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Research Article

Effect of a commercial probiotic (Supersets® and Varna®) on growth performance, hematological indices, and immunological parameters in sturgeon hybrid bester (*Huso huso* ♂× *Acipenser ruthenus* ♀) fingerling

Hosseinpour Zelaty A.[1], Sayed Hassani M.H.[1*], Sohrabi T.[1], Bagherzadeh Lakani F.[1], Mohseni M.[1], Pajand Z.[1]

1 International Sturgeon Research Institute, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran
* Correspondence: mirhamedhassani@yahoo.com

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Abstract

The aim of this study was to investigate the effects of dietary supplementation of a commercial probiotic containing *Bacillus subtilis*, *Lactobacillus acidophilus*, *L. delbrueckii*, *L. rhamnosus*, and *L. plantarum* on growth performance, hematological and immunological parameters of bester fingerlings. Two-hundred and forty fingerlings (10±0.9 g) were randomly distributed into 12 fiberglass tanks. The experimental diets were formulated using 0 (control), 100 (T100), 200 (T200), and 300 (T300) mg/kg of the probiotic. The fish were fed at 3% of body weight for 8 weeks. The result revealed that dietary probiotics increased final weight, condition factor, daily growth rate, specific growth rate, and body weight increase (p>0.05). Also, we observed a significant increase in feed efficiency ratio and protein efficiency ratio for fish fed with T300 compared to T200 (p<0.05). Hematological parameters were not influenced by dietary probiotic levels (p>0.05). There were no significant differences in total immunoglobulin (p>0.05), while alternative complement activity was significantly enhanced by the inclusion of probiotics in diets (p<0.05). The present study showed that feed efficiency, protein efficiency, and immunological parameters of bester fingerling have been improved by adding the commercial probiotic at 200 mg/kg.

Introduction

Today, general use of antibiotics in animal feed is banned and use of various additives is recommended. Probiotics are one of the best dietary supplements (Banerjee and Ray, 2017). Beluga (*Huso huso*) and Siberian sturgeon (*Acipenser baerii*) belong to the Acipenseridea family. This species has a high tolerance for unfavorable environmental conditions and rapid growth, thus being widely raised in cement and fiberglass tanks in Iran (Kalbassi *et al.*, 2013). In recent years, Bester has been introduced to the sturgeon farming industry. However, intensive culture and stocking density can significantly affect fish welfare and cause health problems, poor growth and diseases (Dalsgaard *et al.*, 2013; Abarike *et al.*, 2018). Probiotics are living organisms that are added to aqua feed or water to increase growth and resistance against adverse environmental conditions via improving microbial balance in the gastrointestinal tract of fish or rearing water (Merrifield *et al.*, 2010). probiotics can improve digestibility, non-specific immune system, growth rate, and feed conversion ratio in fish (Gatesoupe, 2008). Investigation about probiotic effect on sturgeon welfare is negligible (Askarian *et al.*, 2011; Faramarzi *et al.*, 2011; Faramarzi *et al.*, 2012; Iranshahi *et al.*, 2012; Soltani, 2016). Soltani *et al*. (2019) investigated the genetic diversity of autochthonous LAB isolated from Persian sturgeon fingerlings intestine (~3.2 g) and found that *Lactococcus garvieae* and *Lacbacillus lactis* constituted 42.5% and 36.1% of LAB population. Geraylou *et al*. (2012) reported that the gut microbiota of Siberian sturgeon juvenile has several bacteria including *Candidatus Arthromitus, Plesiomonas shigelloides, Cetobacterium somerae, Clostridium ruminantium), C.sardiniense, B. circulans.* Also, six Lactobacillus families have been described in the stomach or intestine. Although many LAB are harmless, some strains are beneficial for fish health (Gatesoupe, 2010). Literature has shown that Bacillus species application alone or in combination with other bacteria leads to improve growth, food consumption, and immune system in the host. Burr *et al*. (2005) stated that the production of a specific probiotic derived from sturgeon microflora is available (Hoseinifar *et al*., 2016). but an experiment conducted by Elsabagh *et al*. (2018) to evaluate a combination of Bacillus strains (*B. subtilis, B. licheniformis*, and *B. pumilus*) on Nile tilapia (*Oreochromis niloticus*) showed that the combination of probiotic bacteria will have a better effect on growth index and immune system. Therefore, the present study looked at the effect commercial aquaculture (CA) probiotic mixtures of *Bacillus* and *Lactobacillus* (*B. subtilis, L. acidophilus, L.delbrueckii, L. rhamnosus, and L. plantarum*) on growth performance, hematology, and immunological parameters of bester fingerling.

Materials and methods

The commercial aquaculture multi-strain probiotic with a 1×10^{10} CFU mixture of *B. subtilis, L. acidophilus, L. delbrueckii, L. rhamnosus,* and *L. plantarum* was prepared

from Superzist, Varena Company, Rasht, Iran.

Preparation of diets and probiotics

The experimental diets were as follows: the control (a commercial sturgeon diet; 1.9 mm diameter, Biomar, France), PR_{100}, PR_{200}, and PR_{300} diets. The proximate composition of the diets included 47% crude protein, 11% lipid, 1.16% phosphorus, 11% moisture, 10% ash, and 3% fiber as provided by Abzyco Company, Iran. To prepare $PR_{100\text{-}300}$ diets, the desired amount of bacterial powder (1×10^{10} CFU) was mixed with 50 ml of sterile physiological serum and sprayed into 1 kg of the control diet to obtain 1×10^{6}, 2×10^{6} and 3×10^{6} CFU g of probiotic bacteria in diet. Then Diets were dried at room temperature (24±1°C) (Merrifield *et al.*, 2010). The fish were fed twice a day for 8 weeks at 3% of the body weight (Mohseni *et al.*, 2008).

Rearing conditions and growth performance

Bester fingerlings were from the Caspian Sea International Sturgeon Research Institute, Rasht, Iran. All fish (~2 g) were acclimated in fiberglass tanks for two weeks and fed three times in day (Biomar, France). After this period, 240 fish were distributed in twelve fiberglass tanks (2 m^3) (30 fish per tank) with body weight and total length of 10.52±0.67 g and 13.26±0.11 cm (p>0.05), respectively. During the investigation, water temperature, pH (8.9), dissolved oxygen (6.4 mg/L), ammonia and photoperiod were estimated. For all experimental tanks, water flow was set at 2 L/min. Fish were fed 3 % of the body with experimental diets for 8 weeks. The fish were monitored every two weeks. For this purpose, individual fish were anesthetized using clove powder (150 mg/L) (Hallajian *et al.*, 2011) and their weight and total length were determined using a digital balancer (Mahak, Iran). Growth performance and feed utilization were calculated using the following formula:

CF= (Final Body Weight÷ Total length^3) × 100; WG = (Final weight − Initial weight); PWG=((Final weight − Initial weight (g)) / (initial weight)×100; FCR=Feed fed / Fish weight gain; S.G.R=(ln Final Body Weight −ln Initial Body Weight)/duration of rearing × 100; PER=Mean weight gain (g)/Protein intake

Where, PI = Feed intake ×% of protein in the diet.

Hematological and immune parameters assays

At the end of the feeding trial, fish were fasted for 24 h before blood sampling. Anesthetized and blood samples were collected using a syringe from the caudal vein. The blood sample was divided into two parts. One half was transferred to 2mL heparinized Eppendorf tubes for hematological assay, and other part was immediately centrifuged to separate serum (Labfuge 200, Frankfort, Germany) at 3000 ×g for 10 min at 4°C (Anderson *et al.*, 1997). White blood cells (WBCs) were counted by a Neubauer hemocytometer using an isotonic solution (Turk solution).

leukocyte (lymphocyte, monocyte, neutrophil, and eosinophil) were detected according to Amlashi *et al*. (2011) with a light microscope. Red blood cells (RBC) were counted using a Neubauer chamber. Hematocrit (Hct) was determined by the standard microhematocrit method and expressed as a percentage. Hemoglobin (Hb) was determined by spectrophotometer (U.V./Vis-6505, Junway, England) by Blaxhall and Daisley (1973) method. Erythrocyte index (mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and mean cell volume (MCV) were calculated using the following equations (Potki *et al.*, 2018):

$$\text{MCH (pg)} = \text{Haemoglobin (g/L)} \times \text{Red blood cell } (10^{12}/\text{L})$$
$$\text{MCHC (g/L)} = \text{Haemoglobin (g/L)} / \text{Haematocrit (\%)}$$
$$\text{MCV (fl)} = \text{Haematocrit} \times 10 / \text{Red blood cell } (10^{12}/\text{L})$$

The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured according to of Thomas (1998) method using Pars Azmun kits (Karaj, Iran) at 540 nm. Lysozyme activity was measured by a turbidimetric method according to method described by (Ellis, 1990). Alternative complement pathway activity (ACH_{50}) was measured according to Yano (1992) method based on rabbit hemolysis red blood cells. Serum immunoglobulin (IgM) value was measured by Siwicki (1993) method.

Feed and intestinal bacteria

Feed and intestinal tissues (10 g) (after removal of gut content) were transferred to sterile glass containers and 50 mL physiological serum (Mahban Darou., Tehran, Iran) was added. One-hundred µl of the above solution was cultured on MRS agar (Man Rogson Sharp). The plates were incubated at 30°C for 96 h in anaerobic conditions (Mermment, Germany) and the number of bacteria was recorded (Merrifield *et al.*, 2010).

Statistical analysis

Statistical analyses were done by SPSS statistical package version 20 (SPSS Inc., Chicago IL, USA). One-way analysis of variance followed by Tukey's post hoc test was used for data analysis with a confidence interval of 95% after checking for the normality of data and homogeneity of variance. Data were presented as mean ± SD of experiments performed in triplicate.

Results

Growth performance

Bester Growth performance is shown in Table 1. The fish fed T_{300} showed the highest FW and CF (p>0.05) and lowest FCR (p<0.05). There were no differences in specific growth rate and weight gain in fish fed different experimental diets (p>0.05). Also, fingerlings with the best feed and protein efficiency ratio belonged to 300 mg/kg probiotic group (p<0.05). In contrast, fingerlings fed with 200 mg/kg probiotics showed the lowest FE and PER (p<0.05).

Hematological parameters

CA probiotic effects on hybrid bester hematological and immunological parameters are shown in Tables 2 to 4. Probiotics in the diets (200 and 300 mg/kg) increased hematocrit, hemoglobin, white blood cells, red blood cells, and monocyte compared to other treatments. But differences were not significant (p>0.05). An increase in probiotic level did not change MCV, MCH and MCHC, neutrophil, and lymphocytes (p>0.05). Probiotic supplementation has a positive effect on fish immune system indices (P>0.05). However, no significant differences were observed in IGM and lysozyme in fish fed different levels of probiotics. However, dietary probiotics significantly increased serum ACH_{50}. The highest value (135±5.5 un/%) was observed in fish fed on T_{200} diet (p<0.05).

Table 1: Growth index and feed conversion ratio of bester fed with different levels of commercial aquaculture probiotic comprising *B. subtilis, L. acidophilus, L. delbrueckii, L.rhamnosus,* and *L.plantarum* for 8 weeks.

Indices	T_{300}	T_{200}	T_{300}	T_0
Initial weight (g)	10.54 ± 0.06	10.38 ± 0.25	10.49 ± 0.12	10.69 ± 0.24
Final weight (g)	78.44 ± 2.14	70.37 ± 4.66	70.26 ± 8.09	72.15 ± 3.07
Initial Length (cm)	13.48± 0.17	13.42 ± 0.08	12.57 ± 0.07	13.59 ± 0.12
Final Length (cm)	27.85± 0.53	27.30± 0.56	27.47± 0.56	27.59± 0.75
Initial biomass (gr)	210.80± 1.23	207.50± 4.99	209.63± 2.30	213.70± 4.75
Final biomass (gr)	1568.73 ± 42.89	1407.40± 93.25	1405.20 ± 161.73	1443.07± 61.43
Product (gr)	1357.93± 43.16	1199.90± 92.35	1195.57± 161.60	1229.37± 56.92
FI (gr)	1234.53± 57.24	1197.32± 63.15	1131.52± 66.23	1206.30± 28.82
CF	0.36 ± 0.01	0.35 ± 0.01	0.34 ± 0.03	0.34 ± 0.02
FCR	0.91± 0.02 ^{b}	1.00± 0.03^{a}	0.95± 0.07^{ab}	0.98± 0.03^{ab}
DGR (gr/ day)	1.21 ± 0.04	1.07± 0.09	1.07± 0.14	1.10 ± 0.05
SGR (% /day)	3.58± 0.06	3.42± 0.12	3.39± 0.20	3.41± 0.04
BWI (% / total experiment)	643.98± 21.74	578.36 ± 44.85	570.33 ± 76.96	575.09± 15.08
FE	110.05 ± 1.71^{a}	100.14 ± 2.93 ^{b}	105.35 ± 7.88^{ab}	101.87 ± 2.31^{ab}
PER	2.45 ± 0.04^{a}	2.22 ± 0.06^{b}	2.34± 0.17^{ab}	2.27± 0.05^{ab}

Data are given as mean ± SD. The mean values in the same row with different letters are significantly different (p<0.05). FI: Food intake; CF: Condition factor; FCR: feed conversion ratio; DGR: daily growth rate; SGR: specific growth rate; BWI: Body weight increase; FE: Feed efficiency; PER: Protein efficiency ratio

Table 2: Hematological parameters of bester fed with different levels of commercial aquaculture (CA) probiotic comprising *B. subtilis, L. acidophilus, Lactobacillus delbrueckii, Lactobacillus rhamnosus,* and *Lactobacillus plantarum* for 8 weeks.

Indices	T_0	T_{100}	T_{200}	T_{300}
Hematocrit (%)	29.7± 1.5	28.0± 1.0	30.0± 6.0	30.3± 5.1
Hemoglobin (g/dl)	6.1± 0.4	5.8± 0.2	6.1± 1.0	6.1± 1.0
RBC (µl $\times 10^6$)	0.768± 0.047	0.726± 0.022	0.78± 0.14	0.79± 0.16
WBC (µl $\times 10^3$)	9.96±0.568	11.0± 0.5	10.9± 2.1	11.6± 2.30
MCV (fl)	386.3± 5.5	385.0± 1.7	379.7± 10.1	381.3± 12.9
MCH (pg)	79.0± 1.7	80.3± 0.6	77.7± 4.0	76.3± 4.0
MCHC (gr/dl)	20.7± 0.6	20.7± 0.6	20.3± 1.2	19.7± 0.6
Neutrophil (%)	22.3± 1.5	23.7± 1.5	22.3± 1.5	23.7± 2.1
Lymphocytes (%)	73.0± 3.0	71.7± 2.5	72.7± 2.3	69.7± 2.5
Monocyte (%)	4.0± 1.0	4.0± 1.0	5.0± 1.0	5.7± 1.5
Eosinophil (%)	0.7± 0.6	0.7± 0.6	0.0± 0.0	1.0± 1.0

Data are presented as mean ± SD. Data in the same row with different superscripts are significantly different (p>0.05). hematocrit (Hct), hemoglobin (Hb), red blood cell (RBC), mean erythrocyte cell volume (MCV), mean erythrocyte cell hemoglobin content (MCH), mean erythrocyte cell hemoglobin concentration (MCHC), monocytes, neutrophil, white blood cell (WBC), lymphocyte, and eosinophils and biochemical parameters: Cholesterol, Total Lipid, Albumin, and Total Protein.

Table 3: Immune system index of bester fed with different levels of commercial aquaculture (CA) probiotic comprising *B. subtilis, L.acidophilus, Lactobacillus delbrueckii, Lactobacillus rhamnosus,* and *Lactobacillus plantarum* for 8 weeks.

Indices	T_0	T_{100}	T_{200}	T_{300}
IGM (mg/dl)	60.7± 3.1	54.7±6.5	74.0±11.0	66.0± 9.0
Lysozyme (un/mL/min)	33.5 ± 0.5^{ab}	33.0 ± 2.0^{b}	34.0 ± 1.0^{a}	33.0 ± 1.0^{ab}
ACH_{50} (un/%)	118.0 ± 5.0^{b}	133.0 ± 5.0^{a}	135.0 ± 5.0^{a}	130.0 ± 5.0^{a}

Data presented as mean ± SD. Data in the same row with different superscripts are significantly different (p<0.05). Total immunoglobulin (IgM), lysozyme activity, and alternative complement activity (ACH_{50}).

Table 4: Total count of commercial aquaculture probiotic comprising *B. subtilis, L. acidophilus, Lactobacillus delbrueckii, Lactobacillus rhamnosus,* and *Lactobacillus plantarum* on intestine bacterial total count of bester fingerling for 8 weeks.

Indices	Diet 1 T_0	Diet 2 T_{100}	Diet 3 T_{200}	Diet 4 T_{300}
bacterial total count (log CFU/g)	3.38 ± 0.1^{b}	3.48 ± 0.15^{b}	3.81 ± 0.1^{ab}	4.6 ± 0.1^{a}

Data presented as mean ± SD. Data in the same row with different superscripts are significantly different (p<0.05).

Discussion

The isolation and characterization of cultivated allochthones lactobacilli were carried out on beluga (Askarian *et al.*, 2011) and *Acipenser persicus* (Ghanbari *et al.,* 2009). The stabilized bacteria in the intestine resulted in growth indices improvement in this species (Hoseinifar *et al.,* 2016). In the present study, we showed that fish fed by a non-isolated native protein had more food consumption than control. Also, we showed that the final biomass, FI, CF, DGR, SGR, and BWI had increased trends while this progress was not significant. Similarly, some other studies that worked on Siberian sturgeon (Hassani *et al.,* 2020), Persian sturgeon (Askarian *et al.*, 2011; Soltani, 2013), Nile tilapia (Shelby *et al.*, 2006), and channel catfish (*Ictalurus punctatus*) (Shelby *et al.*, 2007) reported the same results. The results of SGR and FCR in fish fed T_{300} are approved by the studies that worked on beneficial microbiota in feed and reported improved growth parameters, intestinal microflora, digestion enzymes, and increased appetite in Zebrafish (*Danio rerio*) (Rawles *et al.*, 2004) and *Cyprinus carpio* (Ran *et al.*, 2018). Also, the higher total numbers of colonies in the intestine of fishes fed T_{100}, T_{200}, and T_{300} compared to T_0 indicated that our current probiotic comprising *B. subtilis*, *L. acidophilus*, *L. delbrueckii*, *L. rhamnosus* and *L.plantarum* similar to sturgeon native probiotic bacteria (*L.lactis* and *weissella cibaria*) (unpublished data) can substitute in bester intestine as microflora, that lead to protease, lipase and amino acids secretion, high digestion ability, growth improvement, and low food conversion ratio (Askarian *et al.*, 2011).Unfortunately, in this study, intestinal microflora and gastrointestinal enzymes were not measured, but the increased growth rate and decreased FCR can confirm the above issues. Furthermore, in the present study, the hybrid sturgeon fingerlings fed with non-isolated native probiotics had faster swimming behavior, high final biomass, FI, CF, DGR, SGR, and BWI indices, that were consistent with previous studies on Siberian sturgeon (Hassani *et al.,* 2020), Persian sturgeon (Askarian *et al.,* 2011; Soltani, 2016), Nile tilapia (Shelby *et al.,* 2006) and channel catfish (Shelby *et al.,* 2007) fed probiotic

supplemented diets. A wide range of exo-enzymes secretion by *Lactobacillus* has been reported (Suzer *et al.*, 2008). In addition to bester fingerlings' improved SGR and FCR indices that represented in this study, microbiota replacement in the intestine can lead to gut microflora enhancement, more efficient digestion enzymes activity, and more appetite that was noticed in Zebrafish (Rawles *et al.*, 2004) and Common carp (Ran *et al.*, 2018). In this study, we observed Higher counts of *B. subtilis*, *L.acidophilus*, *L. delbrueckii*, *L. rhamnosus*, and *L. plantarum* in the intestine of fish fed T_{100}, T_{200} and T_{300} compared to T_0. This indicated that CA probiotics successfully settled in bester intestine, which led to higher enzyme secretion (protease and lipase) for food digestion and improved growth and FCR (Askarian *et al.*, 2011). Although, in this study, enzyme digestion was not measured in hybrid bester sturgeon, the probable produced intestinal microflora by probiotics may contributed to better digestibility and a higher growth performance. Hematological parameters are special indicators of fish health status and they are affected by environmental stress (Schütt *et al.*, 1997). In this study, there were no significant differences in hematological indices between treatments but hematocrit, RBC, WBC, neutrophil, monocyte, and eosinophil values of the fish in T_{300} treatment were higher than other groups. Hemoglobin and erythrocyte numbers are good indicators of the oxygen transportation capacity of fish (Lamas *et al.*, 1994). On the other hand, an increase in hemoglobin and hematocrit plays an important role in improving fish health, enhancing fish immunity and growth (Talpur *et al*., 2012). On the other hand, rising in the number of RBCs can intensify the concentration of hemoglobin and eventually leads to a high oxygen-carrying capacity in fish fed probiotic. Our daily observations showed that fish fed T_{300} had more freshness and vitality compared to other treatments. Also, in agreement with Hassani *et al*. (2020) and Soltani *et al*. (2016) that asserted the supplementation of feed with probiotics (lactic acid bacteria) leads to RBC, hemoglobin, and WBC increase in fingerlings Siberian sturgeon and Persian sturgeon. Some scientists believe that higher counts (%) of phagocytic cells (neutrophils and monocytes) and lymphocytes are infection occurrence indices (Mohapatra *et al*., 2012), and many studies showed that probiotics have stimulation effect on phagocytic cell proliferation in *Oncorhynchus mykiss* (Panigrahi *et al.*, 2004), *Labeo rohita* (Nayak *et al.*, 2007), Nile tilapia (Pirarat *et al*., 2011), and Persian sturgeon (Soltani, 2016). Also, probiotics have a multiplier factor in leukocytes (neutrophils) and NK cells in Rainbow trout and Rohu that leads to innate immune responses improvement (Nikoskelainen *et al*., 2001; Irianto and Austin, 2002; Kumar *et al*., 2008).In the present study, we also found an increase in neutrophils and monocytes number in T_{300} group, which may be due to phagocytic activity stimulant of our probiotic in bester, but the number of lymphocytes in T_{300} treatment decreased compared to other treatments, respectively and, the reason is not clear.

In fish like other vertebrates, intrinsic defense mechanisms include epithelial barriers such as skin, gills, intestines, and mucosa, lectin, lysozyme, C-reactive protein, interferon, complement system, and inflammatory reactions that have prophylactic effects on disease outbreaks in fish. Many studies have shown that probiotic bacteria are known to have immunestimulant effects (Gatesoupe, 1999; Verschuere *et al*., 2000). In the present study, probiotics caused a non-significant increase in lysozyme and IgM and a significant increase in complement in fish fed T_{300}. Lysozyme may act on the peptidoglycan layer of the bacterial cell walls resulting in the lysis of the bacterium. Lysozyme was found in mucus and ova (Kiron, 2012), and the serum lysozyme was used as a non-specific immune response indicator. Some studies indicated lysozyme enhancement level in Siberian sturgeon fed (3×10^6 CFU g *B. subtilis*, *L. acidophilus*, *L. delbrueckii*, *L. rhamnosus*, and *L. plantarum*) (Hassani *et al*., 2020), Persian sturgeon fed (10^8 CFU/g LAB) (Soltani *et al*., 2016) and Russian sturgeon (*Acipenser gueldenstaedtii*) fed (symbiotic Biomin IMBO,1.5-5 g/kg diet) (Jafarzadeh *et al.*, 2015). Despite the fact that leukocytes are the source of lysozyme production, WBC increase (Soltani *et al*., 2016) or stimulation of innate immune system triggers lysozyme secretion and leads to lysozyme increase (Ibrahim, 2015; Jafarzadeh *et al*., 2015).

Complement roles in sturgeons are not known clearly as teleost fish. The complement system in teleost fish serum is one of the most potent non-effector cell responses of the immune system and may be activated by antigen-specific antibodies, a microbial cell surface, or a lectin (Hellio *et al*., 2007). Probiotics can enhance the natural complement activity of teleost fish (Ellis, 1999; Panigrahi *et al*., 2007). Similar results were reported in Siberian sturgeon (Hassani *et al*., 2020), Persian sturgeon, (Soltani *et al*., 2016), Orange-spotted grouper (*Epinephelus coioides*) (Son *et al*., 2009), Striped beakfish (*Oplegnathus fasciatus*) (Harikrishnan *et al*., 2010), Cobia (*Rachycentron canadum*) (Geng *et al*., 2011), and Persian sturgeon (Soltani *et al*., 2016). It seems that commercial probiotics have been able to significantly stimulate complement secretion in the blood serum of fish, although more studies are needed to understand their mechanism of action.

Immunoglobulins are natural antibodies that are fully regulated in the absence of foreign antigens and have extensive protection against pathogens. This feature makes them a vital part of the non-specific fish immune system (Magnadottir, 2006). In this study, there was no significant difference in the ranges of immunoglobulin in different treatments, but the highest level of immunoglobulin was observed in fish at T_{200} treatment. Ghiasi *et al*. (2018) confirmed that the total serum immunoglobulin (Ig) was elevated in Beluga (248.32±10.21 g) fed probiotic *Pediococcus acidilactici* for eight weeks. Similarly, Hassani *et al*. (2020) observed a steadily augmented trend of IgM activities by elevating lactic acid bacteria (Superzist® and Varena®) up to 3×10^6 CFU/g in Siberian sturgeon fingerlings during 56 days.

Although, our result was inconsistent with previous findings, which showed no

significant differences in the IgM levels. It is necessary to note that the feeding duration of probiotics is another important factor to affects the establishment, persistence, and subsequent induction of immune responses in the host. In fish, the most beneficial effects like high weight gain, improved immunity, and disease resistance have been recorded through a dietary probiotic feeding regime in 1-10 weeks (Nayak, 2010). However, the duration of our study was only 8 weeks and the stimulating innate immunity effects of different probiotic strains and their feeding duration can vary in the same family (Hoseinifar *et al*., 2016).

Finally, we concluded that adding the commercial probiotic at 200 mg/kg had beneficial effects on growth rate and some hematological and immunological indices in hybrid bester sturgeon. Based on the present results, it is recommended to use this product at high levels, due to the improvement of growth indices especially in T_{200}.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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