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Enhancement of growth performance and hematological changes in rainbow trout (*Onchorhynchus mykiss*) alevins fed with *Bifidobacterium* bacteria

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Abstract

The study of probiotic application as an important rearing strategy was started more than 30 years ago and most of these studies were carried out to increase growth and survival of larvae. Effect of *Bifidobacterium animalis* PTTC-1631 and *B. lactis* PTTC-1736 as probiotic supplement has been studied on growth performance and hematological changes on rainbow trout, *Oncorhynchus mykiss* alevins with initial body weight of 0.583 ± 0.197 g. The commercial diet was supplemented with graded levels of probiotics (1×10^7, 2×10^7 and 3×10^7 CFU g^{-1} dry feed) to obtain 3 sets of experimental diets (T_1, T_2, and T_3 respectively) and fed four times a day for 60 days. T_1 alevin showed the best growth performance in terms of specific growth rate, weight gain, metabolic growth rate, feed conversion ratio and survival rate. The highest red and white blood cell concentrations were noticed in fish fed T_2 and T_3 diets, respectively, no significant difference was observed in hemoglobin content. T_1 showed the significant elevation of serum biochemical parameters and reduction of cortisol level. The results of present study might suggest likely positive effects of probiotic supplements with concentration of 1×10^7 CFU g^{-1} dry feed on growth and hematology on rainbow trout alevins.

Keywords: Rainbow trout; alevins; probiotic; blood; *Bifidobacterium*; feed.

1 | INTRODUCTION

Probiotics possess several important properties and therefore can be used as effective organism. These features are contain efficient adherence to intestinal epithelial cells to reduce or prevent colonization of pathogens (Kirjavainen *et al.* 1998), competitive growth (Holzapfel *et al.* 1998), production of metabolites to inhibit or kill pathogen (Reid and Burton 2002), and non-pathogen (Gonzalez *et al.* 1995). Recently, efforts have been made to develop strategies for microbial control in order to decrease the use of therapeutic chemicals and antibiotics (Cabello 2006; Sahandi *et al.* 2012). Probiotic supplements have special relevance in development of rearing technologies for fish alevin. Probiotic is fast being recognized as an alternative therapy for health management, considering the encouraged restriction on antibiotics and

limitations of vaccination and chemotherapy (Panigrahi *et al.* 2005). Despite its wider acceptance in aquaculture, many questions remain unanswered about its influence on fish physiology. Recently, some studies have looked into the some kinds of probiotics increased immune response and growth performance in rainbow trout (Panigrahi *et al.* 2010; Ramesh *et al.* 2015). The administration of probiotic in adequate amounts confers health benefit on the host in reduction of mortality (Jafaryan *et al.* 2007; Gupta *et al.* 2014). However, due to various applications of probiotics, there is an attempt to screen and identify new probiotic strain with specific characteristic to be appropriate for growth performance. The use of *Bifidobacterium* sp. as probiotic has been recognized and accepted (Charteris *et al.* 1998; Gill *et al.* 2001), but very few studies were published. Also, there is no report about effect of *B. animalis* PTCC-1631 and *B. lactis* PTTC-1736 on growth and hematology of rainbow trout. *Bifidobacterium* are genus of gram-positive bacteria that ferment various carbohydrates mainly to lactate and acetate. These bacteria are able to produce biogenic compounds such as bioactive peptides and fatty acids. These components were shown to be possible mechanism for their health enhancing properties (Gobbetti *et al.* 2010). The present study investigated effect of dietary *B. animalis* PTCC-1631 and *B. lactis* PTTC-1736 as probiotic on growth performances and hematological profile of rainbow trout alevins.

2 | METHODOLOGY

2.1 | Experimental design

The present experiment was carried out for 60 days. The rainbow trout (*Oncorhynchus mykiss*) alevins were obtained from a private Coldwater fish farm (Amol, Mazandaran- Iran). Alevins were transferred with nylon bags (one part water and two parts oxygen) to the laboratory. They were acclimatized to the laboratory condition for two weeks during which they were fed commercial diet. After acclimatization 30% of total fish were weighted to determine the initial body weight (mean [± SE] initial weight, 0.583 ± 0.197 g). A total of 480 alevins were randomly divided in 4 treatments (3 experimental treatments and a control), each with three replicates. Fish rearing system was consisting of 16 self-cleaning 20-L plastic tanks that filled with 15 liters of water and the alevins were stocked a density of 2 fish per liter. The experiment was conducted in temperature (17.66 °C) and photoperiod (12 h light and 12 h darkness) controlled condition. Each tank had permanent water exchange (2 L min) and aerated with air stone. The water quality parameters were found to be in the range of temperature (17.66 ± 1.33 °C), pH (7.63 ± 0.08), TDS (2.01 ± 0.13 mg L^{-1}), alkalinity (240 mmol L^{-1}) and total hardness (391.6 mg L^{-1}) throughout the experiment period.

2.2 | Bacterial strains and preparation

Bifidobacterium animalis PTCC-1631 and *B. lactis* PTCC-1736 were obtained from the Persian Type Culture Collection (Tehran-Iran). *Bifidobacterium* species were cultivated separately in MRS plate and incubated at 37 °C for 24–48 hrs. The cell free supernatants (CFS) for each bacterial species were obtained separately according to optimal density (OD) method, reading against Mc-Farland turbidity standard solution using Bio-chrome spectrophotometer (Libera-S22) at 625 nm wavelength (Dehghan 2012). In this procedure, Mc-Farland turbidity standard was used as reference to adjust turbidity of bacterial suspensions so that the number of bacteria will be within given range to standardize microbial testing. Three concentrations of 1×10^7 (T_1), 2×10^7 (T_2) and 3×10^7 (T_3) CFU g^{-1} dry feed of probiotics were employed with equal-proportional amounts.

2.3 | Preparation of diets

A commercial diet (BEYZA, Pars province- Iran) containing 50% crude protein, 14% crude fat, 9.7% crude ash and 4300 Kcal/kg digestible energy was supplemented with graded levels of probiotics (1×10^7, 2×10^7 and 3×10^7 CFU g^{-1} dry feed) to obtain 3 sets of experimental diets and the control (without probiotic). To prepare experimental diets bacterial colonies were scratched from the surface of plates and mixed with physiological solution (0.9%, w/v), to prepare an equal volume for all treatment sets and sprayed into the diet and mixed part by part. Feed with different concentrations of probiotic were prepared every week and kept in refrigerator and manually administered for four times daily, during 60 days of study. Alevins were fed according to 4–8% of body weight and daily record was kept of feed offered.

2.4 | Examination of growth performance

At the end of the experiment total alevins from each tank were captured for measurement of growth performance as per the following formulae.

Weight gain (WG) = final weight – initial weigh (Tacon 1990)

Specific growth rate for weight (SGR %) = (Ln final weight of fish – Ln initial weight of fish) ×100/days of feeding trial (Helland *et al.* 1996)

Metabolic growth rate = ((weight gain (g)) / [{$(W_{initial}/1000)^{0.8}$ + W_{final} / $1000)^{0.8}$}/2]/study duration (Dabrowski *et al.* 1986)

Feed conversion ratio (FCR) = food intake (g) / living weight gain (g) (Helland *et al.* 1996)

2.5 | Hematological parameters

Alevins were starved for 24 h, then five alevins from each tank were randomly sampled, thereby total of fifteen fish were taken from each treatment. For evaluation of blood parameters each sample of fish were analyzed separately and for biochemical parameters pooled samples of five fish were used for each replicate (three pooled sample for each treatment). Blood was drawn with an insulin siring from caudal vein of individual fish after anaesthetization with 100 mg L clove flower powder. Blood samples were got in heparinized (20 µl) vial of 1.5 ml capacity and following parameters were measured: Red (RBC) and White Blood Cells (WBC) were counted under Neubaur hemocytometer (Bullis 1993). Hemoglobin content was determined after cyanmethemoglobin method (Drobkin 1945). Hematocrit value was measured after Svetina *et al.* (2002). Following blood cell indices were assessed (Campbell and Ellia 2007):

Mean corpuscular volume (MCV) = (Hct×10)/RBC (fL)

Mean corpuscular hemoglobin (MCH) = (Hb×10)/RBC (pg)

Mean corpuscular hemoglobin concentration (MCHC) = (MCH/MCV) × 100 (g/dL)

2.6 | Blood serum biochemical changes

Blood samples which were collected without anticoagulant were incubated in an upright position at room temperature for 30 min to allow clotting. After incubation time samples were centrifuged for 15 min at 5000 rpm. The obtained serum were used to estimate the biochemical changes as follow: total protein (g/dL), albumin (g/dL), lipase (U/L), amylase (U/L), alkaline phosphatase (U/L), ALT and AST (U/L) were determined using Pars Azmoon Kit (Tehran-Iran) according to instruction of producer. Also cortisol content in serum samples were determined using Monobind ELA assay Kit (USA) according to instruction of producer.

2.7 | Statistical analyses

The differences in growth rates and parameters among the different treatments were calculated by employing one way ANOVA followed by Duncan's multiple range test by using SPSS (version 19).

3 | RESULTS

After 60 days, mean weight varied significantly among groups, T_1–T_3. The mean weight of each treatment was significantly higher than the control and T_3. The lowest FCR was observed in T_2 and T_1 respectively. The values of WG and SGR in all groups, fed with probiotic at all concentrations were significantly higher than those of the Control and T_3. However, mean values of WG, MGR and SGR were significantly different among treatments. These results showed that different concentrations of *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736 composed of equal concentration in each treatment and increased growth performance on rainbow trout alevins (Table 1).

TABLE 1 Growth response of alevins fed diets supplemented with *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736

Growth parameters	*Treatments*			
	Control	*T_1*	*T_2*	*T_3*
Mean initial weight (g)	0.57± 0.02	0.50 ± 0.01	0.52 ± 0.01	0.54 ± 0.01
Mean final weight (g)	20.98 ± 0.42^c	24.98 ± 0.62^a	23.06 ± 0.47^b	20.70 ± 0.47^c
Survival (%)	97.29 ± 0.52^c	99.58 ± 0.41^a	98.33 ± 0.96^b	98.95 ± 0.52^b
WG (g)	20.41 ± 0.42^c	24.48 ± 0.62^a	22.55 ± 0.47^b	20.15 ± 0.40^c
SGR (%)	5.95± 0.03^c	6.46 ± 0.04^a	6.29 ± 0.03^b	6.01 ± 0.03^c
FCR	1.20 ± 0.03^a	1.01 ± 0.02^b	1.08 ± 0.02^b	1.21 ± 0.02^a
Metabolic growth rate (g/kg/day)	14.06 ± 0.08	14.72 ± 0.09	14.44 ± 0.07	14.01 ± 0.07

Data are expressed as mean (± SE). Different means in the same row sharing the same superscript letter are not significantly different determined by Duncan's test ($P > 0.05$).

Table 2 represents hematological parameters and differential counting of red and white blood cell respectively. The highest RBC and WBC count within 60 days were observed in T_3 and T_2 respectively. There were no significant difference between experimental groups and the control in hemoglobin level and similarly MCV and MCH ($P > 0.05$).

Table 3 represents fish blood serum biochemical changes and differential determining of total protein, amylase and alkaline phosphatase respectively that found in rainbow trout supplemented various concentrations of *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736. The highest content of total protein and alkaline phosphatase level within 60 days were observed in T_1. No significant difference was observed in albumin content and ALT content between treatments ($P > 0.05$). Control group showed the highest content of cortisol.

4 | DISCUSSION

One of the main objectives of developing larval rearing strategies is the establishment of feeding regimen with probiotic supplementation that will resulted in optimal growth, survival and health. High growth performance of T_1 group fed diet containing the lowest concentration of *Bifidobacterium* in comparison with other exprimental groups might showed that intestinal layers is suitable for

given low concentration of *Bifidobacterium* to adheres and grow, in addition high concentration of probiotics necessarily did not cause better growth (Swain *et al.* 1996; Sahandi *et al.* 2012).

TABLE 2 Hematological profile of alevins fed diets supplemented with *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736

Parameters	Treatment			
	Control	T_1	T_2	T_3
Hematocrit (%)	41.33 ± 3.17^{a}	30.33 ± 3.84^{b}	22.66 ± 2.60^{b}	30.33 ± 1.45^{b}
Hemoglobin (g dL^{-1})	5.139 ± 0.21	6.145 ± 1.60	5.765 ± 1.66	5.581 ± 0.74^{NS}
RBC (10^6 cell mm3)	1.53 ± 0.07^{ab}	0.99± 0.31^{b}	0.90 ± 0.12^{c}	1.63 ± 0.12^{a}
WBC (10^3 cell µL)	34.61 ± 5.71^{c}	36.37 ± 4.17^{b}	59.10 ± 10.48^{a}	43.56 ± 4.703^{b}
MCV (fl)	268.35 ± 10.07	368.25 ± 120.68	254.71 ± 23.97	186.199 ± 6.02^{NS}
MCH (pg)	33.71 ± 2.85	65.74 ± 11.95	63.787 ± 16.46	34.091 ± 4.06^{NS}
MCHC (g dL^{-1})	12.65 ± 1.42^{c}	20.14 ± 4.38^{b}	24.51 ± 4.41^{a}	18.295 ± 1.97^{b}

Data are expressed as mean (± SE). Means in the same row sharing the same superscript letter and NS are not significantly different determined by Duncan's test (P > 0.05)

TABLE 3 Blood Serum biochemical changes of alevins fed diets supplemented with *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736

Parameters	Treatment			
	Control	T_1	T_2	T_3
Total protein (g/dL)	4.18 ± 0.24^{b}	6.60 ± 0.60^{a}	5.31 ± 0.54^{ab}	4.39 ± 0.40^{b}
Albumin (g/dL)	2.97 ± 0.28	3.60 ± 0.39	3.55 ± 0.45	3.14 ± 0.42^{NS}
Alkaline phosphatase (U/L)	678.75 ± 100.51^{ab}	838.12 ± 102.41^{a}	522.25 ± 25.91^{b}	508.87 ± 61.51^{b}
Amylase (U/L)	738.50 ± 76.20^{b}	913.12 ± 46.26^{a}	837 ± 49.89^{ab}	743 ± 26.99^{b}
AST (U/L)	22.62 ± 5.49^{a}	6.25 ± 0.83^{b}	18.75 ± 3.20^{a}	21.12 ± 4.18^{a}
ALT (U/L)	306.87 ± 79.65	271.87 ± 32.61	381.25 ± 28.95	261.12 ± 27.78^{NS}
Lipase (U/L)	14.87 ± 1.65^{c}	45.87 ± 3.14^{a}	29 ± 7.78^{b}	19.37 ± 1.46^{c}
Cortisol (µg/dL)	18.80 ± 0.22^{a}	5.05 ± 0.11^{b}	6.55 ± 0.08^{b}	5.62 ± 0.11^{b}

Data are expressed as mean (± SE). Means in the same row sharing the same superscript letter and NS are not significantly different determined by Duncan's test (P > 0.05)

Probiotics produce extra cellular enzymes which help the alevins in digestion (Jafaryan *et al.* 2007). So it can be suggested that administration of *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736 in feed of alevins resulted in enhancing of nutrient utilization and improving of growth including low FCR, high SGR and FCE. Gram-positive bacteria, secreted a wide range of exon-enzymes (Jafaryan *et al.* 2007), so better enzyme activities obtained with supplemented diet improved digestibility (Ramesh *et al.* 2015), and caused fast maturation of digestive system (Waché *et al.* 2006) which might in turn explain the better growth performances. In addition probiotics can break down proteins and carbohydrates (Farzanfar 2006) whereby it could improve absorption of broken nutrients. Therefore, it can be suggested that distribution of *Bifidobacterium* bacteria on rainbow trout alevins resulted in enhanced digestion of feed and improved growth performance (Gupta *et al.* 2014; Ramos *et al.* 2013).

The effect of probiotic on blood parameters have been studied in a number of studies (e.g. Rawling *et al.* 2009; Merrifield *et al.* 2010). In present study white blood cell count were increased in experimental groups in comparison with the control and this was an inevitable result because probiotics effect on immune system which might appear in white blood cell density (Panigrahi *et al.* 2005) and may increase phagocytosis and cytotoxic activity (Picchietti *et al.* 2007). The use of *Micrococcus luteus* on Nile tilapia resulted in increasing of RBC level (Kumat *et al.* 2008; Abdel-Rahman 2009; Nayak *et al.* 2010) and it's similar with our finding. Major hematological changes in experimental groups were observed in T_2 groups which showed statistically noticeable rise of total numbers of MCHC and WBC ($P < 0.05$). Total protein increased significantly in fish fed supplemented diet containing probiotics with the lowest concentration ($P < 0.05$). The present findings confirmed by those of Marzouk *et al.* (2008); Zhou *et al.* (2010) and Chelladurai *et al.* (2013) who reported that total protein level were significantly increased by using probiotics. The use of 1×10^7 CFU g^{-1} *Bifidobacterium* was increased the ALP content and it is probably related to increasing of nutrient absorption of alevins (Kumar *et al.* 2006; Marzouk *et al.* 2008). Also Panigrahi *et al.* (2010) reported that use of *Lactobacillus rhamnosus* significantly elevated ALP in rainbow trout after 30 days. The use of *Bifidobacterium* significantly increased the lipase and amylase and its might be occurred by the mucusal activity of inoculated probiotic bacteria. Balcazar *et al.* (2006) suggested that probiotics have beneficial effect on digestive operation because probiotic strains synthesize extracellular enzymes such as proteases, amylases and lipases (Ramesh *et al.* 2015). Therefore, nutrients are absorbed more efficiently when the feed was supplemented with probiotics (El-Haroun *et al.* 2006). Stress is one of the main causes affecting growth with cortisol being primary mediator for stress response in fish (Vijayan *et al.* 2003). Its means that any stress in fish would be ended to secretion of cortisol as its confirmed by Barton *et al.* (1985) before in rainbow trout and Coho. In present

study experimental treatments showed the lowest cortisol level in comparison with the control. Similar results were reported by Ramos *et al.* (2015) and Rollo *et al.* (2006) on sea bass alevins. Also this result was consistent with survival rate in different groups. Survival rate in experimental groups were significantly higher than the control and this finding was in agreement with Gatesoupe (1994), Robertson *et al.* (2000), and Nikoskelanin *et al.* (2001). The results of this study showed that dietary *B. animalis* PTTC-1631 and *B. lactis* PTTC-1736 of 1×10^7 would give rise to positive effects on hematological factors of the rainbow trout alevins, and improvement of growth performance and reduction in feed conversion ratio. Also the use of *Bifidobacterium* as new probiotic strains in aquaculture caused the decreasing of cortisol and increasing of rainbow trout survival rate in comparison with the control.

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JS primary data collection; **HJ** research supervision; **JS, MS & PE** manuscript preparation.