Animal Nutrition 7 (2021) 539–547

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Effects of dietary sodium butyrate on growth, digestive enzymes, body composition and nutrient retention-related gene expression of juvenile yellow catfish (*Pelteobagrus fulvidraco*)

Hongxia Zhao [a, b, c, *], Guoxia Wang [a, b, c], Hairui Wang [a, b, c], Wenyan Mo [a, b, c], Yanhua Huang [a, b, c], Junming Cao [a, b, c, d, *], Peijia Li [a, b, c, d]

[a] Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
[b] Key Laboratory of Animal Nutrition and Feed Science in South China, Ministry of Agriculture and Rural Affairs, Guangzhou 510640, China
[c] Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, China
[d] Guangdong Ocean University, Zhanjiang 524088, China

ARTICLE INFO

Article history:
Received 18 May 2020
Received in revised form
15 December 2020
Accepted 20 December 2020
Available online 30 March 2021

Keywords:
Sodium butyrate
Growth
Digestion
Absorption
Target of rapamycin
Lipoprotein lipase

ABSTRACT

An 8-week feeding trial was conducted to evaluate the effects of sodium butyrate (SB) on growth, digestive enzymes, body composition and nutrient retention-related gene expression of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Five isonitrogenous and isolipidic diets (420 g/kg protein and 90 g/kg lipid) were formulated to contain 0 (control), 250, 500, 1,000 or 2,000 mg/kg SB. Triplicate groups of 40 fish (BW = 1.26 ± 0.01 g) per tank (300-L cylindrical fiberglass tanks) for each diet were fed to apparent satiation twice daily. Stomach, hepatopancreas and intestine samples were obtained for digestive enzymes activities analyses. A real-time quantitative PCR analysis was performed to determine the relative expression of target of rapamycin (*TOR*) and lipoprotein lipase (*LPL*) in the hepatopancreas and intestine. Fish fed the diets supplemented with SB at 500 and 1,000 mg/kg showed significantly higher specific growth rate and significantly lower feed conversion ratio compared to the control ($P < 0.05$). Dietary SB inclusion did not alter activities of intestinal amylase, creatine kinase and sodium–potassium adenosine triphosphatase (Na^+/K^+-ATPase), but increased activities of hepatic trypsin, stomachic lipase, intestinal lipase, alkaline phosphatase and γ-glutamyl transpeptidase for fish fed 1,000 mg/kg SB compared to the control ($P < 0.05$). Intestine length index, intestine somatic index, fold height and muscular thickness of distal intestine were significantly higher in 1,000 mg/kg SB groups compared to the control ($P < 0.05$). Significantly higher levels of whole-body crude protein, ash, calcium, phosphorus, nutrition retention and relative mRNA of intestinal *TOR* were observed in 1,000 mg/kg SB group ($P < 0.05$). Whole-body lipid content and hepatopancreas *LPL* mRNA expression in 2,000 mg/kg SB group were significantly higher than the control ($P < 0.05$). Relative mRNA levels of intestinal *LPL* and hepatopancreas *TOR* were significantly higher in the 500 mg/kg SB group compared to those in other groups ($P < 0.05$). The increased growth performance, digestive enzymes and nutrient retention in fish fed the diets supplemented with SB at 500 and 1,000 mg/kg suggests that SB can be a desirable growth promoter as an antibiotic alternative in diets.

© 2021 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Organic acids are organic carboxylic compounds including acetic, butyric, citric, formic, lactic, propionic and sorbic acids (Hoseinifar et al., 2017; Ng and Koh, 2017). Feed supplementation with organic acids has been shown to improve health and growth performance in various fish and crustacean species by altering the gastrointestinal tract function and energy metabolism, improving nutrition retention, increasing the availability of nutrients and

* Corresponding authors.
E-mail addresses: zhaohongxia8866@163.com (H. Zhao), junmcao@163.com (J. Cao).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2020.12.007

2405-6545/© 2021 Chinese Association of Animal Science and Veterinary Medicine. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

inhibiting the growth of pathogenic bacteria (Ng and Koh 2017; Hoseinifar et al., 2017). Butyrate is one of the most important short-chain fatty acids, which presents in the gastrointestinal tract as a main end product of anaerobic bacterial fermentation of carbohydrates. Sodium butyrate (SB) is solid, stabile and much less odorous compared to butyrate, and SB is more commonly added to animal feeds (Guilloteau et al., 2009). Increasingly, SB is being investigated as a dietary additive in various aquatic animals, to improve their growth, nutrient utilization and intestinal health (Abdel-Latif et al., 2020). Positive effects of dietary SB on growth performance have been confirmed in fish, such as sea bream *Sparus aurata* (Robles et al., 2013), common carp *Cyprinus carpio* (Liu et al., 2014), tilapia *Oreochromis niloticus* (Ahmed and Sadek, 2015), grass carp *Ctenopharyngodon idellus* (Liu et al., 2017), European sea bass *Dicentrarchus labrax* (Abdel-Mohsen et al., 2018), golden pompano *Trachinotus ovatus* (Zhou et al., 2019), Nile tilapia *O. niloticus* (Jesus et al., 2019a), turbot *Scophthalmus maximus* L. (Liu et al., 2019) and Asian seabass *L. calcarifer* (Aalamifar et al., 2020).

Fish growth is closely related to the digestive and absorptive abilities of digestive organs (Mitra et al., 2008). Jesus et al. (2019b) reported that protected forms of SB improved absorption capacity of nutrients through increasing length, perimeter and villus area of the anterior region of the intestinal tract during the sexual reversion period. The improvement of digestion and absorption of nutrients was characterized by the increase in activity of digestive enzymes, e.g., trypsin, chymotrypsin, lipase and amylase, and brush-border membrane enzymes, e.g., alkaline phosphatase, sodium–potassium adenosine triphosphatase (Na^+/K^+-ATPase) and creatine kinase (Krogdahl and Marie Bakke-McKellep, 2005; Tibaldi et al., 2006). Alkaline phosphatase is used as a marker of nutrient absorption and takes part in the absorption of lipid, glucose, calcium and inorganic phosphate (Villanueva et al., 1997). Na^+/K^+-ATPase created the potential energy of the Na gradient for many transport systems and the activity of this enzyme could indirectly reflect the absorption ability of the nutrient, such as amino acids, phosphate or glucose (Hakim et al., 2009). Creatine kinase is associated with energy metabolism and the coupling of ATP and kinase (Wallimann and Hemmer, 1994) and γ-glutamyl transpeptidase is related to peptide transport (Griffith and Meister, 1980). Dietary SB could enhance the activities of intestinal protease, amylase, alkaline phosphatase and Na^+/K^+-ATPase of golden pompano (Zhou et al., 2019). Furthermore, dietary SB enhanced intestinal physical barrier function referring to NF-E2-related factor 2 (*Nrf2*), c-Jun N-terminal protein kinase (*JNK*) and myosin light chain kinase (*MLCK*) signaling pathways of young grass carp (Wu et al., 2018). The syntheses of proteins and lipids are key processes involved in growth response (Mommsen, 2001). In gilthead sea bream and yellow drum (*Nibea albiflora*) (Richardson), the improvement of growth performance may be due to the increase in the availability of essential amino acids of fish fed SB diets (Robles et al., 2013; Wu et al., 2019). Wu et al. (2019) suggested that supplementation of 0.15% SB increased crude lipid content of the whole body in yellow drum. The limiting step in protein synthesis is translation initiation, which is regulated by the signaling pathway of target of rapamycin (*TOR*) (Seiliez et al., 2008). Similarly, lipoprotein lipase (*LPL*) is an important lipid regulatory enzyme involved in supplying free fatty acids for storage in adipocytes or for oxidation in other tissues (Zheng et al., 2013). Rainbow trout genetically selected for greater muscle fat content displays increased activation of liver *TOR* signaling and lipogenic gene expression (Skiba-Cassy et al., 2009). Although some preliminary work has been conducted in fish species previously, studies concerning intestinal health and nutrient retention of dietary SB in fish are still limited. Hence, it is necessary to address the effects of SB on intestinal health and nutrient retention of fish.

Yellow catfish (*Pelteobagrus fulvidraco*) is one of omnivorous freshwater fish species, which has a delicious quality and high nutritional value. Considering the increasing interest in dietary SB, and increasing popularity of yellow catfish as a food fish that is a promising farmed fish in aquaculture, evaluating the relationship of SB to growth, feed utilization and nutrient retention of this commercially important species would likely benefit the industry. Thus, this study was conducted to study the effects of SB on growth, digestive enzymes, body composition and nutrient retention related gene expression of juvenile yellow catfish (*P. fulvidraco*).

2. Materials and methods

All experimental procedures were carried out in accordance with the Guidelines for Experimental Animals by the Guangdong Academy of Agricultural Sciences, China. The animal use and care protocol was reviewed and approved by the Animal Care and Use Committee of Guangdong Academy of Agricultural Sciences (SC-GDAAS-2019-018).

2.1. Experimental design and diets

Formulation and proximate composition of experimental diets are presented in Table 1. Fishmeal, soybean, rapeseed meal and corn gluten meal were used as protein sources. Wheat flour, fish oil and soybean oil were used as carbohydrate and lipid sources. The levels of nutrients met the requirements of yellow catfish according to previous studies conducted in our laboratory (Cao et al., 2012; Chen et al., 2016; Zhao et al., 2020). Five isonitrogenous and isolipidic diets were formulated to contain SB at 0 (control), 250, 500, 1,000 or 2,000 mg/kg, by supplementing SB (purity ≥ 98%). All ingredients were ground through a 60-mesh screen. SB was mixed with dietary water sources and then mixed with the other feedstuffs. The diets were prepared by mixing the ingredients before adding fish oil, soybean oil and water using a kneading machine (NH-10, South China University of Technology, Guangzhou, China). The feed ingredients were thoroughly mixed with fish oil, soybean oil and the appropriate amount of water in a strong stirrer (B20, Guangzhou Panyu Lifeng Food Machinery Factory, Guangzhou, China) and the mixture was processed into 1.5-mm diameter pellets using a twin screw extruder (SLX-80, South China University of Technology, Guangzhou, China) and dried at 55 °C for 6 h. After drying, diets were stored at −20 °C in plastic bags until being used.

2.2. Fish and experimental conditions

Yellow catfish were obtained from Guangzhou Jinglong Fisheries (Guangzhou, China). The fish were acclimatised with the control diet for 2 weeks prior to this trial. The feeding trial was conducted in an indoor re-circulating aquaculture system at the Animal Science Institute of Guangdong Academy of Agricultural Sciences (Guangzhou, China). The circling waterflow rate in each aquarium was maintained at 1.5 L/min. Fish (1.26 ± 0.01 g) were randomly stocked into fifteen 330-L cylindrical fiberglass tanks (the water volume was 300 L) at 40 fish per tank in triplicate. Fish were fed 2 times per day at 8:30 and 18:30 to apparent satiation. The uneaten feed was collected to calculate total consumption. The amounts of diets consumed by the fish in each tank were recorded daily, and adjusted according to the amounts consumed the day before. The water source was drawn from underground and one-third of the water in the tank was exchanged weekly. Aeration was also provided to maintain enough dissolved oxygen. Water samples were collected twice a week for chemical analysis. During the 56-d feeding trial, natural water temperature ranged from 28 to 32 °C, pH 7.4 to 7.9, ammonia ≤0.02 mg/L, nitrite ≤0.2 mg/L and

Table 1
Formulation and proximate composition of experimental diets (g/kg, DM basis).

Item	Dietary SB[1] supplementation				
	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Ingredients					
Peru fish meal[2]	250.0	250.0	250.0	250.0	250.0
Soybean meal[2]	300.0	300.0	300.0	300.0	300.0
Rapeseed meal[2]	90.0	90.0	90.0	90.0	90.0
Corn gluten meal[2]	60.0	60.0	60.0	60.0	60.0
Wheat flour[2]	223.0	223.0	223.0	223.0	223.0
Menhaden oil[2]	25.0	25.0	25.0	25.0	25.0
Soybean oil[2]	25.0	25.0	25.0	25.0	25.0
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0
Mineral premix[4]	5.0	5.0	5.0	5.0	5.0
$Ca(H_2PO4)_2$[2]	15.0	15.0	15.0	15.0	15.0
Vitamin C ester[2]	1.0	1.0	1.0	1.0	1.0
Choline chloride[2]	3.0	3.0	3.0	3.0	3.0
Microcrystalline Cellulose[2]	2.0	1.75	1.5	1.0	0
SB	0.0	0.25	0.5	1.0	2.0
Proximate nutrition composition					
Crude protein	426	423	420	425	422
Crude lipid	90.1	87.7	90.1	85.6	84.5
Ash	82.5	81.9	81.9	83.0	84.2
Moisture	77.9	86.9	85.7	80.7	81.8

SB = sodium butyrate.
[1] Provided by Sigma–Aldrich, MO, USA. The actual SB concentrations were 0.0 (control), 158.1, 314.7, 612.3 and 1,193.5 mg/kg diet, which were determined by high-performance liquid chromatography (HPLC) (Liu et al., 2014).
[2] Provided by Guangzhou Fishtech Fisheries Science & Technology Co., Ltd (Guangzhou, China).
[3] One kilogram of vitamin premix contained the following: vitamin A 3,200,000 IU, vitamin B_1 4 g, vitamin B_2 8 g, vitamin B_6 4.8 g, vitamin B_{12} 0.016 g, vitamin D 1,600,000 IU, vitamin E 16 g, vitamin K 4 g, nicotinic acid 28 g, calcium pantothenate 16 g, folic acid 1.28 g, inositol 40 g, biotin 0.064 g, wheat middling, 876.84 g. Moisture ≤10%.
[4] One kilogram of mineral premix contained the following: $MgSO_4 \cdot H_2O$ 12 g, $Ca(IO_3)_2$ 9 g, KCl 36 g, Met-Cu 1.5 g, $ZnSO_4 \cdot H_2O$ 10 g, $FeSO_4 \cdot H_2O$ 1 g, Met-Co 0.25 g, $NaSeO_3$ 0.0036 g, zeolite 930.25 g. Moisture ≤10%.

dissolved oxygen >6.0 mg/L. The experimental units were under a natural light and dark cycle (approximately 12 h light:12 h dark).

2.3. Sample collection and analysis

After a fasting period of 24 h, fish in each tank were individually weighed and counted at the end of the feeding trial. Prior to sampling, fish were anesthetized in tricaine methane sulfonate (120 mg/L). Ten fish before the experiment and 5 fish at the end of the experiment per tank were obtained to determinate the initial and final whole-body composition, respectively. The intestine, hepatopancreas and stomach of 6 fish per tank were collected and frozen in liquid nitrogen, then stored at −70 °C until analysis. Another 3 fish per tank were randomly selected to measure hepatosomatic index (HSI) and intestine somatic index (ISI).

The whole intestine, hepatopancreas and stomach samples from each tank were homogenized in ice-cold physiological saline solution and centrifuged at 6,000 × *g* at 4 °C for 20 min. The supernatant was stored at −70 °C for analysis of protein and enzyme activities. The protein concentrations of homogenates were determined by the method of Bradford (1976).

The moisture, crude protein, crude lipid and ash contents in the diets and whole body of fish were determined according to the Association of Official Analytical Chemists (Association of Official Analytical Chemists (AOAC), 1995). Moisture was determined by drying the samples to a constant weight at 105 °C. Crude protein (N × 6.25) was determined by the Kjeldahl method using a semi-automatic Kjeldahl System after acid digestion. Crude lipid was determined by using the Soxhlet extraction method. Crude ash was determined after burning at 550 °C in a muffle furnace. Commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used to determine intestine enzyme activities of protease, lipase, amylase, alkaline phosphatase (AKP), γ-glutamyl transpeptidase (γ-GT), creatine kinase (CK) and Na^+/K^+-ATPase, following the corresponding manufacturer's instructions.

Three fish per tank were randomly sampled and dissected to obtain the proximal intestine (1 cm after the stomach), mid intestine and distal intestine (1 cm before the anus) according to the method described by Anguiano et al. (2013). The samples were dissected and fixed immediately in the 4% buffered formalin solution for 24 h, and then transferred to 70% ethanol. Subsequently, the tissues were sliced, embedded in paraffin and processed via standard hematoxylin and eosin staining (Cheng et al., 2011). Examination was done by light microscopy (Eclipse E100, Tokyo, Japan) with a photomicrograph attached to a computer with Image-Pro Plus 6 software (Media Cybernetics, Maryland, USA). Image collection, slices measurement ($n = 9$) and each slice ($n = 8$) for fold height and muscular thickness were determined.

2.4. RNA extraction and real-time quantitative PCR analysis

Total RNA was extracted from the intestine and hepatopancreas according to the Trizol protocols (Invitrogen, USA). Total RNA was incubated with RNase-free DNase (Dalian Takara Co. Ltd., China) to remove the contaminating genomic DNA. Then, the quality and quantity were assessed using agarose gel (1.2%) electrophoresis and spectrophotometric (a 260:280 nm ratio) analysis, respectively. The RNA was then reverse transcribed to cDNA using PrimeScript RT reagent kit (Takara, Japan). The real-time quantitative PCR (RT-qPCR) was performed in an ABI 7500 Real-Time PCR machine (Applied Biosystems, USA). The amplification was performed in a final volume of 20 μL containing 1 μL cDNA product, 10 μL SYBR Premix ExTaq II (Takara, Japan), 0.4 μL of each respective primer, and 8.2 μL dH_2O. Primers (shown in Table 2) for *TOR* and lipoprotein lipase (*LPL*) were designed using primer 5.0 (PREMIER Biosoft International, Palo Alto, CA, USA) based on the sequences obtained from the published sequences of yellow catfish. A melting curve analysis was generated following the amplification to check and verify the specificity of RT-qPCR products. Beta-actin was used as a housekeeping gene to normalize target gene transcript levels. The target and housekeeping

Table 2
Primers used in real-time quantitative PCR.

Target	GenBank ID	Forward primer (5′- 3′)	Reverse primer (5′- 3′)
TOR	KY072931	GTGAAGGACCTGACTCAAGCC	TGATAGACTGGATGCGTATGATTGG
LPL	JX992743	GACCAGAGAGATGATGCCGT	TAGCTTAGCTGGCTCTTGCTG
β-Actin	XM027148463	TTCGCTGGAGATGATGCT	CGTGCTCAATGGGGTACT

TOR = target of rapamycin; *LPL* = lipoprotein lipase.

gene amplification efficiency were calculated according to the specific gene standard curves generated from 10-fold serial dilutions. After verifying that the primers amplified with an efficiency of approximately 100%, the $2^{-\Delta\Delta CT}$ method was used for calculating the expression results according to Livak and Schmittgen (2001).

2.5. Statistical analysis

Statistical analyses were carried out using the SPSS version 20.0 software package (SPSS Inc., Chicago, IL, USA). All data were subjected to the homogeneity of variance tests before one-way ANOVA and a multiple range test (Tukey's test) was used. The significance criterion was $P < 0.05$. Data were presented as means ± SD.

3. Results

3.1. Growth performance

Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR) are presented in Table 3. The WG, SGR and FCR were affected by the dietary SB levels ($P < 0.05$). There was a significant increase in WG in 500 mg/kg SB group compared with that of the control ($P < 0.05$). Fish in 500 and 1,000 mg/kg SB groups showed a significantly higher SGR and a significantly lower FCR than that of control ($P < 0.05$). The SR was not influenced by the inclusion of SB in the diets ($P > 0.05$).

3.2. Enzyme activities in stomach, hepatopancreas and intestine

Protease, trypsin, lipase, amylase, AKP, γ-GT (γ-glutamyl transpeptidase), CK (creatine kinase) and Na^+/K^+-ATPase activities are shown in Tables 4 and 5. Lipase activity of stomach in SB groups was significantly higher than that in the control group ($P < 0.05$). Trypsin activity in hepatopancreas of fish in 500 and 1,000 mg/kg SB groups was significantly higher as compared with that in control and 250 mg/kg SB groups ($P < 0.05$). Lipase activity in intestine of fish in 1,000 mg/kg SB group was significantly higher than that in control ($P < 0.05$). There was a significant increase of intestinal AKP activity in 500 and 1,000 mg/kg SB groups compared with that of control and 2,000 mg/kg SB groups ($P < 0.05$). The activity of γ-GT in intestine of fish in 1,000 and 2,000 mg/kg SB groups was higher as compared with that in control ($P < 0.05$). The activities of protease in stomach, trypsin, CK and Na^+/K^+-ATPase in intestine, lipase in hepatopancreas, amylase in stomach, hepatopancreas and intestine were not influenced by the inclusion of SB in the diets ($P > 0.05$).

3.3. Hepatopancreas and intestine morphometric parameters

Hepatopancreas and intestine morphometric parameters are shown in Table 6. Intestine somatic index in 500, 1,000 and 2,000 mg/kg SB groups was significantly higher as compared with control and 250 mg/kg groups ($P < 0.05$). Intestine length index (ILI), intestinal protein content (IPC) and hepatopancreatic protein content (HPC) in 1,000 mg/kg SB group were significantly higher as compared with those in control ($P < 0.05$). There was a significant increase of fold height in proximal intestine (PI) in 500 and 1,000 mg/kg SB groups compared with that of control ($P < 0.05$). Muscular thickness in PI was significantly higher in 500 mg/kg SB group than that in control ($P < 0.05$). Significantly higher values of fold height and muscular thickness in distal intestine (DI) were observed in 250, 500 and 1,000 mg/kg SB groups ($P < 0.05$).

3.4. Whole body composition and nutrition retention value

Dry matter, crude protein, crude lipid, ash, calcium and phosphorus contents are shown in Table 7. Significantly higher contents of crude protein and phosphorus were observed in the 1,000 mg/kg SB group compared to those in control and 2,000 mg/kg SB groups

Table 3
Effects of different levels of sodium butyrate (SB) supplementation on growth performance and nutrition retention of juvenile yellow catfish.[1]

Item	Dietary SB supplementation				
	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
WG[2], g	11.61 ± 0.57^{a}	12.56 ± 1.24^{ab}	13.36 ± 0.22^{b}	13.08 ± 0.56^{ab}	12.46 ± 0.88^{ab}
SGR[3], %/day	4.16 ± 0.08^{a}	4.28 ± 0.15^{ab}	4.38 ± 0.03^{b}	4.35 ± 0.06^{b}	4.27 ± 0.11^{ab}
FCR[4]	1.20 ± 0.04^{b}	1.08 ± 0.09^{ab}	1.03 ± 0.01^{a}	0.99 ± 0.09^{a}	1.13 ± 0.13^{ab}
SR[5], %	92.50 ± 4.33^{a}	95.83 ± 1.44^{a}	92.50 ± 5.00^{a}	91.67 ± 8.04^{a}	94.17 ± 2.89^{a}
PRV[6], %	25.48 ± 1.75^{a}	29.99 ± 3.10^{b}	30.44 ± 2.23^{b}	31.98 ± 1.24^{b}	27.66 ± 2.72^{ab}
LRV[7], %	56.44 ± 3.79^{a}	69.55 ± 4.75^{b}	66.75 ± 1.16^{b}	65.28 ± 5.15^{b}	68.21 ± 7.05^{b}
CaRV[8], %	47.71 ± 5.51^{a}	55.48 ± 9.34^{ab}	56.48 ± 2.82^{ab}	64.61 ± 2.61^{b}	49.48 ± 2.31^{a}
PhRV[9], %	52.12 ± 2.28^{a}	62.51 ± 10.45^{abc}	65.34 ± 4.94^{bc}	70.59 ± 6.46^{c}	56.29 ± 6.16^{ab}

WG = weight gain; SGR = specific growth rate; FCR = feed conversion ratio; SR = survival rate; PRV = protein retention value; LRV = lipid retention value; CaRV = calcium retention value; PhRV = phosphorus retention value.
[a, b, c] Within a row, means with different superscripts represent significant difference by Tukey's test ($P < 0.05$). Data presented are means ± SD of 3 replicates.
[1] Initial fish average weight = 1.26 ± 0.01 g; initial fish whole-body composition (% wet weight): protein, 15.13; lipid, 3.29; calcium, 1.43; phosphorus, 0.92.
[2] WG (g) = Final weight (g) − Initial weight (g).
[3] SGR (%/day) = 100 × [ln (final weight) (g) − ln (initial weight) (g)]/Number of days.
[4] FCR = Dry diet fed (g)/Wet weight gain (g).
[5] SR (%) = 100 × (Finial number of fish)/(Initial number of fish).
[6] PRV (%) = 100 × [Final weight (g) × Final fish protein (%) − Initial weight (g) × Initial fish protein (%)]/[Feed intake (g) × Feed protein (%)].
[7] LRV (%) = 100 × [Final weight (g) × Final fish lipid (%) − Initial weight (g) × Initial fish lipid (%)]/[Feed intake (g) × Feed lipid (%)].
[8] CaRV (%) = 100 × [Final weight (g) × Final fish calcium (%) − Initial weight (g) × Initial fish calcium (%)]/[Feed intake (g) × Feed calcium (%)].
[9] PhRV (%) = 100 × [Final weight (g) × Final fish phosphorus (%) − Initial weight (g) × Initial fish phosphorus (%)]/[Feed intake (g) × Feed phosphorus (%)].

Table 4
Effects of different levels of sodium butyrate (SB) supplementation on digestive enzymes activities in stomach, hepatopancreas and intestine of yellow catfish.

Item	Dietary SB supplementation				
	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Stomach					
Protease, U/g protein	15.50 ± 1.19[a]	15.00 ± 3.06[a]	14.39 ± 1.70[a]	19.05 ± 0.46[a]	15.56 ± 3.96[a]
Lipase, U/g protein	33.19 ± 2.45[a]	39.74 ± 3.11[b]	42.39 ± 4.23[b]	44.63 ± 4.31[b]	42.03 ± 0.53[b]
Amylase, U/mg protein	0.64 ± 0.13[a]	0.80 ± 0.25[a]	0.62 ± 0.02[a]	0.65 ± 0.13[a]	0.67 ± 0.04[a]
Hepatopancreas					
Trypsin, U/g protein	171.6 ± 14.31[a]	181.8 ± 15.12[a]	231.0 ± 30.40[b]	239.9 ± 16.21[b]	203.8 ± 24.78[ab]
Lipase, U/g protein	8.19 ± 0.31[a]	9.97 ± 1.20[a]	8.51 ± 0.31[a]	9.00 ± 0.90[a]	9.05 ± 1.72[a]
Amylase, U/mg protein	0.64 ± 0.10[a]	0.81 ± 0.16[a]	0.67 ± 0.06[a]	0.64 ± 0.12[a]	0.81 ± 0.24[a]
Intestine					
Trypsin, U/g protein	389.9 ± 31.69[a]	421.1 ± 69.18[a]	423.8 ± 68.27[a]	429.4 ± 58.45[a]	419.9 ± 42.23[a]
Lipase, U/g protein	17.85 ± 1.25[a]	19.30 ± 1.93[a]	22.80 ± 3.13[ab]	24.63 ± 1.49[b]	21.07 ± 3.89[ab]
Amylase, U/mg protein	1.62 ± 0.07[a]	1.38 ± 0.26[a]	1.55 ± 0.06[a]	1.60 ± 0.27[a]	1.47 ± 0.05[a]

[a, b] Within a row, means with different superscripts represent significant difference by Tukey's test ($P < 0.05$). Data presented are means ± SD of 3 replicates.

Table 5
Effects of different levels of sodium butyrate (SB) supplementation on intestinal brush-border membrane enzymes activities of yellow catfish (U/mg protein).

Item	Dietary SB supplementation				
	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
AKP	78.68 ± 10.92[a]	96.33 ± 10.50[ab]	119.05 ± 18.99[b]	105.72 ± 6.83[b]	81.35 ± 10.03[a]
γ-GT	16.13 ± 1.85[a]	19.13 ± 1.57[ab]	18.26 ± 2.14[ab]	21.12 ± 1.86[bc]	24.14 ± 3.40[c]
CK	0.40 ± 0.03[a]	0.38 ± 0.02[a]	0.42 ± 0.08[a]	0.41 ± 0.07[a]	0.36 ± 0.04[a]
Na+/K + -ATPase	3.64 ± 0.81[a]	3.49 ± 0.61[a]	4.02 ± 0.93[a]	4.39 ± 0.68[a]	3.91 ± 0.66[a]

AKP = alkaline phosphatase; γ-GT = γ-glutamyl transpeptidase; CK = creatine kinase; Na^+/K^+-ATPase = sodium–potassium adenosine triphosphatase.
[a, b, c] Within a row, means with different superscripts represent significant difference by Tukey's test ($P < 0.05$). Data presented are means ± SD of 3 replicates.

Table 6
Effects of different levels of sodium butyrate (SB) supplementation on morphology and nutrient content of intestine and hepatopancreas of yellow catfish.

Item	Dietary SB supplementation				
	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
ILI[1]	0.81 ± 0.08[a]	0.83 ± 0.09[ab]	0.84 ± 0.08[ab]	0.88 ± 0.06[b]	0.83 ± 0.07[ab]
ISI[2], %	1.88 ± 0.26[a]	1.90 ± 0.30[a]	2.18 ± 0.56[b]	2.34 ± 0.44[b]	2.18 ± 0.33[b]
IPC[3], %	4.13 ± 0.34[a]	4.47 ± 0.41[a]	4.72 ± 0.37[ab]	5.43 ± 0.72[b]	4.52 ± 0.47[ab]
HSI[4], %	1.77 ± 0.26[a]	1.96 ± 0.32[a]	1.84 ± 0.25[a]	1.88 ± 0.36[a]	1.89 ± 0.37[a]
HPC[5], %	7.87 ± 0.27[a]	7.98 ± 0.55[ab]	8.26 ± 0.65[ab]	8.79 ± 0.36[b]	8.38 ± 0.30[ab]
Fold height, μm					
PI	438.49 ± 26.64[a]	535.53 ± 57.52[ab]	580.30 ± 47.15[b]	600.67 ± 33.26[b]	512.99 ± 81.82[ab]
MI	280.34 ± 38.49[a]	346.78 ± 45.66[a]	351.76 ± 38.47[a]	313.57 ± 30.67[a]	314.46 ± 26.37[a]
DI	251.72 ± 30.27[a]	303.91 ± 19.29[b]	321.35 ± 30.46[b]	327.42 ± 27.99[b]	281.77 ± 21.63[ab]
Muscular thickness, μm					
PI	91.32 ± 18.97[a]	102.31 ± 6.87[ab]	124.19 ± 11.40[b]	106.53 ± 7.49[ab]	107.356 ± 9.38[ab]
MI	52.06 ± 9.52[a]	63.52 ± 10.53[a]	58.82 ± 9.73[a]	64.51 ± 7.71[a]	54.38 ± 13.43[a]
DI	54.75 ± 6.20[a]	75.77 ± 12.03[b]	70.36 ± 7.528[b]	72.81 ± 6.95[b]	62.94 ± 6.68[ab]

ILI = intestine length index; ISI = intestine somatic index; IPC = intestinal protein content; HSI = hepatosomatic index; HPC = hepatopancreatic protein content; PI = proximal intestine; MI = mid intestine; DI = distal intestine.
[a, b] Within a row, means with different superscripts represent significant difference by Tukey's test ($P < 0.05$). Data presented are means ± SD of 3 replicates.
[1] ILI = Intestine length (cm)/Total body length (cm).
[2] ISI (%) = 100 × Intestine weight (g)/Body weight (g).
[3] IPC (%) = 100 × Intestine protein (g)/Intestine weight (g).
[4] HSI (%) = 100 × Hepatopancreas weight (g)/Body weight (g).
[5] HPC (%) = 100 × Hepatopancreas protein (g)/Hepatopancreas weight (g).

Table 7
Effects of different levels of sodium butyrate (SB) supplementation on whole body composition of juvenile yellow catfish (% wet weight).

Item	Dietary SB supplementation				
	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg	2,000 mg/kg
Dry matter	25.22 ± 0.47[a]	25.01 ± 0.14[a]	24.98 ± 0.34[a]	25.38 ± 0.65[a]	25.24 ± 0.19[a]
Crude protein	14.16 ± 0.26[a]	14.38 ± 0.22[ab]	14.41 ± 0.27[ab]	14.91 ± 0.22[b]	14.16 ± 0.20[a]
Crude lipid	6.29 ± 0.11[a]	6.71 ± 0.32[ab]	6.39 ± 0.24[ab]	6.16 ± 0.35[a]	6.98 ± 0.18[b]
Ash	2.81 ± 0.21[a]	2.88 ± 0.20[ab]	2.99 ± 0.19[ab]	3.21 ± 0.12[b]	2.92 ± 0.18[ab]
Calcium	0.79 ± 0.05[a]	0.78 ± 0.03[a]	0.79 ± 0.05[a]	0.87 ± 0.02[b]	0.76 ± 0.04[a]
Phosphorus	0.53 ± 0.02[a]	0.45 ± 0.03[ab]	0.55 ± 0.03[ab]	0.59 ± 0.04[b]	0.52 ± 0.03[a]

[a, b] Within a row, means with different superscripts represent significant difference by Tukey's test ($P < 0.05$). Data presented are means ± SD of 3 replicates.

($P < 0.05$). Crude lipid content in the control and 1,000 mg/kg SB groups were significantly lower than that in 2,000 mg/kg SB group ($P < 0.05$). Ash in 1,000 mg/kg SB group was higher as compared with control ($P < 0.05$). Calcium content in 1,000 mg/kg SB group was higher as compared with those in other groups ($P < 0.05$). Dry matter was not influenced by the inclusion of SB in the diets ($P > 0.05$).

Dietary SB levels had a significant effect on protein, lipid, calcium and phosphorus retention in the whole body of juvenile yellow catfish ($P < 0.05$) (Table 1). A significantly higher value of protein retention was observed in 250, 500 and 1,000 mg/kg SB groups ($P < 0.05$). Lipid retention in 250, 500, 1,000 and 2,000 mg/kg SB groups was significantly higher as compared with control ($P < 0.05$). Calcium retention in 1,000 mg/kg SB group was significantly higher as compared with those in control and 2,000 mg/kg SB groups ($P < 0.05$). Phosphorus retention in 500 and 1,000 mg/kg SB groups was significantly higher than that in control ($P < 0.05$).

3.5. Relative expression of TOR and LPL in intestine and hepatopancreas

Relative expression of *TOR* and *LPL* mRNA in intestine and hepatopancreas are shown in Fig. 1. Relative mRNA levels of intestinal *TOR* in 500, 1,000 and 2,000 mg/kg SB groups were significantly up-regulated ($P < 0.05$). Relative mRNA levels of intestinal *LPL* and hepatopancreas *TOR* were significantly up-regulated and the values were higher in 500 mg/kg SB group as compared with those in other groups ($P < 0.05$). The relative mRNA level of hepatopancreas *LPL* was significantly up-regulated and higher in the 2,000 mg/kg SB group as compared with that in control, 250 and 1,000 mg/kg groups ($P < 0.05$).

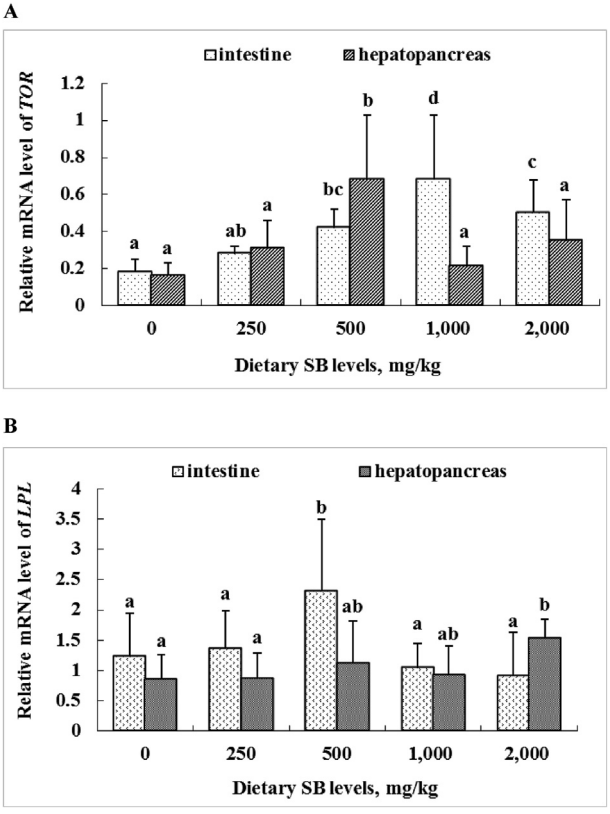

Fig. 1. Effects of different levels of sodium butyrate (SB) supplementation on relative expressions of (A) *TOR* and (B) *LPL* genes in intestinal tissue of juvenile yellow catfish. *TOR* = target of rapamycin; *LPL* = lipoprotein lipase. [a, b, c, d] Bars with different superscripts represent significant difference by Tukey's test ($P < 0.05$). Data presented are means ± SD of 3 replicates.

4. Discussion

4.1. Growth performance

Sodium butyrate was shown to have beneficial effects on growth and feeding efficiencies of yellow catfish. Fish weight gain (WG) and specific growth rate (SGR) increased with SB supplementation, which suggested that growth performance can be improved using SB in the diets. Growth benefit observed in this study was attributed to an improvement in feed utilization, which significantly increased with SB supplementation. Maximum growth performance and feed efficiency were observed at dietary SB levels of 500 and 1,000 mg/kg. The value of SGR was higher than those reported in some previous studies on yellow catfish (Luo et al., 2016; Zhao et al., 2017), but was lower than those observed in more recent studies on yellow catfish (Zhao et al., 2019). This is probably due to differences in the initial weight of the fish in these experiments. Based on the results of previous research, dietary supplementation of SB at 2,000 mg/kg improved the growth or feed utilization of juvenile grass carp (Liu et al., 2017), golden pompano (Zhou et al., 2019), and turbot (Liu et al., 2019). However, supplementation of 200 or 2,000 mg/kg SB did not improve the growth performance and feed utilization of African catfish and red hybrid tilapia (Owen et al., 2006; Ebrahimi et al., 2017). In Atlantic salmon (Bjerkeng et al., 1999) and rainbow trout (Gao et al., 2011), 0.5% to 2% dietary SB had no significant effect on growth performance. Studies containing different sustained-release times of microencapsulated SB did not demonstrate significantly improved growth in common carp (Liu et al., 2014). Protected forms of SB improved the biomass gain of Nile tilapia fingerlings during sexual reversion, but there was no significant difference between pure and protected forms of SB (Jesus et al., 2019a). This suggests that there is a dose–effect response in SB and growth performance. Therefore, the efficacy of SB to fish is mainly dependent on the dosage and protected form as well as species.

4.2. Enzyme activities in stomach, hepatopancreas and intestine

The activities of digestive enzymes are generally associated with digestive capacity and affect fish growth rate (Krogdahl and Marie Bakke-McKellep, 2005). In this study, SB improved lipase activity in stomach and intestine, as well as the trypsin activity in hepatopancreas of yellow catfish. Similar results were reported for golden pompano in which intestinal digestive enzyme activity was increased by 2,000 mg/kg SB in diets (Zhou et al., 2019). Silva et al. (2016) found that diets supplemented with 2% SB increased intestinal lipase of white prawns as compared with the non-supplemented group. However, Jesus et al. (2019b) supplemented Nile tilapia with different forms and concentrations of SB and found no change in intestinal digestive enzymes activities of fish. The variation among results may be due to the forms and concentrations of SB used, structural characteristics of digestive organs, variations in the types of enzymes and experimental conditions (Yao et al., 2019). Absorption of nutrients is affected by the brush-border membrane enzymes of the intestine, such as AKP, Na^+/K^+-ATPase and CK (Tibaldi et al., 2006). In golden pompano, diets supplemented with SB increased intestinal AKP, CK and Na^+/K^+-ATPase activities (Zhou et al., 2019). In the present study, an increase of intestinal AKP and γ-GT activities

was observed in yellow catfish fed with SB diets. SB enhanced intestinal enzymes activities of yellow catfish possibly partly due to its slight acidifying properties (Castillo et al., 2014; Silva et al., 2016).

4.3. Hepatopancreas and intestine morphometric parameters

The improvement of intestinal enzyme activities of fish fed with SB diets might be attributed to the integrity and growth of digestive organs. In Jian carp (*C. carpio* var. Jian) and white shrimp (*Penaeus setiferus*), digestive enzymes activity was correlated with growth and development of the hepatopancreas (Chen et al., 2012; Lovett and Felder, 1990). The present study showed that the weight and protein content of hepatopancreas had a similar trend to digestive enzyme activity, indicating there was a beneficial effect of SB on the hepatopancreas. Similarly, HSI was increased as dietary SB levels increased in golden pompano (Zhou et al., 2019). The intestine is the main site for nutrient absorption in fish. An increase in intestine length, weight and protein content was observed in fish fed with SB diets, indicating that SB promoted intestinal growth of yellow catfish. In Nile tilapia, protected forms of SB increased length, perimeter and villus area of the proximal intestine (Jesus et al., 2019b). In this study, dietary SB increased fold height and muscular thickness of proximal and distal intestine in yellow catfish. The observed increase in fold height and muscular thickness would presumably be conducive to increased absorption in the intestine of yellow catfish as in Jian carp (Chen et al., 2012). This finding was consistent with the corresponding feed conversion rate, weight gain and nutrient retention in yellow catfish fed with SB diets. Therefore, well-developed intestine, as those observed in this study for yellow catfish fed SB diets, correlates well with an improved nutrient uptake which was illustrated by significantly improved feed efficiency and final weight.

4.4. Chemical composition of whole fish and nutrition retention value

In the present study, whole-body composition and nutrient retention of yellow catfish were affected by dietary SB levels. Significantly higher levels of crude protein, ash, calcium, phosphorus in whole body and the retention of protein, lipid, calcium and phosphorus were found in fish fed with 1,000 mg/kg SB diet. Dietary SB is an important energy source for intestinal epithelial cells (Piva et al., 2009). An increase of whole-body crude protein and lipid contents in fish fed with SB diets may be attributed to SB's energy-supplying effect for animal metabolism, and it is not necessary to use more protein as an energy source (Jesus et al., 2019b). Thus, SB can spare the protein to ensure its use for fish growth (Lee, 2015). The increase in retention of calcium and phosphorus may be related to nutrient digestibility. Lin and Cheng (2017) suggested that 1% dietary butyrate increased the calcium and phosphorus digestibility in groupers. The respective addition of 5% and 3% dietary citric acid improved the absorption of calcium and phosphorus in rainbow trout (Sugiura et al., 2001) and Indian major carp (Baruah et al., 2005). Dietary SB may solubilize bone minerals in fish meal and reduce the antagonistic interaction between calcium and phosphorus at the intestinal brush border (Sugiura et al., 1998; Baruah et al., 2007).

4.5. Relative expression of TOR and LPL in intestine and hepatopancreas

Protein synthesis is a key component of fish growth. The present study showed that protein content in hepatopancreas, intestine and whole body increased with dietary SB, indicating the improvement of protein synthesis and nitrogen deposition. The *TOR* signaling pathway is involved in the cell growth and proliferation by regulating protein synthesis (Hay and Sonenberg, 2004). The relationship between nutrition retention and *TOR* signaling pathway has been reported in fish (Liang et al., 2016; Wu et al., 2019). However, the effect of SB on *TOR* signaling pathway of fish has not been reported. Relative expression of *TOR* mRNA in intestine and hepatopancreas was significantly up-regulated by dietary SB in this study, which indicated a similar trend with whole-body protein content of yellow catfish. This suggested that dietary SB might activate *TOR* signaling pathway, which improved protein synthesis of yellow catfish. SB could promote the proliferation of enterocytes and increase the expressions of intestinal total binding proteins by enlarging the absorption surface of the intestine (Scholzahrens et al., 2007). Liu et al. (2017) reported that butyrate could increase protein absorption by upregulation of the mRNA expression of intestinal amino acid transporters in grass carp. Butyrate, providing an energy source for animals, is a signaling molecule regulating animal energy metabolism (Samuel et al., 2008; Yu et al., 2017). To date, reports concerning the effect of SB on lipid metabolism of fish are scarce. In piglets, butyrate has been shown to promote differentiation and metabolic adaptations of adipocytes by down-regulating fatty acid synthesis genes and up-regulating fatty acid oxidation genes (Li et al., 2014; Lu et al., 2012). Although lipid metabolism of pigs was regulated in vivo or in vitro by SB, the effects of SB in fish have not been reported. Lipoprotein lipase is an important lipid regulatory enzyme involving hydrolyzing triglyceride of plasma lipoproteins and supplying free fatty acids for storage in adipocytes or for oxidation in other tissues (Zheng et al., 2013). In this study, the expression of hepatopancreas *LPL* was significantly up-regulated in the 2,000 mg/kg SB group, which is in agreement with the increase of whole-body fat in yellow catfish. This indicates that dietary SB might have a role in increasing lipid synthesis and decreasing hydrolysis of lipoproteins and triglyceride, and this explains the positive effects of SB on whole body lipid deposition of the fish. Butyrate has a direct impact on the lipid storage regulation of porcine adipocytes through inhibiting lipolysis and enhancing fatty acid synthesis (Yan and Ajuwon, 2015). However, Lu et al. (2012) reported that SB might decrease fat deposition of loin muscle in vitro though up-regulating the expression of oxidative genes. The exact mechanism by which SB regulates lipid metabolism of fish is still unknown and needs further investigation.

5. Conclusions

In conclusion, dietary SB improved the growth performance, digestive enzymes, body composition and nutrient retention of yellow catfish. The fish fed 500 or 1,000 mg/kg SB diet significantly increased weigh gain, feed efficiency, digestive tract health, and crude protein, ash, calcium, and phosphorus content of the whole body. However, a high level of SB (2,000 mg/kg) increased the lipid content in the whole body and the lipoprotein lipase mRNA expression in the hepatopancreas of fish. The addition of SB in diets of *P. fulvidraco* in the range of 500 to 1,000 mg/kg is recommended. This study brought new insights into the role of SB in improving growth performance, intestinal health and nutrient retention. Moreover, it is confidently predicted that SB could be widely used for increasing growth performance, nutrient retention and improving the health status of the digestive tract in other animals and humans.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that might inappropriately

influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (31402307), modern agricultural technology extension system construction project of Guangdong Province (2019KJ115), Science and Technology Planning Project of Guangdong Province (2019A050505007) and discipline team building projects of Guangdong Academy of Agricultural Sciences in the 13th Five-Year Period (201614TD).

References

Aalamifar H, Soltanian S, Vazirzadeh A, Akhlaghi M, Morshedi V, Gholamhosseini A, Mozanzadeh MT. Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). Aquacult Nutr 2020;26(1):156–64.

Abdel-Latif HMR, Abdel-Tawwab M, Dawood MAO, Menanteau-Ledouble S, El-Matbouli M. Benefits of dietary butyric Acid, sodium butyrate, and their protected forms in aquafeeds: a review. Rev Fish Sci Aquacult 2020. https://doi.org/10.1080/23308249.2020.1758899.

Abdel-Mohsen HH, Wassef EA, El-Bermawy NM, Abdel-Meguid NE, Saleh NE, Barakat KM, Shaltout OE. Advantageous effects of dietary butyrate on growth, immunity response, intestinal microbiota and histomorphology of European Seabass (*Dicentrarchus labrax*) fry. Egypt J Aquat Biol Fish 2018;22(4):93–110.

Ahmed HA, Sadek KM. Impact of dietary supplementation of sodium butyrate and/or protexin on the growth performance, some blood parameters, and immune response of *Oreochromis niloticus*. Int J Agric Innovat Res 2015;3:985–91.

Anguiano M, Pohlenz C, Buentello A, Gatlin DM. The effects of prebiotics on the digestive enzymes and gut histomorphology of red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops* × *M. saxatilis*). Br J Nutr 2013;109:623–9.

Association of Official Analytical Chemists (AOAC). Official methods of analysis of official analytical Chemists international. 16th ed. Arlington, VA: Association of Official Analytical Chemists; 1995.

Baruah K, Pal AK, Sahu NP, Jain KK, Mukherjee SC, Debnath D. Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of *Labeo rohita* (Hamilton) juveniles. Aquacult Res 2005;36:803–12.

Baruah K, Sahu N, Pal AK, Jain KK, Debnath D, Mukherjee SC. Dietary microbial phytase and citric acid synergistically enhances nutrient digestibility and growth performance of *Labeo rohita* (Hamilton) juveniles at sub-optimal protein. Aquacult Res 2007;38:109–20.

Bjerkeng B, Storebakken T, Wathne E. Cholesterol and short-chain fatty acids in diets for Atlantic salmon *Salmo salar* (L.): effects on growth, organ indices, macronutrient digestibility, and fatty acid composition. Aquacult Nutr 1999;5:181–91.

Bradford M. A rapid and sensitive method for the quantification of microgramquantities of protein utilizing the principle of protein dye-binding. Anal Biochem 1976;72:248–56.

Cao J, Chen Y, Zhu X, Huang Y, Zhao H, Li G, Lan H, Chen B, Pan Q. A study on dietary L-lysine requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquacult Nutr 2012;18:35–45.

Castillo S, Rosales M, Pohlenz C, Gatlin III DM. 2014. Effects of organic acids on growth performance and digestive enzyme activities of juvenile red drum *Sciaenops ocellatus*. Aquaculture 2014;433:6–12.

Chen GF, Feng L, Kuang SY, Liu Y, Jiang J, Hu K, Jiang W, Li S, Tang L, Zhou XQ. Effect of dietary arginine on growth, intestinal enzyme activities and gene expression in muscle, hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian). Br J Nutr 2012;108:195–207.

Chen Q, Zhao H, Huang Y, Cao J, Wang G, Sun Y, Li Y. Effects of dietary arginine levels on growth performance, body composition, serum biochemical indices and resistance ability against ammonia-nitrogen stress in juvenile yellow catfish (*Pelteobagrus fulvidraco*). Anim Nutr 2016;2(3):204–10.

Cheng ZY, Buentello A, Gatlin III DM. Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, *Sciaenops ocellatus*. Aquaculture 2011;319:247–52.

Ebrahimi M, Daeman NH, Chong CM, Karami A, Kumar V, Hoseinifar SH, Romano N. Comparing the effects of different dietary organic acids on the growth, intestinal shortchain fatty acids, and liver histopathology of red hybrid tilapia (*Oreochromis* sp.) and potential use of these as preservatives. Fish Physiol Biochem 2017;43:1195–207.

Gao YL, Storebakken T, Shearer KD, Penn M, Øverland M. Supplementation of fishmeal and plant protein-based diets for rainbow trout with a mixture of sodium formate and butyrate. Aquaculture 2011;311:233–40.

Griffith OW, Meister A. Excretion of cysteine and gamma-glutamylcysteine moieties in human and experimental animal gamma-glutamyl transpeptidase deficiency. Proc Natl Acad Sci U S A 1980;77:3384–7.

Guilloteau P, Zabielski R, David JC, Blum JW, Morisset JA, Biernat M, Wolinski J, Laubitz D, Hamon Y. Sodium-butyrate as a growth promoter in milk replacer formula for young calves. J Dairy Sci 2009;92:1038–49.

Hakim Y, Harpaz S, Uni Z. Expression of brush border enzymes and transporters in the intestine of European sea bass (*Dicentrarchus labrax*) following food deprivation. Aquaculture 2009;290:110–5.

Hay N, Sonenberg N. Upstream and downstream of mTOR. Gene Dev 2004;18:1926–45.

Hoseinifar SH, Sun YZ, Caipang CM. Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquacult Res 2017;48:1380–91.

Jesus GFA, Pereira SA, Owatari MS, Syracuse N, Silva BC, Silva A, Pierri BS, Lehmann NB, Figueiredo HCP, Fracalossi DM, Mouriño JLP, Martins ML. Protected forms of sodium butyrate improve the growth and health of Nile tilapia fingerlings during sexual reversion. Aquaculture 2019a;499:119–27.

Jesus GFA, Pereira SA, Owatari MS, Addam K, Silva BC, Sterzelecki FC, Sugai JK, Cardoso L, Jatobá A, Mouriño JLP, Martins ML. Use of protected forms of sodium butyrate benefit the development and intestinal health of Nile tilapia during the sexual reversion period. Aquaculture 2019b;504:326–33.

Krogdahl A, Marie Bakke-McKellep A. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon *(Salmo salar L.)*. Comp Biochem Physiol 2005;141A:450–60.

Lee CS. Dietary nutrients, additives and fish health. John Wiley & Sons; 2015.

Li G, Yao W, Jiang H. Short-chain fatty acids enhance adipocyte differentiation in the stromal vascular fraction of porcine adipose tissue. J Nutr 2014;144:1887–95.

Liang H, Ren M, Habte-Tsion H, Ge X, Xie J, Mi H, Xi B, Miao L, Liua B, Zhou Q, Fang W. Dietary arginine affects growth performance, plasma amino acid contents and gene expressions of the TOR signaling pathway in juvenile blunt snout bream, *Megalobrama amblycephala*. Aquaculture 2016;461:1–8.

Lin YH, Cheng MY. Effects of dietary organic acid supplementation on the growth, nutrient digestibility and intestinal histology of the giant grouper *Epinephelus lanceolatus* fed a diet with soybean meal. Aquaculture 2017;469:106–11.

Liu W, Yang Y, Zhang J, Gatlin DM, Ringø E, Zhou Z. Effects of dietary microencapsulated 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. Br J Nutr 2014;112:15–29.

Liu M, Guo W, Wu F, Qu Q, Tang Q, Gong W. Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp *(Ctenopharyngodon idellus)*. Aquacult Res 2017;48:4102–11.

Liu Y, Chen Z, Dai J, Yang P, Xu W, Ai Q, Zhang W, Zhang Y, Zhang Y, Mai K. Sodium butyrate supplementation in high-soybean meal diets for turbot *(Scophthalmus maximus L.)*: effects on inflammatory status, mucosal barriers and microbiota in the intestine. Fish Shellfish Immunol 2019;88:65–75.

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 2001;25:402–8.

Lovett DL, Felder DL. Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea Decapoda, Penaeidae). Biol Bull 1990;178:144–59.

Lu H, Su S, Ajuwon KM. Butyrate supplementation to gestating sows and piglets induces muscle and adipose tissue oxidative genes and improves growth performance. J Anim Sci 2012;90:430–2.

Luo Z, Wei CC, Ye HM, Zhao HP, Song YF, Wu K. Effect of dietary choline levels on growth performance, lipid deposition and metabolism in juvenile yellow catfish *Pelteobagrus fulvidraco*. Comp Biochem Physiol, B 2016;202:1–7.

Mitra G, Mukhopadhyay PK, Ayyappan S. Modulation of digestive enzyme activities during ontogeny of Labeo rohita larvae fed ascorbic acid enriched zooplankton. Comp Biochem Physiol, A 2008;149:341–50.

Mommsen TP. Paradigms of growth in fish. Comp Biochem Physiol, B 2001;129:207–19.

Ng WK, Koh CB. The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Rev Aquacult 2017;9:342–68.

Owen MAG, Waines P, Bradley G, Davies S. The effect of dietary supplementation of sodium butyrate on the growth and microflora of *Clarias gariepinus* (Burchell 1822). In: Proceedings of the XII international symposium fish nutrition and feeding; 2006. p. 149.

Piva A, Morlacchini M, Casadei G, Gatta PP, Biagi G, Prandini A. Sodium butyrate improves growth performance of weaned piglets during the first period after weaning. Ital J Anim Sci 2009;1:35–42.

Robles R, Lozano AB, Sevilla A, Marquez L, Nuez-Ortin W, Moyano FJ. Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*). Fish Physiol Biochem 2013;39:1567–80.

Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G proteincoupled receptor, Gpr41. Proc Natl Acad Sci USA 2008;105:16767–72.

Scholzahrens KE, Ade P, Marten B, Weber P, Timm W, Acil Y, Glüer C, Schrezenmeir J. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. J Nutr 2007;137:838–46.

Seiliez I, Gabillard JC, Skiba-Cassy S, Garcia-Serrana D, Gutiérrez J, Kaushik S, Panserat S, Tesseraud S. An in vivo and in vitro assessment of TOR signaling cascade in rainbow trout (*Oncorhynchus mykiss*). Am J Physiol Regul Integr Comp Physiol 2008;295:329–35.

Silva BC, Jesus GFA, Seiffert WQ, Vieira FN, Mouriño JLP, Jatobá A, Nolasco-Soria H. The effects of dietary supplementation with butyrate and polyhydroxybutyrate on the digestive capacity and intestinal morphology of Pacific White Shrimp (*Litopenaeus vannamei*). Mar Freshw Behav Physiol 2016;49:447–58.

Skiba-Cassy S, Lansard M, Panserat S, Médale F. Rainbow trout genetically selected for greater muscle fat content display increased activation of liver TOR signaling and lipogenic gene expression. Am J Physiol Regul Integr Comp Physiol 2009;297:1421–9.

Sugiura S, Dong F, Hardy R. Effects of dietary supplements on the availability of minerals in fish meal: preliminary observations. Aquaculture 1998;160:283–303.

Sugiura S, Gabaudan J, Dong F, Hardy R. Dietary microbial phytase supplementation and the utilization of phosphorus, traceminerals and protein by rainbow trout [*Oncorhynchus mykiss* (Walbaum)] fed soybean meal-based diets. Aquacult Res 2001;32:583–92.

Tibaldi E, Hakim Y, Uni Z, Tulli F, de Francesco M, Luzzana U, Harpaz S. Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass *(Dicentrarchus labrax)*. Aquaculture 2006;261:182–93.

Villanueva J, Vanacore R, Goicoechea O, Amthauer R. Intestinal alkaline phosphatase of the fish *Cyprinus carpio*: regional distribution and membrane association. J Exp Zool 1997;279:347–55.

Wallimann T, Hemmer W. Creatine kinase in non-muscle tissues and cells. Mol Cell Biochem 1994;33:93. 20.

Wu P, Tian L, Zhou X, Jiang W, Liu Y, Jiang J, Xie F, Kuang S, Tang L, Tang W, Yang J, Zhang Y, Shi H, Feng L. Sodium butyrate enhanced physical barrier function referring to Nrf2, JNK and MLCK signaling pathways in the intestine of young grass carp (*Ctenopharyngodon idella*). Fish Shellfish Immunol 2018;73:121–32.

Wu X, Wang L, Xie Q, Tan P. Effects of dietary sodium butyrate on growth, diet conversion, body chemical compositions and distal intestinal health in yellow drum (*Nibea albiflora*, Richardson). Aquacult Res 2019:1–11.

Yan H, Ajuwon KM. Mechanism of butyrate stimulation of triglyceride storage and adipokine expression during adipogenic differentiation of porcine stromovascular cells. PloS One 2015;10:e0145940.

Yao W, Li X, Kabir Chowdhury MA, Wang J, Leng X. Dietary protease, carbohydrase and micro-encapsulated organic acid salts individually or in combination improved growth, feed utilization and intestinal histology of Pacific white shrimp. Aquaculture 2019;503:88–95.

Yu S, Ren E, Xu J, Su Y, Zhu W. Effects of early intervention with sodium butyrate on lipid metabolismrelated gene expression and liver metabolite profiles in neonatal piglets. Livest Sci 2017;195:80–6.

Zhao HX, Cao JM, Huang YH, Zhou CP, Wang GX, Mo WY, Chen XY. Effects of dietary nucleotides on growth, physiological parameters and antioxidant responses of Juvenile Yellow Catfish *Pelteobagrus fulvidraco*. Aquacult Res 2017;48:214–22.

Zhao HX, Qiao GX, Cao JM, Huang YH, Wang GX, Chen B, Chen XY, Mo WY. Dietary supplementation of N-Carbamylglutamate and effects on growth, intestinal enzyme activities, immunological and antioxidant abilities of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Aquacult Nutr 2019;25:1250–60.

Zhao H, Chen B, Huang Y, Cao J, Wang G, Chen X, Mo W. Effects of dietary vitamin B_1 on growth performance, blood metabolites, body composition, intestinal enzyme activities and morphometric parameters of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Aquacult Nutr 2020;26(5):1681–90.

Zheng JL, Luo Z, Zhu QL, Tan XY, Chen QL, Sun LD, Hu W. Molecular cloning and expression pattern of 11 genes involved in lipid metabolism in yellow catfish *Pelteobagrus fulvidraco*. Gene 2013;531(1):53–63.

Zhou C, Lin H, Huang Z, Wang J, Wang Y, Yu W. Effect of dietary sodium butyrate on growth performance, enzyme activities and intestinal proliferation-related gene expression of juvenile golden pompano *Trachinotus ovatus*. Aquacult Nutr 2019;25:1261–71.