Beneficial effects of a sodium butyrate source on growth performance, intestinal bacterial communities, digestive enzymes, immune responses and disease resistance in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The aim of the present study was to investigate the effects of dietary sodium butyrate on growth performance, intestinal microbiota, digestive enzymes, humoral immune responses and resistance against *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*) juveniles (39.5±1.12 g). The fish were fed diets supplemented with 0 (SB0), 1.5 (SB1.5), 2.5 (SB2.5) and 5 (SB5.0) g/kg sodium butyrate for 45 days and challenged with *A. hydrophila* thereafter. The results showed that 2.5 and 5 g/kg sodium butyrate significantly increased growth rate and feed efficiency, compared to the SB0 group. Red blood cell (SB2.5, SB5.0), white blood cell (SB1.5, SB2.5, SB5.0), hematocrit (SB2.5) and hemoglobin (SB2.5), plasma total protein (SB2.5), lysozyme (SB2.5), bactericidal activities (SB1.5, SB2.5, SB5.0), intestinal total bacteria (SB1.5, SB2.5, SB5.0), lactic acid bacteria (SB2.5, SB5.0), stomach pepsin activity (SB2.5), intestinal trypsin (SB1.5, SB2.5, SB5.0), chymotrypsin (SB2.5), protease (SB1.5, SB2.5, SB5.0), and amylase (SB2.5, SB5.0) activities significantly increased by dietary sodium butyrate inclusion. Post-challenge survival showed no significant difference among the treatments; however, mortality was numerically in the following order: SB5.0 (64.4±7.07%), SB2.5 (57.8±2.67%), (SB1.5 51.1±7.07%) and SB0 (44.4±7.07%). In conclusion, dietary sodium butyrate at 2.5-5 g/kg is recommended as a feed supplement for rainbow trout to augment the fish growth and disease resistance. Such beneficial effects of dietary sodium butyrate in rainbow trout might be due to the changes in intestinal microbial communities, which in turn improve nutrient availability and immune function.

Keywords: Blood, Health, Intestinal microbiota, Nutrition, Organic acid

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Introduction

Aquaculture industry is a rapid growing sector in human food production all-over the world. Rainbow trout (*Oncorhynchus mykiss*) is an important aquaculture species in the world with more than 160,000 tones annual production in Iran. The species has an important role in aquatic protein supply in Iran (Zorriehzahra *et al.*, 2018).

Fish farmers are willing to feed the fish with improved aquafeeds to guarantee maximum growth rate and boosted immune strength. Increased growth rate markedly suppresses the cost of fish production; thus, numerous studies have been conducted to find different feed additives for aquafeed supplementation. In this regard, amino acids (Hoseini *et al.*, 2019a; Hoseini *et al.*, 2020a; Hoseini *et al.*, 2020b), phytochemicals (Hoseini *et al.*, 2018a; Hoseini *et al.*, 2018b; Biabani Asrami *et al.*, 2019), prebiotics (Ringø *et al.*, 2010), and probiotics (Merrifield and Carnevali, 2014) were found effective.

Disease outbreaks threaten the aquaculture industry, leading to a huge economic loss. Besides, antibiotic therapy is a common way to treat bacterial diseases, which leads to the raise of antibiotic-resistance pathogens, environmental pollution, and fish health deterioration (Alderman and Hastings, 1998; Hoseini and Yousefi, 2019; Zargar *et al.*, 2020). Accordingly, researchers have been searching for new methods to boost fish health and control the diseases. Dietary additives, such as amino acids, herbal products, vitamins, nucleotides, prebiotics and probiotics were found beneficial in boosting the fish innate immune system and disease resistance (Lee *et al.*, 2015).

Intestinal microflora, among them lactic acid bacteria in particular, plays important roles in fish immune strength (Ringo *et al.*, 2018). Digestive tract folding and development markedly affect site availability for microbial communities. On the other hand, pH of digestive tract plays an important role in microbial community domination, as low pH favors the conditions for beneficial bacteria (Ringo *et al.*, 2018). Organic acids and their salts target the fish intestine and are important feed additives to augment fish growth performance and immune responses (Lee *et al.*, 2015). Butyrate is an organic acid salt and the main energy source for intestinal epithelium, which, stimulates growth and folding of the digestive tract (Robles *et al.*, 2013). This provides more adhesive sites for microbial communities. Besides, butyrate, similar to other organic acids (Castillo *et al.*, 2014), may reduce intestinal pH that favors the conditions for domination of beneficial bacteria (e.g. lactic acid bacteria). In this regard, a significant elevation was observed in the digestive enzymes' activity in butyrate-treated grass carp (*Ctenopharyngodon idella*), which might be due to the domination of beneficial bacteria population (Tian *et al.*, 2017). Similar results were reported by Aalamifar *et al.* (2020), when butyrate was added to the diet of Asian sea bass (*Lates calcarifer*). Studies on fish showed that the growth response to dietary butyrate administration was not

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constant among the studies, as some reported improved growth (Robles *et al.*, 2013; Ahmed and Sadek, 2014; Liu *et al.*, 2014; Tian *et al.*, 2017) and others not (Estensoro *et al.*, 2016; Ebrahimi *et al.*, 2017; Zhou *et al.*, 2019). There are no data about fish resistance to pathogenic bacteria, when orally administered by butyrate; nevertheless, it has been shown that butyrate improved various immune responses in fish (Tian *et al.*, 2017). According to the above, in this study, it was hypothesized if dietary butyrate administration improves rainbow trout growth performance, intestinal microbial communities, humoral immune responses, and resistance against *A. hydrophila*.

Materials and methods

Experimental design and feed preparation

Two hundred and forty trout, with average weight of 39.5±1.12 g, were randomly distributed in 12 tanks (500 L) and were fed with a control diet (SB0; Table 1) for 10 days to acclimatize to the conditions. The fish were fed based on 4% of body weight, in three meals per day. After the acclimation, the fish were randomly assigned to four triplicated treatments, fed diets supplemented with 0 (SB0), 1.5 (SB1.5), 2.5 (SB2.5) and 5 (SB5.0) g/kg sodium butyrate (Butirex® C4; Novation Co., Spain).

The feedstuffs were mixed and moisturized to give a dough. The dough was passed through a die to form sticks that were died overnight, crushed into appropriate size, and kept at 4°C until use. A portable apparatus (HI 14198; Hana Co.) was used to measure water quality parameters. Water temperature, dissolved oxygen and pH were were 17±1.2˚C, 6.6±0.3 mg/L and 7.0±0.1, respectively.

Table 1: Dietary formulations (g/kg as air dry basis) and proximate composition of basal diet ingredients.

Ingredients	g/kg
Fish meal[1]	455
Soybean meal	180
Corn starch	213
Soybean oil	48.8
Fish oil	23.1
Vitamin[2]	30
Mineral[3]	30
CMC[4]	20
Proximate composition	
Crude Protein	453.2
Crude Lipid	91.4
Ash	110.2
GE (MJ/kg)	20.71

[1] Kilka fish meal.

[2] Vitamin contains the following (kg^{-1} dry weight): vitamin A: 50.000 MIU; vitamin D3: 10 MIU; vitamin E: 130 g; vitamin K3: 10 g; vitamin B1: 10 g; vitamin B2: 25 g; vitamin B6: 16 g; vitamin B12: 100 mg; niacin: 200 g; pantothenic acid: 56 g; folic acid: 8 g; biotin: 500 mg; antioxidant: 0.2 g; anticake: 20 g.

[3] Mineral premix contains the following (kg^{-1} dry weight): calcium phosphate 397 g; calcium lactate 327 g; ferrous sulfate 25 g; magnesium sulfate 137 g; potassium chloride 50 g; sodium chloride 60 g; potassium iodide 150 mg; copper sulfate 780 mg; manganese oxide 800 mg; cobalt carbonate 100 mg; zinc oxide 1.5 g; sodium selenite 20 mg.

[4] Carboxyl methyl cellulose (sodium salt), binder.

Sampling

After 45 days rearing, nine fish were blood-sampled per treatment (three fish per replicate) after 24 h fasting (Hoseini *et al.*, 2014). The fish were anesthetized using clove powder 500 mg/L for sampling (Hoseini *et al.*, 2011). The blood samples were divided into two

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portions; one used for hematological study and the other for plasma separation and biochemical studies. After the blood sampling, the fish were killed by a sharp blow on the head and washed with 0.1% benzalkonium chloride. The fish stomach and intestine were dissected immediately after opening ventral side of the fish. The stomach was immediately frozen in liquid nitrogen and kept at -70°C for pepsin activity assay. The intestine (posterior part) was divided into two portions; one was immediately frozen in liquid nitrogen for digestive enzymes' activity assay and the other used for microbiological assessments.

Hematological parameters

Fresh blood samples were used for counting of the blood cells and measurement of hematocrit and hemoglobin. Red blood cell and white blood cell were counted under light microscopy using a Neubauer chamber. Differential WBC counting was conducting after preparation blood slides and Wright-Giemsa staining (Blaxhall, 1972). Blood hematocrit percentage was determined after 7 min centrifugation of capillary tubes. A commercial kit (ZistChem Co., Tehran Iran) was used to measure blood hemoglobin content, as previously reported (Hoseini and Ghelichpour, 2012).

Plasma analyses

Plasma total protein levels were determined using a commercial kit supplied by Pars Azmun (Tehran, Iran). Plasma lysozyme activity was determined based on turbidimetric approach using *Micrococcus luteus* as target (Ellis, 1990). The reaction medium was 50 mM phosphate buffer (pH 6.2). Complement activity was determined based on hemolytic activity of the plasma, using sheep RBC in veronal buffer (pH 7) containing magnesium ion as activator. Plasma bactericidal activity was determined using *A. hydrophila* as target, based on inhibition of CFU forming on nutrient agar plates as described previously (Hoseini *et al.*, 2019b). For this, the bacterial suspension were mixed with plasma sample and incubated, then cultured on nutrient agrer plate. CFU were counted and the results were expressed as percentage inhibition, compared to a control plate (not incubated with plasma). Hemagglutination titer was studied against *A. hydrophila* by determining the highest plasma dilution giving agglutination. One unit of hemagglutination titer was equal to log-2 of the highest plasma dilution inducing hemagglutination. For this, the plasma samples were serially diluted (2-32 times) and 50 µL of them were mixed with equal volume of the bacterium suspension (10^7 cell per mL). After 24-h incubation at room temperature, the highest dilution that produced agglutination was determined and expressed as $\log_2$ (Yarahmadi *et al.*, 2014).

Intestinal microbiota study

Fresh samples of the fish intestine were washed with physiological saline and

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homogenized well. The homogenates were diluted to 10^{-7} using sterile physiological saline. One hundred µL of the diluted samples were cultured to determine total viable bacterial count (plate count 125agar) and lactic acid bacteria. After 5 days incubation at 25°C, CFU were counted on each plate (Taheri Mirghaed *et al.*, 2018).

Digestive enzymes' activities

Alkaline phosphatase activity was determined using a commercial kit (Pars Azmun Co., Tehran, Iran) according to a previous study (Taheri Mirghaed *et al.*, 2018). Protease activity was conducted using casein as substrate and Folin's reagent as chromogenic agent. One unit of protease activity was defined by µM tyrosine released per min per mg protein (GarcÍA-CarreÑO and Haard, 1993).

Stomach and intestine samples were homogenized well in 0.2 M NaCl. After centrifugation, the supernatants were used for enzymatic activity determination. The stomach samples were used for pepsin activity determination using 2% hemoglobin solution (in 0.3 N HCl at pH=2.0) at 280 nm. One unit of pepsin activity was equivalent to µg tyrosine per mL in one min (Worthington, 1991). Activities of the other enzymes were determined in the intestine samples. Benzoyl-DLarginine-p-nitroanilide and Suc-Ala-Ala-Pro-Phe-p-nitroanilide were used as substrate for trypsin and chymotrypsin activity, respectively. Wavelength was 410 nm and activity of the enzymes were calculated according to follow (Erlanger *et al.*, 1961):

$$unit\ per\ mg\ protein = \frac{(Abs_{410}/min) \times 1000 \times ml\ of\ reaction\ mixture}{8800 \times mg\ protein\ in\ reaction\ mixture}$$

Amylase activity was measured using starch as substrate and dinitrosalicylic acid as reductive compound. Maltose was used to draw a standard graph. One unit of the enzyme activity was defined by µM maltose produced per min per mg protein (Worthington, 1991). Lipase activity was measured using n-nitrophenyl myristate as substrate at 405 nm. One unit of lipase activity was defined as 1 µmol of n-nitrophenol released per min (Iijima *et al.*, 1998).

Bacterial challenge

A. hydrophila was grown on tryptic soy broth (TSB, Difco Laboratories, Sparks, MD) for 24 h at 30°C. Then, the cells were harvested (centrifugation; 4000 *g* for 10 min at 4°C) and was washed by 0.9% (w/v) NaCl and set for a cell concentration with optical density of 0.132 at 600 nm (Yarahmadi *et al.*, 2014). After adjusting the cell concentration at 2×10^7 cells/mL, all fish were challenged by intraperitoneal injection. The injected suspension volume was 0.1 mL per fish and mortality was recorded for 14 days. During the challenge test the fish fed with control diet (SB 0.0).

Statistical analyses

Normality of the data was check by Shapiro-Wilk test; when normal

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distribution was not confirmed (intestine pepsin activity, plasma total protein, ACH50, hemagglutination and bactericidal activity), log-transformation was used before one-way ANOVA test. Duncan test was used to find significant differences (p<0.05) among the treatments. The data were analyzed in SPSS v.22 and expressed as mean±SEM.

Results

Growth performance in different treatments is presented in Table 2. Final weight of the Butriex-treated fish were significantly higher than the SB0 and the highest weight was related to SB2.5 treatment (p=0.008). Weight gain (p=0.018) and SGR (p=0.012) of the SB2.5 and SB5.0 treatments were significantly higher than those of the SB0 diet. FCR of the SB2.5 treatment was significantly (p=0.032) lower than the SB0 group.

Total autochthonous bacterial count significantly increased in the Butriex-treated fish compared to the SB0 group (p<0.001), and the SB2.5 and SB5.0 groups had the highest bacterial counts. There was no significant difference in lactic acid bacteria count between the SB0 and SB1.5 treatments, and the highest count was observed in the SB2.5 and SB5.0 treatments (p<0.001) (Fig. 1).

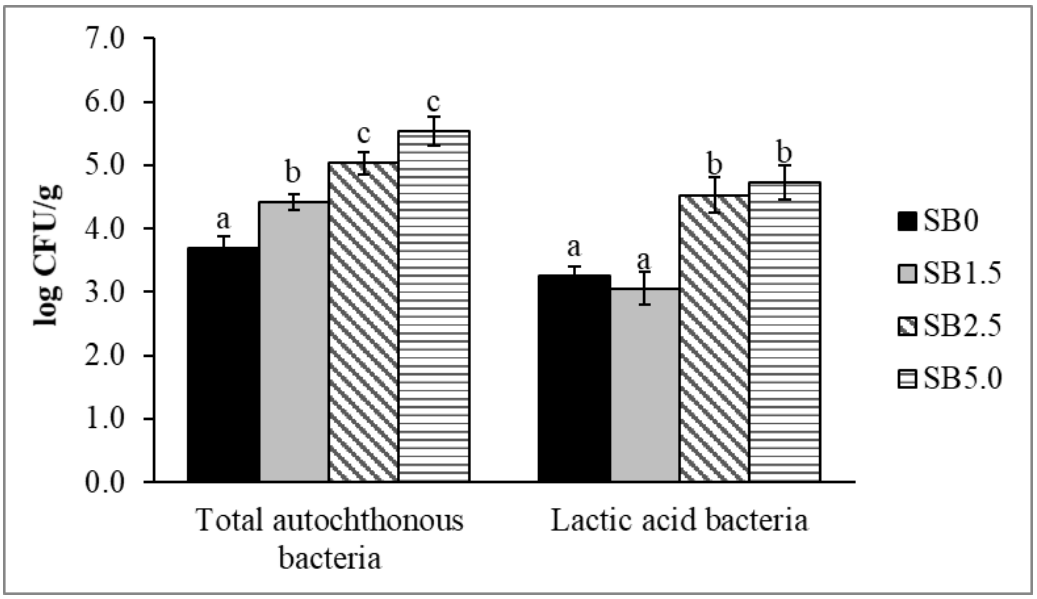

Figure 1: Intestinal bacterial counts in rainbow trout fed sodium butyrate -supplemented diets for 45 days. Different letters above the bars indicate significant difference among the treatments (n = 9; Duncan test). Values are presented as the mean ± SEM.

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Table 2: Growth performance in rainbow trout after 45 days feeding with the sodium butyrate -supplemented diets. Different letters within each column indicate significant difference among the treatments (n = 3; Duncan test). Values are presented as the mean ± SEM.

	SB0	SB1.5	SB2.5	SB5.0	P-value
Initial weight (g)	39.5 ± 0.21	39.9 ± 1.43	39.2 ± 0.86	39.2 ± 0.64	0.952
Final weight (g)	114 ± 1.88a	121 ± 1.91b	129 ± 2.29c	127 ± 2.18bc	0.008
WG (%)	190 ± 3.62a	204 ± 6.75ab	231 ± 12.3b	220 ± 8.05b	0.018
SGR (%/d)	2.53 ± 0.03a	2.65 ± 0.05ab	2.85 ± 0.09b	2.77 ± 0.06b	0.012
FCR	1.14 ± 0.03b	1.06 ± 0.03ab	0.95 ± 0.05a	1.03 ± 0.02ab	0.039

Pepsin (p=0.021) and chymotrypsin (p=0.025) activities of the SB2.5 treatment were significantly higher than the other treatments; there was no significant difference in activity of the enzymes among the SB0, SB1.5 and SB5.0 treatments. The Butirex-treated fish had significantly higher trypsin (p<0.001) and protease (p<0.001) activities compared to the SB0 group.

The highest activity of the enzymes was observed in the SB2.5 and SB5.0 treatments. The highest amylase activity was observed in the SB2.5 and SB5.0 treatments (p=0.039) and there was no significant difference in the enzyme's activity between the SB0 and SB1.5 treatments. Alkaline phosphatase and lipase showed no significant difference among the treatments (Fig. 2).

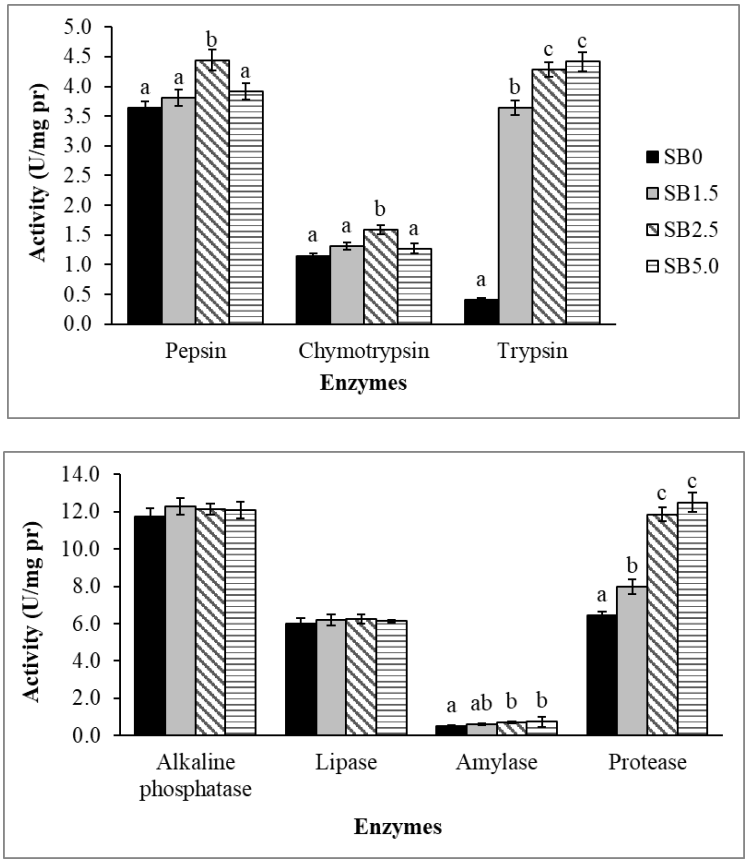

Figure 2: Activities of digestive enzymes in rainbow trout fed sodium butyrate -supplemented diets for 45 days. Different letters above the bars indicate significant difference among the treatments (n = 9; Duncan test). Values are presented as the mean ± SEM.

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Hematological data are presented in Table 3. The SB2.5 and SB5.0 treatments had significantly (p=0.011) higher blood RBC compared to the SB0 group. Blood hematocrit (p=0.012) and hemoglobin (p=0.006) in the SB2.5 treatment was significantly higher than the SB0 group. All the Butirex-treated fish had significantly higher blood WBC (p<0.001), compared to the SB0 group and the SB2.5 treatment had significantly higher lymphocyte (p=0.046) compared to the other treatments. There was no significant difference in blood neutrophil and monocyte among the treatments.

Table 3: Blood hematological parameters in rainbow trout fed sodium butyrate -supplemented diets for 45 days. Different letters within each row indicate significant difference among the treatments (n = 9; Duncan test). Values are presented as the mean ± SEM.

	SB0	SB1.5	SB2.5	SB5.0	P-value
RBC (million/mm^3)	1.5 ± 0.05a	1.6 ± 0.04ab	1.8 ± 0.07c	1.7 ± 0.04bc	0.011
Hematocrit (%)	38.9 ± 1.24a	40.5 ± 1.70a	45.4 ± 0.98b	41.6± 1.03ab	0.012
Hemoglobin (mg/dl)	10.4 ± 0.23a	11.1 ± 0.24ab	11.8 ± 0.31b	11.1 ±0.23ab	0.006
WBC (thousand/mm^3)	32.4 ± 1.63a	46.2 ± 1.86b	47.8 ± 1.20b	46.1 ± 1.03b	<0.001
Lymphocyte (%)	62.3 ± 1.11a	62.2 ± 1.53a	66.5 ± 0.99b	61.7 ± 1.30a	0.046
Monocyte (%)	1.3 ± 0.04	1.3 ± 0.06	1.5 ± 0.07	1.4 ± 0.11	0.175
Neutrophil (%)	30.7 ± 1.14	31.0 ± 1.06	35.2 ± 1.22	32.0 ± 1.31	0.056

The SB2.5 treatment had significantly (p=0.026) higher plasma total protein level compared to the SB0 treatment. The highest plasma lysozyme activity (p<0.001) was observed in the SB2.5 treatment; the SB1.5 treatment had significantly higher lysozyme activity compared to the SB0 treatment; and SB5.0 had the lowest value. All the Butirex-treated fish had significantly (p<0.001) higher plasma bactericidal activities compared to the SB0 treatment and the highest activity was observed in the SB5.0 treatment. There was no significant difference in plasma ACH50 activity and hemagglutination titer among the treatments (Table 4).

Table 4: Plasma immunological parameters in rainbow trout fed sodium butyrate -supplemented diets for 45 days. Different letters within each row indicate significant difference among the treatments (n = 9; Duncan test). Values are presented as the mean ± SEM.

	SB0	SB1.5	SB2.5	SB5.0	*P*-value
Total protein (g/dl)	3.13 ± 0.10a	3.34 ± 0.06ab	3.42 ± 0.06b	3.10 ± 0.11a	0.026
Lysozyme (U/ml)	414 ± 8.52b	457 ± 7.81c	498 ± 12.61d	352 ± 9.26a	<0.001
Bactericidal activity (%)	38.7 ± 2.53a	44.2 ± 1.87b	49.7 ± 1.36bc	51.8 ± 1.85c	<0.001
ACH50 (U/ml)	12.05 ± 0.42	11.58± 1.28	12.81 ± 0.46	10.53 ± 0.50	0.428
Hemagglutination titer (unit)	1.56 ± 0.24	1.77 ± 0.28	2.11 ± 0.26	2.22 ± 0.28	0.284

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Post-challenge mortality started two days after challenge in the SB0 group and six days after challenge in the SB2.5 treatment. Fourteen days after challenge, survival in the SB0, SB1.5, SB2.5 and SB5.0 treatments were 44.4±7.07, 51.1±7.07, 57.8±2.67 and 64.4±7.07 %, respectively, but the differences were not statistically significant (Fig. 3).

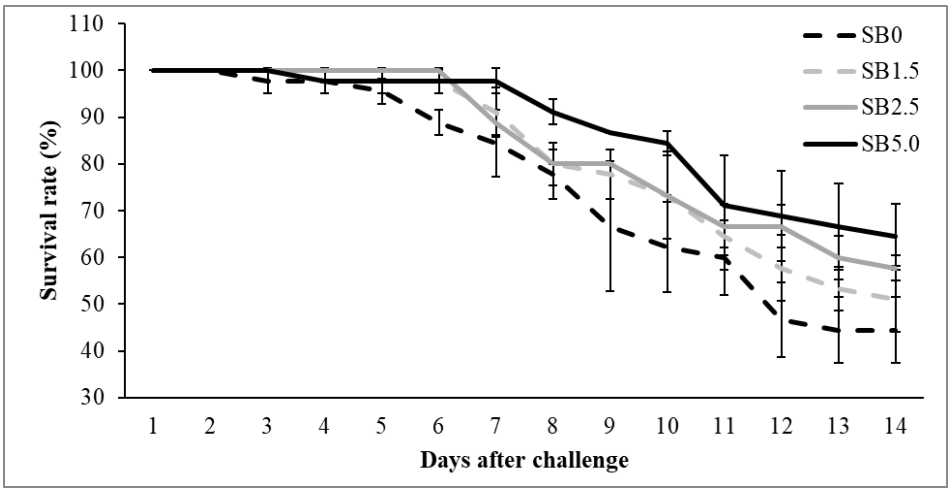

Figure 3: Survival rate of rainbow trout fed sodium butyrate -supplemented diets for 45 days and challenged with *Aeromonas hydrophila* (n = 3).

Discussion

There are inconsistencies in the growth responses of fish to butyrate administration among different studies. While Robles *et al.* (2013), Ahmed and Sadek (2014), Liu *et al.* (2014), and Tian *et al.* (2017) reported improve in growth performance of gilthead sea bream (*Sparus aurata*), Nile tilapia (*Oreochromis niloticus*) and grass carp Estensoro *et al.* (2016), Ebrahimi *et al.* (2017) and Zhou *et al.* (2019) found no significant effects of dietary butyrate supplementation on growth performance of the same species. Butyrate growth promoting effects in fish might be due to providing more amino acids, vitamins and nucleotides available to host (Robles *et al.*, 2013) and/or improved intestinal health and digestive enzymes' activities leading to a better nutrient digestion and absorption (Castillo *et al.*, 2014; Tian *et al.*, 2017), as discussed below.

Butyrate is the main energy source for fish intestinal epithelium leading to growth of intestinal tissue and increase in the tissue folding (Liu *et al.*, 2014). On the other hand, butyrate improve intestine health conditions by augmenting antioxidant capacity of the cells (Ebrahimi *et al.*, 2017); this may improve the cells response to harmful bacteria, providing a suitable medium for beneficial bacteria such as lactic acid bacteria (Rombout *et al.*, 2011). Establishing of lactic acid bacteria in the fish gut leads to lower pH and further reduction in harmful bacteria population (Lee *et al.*, 2015). In the present study, sodium butyrate administration led to increased total bacterial count and lactic acid bacteria count in trout intestine. Data on sodium butyrate /butyrate

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effects on fish bacterial community are scarce. Liu *et al.* (2014) found no significant effects of sustained-release microencapsulated butyrate on intestinal microflora in common carp (*Cyprinus carpio*). Tian *et al.* (2017) found increase in lactic acid bacteria in grass carp intestine, when administered with microencapsulated sodium butyrate. Such differences might be due to the type of butyrate administered and species difference. Moreover, Zhou *et al.* (2019) showed that type of dietary butyrate supplements significantly affects its effects in fish.

Sodium butyrate administration significantly increased activity of digestive enzymes. Increase in lactic acid bacteria population in aquatic animals' intestine has been reported to increase digestive enzyme activities (Nedaei *et al.*, 2019). Thus, the increase in the fish microflora might be a reason for the increase in activity of the enzymes. Similarly, Tian *et al.* (2017) showed that dietary butyrate supplementation led to increase in digestive enzymes' activities along with increase in intestinal lactic acid bacteria count in grass carp. Castillo *et al.* (2014) reported increases in digestive enzymes' activities due to dietary supplementation with different organic acids in red drum (*Sciaenops ocellatus*). Increased pepsin activity in the fish treated by organic acids might be due to lower pH in the animal stomach, leading to accelerated conversion of pepsinogen to pepsin (Castillo *et al.*, 2014). However, it is not clear how organic acids increase activities of digestive enzymes in the fish intestine. One theory might be improved intestinal health in the fish treated by organic acids. As mentioned above, organic acids have antioxidant effects (Ebrahimi *et al.*, 2017) and augment beneficial bacteria population in fish intestine (Tian *et al.*, 2017), which lead to boosted intestine health; healthier intestine might have better function and enzyme production.

In the present study, sodium butyrate supplementation (2.5 g/kg) significantly increased RBC, hemoglobin and hematocrit. Increased blood RBC might be due to antioxidant effects of butyrate (Ebrahimi *et al.*, 2017), because it has been reported that pro-oxidants attack RBC membrane and cause hemolysis and improved fish health was accompanied by increase in blood RBC (Taheri Mirghaed *et al.*, 2017; Ghelichpour *et al.*, 2021). WBC are important in both innate and adaptive immune responses (Lee *et al.*, 2015), thus increase in their number might help fish to have better responses to pathogens. In the present study, blood WBC and lymphocyte count increased by sodium butyrate treatment, which means boosted immune system. Plasma proteins are important in fish immune function (Hoseini *et al.*, 2016) and increase in plasma total protein is in line with previous study on Nile tilapia fed with butyrate-supplemented diet (Ahmed and Sadek, 2014). Lysozyme is an important enzyme with bactericidal activity and increase in this enzyme activity helps fish to have stronger responses to pathogens and butyrate was reported to augment lysozyme activity in

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grass carp (Tian *et al.*, 2017). Bactericidal activity is a suitable indicator of fish strength against pathogens. In the present study, plasma bactericidal activity significantly increased along with increase in dietary sodium butyrate levels, and this was accompanied by numerical increase in survival against *A. hydrophila*. There is no study about the effects of butyrate on fish resistance against bacterial challenge to make comparison. However, increase in plasma bactericidal activity and survival against bacterial challenge might be due to the effects of butyrate on intestinal microbiota. It has been previously reported that increase in lactic acid bacteria in fish intestine resulted in higher immune responses and disease resistance (Ringo *et al.*, 2018). On the other hand, butyrate administration was found to augment availability of some immunostimulant compounds such as nucleotide, tryptophan, arginine and glutamine in fish intestine (Robles *et al.*, 2013), which might be due to the changes in the intestinal microbial communities.

Conclusion

Dietary sodium butyrate at 2.5 and 5 g/kg (SB 2.5 and SB 5.0) is recommended for dietary inclusion to augment rainbow trout growth performance and disease resistance. Such effects seen to be mediated by change in intestinal microbiota, which increases digestive enzymes' activity and availability of nutrient for fish growth. On the other hand, such a change in microbial community might provide higher amounts of micronutrients, such as nucleotides and amino acids, which are known as strong immunostimulants.

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References

Aalamifar, H., Soltanian, S., Vazirzadeh, A., Akhlaghi, M., Morshedi, V., Gholamhosseini, A. and Torfi Mozanzadeh, M., 2020. Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). *Aquaculture Nutrition* (**26**), 156-164.

Ahmed, H., and Sadek, K., 2014. Impact of dietary supplementation of sodium butyrate and/or protexin on the growth performance, some blood parameters, and immune response of *Oreochromis niloticus*. *International journal of agriculture innovations and research* (**3)**, 985-991.

Alderman, D., and Hastings, T., 1998. Antibiotic use in aquaculture: development of antibiotic resistance–potential for consumer health risks. *International journal of food science & technology* (**33**), 139-155.

Biabani Asrami, M., Sudagar, M., Shahraki, N. and Vahdat, S., 2019. Effect of extracted phycocyanin from Spirulina platensison growth parameters, colorations, digestive enzymes and body chemical

[DOI: 10.18331/SFS2022.8.3.1]

compositions of Guppy fish (*Poecilia reticulata*). *Survey in Fisheries Sciences* (**6**), 1-8.

Blaxhall, P.C., 1972. The haematological assessment of the health of freshwater fish. *Journal of Fish Biology* (**4)**, 593-604.

Castillo, S., Rosales, M., Pohlenz, C. and Gatlin III, D.M., 2014. Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. *Aquaculture (Amsterdam, Netherlands)* (**433**), 6-12.

Ebrahimi, M., Daeman, N.H., Chong, C.M., Karami, A., Kumar, V., Hoseinifar, S.H. and Romano, N., 2017. Comparing the effects of different dietary organic acids on the growth, intestinal short-chain fatty acids, and liver histopathology of red hybrid tilapia (*Oreochromis* sp.) and potential use of these as preservatives. *Fish Physiology and Biochemistry* (**43**), 1195-1207.

Ellis, A.E., 1990. Lysozyme assays. pp. 101-103 in J.S. Stolen, ed. Techniques in fish immunology. SOS publication, Fair Haven.

Erlanger, B.F., Kokowsky, N. and Cohen, W., 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics* (**95**), 271-278.

Estensoro, I., Ballester-Lozano, G., Benedito-Palos, L., Grammes, F., Martos-Sitcha, J.A., Mydland, L.-T., Calduch-Giner, J.A., Fuentes, J., Karalazos, V. and Ortiz, Á., 2016. Dietary butyrate helps to restore the intestinal status of a marine teleost (*Sparus aurata*) fed extreme diets low in fish meal and fish oil. *PloS One* (**11**), e0166564.

GarcÍA-CarreÑO, F.L., and Haard, N.F., 1993. Characterization of proteinase classes in langostilla (*Pleuroncodes planipes*) and crayfish (*Pacifastacus astacus*) extracts. *Journal of Food Biochemistry* (**17**), 97-113.

Ghelichpour, M., Mirghaed, A.T., Dawood, M.A., Hoseinifar, S.H. and Van Doan, H., 2021. Alteration of haematological and antioxidant parameters in common carp (*Cyprinus carpio*) fed olive (*Olea europea*) leaf extract after exposure to Danitol®. *Aquaculture Research* (**52**), 1088-1095.

Hoseini, S.M., Hosseini, S. and Nodeh, A., 2011. Serum biochemical characteristics of Beluga, *Huso huso* (L.), in response to blood sampling after clove powder solution exposure. *Fish Physiology and Biochemistry* (**37**), 567-572.

Hoseini, S.M., and Ghelichpour, M., 2012. Efficacy of clove solution on blood sampling and hematological study in Beluga, *Huso huso* (L.). *Fish Physiology and Biochemistry* (**38**), 493-498.

Hoseini, S.M., Yousefi, M., Rajabiesterabadi, H. and Paktinat, M., 2014. Effect of short-term (0–72 h) fasting on serum biochemical characteristics in rainbow trout *Oncorhynchus mykiss*. *Journal of applied ichthyology* (**30**), 569-573.

[DOI: 10.18331/SFS2022.8.3.1]

Hoseini, S.M., Rajabiesterabadi, H. and Kordrostami, S., 2016. Chronic exposure of *Rutilus rutilus caspicus* fingerlings to ambient copper: Effects on food intake, growth performance, biochemistry and stress resistance. *Toxicology and Industrial Health* (**32**), 375-383.

Hoseini, S.M., Hoseinifar, S.H. and Doan, H.V., 2018a. Effect of dietary eucalyptol on stress markers, enzyme activities and immune indicators in serum and hematological characteristics of common carp (*Cyprinus carpio*) exposed to toxic concentration of ambient copper. *Aquaculture Research* (**49**), 3045-3054.

Hoseini, S.M., Mirghaed, A.T., Iri, Y. and Ghelichpour, M., 2018b. Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture (Amsterdam, Netherlands)* (**495**), 766-772.

Hoseini, S.M., and Yousefi, M., 2019. Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition* (**25**), 298-309.

Hoseini, S.M., Pérez-Jiménez, A., Costas, B., Azeredo, R. and Gesto, M., 2019a. Physiological roles of tryptophan in teleosts: current knowledge and perspectives for future studies. *Reviews in Aquaculture* (**11**), 3-24.

Hoseini, S.M., Rajabiesterabadi, H., Khalili, M., Yousefi, M., Hoseinifar, S.H. and Van Doan, H., 2019b. Antioxidant and immune responses of common carp (*Cyprinus carpio*) anesthetized by cineole: Effects of anesthetic concentration. *Aquaculture (Amsterdam, Netherlands)*, 734680.

Hoseini, S.M., Khan, M., Yousefi, M. and Costas, B., 2020a. Roles of arginine in fish nutrition and health: Insights for future researches. *Reviews in Aquaculture* (**12**), 2091-2108.

Hoseini, S.M., Taheri Mirghaed, A., Ghelichpour, M., Pagheh, E., Iri, Y. and Abdolvahhab, K., 2020b. Effects of dietary tryptophan supplementation and stocking density on growth performance and stress responses in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture (Amsterdam, Netherlands)* (**519**), 734908.

Iijima, N., Tanaka, S. and Ota, Y., 1998. Purification and characterization of bile salt-activated lipase from the hepatopancreas of red sea bream, *Pagrus major*. *Fish Physiology and Biochemistry* (**18**), 59-69.

Lee, C.S., Lim, C. and Webster, C.D., 2015. Dietary nutrients, additives, and fish health. Wiley-Blackwell, NJ, USA.

Liu, W., Yang, Y., Zhang, J., Gatlin, D.M., Ringø, E. and Zhou, Z., 2014. Effects of dietary microencapsulated

[DOI: 10.18331/SFS2022.8.3.1]

sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidised oil. *British Journal of Nutrition* (**112**), 15-29.

Merrifield, D.L., and Carnevali, O., 2014. Probiotic modulation of the gut microbiota of fish. *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics*, 185-222.

Nedaei, S., Noori, A., Valipour, A., Khanipour, A.A. and Hoseinifar, S.H., 2019. Effects of dietary galactooligosaccharide enriched commercial prebiotic on growth performance, innate immune response, stress resistance, intestinal microbiota and digestive enzyme activity in Narrow clawed crayfish (*Astacus leptodactylus* Eschscholtz, 1823). *Aquaculture (Amsterdam, Netherlands)* (**499**), 80-89.

Ringø, E., Olsen, R., Gifstad, T., Dalmo, R., Amlund, H., HEMRE, G.I. and Bakke, A., 2010. Prebiotics in aquaculture: a review. *Aquaculture Nutrition* (**16**), 117-136.

Ringø, E., Hossein, S., Ghosh, K., Doan, H.V., Beck, B.R. and Song, S., 2018. Lactic acid bacteria in finfish–an update. *Frontiers in microbiology* (**9**), 1818.

Robles, R., Lozano, A., Sevilla, A., Márquez, L., Nuez-Ortin, W. and Moyano, F., 2013. Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*). *Fish Physiology and Biochemistry* (**39**), 1567-1580.

Rombout, J.H., Abelli, L., Picchietti, S., Scapigliati, G. and Kiron, V., 2011. Teleost intestinal immunology. *Fish & Shellfish Immunology* (**31**), 616-626.

Taheri Mirghaed, A., Ghelichpour, M., Hoseini, S.M. and Amini, K., 2017. Hemolysis interference in measuring fish plasma biochemical indicators. *Fish Physiology and Biochemistry* (**34**), 1143-1151.

Taheri Mirghaed, A., Yarahmadi, P., Hosseinifar, S.H., Tahmasebi, D., Gheisvandi, N. and Ghaedi, A., 2018. The effects singular or combined administration of fermentable fiber and probiotic on mucosal immune parameters, digestive enzyme activity, gut microbiota and growth performance of Caspian white fish (*Rutilus frisii kutum*) fingerlings. *Fish & Shellfish Immunology* (**77**), 194-199.

Tian, L., Zhou, X.Q., Jiang, W.D., Liu, Y., Wu, P., Jiang, J., Kuang, S.Y., Tang, L., Tang, W.N. and Zhang, Y.A., 2017. Sodium butyrate improved intestinal immune function associated with NF-κB and p38MAPK signalling pathways in young grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology* (**66**), 548-563.

Worthington, C., 1991. Worthington enzyme manual related biochemical. *Freehold. New Jersey* (**34**), 212-215.

Yarahmadi, P., Farahmand, H., Miandare, H.K., Mirvaghefi, A. and Hoseinifar, S.H., 2014. The

[DOI: 10.18331/SFS2022.8.3.1]

effects of dietary Immunogen® on innate immune response, immune related genes expression and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* (**37**), 209-214.

Zargar, A., Taheri Mirghaed, A., Mirzargar, S.S., Ghelichpour, M., Yousefi, M. and Hoseini, S.M., 2020. Dietary ginger administration attenuates oxidative stress and immunosuppression caused by oxytetracycline in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research* (**51**), 4215-4224.

Zhou, J.S., Guo, P., Yu, H.B., Ji, H., Lai, Z.W. and Chen, Y.A. 2019. Growth performance, lipid metabolism, and health status of grass carp (*Ctenopharyngodon idella*) fed three different forms of sodium butyrate. *Fish Physiology and Biochemistry* (**45**), 287-298.

Zorriehzahra, M., Mohd Daud, H., Sharifpour, I., Soltani, M., Mehrabi, M., Kies, F. and Sheikh Asadi, M., 2018. Histopathology survey of rainbow trout (*Oncorhynchus mykiss*) fry mortality syndrome in coldwater hatcheries and reared farms in Iran. *Survey in Fisheries Sciences* (**4**), 58-72.

[DOI: 10.18331/SFS2022.8.3.1]