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Dietary sodium butyrate (Butirex® C4) supplementation modulates intestinal transcriptomic responses and augments disease resistance of rainbow trout (*Oncorhynchus mykiss*)

Ali Taheri Mirghaed[a,*], Peyman Yarahmadi[a], Mehdi Soltani[a], Hamed Paknejad[b], Seyyed Morteza Hoseini[c]

[a] Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[b] Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
[c] Inland Waters Aquatics Resources Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research, Education and Extension Organization, Gorgan, Iran

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ABSTRACT

Intestine in fish is a complex multifunctional organ, not only plays roles in digestion and absorption of nutrient, but also has critical role in immunity. The present study evaluated the effects of different levels of dietary sodium butyrate [Butirex® C4 (Butirex)] on intestinal immune-,antioxidant-and tight junction-related gene expression injuvenile rainbow trout(*Oncorhynchusmykiss*). 240 healthy rainbow trout were dispensed in 12 fiberglass tanks appointed to four treatments [0 (control), 1.5 (B1.5), 2.5 (B2.5) and 5 (B5)g Butirex per kg diet]. After a 45-day feeding trial, the fish fed with the Butirex-supplemented diets showed higher intestinal lysozyme (LYZ), complement(ACH50) and bactericidal activities; the elevations in ACH50 and bactericidal activities depended on Butirex levels ($P < 0.05$). The Butirex-supplemented groups, particularly the B2.5 group, had significantly higher LYZ gene expression compared to the control group ($P < 0.05$). Butirex at 2.5 and 5 g/kg levels led to significantly higher IL-1β gene expression. B2.5 and B5 had significantly lower and higher TNF-α gene expression compared to the control group ($P < 0.05$). The B2.5 group had significantly higher TGF-B, and significantly lower IL-8 compared to the control group ($P < 0.05$). The B1.5 and B2.5 group had significantly higher IL-10 gene expression compared to the control group ($P < 0.05$). The B2.5 and B5 groups had significantly higher SOD gene expression compared to the other groups; the highest expression was related to the B2.5 group ($P < 0.05$). Dietary Butirex supplementation significantly up-regulated CAT and GPx genes expression compared to the control group; the highest expression as related to the B2.5 and B5 groups ($P < 0.05$). The B2.5 group had significantly lower CLD12 gene expression compared to the control group ($P < 0.05$). The B2.5 and B5 groups had significantly higher CLD3, OCLD and ZO-1 gene expression compared to the control. The highest CLD3, ZO-1 gene expressions was related to the B2.5, and B5 groups respectively ($P < 0.05$). After challenge with *Streptococcus iniae*, B2.5 and B5 had significantly higher survival compared to the control group (55.6 ± 7.70 and 68.9 ± 10.2 vs. 33.3 ± 6.67). In conclusion, Butirex is efficient immune stimulant and health booster in rainbow trout, which augments the fish resistance to disease. Modulation of immune components, cytokines, antioxidant system and intestinal integrity might involve in improving disease resistance in Butirex-treated fish. Although most of the examined genes were modulated by 2.5 g/kg Butirex under normal conditions, 5 g/kg level is recommended under pathogenic state to mitigate mortality.

1. Introduction

Most of the aquaculture production in Iran is allocated to rainbow trout (*Oncorhynchus mykiss*), so it is one of the most economically important fish species [1]. In intensive and modern culture systems, fish may face different stressors such as crowding, handling and bad water quality that could negatively affect the fish health status [2]. Different stresses subsequently decrease growth performance and efficiency, increases its susceptibility to outbreak of infectious diseases and finally reduces profitability [3–6]. In aquaculture, the common way to treat

* Corresponding author.
E-mail address: mirghaed@ut.ac.ir (A.T. Mirghaed).

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diseased fish is use of antibiotic and chemical drugs. The administration of chemical drugs and antibiotics treats fish health [7] and suppresses immune functions [8].Moreover, application ofantibiotics results in food and environmental pollutions and development of antibiotic resistant pathogen [9,10]. Besides, antibiotics cause immune suppression, oxidative stress and tissue damage in fish [11]. Therefore, finding organic materials with health- and immune-boosting potentials is key factor to prevent outbreak of disease and reduce use of antibiotics in aquaculture [12].

Innate immune system in fishes is first line of defense in preservation of fish against infectious diseases [10].The innate immune responses in fish are divided into physical barriers, cellular and humoral components [13,14]. The intestine of fishes is a complex multifunctional organ, not only plays role in digestion and absorption of nutrients, but also has critical role in immune responses [15,16]. The physicalbarrier ofintestine controls uptake nutritionals material across the mucosa and serves as the front-line defense to prevent pathogens entering to the body [15,17]. Tight junction proteins and epithelial cells are also important to maintain integrity and function of intestinal physical barrier for optimum intestinal health [17,18]. Gut associated lymphoid tissue (GALT) contains numerous immune cell types that are involved in both innate and adaptive immune responses and immune activity control of the intestine [19]. Lysozyme (muramidase, EC 3.2.1.17) is a lytic enzyme that hydrolyzes glycosidic bonds and its level or activity has been commonly measured as an important innate immune parameter [33]. Cytokines are key regulators of the immune system [20] and expressions of different cytokine genes are important indicators of intestinal health statues [16]. Previous study demonstrated that deficiency or excess of some nutritional factors affect cytokine genes expression in intestine of some species [16,20,21]. Lipid peroxidation and protein oxidation may result in excessive reactive oxygen species (ROS) production in the intestine. ROS can affect the epithelial cells and eliminate the integrity of the intestinal tissue.ROS negatively can affect epithelial and integrity of intestine.The parameters of the antioxidant system in the intestinal tract are important factors in controlling intestinal health, because oxidative damage causes disturbances in the tight junction proteins through the reduction of expression ZO-1 and Claudine genes in intestine [22]. Therefore, the analysis of antioxidant enzymes such as SOD, CAT, GPx and GR in fish, may use as indicators of fish health.

It is now widely accepted that nutritional approaches are acceptable strategy to stimulate immune system and improve health to prevent disease among farmed aquatic animals [23]. Different studies have demonstrated that feed additives such as probiotic, prebiotic and non-digestible fibers potentiate to improve intestine tissue integrity and immune responses through modulation of gastrointestinal microbiota [27,31]. Short chain fatty acids (SCFAs)are alternatives for antibiotics and other chemical drugs in aquaculture because the organic acids have multiple effects on aquatic animals [24]. SCFAs have 1 to 6 carbon molecules (C1–C6) and weak carboxylic acids with one or more carboxyl groups in their structure [25]. Several studies have reported positive effects of different organic acids such as benzoic, formic, lactic and propionic acids on reducing the pH and microbial population of thefood and feed ingredients, so commercial mixtures of organic acids can be used as storage preservative for food and feed [26].

Butyric acid is formed by microbial fermentation of non-digestible carbohydrates in gastrointestinal tract of animals. Among SCFAs, butyrate has received particular attention for its multiple beneficial effects on intestinal tract and peripheral tissues [27]. Based on our knowledge some previous studies reported the positive effect of butyrate on growth performance, feed efficiency, antioxidant factors and immunological responses in different aquatic animal species [18,28–30]. However, few studies have clarified the effects of butyrate on the fish intestine [31,32].

Despite the mentioned studies that report the effects of dietary butyrate supplementation on growth performance, physiological and immunological responses indifferent species, there is limited information about effect of butyrate on regulation of intestinal immune related genes in rainbow trout. Therefore, the aim of the present study was to investigate the effects of different levels of sodium butyrate [Butirex® C4 (Butirex)] on immune, antioxidant defense and tight junction related genes expression in intestine of rainbow trout.

2. Materials and methods

2.1. Experimental design and feed preparation

Two hundred and forty rainbow trout (34.97 ± 1.87 g) were obtained from a private farm (Tehran, Iran), transferred tothe department of aquatic animal health and disease, University of Tehran and were acclimated with the experimental conditions for 10 days. During the acclimation period, the fish were fed control diet (Faradaneh Co.) at rate of 4% of body weight, thrice a day. Thereafter, the fishwere randomly assigned to each of 12 fiberglass tanks (500 L); triplicate tanks per treatment. Four levels [0 (control), 1.5 (B1.5), 2.5 (B2.5) and 5 (B5) g/kg) of Butirex® C4 (Butirex: commercial sodium butyrate; Novation Co; Spain) wereused for preparation of the experimental diets. Butirex contained 54% sodium butyrate protected by a physical and chemical matrix of buffer salts.

The ingredients and proximate composition of the experimental diets are shownin Table 1. During the experiment (45 days), the fish were hand-fed at rate of 4% of BW,thrice a day (08:00, 12:00 and 17:00). The amount of feed for each tank was calculated based on feeding ration and biomass of each tank that was corrected every 2 weeks following a 24-h starvation period and batch weighing.

Table 1
Dietary formulations (g kg1 dry matter) and proximate composition of basal diet Ingredients.

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

[a] Danish fish meal.
[b] 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.
[c] 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.
[d] Carboxyl methyl cellulose (sodium salt), binder.

2.2. Water quality parameters

Water temperature, dissolved oxygen and pH were monitored daily using portable multiparameter (HI 14198; Hana Co) and maintained at 17 ± 1.2 °C, 6.6 ± 0.3 mg L^{-1} and 7.0 ± 0.1, respectively. Continuous aeration was provided to each tank through an air stone connected to a central air compressor.

2.3. Sampling

Three specimens per tank were randomly sampled at the end of experiment. The fish were starved 24 h before sampling and then anesthetized using clove powder (200 ppm, 20 min) for tissue sampling. Animal study protocol was approved by The Animal Ethic Committee based on International Laws [33]. The intestine samples were collected immediately under sterile condition, transferred to liquid nitrogen, and stored at −80 °C for biochemical analysis and RNA extraction.

2.4. Intestinal biochemical and immunological analysis

The intestinal samples were homogenized in 10 vol (w v^{-1}) of ice-cold physiological saline and centrifuged at 6000 g for 20 min at4°C, and then the collected supernatant was stored at −80 °C for the analysis of related parameters as described by Pérez-Jiménez, Cardenete [34].

2.5. Intestinal alternative complement activity

The alternative complement activity was determined based on protocol that described by Ortono et al. [35] using sheep red blood cells (SRBC). The volume of extract yielding 50% hemolysis was determined and used to calculate the complement activity of the samples (value of ACH50 is in units per ml).

2.6. Intestinal lysozyme activity

Intestinal lysozyme activity was measured after Demers and Bayne [29] based on turbidmetric assay and lysis of the lysozyme-sensitive Gram-positive bacterium *Micrococcus luteus* (Sigma).

2.7. Intestinal bactericidal activity

The intestinal bactericidal activity was determined according to the method previously described by Ahmadi, Farahmand [36].

2.8. Total RNA extraction and cDNA synthesis

At the end of the experiment 50–100 mg of intestine samples were collectedfrom three fish per replicate (i.e. 9 per treatment) and samples were immediately stored in liquid nitrogen and then transferred to −80 C until analysis. The totalRNA extraction was performed from 100 mg of all homogenized body tissue using 1 ml Biozol Rigent (Bio flux; Chaina) based on company's protocol that previously described by Yarahmadi et al., [37].

The quantity and quality of RNA were evaluated by spectrophotometer at 260/280 nm and 1.5% agarose, respectively. The synthesis of the first-strand cDNAs were carried out using GenetBio®cDNA synthesis kit (Korea) following the manufacture instruction. RT-PCR reactions were run in triplicate and using a standard protocol and especial primers (see Table 2).

2.9. Challenge test

Frozen stock-culture of strain *Streptococcus iniae* isolated from diseased Rainbow trout that identified by Phenotyping tests, was grown in tryptic soy broth (TSB, Difco Laboratories, Sparks, MD) for 24 h at 30 °C harvested by centrifugation at 4000*g* for 10 min at 4 °C, and was washed three times in 0.9% (w/v) saline and was prepared a suspension in 0.9% (w/v) saline to achieve an absorbance of 0.132 at 600 nm (0.5 McFarland Standard) [28]. The resultant suspension adjusted to 2×10^7 cells ml^{-1}. All fish in each tank were challenged by intraperitoneal injection with 10 mL of *S. iniae* culture containing 2 *10^7 CFU/ml. During the challenge period, fish were continually fed their respective diets. Fish mortality was monitored twice a day for 14 days. Total cumulative mortalities were accounted and data presented as survival rate and finally relative percent survival (RPS) values were calculated as follows.

$$RPS = 100 - [(test\ mortality/control\ mortality) * 100]$$

Table 2
Primers sequences for the study of selected immune, oxidative and tight junction related genes expression in rainbow trout.

qPCR primers	Accession Number	Gen name
LYZ	X59491.1	ACAGCCGCTACTGGTGTGACG
		GCTGCTGCCGCACATAGAC
IL-1β	AJ278242	ACAGACATGGATTTTGAGTCA
		CTCATACTGTGATGTACTGCTGA
IL-10	NM_001246350.1	CGACTTTAAATCTCCCATCGAC
		GCATTGGACGATCTCTTTCTTC
TGF-β	X99303.1	AGATAAATCGGAGAGTTGCTGTG
		CCTGCTCCACCTTGTGTTGT
TNF-α	NM_001124374.1	CAAGAGTTTGAACCTCATTCAG
		TGGCAACGATGCAGGACGGAA
CAT	BE669040	GAGGGCAACTGGGACCTTACT
		GGACGAAGGACGGGAACAG
SOD	NM_001160614.1	TGGTCCTGTGAAGCTGATTG
		TTGTCAGCTCCTGCAGTCAC
GPx	BG934453	GATTCGTTCCAAACTTCCTGCTA
		GCTCCCAGAACAGCCTGTTG
CLD3	BK007964.1	CTTCATCGGCAGCAACATCG
		ATGGCCAGGAGGATGGAGAT
CLD12	BK007967.1	TCTCCCCACACTTCCTAACTC
		GTAGCCCTGGGAGGAGG
OCLD	NM_001190446.1	CAGCCCAGTTCCTCCAGTAG
		GCTCATCCAGCTCTCTGTCC
ZO-1	HQ656020.1	CTGGCCCAGAAGAAGAAGGA
		CGTTGTAGAGGGTGTCCACT
Beta actin	NM_001124235.1	ATGGGCCAGAAAGACAGCTACGTG
		CTTCTCCATGTCGTCCCAGTTG

2.10. Statistical analysis

β-actin gene was used as the housekeeping gene to normalize the expression of the target genes. The iQ5 optical system software (Bio-Rad) and ΔΔCt method was used for data analysis. All values were expressed as means ± SD. Differences in normalized mRNA levels.

β-actin gene was used as the housekeeping gene to normalize the expression of lysozyme (LYZ), TNF-α, IL-1β and IL-8, IL-10, TGF-β, SOD, CAT, GPx, cluadin-3 (CLD3), cluadin-12 (CLD12), ocludin(OCLD) and ZO-1 target genes and immune parameters of rainbow trout in different treatments were analyzed using one-way analysis of variance [38] followed by Duncan's test. Differences were considered statistically significant at $P < 0.05$. SPSS software (version 23.0) was applied for statistical analysis.

3. Results

3.1. Intestinal innate immune responses

The effects of dietary Butirex on innate immune parameters in the intestine of juvenile rainbow trout are presented in Table 3. Dietary Butirex supplementation led to significantly ($P < 0.05$) higher LYZ, ACH50 and bactericidal activities compared to the control. The highest LYZ activity was related to the B2.5 and B5 groups; whereas, ACH50 and bactericidal activities increased along with the dietary Butirex

Table 3
Intestinal innate immune responses of rainbow trout fed 0 (control), 1.5, 2.5 and 5 g/kg Butirex for 45 days. Values in a row with different superscripts denote significant difference ($P < 0.05$). Values are presented as the mean ± SE.

	Dietary Butirex levels (g/kg)			
	Control	1.5	2.5	5
Lysozyme (U/ml)	168.44 ± 2.22^{a}	193.67 ± 2.12^{b}	218.11 ± 2.36^{c}	221.33 ± 2.04^{c}
ACH50(U/ml)	3.17 ± 0.20^{a}	4.28 ± 018^{b}	8.97 ± 0.26^{c}	11.19 ± 0.29^{d}
Bactericidal activity (% control)	29.33 ± 0.88^{a}	34.66 ± 1.33^{b}	49.11 ± 2.1^{c}	56.11 ± 1.53^{d}

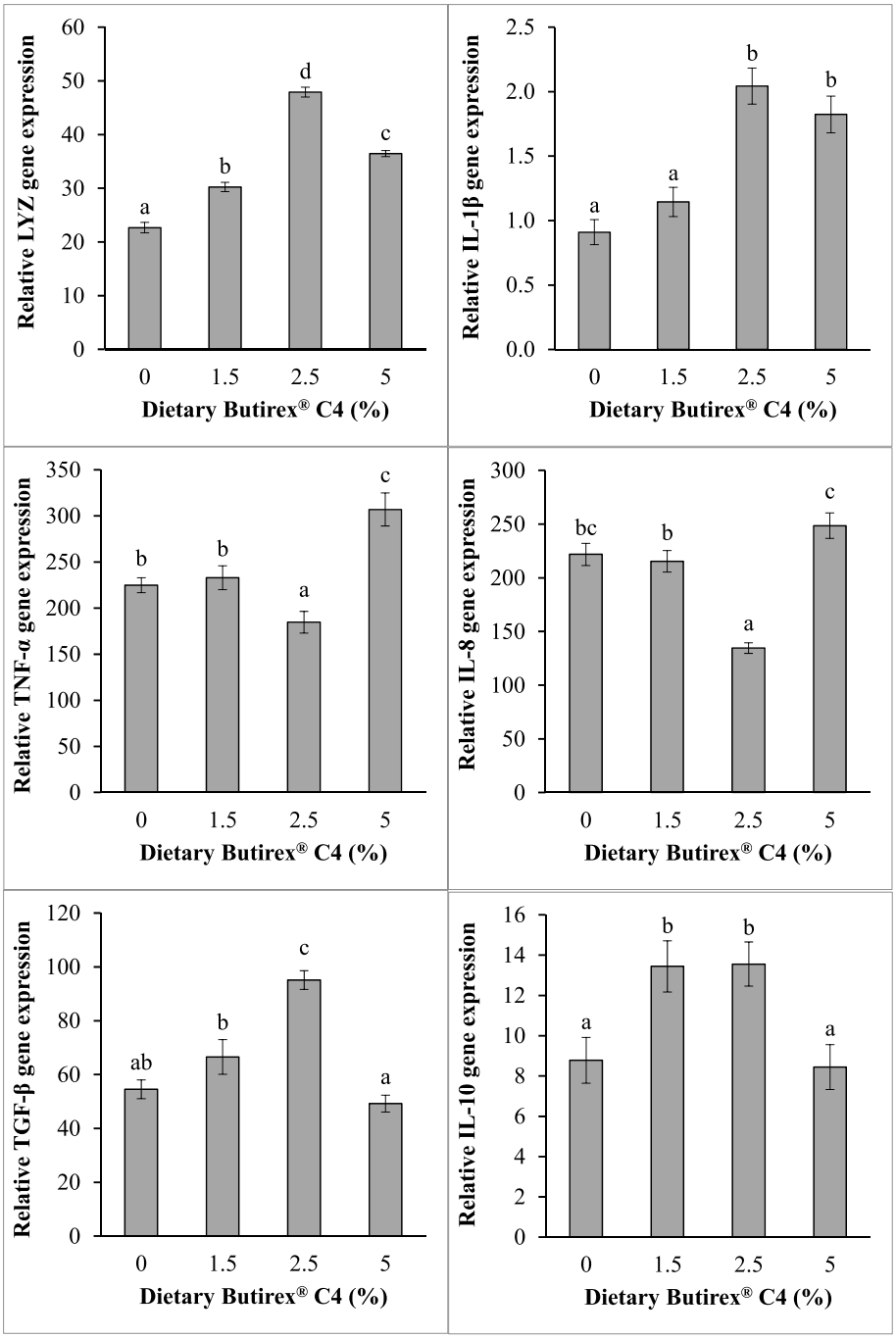

Fig. 1. Relative expression of LYZ, IL-1β, TNF-α, IL- 8, TGF-β and IL-10 genes in intestine of rainbow trout fed different levels ofButirex. Data are expressed as mean ± SE. Error bars with different superscripts denote show significant difference at $P < 0.05$.

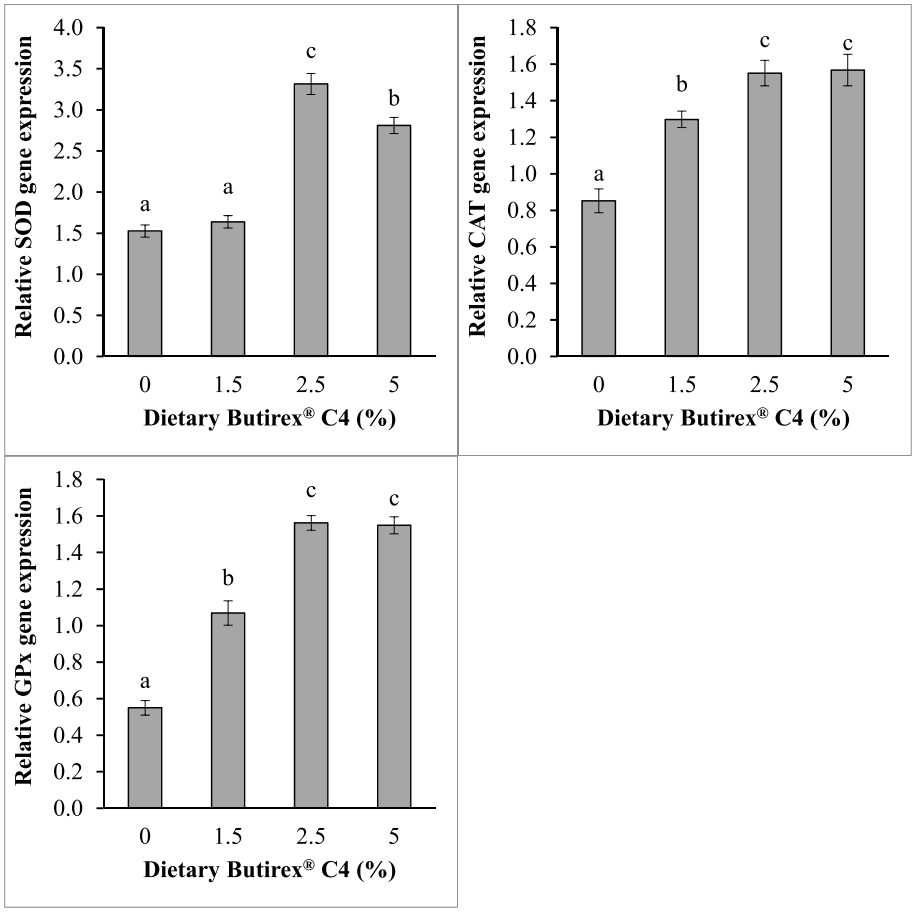

Fig. 2. Relative expression of SOD, CAT and GPX genes in intestine of rainbow trout fed different levels of Butirex. Data are expressed as mean ± SE. Error bars with different superscripts denote show significant difference at $P < 0.05$.

levels in dose-depending manner.

3.2. Intestinal immune-related genes expression

The immune-related genes expression are shown in Fig. 1. The Butirex-supplemented groups had significantly higher LYZ gene expression compared to the control group and the highest expression was related to the B2.5 group ($P < 0.05$). Butirex at 1.5 g/kg levels had no effects on IL-1B expression, but at 2.5 and 5 g/kg levels, it led to significantly higher gene expression. Expression of TNF-α gene significantly decreased in B2.5 and increased in B5 groups, compared to the control. There were no significant differences in IL-8 and TGF-β gene expression between the control group with B1.5 and B5 groups. The B2.5 group had significantly higher TGF-β, and significantly lower IL-8 gene expression compared to the control group ($P < 0.05$). There was no significant difference in IL-10 gene expression between the control and B5 groups, both were significantly lower than those of the B1.5 and B2.5 groups ($P < 0.05$).

3.3. Intestinal antioxidant-related genes expression

Antioxidant-related gene expressions are presented in Fig. 2. There was no significant difference in SOD gene expression between the control and B1.5 groups, but the B2.5 and B5 groups had significantly higher SOD gene expression compared to the other groups; the highest expression was related to the B2.5 group ($P < 0.05$). Dietary Butirex supplementation significantly up-regulated CAT and GPx gene expression compared to the control group; the highest expression as related to the B2.5 and B5 groups ($P < 0.05$).

3.4. Intestinal tight junction protein genes expression

The expression of tight junction protein genes are presented in Fig. 3. There were no significant differences in CLD12 gene expression between the control, B1.5 and B5 groups, but B2.5 group had significantly lower gene expression compared to the control group ($P < 0.05$). The B2.5 and B5 groups had significantly higher CLD3, OCLD and ZO-1 gene expression compared to the control and B1.5 groups. The highest CLD3 and ZO-1 gene expressions were related to the B2.5 and B5 groups respectively ($P < 0.05$). However, there was no significant difference in OCLD gene expression between the B2.5 and B5 groups.

3.5. Challenge test

14-day the survival rates of rainbow trout fed different dietsand challenged with *S. iniae* are shown in Fig. 4. RPS was markedlyhigher in B2.5 and B5 (33.3% and 53.3%) compared to the B1.5 (10%).

4. Discussion

Previous studies have well documented the multiple function of intestine in fishes [15,39]. One of the important roles of the intestine is immune responses and eliminating pathogens by physical barrier, chemical and cellular components, which finally prevent infection [40]; however, innate and adaptive immune responses can be initiated in the gastrointestinal tract as part of a mucosal immune responses [41].The digestive tract including intestine is known to be an important site that exposed to a heavy load of antigenic molecules and aquatic microbes,

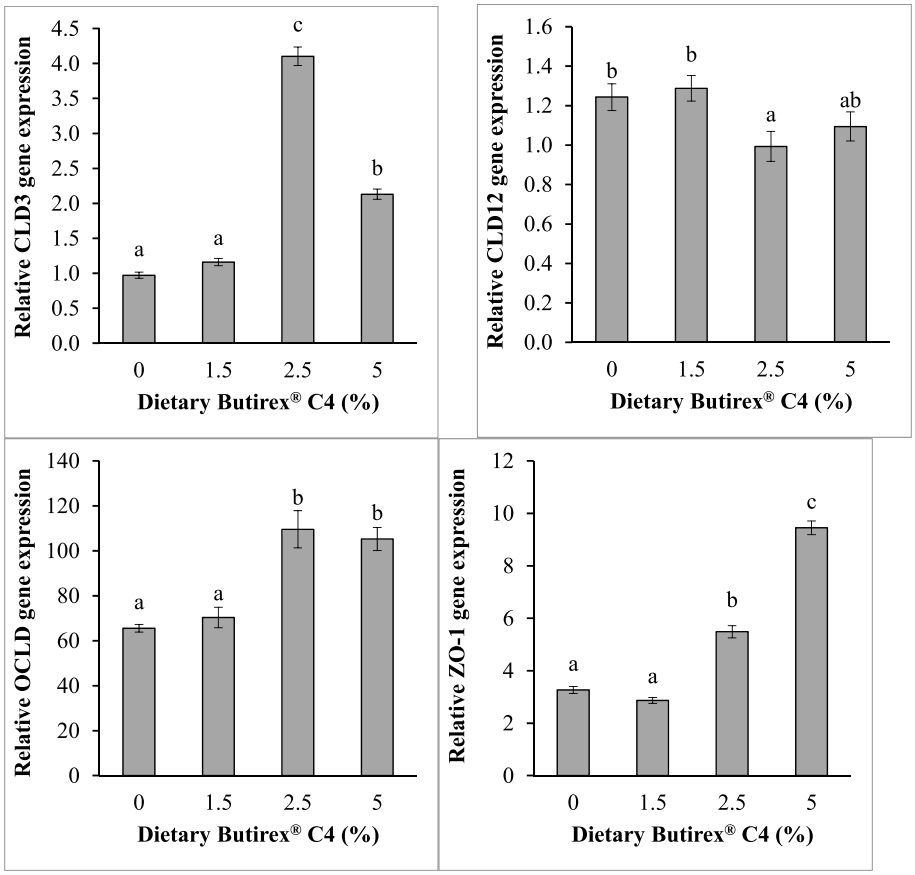

Fig. 3. Relative expression of CLD12, CLD3, OCLD and ZO-1 genes in intestine of rainbow trout fed different levels of Butirex. Data are expressed as mean ± SE. Error bars with different superscripts denote show significant difference at $P < 0.05$.

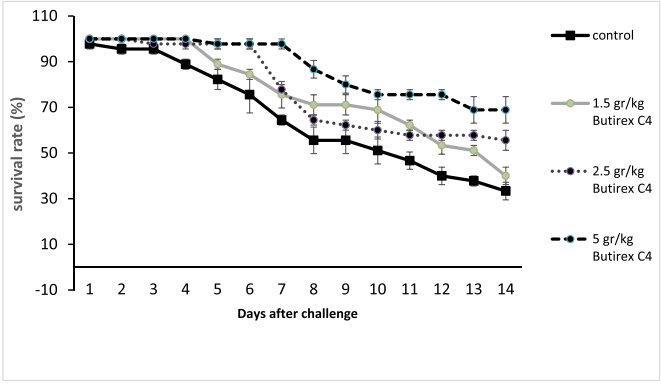

Fig. 4. Survival rate values (%) against *S. iniae* of rainbow trout fed different levels of Butirex. Data are expressed as mean ± SE. Error bars with different superscripts denote show significant difference at $P < 0.05$.

so requires innate and adaptive immune systems to be presented in the gut that act rapidly to prevent infection [42].

4.1. Intestinal innate immune responses

LYZ activity is an important index of non-specific immunity in fishes [43]. It is well documented that fish lysozyme possess lytic activity against both Gram-positive and Gram-negative bacteria [43]. In line with the present results, there are some studies indicating the positive effects of different SCFAs on immunological and physiological aspect of fishes. Tian et al. [32] reported that dietary butyrate supplementation significantly increased gut lysozyme activity, and C3, C4 and IgM levels in *Ctenopharyngodon idella*. It is known that SCFAs can improve fish gut microbiota [44–46]. It is also known that improved microbiota is followed by improved immune responses [47,48]. Therefore it can be concluded that improved immune responses observed in this experiment is because of the improvement in the gut microbiota by administration of Butirex® C4 in the feed. Another mechanism that can be considered is the improvement of the intestinal tissue, which will further improve the mucus secretion. Mucus contains several immune components and elevated mucus secretion might lead to improved immune responses [32].

4.2. Lysozyme and cytokine genes

To our knowledge, this study is the first attempt to investigate the effects of dietary Butirex supplementation on immune and cytokine genes expressionin the intestine of rainbow trout. Our results revealed that administration of Butirex significantly modified immune and cytokine gene expression in intestine of rainbow trout.

Administrations of different level of Butirex in the present study upregulated lysozyme gene expression, which is the reason for increased LYZ activity in the fish intestine (see above). There is no published data on the effects of dietary butyratesupplementation onintestinal lysozyme gene expression in fish.The mechanism of butyrate effect on lysozyme production is not clear but butyrate might stimulate monocytes to improve lysozyme production [49].

Cytokines are key regulators of the immune system.Inflammatory responses in the intestine are an important category of immune responses that together with proteins and antimicrobial peptides play an important role in controlling pathogens [20]. Cytokines responses are divided into pro-inflammatory and anti-inflammatoryresponses. Previous study confirmed that inflammation responses could be weaken

via down-regulating pro-inflammatory cytokines (like IL-1β and TNFα) and up-regulating anti-inflammatory cytokines (like TGF-β and IL-10) mRNA levels in fish [20]. Based on the present results, Butirex had anti-inflammatory effects at 2.5 g/kg level. The results are in line with previous studies showing that butyrate had anti-inflammatory effects by up-regulating anti-inflammatory cytokines (e.g. IL-10 and TGF-β) and down-regulating pro-inflammatory cytokines (TNF-α, IL-1β and IL-8) gene expression in intestine of *C. idella* [32]. It is because that butyrate and other SCFAs have anti-inflammatory potential [50] and this effect may be associated with suppression of NF-KB-P65 pathway activity [51]. However, sodium butyrate suppressed inflammation in the fish intestinal tissues, along with up-regulation in TNF-α gene expression and no change in IL-1β, IL-8 and IL-10 gene expression in European sea bass fed soybean-based diet [52]. Another study on European sea bass showed that supplementation of a plant-based diet with sodium butyrate led to up-regulation of IL-10 gene expression in the fish liver not intestine; moreover, sodium butyrate had no significant effects on IL-8, IL-1β and TNF-α gene expression [28]. Such contradictions encourage further researches on butyrate-modulated inflammation responses in different fish species.

4.3. Antioxidant genes

Oxidative statues in intestine of fish is key factor to monitoring intestine health status because oxidative damages induced disruption of TJ proteins by reducing the ZO-1 and occluding complexstructural integrity of intestinal epithelial cells [53]. Results of present study indicated that optimal level of Butirex improved antioxidant enzymes gene expressionin intestine of rainbow trout. In line with our results, previous study revealed that sodium butyrate administration increased SOD, CAT, GPx, GST and GR gene expression, and consequently these enzyme activities in intestine of grass carp, which led to lower ROS formation in the intestinal tissues [18]. Similarly, Liu et al. [54] found increased SOD and GPx activities in liver of grass carp. Antioxidant effects of butyrate in fish seems to be related to modulation of nrf2 an keap1 systems, as they are key regulators of antioxidant systems and are modulated by butyrate administration in fish [18]. On the other hand, some species of fish gut microbiota improve antioxidant defense of the host [26,55] and SCFAs are capable to dominate these beneficial microbes [56]. Therefore, the results of this study suggest that administration Butirex® C4, increase antioxidant responses in intestine through improve intestinal physiology responses.

4.4. Tight junction proteins' genes

Intestinal barrier function regulates transport and host defense mechanisms at the mucosal interface with the outside world. The TJs proteins play a key role in holding the intestinal epithelium integrity and were important to preserve the physical intestinal barrier in fish [41]. It is well known that administration of some feed additives up-regulates the barrier-forming tight junction proteins like claudin-c, CLD3, OCLD and ZO-1 and in some cases down-regulatestheCLD12 and CLD15, followed by improved physical barrier in intestine [18,21,57–59]. There is limited information about effect of dietary sodium butyrate on intestinal tight junction protein genes expression of rainbow trout. A previous study indicated that optimal sodium butyrate supplementation up-regulated OCLD, ZO-1, ZO-2, claudin-b, claudin-c, claudin-f, claudin-3c, claudin-7a, claudin-7b and CLD11 mRNA levels, and down-regulated CLD12, claudin-15a and claudin-15b mRNA levels in three intestinal segments of young *C. Idella* [18]. In gilthead sea bream, dietary butyrate supplementation to a plant-based diet significantly restored gut OCLD gene expression, but had no effects on CLD12 and ZO-1 gene expression [60]. There are other studies showing butyrate potentiates to improve fish gut structure integrity [32,52,61]; such improvements might be related to modulation of TJ proteins. Our results showed that different levels of dietary sodium butyrate supplementation improved tight junction protein genes expression and finally physical barrier of intestine which is possibly due to increase antioxidant activity in the intestine (see the results of antioxidant enzymes gene). Previous studies showed that oxidative stresshad negativeeffect on physical intestinal barrier [62,63]. On the other hand, it should be noted that SCFAs, especially butyric acid, can provide energy source for the regeneration and repair of the intestine epithelial cells [64].

4.5. Disease resistance

The results of present study revealed that dietary sodium butyrate supplementation increased rainbow trout resistance against *S. iniae*. There is limited information about effect of butyrate on disease resistance in fishes. previous study reported that mixture of organic acids include formic acid, propionic acid and calcium propionate improve resistance of Nile Tilapia (*Oreochromis niloticus*) against *Aeromonas Sobria* [65]. As well as in line with our results dietary supplemented with mixture of organic acids and essential oils increase resistance of Pacific white shrimp (*Litopenaeus vannamei*) against *Vibrio parahaemolyticus* [56]. The increase disease resistance fallowing organic acids administration in aquatic animals is possibly due to improvement of immunological parameters, antioxidant defense and gut integrity, which discussed above.

5. Conclusion

In conclusion, dietary Burirex modulated cytokines gene expression in the fish intestine. Besides, Butirex augmented antioxidant defense and improved integrity of the fish gut by modulation antioxidant enzymes and TJ proteins gene expression. The fish treated by Butirex would be more resistance to bacterial infection, which is due to improved immune responses, antioxidant defense and gut health. Based on the results, 5 g/kg Butirexis recommended to be incorporated in rainbow trout diet to augment fish health and disease resistance.

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