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Research Article

The effect of probiotic Bio-Aqua® on growth performance, haematological and biochemical parameters of bighead carp (*Hypophthalmichthys nobilis*)

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Abstract

The main objective of this study was to evaluate the effect of probiotic Bio-Aqua® on growth performance, carcass composition, haematological and biochemical parameters of bighead carp (*Hypophthalmichthys nobilis*) as well as the physico-chemical parameters of earthen ponds waters. For this purpose, bighead carp was cultured with initial weight of 0.5±0.05 g with selective experimental treatments for 4 months. In this study, treatments included: control (0 g / ha Bio-Aqua ®), T1 (250 g/ha Bio-Aqua ®), T2 (350 g/ha Bio-Aqua®) and T3 (450 g/ha Bio-Aqua®) which they were added to water of earthen ponds. Based on these results, significant difference was observed in specific growth rate, weight gain, daily growth rate, feed conversion ratio and survival in T3 treatment (450 g/ha) compared to the other treatments (p<0.05). Also, there was a significant difference in the crude protein and moisture contents in T3 group compared to control and T1 groups (p<0.05). Moreover, in T3 treatment, total protein, number of red and white blood cells, hemoglobin, PCV, monocytes and neutrophils were significantly higher than the other treatments (p<0.05). Bio-Aqua ®, in T3 (450 g/ha concentration) significantly reduced TSS, BOD, COD, nitrite and phosphate of water *in* earthen ponds compare to other treatments (p<0.05). This study demonstrated that Bio-Aqua® supplement, especially in T3 group (450 g/ha) improved growth performance, nutritional value, survival rate, chemical composition and haematological parameters of bighead carp.

Keywords: Bio-Aqua®, Bighead carp, Growth performance, Chemical composition, Haematological parameters, Biochemical profile, Physico-chemical parameters of waters

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Introduction

Bighead carp (*Hypophthalmichthys nobilis*) is one of the economically important fish which is widely distributed in the world especially in the Middle East (Eddy and Underhill, 1974). High stocking density, especially the intensive system has generated potential environmental stressors to the fishes, which leads to a high susceptibility to various disease agents including bacteria, fungi, viruses and parasites causing severe damages and huge economic losses. Using antibiotics and other chemotherapeutics to control infection may lead to drug resistant strains, environmental pollution, accumulation of residues in tissues and food safety concerns (Wang, 2014; Lazado and Caipang *et al*., 2014). Consequently, using of natural and eco-friendly therapeutics as growth promoters and immunostimulants recommend in aquaculture.

Probiotics are live microorganisms such as bacteria, fungi and bacteria which are used as complementary feed and have beneficial effects on the host through the balance and improve the useful microbial intestine. *Lactobacillus, Lactococcus, Leuconostoc, Enterococcus, Bifidobacter, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Pseudomonas, Clostridium* and *Saccharomyces* are the common probiotics that are used in aquaculture (Nayak, 2010; Cruz *et al*., 2012; Eissa *et al*., 2014; Poolsawat *et al*., 2019). Probiotics can increase the growth performance of the fish by producing digestive enzymes, promote their nutritional value and survival rate. The positive effects of probiotics as a feed additive on growth performance, innate immune system, intestinal microbiota, stress and disease resistance and improve the water quality have been confirmed in rainbow trout (Kumar *et al*., 2006; Wang, 2011; Asadian *et al*., 2015; Adel *et al*., 2017; Mehrabi *et al*., 2018). Probiotics can stimulate the immunity system of fish by up-regulation of the pro-inflammatory cytokine genes and increasing the phagocytic activity of leucocytes (Pirarat *et al*. 2006; Panigrahi *et al*., 2010).

Bio-Aqua® supplement is a multi-strain probiotic supplement including *Lactobacillus* spp., *Bacillus* spp., *Bifidobacterium bifidum* and *Saccharomyces cerevissiae* that are commonly identified as safe and eco-friendly substances among the main candidates to improve the health status. Study of Akbari Nargesi *et al*. (2018) showed that using of Bio-Aqua® supplement (2 g/kg diet) in rainbow trout diet increased the growth parameters including weight gain, specific growth rate and final weight. The main objectives of this study was to evalute the effects of dietary Bio-Aqua® supplement on growth performance, chemical composition, haematological and biochemical parameters of bighead carp as well as physico-chemical parameters of water in earthen ponds.

Materials and methods

Experimental fish

A number of 2400 bighead carps with the mean weight 0.5±0.05 g were obtained from a commercial fish farm in Mazandaran province, Iran. Fish were acclimatized for 2 weeks in earthen ponds (each with 500 cubic meters capacity). Fish were distributed randomly into 12 earthen ponds, each with 200 fish.

Preparations of probiotic and experimental diets

Bio-Aqua® supplement was obtained from Zist Darman Mahan Company (Tehran, Iran). This supplement included: *Enterococcus faecium*, *Bacillus subtilis*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Pediococcus acidilactici*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum* and *Saccharomyces cerevissiae* (3×10^6 CFU/g bacteria). In this study, treatments included: control (0 g/ha Bio-Aqua®), T1 (250 g/ha Bio-Aqua®), T2 (350 g/ha Bio-Aqua®) and T3 (450 g/ha Bio-Aqua®) which were added to the water of earthen ponds. The experiments were performed with three replications. In the second of two months, the amounts of this supplement were doubled.

Carcass composition

At the end of the experimental period, 9 fish from each individual *earthen pond* (27 fish per group) were selected randomly. Carcass composition was analysed according to the standard methods described by the Association of Official Analytical Chemists (AOAC, 2005). Moisture was determined by drying the samples in a hot air oven (Behr, Germany) at 105 °C. Crude lipid, protein and ash were determined by chloroform methanol extraction (2:1, v/v), Kjeldahl method (N×6.25) and incineration in a muffle furnace at 500 °C for 6 h, respectively (AOAC, 2005).

Growth performance

All fish were deprived of food for 24 h before weighing and sampling, and the following parameters were measured at the end of feeding trial after 4 months:

Weight gain=W_2 (g)-W_1 (g)

where W1 is the initial weight, W_2 is the final weight and T is the number of days in the feeding period (Tacon 1990).

Specific growth rate (SGR) = 100×(ln W_2-ln W_1)/ T

Daily growth rate (DGR)=(W_2-W_1)/ T

where ln=Natural logarithm, W2= Final weight at time t2, W1=Initial weight at time t1.

Feed conversion ratio (FCR)=Feed intake (g) /weight gain (g).

Survival rate (%) = 100×(final number of fish/initial number of fish).

Blood sampling

At the end of the study, fish were fasted for 24 h prior to sampling and anesthetized with clove oil (100 mg L^{-1}) before sample collection (Saeidi asl *et al.*, 2017). Blood samples (about 1 ml) was drawn from the caudal vein and immediately transferred to non-

heparinized tubes for serum collection (27 fish per group).

Blood samples of 9 fish from each individual tank (27 fish per group) were immediately divided into two half parts. One half was transferred to a tube containing anti-coagulant (heparin) for study the hematological analyses, while the other half was transferred to non-heparinized tubes for biochemical studies. Sera samples were obtained by coagulated blood samples after centrifugation for 15 min at 3000×g at 4°C and stored at -80°C until use (Binaii *et al*., 2014).

Blood analysis

The number of red blood cells (RBC: 10^6 mm^{-3}) and white blood cells (WBC: 10^3 mm^{-3}) were counted in an improved Neubauer hemocytometer using Hayem and Turck diluting fluids (Blaxhall and Daisley, 1973). Haematocrit (Ht %) was determined by the standard microhematocrit method and expressed as percentage. The haemoglobin (Hb, g dl^{-1}) level was determined according to cyanomethemoglobin method. Furthermore, differential leukocyte cells were measured by preparing Giemsa stained smears. Blood smears were studied by light microscopy in order to make blood cell counts.

Biochemical analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, albumin and total protein levels were determined by using commercial kits (Pars Azmoon Company, Tehran, Iran) and a biochemical auto analyzer (Eurolyser, Belgium) (Adel *et al*., 2015).

Physico-chemical analysis of water in earthen ponds

Sampling was done monthly from selected earthen ponds waters. For this purpose, 6 samples from each pond (with three replications) were taken. Water samples were transferred to the laboratory as alongside the ice boxes (4°C) in minimum time. The pH, dissolved oxygen (DO) and temperature (°C) were measured by WTW potable (320-WTW, USA). *Total dissolved solids* (TDS) and total suspended solids (TSS) by weighting method, COD through the titration method, BOD by the BOD Track equipment, alkalinity and soluble phosphate by titration method, ammonium and nitrite using a spectrophotometer were determined (APHA, 1998).

Statistical analysis

The data were subjected to statistical analysis using the SPSS software version 18 (SPSS Inc., Chicago, IL, USA). The statistical analysis was done using One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. *P*-value of <0.05 was considered significant. All the data were performed in triplicate.

Results

Growth parameters

Growth performance and survival of bighead carps at the end of the study are

presented in Table 1. Based on the results, SGR, WG, daily growth rate (DGR, and FCR in T3 (450 g/ha) were significantly different than the other treatments (p<0.05). According to the results, by increasing the probiotic concentration from 250 to 450 g/ha, the growth parameters (exception on FCR) were increased (p<0.05). Also, the highest level of survival rate was observed in T3 treatment, which showed a significant difference with the other treatments (p<0.05).

Table 1: Growth performance of bighead carp in different treatments with probiotic Bio-Aqua® (Mean ±SE).

Parameter	Control	T1	T2	T3
Initial weight (g)	0.5±0.05 ^{b}	0.6±0.04^{b}	0.5±0.05^{a}	0.5±0.02^{a}
WG (g)	38.12±1.1^{c}	39.3±0.9^{c}	42.7±0.5^{b}	48.5±0.7^{a}
SGR (%)	1.16±0.05^{b}	1.24±0.06^{b}	1.56±0.08^{a}	1.67±0.03^{a}
FCR	2.52±0.4^{a}	2.41±0.2^{a}	2.32±0.3^{ab}	2.2±0.2 ^{b}
DGR	1.92±0.1^{d}	2.08±0. 2^{c}	2.43±0.4^{b}	2.67±0.3^{a}
SR%	67.0±1.3^{d}	72.0±1.5^{c}	76.3±0.8^{b}	85.0±2.3^{a}

WG, weight gain; SGR, specific growth rate; FCR, fed conversion ratio. Means in the same rows with different superscript are significantly different (p<0.05).

Carcass composition

Carcass compositions of bighead carps cultured in enriched water contains different levels of dietary Bio-Aqua® after 4 months are presented in Table 2. Based on the results, there was a significant difference in the crude protein and moisture contents in T3 compared to control and T1 groups (p<0.05). Although, no significant difference was observed in crude lipid and ash contents in all treatments (p≥0.05).

Table 2: Carcass composition of bighead carp in different treatments of Bio-Aqua®.

Parameter	Control	T1	T2	T3
Crude protein	15.2±0.2^{b}	15.0±0.4^{b}	15.4±0.2^{ab}	16.1±0.3^{a}
Crude lipid	2.3±0.2^{a}	2.1±0.1^{a}	1.9±0.2^{a}	1.9±0.1^{a}
Ash	2.1±0.1^{a}	2.1±0.1^{a}	2.2±0.1^{a}	2.0±0.1^{a}
Moisture	78.8±0.6^{a}	78.3±0.4^{a}	77.7±0.5^{b}	76.9±0.4^{c}

Means in the same rows with different superscript are significantly different (p<0.05).

Blood analysis

The number of white and red blood cells significantly increased (p<0.05) in Bio-Aqua® treatments (Table 3). The highest numbers of white and red blood cell was observed in T3 treatment which showed significant differences with other treatments, especially control and T (p<0.05). Based on the results, there was a significant difference in neutrophil and monocyte percentages, haemoglobin and hematocrit values in T2 and T3 compared to control and T1 (p<0.05). Although, no significant difference was observed in eosinophil percentage in all treatments (p≥0.05).

Blood serum biochemical changes

Blood serum biochemical changes were presented in Table 4. Based on these results, significant differences were observed in the AST activity of bighead carp cultured in enriched water contained different levels of Bio-Aqua® compare to control (Table 4). Also, there was a significant difference among T3 (450 g/ha) and T2 (350 g/ ha) with control and T1 (250 g/ ha) for ALT activity (p<0.05). Moreover, there was a significant difference in the total protein and serum albumin levels among T3 (450 g/ha) with other treatments, especially control group (p<0.05).

Table 3: Blood analysis of bighead carp cultured in enriched water contains different levels of Bio-Aqua ® after 4 month.

Parameter	Control	T1	T2	T3
RBC (10^6 mm-^{3})	0.92±0.04^b	0.98±0.03^b	1.01±0.04^b	1.24±0.02 ^{a}
WBC (10^3 mm-^{3})	23.11±0.8^b	23.24±1.0^b	24.42±1.1^{ab}	25.73±1.3^a
Hct (%)	26.1±0.5^b	27.24±0.6^{ab}	27.58±0.4^{ab}	28.87±0.8^a
Hb (g dl^{-1})	7.11±0.1^b	7.59±0.1^{ab}	7.82±0.2^a	7.98±0.1^a
Monocyte	3.02±0.07^b	3.14±0.03^b	3.24±0.02 ^{ab}	3.9±0.05 ^{a}
Lymphocyte	72.1±2.1^a	71.92±2.24^a	71.5±2.1 ^{ab}	70.25±0.9 ^{b}
Neutrophil	19.86±1.1^c	21.35±1.5^b	22.68±1.7^a	23.64±1.4^a
Eosinophil	0.46±0.01^a	0.48±0.02^a	0.40±0.01^a	0.47±0.05^a

Note: RBC, red blood cells; WBC, white blood cells; Hct, hematocrit; Hb, haemoglobin concentration. Data are presented as mean ± S.D (n =12 fish from each group). Means in the same rows with different superscripts are significantly different (p<0.05).

Table 4: Blood serum biochemical changes of bighead carp cultured in enriched water Contains different levels of Bio-Aqua ®

Parameter	Control	T1	T2	T3
AST (U l^{-1})	368.0±9.1^a	358.0±7.5^a	326.0±8.1^b	306.1±6.2^c
ALT (U l^{-1})	9.12±0.10^a	8.86±0.1^b	8.72±0.13^b	7.76±0.07^b
Albumin (g dL^{-1})	0.56±0.1^b	0.61±0.1^b	0.73±0.1^b	0.81±0.1^a
Total protein (g dL^{-1})	3.5±0.2^b	3.4±0.1^b	3.6±0.1^b	3.9±0.1^a

*Data are presented as mean ± S.D (n =9 fish from each group). Means in the same rows with different superscripts are significantly different (p<0.05).

Physico-chemical parameters of pond water

The effect of different levels of Bio-Aqua® on physic-chemical parameters of water in earthen ponds are shown in Table 5. Based on the results, Bio-Aqua® in T3 treatment significantly reduced TSS, BOD, COD, nitrite and phosphate of water in earthen ponds compare to the other treatments (p<0.05). Although, no significant differences were observed in temperature, pH, salinity, TDS and ammonium values of water in earthen ponds in all treatments (p≥0.05).

Table 5: Physico-chemical parameters of water in earthen ponds enriched with different levels of Bio-Aqua ® after 120 days.

Parameter	Control	T1	T2	T3	standard
T (°C)	31.2±0.3a	31.5±0.5a	31.5±0.2a	31.6±0. 4 a	16-22
pH	8.5±0.1a	8.3±0.2a	8.4±0.1a	8.6±0.1a	6.5-8.5
Salinity (ppt)	3.1±0.07a	3.2±0.08a	3.4±0.05a	3.2±0.08a	-
TDS (ppt)	2.48±0.03a	2.5±0.01a	2.61±0.02a	2.36±0.03a	<400
TSS (mg/L)	279.0±5.2b	268.5±8.3b	228.5±7.4 c	210.0±5.0 d	-
BOD (mg/L)	4.0±0.1a	3.75±0.12ab	3.55±0.05 ab	3.1±0.05 b	20
COD (mg/L)	14.0±0.8b	13.3±1.2a	12.5±1.2ab	10.17±1.2b	50
Ammonium (mg/L)	0.19±0.02a	0.2±0.01a	0.19±0.01ab	0.18±0.01b	0-3
Nitrite (mg/L)	0.09±0.02a	0.10±0.01a	0.07±0.01a	0.06±0.01a	0.06-0.1
Phosphate (mg/L)	0.062±0.02a	0.060±0.02a	0.050±0.02a	0.034±0.02a	0.01-0.3

Means in the same rows with different superscripts are significantly different (p<0.05).

Discussion

Probiotics are one of the identified alternatives which can lessen the dependence of the aquaculture industry to antibiotics (Akhter *et al*., 2015). One of the most important features of probiotics is increasing the nutritional value and also improves the growth performance. The results of the present study showed that adding different levels of Bio-Aqua® to the water *of* earthen ponds had significant effects on growth performance of bighead carp. At the end of the study, T3 treatment (450 g/ ha) showed the highest levels of weight gain, specific growth factor, daily growth rate and survival. Similar to present results, Akbari Nargesi *et al*. (2018) showed that Bio-Aqua® supplement (2 g/kg diet) in rainbow diet increased the growth parameters (weight gain, SGR and final weight). Also, it is shown that *Bacillus latrospores* and *Bacillus licheniformis* spores added to rearing water improve the growth performance and survival of silver carp (Sahandi *et al*., 2012). Significant differences were observed in FCR, SGR and feed efficiency of bighead carp, common carp and grass carp when fed with *Bacillus* spp. (Jamali *et al*., 2015). Similarly, Qi (2006) reported that using probiotic supplement can reduce 7% of total costs for production of silver carp and bighead carp. Improved growth performance were also reported for bighead, silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*) and black carp (*Mylopharyngodon piceus*) cultured with probiotics supplementation (Hua *et al*., 2001).

The results of the present study showed that Bio-Aqua® supplement increased the survival of bighead carp. The highest level of survival rate was observed in T3 treatment, which had a significant difference with other treatments. The reason for this increase may be related to the elimination of harmful bacteria by beneficial bacteria in this multi-strain probiotic especially *L. plantaru*m, *L. casei*, *L. acidophilus*, and *L. rhamnosus*. Vadstein (1997) showed that lactic acid bacteria produce compounds such as *bacteriocins* and thereby prevent the growth of harmful microorganisms in intestine.

Blood analysis known as valuable biological indicators can reflect the response of body to physiological stresses as well as general health status. Based on the results, there was a significant difference in neutrophil and monocyte percentage, hemoglobin, haematocrit, RBC and WBC counts in T2 and T3 treatments compared to control and T1 treatment. These results confirmed the improvement of immune system of bighead carp cultured in enriched water contains different levels of Bio-Aqua® after 4 months. Similarly, RBC, hematocrit, and hemoglobin levels of common carp (*Cyprinus carpio*) in *Lactobacillus plantarum* groups were higher than the control group (Soltani *et al*., 2017). Also, Kumar *et al*. (2006) showed that *Bacillus subtilis* as a probiotic in Indian carp (*Labeo rohita*) diet has a significant effect on the number of white blood cells, immune parameters and survival rate of fish against *Aeromonas hydrophilia* infection. Al-dohail *et al*. (2009) showed that the addition of probiotic *L. acidophilus* to African catfish (*Clarias gariepinus*) feed increased the hematocrit and hemoglobin values and also total number of red and white blood cells.

Total proteins include albumin and globulin proteins. Increasing the levels of albumin, globulin, and total proteins are thought to be more associated with stimulation of the non-specific immune system (Wiegertjes *et al*., 1996). In the present study, there was a significant increase in serum total protein among T3 (450 g/ha) and T2 (350 g/ha) compared to control and T1 groups (250 g/ha). Increasing the level of total proteins is a good indicator which reflects the evaluating of immune response. In similar study, Soltani *et al*. (2017) showed that common carp fed orally with different concentrations of *L. plantarum* has higher level of serum total protein compare to control. In a study conducted by Safari *et al*. (2016), neutrophil counts significantly increased in all probiotics (*Enterococcus casseliflavus*) groups of juvenile rainbow trout compare to control group.

Liver enzymes are considered as indicators to evaluate the liver status. Changes in liver enzymes activity and their secretion can be affected by water physico-chemical parameters, density, type of diet, age and sex of fish. The present results demonstrated that the activity of liver enzymes (ALT and AST) have not been influenced by different levels of Bio-Aqua®. Generally, increasing the activities of ALT AST are commonly regarded as indicators of hepatic damage (Sheikhzadeh *et al.*, 2012). Therefore, these results suggest that Bio-Aqua® is not toxic to hepatic health. Likely, no significant differences were found in aspartate aminotransferase (AST, ALT, alkaline phosphatase, and lactate dehydrogenase activities of common carp fed with different concentrations of *L. plantarum.*

Based on the current results, there was a significant difference in the crude protein and moisture contents in T3

compared to control and T1 which probably related to the ability of lactic acid bacteria to improve digestion and absorption of protein compounds from the gastrointestinal tract (Fuller and Perdigon, 2003). In similar study, Jafarian *et al*. (2014) confirmed that Bacillus and *S. cerevisiae* isolated from gut of Beluga (*Huso huso*) significantly increased the protein content of carcass compare to control group. Ramachandran and Ray (2007) showed that *Bacillus sp.* isolated from intestine of common carp as probiotic increased the crude protein content of rohu fingerling. Ghosh *et al*. (2003) reported that probiotics would probably reduce lipid level of carcass and increase their quality through increased digestibility of fat and protein contents.

Water quality plays an important role in growth performance and survival rate of fish. Probiotics could increase the organic matter decomposition, algal growth and dissolved oxygen and also, help to control and decrease the ammonia, nitrite and hydrogen sulfide and outbreak diseases (Boyd and Gross, 1998). In the present study, Bio-Aqua®, in T3 treatment significantly reduced TSS, BOD, COD, nitrite and phosphate of cultivated pond water compare to other treatments. Probiotics increased the water quality by accelerating natural processes such as nitrification, ammonification, denitrification, oxidation of sulfide and decomposition of poisonous compounds (Boyd and Gross, 1998). Zhou *et al*. (2009) and Wang *et al*. (2005) showed that use of commercial probiotics significantly reduce the amount of nitrogen and phosphorus in the pond and improved the water quality parameters. De-Paiva-Maia *et al*. (2013) showed that the use of commercial probiotics in earthen ponds improve the quality of water by decreasing the pathogenic bacteria in the cultivated environment. These results were similar to current results and confirm the positive effects of Bio-Aqua®.

In conclude, Bio-Aqua® supplement, especially in the concentration of 450 g/ha, has a positive and significant effects on growth performance, nutritional value, survival, and chemical composition of bighead carp and also on physico-chemical parameters of water in earthen ponds. Therefore, it is recommended to use probiotics in earthen ponds to culture of bighead carp. Although, more studies are needed to find the effect of Bio-Aqua® on non-specific immune response, disease resistance and intestine enzymes activity of bighead carp.

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References

Adel, M., Safari, R., Pourgholam, R., Zorriehzahra, J. and Esteban, M.A., 2015. Dietary peppermint (*Mentha piperita*) extracts promote growth performance and increase the

main humoral immune parameters (both at mucosal and systemic level) of Caspian brown trout (*Salmo trutta caspius Kessler*, 1877). *Fish and Shellfish Immunology*, 47, 623-629. DOI:10.1016/j.fsi.2015.10.005

Adel, M., Lazado, C.C., Safari, R., Yeganeh, S. and Zorriehzahra, M.J., 2017. Aqualase®, a yeast-based in-feed probiotic, modulates intestinal microbiota, immunity and growth of rainbow trout *Oncorhynchus mykiss. Aquaculture Research*, 48(**4**), 1815-1826. DOI:10.1111/are.13019

Akbari Nargesi., E., Falahatkar, B. and Sajadi, M.S., 2018. The effect of probiotic on growth performance and blood indices of rainbow trout. *Aquatic Ecology Journal*, 8(**2**), 51-60. DOI: 10.22092/ISFJ.2019.119093

Akhter, N., Wu, B., Memon, A.M. and Mohsin, M., 2015. Probiotics and prebiotics associated with aquaculture: a review. *Fish and Shellfish Immunology*, 45(**2**), 733-741. DOI:10.1016/j.fsi.2015.05.038

Al-dohail, M.A., Hashim, R. and Aliyu-Paiko., M., 2009. Effects of the probiotic, Lactobacillus acidophilus on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus* Burchell, 1822) fingerling. *Aquaculture Research*, 40,1642-1652. DOI :10.1111/j.1365-2109.2009.02265.x

American Public Health Association (APHA), 1998. Standard methods for the examination of water and wastewater. 20th edn, New York.

AOAC, 2005. Official Methods of Analysis. 17th Edn., Association of Official Analytical Chemistry, Arlington, Virginia, USA.

Asadian, M., Shahsavani, D. and Kazerani, H.R., 2015. Growth promoting effects of a multi-strain probiotic on common carp (Cyprinus carpio) fingerling. *Iranian Journal of Veterinary Science and Technology*, 7(**2**), 63-74. DOI: 10.22067/VETERINARY.V7I2.44554

Binaii, M., Ghiasi, M., Farabi, S.M.V., Pourgholam, R., Fazli, H., Safari, R., Alavi, S.E., Taghavi, M.J. and Bankehsaz, Z., 2014. Biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*) following the diet supplemented with nettle (*Urtica dioica*). *Fish and Shellfish Immunology*, 36, 46-51. DOI: 10.1016/j.fsi.2013.10.001

Blaxhall, P.C. and Daisley, K.W., 1973. Routine hematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.

Boyd, C.E. and Gross, A., 1998. Use of probiotics for improving soil and water quality in aquaculture ponds, In Flegel TW (ed) Advances in shrimp biotechnology. National Center for Genetic Engineering and Biotechnology, Bangkok.

Cruz, P.M., Ibanez, A.L., Hermosillo, O.A. and Ramirez Saad, H.C., 2012. Use of Probiotics in Aquaculture. *ISRN Microbiology,* 1, 1-13. DOI: 10.5402/2012/916845

De Paiva-Maia, E., Alves-Modesto, G., Otavio-Brito, L., Vasconcelos-Gesteira, T.C. and Olivera, A., 2013. Effect of a commercial probiotic on bacterial and phytoplankton concentration in intensive shrimp farming (*Litopenaeus vannamei*) recirculation systems. Lat. *American Journal of Aquatic* Research, 41(**1**), 126-137. DOI: 10.3856/vol41-issue1-fulltext-10

Eddy, S. and Underhill, J.C., 1974. Northern fishes with special reference to the upper Mississippi Valley. Third Edition. University of Minnesota Press, pp. 32-41.

Eissa, N., Abou El-Gheit, E. and Shaheen, A.A., 2014. Protective effect of *Pseudomonas fluorescens* as a probiotic in controlling fish pathogens. *American Journal of BioScience,* 2(**5**), 175-181. DOI : 10.11648/j.ajbio.20140205.12

Fuller, R. and Perdigon, G. 2003. Gut flora, immunity and health. Blackwell publishing. 276 P.

Ghosh, S., Moore, S., Bell, R.M. and Dush, M., 2003. Functional analysis of a phosphatidic acid binding domain in human Raf-1 kinase: Mutations in the phosphatidate binding domain lead to tail and trunk abnormalities in developing zebrafish embryos. *The Journal of biological chemistry,* 278(**46**), 45690-45696. DOI: 10.1074/jbc.M302933200

Hua, R.S., Ge, L.L., Wang, J.P. and Guo, X.D., 2001. The application of EM in aquaculture. Sci. *Fish Farming*, 6, 41–42.

Jafarian, H., Khosravi, Kh., Abdollahi, D. and Tavana, S., 2014. The effect of blend of isolated bacillus and *Saccharomyces cerevisiae* from gut of Beluga (*Huso huso*) on growth and feeding parameters of silver carp (*Hypophthalmichthys molitrix*) larvae. *Nutrition and Biochemistry of Aquaculture,* 2(**1**), 1-12.

Jamali, H., Jafaryan, H., Nazerian, S. and Sadegh Aramli, M., 2015. Effect of probiotic *Bacillus spp.* on growth and feed efficiency of common carp, grass carp and bighead larvae feed with nauplii of different Artemia species. *Fisheries Sciences and Technology,* 4(**2**), 27-43.

Kumar, R., Mukherjee, S.C., Prasad, K.P. and Pal, A.K., 2006. Evaluation of *Bacillus subtilis* as a probiotic to Indian major carp *Labeo rohita* (Ham.). *Aquaculture Research,* 37, 1215-1221. DOI: 10.1111/j.1365-2109.2006.01551.x

Lazado, C.C. and Caipang, C.M.A., 2014. Atlantic cod in the dynamic probiotics research in aquaculture. *Aquaculture,* 424–425; 53-62. DOI: 10.1016/j.aquaculture.2013.12.040

Mehrabi, f., Khalesi, M.K. and Hazaie, K., 2018. Effects of Pre- and probiotics on growth, survival, body

composition, and hematology of common carp (*Cyprinus carpio* L.) fry from the Caspian Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, 597-602. DOI: 10.4194/1303-2712-v18_4_11

Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. *Fish and Shellfish Immunology*, 29, 2-14. DOI: 10.1016/j.fsi.2010.02.017

Panigrahi, A.,Kiron, V., Satoh, S. and Watanabe, T., 2010. Probiotic bacteria Lactobacillus rhamnosus influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Fish physiology and biochemistry*, 36, 969-77. DOI: 10.1007/s10695-009-9375-x

Pirarat, N.,Kobayashi, T.,Katagiri, T.,Maita, M. Endo, M.,2006. Protective effects and mechanisms of a probiotic bacterium *Lactobacillus rhamnosus* against experimental Edwardsiella tarda infection in tilapia (*Oreochromis niloticus*). *Veterinary immunology and immunopathology*, 113, 339-47. DOI: 10.1016/j.vetimm.2006.06.003

Poolsawat, L., Li, X., He, M., Ji, D. and Leng, X., 2019. *Clostridium butyricum* as probiotic for promoting growth performance, feed utilization, gut health and microbiota community of tilapia (*Oreochromis niloticus* × *O. aureus*). *Aquaculture Nutrition*, 35, 1–14. DOI: 10.1111/anu.13025

Qi, Z., Zhang, X.-H., Boon, N., & Bossier, P., 2009. Probiotics in aquaculture of China-Current state, problems and prospect. *Aquaculture*, 290(**1**),15-21. Doi: 10.1016/j.aquaculture.2009.02.012

Ramachandran, S. and Ray, A.K., 2007. Nutritional evaluation of fermented black gram (*Phaseolus mungo*) seed mealin compound diets for rohu, *Labeo rohita* (Hamilton), fingerling. *Journal of Applied Ichthyology*, 23, 74-79. DOI: 10.1111/j.1439-0426.2006.00772.x

Saeidi asl, M.R., Adel, M., Marlowe Caipang, A.C. and Dawood, M.A.O., 2017. Immunological responses and disease resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles following dietary administration of stinging nettle (*Urtica dioica*). *Fish and Shellfish Immunology*, 71, 230–238. DOI: 10.1016/j.fsi.2017.10.016

Safari, R., Adel, M., Lazado, C. C., Caipang C. M. A. and Dadar, M.,2016. Host-derived probiotics *Enterococcus casseliflavus* improves resistance against *Streptococcus iniae* infection in rainbow trout (*Oncorhynchus mykiss*) via immunomodulation. *Fish & Shellfish Immunology*, 52, 198-205. DOI: 10.1016/j.fsi.2016.03.020

Sahandi, J., Jafariyan, H., Roozbehfar, R., Babaei, S. and Dehestani, M., 2012. The use of two enrichment forms (*Brachionus plicatilis* enrichment and rearing water enrichment) with probiotic bacilli spore on growth and survival of Silver carp (*Hypophthalmichthys molitrix*). *Iranian Journal of Veterinary Research, Shiraz*

University, 13(**4**), 282-295. DOI: 10.1007/s12602-018-9455-2

Sheikhzadeh, N.,Karimi Pashaki, A., Nofouzi, K., Heidarieh, M. and Tayefi-Nasrabadi, H., 2012. Effects of dietary Ergosan on cutaneous mucosal immune response in rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, 32, 407-410. DOI: 10.1016/j.fsi.2011.11.028

Soltani, M., Abdy, E., Alishahi, M., Mirghaed, A.T. and Hosseini-Shekarabi, P., 2017. Growth performance, immune-physiological variables and disease resistance of common carp (*Cyprinus carpio*) orally subjected to different concentrations of *Lactobacillus plantarum*. *Aquaculture International*, 25(**5**), 1913–1933. DOI: 10.1007/s10499-017-0164-8

Tacon, AG., 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. Feeding methods. Agent Laboratories Press, Redmond, Taoka, 138 P.

Vadstein, O., 1997. The use of immunostimulation in marine larvicular: possibilities and challenges. *Aquaculture*, 155, 401-417.

Wang, Y., Xu, Z. and Xia, M.S., 2005. The effectiveness of commercial probiotics in Northern White Shrimp (*Penaeus vannamei*) ponds. *Fisheries Science*, 71, 1034–1039. DOI: 10.1111/j.1444-2906.2005.01061.x

Wang, Y., 2011. Use of probiotics Bacillus coagulans, Rhodopseudomonas palustris and Lactobacillus acidophilus as growth promoters in grass carp, Ctenopharyngodon idella fingerlings. *Aquacalture Nutrition*, 17, 372–378. DOI: 10.1111/j.1365-2095.2010.00771.x

Wang, F.H., 2014. High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation. *Environmental Science and Technology,* 48, 9079–9085. DOI: 10.1021/es502615e

Wiegertjes, G. F., Stet, R. M., Parmentier, H. K. and Muiswinkel, W. B., 1996. Immunogenetics of disease resistance in fish: a comparative approach. Developmental & Comparative Immunology, 20, 365-381.

Zhou, X., Wang Y. and Li, E., 2009. Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. *Aquaculture*, 287, 349–353. DOI: 10.1016/j.aquaculture.2008.10.046