Aquaculture International (2020) 28:377–387
https://doi.org/10.1007/s10499-019-00468-1

# Effects of commercial superzist probiotic on growth performance and hematological and immune indices in fingerlings *Acipenser baerii*

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Received: 11 June 2019 / Accepted: 24 September 2019 / Published online: 28 October 2019


**Abstract**
This study was carried out to evaluate the effects of using superzist probiotic (a mixture of *Lactobacillus* spp., *Bacillus subtilis*, and *Bifidobacterium bifidum*) on growth performance and hematological and some immunological indices in fingerling *Acipenser baerii*. In total, 240 *Acipenser baerii* fingerlings with mean weight 10.5 ± 0.14 $g^{-1}$ were stocked in 12 tanks, 20 per each tank and each treatment in triplicate. Diets were prepared by spraying slowly the mixture of 50 ml saline serum with 100, 200, and 300 mg probiotic powder per 1-kg diet to make the concentrations $1 \times 10^6$, $2 \times 10^6$, and $3 \times 10^6$ CFU $g^{-1}$ of probiotic bacteria in diet. Results showed that there was a significant increase in the final weight, weight gain (WG), percentage weight gain, condition factor (CF), and specific growth rate (SGR) in fish fed $PB_{300}$ treatment compared with the control at the 8th week ($p < 0.05$); also, the lowest feed conversion ratio (FCR) belonged to fish fed $PB_{300}$ that showed a significant difference compared with fish fed control diet ($p < 0.05$). Except for neutrophil, lymphocyte, and hematocrit ($p < 0.05$), values of all hematological parameters of fish fed different concentrations of probiotic diet did not differ from the values of fish fed the control diet ($p > 0.05$), but there was a significant increase in lysozyme and IgM fish fed probiotic ($PB_{300}$) and compliment ($ACH_{50}$) ($PB_{200}$ and $PB_{300}$) compared with control treatment during 56 days. Therefore, results indicated that 300 mg $kg^{-1}$ probiotic (*Lactobacillus* and *Bifidobacterium*) can be used as a proper probiotic in sturgeon aquaculture to enhance fish health and growth performance.

**Keywords** *Acipenser baerii* · Probiotic · Growth rate · Hematological indices · Immune system

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## Introduction

Siberian sturgeon, *Acipenser baerii* is one of the most important sturgeon species that is used for caviar and meat production in sturgeon aquaculture (Bronzi et al. 2011). However, high-density rearing seems to lead to poor physiological environment that adversely affects fish health and growth rate in fingerlings and Juvenile periods (Yazdani et al. 2010) and therefore lead to a high degree of mortality. In the past decades, heavy reliance on vaccines and antibiotics to combat these diseases is intensive culture (Broch et al. 2015), but adverse effects on the use of antibiotics to control diseases have created some problems. These effects include accumulation of antibiotics in the tissue, fish immune-suppression (Tukmechi et al. 2007; Nayak et al. 2007; El-Haroun et al. 2006), and ecological threat to coastal areas that caused by heavily exploited for industrial cultivation of fish and shell fish (Gildberg et al. 1997). The probiotic is alive or dead or is a component of a microbial cell that when included to the feed or to the rearing water has benefits for the host by improving its microbial balance of digestive tract or microbial balance of the ambient environment (Merrifield et al. 2010C); in addition, probiotic has beneficial effect on diseaces controls via enhancement of the non-specific innate immunity system (Gatesoupe 2008) and improve digestibility (Cruz et al. 2012) in fish that leads to the increase growth of the cultured organisms (Balcazar et al. 2006).

During the last decade, an improved understanding arose of the importance of commensal microbiota in the fish intestine and production of indigenous probiotics (Burr et al. 2005). Askarian et al. (2009) isolated two lactic acid bacteria (LAB) species, *Lactobacillus curvatus* and *Leuconostoc mesenteroides*, from the GI tract of *Huso huso* and *A. baerii*, respectively. In other study, Ghanbari et al. (2009) carried out isolation and characterization of cultivable *allochthonous lactobacilli* in this species. Also, Hoseinifar et al. (2016) addressed the effects of *Lactococcus lactis* JF831150 administration on intestinal microbiota (TVC and presumptive LAB levels) of *A. baerii* in a 56-day feeding trial, but little information is available on the use of commercial probiotics in sturgeon studies. For example, some studies have evaluated the effects of probiotic on sturgeon growth performance, physiology, and health status (Faramarzi et al. 2011, 2012a, b; Iranshahi et al. 2011) and modulation of the intestinal microbiota (Askarian et al. 2011). Therefore, the present study was undertaken to evaluate the potential effect of the dietary administration of the commercial aquaculture probiotic (superzist, Varena Co., Iran) *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum* on growth parameters, blood, biochemical, and immune indices in this species.

## Materials and methods

### Probiotics and preparation of diets

The probiotic powder was prepared from Zistyar Varena Co. (Rasht, Gilan Province, Iran) with $10 \times 10^{10}$ CFU mixture of *Bacillus subtilis*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium bifidum*. A commercial diet composed of 47% crude protein, 11% lipid, 1.16% phosphorus, 11% moisture, 10% ash, and 3% fiber was used as a control diet (1.9-mm pellets, BioMar, France). Diets were prepared by spraying slowly the mixture of 50 ml saline serum with 100, 200, and

300 mg probiotic powder per 1-kg diet to make the concentrations $1 \times 10^6$, $2 \times 10^6$, and $3 \times 10^6$ CFU $g^{-1}$ of probiotic bacteria in diet except for the control diet and named $PR_{100}$, $PR_{200}$, and $PR_{300}$.

## Fish and rearing conditions

The fish were obtained from the breeding of 5 Siberian sturgeon breeders (3 female and 2 male fish) in International Sturgeon Research Institute. One thousand larvae absorbed yolk sac were transferred to 15 fiberglass tanks ($1.5 \times 0.5 \times 0.3$ $mm^3$). After larva transferring, feeding was done under the same culture conditions with *Artemia* (stage Instar I) and *Daphnia* (for 20 days); then for another 15 days, larvae were fed by dry diet mixed with different percentages of *Gammarus* for adaptation for commercial diet. During the adaptation period, the fish fed commercial diet well and had low mortality rate (3 to 5%). Fingerlings adapted to experimental diets for 7 days. During the adaptation period, the feed efficiency in fish fed by dry diet was high with low mortality rate (survival, 95%; and mortality, 3 to 5%). Also, fingerlings had fast swimming, had no lesions in the body morphologically, and fed dry diet well. After adaptation, a total of 240 fingerlings (average weight $10.5 \pm 0.14$ $g^{-1}$) were stocked into 12 circular fiberglass tanks (diameter 200 cm, water volume 2000 l, water flow 4.75 l $min^{-1}$) and fed with diet (1.9-mm pellets, BioMar, France) supplemented with varying inclusions of probiotic (100, 200, and 300 g $kg^{-1}$) ($PR_{100}$, $PR_{200}$, and $PR_{300}$) for 8 weeks. Fish were fed twice daily by hand in the rate of 3% of body weight for 8, 13, and 22 h. Each of 2 weeks, the weights of each experimental fish were determined using a digital balancer (Mahak, Iran). Water quality was monitored daily and was within acceptable limits throughout the experiment. Dissolved oxygen concentrations, temperature, and pH were ranged 6.0–6.5 $mg/l^{-1}$, 20–23 °C, and 6.5–6.8, respectively. Growth performance of the *A. baerii* was calculated using the following formula (Hung et al. 1989):

$$\mathrm{CF} = \left(\textit{Final Body Weight}/\textit{Total length}^3\right) \times 100$$

$$\mathrm{WG} = (\textit{Final weight} - \textit{Initial weight})$$

$$\mathrm{PWG} = ((\textit{Final weight} - \textit{Initial weight}\ (\mathrm{g}))/(\textit{initial weight}) \times 100$$

$$\mathrm{FCR} = \textit{Feed}\ \mathrm{fed}/\textit{Fish weight gain}$$

$$\mathrm{SGR} = (\ln \textit{Final Body Weight} - \ln \textit{Initial Body Weight})/\textit{duration of rearing} \times 100$$

$$\mathrm{PER} = \textit{Mean weight gain}\ (\mathrm{g})/\textit{Protein intake}$$

where PI = Feed intake × % of protein in diet

## Blood index assays and total bacterial count in the intestine and food

At the end of trial feeding, fish were fasted for 24 h immediately prior to blood sampling and 30% of fish per each tank were randomly chosen and anaesthetized with clove powder (150 mg $l^{-1}$) (Hallajian et al. 2011). The blood samples were collected through a syringe by caudal vein and stored in non-heparinized tubes. For biochemical assays, blood samples were immediately centrifuged (3000 *g*, 10 $min^{-1}$) at room temperature and then serum was separated and stored at − 20 °C until analysis (Anderson et al. 1997). The hematological indices included white blood cell (WBC), red blood cells (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were measured by a spectrophotometer at 450 nm (UV/Vis-6505 N, Junway Company, England) using commercial kits (Pars Azmun Co. Ltd., Tehran, Iran). Lysozyme and compliment were measured by AutoAnalyzer Technicon (R.A.1000, Junway Company, England) using commercial kits (Pars Azmun Co. Model ISC and ILT., Tehran, Iran) described by Ellis 1990, and IgM were determined by the nephelometric method using the Binding Site Kit (Yousefi Jourdehi et al. 2014).

Also for determination of viability and counting of bacteria in food and the intestine, at first, 10 g of enrichment food and the intestine were weighted separately; contents of the intestine were washed three times with physiological serum. Then, the weighted material was transferred to sterile glass containers and physiological serum was added to give the desired dilution. In the following, 100 μl of solution was cultured on a culture medium of MRS (Man Rogson Sharp) bacteria, respectively. Plates were incubated at 30 °C for 96 h in anaerobic conditions, and after incubation, bacteria were counted (Merrifield et al. 2011).

## Statistical analysis

Statistical analyses were conducted using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Duncan's test was used for data analysis after checking the normality of data and homogeneity of variance (Table 1). Mean values were considered significantly different at $p < 0.05$. Data are presented as mean values ± SD.

**Table 1** Statistical results of one-way ANOVA for effect of superzist probiotic on growth indices of *Acipenser baerii* during 8-week feeding

| Indices | Diet 1 ($PB_0$) | Diet 2 ($PB_{100}$) | Diet 3 ($PB_{200}$) | Diet 4 ($PB_{300}$) |
|---|---|---|---|---|
| Initial weight ($W_1$) (g) | $10.36 \pm 9.1^{a}$ | $10.57 \pm 0.55^{a}$ | $10.61 \pm 0.87^{a}$ | $10.45 \pm 0.13^{a}$ |
| Final weight ($W_2$) (g) | $66 \pm 4.96^{b}$ | $68.00 \pm 3.8^{b}$ | $70.36 \pm 4.8^{ab}$ | $77.24 \pm 1.48^{a}$ |
| Condition factor | $0.24 \pm 0.018^{b}$ | $0.28 \pm 0.017^{ab}$ | $0.28 \pm 0.016^{ab}$ | $0.3 \pm 0.029^{a}$ |
| Weight gain | $55.84 \pm 2.7^{b}$ | $57.62 \pm 2.23^{b}$ | $59.75 \pm 2.82^{ab}$ | $66.79 \pm 0.81^{a}$ |
| Percentage weight gain (%) | $539.55 \pm 55.16^{b}$ | $544.88 \pm 38.59^{b}$ | $563.31 \pm 47.25^{ab}$ | $638.97 \pm 11.68^{a}$ |
| Specific growth rate (%) | $3.14 \pm 0.14^{b}$ | $3.5 \pm 0.17^{a}$ | $3.2 \pm 0.12^{ab}$ | $3.38 \pm 0.026^{a}$ |
| Feed conversion ratio | $1.63 \pm 0.2^{a}$ | $1.6 \pm 0.05^{a}$ | $1.56 \pm 0.04^{a}$ | $1.34 \pm 0.01^{b}$ |
| Protein efficiency ratio | $1.37 \pm 0.17^{b}$ | $1.38 \pm 0.04^{b}$ | $1.42 \pm 0.04^{b}$ | $1.65 \pm 0.01^{a}$ |

* Different superscript letters indicated significant difference (±SD) ($P < 0.05$)

**Table 2** Statistical results of one-way ANOVA for effect of superzist probiotic on the hematological and immune systems of fingerling *Acipenser baerii* during 8-week feeding

| Indices | Diet 1 ($PB_0$) | Diet 2 ($PB_{100}$) | Diet 3 ($PB_{200}$) | Diet 4 ($PB_{300}$) |
|---|---|---|---|---|
| Hematocrit (%) | $27.66 \pm 0.33^{b}$ | $29.66 \pm 1.2^{ab}$ | $29 \pm 0.57^{ab}$ | $32.33 \pm 2.1^{a}$ |
| WBC ($mm^3$) | $10866.66 \pm 696.02^{a}$ | $14{,}933.3 \pm 1980.17^{a}$ | $15{,}433.3 \pm 633.3^{a}$ | $16{,}233.3 \pm 1134.8^{a}$ |
| Hemoglobin (g $dl^{-}$) | $5.63 \pm 0.38^{a}$ | $5.6 \pm 0.057^{a}$ | $5.96 \pm 0.5^{a}$ | $6.36 \pm 0.029^{a}$ |
| RBC ($mm^3$) | $771{,}666.6 \pm 19{,}220.93^{a}$ | $786{,}251.3 \pm 21{,}880.9^{a}$ | $795{,}240.3 \pm 28{,}168.14^{a}$ | $863{,}255.3 \pm 26{,}117.25^{a}$ |
| MCV (fl) | $385.53 \pm 1.1^{a}$ | $385.26 \pm 1.2^{a}$ | $383.66 \pm 1.85^{a}$ | $387.66 \pm 2.1^{a}$ |
| MCH (pg) | $79.00 \pm 1.00^{a}$ | $78.00 \pm 1.15^{a}$ | $79.6 \pm 0.66^{a}$ | $81.00 \pm 1.15^{a}$ |
| Neutrophil (%) | $15.66 \pm 0.2^{b}$ | $19.66 \pm 1.2^{a}$ | $21.33 \pm 0.88^{a}$ | $20.00 \pm 1.52^{a}$ |
| Lymphocyte (%) | $74.66 \pm 2.96^{b}$ | $75.66 \pm 1.7^{b}$ | $79.66 \pm 1.5^{ab}$ | $84 \pm 1.00^{a}$ |
| Monocyte (%) | $3.66 \pm 0.57^{a}$ | $4.00 \pm 1.5^{a}$ | $4.66 \pm 0.57^{a}$ | $4.33 \pm 1.52^{a}$ |
| Lysozyme (u/ml/min) | $24.33 \pm 0.57^{b}$ | $25.33 \pm 3.7^{ab}$ | $30.66 \pm 2.08^{ab}$ | $34.00 \pm 2.29^{a}$ |
| Compliment (ACH50) (U %) | $118.66 \pm 10.96^{b}$ | $126.00 \pm 1.73^{ab}$ | $132.33 \pm 4.93^{a}$ | $134.33 \pm 4.04^{a}$ |
| IgM (g $dl^{-}$) | $22.66 \pm 2.08^{b}$ | $27 \pm 3.6^{ab}$ | $33.3 \pm 3.21^{ab}$ | $36 \pm 3.3^{a}$ |

* Different superscript letters indicated significant difference (±SD) ($P < 0.05$)

## Results

The growth performance of *Acipenser baerii* fed diets containing various probiotic levels is shown in Table 2. After 8-week feeding, fish fed the basal diet had a significant lower growth performance than those fed diet 4 ($PB_{300}$). Fish fed with $PB_{300}$ have the highest final weight (FW), weight gain (WG), and percentage weight gain (PWG). Also, the fish treated with $PR_{300}$ were more interested in food than the control. Increase of commercial probiotic in diet leads to an increase of protein efficiency ratio. The highest parameter showed belonged to the fish fed with diet containing 300 mg $kg^{-1}$ probiotic. Also, specific growth rate (SGR) in fish fed with dietary $PB_{100}$ and $PB_{300}$ were significantly higher than that in fish fed basal diet and $PB_{200}$. Feed conversion ratio (FCR) in fish fed with basal diet showed no significant difference ($p > 0.05$) from that in those fed diets $PB_{100}$ and $PB_{200}$, but significantly different ($p < 0.05$) from that in fish fed $PB_{300}$ (300 mg $kg^{-1}$ probiotic) (Table 2). The best of protein efficiency ratio of fingerling *Acipenser baerii* during 8-week feeding belonged to diet 4 ($PB_{300}$) ($p < 0.05$).

Blood samples collected at the end of the feeding trial during the experiment indicated that the hematocrit in fish fed with control diet has a significant difference ($p < 0.05$) compared with that in fish fed $PB_{300}$ (300 mg $kg^{-1}$) but no significant difference ($p > 0.05$) showed in $PB_{100}$ and $PB_{200}$ groups. There was no significant difference in WBC of fish fed diets $PB_0$, $PB_{100}$, $PB_{200}$, and $PB_{300}$. The red blood cell count (RBC) showed no significant difference ($p > 0.05$) in fish fed $PB_0$, $PB_{100}$, $PB_{200}$, and $PB_{300}$; however, with increasing probiotic in diet, WBC and RBC showed an increasing trend. The highest hemoglobin was measured in fish fed diet $PB_{300}$, and the lowest was observed in fish fed with control diet without significant difference ($p > 0.05$). There were no significant differences ($p > 0.05$) in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), but the percentage of neutrophil in all fish fed had varying concentrations. In the other hand, at the end of 8-week fish feeding, the highest percentage of neutrophil was recorded for fish fed with $PB_{300}$ and showed a significant difference in fish fed $PB_0$, but no significant difference in fish fed $PB_{100}$ and $PB_{200}$. The lowest lymphocyte was recorded in fish fed with $PB_0$ and the highest was recorded in fish fed $PB_{300}$ ($p < 0.05$). RBC showed no significant difference in all diets ($p > 0.05$). There was no significant difference ($p < 0.05$) in the monocyte of all fish fed varying quantity of probiotic. At the end of the experimental period (8 weeks), the fish fed $PB_{300}$ (300 mg $kg^{-1}$ probiotic) had better secretion of lysozyme, compliment ($ACH_{50}$), IgM and was higher than the fish fed the control diet ($p < 0.05$), whereas fish fed diets $PB_0$, $PB_{100}$, and $PB_{200}$ had no significant difference in lysozyme, compliment ($ACH_{50}$), and IgM levels ($p > 0.05$) (Table 2).

Mean values were considered significantly different at $p < 0.05$. Means within column with different superscript letters are significantly different (± SD) ($p < 0.05$)

**Table 3** Statistical results of one-way ANOVA superzist probiotic on intestine bacterial total count m of fingerling *Acipenser baerii* during 8-week feeding

| Indices | (Diet 1) ($PB_0$) | (Diet 2) ($PB_{100}$) | (Diet 3) ($PB_{200}$) | (Diet 4) ($PB_{300}$) |
|---|---|---|---|---|
| Bacterial total count (log CFU $g^{-1}$) | $3.26 \pm 0.1^{b}$ | $3.27 \pm 0.15^{b}$ | $3.56 \pm 0.2^{ab}$ | $4.2 \pm 0.1^{a}$ |

* Different superscript letters indicated significant difference (±SD) (P < 0.05)

The results of total bacterial count in the *H. huso* intestine fed with $PB_0$, $PB_{100}$, $PB_{200}$, and $PB_{300}$ were calculated which the highest bacterial count was observed in the intestine of fish fed the $PB_{300}$ that showed a statistically significant difference with the bacterial intestine fed the $PB_{200}$ and $PB_{100}$ diets ($p < 0.05$). Also, the approximate total bacterial count in diets was being CFU/$g^{-1}$ (Table 3).

Mean values were considered significantly different at $p < 0.05$. Means within column with different superscript letters are significantly different (± SD) ($p < 0.05$)

## Discussion

All fish were well fed with the given feed and no mortality was observed; also, the fish treated with $PB_{300}$ were more interested in food than the control and had fast swimming. At the end of the experimental period (8 weeks), the fish fed 300 mg $kg^{-1}$ probiotic grew better than the fish fed the control diet, whereas the final body weight, weight gain, percentage weight gain, and specific growth rate in fish fed diet $PB_{300}$ were higher significantly ($p < 0.05$) than those in the fish fed diet control, but the lowest feed conversion ratio (FCR) was achieved in fish fed diet $PB_{300}$ that indicated an increase of probiotic in diet leads to that growth rate and feed efficiency ratio was improved. Results of our study were in agreement with numerous studies that have shown the application of probiotics can improve feed conversion, growth rates, and weight gain of *Salmonidae* (Merrifield et al. 2010b) and available information in *Acipenseridae* (Hoseinifar et al. 2016). Application of *B. subtilis* and *Bacillus licheniformis* resulted in a significant improvement of *Oncorhynchus mykiss* in feed conversion ratio (FCR), specific growth rate (SGR), weight gain, and protein efficiency ratio (PER) after 2-month feeding trial (Bagheri et al. 2008). Similar results were reported by Askarian et al. (2011) after feeding *A. persicus* and *H. huso* with Chironomidae incorporated with *Lactobacillus curvatus* and *Leuconostoc mesenteroides* for 50 days. Results of other investigations about probiotic indicated that these organisms are promoting growth rate and increasing the efficiency of feed conversion (Young-Hyo et al. 2001) by competitive adhesion to the digestive tract wall to prevent colonization with pathogenic microorganisms (Ibrahem 2015) and enhancement of nutrition of host species through the production of supplemental digestive enzymes (Kesarcodi-Watson et al. 2008). Askarian et al. (2011) and Soltani et al. (2015) attributed growth improvement in *A. persicus* and *H. huso* on some vitamins like vitamin K, and $B_{12}$, as well as Lara-Flores et al. (2003) indicated the role of probiotic that helps to improve feed utility and digestion of proteins and increase the digestibility of feed that led to increase of growth and feed efficiency in *Oreochromis niloticus*. Unfortunately, we did not measure the enzyme digestion of *A. baerii* fed different levels of probiotic superzist, but possibility seems that suitable intestinal microflora produced by probiotic (in diet of $PB_{200}$ and $PB_{300}$) leads to a good digestibility and a high growth performance (Adineh et al. 2013) in juvenile *A. baerii*.

RBC and hemoglobin level were improved in $PB_{300}$ compared with those in control diet ($PB_0$) ($p > 0.05$), but hematocrit of fish fed the control diet was significantly lower than that of fish fed $PB_{300}$ ($p < 0.05$). Hemoglobin and erythrocyte count are good indicators for oxygen transportation capacity of fish (Lamas et al. 1994). On the other hand, increase of hemoglobin and hematocrit plays an important role for improving the well-being of fish and consequently enhancing the immunity and growth of fish (Talpur

and Ikhwanuddin 2012). At a similar manner, WBC count of fish was increased ($p > 0.05$). But, the highest percentage of neutrophil and lymphocyte was recorded for fish fed with $PB_{300}$ that was significantly higher than the percentage of neutrophil and lymphocyte of fish fed with $PB_0$ ($p < 0.05$). Some researchers believed that higher counts (%) of phagocytic cells (neutrophils and monocytes) and lymphocytes are also indicative of infection in fish (Mohapatra et al. 2012); however, others suggested that probiotics actively stimulate the proliferation of B lymphocytes in fish such as *O. mykiss* (Panigrahi et al. 2004), *L. rohita* (Nayak et al. 2007), *O. niloticus* (Pirarat et al. 2011), and *A. persicus* (Soltani et al. 2015) and are a multiplier factor in leukocytes (neutrophils) and NK cells in *O. mykiss* and *L. rohita* that leads to enhancing innate immune responses (Irianto and Austin 2002; Nikoskelainen et al. 2001; Kumar et al. 2008). In this study, there was a significant increase in lysozyme and IgM in fish fed probiotic ($PR_{300}$) and compliment ($ACH_{50}$) ($PR_{200}$ and $PR_{300}$) compared with control treatment ($p < 0.05$) that supported the hypothesis that probiotic can modulate the non-specific immune responses and could increase the threshold of fish on disease and high density (Balcazar et al. 2006; Gatesoupe 2008).

Lysozyme, one of the important bactericidal enzymes of innate immunity, is an indispensable tool of fish to fight against infectious agents (Lindsay 1986). Probiotics either single or in combination are found to trigger the lysozyme level in teleost fish (Ibrahem 2015). The enhancement of lysozyme level was recorded by various types of probiotics in *O. mykiss* (Panigrahi et al. 2004; Kim and Austin 2006), *Miichthys miiuy* (Song et al. 2006), and *A. persicus* (Soltani et al. 2015), respectively. In the teleost fish, compliment system is a component of the non-specific immune response, plays a key role in adaptive immune responses, involves in chemotaxis, opsonization, phagocytosis, and degradation of pathogens, and has effector mechanisms like direct killing of microorganisms by lysis (Ellis 1999). Probiotics can enhance the natural complement activity of teleost fish (Ellis 1999; Panigrahi et al. 2007). Similar results were reported in *O. mykiss* (Panigrahi et al. 2005), *Epinephelus coioides* (Son et al. 2009), *Oplegnathus fasciatus* (Harikrishnan et al. 2010), *Rachycentron canadum* (Geng et al. 2011), and *A. persicus* (Soltani et al. 2015). Also, the result of Soltani et al. (2015) showed an increase in the level of total IgM in the treated *A. persicus* by LAB compared with control. Also, stimulated lymphocyte population for IgM production as already reported by other researchers in *Sparus aurata* (Salinas et al. 2008) and orange-spotted grouper (*Epinephelus coioides*) (Sun et al. 2010) is similar to our results.

Finally, we concluded that adding 300 mg $kg^{-1}$ superzist probiotic to diet had benefit effects on growth rate and some hematological and immunological indices in *A. baerii*, but further researches must focus on the effect of superzist probiotic on gut microflora, digestive enzymes, and challenge tests in *A. baerii* at different ages.

**Acknowledgments** The authors thank the experts and directors in International Sturgeon Research Institute (Iran) for cooperation, especially Alireza Alipour, Reza Ghorbani, Ali Hoshyar, and Arash Ghorbani.

## Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

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