

Interaction of dietary *Pediococcus acidilactici* and folic acid on growth performance, haematological parameters and non-specific immune response of finger barbel, *Acipenser nudiventris*

Delsoz N.¹; Khara H.^{1*}; Shenavar A.R.²; Mohseni M.²

Received: March 2015

Accepted: March 2016

Abstract

This study was conducted to investigate the effects of dietary *Pediococcus acidilactici* and folic acid (FA) and their combinations on growth performance, haematological parameters and non-specific immune response of *Acipenser nudiventris*. 210 individuals (initial body weight: 12.84 ± 1.53 g) were fed seven practical diets: the basal diet as the control diet was supplemented with two levels of *P. acidilactici* (2 and 3 g per kg diet), FA (2 and 4 mg per kg diet) and their combinations. The results showed that the group fed a combined diet of *P. acidilactici* and FA (4 mg FA+3 g *P. acidilactici* per kg diet) showed significant increase in body weight increase (BWI), specific growth rate (SGR), feed conversion ratio (FCR) and condition factor (CF). However, experimental diets had no significant effects on survival rate. Both haematological indices and leucocyte counts were significantly ($p < 0.05$) influenced by dietary *P. acidilactici* and FA and their combinations. In the group fed 4 mg FA+3 g *P. acidilactici*, lysozyme activity and total immunoglobulin (Ig) levels significantly increased compared to those fed other diets ($p < 0.05$). Moreover, the fish fed diets supplemented with 2 mg FA+ 3 g *P. acidilactici* per kg diet had considerably higher immunoglobulin (IgM) level ($p < 0.05$). Under the experimental conditions, dietary *P. acidilactici* and FA had a synergistic effect on enhancing growth performance and immunity of *A. nudiventris*.

Keywords: *Acipenser nudiventris*, Probiotic, Physiological measurements, Immune response

1- Department of Fishery, Lahijan Branch, Islamic Azad University, Lahijan, Iran, P.O. Box: 1616

2- International Sturgeon Research Institute, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Rasht, Iran, P.O. Box: 41635-3464

*Corresponding author's Email: h.khara1974@yahoo.com

Introduction

The demand for animal protein in the world is expected to increase progressively with each increase in human population. Aquaculture is one of the most important options for animal protein production and requires high quality feeds with high protein content as well as some complementary additives to keep organisms healthy and to favor growth. The supplementation of probiotics through feeds is the best method to reduce feed costs (Lara - Flores *et al.*, 2003). The use of probiotics as farm animal feed supplements dates back to the 1970.

Probiotics are recently used in aquaculture to promote growth rate, inhibit growth of pathogens (Rengpipat *et al.*, 1998) and improve feed efficiency, reproduction and health (Gatesoupe, 1999; Irianto and Austin, 2002; Marzouket *et al.*, 2008; Kesarcodi-Watson *et al.*, 2008).

The commonly used probiotics in fish culture practices belong to *Saccharomyces*,

Clostridium, *Bacillus*, *Enteroccus*, *Lactobacillus*, *actococcus*, *Aeromonas* and several other genera (Ghosh *et al.*, 2008; Capkin and Altinok 2009; Boonthai *et al.*, 2011). *P. acidilactici* is a species of probiotic bacteria which belonged to the genus *Lactobacillus*. *P. acidilactici* has emerged as a potential probiotic that has improved growth efficiency and enhanced immune response (Perdigo'n *et al.*, 1988). In green terror, *Aequidens rivulatus*, use of dietary *P. acidilactici* improved growth

and innate immunity parameters including lysozyme activity, total immunoglobulin and alternative complement activity (Neissi *et al.*, 2013). In tilapia, *Oreochromis niloticus*, dietary *P. acidilactici* has had a protective action on the intestinal mucosal cells, stimulating the innate immune response after feeding for a period of six weeks (Standen *et al.*, 2013).

Vitamins including folic acid are used widely in aquaculture as immunostimulants and are important in modulating the fish immune system. Folic acid deficiency has resulted in appropriate growth (Smith 1968); health (Smith 1968; Esmaeili and Khara 2014) and pathological symptoms (Smith 1968) in cultured fish. 2.5 mg kg⁻¹ folic acid had positive effects on growth, feed efficiency and immunity (total immunoglobulin) of rainbow trout, *Oncorhynchus mykiss* (Esmaeili and Khara, 2014). Again in rainbow trout, dietary folic acid resulted in an improvement in immunological functions (Abbasian *et al.*, 2013).

To the best of our knowledge, there are no studies on the use of *P. acidilactici* as a dietary probiotic in sturgeons especially in barbel sturgeon, *A. nudiventris*. This is also the case with folic acid. The barbel sturgeon, *A. nudiventris* is a critically endangered anadromous species. The rearing of these species has seen considerable progress in the past years. Despite the economic importance of barbel sturgeon, there is little information on

its nutrient requirements. Thus, in the present study, we decided to examine the effects of dietary *Pediococcus acidilactici* and folic acid (FA) and their combinations on growth performance, haematological parameters and non-specific immune response in *Acipenser nudiventris*.

Materials and methods

Experimental fish

The experiment was carried out in Shahid Beheshti Sturgeon Propagation and Rearing Center, Rasht, Iran. The experimental fish were acclimatized at ambient laboratory conditions for 15 days and starved for 24 h before the beginning of the feeding trial. A total of 210 fish were used during the experiment (30 fish per treatment) which was distributed randomly in 21 fiberglass tanks. Each treatment included 3 replicates (tank) with 10 fish stocked in each tank. Each tank was supplied with an air pump with air stones for aeration. Tap water was stored for 24 hours in fiberglass tanks for dechlorination. Tanks were filled after replacing 100% of water, daily. Water temperature (via a thermometer) was measured daily and the average was between 16-18 °C during the experimental period. Water quality parameters were measured weekly. The pH (using WTW, Model PH330i-pH-meter) and dissolved oxygen (using WTW, Model PH330i-dissolved oxygen meter) were also determined.

Food preparation

A commercial probiotic product entitled Bactocell containing *Pediococcus acidilactici* (Lallemand Company, Toulouse, France) and the FA (Dae Jung Chemical Company, Korea) [25 g FA ($C_{19}H_{19}N_7O_6$)] as FA donor were added to test diets. The proportion of each ingredient required was calculated precisely providing allowance for the premix (Table 1). The dough was steam cooked and cooled to room temperature. Then, different concentrations of *P. acidilactici*, FA and their combinations were mixed with the dough to provide seven treatments as follows: T1 (4 mg FA per kg diet), T2 (3 g *P. acidilactici* per kg⁻¹ diet), T3 (4 mg FA+2 g *P. acidilactici* per kg⁻¹ diet), T4 (2 mg FA+ 3 g *P. acidilactici* per kg⁻¹ diet), T5 (2 mg FA+ 2 g *P. acidilactici* per kg⁻¹ diet), T6 (4 mg FA+3 g *P. acidilactici* per kg⁻¹ diet) and T7 which served as the control (0% of probiotic and FA). The diets were pelletized by pressing through 2 mm diameter pelleting unit. The pellets were dried in a drying oven at 40°C for 24 hours and stored at - 4°C until use during the trial to avoid oxidation and rancidity. The proximate body composition (moisture, crude protein, crude lipid, and ash) was determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1995).

Table 1: Composition of the experimental diet (dry weight).

Ingredients	% kg⁻¹ dry weight
Fish meal	40
Wheat meal	20
Corn meal	5
Fish oil	6.5
Soybean meal	20
Beet molasses	2
Salt	1
Vitamin mixture ^a	2
Mineral mixture ^b	1
Lysine	1.5
Methionine	1.5
Proximate composition	%
Crude protein	40
Crude lipid	9
Moisture	5.5
Ash	55.2
Crude Energy (kcal kg ⁻¹)	1897

^a Vitamin mixture was manually provided according to feed requirements of the fish and ingredients were obtained from Science Laboratories (Ghazvin, Iran); which each 1000 g vitamin mixture provides vitamin A, 1,600,000 I.U; vitamin D3, 400 000 I.U; thiamin, 6 g; riboflavin, 8 g; niacin, 12 g; pantothenic acid, 40 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 8 mg; vitamin C, 60 g; vitamin K3, 2 g; biotin, 240 mg and inositol, 20 g.

^b Aquatic mineral premix, for cold and warm water fish, is manufactured by Science Laboratories (Ghazvin, Iran); which each 1000 g contains mineral trace elements such as ferrous, 6000 mg; zinc, 10 000 mg; selenium, 20 mg; cobalt, 100 mg; copper, 600 mg; magnesium, 5000 mg; iodine, 600 mg. In addition, choline chloride (6000 mg), which is vital for fish and cannot be combined with other vitamins, is also included in mineral premix.

Protein by estimating the Kjeldahl nitrogen (6.25×), moisture by heating at 105°C to constant weight, ash by incinerating in a crucible at 600°C for 18 h and crude lipid was determined by using Soxhlet apparatus.

Feeding trial

During 60 days, *A. nudiventris* with an initial weight of 12.84 ±1.53g was used for the feeding trial. The fish were randomly distributed in twenty one 400 L fiberglass tanks (30 per tank in each triplicate) continuously aerated in order to maintain the optimal oxygen level.

Then experimental fish were fed with experimental diets in each treatment. Feeding operation was done two times a day (9:00 am and 9:00 pm). The daily amount of feed was calculated based on 5% of body weight. Unfed feed and feces were collected daily after feeding.

Growth parameters

After the feeding trial, the growth parameters including survival rate, body weight gain (BWI), specific growth rate (SGR), feed conversion rate (FCR), condition factor and survival rate (%) was individually determined by the

following equations (Tekinay and Davis, 2001):

FCR = dry feed intake (g)/wet WG (g)

SGR (% day⁻¹) = (Ln W_f - Ln W_i) × 100/t

CF = 100 × [wet weight (g)/TL (cm)³]

Where W_f and W_i were final and initial fish weights, respectively; TL was total length and t is the experimental duration in day.

Blood analysis

The fish from each tank were quickly captured and sampled after being anesthetized. Then, blood samples were collected from the caudal vein of each fish using a 2 mL heparinized syringe. Blood samples were divided into two portions. Half of the blood was used for separating plasma from it and the remaining was used for hematological analysis. Plasma was separated by centrifugation (1500 × g for 10 min) and stored at -80 °C until further analysis. Hemoglobin concentration was determined using the cyanmethemoglobin method. The numbers of white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration were calculated according to Ranzani-Paiva *et al.* (2004). To determine differential count of leukocyte, that is, measure of lymphocyte, neutrophil, eosinophil and monocyte, the obtained smears were first air dried, fixed in 96% ethanol for 30 min, stained by Giemsa staining for

30 min and were examined for leukocyte differential count under light microscope (Klontz, 1994). Lysozyme level was determined by turbidometric assay according to the method of Sankaran and Gurnani (1972) with slight modifications. Aliquots (1.75 mL⁻¹) of *Micrococcus lysodeikticus* suspension (Sigma) (0.375 mg mL⁻¹, 0.05 M PBS, pH 6.2) were mixed with 250 mL⁻¹ of each sample and the optical density was measured after 15 and 180s by spectrophotometer (Biophotometer Eppendorf) at 670 nm. PBS was used as the blank and results were expressed in amounts of lysozyme (mg) per 1 mg of sample calibrated using a standard curve determined with hens egg white lysozyme (Sigma) in sterile sodium phosphate buffer. The serum IgM was measured according to Fuda *et al.* (1991). Antisera for fish were prepared by immunizing rabbits as previously described by Fuda *et al.* (1991). The procedure for labeling antibody fragment with enzyme was performed. Plasma total immunoglobulin (Ig) content was determined following the method of Puangkaew *et al.* (2004) with slight modification.

Statistical analyses

One way analysis of variance (ANOVA; SPSS, 13.0) was used to determine whether significant variation between the treatments existed. Differences between means were determined and compared by post hoc multiple comparison tests. All the tests used a significance level of $p < 0.05$.

Data are reported as means \pm standard deviations.

Results

Data presented in Table 2 indicated that the highest final body weight was obtained in T6 group (60 ± 2.66 g) which received the diet supplemented with 4 mg FA+3 g *P. acidilactici* followed by T4 (58.1 ± 3.3 g), T2 (56.4 ± 4.14 g) and T1 (54.5 ± 8.49 g) groups. The lowest values were obtained in the control. The results showed that FCR, SGR, BWI and CF were significantly affected by *P. acidilactici* and FA supplementation (Tables 2 and 3). During the whole experimental period, T6 fish produced the lowest FCR (1.08 ± 0.011) and also had highest SGR (3.18 ± 0.04) and BWI ($347.2 \pm 9.83\%$). The values of survival

rate and condition factor were higher in fish supplemented with *P. acidilactici* and FA compared with the control (Table 3, $p < 0.05$). Data in Table 4 showed significant ($p < 0.05$) increase in white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) and hematocrit (Ht) in fish supplemented with a *P. acidilactici* and FA compared with the control. All immune parameters showed significant ($p > 0.05$) difference among dietary treatments. Lysozyme activity and Ig level were higher in T6 which received diet supplemented with 4 mg FA+3 g *P. acidilactici* compared with the other treatments (Figs. 1 and 2), whereas IgM content of fish fed 2 mg FA+ 3 g *P. acidilactici* per kg^{-1} diet (T4) was significantly ($p < 0.05$) higher than that of the other groups.

Table 2: Combined effects of dietary *Pediococcus acidilactici* and folic acid on the growth performance of *Acipenser nudiventris*.

Treatments	Initial body weight	Final body weight	BWI (%)	FCR	SGR
T 1	12.48 ± 1.53	54.5 ± 8.49	327.9 ± 9.98^c	1.13 ± 0.017^a	2.97 ± 0.047^c
T 2	12.38 ± 1.41	56.4 ± 4.14^{de}	340.5 ± 6.93^c	1.11 ± 0.015^a	3.03 ± 0.03^{cd}
T 3	12.39 ± 1.24	50.7 ± 7.4^{bc}	288.3 ± 19.08^b	1.21 ± 0.036^b	2.77 ± 0.099^b
T 4	12.36 ± 1.19	58.1 ± 3.3^{de}	358.6 ± 26.23^{cd}	1.09 ± 0.006^a	3.11 ± 0.11^{cd}
T 5	12.40 ± 1.62	48.9 ± 7.39^{ab}	278.3 ± 27.53^{ab}	1.26 ± 0.074^b	2.71 ± 0.15^{ab}
T 6	12.43 ± 1.58	60 ± 2.66^e	347.2 ± 9.83^d	1.08 ± 0.011^a	3.18 ± 0.04^d
Control	12.49 ± 1.73	45.5 ± 5.06^a	255.2 ± 7.16^a	1.34 ± 0.019^c	2.59 ± 0.041^a

The values with different letters in the figure are significantly different ($p < 0.05$).

Table 3: Combined effects of dietary *Pediococcus acidilactici* and folic acid on CF and survival rate.

Treatments	Condition factor (CF)	Survival rate (%)
T 1	0.52±0.01 ^{bc}	82.5±5.77 ^{ab}
T 2	0.53±0.005 ^c	84±1.67 ^a
T 3	0.53±0.03 ^c	86±1.67 ^b
T 4	0.48±0.02 ^{abc}	87±3.33 ^{ab}
T 5	0.49±0.03 ^{abc}	81±3.33 ^a
T 6	0.44±0.02 ^a	87±1.67 ^{ab}
Control	0.47±0.06 ^{ab}	78±2.54 ^c

The values with different letters in the figure are significantly different ($p<0.05$).

Table 4: Combined effects of dietary *Pediococcus acidilactici* and folic acid on haematological indices of *Acipenser nudiventris*.

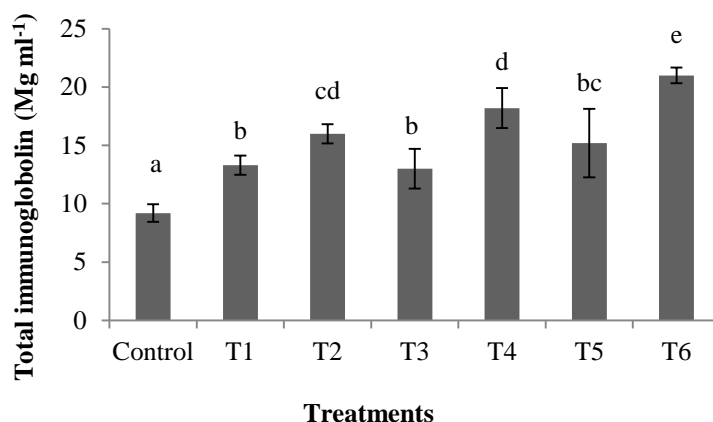
Treatments	WBC ($\times 10^3$ mm)	RBC ($\times 10^6$)	Hb (g/dL)	Ht (%)	MCV (fl)
T 1	65.25±6.7 ^{ab}	8.03±2.6 ^b	6.7±0.17 ^b	33±0.82 ^b	441.25±5.91 ^a
T 2	89.2±17.08 ^{cd}	8.6±4.02 ^c	7.2±0.26 ^c	35.7±1.71 ^c	412.75±5.56 ^a
T 3	81±18.2 ^{bc}	7.9±3.4 ^b	6.6±0.30 ^b	33.5±1.29 ^b	419.75±3.4 ^{ab}
T 4	105.7±6.7 ^d	8.7±4.2 ^c	7.3±0.36 ^c	37.2±1.71 ^c	424.75±3.9 ^b
T 5	130.2±19.3 ^e	8.05±1.9 ^b	6.7±0.17 ^b	33.2±0.96 ^b	412.5±6.35 ^a
T 6	131.7±20.3 ^e	8.9±4.3 ^c	7.4±0.3 ^c	37.2±1.26 ^c	423.75±10.44 ^b
Control	62.2±8.5 ^a	6.5±3.4 ^a	5.4±0.26 ^a	27.5±1.36 ^a	417.25±0.06 ^{ab}

The values with different letters in the figure are significantly different ($p<0.05$).

Table 5: Combined effects of dietary *Pediococcus acidilactici* and folic acid on differential count of leukocyte of *Acipenser nudiventris*.

Treatments	MCH (pg)	MCHC (g/dL)	Lymphocyte (%)	Neutrophil (%)	Monocyte (%)
T 1	84±0.82 ^e	8.9±4.3	131.7±20.3 ^b	8.9±4.3 ^a	131.7±20.3 ^a
T 2	83.2±0.96 ^a	8.9±4.3 ^a	131.7±20.3 ^a	8.9±4.3 ^{bc}	131.7±20.3 ^a

The values with different letters in the figure are significantly different ($p<0.05$).

**Figure 1: Plasma Ig level of *Acipenser nudiventris* fed the experimental diets for 60 days.**

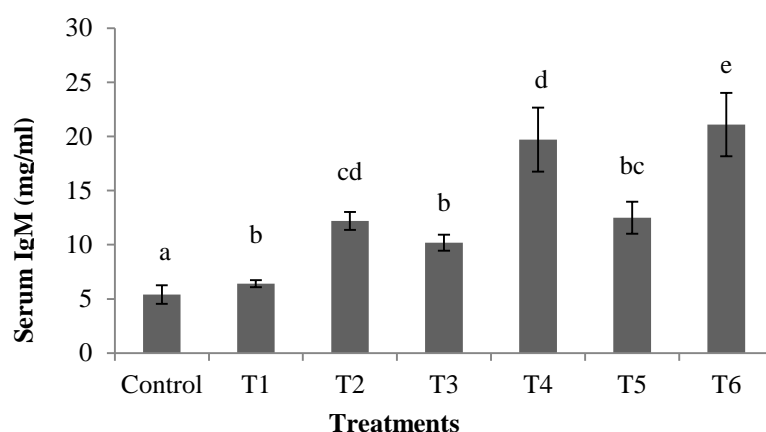


Figure 2: Plasma IgM level of *Acipenser nudiventris* fed the experimental diets for 60 days.

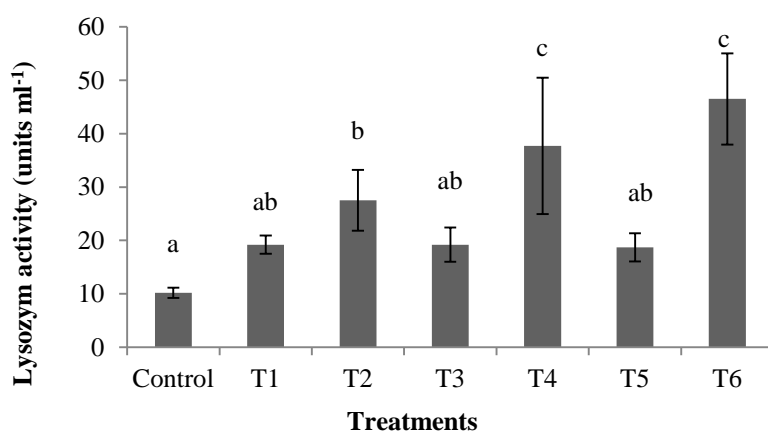


Figure 3: Lysozyme activity of *Acipenser nudiventris* fed the experimental diets for 60 days.

Discussion

Several studies on probiotics have been published in recent years which suggest that probiotics provide nutritional benefits (Balca'zaret *et al.*, 2006; Kesarcodi-Watson *et al.*, 2008; Merrifield *et al.*, 2010; Naseri *et al.*, 2013; Zare *et al.*, 2017). In the present study for first time, the effects of probiotic, *P. acidilactici*, folic acid (FA) and their combinations were investigated on three biological aspects of barbel sturgeon including Growth performance and survival,

haematological and immunological parameters:

Growth performance and survival

In this study, we observed a tendency in fish fed a probiotic-supplemented diet towards better growth performance which may be due to the several mechanisms stimulated by *P. acidilactici* including increasing nutrients availability for absorption and activity of digestive enzymes in gastrointestinal tract (Doeschate and Coyne, 2008). As a result, it increases

availability of nutrients and digestives. Better digestibility and absorption capacity could cause an increasing trend in growth indices (and survival) or a decreasing trend in FCR (Goldin 1998; Doeschate and Coyne 2008; Ghosh *et al.*, 2008). Previous studies also support this fact for *P. acidilactici* in larval Pollock, *Pollachius pollachius* when fed with artemia feed enriched with *P. acidilactici* (Gatesoupe, 2002) and carp fed with the probiotic *Streptococcus faecium* (Noh *et al.*, 1994). However, no influences have been observed (Gatesoupe, 2002; Shelby *et al.*, 2007; Ferguson *et al.*, 2010; Zhou *et al.*, 2010) in some studies that might be attributed to the differences in concentration of probiotics added to the diet, the bacterial strains, experimental design and the species of organisms examined. In our study, higher survival rate and growth were observed in fish fed diets supplemented with FA or FA+*P. acidilactici*. Similar results were found in grouper, *Epinephelus malabaricus* (Lin *et al.*, 2011). Stimulated growth by FA is attributed usually to the role of this vitamin in enhancing oxygen transporting capacity in blood through participating in formation of red blood cells and thus maintaining the metabolism in normal state (Hosokawa 1989). In addition to this, numerous studies have shown that microorganisms are a significant source of folic acid for some species including common carp, *Cyprinus carpio* (Kashiwada *et al.*, 1971) and channel catfish (Duncan *et al.*, 1993). It seems

that probiotic in combination with FA in diets may improve nutritional and physiological function, but further studies are needed to prove this synergic effect.

Haematological parameters

Haematological indices are considered as valuable tools for assessing fish health and are reported to be effected by dietary probiotic (Irianto and Austin, 2002). In this study, significant ($p<0.05$) increase in WBC, RBC, Hb and Ht in fish fed the probiotic and FA-supplemented diet compared to the control. On the effects of probiotics on haematology different results have been reported. Dias *et al.* (2012) found no differences in RBC, MCV and Hb concentration in *Brycon amazonicus* breeders in relation to dietary supplementation with *B. subtilis*, while Faramazi *et al.* (2011) found higher RBC, Hb and Ht. This suggests that fish fed probiotic-supplemented diets are healthier than controls, possibly because of decreased blood cortisol levels, as reported by Carnevali *et al.* (2006) and Rollo *et al.* (2006) in sea bream (*Sparus aurata*). In our study, increases in WBC in fish fed probiotic-supplemented diets may be due to the stimulation of immunity by bacteria, *P. acidilacticias* reported in relation to other dietary probiotic bacteria (Borchers *et al.*, 2009). Ferguson *et al.*, (2010) showed that significant increases in the total number of leucocytes were observed in tilapia fed *P. acidilactici* supplemented diets. However, contrary

to these findings, other probiotic studies observed either no differences (Aly *et al.*, 2008a, El-Rhman *et al.*, 2009) or elevated haematocrit levels (Aly *et al.*, 2008b), but the reason for this is not clear. Anemia was observed in relation to dietary FA deficiency in some examined fish (Smith 1968; Hosokawa 1989). This fact was reinforced when we observed more RBC, Hb and Ht in FA-supplemented fish compared to the control group. It was confirmed that FA has an important role in production of blood cells of fish species (Hosokawa, 1989; Abbasian *et al.*, 2013).

Immunological parameters

Probiotics act as the stimulators of innate immune response and in this regard, the primary effect of *P. acidilactici* on the immune system of barbel sturgeon was evaluated based on two indices including total immunoglobulin and lysozyme activity (Rengpipat *et al.*, 2000; Heyman and Me'nard, 2002; Taoka *et al.*, 2006; Nayak, 2010). In the present study, the IgM and lysozyme levels showed significantly higher values in fish fed with combined probiotic and FA groups compared to the control. This confirms that the probiotic bacteria can stimulate the antibody production and lysozyme activity in fish (Panigrahi *et al.*, 2004). Our results were in accordance with those reported with *L. rhamnosus*, *Carnobacterium maltaromaticum*, *Carnobacterium divergens* in rainbow trout (Panigrahi *et al.*, 2004; Kim and Austin, 2006), *P. acidilactici* in red

tilapia, *Oreochromis niloticus* (Ferguson *et al.*, 2010), *B. subtilis* in *Labeo rohita* (Nayak *et al.*, 2007) and yeast cells in seabream, *Sparus aurata* (Cuesta *et al.*, 2004).

In contrast, diets containing *Aeromonas sobria* fed to *O. mykiss* (Brunt *et al.*, 2007) and to tilapia, *Oreochromis niloticus* (Wang *et al.*, 2008) failed to improve the level of lysozyme. These differences in results can be caused by various factors like source, type, dose and duration of supplementation of probiotics which can significantly affect the immunomodulatory activity of probiotics (Nayak, 2010).

In general, in the probiotic and FA combined group the level of various immune parameters was significantly enhanced compared to the individual probiotic and FA treated groups. This might be due to the establishment of the *P. acidilactici* in the gastrointestinal tract of *A. nudiventis* because the co administration of FA along with probiotic bacterium might have promoted the growth of other gut bacteria, which either utilized or reduced the FA.

Generally, the results of the present study indicated clearly that the supplementation of *P. acidilactici* and FA enhanced not only the growth performance of *A. nudiventis*, but also the non-specific immune responses. Further studies regarding the application of *P. acidilactici* and FA in aquatic animals, especially their appropriate inclusion levels in specific

species and specific rearing conditions, are needed.

Acknowledgements

The authors express their sincere appreciation to the people who gave their time, advice, and support to this study. The author also thanks the staff of Artificial Sturgeon Propagation and Rearing Center for their technical help during this trial.

References

- Abbasian, F., Lameeihassankiadeh, S., Khara, H., Nezamibaloochi, Sh. A., Borani, M. and Mohammadalikhani, M., 2013.** The effects of folic acid treatment on biometric and blood parameters of fingerling rainbow trout fishes (*Oncorhynchus mykiss*). *Journal of Aquaculture Research and Development*, 4, 175. doi:10.4172/2155-9546.1000175
- Aly, S.M., Mohamed, M.F. and John, G., 2008a.** Effect of probiotics on the survival, growth and challenge infection in *Tilapia nilotica* (*Oreochromis niloticus*). *Aquaculture Research*, 39, 647–656.
- Aly, S.M., Abdel-Galil Ahmed, Y., Abdel-Aziz Ghareeb, A. and Mohamed, M.F., 2008b.** Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. *Fish and Shellfish Immunology*, 25, 128–136.
- Association of Official Analytical Chemists (AOAC), 1995.** Official methods of analysis of the AOAC. 16th ed. Arlington (VA): AOAC.
- Balca'zar, J.L., Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D. and Mu' zqui, J.L., 2006.** The role of probiotics in aquaculture. *Veterinary Microbiology*, 114, 173–186.
- Boonthai, T., Vuthhiphandchai, V. and Nimrat, S., 2011.** Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaeus monodon*). *Aquaculture Nutrition*, 6, 634–644.
- Borchers, A., Selmi, C., Meyers, F., Keen, C. and Gershwin M., 2009.** Probiotics and immunity. *Journal of Gastroenterology*, 44, 26–46.
- Brunt, J., Newaj-Fyzul. A. and Austin, B., 2007.** The development of probiotics for the control of multiple bacterial diseases of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Disease*, 30, 573–579.
- Capkin, E. and Altinok, I., 2009.** Effects of dietary probiotic supplementations on prevention / treatment of yersiniosis disease. *Journal Applied Microbiology*, 106, 1147–1153.
- Carnevali, O., Vivo, L., Sulpizio, R., Gioacchini, G.I., Olivotto, I., Silvi, S. and Cresci, A., 2006.** Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1,

- myostatin and cortisol gene expression. *Aquaculture*, 258, 430–438.
- Cuesta, A., Meseguer, J. and Esteban, M.A., 2004.** Total serum immunoglobulin M levels are affected by immunomodulators in seabream (*Sparus aurata* L.). *Veterinary Immunology and Immunopathology*, 101, 203-210.
- Dias, D.C., Leonardo, A.F.G., Tachibana, L., Correa, C.F., Bordon, C.A.C., Romagosa, E. and Ranzani-Paiva, M.J.T., 2012.** Effect of incorporating probiotics into the diet of matrinxá (*Brycon amazonicus*) breeders. *Journal of Applied Ichthyology*, 28, 40–45.
- Doeschate, K.I.T. and Coyne, V.E., 2008.** Improved growth rate in farmed Halotiidae through probiotic treatment. *Aquaculture*, 284, 174-179.
- Duncan, P.L., Lovell, R.T., Butterworth, C.E., Freeberg, L.F. and Tamura, T., 1993.** Dietary folate requirements determined for Channel catfish, *Ictalurus punctatus*. *Journal of Nutrition*, 123, 1888–1897.
- El-Rhman, A.M.A., Khattab, Y.A.E. and Shalaby, A.M.E., 2009.** *Micrococcus luteus* and *Pseudomonas* species as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology*, 27, 175–180.
- Esmaceli, B., Khara, H., 2014.** Growth Performance, Hematology And Immunological Parameters Of Rainbow Trout, *Oncorhynchus Mykiss*, Fed With Diets Containing Different Levels Of Vitamin E And Folic Acid. *Iranian Journal of Fisheries Science*, 13, 931-943.
- Faramazi, M., Kiaalvandi, S., Lashkarbolooki, M. and Iranshahi, F., 2011.** The investigations of *Lactobacillus acidophilus* as probiotics on grown performance and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *American-Eurasian Journal of Scientific Research*, 6, 32–38.
- Ferguson, R., Merrifield, D.L., Harper, G.M., Rawling, M.D., Mustafa, S., Picchietti, S., Balcazar, J.L. and Davies, S.J., 2010.** The effect of *Pediococcus acidilactici* on the gut microbiota and immune status of on-growing red tilapia (*Oreochromis niloticus*). *Journal Applied Microbiology*, 109, 851-862.
- Fuda, H., Sayano, K. and Yamaji, F., 1991.** Serum immunoglobulin M (IgM) during early development of masu salmon *oncorhynchus masou*. *Comparative Biochemistry and Physiology*, 99, 637-643.
- Gatesoupe, F.J., 1999.** The use of probiotics in aquaculture. *Aquaculture*, 180, 147-165.
- Gatesoupe, F.J., 2002.** Probiotic and formaldehyde treatments of *Artemia* nauplii as food for larval pollack,

- Pollachius pollachius*. *Aquaculture*, 212, 347–360.
- Ghosh, S., Sinha, A. and Sahu, C., 2008.** Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. *Aquaculture Nutrition*, 14, 289-299.
- Goldin, B.R., 1998.** Health benefits of probiotics. *British Journal of Nutrition*, 80, 203-207.
- Heyman, M. and Me´nard, S., 2002.** Probiotic microorganisms: how they affect intestinal pathophysiology. *Cellular and Molecular Life Science*, 59, 1-15.
- Hosokawa, H., 1989.** The vitamin requirements of fingerling yellowtail, *Seriola quinqueradiata*. Ph.D. Dissertation, Kochi University, Japan.
- Kesarcodi-Watson, A., Kaspar, H., Lategan, M.J. and Gibson L., 2008.** Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. *Aquaculture*, 274, 1–14.
- Kim, D. and Austin, B., 2006.** Innate immune responses in rainbow trout (*Oncorhynchus mykiss*, Walbaum) induced by probiotics. *Fish and Shellfish Immunology*, 21, 513-524.
- Klontz, G.W., 1994.** Fish hematology. In: Stolen J.S., Fletcher T.C., Rowley A.F., Kelikoff T.C., Kaattari S.L., Smith S.A., editors. *Techniques in fish immunology*. Poland: SOS Publications. pp. 121-32.
- Lara-Flores, M., Olvera-Novoa, M.A., Guzman- Mendez, B.E. and Lopez-Madrid, W., 2003.** Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216, 193-201.
- Lin, Y.H., Lin, H.Y. and Shiau, S.Y., 2011.** Dietary folic acid requirement of grouper, *Epinephelus malabaricus*, and its effects on non-specific immune responses. *Aquaculture*, 317, 133-137.
- Irianto, A. and Austin, B., 2002.** Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Disease*, 25, 333-342.
- Marzouk, M.S., Mostafa, M.M. and Mohamed, N.M., 2008.** Evaluation of immunomodulatory effects of some probiotics on cultured (*Oreochromis niloticus*). 8th international Symposium on Tilapia in aquaculture, Cairo Egypt 2, 1043-1058.
- Merrifield, D.L., Dinitroglou, A., Foey, A., Davies, S.J., Baker, R.T.M., Bogwald, J., Castex, M. and Ringo, E., 2010.** The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, 302, 1–18.
- Naseri, S., Khara, H., Shakoory, Sh., 2013.** Effects of probiotics and Fe ion on the growth and survival and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) fry. *Journal of Applied Animal Research*, 41, 318-325.

- Nayak, S.K., Swain, P. and Mukherjee, S.C., 2007.** Effect of dietary supplementation of probiotic and vitamin C on the immune response of Indian major carp, *Labeorohita* (Ham.). *Fish and Shellfish Immunology*, 23, 892-896.
- Nayak, S.K., 2010.** Probiotics and immunity: A fish perspective. *Fish and Shellfish Immunology*, 29, 2-14.
- Neissi, A., Rafiee, G., Nematollahi, M., Safari, O., 2013.** The effect of *Pediococcus acidilactici* bacteria used as probiotic supplement on the growth and non-specific immune responses of green terror, *Aequidens rivulatus*. *Fish and Shellfish Immunology*, 35, 1976-1980.
- Noh, S.H., Han, K., Won, T.H. and Choi Y.J., 1994.** Effect of antibiotics, enzyme, yeast culture and probiotics on the growth performance of Israeli carp. *Korean Journal of Animal Science*, 36, 480-486.
- Panigrahi, A., Kiron, V., Kobayashi, T., Puangkaew, J., Satoh, S. and Sugita, H., 2004.** Immune responses in rainbow trout *Oncorhynchus mykiss* induced by a potential probiotic bacteria *Lactobacillus rhamnosus* JCM1136. *Veterinary Immunology and Immunopathology*, 102, 379-388.
- Perdigo'n, G.S., Alvarez, M.E., de Macias, N. and Pesce de Ruiz Holgado, A.A., 1988.** Adjuvant activity of lactic bacteria: Perspectives for its use in oral vaccines. *Revista Argentina De Microbiologia*, 20, 141-146.
- Puangkaew, J., Kiron, V., Somamoto, T., Okamoto, N., Satoh, S., Takeuchi, T. and Watanabe, T., 2004.** Non-specific immune response of rainbow trout (*Onchorhynchus mykiss*, Walbaum) in relation to different status of vitamin E and highly unsaturated fatty acids. *Fish and Shellfish Immunology*, 16, 25-39.
- Ranzani-Paiva, M.J.T., Ishikawam C.M., das Eiras, A.C. and da Silveira, V.R., 2004.** Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1757). *Brazilian Archive Biology and Technology*, 6, 945-953.
- Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. and Menasaveta, P., 1998.** Effects of a probiotic bacterium in black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture*, 167, 301-313.
- Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S. and Menasaveta, P., 2000.** Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). *Aquaculture*, 191, 271-288.
- Rollo, A., Sulpizio, R., Nardi, M., Silvi, S., Orpianesi, C., Caggiano, M., Cresci, A. and Carnevali, O., 2006.** Live microbial feed supplement in aquaculture for

- improvement of stress tolerance. *Fish Physiology and Biochemistry*, 32, 167–177.
- Sankaran, K. and Gurnani, S., 1972.** On the variation in the catalytic activity of lysozyme in fishes Indian. *Journal of Biochemistry and Biophysics*, 9, 162–165.
- Shelby, R.A., Lim, C., Yildirim-Aksoy, M. and Klesius, P.H., 2007.** Effects of probiotic bacteria as dietary supplements on growth and disease resistance in young channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal Applied Aquaculture*, 19, 81–91.
- Smith, C.E., 1968.** Hematological changes in coho salmon fed a folic acid deficient diet. *Journal of Fisheries Research Board of Canada*, 25, 151–156.
- Standen, B.T., Rawling, M.D., Davies, S.J., Castex, M., Foey, A., Gioacchini, G., Carnevali, O. and Merrifield D.L. 2013.** Probiotic *Pediococcus acidilactici* modulates both localised intestinal- and peripheral-immunity in tilapia (*Oreochromis niloticus*). *Fish and Shellfish Immunology*, 35(4), 1097–1104.
- Taoka, Y., Maeda, H., Jo, J.Y., Kim, S.M., Park, S.I., Yoshikawa, T. and Sakata, T., 2006.** Use of live and dead probiotic cells in tilapia, *Oreochromis niloticus*. *Fisheries Science*, 72, 755–766.
- Tekinay, A.A. and Davies, S.J., 2001.** Dietary carbohydrate level influencing feed intake, nutrient utilization and plasma glucose concentration in the rainbow trout, *Oncorhynchus mykiss*. *Turkish Journal Veterinary and Animal Science*, 25, 657–666.
- Wang, Y.B., Tian, Z.Q., Yao, J.T. and LI, W.E., 2008.** Effect of probiotics, *Enterococcus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Aquaculture*, 277, 203–207.
- Zare, A., Azari-Takami, G., Taridashti, F., Khara, H. 2017.** The effects of *Pediococcus acidilactici* as a probiotic on growth performance and survival rate of great sturgeon, *Huso huso* (Linnaeus, 1758). *Iranian Journal of Fisheries Science* 16, 150–161.
- Zhou, X., Tian, Z., Wang, Y. and Li, W., 2010.** Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Fish Physiology and Biochemistry*, 36, 501–509.