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Immunostimulatory and growth-promoting potential of poly-β-hydroxybutyrate in rainbow trout (*Oncorhynchus mykiss*) fingerlings culture

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Abstract

In the current study, the effect of dietary supplementation of poly-β-hydroxybutyrate (PHB) on growth performance, digestive enzymes activity, body composition, non-specific immune response and diseases resistance of rainbow trout fingerlings were investigated. Five hundred and fifty-two healthy fingerlings (8.2±1.0 g) were randomly distributed in 12 tanks (150 L) at a density of 46 fish/ tank and fed different levels of dietary PHB (0, 0.5, 1 and 2%) for 70 days. At the end of the experiment, the results showed that PHB affected growth performance and the fish fed 0.5 and 1% PHB diets had a significant higher weight gain and specific growth rate (SGR) than the control (p<0.05). The specific activity for total protease and amylase in 1% PHB was significantly higher than that of both the control and 2% PHB (p<0.05). In addition, PHB significantly affected the muscle fatty acids profile but did not change protein and lipid content. Also, our results indicated that 1 and 2% PHB markedly decreased cumulative mortality of the fingerlings challenged by *Yersinia ruckeri* infection. Overall, the results of this experiment revealed that PHB acts as a growth promoter and immunostimulator in rainbow trout fingerlings and the optimal PHB level in trout diet was estimated at 1% PHB.

Keywords: Poly-β-hydroxybutyrate, Growth promoter, Rainbow trout, Immune response, *Yersinia ruckeri*

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Introduction

The intensive culture of rainbow trout (*Oncorhynchus mykiss*) in Iran has greatly increased during the last decade, resulting in the occurrence of bacterial disease outbreaks in farms. Hitherto, the antibiotics were commonly used to treat many bacterial diseases. The ban on the use of antibiotics in animal production and the global demand for safe food has promoted researchers throughout the world to seek alternative biocontrol strategies (Defoirdt *et al.*, 2011). So far, various alternatives to antibiotics were applied in aquaculture. Amongst them is dietary supplements such as prebiotics that are degraded by animal gut flora thereby producing short chain fatty acids (SCFAs) (Ringø *et al.*, 2010). The SCFAs produced through metabolism of beneficial microorganisms alter the gut environment by promoting the growth of beneficial bacteria over pathogenic bacteria (Van Immerseel *et al.*, 2003; Defoirdt *et al.*, 2007). In general, the growth inhibitory effect is believed to be caused by the undissociated form of the acid which is able to penetrate the bacterial cell membrane. Once inside, the acid releases protons (H^+) in the neutral cytoplasm decreasing the intracellular pH (De Schryver *et al.*, 2009), forcing bacteria to redirect energy towards the efflux of the excess protons, thereby straining the cell metabolism leading to lower cell growth and even cell death (Kato *et al*., 1992).

Several studies addressed the effects of SCFAs on growth performance and health of aquatic species but the results are contradictory which seems to depend on the fish species and/or the type of organic acid tested (Lückstädt, 2006). The promoting effects of SCFAs for growth performance, nutrient digestibility and gut health have been reported in *Artemia franciscana* (Defoirdt *et al.*, 2006), Arctic charr (Ringo, 1991; Ringø *et al.*, 1994), rainbow trout (De Wet, 2005; Pandey and Satoh, 2008; Nazari et al., 2016), red hybrid tilapia (Ramli *et al.*, 2005; Ng *et al.*, 2009; Zhou *et al.*, 2009), red sea bream (Sarker *et al.*, 2005, 2007; Hossain *et al.*, 2007), African catfish (Owen *et al*., 2006), rohu (Baruah *et al.*, 2007a,b) and great sturgeon (Akrami et al., 2018). In contrast, Zhou *et al.* (2008) and Petkam *et al.* (2008) in red tilapia and Sarker *et al.* (2012 a,b) in juvenile yellowtail reported no significant improvement in the growth performance of organic acid/ salt blend was observed.

Polyhydroxybutyrate (PHB) is an important family member of polyhydroxyalkanoates (PHAs) and serves as an intracellular energy and carbon reserve for bacteria (Madison and Huisman, 1999; Tokiwa and Calabia, 2004). PHB is insoluble in water and can be degraded by bacteria and produce β-hydroxybutyric acid as a SCFA (Kato *et al.*, 1992; Patnaik, 2005). Previous studies have demonstrated that dietary PHB exerts a beneficial effect on the growth performance of sea bass juveniles (De Schryver *et al.*, 2009), giant freshwater prawn larvae (Nhan *et al*., 2010) and Siberian sturgeon fingerlings (Najdegerami *et al.*, 2011). In another

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report, PHB was shown to protect *Artemia* nauplii from the virulent *V. campbellii* strain (Defoirdt *et al.,* 2007; Halet *et al.,* 2007) and inhibit the growth of yeast and enterobacteria such as *Salmonella typhimurim*, *Escherichia coli* and *Shigella flexneri*. However, the dietary role of PHB on growth performance, digestive enzymes activity and diseases resistance in rainbow trout fingerlings is uncertain and there is a dearth of supporting information.

Therefore, this study was performed to evaluate the use of different levels of PHB on the growth performance, digestive enzymes activity, body composition, gut pH and fingerlings survival rate after a challenge test by *Yersinia ruckeri*.

Material and methods

Experimental diets

A basal diet was formulated for fingerlings using Kilka meal (Caspian Sea sprat) and soybean meal as protein sources and Kilka oil and soybean oil as lipid sources (Table 1). This basal diet served as the control diet and the experimental diets were prepared by supplementation of varying levels (0.5, 1 or 2%) of PHB (Goodfellow, England) to the basal diet formulation. The ingredients were blended thoroughly in a mixer and pelleted using a meat grinder. The pelleted diets were air-dried, ground and sieved to produce a suitable pellet (ca. 2.2 mm). The chemical composition of the formulated diets were determined according to standard AOAC methodology (AOAC, 2000).

Table 1: Dietary formulations (%) and proximate composition.

Diets	Control	0.5 % PHB	1% PHB	2% PHB
Ingredients (g kg^{-1})				
Fish meal	43	43	43	43
Soybean meal	26	26	26	26
Wheat flour	18.6	18.6	18.6	18.6
Fish oil	3	3	3	3
Soybean oil	3	3	3	3
Mineral mix [a]	1.5	1.5	1.5	1.5
Vitamin mix[b]	1.5	1.5	1.5	1.5
Antifungal	0.25	0.25	0.25	0.25
Cellulose	2	1.5	1	-
PHB[c]	-	0.5	1	2
Dicalcium Phosphate	1	1	1	1
Vitamin C	0.1	0.1	0.1	0.1
Proximate analysis (% dry matter basis)				
Crud protein (%)	48.25	47.6	47.8	48.7
Lipid (%)	18.8	18	17.6	18.1
Ash (%)	12.25	13.1	12.6	13.5
Energy (Kj g^{-1})	20.25	20.6	20.5	21.1

[a] Mineral premix U kg^{-1} of diet: manganese, 2600mg kg^{-1}; copper, 600mg kg-1;iron, 6000mg kg^{-1}; zinc, 600mg kg^{-1}; selenium, 50mg kg^{-1}; iodine, 100mg kg^{-1}; cobalt, 50mg kg^{-1}; choline chloride,100000mg kg^{-1}.

[b] Vitamin premix Ukg^{-1} of diet: vitamin A, 1200000 IU; vitamin D3, 400000IU; vitamin E, 3000IU; vitamin K3,1200mg; vitamin C, 5400mg; vitamin H2, 200mg; vitamin B1, 200mg; vitamin B3, 7200mg; vitamin B5, 9000mg; vitamin B6, 2400mg; vitamin B9, 600mg; vitamin B12, 4mg; antioxidant, 500mg.

[c] Poly-β- hydroxybutyrate (PHB) (Goodfellow, England)

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Experimental setup and animals

Healthy rainbow trout fingerlings were purchased from local propagation centers (Urmia, West Azerbaijan, Iran). The experiment was conducted at the Artemia and Aquaculture Research Institute, Urmia University, Iran, in September 2013. Prior to initiation of the experiment, fingerlings were acclimatized to the experimental conditions for 2 weeks. After the acclimation period, fish (initial weight, 8.2±1.0 g) were randomly distributed to four experimental groups each with three replicates in 150-L tanks supplied with flow-through water (6 L min^{-1}). The fish were stocked at the density of 46 fish per tank. Continuous aeration was provided to all the tanks from a compressed-air pump. Water temperature was maintained at around 16±1°C. Initially, each diet was fed to the fish three times a day with a 5-hour interval (first at 08:00) at 3 % of body weight under the normal light regime (light/dark: 12/12 h). From the second week, the fish were fed ad libitum. Syphoning of fecal matter was done on a daily basis. The feeding was carried out for 70 days.

Sampling and analysis

Weights of all fingerlings from each tank were determined at the beginning and at the end of the experiment, and the fingerlings survival was also determined by observing the individuals in each replicate. The SGR was calculated as follows: SGR (%)=[ln W– ln W0/t]×100, where W is the average weight after 70 days, W0 is the average initial weight (measured at the beginning of the experiment), and t is the experiment period (70 days). The same approach was used to calculate food conversion ratio (FCR), expressed as the given feed (g) divided by the weight increase of the fish (g) per treatment (Najdegerami *et al*., 2011).

Assay of digestive enzymes

Three fish from each replicate with a total of twelve fish from each treatment were randomly sampled, euthanized with clove powder (200 mg L^{-1}), and dissected to collect the whole digestive tract. They were homogenized in 100 mM Tris-HCl buffer with 0.1 mM EDTA and 0.1 % Triton X-100 at 9:1 ratio (pH 7.8) in an electric homogenizer (Heidolph, Instruments Switzerland) so as to prepare a 5% homogenate. All these processes were performed on ice. The homogenate was centrifuged at 25000 g for 20 min at 4 ˚C, the supernatant was collected, and then stored at -80°C for further analysis (Najdegerami, 2012).

Total alkaline protease activity was assayed at 25˚C using 1% (w/v) casein (Sigma, USA) as a substrate in 0.2 M phosphate buffer at pH 7.0 (Walter, 1984). Pepsin was measured at 37˚C using 2% hemoglobin in 0.06 N HCl as a substrate (Zambonino and Cahu, 1994). Tyrosin was used as a standard, and one unit of total alkaline protease activity and pepsin was defined as the amount of enzyme required for the formation of 1 mg of tyrosin per min. Amylase activity was determined according to Langlois *et al*. (1987), using 0.3% soluble starch as substrate dissolved in NaH2PO4 buffer (pH 7.4).

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Amylase activity (U) was defined as the mg of starch hydrolyzed during 30 min per ml homogenate at 37˚C. Lipase activity was measured for15 min at 30˚C using p-nitrophenol myristate as a substrate that is dissolved in 0.25 M Tris-HCl (pH 9.0). One unit of lipase activity (U ml^{-1}) was defined as the µmol of substrate hydrolyzed per minute in 30˚C per ml homogenate (Iijima *et al.,* 1998). Alkaline phosphatase activity was determined at 37˚C using 4- nitrophenyl phosphate (PNPP) as substrate dissolved in 30 mM NaHCO3 buffer (pH 9.8) (Bessey *et al.,* 1946). One unit of enzyme was defined of µmol hydrolyzed PNPP per min at 37 ˚C. Amino peptidase activity was measured according to Maroux *et al*. (1973) using 80 mM phosphate buffer, pH 7 and L-leucine p-nitroanilide as substrate dissolved in 0.1 mM DMSO. One unit of enzyme activity was defined 1 µmol hydrolyzed nitroanilide per min at 37 ˚C.

Total protein concentration in the homogenate was determined according to Bradford (1976) method using bovine serum albumin as standard. The specific activity of the measured enzymes was expressed as unit enzyme activity per mg protein (U mg^{-1} protein).

Proximate composition of the fish muscle

The proximate composition of the fingerlings muscle was analyzed at the end of the experiment following the standard methods. The moisture content of the muscle was determined by drying the samples at 105 ^{o}C to a constant weight. Nitrogen content was measured using Kjeldahl method (AOAC, 2000) and crude protein content was estimated by multiplying nitrogen percentage by 6.25. Ash content was estimated by incinerating the samples in a muffle furnace at 550 ^{o}C for 16h. Body tissues of the fingerlings at the end of the experiment were used for lipid extraction following the method of Folch *et al*. (1957), modified by Ways and Hanahan (1964). Fatty acid composition in muscle was determined by gas chromatography. FAME (fatty acid methyl ester) was prepared following a modified procedure by Lepage and Roy (1984).

Hindgut pH and fecal characterization

Fingerlings hindgut pH was determined according to the method proposed by Baruah *et al.* (2007 a). Briefly, three fish were randomly selected, euthanized as described above, dissected and then an incision was made in the hindgut to remove digesta. The pH of the hindgut was determined by mixing 5 g digesta with 50mL of deionized water for 1 min using a biotrode pH electrode (Hamilton, Switzerland). For determination of fecal ash content, the hindgut contents of three fingerlings in each replicate were mixed and then ash was measured as mentioned above (Najdegerami et al., 2011).

Assay for immunological parameters

At the end of the 70-day feeding trial, eight fingerlings from each treatment were euthanized with clove powder (200 mg L^{-1}), and blood samples were drawn from the caudal vein and allowed

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to clot at room temperature. Serum was separated by centrifugation at 1500×g for 20 min and stored at -20 ˚C for later analysis (Bakhshi *et al*., 2018).

Serum total antibody level

Serum total antibody level was assayed following the method proposed by Siwicki *et al.* (1994). After dilution of fingerlings serum with 0.85% sodium chloride (100 times), total protein was determined by the Bradford (1976) method. 100 µl of serum was mixed with an equal volume of 12% solution of polyethyleneglycol (Sigma) in a micro-tube. The solution was incubated at room temperature. After centrifuging at 5000 g at 4 °C, the supernatant was diluted 50 times with 0.85% of sodium chloride. The protein content was determined by the Bradford method and was then subtracted from the total protein level. The result was equal to the total immunoglobulin concentration of the serum that was expressed as mg ml^{-1} (Najdegerami, 2012).

Bacterial challenge

At the end of the experiment period, trout fingerlings from each replicate were distributed in twelve tanks (20 L) at a density of 20 fingerlings/tanks. Then, the fingerlings were challenged with an I.P. injection of 100 µl of a suspension of *Y. ruckeri* (LMG 3279) (1.1×10^{7} CFU ml^{-1}). Mortality was monitored every day for 2 weeks and dead fish were removed for microbiological tests to confirm the bacterial challenge.

Statistical analysis

All the data were subjected to one-way ANOVA using the statistical software program SPSS version 16.0 (SPSS Inc., IL, USA). Duncan's multiple range tests were used to determine the differences among treatment means at $p<0.05$ (Najdegerami *et al.*, 2011)

Results

Fish performance

The effects of diets containing different levels of PHB were investigated with respect to weight gain, SGR, FCR and survival of trout fingerlings (Fig. 1). The treatments with 0.5 and 1 % PHB resulted in the highest average values for weight gain and SGR which had significant difference with the control and 2% PHB ($p<0.05$). No significant difference was observed between 0.5% and 1% PHB ($p>0.05$). The FCR of fish fed with PHB were also not significantly different from that of fishes fed the control diet ($P > 0.05$).

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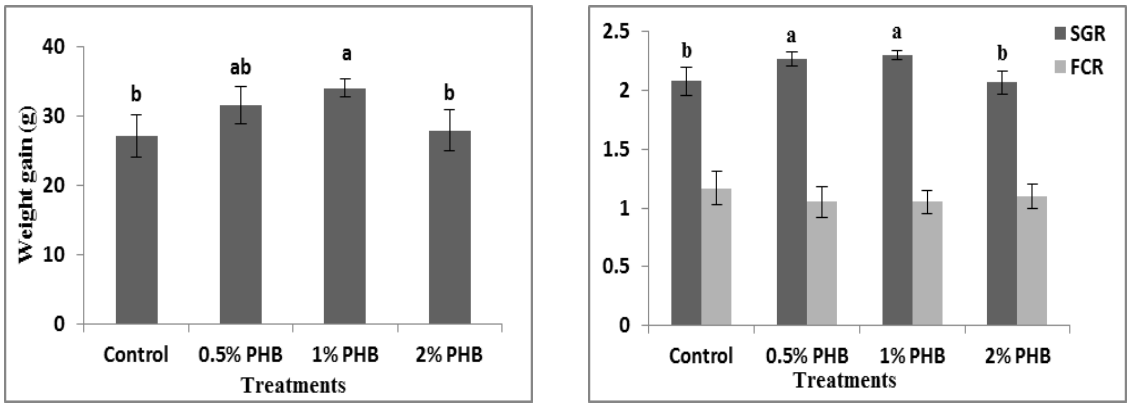

Figure 1: Weight gain (Left), FCR and SGR (Right) of rainbow trout fingerlings at the end of the experiment.

Digestive enzymes

After 70 days feeding with experimental diets, the results indicated that PHB changed the digestive enzymes activity in the fingerlings digestive tract (Table 2). The activity of the total alkaline protease in the fingerlings fed 0.5 and 1 % PHB treated diets were significantly higher than those fed 2% PHB (p<0.05). No significant difference was found between 2% PHB and the control (p>0.05). The highest value for pepsin activity was observed in 1% PHB which differed significantly from the others (p<0.05). Lipase specific activity responds to experimental diets and the 2% PHB significantly decreased lipase activity than the others (p<0.05). The fingerlings fed on 1% PHB treatment differed significantly with those fed other treatments with regard to amylase activity and the highest value was found in 1% PHB (p<0.05). Also, the activity of the alkaline phosphatase in the fingerlings fed 0.5% PHB (highest value) treated diet was significantly higher (p<0.05) than those fed 1%, control and 2% PHB diets. No significant difference was found between the control and 2% PHB (p>0.05).

Table 2: Specific enzyme activities in rainbow trout fingerlings fed experimental diets for 62 days.

	Control	0.5% PHB	1% PHB	2% PHB
Total alkaline protease	6.8 ± 0.09 ^{bc}	9.8 ± 0.3 ^{ab}	11.6 ± 1.0 ^{a}	4.5 ± 0.7 ^{c}
Pepsin	5.6 ± 0.7 ^{ab}	10.5 ± 4.8 ^{ab}	19.6 ± 4.7 ^{a}	8.0 ± 8.0 ^{b}
Lipase	1.6 ± 0.3 ^{ab}	2.4 ± 0.6 ^{a}	1.6 ± 0.4 ^{ab}	0.85 ± 0.0 ^{b}
Amylase	1.1 ± 0.2 ^{b}	0. 7 ± 0.3 ^{b}	5± 1.9 ^{a}	3.0 ± 0.4 ^{b}
Alkaline phosphatase	0.03 ± 0.00 ^{c}	0.13 ± 0.02 ^{a}	0.07 ± 0.00 ^{b}	0.03 ± 0.00 ^{c}

-Activities are expressed as follows: Total alkaline protease and pepsin activities as mmol of tyrosine released min^{-1} mg^{-1} protein; Amylase activity as mg starch hydrolyzed min^{-1} mg^{-1} protein; Lipase activity as mmol of substrate hydrolyzed min^{-1} mg^{-1} protein; Values are averages ± SEM, n = 4.
-Different letters within a row denote significant differences (p<0.05)

Proximate composition of the fish muscle

Dietary supplementation of PHB had no significant effect on the crude protein and lipid contents of the trout fingerlings muscle (p>0.05). However, the fish fed 2 % PHB had the highest ash percentage and showed significant

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difference with the control and 1 % PHB treatments (p<0.05) (Table 3).

Table 3: Muscle composition in rainbow trout fingerlings fed experimental diets.

	Control	0.5% PHB	1% PHB	2% PHB
Protein(%)	53.3 ± 1.7	53.1 ± 0.9	52.3 ± 1.6	53.7 ± 0.1
Lipid(%)	23.9 ± 1.7	24.7 ± 2.3	25.5 ± 2.0	23.2 ± 4.4
Ash(%)	8.8 ± 0.2 ^{bc}	8.9 ± 0.6 ^{ab}	7.9 ± 0.6 ^{c}	9.8 ± 0.8 ^{a}

Different letters within a row denote significant differences (p<0.05)

Muscle fatty acid composition

The fatty acid composition of the fingerlings muscle following the 70 day feeding period is presented in Table 4. The results indicated that using PHB in fish diets did not change the total saturated fatty acid content in fish muscle (p>0.05). Also, PHB changed the total monounsaturated fatty acid profile; the highest value was observed in the fish fed PHB supplemented diets (p<0.05). A significant reduction was found for linoleic acid (C18:2n6), eicosadienoic acid (C20:2n6) and total n6 in fish muscle that fed 1 and 2 % PHB treatments (p<0.05) over the experiment period. PHB did not affect the linolenic acid (C18:3n3), eicosapentaenoic acid (C20:5n3) and total n3 value in fingerlings muscle (p>0.05). However, it significantly decreased docosahexaenoic acid (C22:6n3) in fish fed 0.5 and 1 % PHB (p<0.05). The lowest values for total n3 in fish muscle were observed in 0.5 and 1 % PHB which differed significantly from the control and 2% PHB (p<0.05).

Table 4: The main groups of fatty acids in whole body of rainbow trout fingerlings fed experimental diets. Data are expressed as mg fatty acid g^{-1} lipid.

	Control	0.5% PHB	1% PHB	2% PHB
Total saturated	174 ± 7.2	172.9 ± 27.7	160.5 ± 22.5	164.8 ± 19.8
Total monounsaturated	335.3 ± 11.9 ^{b}	359.8 ± 7.0 ^{a}	359.8 ± 13^{a}	360 ± 5.7^{a}
C18 :2n6	195.9 ± 5.9 ^{a}	187.8 ± 1.8 ^{ab}	183.8 ± 4.0 ^{b}	181.3 ± 8.3 ^{b}
C20 :2n6	11.3 ± 0.7^{a}	11.3 ± 0.4 ^{a}	9.5 ± 0.7^{b}	9.9 ± 0.7 ^{b}
C20 :4n6	6 ± 1.3	6.3 ± 1.3	4.9 ± 0.5	5.9 ± 0.9
Total n6	213.2 ± 6.2 ^{a}	205.5 ± 1.9^{ab}	198.3 ± 4.3 ^{b}	197.3 ± 8.1^{b}
C18 :3n3	7.6 ± 0.4	7 ± 0.5	6.9 ± 0.5	6.8 ± 0.5
C20 :5n3 (EPA)	5 ± 0.5	4.6 ± 0.8	4.4 ± 0.6	5.3 ± 0.3
C22 :6n3 (DHA)	42.6 ± 4.0 ^{a}	35.4 ± 1.8^{b}	34 ± 4.6 ^{b}	42.3 ± 2.7^{a}
Total n3	55.3 ± 4.3 ^{a}	47.1 ± 1.7^{b}	45.4 ± 5.1 ^{b}	54.4 ± 3^{a}

Different letters within a row denote significant differences (p<0.05)

Hindgut pH and fecal characterization

The hindgut pH of the groups fed 1 and 2% PHB supplemented diets were significantly lower than that of those fed the control and 0.5% PHB diets (p<0.05) (Fig. 2). The ash content of fecal matter in the fish fed experimental diets was investigated. The results indicated that the fish fed 0.5 and 1% PHB supplemented diets had a significant higher ash percentage compared to those fed the control and 2% PHB (p<0.05). There was no significant difference between the control and 2% PHB (p>0.05).

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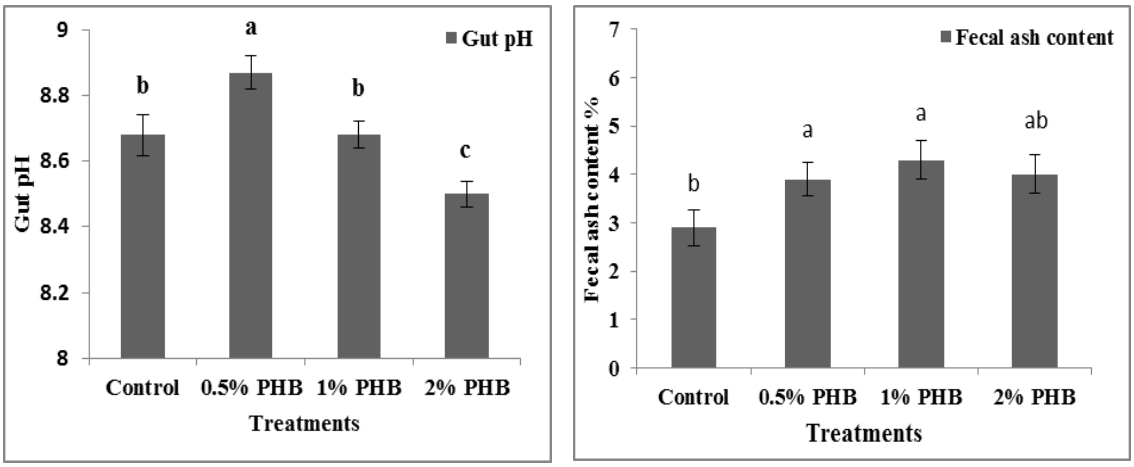

Figure 2: Hindgut pH (Left) and Fecal as content (Right) in rainbow trout fingerlings after a 70 day feeding trial with diets containing different levels of PHB.

Assay for immunological parameters

The effect of dietary supplementation of PHB on the total antibody level and bacterial challenge in trout fingerlings was investigated (Figs. 3, 4). The results indicated that PHB significantly increased total antibody level in fish serum and the highest values were found in the fish fed 1 and 2 % PHB (Fig. 3). Fig. 4 shows the cumulative mortality percentage of trout fingerlings challenged with virulent strain of *Y. ruckeri*. During 14 days of pathogen bacterial challenge, the results indicated that dietary supplementation of 1 and 2% PHB decreased the cumulative mortality rate. The cumulative mortality percentage of the fish in control and 0.5% PHB was higher.

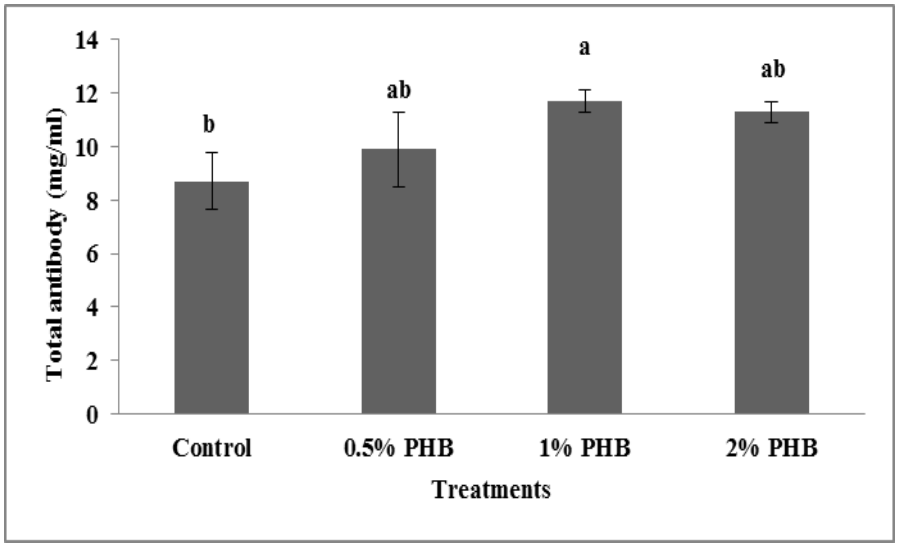

Figure 3: The total antibody level of rainbow trout fingerlings fed with PHB supplemented diets for a period of 70 days.

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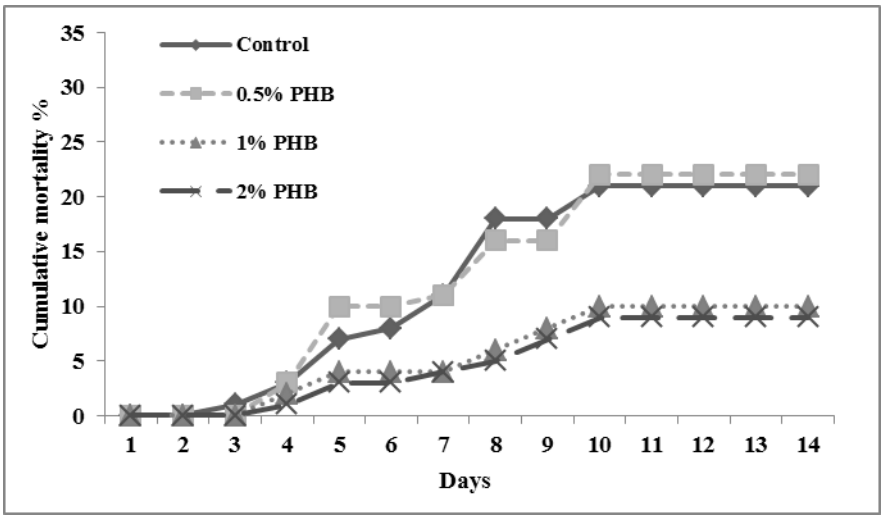

Figure 4: Cumulative mortality rate of rainbow trout fingerlings fed PHB supplemented diets after a virulent strain of *Yersinia ruckeri* challenge.

Discussion

In the present study, we investigated the effects of poly-β-hydroxybutyrate (PHB) supplementation as a microbial control agent on the growth performance, digestive enzymes activity, body composition, hindgut pH, fecal ash and immune response in rainbow trout fingerlings.

Previous studies indicated that PHB, as a bio-control compound, can be degraded by microbial extracellular enzymes to β-hydroxybutyric acid, becoming a carbon and energy source for the fish (Kato *et al.*, 1992; Patnaik, 2005; De Schryver *et al.*, 2009). The high survival rate of axenic *Artemia franciscana* and sea bass larvae fed only on PHB particles (in comparison with starvation) was observed by Defoirdt *et al.* (2007) and De Schryver *et al.* (2009) respectively, although both organisms might not be able to grow on this substrate alone. The results of the current study showed that there is a significant difference between fish fed on PHB supplemented diets and control in terms of final weight and SGR. These results are in agreement with other published data by De Schryver *et al.* (2009) in sea bass fingerlings, Nhan *et al*. (2010) in giant river prawn larvae. Sui *et al.* (2012), in Chinese mitten crab larvae, showed that PHB significantly increases weight gain in these species in different concentration in their diets. In contrast to our result, Najdegerami *et al.* (2013) found that PHB retards growth performance in Siberian and Persian sturgeon larvae (2013, 2015) indicating that growth promoting effects of PHB is species-specific and depends on the development stage.

Digestive enzymes profile is an index of nutritional condition and digestive processes of fish. Therefore, any manipulation of diets causes immediate changes in activities of digestive enzymes (Mohapatra *et al.*, 2011). The promoting effects of SCFAs on the digestive enzymes activity have been documented in several studies (Dibner and Buttin, 2002; Guilloteau *et al*., 2010 a,b). Among various digestive

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enzymes, pancreatic enzymes activity (e.g. amylase, trypsin and lipase) are commonly used as indexes of digestive system function and maturation (Shan *et al.,* 2008). Addition of pancreatic enzymes induces the decomposition of polyhydroxyalkanoates (PHAs) threefold (Defoirdt *et al.,* 2010). Therefore, this point might prove the promoting and beneficial effects of PHAs in digestive enzymes secretion and administration in the animal feed. In the present study, PHB improved total alkaline protease, pepsin, amylase and alkaline phosphatase activity in trout fingerlings fed 0.5 and 1 % PHB treatments, although no significant difference was observed in some enzymes. The latter results are in agreement with results by Xu *et al*. (2009), Guilloteau *et al*. (2010 a,b) who demonstrated that in Crucian carp (*Carassius auratus gibelio*) and young calves, SCFAs increase pancreatic secretion in the gastrointestinal tract, but the results regarding the effects of PHB on the other aquatic species digestive enzymes are contradictory. Najdegerami *et al*. (2015) reported that feeding Persian sturgeon larvae with *Artemia* nauplii enriched with PHB suppresses digestive enzymes activity. Also, PHB did not affect the activity of digestive enzymes in Siberian sturgeon fingerlings when the diets were supplemented with 2 and 5 % PHB. The main reason for this is obscure. However it seems that the effect of PHB on the digestive enzymes activity is related to gut microbial alteration by PHB which is demonstrated in several studies (De Schreyver *et al.,* 2010; Najdegerami *et al.,* 2011, 2013, and 2015).

There are several reports that prove SCFAs enhance body protein content in rainbow trout (Yilmaz *et al.*, 2007) and hybrid tilapia (*Oreochromis niloticus*×*O. aureus*) (Genc *et al.,* 2007). By contrast, PHB did not affect muscle protein content in Siberian sturgeon fingerlings (Najdegerami, 2012) which was consistent with the results obtained in this experiment. To the best of our knowledge, SCFAs concentration play an essential role in lipid metabolism in GI tract (Delzenne and Williams, 2002; Delzenne *et al.*, 2008) and stimulate a simultaneous reduction in the hepatic expression and activity of the lipogenic enzymes such as acetyl-CoA carboxylase, malic enzyme, ATP citrate lyase and fatty acid synthase (Aghelli *et al.,* 1998; Delzenne and Kok, 1999; Delzenne *et al.,* 2008). PHB did not change the muscle lipid content. However, a significant difference was found in fatty acid profile in fingerlings muscle which was consistent with what has been reported in Nile tilapia (EL-Haroun *et al*., 2006), rainbow trout (Bagheri *et al*., 2008) and all the ornamental fishes (Ghosh *et al.,* 2008).

As mentioned above, PHB can be degraded in the intestine and produce β-hydroxybutyric acid as a SCFA that could have similar beneficial effects as have been described for SCFAs (Defoirdt *et al.,* 2009). SCFAs reduce gut pH, thus inducing feed digestibility and nutrient absorption from the feed (Sui *et al.,* 2012). In our study, a significant pH reduction was observed

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in 2% PHB in fingerlings hindgut. It seems PHB is degraded to β-hydroxybutyric acid or other monomers and has a decreased pH. These results are consistent with those obtained in previous studies regarding the effects of PHB on the sea bass and Siberian sturgeon fingerlings hindgut pH by De Schryver *et al*. (2009) and Najdegerami *et al*. (2011), respectively.

In intensive fish culture, animals are faced with numerous opportunistic pathogens; a non-specific immune system such as immunoglobulin is considered to be the first line of defense against a broad spectrum of pathogens (Tukmechi *et al.,* 2011; Geraylou *et al.,* 2012). In our study, PHB affected the total immunoglobulin level in fish serum and significantly increased this parameter in fish fed 0.1 and 2% PHB. Also, the results showed that supplementation of the diets with PHB enhance the survival of fingerlings against *Y. ruckeri* compared to the control group. Results of numerous studies have shown that the SCFAs improve immune systems and support the gut health through decreasing epithelial permeability and modulating cytokines in the intestine (Van Nuenen *et al.,* 2005). Also, SCFAs alter the microbial community in the gut, which can produce variations in epithelial morphology and concentration of immunoglobulin in serum (Mc Cracken and Lorenz, 2001; Guarner and Malagelada, 2003). In agreement with our results, the positive effect of PHB on the immune response in brine shrimp (Defoirdt *et al.,* 2007), Chinese mitten crab (Sui *et al*., 2012), Siberian sturgeon (Najdegerami *et al.,* 2012), Asian tiger shrimp (Laranja *et al*., 2014) and Mozambique tilapia (Suguna *et al.,* 2014) were reported in several studies.

In conclusion, this study demonstrated the potential of PHB supplemented diets in rainbow trout fingerlings culture. PHB in 0.5 and 1% levels tends to improve growth performances which, in turn, are reflected in the high digestive enzymes activity and fish welfare. Also, the results revealed that the fish fed PHB treatments represent high immunoglobulin level and resistance to live virulent *Y. ruckeri*. However, further focused research is necessary to determine the mode of action of PHB containing diets and its effects on the gut microbial structure and histology.

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