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RESEARCH PAPER

Influence of *Lactobacillus plantarum* Inclusion in the Diet of Siberian Sturgeon (*Acipenser baerii*) on Performance and Hematological Parameters

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Abstract

The current study was carried out to evaluate the effects of probiotic, *Lactobacillus plantarum* on growth performance and hematological parameters in Siberian sturgeon (*Acipenser baerii*). Fish (14.6±2.3 g) were fed three experimental diets prepared by supplementing a basal diet with *L. plantarum* at different concentrations (1×10^7, 1×10^8 and 1×10^9 colony-forming units (cfu) g^{-1}) and a control (non-supplemented basal) diet for 8 weeks. At the end of the experiment, 3 fish were sampled randomly from each tank and were anaesthetized. Then, blood samples were introduced to heparinized tubes in order to perform performance and hematological studies. Growth performance indices were increased in fish fed the 1×10^8 cfu g^{-1} *L. plantarum* diet compared to the other groups. Furthermore, the fish fed on various levels of *L. plantarum* significantly showed higher red blood cell, hemoglobin, white blood cell and monocyte compared to those of the control group ($P<0.05$). Although, the effect of probiotic treatments on other hematological parameters were not significant ($P>0.05$); but this value was higher in probiotic supplement treatments compared to control group. These results indicated that dietary supplementation of *L. plantarum* improved growth performance and hematological parameters in Siberian sturgeon.

Keywords: *Acipenser baerii, Lactobacillus plantarum,* probiotic, hematological parameters.

Introduction

Blood parameters have been adopted in aquaculture as an important tool to assess the health status of fish and monitoring stress responses. Hematological parameters including red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (Ht) and their derivative indices such as mean erythrocyte cell volume (MCV), mean erythrocyte cell hemoglobin content (MCH), and mean erythrocyte cell hemoglobin concentration (MCHC) are particularly known to indicate erythrocyte status and oxygen carrying capability in fish (Houston 1997). These indices also have been studied in many fish species to determine their normal range, and any variation from normal was indicative of problems in fish physiological processes and heath (Ranzani-Paiva *et al*., 2000).

Sturgeon culture has developed into a very successful industry, due to the precious eggs (caviar) and meat. Development of effective methods to boost the immune system of these fishes and to avoid infectious diseases in intensive sturgeon culture is vital. Traditional disease control strategies employ antibiotics and chemical disinfectants, but these are no longer recommended practices due to the emergence of bacterial resistance, and also to concerns over environmental impacts and wildlife protection. Recently, some success has been achieved with immunostimulants as a more environmentally friendly approach to disease management (Nomoto 2005; Ellis 1988). In fact, the use of probiotic bacteria has been suggested to become an alternative method for the prevention and control of various diseases in aquaculture (Irianto and Austin 2002).

Probiotics are defined as live microbial feed supplements that affect the host animal beneficially by improving its intestinal microbial balance. Probiotics offer benefits to the host primarily via direct or indirect modulation of the intestinal microbiota, which will promote growth and the immune system as well as stimulate its enzyme activity and increase its disease resistance (Fuller 1989).

To our knowledge, although the use of pro- and prebiotics are now widely accepted in sturgeon aquaculture, only a few studies have focused on the effects of probiotics on hematological parameters of

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sturgeons (Jafarzadeh *et al*., 2015). Therefore, the current study was undertaken to evaluate the probiotic effects of Lactobacillus plantarum, a new class of candidate probiotics on growth performance and hematological parameters of Siberian sturgeon. This probiotic was isolated form digestive tracts of rainbow trout (Oncorhynchus mykiss), obtained from commercial farms of Iran.

Materials and Methods

Experimental Setup

This study was conducted at the International Sturgeon Research Institute (Guilan, Iran). Juvenile Siberian sturgeon (14.6±2.3 g), obtained from the Shahid Beheshti Sturgeon Propagation & Cultivation Center (Guilan province, Iran), were acclimatized to aquarium conditions for 2 weeks and fed the control diet (without L. plantarum) before the experiment. The health status (excess of mucous secretion, normal coloration, erosion of scales or fins, skin, bulging of eyes and presence of cysts, spots or patches over the body and gills) and behavioral signs (swimming and feeding reflexes) were monitored by physical examination. Healthy fish were assigned at random to 12 aquaria (volume 200 l; 15 fish/aquarium) and four diet groups (one control and three tests) were conducted for 8 weeks. LAB ranged from 6.5×108 in control diet to 6.5−7.5×109 in experimental diets. Water quality parameters including dissolved oxygen and pH, were measured daily. Flow rate, temperature, dissolved oxygen and pH of the water were maintained at approximately 400 ml min–1, 14.6(±1.3)ºC, 8.8(±0.59) mg l–1 and 7.6(±0.34), respectively.

Experimental Diets

Four diets containing different doses of L. plantarum, were prepared as described in Table 1. The proximate analysis of the basal diet was: 43% crude protein, 22% crude lipid, 6.97% ash, and 10% moisture. L. plantarum (1.2×109 colony-forming units (cfu) g–1) was added to the basal diet at different amounts of 0.0001, 0.01, and 1 g, resulting in 107, 108, and 109 colony-forming units (cfu) (kg diet) –1 as the respective test diets, with corresponding decreases in the amount of skim milk. The diet materials were formed into pellets using a manually operated pelletizer and air-dried overnight at 50ºC in a Heraeus oven with ventilation. The dry food pellets were stored in sealed plastic bags at 4°C.

Growth Performance Assays

Growth performance was calculated as described by Geraylou *et al*. (2013) and according to the following formulae: Daily growth rate (DGR) g day–1= t–1 (W2−W1); Specific growth rate (SGR) g day–1=100 (Ln Wf – Ln Wi) / t and Feed conversion ratio (FCR) = Dry feed intake (g) / Weight gain (g). Wf is final body weight (g), Wi is initial body weight (g) and t is experimental duration (in days).

Sampling and Hematology Assays

At the end of the 8-week feeding trial, three apparently healthy fish from each replicate aquarium were anesthetized with 120 mg benzocaine (Geraylou *et al*., 2013). Blood samples were collected from the caudal vein using 1.0 ml non-heparinized syringes and placed in a refrigerator at 4°C until analysis.

The RBC and WBC counts were determined using a Neubaeur hemocytometer (Martins *et al*., 2004). Htc was measured using the standard microhematocrit method and reported as percentages. Hb levels were obtained by the cyanomethemoglobin spectrophotometry method (Collier 1999). Differential leukocyte counts (monocyte, lymphocyte and neutrophil) were determined using Giemsa staining method and detected blood smears under light microscope (Ghiasi *et al*., 2010). The blood indices including MCV, MCH and MCHC were calculated according to Seiverd, 1964.

Statistical Analysis

Data are presented as mean ± SD. Normality and homogeneity of variances were checked with Kolmogorov–Smirnov and Levene’s test. One-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to identify significant variations. All statistical analyses used the SPSS

Table 1. Composition of the basal diet (g kg^{-1}) for Siberian sturgeon

Ingredients	Diets			
	Control	1×10^7 cfu g^{-1}	1×10^8 cfu g^{-1}	1×10^9 cfu g^{-1}
Fish meal	650	650	650	650
Squid meal	85	85	85	85
Shrimp meal	60	60	60	60
Starch	190	190	190	190
Mineral mixture	10	10	10	10
Vitamin mixture	4	4	4	4
Skim milk	1	0.9999	0.99	0
Probiotic	0	0.0001	0.01	1

statistical package version 16.0 (SPSS Inc., Chicago, IL, USA). Statistically significant difference was set at P≤0.05.

Results

Growth Performance

The effects of the probiotic L. plantarum on the growth performance of Siberian sturgeon are given in Table 2. Fish fed a diet supplemented with L. plantarum at 1×108 cfu g–1 displayed significantly improved growth performance, including final weight, length, SGR and FCR, compared to the other diets (P<0.05).

Hematological Parameters

The effects of L. plantarum on hematological parameters of Siberian sturgeon at the end of trial are shown in Table 3. The RBC, Hb, WBC and monocyte values of fish fed L. plantarum diet were significantly higher than those of fish fed control diet (P<0.05). There were no significant differences (P>0.05) among other heamatological parameters in different experimental diets.

Discussion

When used as a dietary supplement, probiotics can colonize the host gut and increase feed utilization by the synthesis of growth factors, including vitamins, co-factors, fatty acids and amino acids; further, probiotics can augment the immune response of the target animal leading to increased health of the host (Farzanfar 2006; Talpur *et al*., 2013). In the present study, fish fed diets supplemented with L. plantarum at 1×108 cfu g–1 showed increased growth and feeding parameters. Stimulation of growth by L. plantarum has been reported for several aquatic animals and in teleost fish, including white shrimp (Litopenaeus vannamei) (Kongnum and Hongpattarakere 2012), orange spotted grouper (Epinephelus coioides) (Son *et al*., 2009), olive flounder (Paralichthys olivaceus) (Kim *et al*., 2013), red sea bream (Pagrus major) (Dawood *et al*., 2015) and giant freshwater prawn (Macrobrachium rosenbergii) (Dash *et al*., 2015). It has been reported that live Lactobacilli produce short chain fatty acids in the digestive tract of the host as a by-product of carbohydrate metabolism and these can be used by intestinal epithelial cells as the main sources of energy. They might have an important role in

Table 2. Growth performance parameters of the Siberian sturgeon juvenile treated with different levels of *L. plantarum* as dietary supplement for 8 weeks

Description	Diets			
	Control	1×10^7 cfu g^{-1}	1×10^8 cfu g^{-1}	1×10^9 cfu g^{-1}
Initial weight (g)	14.5±1.2	13.5±0.5	14.25±0.7	15.4±1
Final weight (g)	37.65±2.38^b	35.7±2.37^b	42.34±1.94^a	31.63±0.78^c
Length (cm)	20.12±0.35^b	21.7±0.23^b	24.13±0.42^a	21.12.±0.42^b
DGR	3.33±0.14	3.33±0.21	3.4±0.13	3.39±0.15
SGR	2.26±0.41^b	2.95±0.47^b	3.41±0.24^a	3.25±0.36^a
FCR	1.6±0.34^b	1.5±0.76^b	1.2±1.1^a	1.4±0.98^a

Data are given as mean ± SD. The mean values in the same row with different letters are significantly different (*P*<0.05).
*DGR: daily growth rate; SGR: specific growth rate; FCR: feed conversion ratio.

Table 3. Hematological parameters of the Siberian sturgeon juvenile treated with different levels of *L. plantarum* as dietary supplement for 8 weeks

Description	Diets			
	Control	1×10^7 cfu g^{-1}	1×10^8 cfu g^{-1}	1×10^9 cfu g^{-1}
WBC (mm^3)	22765±1321.3^a	26583±1383.9^b	29734±1234.3^b	29166±510.9^b
RBC (mm^3)	695000± 21908.59^a	804000±21908.9^b	805000± 18393.84^b	802000± 26204.33^b
HB (g/dL)	2.15±0.17^a	2.83±0.51^a	2.87±0.09^b	2.77±0.06^b
HCT (%)	18±0.46	18.66±0.75	b19.33±0.21	18.66±0.81
MCV (fL)	235.84±8.52	225.23±10.16	241.15±10.47	228.49±6.47
MCH (pg)	33.8±1.83	33.97±2.24	35.94±1.91	33.73±1.2
MCHC (g/dL)	14.68±1.03	15.09±0.87	16.47±1.64	14.48±1.42
Neutrophils (%)	8.28±1.41	9.21±0.37	8.53±0.7	8.38±0.93
Lymphocytes (%)	85.64±2.5	86.64±0.38	87.39±0.85	87.29±0.81
Monocytes (%)	2.83±0.6^a	1.15±0.21^b	1.33±0.21^b	1.5±0.22^b
Eosinophils (%)	3.31±0.65	3±0.44	2.75±0.54	2.83±0.74

Data are given as mean ± SD. The mean values in the same row with different letters are significantly different (P<0.05).
WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV, mean erythrocyte cell volume; MCH, mean erythrocyte cell hemoglobin content; MCHC, mean erythrocyte cell hemoglobin concentration.

increasing the height of the villi in the digestive tract, which would increase nutrient absorption by providing a greater absorptive surface area (Pirarat *et al*., 2011).

Blood is a patho-physiological reflector of the entire body and the counts of haematological parameters in blood give an indication to the health status of fish by determining any abnormality occurring owing to the use of immunostimulants (Tewary and Patra 2011). In the current study, we examined for first time the effects of probiotic, L. plantarum on haematological parameters of Siberian sturgeon. The results of the current study indicated that inclusion of L. plantarum in the fish diet increased significantly the RBC count and hemoglobin levels when compared to control diet ($P<0.05$). Similarly, Faramazi *et al*. (2011) found higher RBC and Hb values in Onchorhynchus mykiss fed with Lactobacillus acidophilus, compared with the control diet. While, Dias *et al*. (2012) found no differences in RBC, MCV and Hb values in Brycon amazonicus following fed with Bacillus subtilis. These results also correspond with those by Jafarzadeh *et al* (2015) who reported the counts of RBC were significantly higher in Russian sturgeon, Acipenser guldenstadti fed with synbiotic (Biomin IMBO). The WBC and monocyte values were also significantly increased in fish treated with L. plantarum. Similar to our results, recent studies suggest that the use of probiotic in some species such as Indian Magur, Clarius batrachus (Dahiya *et al*., 2012), Clarias gariepinus (Ayoola *et al*., 2013), Rainbow trout, Oncorhynchus mykiss (Naseri *et al*., 2015) and Kutum, Rutilus frisii kutum (Azarin *et al*., 2015) increased the WBC count. However, our results are also inconsistent with Jafarzadeh et al (2015) that WBC count showed a decreasing trend during the period of the experiment in all synbiotic supplement diets. This contradictory result may be attributable to the low dosage, different duration of probiotic administration, life stage and/or different fish species (Geraylou *et al*., 2013). In addition, the increase in white blood cells shows that probiotic supplement stimulates more innate immunity of Siberian sturgeon other than adaptive immunity. In agreement with our results, some studies have shown that probiotics interact with the immune cells such as monocytes, macrophages, neutrophils and natural killer cells to enhance innate immune responses (Irianto and Austin 2002; Nikoskelainen *et al*., 2003; Kumar *et al*., 2008).

In conclusion, supplementation of immunostimulants in feed can improve fish health and thereby reduce management costs. The increase in hematology parameters such as RBC, WBC counts and Hb following dietary inclusion of L. plantarum diet indicates the immunostimulant effects and anti-infection properties of this probiotic. Consequently, we recommend dietary inclusion of L. plantarum in the diet of Siberian sturgeon.

References

Ayoola, S.O., Ajani, E.K., Fashae, O.F., 2013. Effect of probiotics (Lactobacillus and Bifidobacterium) on growth performance and hematological profile of *Clarias gariepinus* juveniles. World Journal of Fish and Marine Sciences 5:01–08. doi: 10.5829/idosi.wjfms.2013.05.01.6582.

Azarin, H., Aramli, M.S., Imanpour, M.R., Rajabpour, M., 2015. Effect of a Probiotic Containing *Bacillus licheniformis* and *Bacillus subtilis* and Ferroin Solution on Growth Performance, Body Composition and Haematological Parameters in Kutum (*Rutilus frisii kutum*) Fry. Probiotics and Antimicrobial Proteins 7:31–37. doi: 10.1007/s12602-014-9180-4.

Collier, H.B., 1999. The standardization of blood hemoglobin determinations. Canadian Medical Association Journal 50: 550–552.

Dahiya, T., Sihag, R.C., Gahlawat, S.K., 2012. Effect of probiotics on the haematological parameters of Indian Magur (*Clarius batrachus* L.). Journal of Fisheries and Aquatic Science 7:279–290. doi: 10.3923/jfas.2012.279.290

Dash, G., Prakash Raman, R., Pani Prasad, K., Makesh, M., Pradeep, M.A., Sen, S., 2015. Evaluation of paraprobiotic applicability of *Lactobacillus plantarumin* improving the immune response and disease protection in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Fish and Shellfish Immunology 43:167–174. doi:10.1016/j.fsi.2014.12.007.

Dawood, M.A.O., Koshio, S., Ishikawa, M., Yokoyama, S., 2015. Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. Fish and Shellfish Immunology 45: 33–42. doi:10.1016/j.fsi.2015.01.033.

Dias, D.C., Leonardo, A.F.G., Tachibana, L., Correa, C.F., Bordon, C.A.C., Romagosa, E., Ranzani-Paiva, M.J.T., 2012. Effect of incorporating probiotics into the diet of matrinxa (*Brycon amazonicus*) breeders. Journal of Applied Ichthyology 28:40–45. doi: 10.1111/j.1439-0426.2011.01892.x.

Faramazi, M., Kiaalvandi, S., Lashkarbolooki, M., Iranshahi, F., 2011. The investigations of *Lactobacillus acidophilis* as probiotics on grown performance and disease resistance of rainbow trout (*Oncorhynchus mykiss*). American-Eurasian Journal of Scientific Research 6: 32–38.

Farzanfar, A., 2006. The use of probiotics in shrimp aquaculture. FEMS Immunology & Medical Microbiology 48: 149–158. doi: 10.1111/j.1574-695X.2006.00116.x.

Ellis, A.E., 1988. Fish vaccination. London: Academic Press. 255 pp.

Fuller, R., 1989. Probiotics in man and animals. A review. Journal of Applied Microbiology 66: 365–378. doi: 10.1111/j.1365-2672.1989.tb05105.x.

Geraylou, Z., Souffreau, C., Rurangwa, E., De Meester, L., Courtin, C.M., Delcour, J.A., Buyse, J., Ollevier, F., 2013. Effects of dietary arabinoxylan-oligosaccharides (AXOS) and endogenous probiotics on the growth performance, non-specific immunity and gut microbiota of juvenile Siberian sturgeon (*Acipenser baerii*). Fish and Shellfish Immunology

35: 766–775. doi:10.1016/j.fsi.2013.06.014.

Ghiasi, F., Mirzargar, S.S., Badakhshan, H., Shamsi, S., 2010. Effects of low concentration of cadmium on the level of lysozyme in serum, leukocyte count and phagocytic index in *Cyprinus carpio* under the wintering conditions. Journal of Fisheries and Aquatic Science 5: 113–119. doi: 10.3923/jfas.2010.113.119.

Houston, A.H., 1997. Review: are the classical haematological variables acceptable indicators of fish? Transactions of the American Fisheries Society 126: 879–894. doi: 10.1577/1548-8659(1997)126<0879:RATCHV>2.3.CO;2.

Irianto, A., Austin, B., 2002. Probiotics in aquaculture. Journal of Fish Diseases 25:633– 642. doi: 10.1046/j.1365-2761.2002.00422.x.

Jafarzadeh E, Khara H, Ahmadnezhad M (2015) Effects of synbiotic (Biomin IMBO) on haematological and immunological components of Russian sturgeon, *Acipenser guldenstadti*. Comparative Clinical Pathology 24:1317–1323.doi: 10.1007/s00580-015-2071-6.

Kim, D., Beck, B.R., Heo, S.B., Kim, J., Kim, H.D., Lee, S.M., Kim, Y., Oh, S.Y., Lee, K., Do, H., Lee, K., Holzapfel, W.H., Song, S.K., 2013. *Lactococcus lactis* BFE920 activates the innate immune system of olive flounder (*Paralichthys olivaceus*), resulting in protection against *Streptococcus iniae* infection and enhancing feed efficiency and weight gain in large-scale field studies. Fish and Shellfish Immunology 35:1585–1590. doi:10.1016/j.fsi.2013.09.008.

Kongnum, K., Hongpattarakere, T., 2012. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish and Shellfish Immunology 32: 170–177. doi:10.1016/j.fsi.2011.11.008.

Kumar, R., Mukherjee, S.C., Ranjan, R., Nayak, S.K., 2008. Enhanced innate immune parameters in *Labeo rohita* (Ham.) following oral administration of *Bacillus subtilis*. Fish Shellfish and Immunology 24:168–172. doi:10.1016/j.fsi.2007.10.008.

Martins, M.L., Tavares-Dias, M., Fujimoto, R.Y., Onaka, E.M., Nomura, D.T., 2004. Haematological alterations of *Leporinus macrocephalus* (Osteichtyes: Anostomidae) naturally infected by *Goezia leporine* (Nematoda: Anisakidae) in fish ponds. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 5: 640–646.

Naseri, S., Khara, H., Shakoori, M., 2015. Evaluation of *Bacillus licheniformis* and *Bacillus subtilis* as probiotic and Fe ion effects on haematological parameters of rainbow trout, *Oncorhynchus mykiss* frys. Comparative Clinical Pathology 24:1521–1526. doi: 10.1007/s00580-015-2110-3.

Nikoskelainen, S., Ouwehand, A.C., Bylund, G., Salminen, S., Lilius, E.M., 2003. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobacillus rhamnosus*). Fish and Shellfish Immunology 15:443–452. doi:10.1016/S1050-4648(03)00023-8.

Nomoto, K., 2005. Prevention of infections by probiotics. Journal of Bioscience and Bioengineering 100: 583–592. doi:10.1263/jbb.100.583.

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., Maita, M., 2011. Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Research in Veterinary Science 91: 92–97. doi:10.1016/j.rvsc.2011.02.014.

Ranzani-Paiva, M.J.T., Ishikawa, C.M., das Eiras, A.A., Fel izardo, N.N., 2000. Haemotological analysis of ‘chara’ *Pseudoplatystoma fasciatum* in captivity . Aqua 2000. Responsible aquaculture in the new millennium, Nice, France, 2–6 May 2000, European Aquaculture Society special publication 28: 590 pp.

Seiverd, C.E., 1964. Hematology for Medical Technologists, Lea and Febiger, Philadelphia, PA, 946 pp.

Son V.M., Chang C.C., Wu M.C., Guu Y.K., Chiu C.H. & Cheng W. (2009) Dietary administration of the probiotic, *Lactobacillus plantarum*, enhanced the growth, innate immune responses, and disease resistance of the grouper *Epinephelus coioides*. Fish and Shellfish Immunology 26: 691–698. doi:10.1016/j.fsi.2009.02.018.

Talpur, A.D., Ikhwanuddin, M., Abdullah, M.D.D., Bolong, A.A., 2013. Indigenous *Lactobacillus plantarum* as probiotic for larviculture of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758): effects on survival, digestive enzyme activities and water quality. *Aquaculture* 416–417: 173–178. doi:10.1016/j.aquaculture.2013.09.018.

Tewary, A., Patra, B., 2011. Oral administration of baker's yeast (*Saccharomyces cerevisiae*) acts as a growth promoter and immunomodulator in *Labeo rohita* (Ham.). Journal of Aquaculture Research and Development 2: 1–7. doi:10.4172/2155-9546.1000109.