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Influence of prebiotic Immunowall on growth performance, body composition and immunophysiological variables in juvenile great sturgeon, *Huso huso*

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ABSTRACT

The present study was performed to determine the influence of prebiotic Immunowall on growth performance, body composition and immunophysiological variables in juvenile great sturgeon, Huso huso. After a 4-week acclimatization period, a total of 270 juveniles of great sturgeon weighing 95.30 ± 8.99 g were random ly distributed into 9 fiberglass tanks and kept at a density of 30 fish per tank for a period of 8 weeks. Different levels of Immunowall including 0% (Control), 1% and 3% were tested in three replicate groups. At the end of the feeding trial, blood sampling and body composition analysis were conducted. Final weight, final length, body weight increase, specific growth rate, feed conversion ratio, protein efficiency ratio and condition factor were significantly (P<0.05) improved by Immunowall at 1% and 3% compared to the control. Body composition analysis showed significant differences among the experimental groups (P<0.05). Mean corpuscular volume, mean corpuscular hemoglobin and albumin showed significantly (P<0.05) higher levels in Immunowall-fed groups compared to the control. Immunoglobulin M concentration and lysozyme activity in fish fed Immunowall at 3% were higher than the control group. Based on obtained results, it can be declared that Immunowall can enhance growth performance and improve some immunophysiological variables in great sturgeon.

Keywords: Prebiotic, Growth Indices, Hematological Parameters, *Huso huso*.

INTRODUCTION

Sturgeons are valuable fishes, which are currently highly endangered. The culture of these species has seen remarkable development in past decade. Great sturgeon, *Huso huso*, is an important aquaculture species in Russia, Eastern Europe, Japan and Iran. This species is good for aquaculture activities because of its fast growth, ease of reproduction in captivity and tolerance to variable cultural conditions [1].

Fast growth and disease resistance are two of the most important goals in aquaculture. Human necessity to safe food has prompted the search of natural growth enhancers to use in feeding of aquatic animals [2]. 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, which can enhance host health status [3]. Prebiotics are carbohydrates that can be classified into monosaccharides, oligosaccharides and polysaccharides [4]. 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 [5]. Among the common prebiotics, MOS has been recently studied in aquaculture. Immunowall (IW) is a prebiotic derived from the cell wall of a single source of brewers yeast, *Saccharomyces cerevisiae*. This substance contains MOS.

The effects of MOS on growth performance, hematological parameters and immune responses have been studied in various fishes including gulf sturgeon, *Acipenser oxyrinchus* [6], rainbow trout, *Oncorhynchus mykiss* [7,8], hybrid tilapia, *Oreochromis niloticus* × *O. aureus* [2], European sea bass, *Dicentrachus labrax* [9], channel catfish, *Ictalurus punctatus* [10], cobia, *Rachycentron canadum* [11], red drum, *Sciaenops ocellatus* [12], Nile tilapia, *Oreochromis niloticus* [13], Atlantic salmon, *Salmo salar* [14] and rohu, *Labeo rohita* [15]. In all studies mentioned above, different and contradictory results were recorded because of the basal diet, inclusion of various levels of MOS, animal characteristics, circumstances of culture and length of study.

Although the above cited scientists have studied the effects of MOS in different species, data about the effect of MOS on sturgeons is rare. Therefore, the aim of the present study is to provide information about the effects of IW on juvenile great sturgeon in terms of their growth performance, body composition and immunophysiological variables.

MATERIALS AND METHODS

Experimental Design

Juveniles of great sturgeon were obtained from Shahid Beheshti Sturgeon Fish Propagation and Rearing Center, Rasht, Iran. Prior to the feeding trials, fish were fed the basal diet to apparent satiation four times per day for a 4-week acclimatization period. Then, a total of 270 juveniles of great sturgeon with mean body weight of 95.30 ± 8.99 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 equipped with aeration through air stone connected to a central air compressor. Water (filtered from the Sefidroud River) was exchanged in tanks every 12 h to prevent accumulation of feces and uneaten food. During the trial, water quality parameters such as temperature (23.24 ± 3.06 °C), dissolved oxygen (6.73 ± 0.35 mg/l) and pH values (7.92 ± 0.09) were measured. All tanks were kept under natural photoperiod, 11 h light -13 h dark. The completely randomized design of this study consisted of three levels (control (0%), 1% and 3%) each with three replicates. All groups were fed their respective diets four times daily (at 0200, 0800, 1400 and 2000 h) at the same rate (initially 4% of body weight per day and gradually reduced to 2%). The feeding trial was performed for 8 weeks.

Experimental Diets

The ingredients of the experimental diets (based on the formulation of International Sturgeon Research Institute, Rasht, Iran) are presented in Table 1. Immunowall® (IW) was supplied by ICC Industrial Comercio Exportacao E. Importacao LTDA, São Paulo, Brazil. Three levels of IW (control (0%), 1% and 3%) were used in this trial. IW was added to the basal diet in place of cellulose, except in the control diet. All dry ingredients were thoroughly mixed for 30 min in a food mixer. Then, liquid ingredients were added and ingredients were mixed again for 20 min. The mixture was placed in a commercial meat grinder for through mixing, extruded through a 4 mm diameter dye, and dried at 30 °C for 24 h. The pellets were packed in sterile bags, sealed and stored at -15 °C until used.

Table1. Ingredients of the experimental diets in the 8-week feeding trial

Ingredients (%)	Control	IW 1%	IW 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
Immunowall	0	1	3

**Vitamin premix (g/100g 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/100g 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.*

Proximate Composition of Diets

Proximate analysis of the diets was conducted according to [16] (Table 2). Moisture content was estimated by drying the samples to constant weight at 105 °C in an oven (Memmert, Germany). A distillation unit (Buchi,

Switzerland) was used to measure crude protein content (N×6.25) according to Kjeldahl method. Crude lipid content was determined by a Soxhlet system (Buchi, Switzerland) and ash content was measured by weight after incinerating at 550 °C for 6 h in a hotspot furnace (Gallenkamp, England). In order to determine the energy content, bomb calorimeter (Parr, USA) was utilized. All experimental diets were analyzed in the laboratory of veterinary organization, Rasht, Iran.

Table 2. Analyzed proximate composition of experimental diets

Ingredients (%)	Control	IW 1%	IW 3%
Moisture	6.1	6.3	6.1
Crude protein	42	41.8	41.4
Crude lipid	15	15.4	15.2
Fiber	2.4	2.3	2.3
Ash	10.1	10.3	10.2
NFE*	30.5	30.2	30.9
Gross energy (MJ/kg)	14.65	14.70	14.68

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

Growth Performance

All biometric data were taken only after feeding had been ceased for 24 h. Following these bi-weekly inventories feed rates were adjusted to reflect the new biomass gain in 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 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.

Sample Collection and Analysis

At the end of the feeding trial, six fish per treatment (two fish per replicate) were randomly selected and body composition analysis was carried out according to [16]. Livers were excised and weighed in order to calculate hepatosomatic index.

To study immunophysiological variables, nine fish per treatment (three fish per replicate) were randomly captured at the end of the feeding trial and blood samples were collected using a 2–ml syringe from the caudal vasculature. The extracted blood was divided in two sets of microcentrifuge tubes. One set contained heparin for hematology studies and the other (non-heparinized) was centrifuged at 3000 rpm for 10 min in order to measure biochemical and immune indices. All sera were stored at -80 °C until analyzed. Before the blood samplings, all 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. The counts of red blood cells (RBC) and white blood cells (WBC) were carried out in an improved Neubauer hemocytometer. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated. To perform differential leucocyte count, blood smears were prepared, air-dried, fixed in methanol and stained using Giemsa (Merck, Germany). Leucocytes in blood smears were categorized into lymphocytes, neutrophils, eosinophils and monocytes [17].

Total serum protein was evaluated using the biuret reaction [18,19]. Albumin was measured using the bromocresol green binding method [20,19]. In order to assess osmolarity, a digital freezing osmometer (Roebling, Germany) was utilized. Ca^{2+} and Mg^{2+} values were determined using colorimetric method using an autoanalyzer (Technicon RA-1000, USA) according to [21,22]. Na^+ and K^+ concentrations were measured with a flame photometer (Jenway, England). Immunoglobulin M (IgM) content was estimated according to the method described by [23]. Also, lysozyme levels were determined based on the method of [24]. All immunophysiological variables were measured at International Sturgeon Research Institute, Rasht, Iran.

Statistical Analysis

Levene's test was used to determine the homogeneity of variance. The means of all parameters were subjected to one-way ANOVA and comparisons among treatment means were made by Tukey's HSD test using SPSS software (Version 17, SPSS Inc. Chicago, Illinois, USA). Statistical significance was accepted at the $P<0.05$ level. All data in the text are presented as mean ± SD.

RESULTS

According to findings of this study, fish fed IW at 1% and 3% had better growth performance during the 8-week feeding trial (Table 3). Survival rate was 100% in all treatments. Final weight, final length, BWI, SGR, FCR, PER and CF were significantly (P<0.05) affected by IW at both levels of 1% and 3% compared to the control. However, there was no significant difference in HSI among the treatments (P>0.05).

Table 3. Growth indices of juvenile great sturgeon in the 8-week feeding trial

Growth indices	Control	IW 1%	IW 3%
Initial weight (g)	95.08 ± 10.30^{a}	95.93 ± 8.89^{a}	94.90 ± 7.66^{a}
Final weight (g)	290.28 ± 58.23^{a}	344.64 ± 50.91^{b}	343.73 ± 61.84^{b}
Initial length(cm)	30.84 ± 1.09^{a}	30.80 ± 0.96^{a}	30.76 ± 1.34^{a}
Final length (cm)	42.26 ± 2.57^{a}	43.70 ± 1.90^{b}	44.01 ± 2.49^{b}
BWI (%)	207.15 ± 13.85^{a}	261.28 ± 14.57^{b}	265.13 ± 20.14^{b}
SGR (%/day)	1.99 ± 0.87^{a}	2.28 ± 0.70^{b}	2.30 ± 0.10^{b}
FCR	1.70 ± 0.10^{b}	1.39 ± 0.08^{a}	1.32 ± 0.08^{a}
PER	1.39 ± 0.08^{a}	1.70 ± 0.10^{b}	1.81 ± 0.10^{b}
CF	0.38 ± 0.005^{a}	0.43 ± 0.01^{c}	0.40 ± 0.005^{b}
HSI (%)	3.59 ± 0.37^{a}	3.49 ± 0.40^{a}	3.68 ± 0.38^{a}

Values (mean ±SD) in the same row with different superscripts are significantly different (P<0.05).

At the end of the feeding trial, whole body crude protein of fish fed IW 3% was significantly (P<0.05) higher than the fish fed the control diet but was similar to the IW 1% group. In addition, significant differences were observed in crude lipid and moisture contents among the treated groups (P<0.05; Table 4).

Table 4. Body composition of juvenile great sturgeon in the 8-week feeding trial. (N=6 per treatment)

Ingredients (%)	Control	IW 1%	IW 3%
Crude protein	14.69 ± 0.61^{a}	15.03 ± 0.48^{ab}	15.40 ± 0.25^{b}
Crude lipid	9.20 ± 1.04^{a}	11.25 ± 0.80^{b}	9.62 ± 0.49^{a}
Ash	1.06 ± 0.04^{a}	1.01 ± 0.06^{a}	1.06 ± 0.07^{a}
Moisture	73.84 ± 1.46^{b}	71.41 ± 0.84^{a}	73.06 ± 0.81^{b}

Values (mean ±SD) in the same row with different superscripts are significantly different (P<0.05).

Table 5 shows the levels of hematological indices of juvenile great sturgeon during the 8-week feeding trial. There was an insignificant increase in Hct, Hb, WBC, lymphocytes and neutrophil in fish fed IW at 1% and 3% of diet (P>0.05). However, MCV and MCH were significantly (P<0.05) higher in IW 1% and 3% groups compared to control. Differences were observed in the prevalence of monocytes and eosinophils among the treatments (P<0.05), which were significantly higher in the control and IW 1% groups, respectively, compared to fish fed the IW 3%.

Table 5. Hematological indices of juvenile great sturgeon in the 8-week feeding trial. (N=9 per treatment)

Hematological indices	Control	IW 1%	IW 3%
Hct (%)	23.00 ± 1.73^{a}	24.44 ± 3.28^{a}	25.22 ± 3.11^{a}
Hb (g/dl)	5.35 ± 0.61^{a}	5.74 ± 1.05^{a}	5.50 ± 0.58^{a}
RBC ($\times 10^{6}$ mm^{-3})	0.79 ± 0.08^{a}	0.68 ± 0.06^{a}	0.74 ± 0.13^{a}
WBC ($\times 10^{3}$mm^{-3})	64.05 ± 15.58^{a}	72.72 ± 11.99^{a}	63.83 ± 10.16^{a}
MCV (Fl)	292.27 ± 22.05^{a}	360.46 ± 63.34^{b}	340.98 ± 30.91^{b}
MCH (pg)	67.83 ± 5.77^{a}	84.61 ± 17.98^{b}	74.85 ± 10.72^{b}
MCHC (%)	23.20 ± 1.37^{a}	23.34 ± 1.38^{a}	21.88 ± 1.86^{a}
Lymphocyte (%)	47.44 ± 8.29^{b}	43.67 ± 10.54^{a}	53.11 ± 10.03^{a}
Neutrophil (%)	22.67 ± 6.81^{a}	23.22 ± 12.27^{a}	25.66 ± 8.29^{a}
Eosinophil (%)	26.00 ± 5.36^{ab}	29.89 ± 7.07^{b}	19.77 ± 3.99^{a}
Monocyte (%)	3.89 ± 1.90^{b}	3.22 ± 1.71^{b}	1.33 ± 0.86^{a}

Values (means ±SD) in the same row with different superscripts are significantly different (P<0.05).

There were significant differences in levels of albumin in fish fed IW 3% compared to fish fed the control diet (P<0.05) (Table 6). Ca^{2+} concentrations were significantly (P<0.05) higher in the control and IW 1% groups compared to the IW 3%. No differences were observed in other biochemical indices (Table 6) and immune indices, including IgM and lysozyme (Table 7) between the dietary treatments.

Table 6. Biochemical indices of juvenile great sturgeon in the 8-week feeding trial.
(N=9 per treatment)

Biochemical indices	Control	IW 1%	IW 3%
Total protein (g/dl)	1.50 ± 0.10^{a}	1.61 ± 0.20^{a}	1.65 ± 0.21^{a}
Albumin (g/dl)	0.60 ± 0.03^{a}	0.61 ± 0.07^{ab}	0.68 ± 0.08^{b}
Osmolarity (mOsmo/l)	314.88 ± 10.55^{a}	313.56 ± 8.54^{a}	315.89 ± 9.70^{a}
Na^{+} (meq/l)	131.00 ± 2.06^{a}	129.89 ± 2.61^{a}	130.33 ± 2.82^{a}
K^{+} (meq/l)	1.96 ± 0.35^{a}	1.97 ± 0.18^{a}	2.23 ± 0.27^{a}
Ca^{2+} (mg/dl)	5.09 ± 0.60^{b}	5.50 ± 0.72^{b}	3.62 ± 1.18^{a}
Mg^{2+} (meq/l)	1.22 ± 0.21^{a}	1.18 ± 0.12^{a}	1.13 ± 0.19^{a}

Values (means ±SD) in the same row with different superscripts are significantly different (P<0.05).

Table 7. Immune indices of juvenile great sturgeon in the 8-week feeding trial.
(N=9 per treatment)

Immune indices	Control	IW 1%	IW 3%
IgM (mg/dl)	10.13 ± 4.65^{a}	9.94 ± 5.80^{a}	14.12 ± 3.68^{a}
Lysozyme (µg/ml)	0.38 ± 0.78^{a}	0.82 ± 1.67^{a}	1.38 ± 2.80^{a}

Values (means ±SD) in the same row with the same superscript indicate no significant difference (P>0.05).

DISCUSSION

Results indicate that IW at 1% and 3% of diet improved growth performance. Also, whole body protein of fish fed IW 3% was significantly higher than the fish fed the control diet. No mortality was recorded. These findings are consistent with studies on other species. The results of Torrecillas [9] showed that European sea bass fed MOS at two levels of 2 and 4 g/kg showed a significant increase in body weight and total length. Also, a positive correlation was observed between the MOS levels and feed intake. The studies of Staykov [7] demonstrated that 0.2% MOS in rainbow trout diet significantly enhanced body weight and reduced the FCR and mortality in comparison with the control diet. In hybrid tilapia, *Oreochromis niloticus* × *O. aureus*, the body protein content of fish fed diets containing 1.5, 3 and 4.5 g/kg MOS significantly increased compared to the control but no meaningful differences were reported in weight gain (WG), SGR, FCR, PER, HSI and viscerosomatic index (VSI) among experimental groups [2]. In a similar study with the inclusion levels of 1.5, 3 and 4.5 g/kg MOS in rainbow trout diets, it was observed enhanced feed utilization at 1.5 g/kg. In addition, it was proved that carcass protein significantly increased in all inclusion levels [8]. Enrichment of rotifers and Artemia with 0.2% MOS caused a greater ability to endure hyposaline stress in larval cobia [11]. The reports of Andrews [15] showed that diets supplemented with 1%, 2% and 4% MOS improved WG, SGR and FCR in rohu fingerlings.

In contrast, according to [6] no differences in CF, SGR and FCR were observed between control and 3 g/kg MOS supplemented groups in gulf sturgeon. The findings of Grisdale-Helland [14] demonstrated that supplementing the diet with 10 g/kg MOS resulted in a decrease in the protein concentration in the body of Atlantic salmon. Furthermore, inclusion of MOS had no significant effects on digestibility, feed intake and growth.

The use of MOS as prebiotic to enhance growth performance in fish needs further studies for better explanation of contradictory results. It may be because of the different basal diet, inclusion levels, animal characteristics (species and age) and circumstances of culture and length of study. According to [25] the complexity of carbohydrate structure in yeast's cell wall, yeast's various strains, fermentation and processing procedures can modify their functions.

IW is considered as an immunostimulant for containing β-1, 3 glucans. Some materials 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 used as immunostimulants in fish [26]. β-glucans are the most important structural polysaccharides in the cell walls of plants, fungi, algae, yeast and bacteria. They can show immunostimulatory properties and increase survival rate, disease resistance and modulate innate and acquired immunity responses in fish [27]. The analysis of blood parameters is a useful indicator in assessing the physiological conditions of aquatic animals in response to stress, pollutants, nutrition, and also physiological and ecological changes [28]. Leucocytes are one of the most important cells that can stimulate immune responses of fish. These cells produce antibody and engulf foreign cells [29]. In the current study, the leucocyte count was higher in the IW at 1%. The increase in leucocytes is due to glucan. When β-1, 3 glucans settle on the receptors of WBCs, the cells start to swallow bacteria and secrete cytokines that stimulate the establishment of new WBCs [30]. The studies of Welker [10] revealed that WBC counts in channel catfish fed Bio-MOS at 2 g/kg were insignificantly higher compared to fish fed the control diet. In rohu, the leucocyte count in fish treated with MOS at 1%, 2% and 4% was higher than the control [15].

Level of lymphocytes in the group of IW at 3% was higher than the other groups. The increase in lymphocyte count can cause a higher production of antibody [31]. According to [32] the increase in total serum protein and albumin concentrations can be due to stronger non-specific responses in fish. In this study, albumin content was significantly higher in the IW 3% group compared to the control. The levels of albumin in rohu fed the MOS-supplemented diet were significantly higher than the control [15].

Immunization of sturgeons against pathogens has not been developed as it has for cyprinids and salmonids [31]. IgM is an important part of humoral immune system. Inclusion of vitamin A, chitin, yeast cells and levamisole as immunostimulants to the diet of sea bream, *Sparus auratus*, increased IgM values [33]. In the current study, IW at 3% increased IgM levels in juvenile great sturgeon. Administration of IW at 3% resulted in an increase in serum lysozyme which can contribute to the enhancement in the innate immunity. The reports of Staykov [7] showed that rainbow trout treated with MOS at inclusion rate of 0.2% showed significant differences in lysozyme levels. However, in Atlantic salmon, lysozyme concentration was lower in the MOS-fed group compared to the control [14].

IW did not show significant differences in some hematological and biochemical variables of juvenile great sturgeon likely because there were no physical, chemical or bacterial stresses during the experiment. In addition, the trial period was short to show more stimulation of immune responses. Similar to the current results, differences in hematological variables such as RBC, Hb and Hct in channel catfish fed MOS at 2 g/kg were not found [10]. Furthermore, according to Sado [13] diets supplemented with 0.2%, 0.4%, 0.6% 0.8% and 1% MOS had no significant effects on RBC, Hb, Hct, WBC, MCV, MCHC, MCH and total protein in Nile tilapia. On the contrary, the findings of Andrews [15] demonstrated a significant improvement in WBC, RBC, Hb, serum protein, albumin and globulin in rohu fed the MOS-supplemented diet in comparison with the control. It appears that fluctuations in hematological and biochemical parameters may be associated to characteristics of species, inclusion rates of MOS, ingredients of diets, rearing period, etc.

CONCLUSION

The findings of this experiment indicate that IW can enhance growth performance and affect some immunophysiological variables in juvenile great sturgeon. Further investigations are needed to clarify the action mechanisms of MOS, the appropriate inclusion dose and suitable feeding period in great sturgeon.

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