per treatment)

Ingredients (%)	Control	IS 1%	IS 3%	*P*-value
Crude protein	14.69 ± 0.61^a	14.91 ± 0.68^{ab}	15.52 ± 0.20^b	0.049
Crude lipid	9.20 ± 1.04^a	9.74 ± 1.96^a	9.44 ± 1.35^a	0.824
Ash	1.06 ± 0.04^a	1.01 ± 0.04^a	1.00 ± 0.04^a	0.067
Moisture	73.84 ± 1.46^a	73.48 ± 1.80^a	73.06 ± 1.47^a	0.682

Means in the same row with different superscripts are significantly different (P<0.05)

Table 5: Hematological indices of *Huso huso* in the 8-week feeding trial (N= 9 per treatment)

Hematological index	Control	IS 1%	IS 3%	*P*-value
Hct (%)	23.00 ± 1.73^a	24.55 ± 2.00^a	24.55 ± 3.20^a	0.302
Hb (g dL^{-1})	5.35 ± 0.61^a	5.27 ± 0.76^a	5.57 ± 0.62^a	0.624
RBC ($\times 10^6$ mm^{-3})	0.79 ± 0.08^a	0.72 ± 0.10^a	0.69 ± 0.14^a	0.187
WBC ($\times 10^3$ mm^{-3})	64.05 ± 15.58^a	66.66 ± 15.08^a	70.66 ± 16.63^a	0.674
MCV (Fl)	292.27 ± 22.05^a	342.22 ± 51.62^{ab}	364.68 ± 56.83^b	0.009
MCH (pg)	67.83 ± 5.77^a	73.13 ± 11.67^{ab}	84.01 ± 19.48^b	0.042
MCHC (%)	23.20 ± 1.37^a	21.38 ± 1.89^a	22.82 ± 2.58^a	0.153
Lymphocyte (%)	47.44 ± 8.29^b	46.00 ± 4.84^{ab}	37.33 ± 11.22^a	0.040
Neutrophil (%)	22.67 ± 6.81^a	28.77 ± 6.26^a	25.55 ± 8.07^a	0.209
Eosinophil (%)	26.00 ± 5.36^{ab}	22.22 ± 6.18^a	35.66 ± 14.39^b	0.018
Monocyte (%)	3.89 ± 1.90^b	2.77 ± 2.22^{ab}	1.44 ± 1.13^a	0.029

Means in the same row with different superscripts are significantly different (P<0.05)

Biochemical indices are given in Table 6. No significant differences (P>0.05) were recorded in biochemical indices. Table 7 shows immune indices of *Huso huso*. IgM concentration and lysozyme level in the diet containing IS 1% were higher than the control diet.

Table 6: Biochemical indices of *Huso huso* in the 8-week feeding trial (N= 9 per treatment)

Biochemical index	Control	IS 1%	IS 3%	*P*-value
Total protein (g dL^{-1})	1.50 ± 0.10^a	1.56 ± 0.16^a	1.51 ± 0.24^a	0.742
Albumin (g dL^{-1})	0.60 ± 0.03^a	0.62 ± 0.03^a	0.59 ± 0.10^a	0.638
Osmolarity (mOsmo L^{-1})	314.88 ± 10.55^a	314.66 ± 9.24^a	318.55 ± 7.71^a	0.611
Na^+ (meq L^{-1})	131.00 ± 2.06^a	131.66 ± 2.29^a	130.00 ± 2.95^a	0.368
K^+ (meq L^{-1})	1.96 ± 0.35^a	2.04 ± 0.24^a	2.12 ± 0.28^a	0.554
Ca^{2+} (mg dL^{-1})	5.09 ± 0.60^a	5.20 ± 0.53^a	4.96 ± 0.95^a	0.793
Mg^{2+} (meq L^{-1})	1.22 ± 0.21^a	1.15 ± 0.17^a	1.14 ± 0.20^a	0.705

Means in the same row with the same superscript indicate no significant difference (P>0.05)

Table 7: Immune indices of *Huso huso* in the 8-week feeding trial (N= 9 per treatment)

Immune index	Control	IS 1%	IS 3%	*P*-value
IgM (mg dL^{-1})	10.13 ± 4.65^a	11.41 ± 3.46^a	9.71 ± 4.84^a	0.694
Lysozyme (µg mL^{-1})	0.38 ± 0.78^a	1.81 ± 3.65^a	0.45 ± 1.37^a	0.348

Means in the same row with the same superscript indicate no significant difference (P>0.05)

Discussion

Results indicate that IS at 1% and 3% improved growth indices. Crude protein of carcass composition of fish fed on both levels of IS was significantly higher than the fish fed on the control diet. These results are in agreement with the following similar studies: Staykov et al. (2007) declared that a supplement of 0.2% MOS in rainbow trout, *Oncorhynchus mykiss* diet significantly enhanced body weight and reduced the FCR and mortality in comparison with the control diet. Torrecillas et al. (2007) reported a significant increase in body weight and total length as well as a positive correlation between the MOS levels and feed intake in European sea bass, *Dicentrachus labrax*, fed on MOS at two levels of 2‰ and 4‰. Enrichment of live feeds such as rotifers and Artemia with 0.2% MOS caused a greater ability to endure hyposaline stress in larval cobia, *Rachycentron canadum* (Salze et al., 2008). In another report, Andrews et al. (2009) evaluated that diets supplemented with 1%, 2% and 4% MOS improved WG, SGR and FCR in rohu, *Labeo rohita* fingerlings.

In contrast, Pryor et al. (2003) observed no differences in final weight, fork length, CF, SGR and FCR between control and 0.3% MOS supplemented groups in gulf sturgeon, *Acipenser oxyrinchus*. Grisdale-Helland et al. (2008) demonstrated that supplementing the diet with 1% MOS had no significant effects on digestibility, feed intake and growth of Atlantic salmon, *Salmo salar* and causing a decrease in the body protein concentration.

The use of MOS as prebiotic to improve growth indices in different species of fish still needs further surveys to explain contradictory results. It may be because of the different basal diets, inclusion levels, characteristics of the animal under study (species and age), period of trial, and circumstances of culture. Sado et al. (2008) declared that the complexity of carbohydrate structure in yeast's cell wall, yeast's various strains, fermentation, and processing procedures can modify their functions.

IS is considered as an immunostimulant for its potential (having β-1, 3 glucans). The use of immunostimulants is an important strategy in aquaculture. Some food components such as vitamins e.g. C and E, chitin, chitosan and several types of glucans like yeast glucan, peptide-glucan, and β-1, 3 glucan have been utilized as immunostimulants in fish (Sakai, 1999).

The analysis of blood parameters is a precious indicator in evaluating the conditions of aquatic animals. For instance, in response to stress, different sorts of pollutants, nutrition, ecological and physiological changes are involved (Bahmani et al., 2001). Leucocytes are one of the most important cells that can stimulate immune responses of fish. These cells produce antibody and can perform macrophagus activity (Jalali et al., 2009). In the current study, the WBC was higher in the IS 3% but no significant difference was seen. The increase in leucocyte count is due to glucan, which can detect particular receptors on WBCs. When β-1, 3 glucans settle on the receptors, the cells start to swallow bacteria and secrete cytokines that stimulate the establishment

of new WBCs (Raa, 1996 cited in Andrews et al., 2009). In *L. rohita*, the leucocyte count in fish treated with MOS at 1%, 2% and 4% was higher than the control (Andrews et al., 2009). Welker et al. (2007) revealed that WBC counts in channel catfish, *Ictalurus punctatus* fed on Bio-MOS™ at 2 g kg^{-1} were insignificantly higher compared to fish fed on the control diet.

Total serum protein values in fish fed on IS at 1% and 3% and albumin value in IS 1% group were higher than the control group but no significant difference was found. The increase in total serum protein and albumin concentrations can be due to stronger non-specific responses in fish (Wiegertjes et al., 1996 cited in Andrews et al., 2009).

Immunization of sturgeons against pathogens has not been developed as it has for cyprinids and salmonids (Khoshbavar-Rostami et al., 2007). IgM is an important part of the humoral immune system. Administration of vitamin A, chitin, yeast cells and levamisole as immunostimulants to the diet of *Sparus aurata*, increased IgM values (Cuesta et al., 2004). In this study, IS 1% insignificantly enhanced IgM concentration in great sturgeon juveniles. Administration of IS 1% (because of having MOS and β-1, 3 glucans) resulted in an increase in serum lysozyme which can contribute to the enhancement in the non-specific immunity. Staykov et al. (2007) reported that *O. mykiss* treated with MOS at an inclusion rate of 0.2% showed significant differences in lysozyme levels. However, in *S. salar*, lysozyme concentration was lower in the MOS-fed group compared with the control diet-fed group (Grisdale-Helland et al., 2008).

MCV and MCH were significantly (P<0.05) affected by IS at 1% and 3% compared with the control. Sado et al. (2008) demonstrated that supplementing the diet with 0.2%, 0.4%, 0.6%, 0.8% and 1% MOS had no significant effects on MCV and MCH in *Oreochromis niloticus*.

There is no record on the effect of MOS on osmolarity and blood serum ions in fish. The effects of MOS on osmolarity and Na^+, K^+, Ca^{2+} and Mg^{2+} were studied for the first time in this survey. Results indicate that IS had no significant effects on the above mentioned parameters in *H. huso*. Studies on the use of MOS in fish hematology are few and involve immunological factors including lysozyme concentration, antibody titer, bactericidal activity, and alternative complement pathway (Torrecillas et al., 2007; Staykov et al., 2007).

In the present study, the effects of IS did not show significant differences in some hematological and biochemical parameters of *H. huso*. Welker et al. (2007) found no significant differences in the hematological variables such as RBC, Hb, Hct and WBC in *I. punctatus* fed on MOS at 2 g kg^{-1}. Sado et al. (2008) declared that diets supplemented with 0.2%, 0.4%, 0.6%, 0.8% and 1% MOS had no significant effects on RBC, Hb, Hct, WBC and total protein in *O. niloticus*. On the contrary, Andrews et al. (2009) observed a significant improvement in WBC, RBC, Hb, serum protein, albumin, and globulin in *L. rohita* fed on the MOS-supplemented diet in comparison with those fed on the control diet. It appears that fluctuations in hematological and biochemical variables may be associated to characteristics of species, inclusion rates of MOS, ingredients of diets, rearing period, etc.

In conclusion, the results of this trial indicate that IS can enhance growth performance, body composition and affect some immunophysiological variables such as WBC, MCV, MCH, total protein, albumin, IgM and lysozyme in *H. huso*. Further research is needed to clarify the action mechanisms of MOS, as well as the appropriate inclusion dose and feeding period in great sturgeon.

References
Ameri Mahabadi, M., 2000. Laboratory methods of veterinary hematology. Tehran University Press. 126P. (in Persian).

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.

Association of Official Analytical Chemists, 1995. Official methods of analysis of AOAC International. 16th ed. AOAC International, Arlington, Virginia, USA.1298P.

Bahmani, M., 1998. Phylogenic and systematic study on sturgeons. Paper presented at the 1st international student research conference, the Association of the State Universities of the Caspian Littoral Countries. April 12-18th. Astrakhan, Russia.

Bahmani, M., Kazemi, R. and Donskaya, P., 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). *Fish Physiology and Biochemistry*, 24**,** 135-140.

Burr, G., Hume, M., Neill, W. H. and Gatlin III, D. M., 2008. Effects of prebiotics on nutrient digestibility of a soybean-meal-based diet by red drum (*Sciaenops ocellatus*). *Aquaculture Research*, 39**,** 1680-1686.

Cuesta, A., Meseguer, J. and Esteban, M.A., 2004. Total serum immunoglobulin M levels are affected by immunomodulators in sea bream (*Sparus aurata*). *Veterinary Immunology and Immunopathology*, 101**,** 203-210.

Dalmo, R. A. and Bogwald, J., 2008. β-glucans as conductors of immune symphonies. *Fish and Shellfish Immunology*, 25, 384-396.

Doumas, B. T., Watson, W. and Biggs, H. G., 1971. Albumin standards and measurement of serum albumin with bromocresol green. *Clinica Chimica Acta*, 31, 87-96.

Doumas, B. T., Bayse, D. D., Carter, R. J., Peters, T. J. R. and Schaffer, R. A., 1981. Candidate reference method for determination of total protein in serum. I. Development and Validation. *Clinical Chemistry*, 10**,** 42-50.

Ellis, A. E., 1990. Lysozyme assays. *In*: J.S. Stolen; D.P. Fletcher; B.S. Anderson and W.B. Van Muiswinkel (eds). Techniques in Fish Immunology. SOS Publication, USA. pp.101-103.

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.

Grisdle-Helland, B., Helland, S. J. and Gatlin III, D. M., 2008. The effect of dietary supplement with mannan-oligosaccharide, fructo-oligosaccharide or galacto-oligosaccharide on the growth and feed utilization of Atlantic salmon (*Salmo salar*). *Aquaculture*. 283,163-167.

Haghighi, D. T., Saad, C. R., Hosainzadeh Sahafi, H. and Mansouri, D., 2009. The effect of dietary lipid level on the growth of kutum fry (*Rutilus frisii kutum*). *Iranian Journal of Fisheries Sciences*, 8(**1**),13-24.

Hung, S. S. O., Lutes, P. B., Shqueir, A. A. and Conte, F. S., 1993. Effect of feeding rate and water temperature on growth of juvenile white sturgeon (*Acipenser transmontanus*). *Aquaculture*, 115**,** 297-303.

Hung, S. S. O., Storebakken, T., Cui, Y., Tian, L. and Einen, O., 1997. High-energy diets for white sturgeon (*Acipenser transmontanus*). *Aquaculture Nutrition*, 3**,** 281-286.

Jalali, M. A., Ahmadifar, E., Sudagar, M. and Azari Takami, Gh., 2009.

Growth efficiency, body composition, survival and hematological changes in great sturgeon (*Huso huso*) juveniles fed diets supplemented with different levels of Ergosan. *Aquaculture Research*, 40**,** 804-809.

Kazemi, R., Bahmani, M., Hallajian, A., Pourkazemi, M. and Dezhandian, S., 2006. Investigation of blood serum osmo- and ion-regulation of mature and reared juvenile *Acipenser persicus*. *Journal of Applied Ichthyology*, 22, 188-192.

Khoshbavar-Rostami, H. A., Soltani, M. and Hassan, H. M. D., 2007. Immune responses of great sturgeon (*Huso huso*) to *Aeromonas hydrophila* bacterin. *Journal of Fish Biology,* 70**,** 1931-1938.

Klontz, G.W., 1994. Fish hematology. *In*: J.S. Stolen; T.C. Fletcher; A.F. Rowley; T.C. Kelikoff; S.L. Kaatari and S.A. Smith (eds). Techniques in fish Immunology, Vol.3. SOS Publications, Fair Haven, New Jersey, USA. pp.121-132.

Luo, G., Xu, J., Teng, Y., Ding, C. and Yan, B., 2010. Effects of dietary lipid levels on the growth, digestive enzyme, feed utilization and fatty acid composition of Japanese sea bass (*Lateolabrax japonicus*) reared in freshwater. *Aquaculture Research***,** 41**,** 210-219.

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, 278-282.

Pryor, G. S., Royes, J. B., Chapman, F. A. and Miles, R. D., 2003. Mannanoligosaccharides 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.

RingØ, E., Olsen, R. E. Gifstad, T. Ø., Dalmo, R. A., Amlund, H., Hemre, G. I. and Bake, A. M., 2010. Prebiotics in aquaculture: a review. *Aquaculture Nutrition*, *16,*117-136.

Sado, R. Y., Almeida Bicudo, A. J. D. and Cyrino, J. E. P. 2008. Feeding dietary mannan oligosaccharides to juvenile Nile tilapia, *Oreochromis niloticus*, has no effect on hematological parameters and showed decreased feed consumption. *Journal of the World Aquaculture Society*, 39**(6),** 821-826.

Sakai, M., 1999. Current research status of fish immunostimulants. *Aquaculture*. 172, 63-92.

Salze, G., Malean, E., Schwarz, M. H. and Craig, S. R., 2008. Dietary mannan oligosaccharide enhances salinity tolerance and gut development of larval cobia. *Aquaculture*, 274, 148-152.

Siwicki, A.K. and Anderson, D.P., 1993. Non-Specific defence mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. Disease diagnosis and prevention methods, FAO-project GCP/INT/JPA, IFI, Olsztyn, Poland. pp.105-112.

Soltani, M., Sheikhzadeh, N., Ebrahimzadeh-Mousavi, H. A. and Zargar, A., 2010. Effects of *Zataria multiflora* essential oil on innate immune responses of common carp (*Cyprinus carpio*). *Journal of Fisheries and Aquatic Science*, 5**(3),** 191-199.

Staykov, Y., Spring, P., Denev, S. and Sweetman, J., 2007. Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture International*, 15, 153-161.

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 and Shellfish Immunology,* 23, 969-981.

Torrecillas, S., Makol, A., Caballero, M. J., Montero, D., Gines, R., Sweetman, J. and Izquierdo, M. S., 2010. Improved feed utilization, intestinal mucus production and immune parameters in sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides (MOS). *Aquaculture Nutrition*. (In press).

Welker, T., Lim, C., Yildirim-Aksoy, M., Shelby, R. and Klesius, P. H., 2007. Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. *Journal of the World Aquaculture Society*, 38**(1),** 24-35.

Zorriehzahra, M. J., Hassan, M. D., Gholizadeh, M. and Saidi, A. A., 2010. Study of some hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) fry in western part of Mazandaran province, Iran. *Iranian Journal of Fisheries Sciences,* 9**(1),** 185-198.