fishes

MDPI

Article

Effects of Dietary Sodium Butyrate on Growth Performance, Digestive Ability, Blood Biochemistry, and Ammonia Tolerance of Largemouth Bass (*Micropterus salmoides*)

Xuan Chen [1], Wu Chen [1], Yanjie Tang [1], Muzi Zhang [2] and Ming Li [1,*]

[1] School of Marine Sciences, Ningbo University, Ningbo 315211, China; 1139257263@163.com (X.C.); chenwu2025@163.com (W.C.); 1986258957@163.com (Y.T.)
[2] College of Animal Science, Guizhou University, Guiyang 550025, China; mzzhang3@gzu.edu.cn
* Correspondence: liming1@nbu.edu.cn

Abstract: The aim of this study was to investigate the effects of sodium butyrate (SB) on growth performance, digestive ability, blood health, and ammonia tolerance of largemouth bass. During the experiment, largemouth bass were fed different diets (0.00%, 0.50% and 1.00% SB) followed by a 96-h ammonia challenge. In this study, dietary supplementation of SB can improve the growth (weight gain rate increased; *GH* and *IGF 1* genes up-regulated) of largemouth bass. The addition of SB also significantly increased serum total protein, albumin and globulin contents and reduced triglycerides, cholesterol and aspartate transaminase contents. The digestive ability (pepsin, lipase, amylase, alkaline phosphatase, creatine kinase, gamma-glutamyltranspeptidase, sodium-potassium adenosine triphosphatase, villus height and muscular thickness increased) was significantly higher in the 0.50% and 1.00% SB groups. SB also improved the anti-inflammatory capacity (*IL 1* and *IL 8* genes down-regulated) of largemouth bass. The addition of SB to feed significantly reduced the cumulative mortality rate after 96 h of ammonia stress. SB significantly increased liver ammonia metabolism enzyme (arginase, argininosuccinate synthetase, ornithine transcarboxylase and argininosuccinate lyase) and inducible nitric oxide synthase activity, and significantly decreased the neuronal nitric oxide synthase activity. The results indicate that dietary supplementation of SB can promote growth and improve digestive ability, blood health, and ammonia tolerance in largemouth bass.

Keywords: sodium butyrate; growth; digestive ability; ammonia; *Micropterus salmoides*

Key Contribution: Dietary sodium butyrate supplementation significantly enhances growth performance, augments digestive function, optimizes serum biochemistry profiles, and mitigates ammonia-induced stress responses in largemouth bass.

check for updates

Academic Editor: Shao-Yang Hu

Received: 30 April 2025
Revised: 23 May 2025
Accepted: 26 May 2025
Published: 2 June 2025

Citation: Chen, X.; Chen, W.; Tang, Y.; Zhang, M.; Li, M. Effects of Dietary Sodium Butyrate on Growth Performance, Digestive Ability, Blood Biochemistry, and Ammonia Tolerance of Largemouth Bass (*Micropterus salmoides*). *Fishes* **2025**, *10*, 259. https://doi.org/10.3390/fishes10060259

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Short-chain fatty acids (SCFAs) are metabolites of intestinal flora and are involved in metabolism [1]. Many studies on aquatic animals have shown that SCFAs have important roles in growth promotion, intestinal flora function, intestinal mucosal barrier, energy metabolism, and immune functions [2–4]. Consequently, SCFAs are considered a promising additive for aquatic feed [5]. Butyric acid is a type of SCFA, and sodium butyrate (SB) is its sodium salt form [6]. Previous studies have shown that adding SB to the diet can promote fish growth, enhance antioxidant defense, and improve intestinal function and intestinal integrity, such as in grass carp (*Ctenopharyngodon idella*) [7], rainbow trout

(*Oncorhynchus mykiss*) [8], and turbot (*Scophthalmus maximu*) [9]. Furthermore, whether SB in the diet has other physiological functions remains to be further explored.

In recent years, due to the development of a high-density aquaculture model, the harm caused by ammonia stress to farmed fish has become increasingly serious [10]. Ammonia is toxic to most fish, causing growth inhibition, tissue damage, immunosuppression, impaired metabolic function, oxidative stress, and high mortality, such as in yellow catfish (*Pelteobagrus fulvidraco*) [11], common carp (*Cyprinus carpio*) [12], and largemouth bass (*Micropterus salmoides*) [13]. Common ammonia solutions include biological filtration, frequent water changes and nutrient regulation [14]. Nutritional regulation is considered to be the most effective means, such as taurine [15], inulin [16], carotenoid [17], and astaxanthin [18]. Recently, it has been reported that the addition of SB to dietary can alleviate ammonia toxicity in omnivorous fish, such as the case of yellow catfish [19]. However, it is not very clear whether dietary SB can alleviate the ammonia poisoning symptoms in carnivorous fish.

The largemouth bass is a typical carnivorous freshwater fish that was introduced to China in 1983 [20], with a production of 802 486 *t* in 2022. In order to increase production, largemouth bass mainly adopt a high-density culture mode, and ammonia stress becomes an inevitable problem in daily culture management. Ammonia levels in culture water have been reported to be as high as 46 mg/L, which exceeds the ammonia tolerance threshold for largemouth bass [21]. Recent studies have found SB to be increasingly effective in alleviating ammonia poisoning in aquatic animals [22]. Therefore, the current experiment was designed to evaluate the effects of dietary SB supplementation on the growth, digestive ability, blood health, urea cycle capacity, and ammonia tolerance of largemouth bass.

2. Materials and Methods

2.1. Experimental Diets

In studies with Nile tilapia (*Oreochromis niloticus*), the addition of 0.5% SB to the feed was found to improve growth performance [23]. Consequently, in this experiment, we formulated three experimental feeds: control group (add 0% SB), 0.50% SB group (add 0.50% SB) and 1.00% SB group (add 1.00% SB). Feed mill to make 2 mm and 3 mm pellets from well-mixed ingredients (F-26II, South China University of Technology, Guangzhou, China). Finished experimental feeds are air-dried and stored at −20 °C (Table 1).

Table 1. Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients	SB Levels (%)		
	0.00	**0.50**	**1.00**
Fish meal	15.00	15.00	15.00
Soybean meal	25.00	25.00	25.00
Soy protein concentrate	16.00	16.00	16.00
Corn gluten meal	9.00	9.00	9.00
Rapeseed meal	3.00	3.00	3.00
Cottonseed meal	2.00	2.00	2.00
Fish oil	2.50	2.50	2.50
Soyabean oil	2.50	2.50	2.50
Starch	19.00	19.00	19.00
Vitamin premix [a]	0.50	0.50	0.50
Mineral premix [b]	0.50	0.50	0.50
Monocalcium phosphate	1.00	1.00	1.00
Sodium chloride	2.00	2.00	2.00
Zeolite powder	2.00	1.50	1.00

Table 1. *Cont.*

Ingredients	SB Levels (%)		
	0.00	**0.50**	**1.00**
Sodium butyrate	0.00	0.50	1.00
Proximate composition			
Protein	39.48	39.12	39.01
Lipid	7.58	7.93	7.41

[a] Vitamin premix (per kg diet): VA, 8,000,000 IU; VD, 2,000,000 IU; VE, 5000 UI; VK, 1000 mg; VB_1, 1500 mg; VB_2, 1500 mg; VB_6, 800 mg; VB_{12}, 20 mg; nicotinamide, 400 mg; calcium pantothenate, 25 mg; folic acid, 25 mg; biotin, 8 mg; inositol, 100 mg. [b] Mineral premix (per kg diet): $MnSO_4 \cdot H_2O$, 50 mg; KI, 100 mg; $CoCl_2$ (1%), 100 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; $FeSO_4 \cdot H_2O$, 260 mg; $ZnSO_4 \cdot H_2O$, 150 mg; Na_2SeO_3 (1%), 50 mg.

2.2. Animals and Experimental Conditions

Largemouth bass were obtained from Xinyang and acclimatized for 2 weeks. Consistently sized fish weighing 7.40 ± 0.30 g were randomly assigned to 9 tanks with 30 fish and 300 L of water in each tank, with 3 replicates per group. One-third of the water in the fish tank is refreshed daily. During the 56-day experiment, dechlorinated tap water was used, water temperature was 28.00 ± 0.20 °C, pH 6.50–7.00, dissolved oxygen > 7.0 mg/L, nitrate ≤ 0.5 mg/L, total ammonia nitrogen (T-AN) ≤ 0.2 mg/L. Feed the fish regularly twice a day (8:00 and 18:00) until the fish are full and record the consumption of feed.

2.3. Sampling

After 56 days of feeding and 24 h of fasting, the fish were anesthetized by adding MS-222 (120 mg/L) to each tank, then weighed, measured and counted. Store intact fish (3 fish per tank) at −20 °C. Blood samples were collected from the caudal vein of fish (3 fish per tank), then subjected to centrifugation (3500× *g*, 10 min, 4 °C), followed by storage of the serum. Following euthanasia, the fish livers were isolated and their weights were measured. Store a portion of the liver at a temperature of −80 °C, while keeping the remaining part at −20 °C for storage purposes. Separate the intestines and store one part at −20 °C and the other part in a 4% paraformaldehyde solution. Isolation of intestinal content, stored at −20 °C. The research protocols and methodologies for the largemouth bass study were authorized by the Animal Ethics Committee at Ningbo University (AEC-LA-2025-E0006).

2.4. Ammonia Challenge

At the completion of feeding, 20 fish from each tank were placed in 33.24 mg/L T-AN (96 h LC_{50}) water for 96 h ammonia stress experiment [13]. Maintain ammonia concentration with 10 g/L NH_4Cl solution every 12 h. Daily monitoring of ammonia concentration with YSI ProPlus Multi-Parameter Water Quality Instrument (YSI Inc., Yellow Springs, OH, USA). Dissolved oxygen > 7.0 mg/L, water temperature 28.00 ± 0.20 °C, pH 6.50–7.00, nitrate ≤ 0.5 mg/L. During the experiment, deaths were recorded every 12 h and mortality was calculated. Furthermore, dead fish are removed in a timely manner, and fish exhibiting dead behavior are humanely euthanized. After 96 h, the fish were anesthetized with MS-222 (120 mg/L). Subsequently, three fish from each tank were selected and their livers were extracted and then stored at −20 °C.

2.5. Biochemical Analysis

Proximate composition of experimental samples and diets analyzed using AOAC (2003) standard techniques [24]. The sample was dried at 105 °C to a constant weight to obtain the moisture content. The lipid content was determined by soxtec system (2055 Soxtec Avanti; Foss Tecator, Hoganas, Sweden). The protein content was determined by

the FP-528 nitrogen analyzer of Leco Company in the United States. The ash content of the samples was determined by incinerating them in a muffle furnace (550 °C).

Serum biochemical components, including total protein (TP), albumin (ALB), globulin (GLO), triglyceride (TG), cholesterol (CHOL), alanine transaminase (ALT), and aspartate transaminase (AST) were determined by automatic chemical analyzer (7600-110, Hitachi, Tokyo, Japan).

Accurately weigh the tissues (liver, intestine and intestinal content), add 9 times the volume of phosphoric acid buffer (PBS), mix with a homogenizer (IKA T10 BASIC, IKA Works, Inc., Staufen, Germany) in an ice-water bath, and finally centrifuge (3500× *g*, 10 min, 4 °C) to obtain the supernatant. Lipase, pepsin, amylase, alkaline phosphatase (AKP), creatine kinase (CK), gamma-glutamyl transpeptidase (GGT), and sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) were assayed using commercial kit (Nanjing Jiancheng, Nanjing, China).

The PBS (9 times the volume) was added to the frozen livers for homogenization and then centrifuged (3000× *g*, 10 min, 4 °C). Argininosuccinate lyase (ASL), arginase (ARG), ornithine transcarboxylase (OTC), argininosuccinate synthetase (ASS), neuronal nitric oxide synthase (nNOS), and inducible nitric oxide synthase (iNOS) were determined with ELISA kits (Nanjing Jiancheng, China).

2.6. Histological Analysis

Fixed intestinal samples were paraffin-embedded, dehydrated and sectioned [25]. Tissue samples were stained for H&E (three slices were made for each sample) and then photographed using a Nikon TS100 optical microscope (Nikon, Tokyo, Japan). Villus height (VH) and intestinal muscular thickness (MT) were measured by organizational analysis software (Image J V 10.0) [26].

2.7. Q-PCR Analysis

Total RNA was isolated by RNAiso Reagent kit (Takara, Dalian, China), and first-strand cDNA was synthesized by Prime Script™ RT kit (Vazyme, Nanjing, China). The primers used are detailed in Table 2.

Table 2. All the primers used in this experiment.

Target Gene	Primer Sequence (5′–3′)	Size (bp)
GH	F: ATCAGAGCCAATCAGGACGG R: TACGTTCGTCTCAGCGACTC	112
IGF 1	F: AGTGCGATGTGCTGTATCTC R: TTGCTAGTCTTGGCAGGTG	281
IL 1	F: ACCTCTAACCCAGGGCTGAT R: GGTTCCATTCAGTGGCGAGA	102
IL 8	F: CAGTGGTGTCGCTGCATACG R: AGAGCCGTTTCTCCTGGTGA	129
β-actin	F: CTCTGCATACATGCCTACAC R: GTAGAGTTTCTCCCCATCAGG	117
GAPDH	F: GTGCCAGCCAGAACATCATCC R: GGACCGTCAAGTCAACCACTGA	141

PCR reactions were performed by SYBR Premix Ex Taq (Takara, Dalian, China) and Roche Light Cycler®480 II real-time PCR instrument (Roche, Basel, Switzerland). The 20 μL reaction system, including 8.8 μL dd H_2O, 10 μL 2 × Magic SYBR Mixture, 0.4 μL forward primer, 0.4 μL reverse primers and 0.4 μL cDNA. Reaction program: 95 °C 30 s, followed by 40 cycles: 95 °C 5 s, 60 °C 30 s. *β-actin* and *GAPDH* are housekeeping genes for gene expression calculations using the $2^{-\Delta\Delta CT}$ method [27].

3. Results

3.1. Dietary SB Supplementation on Growth

As shown in Table 3, final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), feed intake (FI), and condition factor (CF) were significantly higher in the 0.50% and 1.00% SB groups than those in the control group ($p < 0.05$). No significant variations were detected in the hepatosomatic index (HSI) and survival rate (SR) ($p > 0.05$).

Table 3. Effects of different dietary levels of SB on growth performance of largemouth bass.

	SB Levels (%)		
	0.00	**0.50**	**1.00**
FBW (g)	34.73 ± 0.37 [a]	39.47 ± 0.69 [b]	39.01 ± 0.36 [b]
WGR (%)	369.32 ± 5.05 [a]	433.38 ± 9.26 [b]	427.12 ± 4.85 [b]
SGR (%/d)	2.76 ± 0.02 [a]	2.99 ± 0.03 [b]	2.96 ± 0.02 [b]
FCR	1.47 ± 0.04	1.46 ± 0.02	1.47 ± 0.02
FI (g/fish)	37.88 ± 0.23 [a]	47.94 ± 0.83 [b]	47.70 ± 0.79 [b]
CF (g/cm^3)	1.57 ± 0.02 [a]	1.75 ± 0.04 [b]	1.67 ± 0.06 [b]
HSI (%)	1.74 ± 0.02	1.77 ± 0.05	1.75 ± 0.02
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Nine fish in each treatment group were utilized in the data analysis, and samples from each fish were analyzed three times ($n = 27$). Means with the same superscript letter on a line were not found to be significantly different based on Tukey's test ($p > 0.05$). Weight gain rate (WGR, %) = 100 × (final weight (g) − initial weight (g))/initial weight (g); Specific growth rate (SGR, %/day) = 100 × [ln (final weight (g)) − ln (initial weight (g))]/days; Feed conversion ratio (FCR) = dry diet fed (g)/wet weight gain (g); Feed intake (FI, g/fish) = total feed intake/[(initial number of fish in each tank + final number of fish in each tank)/2]; Condition factor (CF, g/cm^3) = 100 × [finial individual body weight (g)/finial individual body length (cm)^3]; Hepatosomatic index (HSI, %) = 100 × liver mass (g)/body mass (g); Survival rate (SR, %) = 100 × (number of final fish/numbers of initial fish); FBW (g): Final body weight.

Dietary SB supplementation can significantly increase the whole-body protein and ash contents ($p < 0.05$; Table 4). There was no significant effect of SB on whole-body lipid and moisture contents ($p > 0.05$).

Table 4. Effects of different dietary levels of SB on whole body proximate components of largemouth bass.

	SB Levels (%)		
	0.00	**0.50**	**1.00**
Protein (% dry matter)	57.70 ± 0.28 [a]	59.68 ± 0.42 [b]	58.98 ± 0.45 [b]
Lipid (% dry matter)	27.13 ± 0.63	27.49 ± 0.37	27.75 ± 0.24
Ash (% dry matter)	11.22 ± 0.44 [a]	12.48 ± 0.20 [b]	11.97 ± 0.28 [b]
Moisture (%)	69.83 ± 0.57	69.71 ± 0.40	69.40 ± 0.26

Nine fish in each treatment group were utilized in the data analysis, and samples from each fish were analyzed three times ($n = 27$). Means with the same superscript letter on a line were not found to be significantly different based on Tukey's test ($p > 0.05$).

Liver pepsin and lipase activities were significantly lower in the control group than those in the 0.50% and 1.00% SB groups ($p < 0.05$; Table 5). The pepsin, lipase and amylase activities in intestinal and intestinal content were significantly higher in the 0.5% SB and 1.00% SB groups than those in the control group ($p < 0.05$).

Table 5. Effects of different dietary levels of SB on digestive enzyme activities of largemouth bass.

	SB Levels (%)		
	0.00	**0.50**	**1.00**
	Liver		
Pepsin (U/mg prot)	6.69 ± 0.29 [a]	9.02 ± 0.53 [b]	8.80 ± 0.50 [b]
Lipase (U/g prot)	3.78 ± 0.05 [a]	3.95 ± 0.01 [b]	3.91 ± 0.03 [b]
Amylase (U/mg prot)	0.48 ± 0.02	0.46 ± 0.043	0.44 ± 0.04

Table 5. *Cont.*

	SB Levels (%)		
	0.00	**0.50**	**1.00**
Intestine			
Pepsin (U/mg prot)	0.75 ± 0.06 [a]	0.93 ± 0.05 [b]	0.91 ± 0.01 [b]
Lipase (U/g prot)	1.20 ± 0.01 [a]	1.46 ± 0.05 [b]	1.47 ± 0.04 [b]
Amylase (U/mg prot)	0.21 ± 0.01 [a]	0.24 ± 0.01 [b]	0.24 ± 0.03 [b]
Intestinal content			
Pepsin (U/mg prot)	6.68 ± 0.45 [a]	8.68 ± 0.36 [b]	8.92 ± 0.65 [b]
Lipase (U/g prot)	2.98 ± 0.05 [a]	3.12 ± 0.04 [b]	3.19 ± 0.01 [b]
Amylase (U/mg prot)	0.71 ± 0.01 [a]	0.81 ± 0.01 [b]	0.79 ± 0.03 [b]

Nine fish in each treatment group were utilized in the data analysis, and samples from each fish were analyzed three times (n = 27). Means with the same superscript letter on a line were not found to be significantly different based on Tukey's test ($p > 0.05$).

3.2. Dietary SB Supplementation on Health

The AKP, Na^+/K^+-ATPase, GGT, and CK activities were significantly higher in the 0.50% SB group, followed by the 1.00% SB group, and the lowest was in the control group ($p < 0.05$; Table 6).

Table 6. Effects of different dietary levels of SB on intestinal brush border membrane enzyme activity of largemouth bass.

	SB Levels (%)		
	0.00	**0.50**	**1.00**
AKP (U/mg prot)	37.12 ± 0.33 [a]	46.06 ± 0.70 [c]	45.44 ± 0.80 [b]
Na^+/K^+-ATPase (U/mg prot)	3.83 ± 0.37 [a]	6.86 ± 0.44 [c]	4.06 ± 0.35 [b]
GGT (U/mg prot)	8.75 ± 0.05 [a]	11.12 ± 0.09 [c]	10.01 ± 0.11 [b]
CK (U/mg prot)	19.88 ± 0.06 [a]	25.33 ± 0.15 [c]	21.12 ± 0.11 [b]

Nine fish in each treatment group were utilized in the data analysis, and samples from each fish were analyzed three times (n = 27). Means with the same superscript letter on a line were not found to be significantly different based on Tukey's test ($p > 0.05$). AKP: alkaline phosphatase; Na^+/K^+-ATPase: sodium-potassium adenosine triphosphatase; GGT: gamma-glutamyl transpeptidase; CK: creatine kinase.

Serum TP, ALB and GLO contents in the control group were the lowest, but TG, CHOL and AST contents were the opposite ($p < 0.05$; Table 7). No significant effect of SB on ALT was observed ($p > 0.05$).

Table 7. Effects of dietary SB supplemented with different levels for 56 days on serum biochemistry of largemouth bass.

	SB Levels (%)		
	0.00	**0.50**	**1.00**
TP (g/L)	33.63 ± 1.13 [a]	36.80 ± 1.30 [b]	36.83 ± 0.68 [b]
ALB (g/L)	11.53 ± 0.38 [a]	12.25 ± 0.15 [b]	12.65 ± 0.15 [b]
GLO (g/L)	21.04 ± 0.44 [a]	23.28 ± 0.74 [b]	23.05 ± 0.75 [b]
TG (mmol/L)	10.98 ± 0.52 [b]	8.60 ± 0.37 [a]	8.50 ± 0.08 [a]
CHOL (mmol/L)	0.13 ± 0.03 [b]	0.05 ± 0.01 [a]	0.06 ± 0.01 [a]
ALT (U/L)	2.33 ± 0.58	2.25 ± 0.25	2.50 ± 0.50
AST (U/L)	77.63 ± 4.13 [b]	62.88 ± 4.88 [a]	64.33 ± 2.31 [a]

Nine fish in each treatment group were utilized in the data analysis, and samples from each fish were analyzed three times (n = 27). Means with the same superscript letter on a line were not found to be significantly different based on Tukey's test ($p > 0.05$). TP: total protein; ALB: albumin; GLO: globulin; TG: triglyceride; CHOL: cholesterol; ALT: alanine transaminase; AST: aspartate transaminase.

Intestinal VH and MT were significantly increased in the SB groups compared to the control group ($p < 0.05$; Figure 1).

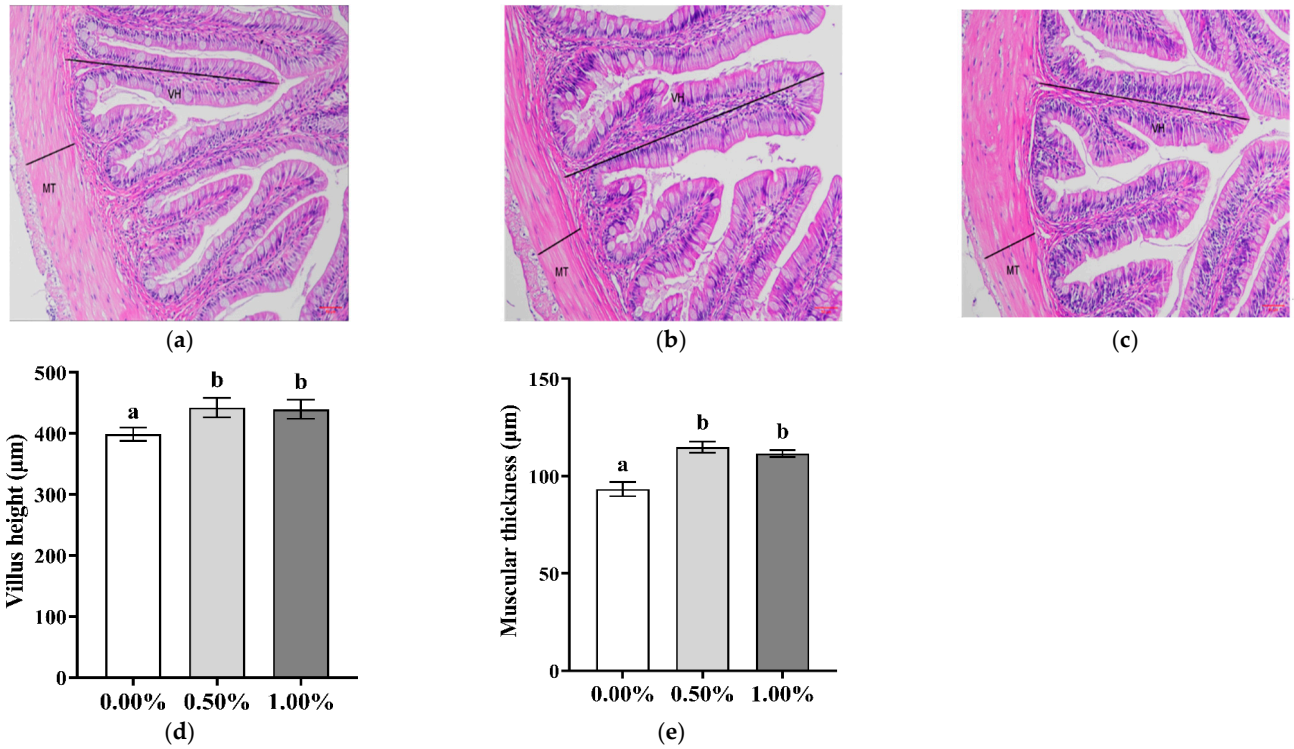


Figure 1. Effects of dietary SB at different levels for 56 d on intestinal morphology of largemouth bass. (**a**) 0.00% SB; (**b**) 0.50% SB; (**c**) 1.00% SB; (**d**) villus height (VH); (**e**) muscular thickness (MT). Nine fish in each treatment group were utilized in the data analysis, three slices were made for each fish, and each slice was measured three times (n = 81). Identical lowercase letters indicate no significant differences between groups ($p > 0.05$).

Both the 0.50% and 1.00% SB groups had significantly higher mRNA expression levels of *GH* and *IGF 1* genes in the liver than those in the control group ($p < 0.05$; Figure 2). No significant differences were found between the 0.50 and 1.00% SB groups ($p > 0.05$).

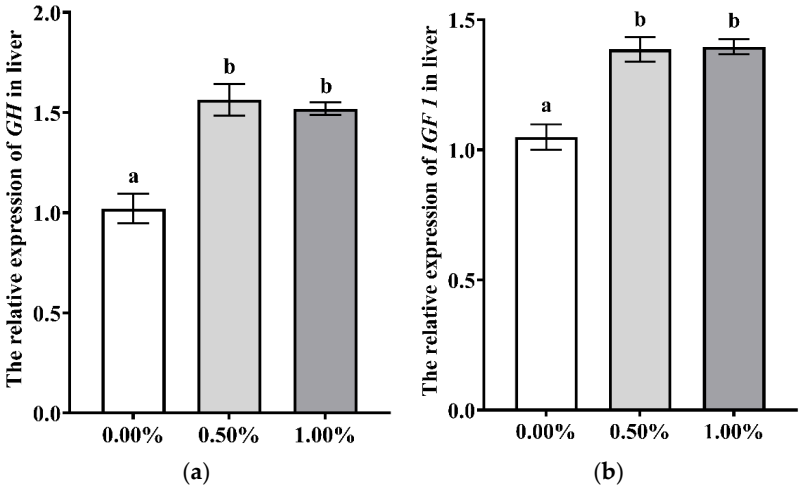


Figure 2. Relative expression levels of growth-related genes *GH* (**a**) and *IGF 1* (**b**) in the liver of largemouth bass after 56 days of culture. The relative expression of the transcript from qRT-PCR was calculated based on the standard curve and normalized to the *β-actin* and *GAPDH* mRNA level (n = 9). The same lowercase uppercase letters are not significantly different among groups ($p > 0.05$).

The mRNA expression levels of *IL 1* gene in the liver of the 0.50% SB group were significantly lower than that in the control group ($p < 0.05$; Figure 3). The mRNA expression

levels of *IL 8* gene in the liver were significantly lower in the 0.50% and 1.00% SB groups than in the control group ($p < 0.05$).

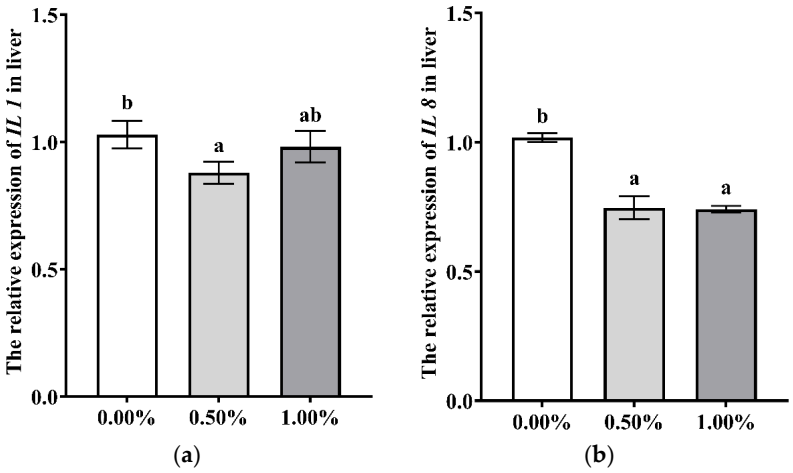


Figure 3. Relative expression levels of inflammation-related genes *IL 1* (**a**) and *IL 8* (**b**) in the liver of largemouth bass after 56 days of culture. The relative expression of the transcript from qRT-PCR was calculated based on the standard curve and normalized to the β-actin and GAPDH mRNA level ($n = 9$). The same lowercase uppercase letters are not significantly different among groups ($p > 0.05$).

3.3. Dietary SB Supplementation on Ammonia Tolerance

After ammonia stress, cumulative mortality rate (CMR) was significantly lower in the 0.50% and 1.00% SB groups than that in the control group, and lowest in the 0.50% SB group ($p < 0.05$; Figure 4).

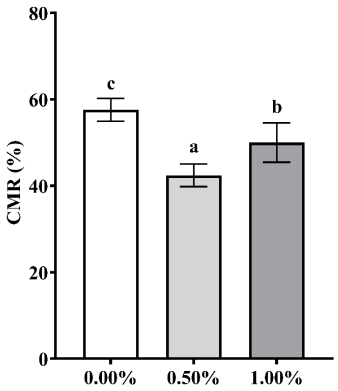


Figure 4. Effect of SB on cumulative mortality rate (CMR) of largemouth bass under 96 h of ammonia stress. The same lowercase uppercase letters are not significantly different among groups ($p > 0.05$).

After ammonia stress, liver ASS, ASL, ARG, OTC and iNOS activities in the 0.50% and 1.00% SB groups were significantly higher than those in the control group ($p < 0.05$; Table 8). The nNOS activity in the liver was significantly lower in the 0.50% and 1.00% SB groups than in the control group ($p < 0.05$).

Table 8. Effects of 96 h of ammonia exposure on the activities of ammonia metabolism enzymes and nitric oxide synthase in the liver of largemouth bass.

	SB Levels (%)		
	0.00	**0.50**	**1.00**
ASS (U/g prot)	9.33 ± 0.09 ^{a}	11.12 ± 0.05 ^{b}	11.08 ± 1.01 ^{b}
ASL (U/g prot)	15.35 ± 1.01 ^{a}	17.66 ± 0.05 ^{b}	17.35 ± 0.07 ^{b}
ARG (U/g prot)	6.58 ± 0.01 ^{a}	7.15 ± 0.26 ^{b}	7.09 ± 0.05 ^{b}

Table 8. *Cont.*

	SB Levels (%)		
	0.00	**0.50**	**1.00**
OTC (U/g prot)	15.35 ± 0.15 [a]	16.47 ± 0.09 [b]	16.89 ± 0.12 [b]
nNOS (U/g prot)	0.43 ± 0.04 [b]	0.35 ± 0.01 [a]	0.31 ± 0.03 [a]
iNOS (U/g prot)	0.66 ± 0.01 [a]	1.12 ± 0.01 [b]	1.15 ± 0.05 [b]

Nine fish in each treatment group were utilized in the data analysis, and samples from each fish were analyzed three times (n = 27). Means with the same superscript letter on a line were not found to be significantly different based on Tukey's test ($p > 0.05$). ASS: argininosuccinate synthetase; ASL: argininosuccinate lyase; ARG: arginase; OTC: ornithine transcarboxylase; nNOS: neuronal nitric oxide synthase; iNOS: inducible nitric oxide synthase.

4. Discussion

4.1. Dietary SB Supplementation on Growth

Previous studies have found that the addition of SB to feed can improve the growth performance of grass carp [28], golden pompano (*Trachinotus ovatus*) [29], yellow catfish [30] and Nile tilapia [31]. In this study, FBW and SGR were significantly improved by the addition of SB to the feed. In mammals, SB has been found to improve growth by increasing FI [32]. In this study, we also obtained the same finding that the addition of 0.50–1.00% SB to the dietary significantly increased the FI of largemouth bass.

It has been reported that the growth-promoting potential of SCFAs is mainly characterized by an increase in muscle protein content [33]. This is because SCFAs can be used directly as metabolic energy for a variety of physiological activities without the need to consume proteins for energy, leading to an increase in whole-body protein content [22,34]. It has been demonstrated that the addition of isobutyric acid can significantly increase the whole-body protein and ash contents of yellow catfish, which was in agreement with the results of our study [35].

Increasing intestinal digestive enzyme activity benefits fish nutrient digestion, including pepsin, lipase, amylase, AKP, CK, GGT and Na^+/K^+-ATPase [36,37]. A prior study indicated that SB promoted the activity of AKP, CK, GGT and Na^+/K^+-ATPase in the intestine of the golden pompano [29]. Similar findings were found in thinlip grey mullet (*Liza ramada*), where SB significantly increased intestinal pepsin, lipase and amylase activities [38]. In this study, dietary SB supplementation improved intestinal digestive enzyme activity in largemouth bass. This is because SB increases the absorptive capacity of the intestinal epithelial layer, increases the energy required by the epithelial lining, and lowers the intestinal pH, thereby increasing digestive enzyme activity [38]. It was also found that the ability of SB to promote digestion, absorption and utilization of feed is achieved by regulating the abundance of beneficial microorganisms secreted by digestive enzymes [39]. The study findings indicate that SB can enhance digestive enzyme activity and promote nutrient absorption, thus improving the largemouth bass growth performance. Intestinal digestion and absorption are also closely related to intestinal morphology [40]. VH and MT are important indicators for evaluating the digestive capacity of the animal intestine [41]. Our findings show that the addition of SB to the dietary increased intestinal VH and MT, in agreement with the results of studies in grass carp [42], Pengze crucian carp (*Carassius auratus* Pengze) [43] and thinlip grey mullet [38]. It can be seen that SB improves largemouth bass intestinal digestion and absorption through intestinal morphologic improvement.

Fish growth and development are also regulated by various growth factors, including growth hormone (*GH*) and insulin-like growth factor 1 (*IGF I*) [44,45]. A previous study reported that sodium butyrate nanoparticles (SB-NP) increased *GH* and *IGF I* gene expression levels in juvenile Nile tilapia, which is consistent with the results of the present study [46]. Our results suggest that the addition of SB benefits the expression of growth-related genes, thereby promoting growth.

4.2. Dietary SB Supplementation on Health

Blood parameters are important indicators for monitoring the health status of fish and help determine the response of fish to feed additives health status [47,48]. In this study, SB significantly increased TP, ALB and GLO levels. The most important compounds in serum are proteins, mainly ALB and GLO [49]. Research indicates that elevated serum TP levels can enhance the metabolism and immunity of fish [50]. AST and ALT are vital aminotransferases in fish, crucial for amino acid metabolism and serving as key indicators for a liver health assessment (Rahimnejad and Lee 2013) [51]. Elevated AST and ALT levels typically indicate liver dysfunction and have been utilized to evaluate stress reactions in fish [52]. When liver lipid metabolism and transport functionality are impaired, serum TG content is elevated [53]. Approximately 70% of serum CHOL originates from the liver, with the remaining 30% derived from the digestive tract [54]. Elevated serum CHOL levels are indicative of liver cell damage [16]. This study shows that SB improves blood health in largemouth bass.

Inflammation is an important component of tissue repair and is a defense mechanism in response to tissue damage [55]. The IL 1, IL 8, and TNF-α are key pro-inflammatory factors in fish, contributing significantly to the intestinal immune response [56,57]. Previous studies have found significant anti-inflammatory effects of SB on fish [7,43]. Aguilar et al. (2014) [58] suggest that SB may enhance anti-inflammatory capacity by inhibiting the *NF-κB* pathway and apoptosis. The results of this study indicated that SB could enhance largemouth bass's anti-inflammatory ability by down-regulating *IL 1* and *IL 8* gene mRNA expression levels.

4.3. Dietary SB Supplementation on Ammonia Tolerance

After ammonia stress, the CMR was significantly lower in the SB groups. The study findings demonstrated that SB effectively enhanced largemouth bass resistance to ammonia stress. Consistent with previous studies, dietary supplementation with SB enhances ammonia tolerance in fish [19]. The addition of SB reduced ammonia stress-induced mortality, which may be achieved through enhanced antioxidant, anti-inflammatory and anti-apoptotic properties [59,60]. Previous studies have shown that most fish can effectively eliminate endogenous ammonia via the urea cycle pathway [61]. ASS, ASL, ARG, and OTC are four key enzymes in the urea cycle [62]. The data outcomes showed that the addition of SB significantly increased urea cycle capacity. The nitric oxide cycle involves nitric oxide synthase (NOS), ASS and ASL, which play an important role in the immune response [63]. There are two types of NOS in fish: iNOS and nNOS [64]. iNOS has the ability to reduce mitochondrial oxidative stress-generated superoxide anions [65]. Nitric oxide produced by nNOS is involved in many pathophysiological processes [66]. In this study, we found that SB significantly increased iNOS activity and decreased nNOS activity. In conclusion, adding SB to the diet enhances the ammonia tolerance of largemouth bass by increasing the activity of enzymes related to the urea cycle. However, the enzyme activity is still affected by environmental factors, such as temperature and ph. Therefore, it is very important to further determine the appropriate addition amount of SB in the diet.

5. Conclusions

This study showed that dietary supplementation with SB can improve the growth performance of largemouth bass by increasing the FI, promote growth by increasing the activity of digestive enzymes and improving the intestinal morphology, improve blood health by increasing the content of TP, ALB and GLO, and by decreasing the content of TG, CHOL, and AST, and increase the activity of ammonia metabolism enzymes and iNOS, decrease nNOS, and enhance the capacity of urea cycle, thereby increasing ammonia

tolerance. The determination of the optimal addition amount of SB in the diet is an important study that urgently needs to be carried out.

Author Contributions: Conceptualization, X.C. and M.L.; methodology, W.C. and M.Z; investigation, X.C. and Y.T.; data curation, X.C. and W.C.; writing—original draft preparation, X.C.; writing—review and editing, X.C. and M.L.; supervision, M.L.; project administration, M.L.; funding acquisition, M.L. and M.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 32473130, 32202908, 32072948; the Science and Technology Cooperation Plan of Zhejiang Province of China, grant number 2024SNJF059.

Institutional Review Board Statement: The research protocols and methodologies for the largemouth bass study were authorized by the Animal Ethics Committee at Ningbo University (AEC-LA-2025-E0006, approval date: 30 October 2024).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Tahara, Y.; Yamazaki, M.; Sukigara, H.; Motohashi, H.; Sasaki, H.; Miyakawa, H.; Haraguchi, A.; Ikeda, Y.; Fukuda, S.; Shibata, S. Gut Microbiota-Derived Short Chain Fatty Acids Induce Circadian Clock Entrainment in Mouse Peripheral Tissue. *Sci. Rep.* **2018**, *8*, 1395. [CrossRef] [PubMed]
2. Li, S.; Heng, X.; Guo, L.; Lessing, D.J.; Chu, W. SCFAs improve disease resistance via modulate gut microbiota, enhance immune response and increase antioxidative capacity in the host. *Fish Shellfish Immunol.* **2022**, *120*, 560–568. [CrossRef]
3. Zhang, H.; Ding, Q.; Wang, A.; Liu, Y.; Teame, T.; Ran, C.; Yang, Y.; He, S.; Zhou, W.; Olsen, R.E.; et al. Effects of dietary sodium acetate on food intake, weight gain, intestinal digestive enzyme activities, energy metabolism and gut microbiota in cultured fish: Zebrafish as a model. *Aquaculture* **2020**, *523*, 735188. [CrossRef]
4. Zhou, W.-H.; Limbu, S.M.; Luo, Y.; Li, R.-X.; Ren, J.; Qiao, F.; Zhang, M.-L.; Du, Z.-Y. Dietary acetate promotes growth and nutrients deposition in *Nile tilapia* (*Oreochromis niloticus*) through increasing acetyl-CoA-triggered energy production. *Aquaculture* **2023**, *575*, 739750. [CrossRef]
5. Abdel-Latif, H.M.R.; Abdel-Tawwab, M.; Dawood, M.A.O.; Menanteau-Ledouble, S.; El-Matbouli, M. Benefits of Dietary Butyric Acid, Sodium Butyrate, and Their Protected Forms in Aquafeeds: A Review. *Rev. Fish. Sci. Aquac.* **2020**, *28*, 421–448. [CrossRef]
6. Hoseinifar, S.H.; Sun, Y.-Z.; Caipang, C.M. Short-chain fatty acids as feed supplements for sustainable aquaculture: An updated view. *Aquac. Res.* **2017**, *48*, 1380–1391. [CrossRef]
7. Tian, L.; Zhou, X.Q.; Jiang, W.D.; Liu, Y.; Wu, P.; Jiang, J.; Kuang, S.Y.; Tang, L.; Tang, W.N.; Zhang, Y.A.; et al. Sodium butyrate improved intestinal immune function associated with NF-kappaB and p38MAPK signalling pathways in young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol.* **2017**, *66*, 548–563. [CrossRef] [PubMed]
8. Mirghaed, A.T.; Yarahmadi, P.; Soltani, M.; Paknejad, H.; Hoseini, S.M. Dietary sodium butyrate (Butirex® C4) supplementation modulates intestinal transcriptomic responses and augments disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.* **2019**, *92*, 621–628. [CrossRef] [PubMed]
9. Yu, G.; Ou, W.; Ai, Q.; Zhang, W.; Mai, K.; Zhang, Y. In vitro study of sodium butyrate on soyasaponin challenged intestinal epithelial cells of turbot (*Scophthalmus maximus* L.) refer to inflammation, apoptosis and antioxidant enzymes. *Fish Shellfish Immunol. Rep.* **2021**, *2*, 100031. [CrossRef]
10. Yuan, D.; Wang, S.; Li, X.; Zhang, M.; Li, M. Effects of ammonia and roxithromycin exposure on skin mucus microbiota composition and immune response of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Fish Shellfish Immunol.* **2023**, *141*, 109048. [CrossRef]
11. Zhang, L.; Xiong, D.M.; Li, B.; Zhao, Z.G.; Fang, W.; Yang, K.; Fan, Q.X. Toxicity of ammonia and nitrite to yellow catfish (*Pelteobagrus fulvidraco*). *J. Appl. Ichthyol.* **2012**, *28*, 82–86. [CrossRef]
12. Yousefi, M.; Vatnikov, Y.A.; Kulikov, E.V.; Plushikov, V.G.; Drukovsky, S.G.; Hoseinifar, S.H.; Van Doan, H. The protective effects of dietary garlic on common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity. *Aquaculture* **2020**, *526*, 735400. [CrossRef]
13. Egnew, N.; Renukdas, N.; Ramena, Y.; Yadav, A.K.; Kelly, A.M.; Lochmann, R.T.; Sinha, A.K. Physiological insights into largemouth bass (*Micropterus salmoides*) survival during long-term exposure to high environmental ammonia. *Aquat. Toxicol.* **2019**, *207*, 72–82. [CrossRef] [PubMed]

14. Li, C.; Zhang, M.; Li, M.; Zhang, Q.; Qian, Y.; Wang, R. Effect of dietary alanyl-glutamine dipeptide against chronic ammonia stress induced hyperammonemia in the juvenile yellow catfish (*Pelteobagrus fulvidraco*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2018**, *213*, 55–61. [CrossRef]
15. Li, M.; Lai, H.; Li, Q.; Gong, S.; Wang, R. Effects of dietary taurine on growth, immunity and hyperammonemia in juvenile yellow catfish *Pelteobagrus fulvidraco* fed all-plant protein diets. *Aquaculture* **2016**, *450*, 349–355. [CrossRef]
16. Li, M.; Zhang, M.; Jiang, H.; Qin, C. Comparison of dietary arginine or/and inulin supplementation on growth, digestive ability and ammonia tolerance of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquac. Rep.* **2023**, *30*, 101543. [CrossRef]
17. Pan, C.H.; Chien, Y.H.; Wang, Y.J. Antioxidant defence to ammonia stress of characins (*Hyphessobrycon eques* Steindachner) fed diets supplemented with carotenoids. *Aquac. Nutr.* **2011**, *17*, 258–266. [CrossRef]
18. Jiang, X.; Zu, L.; Wang, Z.; Cheng, Y.; Yang, Y.; Wu, X. Micro-algal astaxanthin could improve the antioxidant capability, immunity and ammonia resistance of juvenile Chinese mitten crab, *Eriocheir sinensis*. *Fish Shellfish Immunol.* **2020**, *102*, 499–510. [CrossRef]
19. Zhao, H.; Peng, K.; Wang, G.; Mo, W.; Huang, Y.; Cao, J. Metabolic changes, antioxidant status, immune response and resistance to ammonia stress in juvenile yellow catfish (*Pelteobagrus fulvidraco*) fed diet supplemented with sodium butyrate. *Aquaculture* **2021**, *536*, 736441. [CrossRef]
20. Bai, J.; Lutz-Carrillo, D.J.; Quan, Y.; Liang, S. Taxonomic status and genetic diversity of cultured largemouth bass *Micropterus salmoides* in China. *Aquaculture* **2008**, *278*, 27–30. [CrossRef]
21. Cheng, C.-H.; Yang, F.-F.; Ling, R.-Z.; Liao, S.-A.; Miao, Y.-T.; Ye, C.-X.; Wang, A.-L. Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (*Takifugu obscurus*). *Aquat. Toxicol.* **2015**, *164*, 61–71. [CrossRef]
22. Li, X.; Wang, S.; Zhang, M.; Li, M. Sodium butyrate improves the growth performance, intestinal immunity, and ammonia tolerance of yellow catfish *Pelteobagrus fulvidraco* by increasing the relative abundance of *Candidatus_Arthromitus*. *Aquaculture* **2024**, *580*, 740359. [CrossRef]
23. Abd El-Naby, A.S.; Khattaby, A.E.-R.A.; Samir, F.; Awad, S.M.M.; Abdel-Tawwab, M. Stimulatory effect of dietary butyrate on growth, immune response, and resistance of *Nile tilapia*, *Oreochromis niloticus* against *Aeromonas hydrophila* infection. *Anim. Feed Sci. Technol.* **2019**, *254*, 114212. [CrossRef]
24. AOAC International. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, USA, 2003.
25. Mu, J.; Song, J.; Li, R.; Xue, T.; Wang, D.; Yu, J. Isovitexin prevents DSS-induced colitis through inhibiting inflammation and preserving intestinal barrier integrity through activating AhR. *Chem.-Biol. Interact.* **2023**, *382*, 110583. [CrossRef] [PubMed]
26. Egan, K.P.; Brennan, T.A.; Pignolo, R.J. Bone histomorphometry using free and commonly available software. *Histopathology* **2012**, *61*, 1168–1173. [CrossRef] [PubMed]
27. Schmittgen, T.D.; Livak, K.J. Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* **2008**, *3*, 1101–1108. [CrossRef]
28. Liu, M.; Guo, W.; Wu, F.; Qu, Q.; Tan, Q.; Gong, W. Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp (*Ctenopharyngodon idellus*). *Aquac. Res.* **2017**, *48*, 4102–4111. [CrossRef]
29. 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*. *Aquac. Nutr.* **2019**, *25*, 1261–1271. [CrossRef]
30. Zhao, H.; Wang, G.; Wang, H.; Mo, W.; Huang, Y.; Cao, J.; Li, P. Effects of dietary sodium butyrate on growth, digestive enzymes, body composition and nutrient retention-related gene expression of juvenile yellow catfish (*Pelteobagrus fulvidraco*). *Anim. Nutr.* **2021**, *7*, 539–547. [CrossRef]
31. Monier, M.N.; Abd El-Naby, A.S.; Samir, F.; Abdel-Tawwab, M. Positive effects of dietary nanosized sodium butyrate on growth performance, immune, antioxidant indices, and resistance of *Nile tilapia* to waterborne copper toxicity. *Aquac. Rep.* **2022**, *26*, 101323. [CrossRef]
32. Piva, A.; Morlacchini, M.; Casadei, G.; Gatta, P.P.; Biagi, G.; Prandini, A. Sodium butyrate improves growth performance of weaned piglets during the first period after weaning. *Ital. J. Anim. Sci.* **2016**, *1*, 35–41. [CrossRef]
33. Luz, J.R.; Ramos, A.P.S.; Melo, J.F.B.; Braga, L.G.T. Use of sodium butyrate in the feeding of *Arapaima gigas* (Schinz, 1822) juvenile. *Aquaculture* **2019**, *510*, 248–255. [CrossRef]
34. Jesus, G.F.A.; Pereira, S.A.; Owatari, M.S.; Addam, K.; Silva, B.C.; Sterzelecki, F.C.; Sugai, J.K.; Cardoso, L.; Jatobá, A.; Mouriño, J.L.P.; et al. Use of protected forms of sodium butyrate benefit the development and intestinal health of *Nile tilapia* during the sexual reversion period. *Aquaculture* **2019**, *504*, 326–333. [CrossRef]
35. Li, Z.; Wang, S.; Li, X.; Zhang, M.; Qian, Y.; Li, M. Effects of three short-chain fatty acids on growth, intestinal microbiota composition, and ammonia tolerance of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquac. Rep.* **2024**, *36*, 102066. [CrossRef]
36. Krogdahl, A.; Bakke-McKellep, A.M. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of *Atlantic salmon* (*Salmo salar* L.). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2005**, *141*, 450–460. [CrossRef]

37. 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*, 182–193. [CrossRef]
38. El-Sharkawy, E.A.; El-Razek, I.M.A.; Amer, A.A.; Soliman, A.A.; Shukry, M.; Gewaily, M.S.; Téllez-Isaías, G.; Kari, Z.A.; Dawood, M.A.O. Effects of sodium butyrate on the growth performance, digestive enzyme activity, intestinal health, and immune responses of Thinlip grey mullet (*Liza ramada*) juveniles. *Aquac. Rep.* **2023**, *30*, 101530. [CrossRef]
39. Taheri Mirghaed, A.; Fayaz, S.; Hoseini, S.M. Effects of dietary 1,8-cineole supplementation on serum stress and antioxidant markers of common carp (*Cyprinus carpio*) acutely exposed to ambient ammonia. *Aquaculture* **2019**, *509*, 8–15. [CrossRef]
40. Liu, W.; Yang, Y.; Zhang, J.; Gatlin, D.M.; Ringo, 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. [CrossRef]
41. Li, P.; Hou, D.; Zhao, H.; Peng, K.; Chen, B.; Guo, H.; Cao, J. Effects of dietary arginine levels on intestinal morphology, digestive enzyme activity, antioxidant capacity and intestinal flora of hybrid snakehead(*Channa maculata* ♀×*Channa argus* ♂). *Aquac. Rep.* **2022**, *25*, 101244. [CrossRef]
42. Zhou, J.S.; Guo, P.; Yu, H.B.; Ji, H.; Lai, Z.W.; Chen, Y.A. Growth performance, lipid metabolism, and health status of grass carp (*Ctenopharyngodon idella*) fed three different forms of sodium butyrate. *Fish Physiol. Biochem.* **2019**, *45*, 287–298. [CrossRef] [PubMed]
43. Fang, L.; Wang, Q.; Guo, X.; Pan, X.; Li, X. Effects of dietary sodium butyrate on growth performance, antioxidant capacity, intestinal histomorphology and immune response in juvenile Pengze crucian carp (*Carassius auratus* Pengze). *Aquac. Rep.* **2021**, *21*, 100828. [CrossRef]
44. Duan, C.; Hirano, T. Effects of insulin-like growth factor-I and insulin on the in-vitro uptake of sulphate by eel branchial cartilage: Evidence for the presence of independent hepatic and pancreatic sulphation factors. *J. Endocrinol.* **1992**, *133*, 211–219. [CrossRef]
45. Yan, J.; Herzog, J.W.; Tsang, K.; Brennan, C.A.; Bower, M.A.; Garrett, W.S.; Sartor, B.R.; Aliprantis, A.O.; Charles, J.F. Gut microbiota induce IGF-1 and promote bone formation and growth. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E7554–E7563. [CrossRef]
46. Abdel-Tawwab, M.; Shukry, M.; Farrag, F.A.; El-Shafai, N.M.; Dawood, M.A.O.; Abdel-Latif, H.M.R. Dietary sodium butyrate nanoparticles enhanced growth, digestive enzyme activities, intestinal histomorphometry, and transcription of growth-related genes in *Nile tilapia* juveniles. *Aquaculture* **2021**, *536*, 736467. [CrossRef]
47. Burgos-Aceves, M.A.; Lionetti, L.; Faggio, C. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. *Sci. Total. Environ.* **2019**, *670*, 1170–1183. [CrossRef]
48. Fazio, F. Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture* **2019**, *500*, 237–242. [CrossRef]
49. Kumar, S.; Sahu, N.; Pal, A.; Choudhury, D.; Yengkokpam, S.; Mukherjee, S. Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in juveniles. *Fish Shellfish Immunol.* **2005**, *19*, 331–344. [CrossRef] [PubMed]
50. Lin, H.; Tan, X.; Zhou, C.; Niu, J.; Xia, D.; Huang, Z.; Wang, J.; Wang, Y. Effect of dietary arginine levels on the growth performance, feed utilization, non-specific immune response and disease resistance of juvenile golden pompano *Trachinotus ovatus*. *Aquaculture* **2015**, *437*, 382–389. [CrossRef]
51. Rahimnejad, S.; Lee, K.-J. Dietary valine requirement of juvenile red sea bream *Pagrus major*. *Aquaculture* **2013**, *416–417*, 212–218. [CrossRef]
52. Liu, B.; Xie, J.; Ge, X.-p.; Miao, L.-h.; Wang, G. Effect of high dietary carbohydrate on growth, serum physiological response, and hepatic heat shock cognate protein 70 expression of the top-mouth culter *Erythroculter ilishaeformis* Bleeker. *Fish. Sci.* **2012**, *78*, 613–623. [CrossRef]
53. Tan, X.; Sun, Z.; Chen, S.; Chen, S.; Huang, Z.; Zhou, C.; Zou, C.; Liu, Q.; Ye, H.; Lin, H.; et al. Effects of dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile golden pompano *Trachinotus ovatus*. *Fish Shellfish Immunol.* **2017**, *66*, 198–206. [CrossRef] [PubMed]
54. Iqbal, M.; Yaqub, A.; Ayub, M. Partial and full substitution of fish meal and soybean meal by canola meal in diets for genetically improved farmed tilapia (*O. niloticus*): Growth performance, carcass composition, serum biochemistry, immune response, and intestine histology. *J. Appl. Aquac.* **2021**, *34*, 829–854. [CrossRef]
55. Li, M.Y.; Gao, C.S.; Du, X.Y.; Zhao, L.; Niu, X.T.; Wang, G.Q.; Zhang, D.M. Effect of sub-chronic exposure to selenium and astaxanthin on Channa argus: Bioaccumulation, oxidative stress and inflammatory response. *Chemosphere* **2020**, *244*, 125546. [CrossRef]
56. Li, M.; Zhang, M.; Qian, Y.; Shi, G.; Wang, R. Ammonia toxicity in the yellow catfish (*Pelteobagrus fulvidraco*): The mechanistic insight from physiological detoxification to poisoning. *Fish Shellfish Immunol.* **2020**, *102*, 195–202. [CrossRef]
57. Zhao, L.; Liang, J.; Chen, F.; Tang, X.; Liao, L.; Liu, Q.; Luo, J.; Du, Z.; Li, Z.; Luo, W.; et al. High carbohydrate diet induced endoplasmic reticulum stress and oxidative stress, promoted inflammation and apoptosis, impaired intestinal barrier of juvenile largemouth bass (*Micropterus salmoides*). *Fish Shellfish Immunol.* **2021**, *119*, 308–317. [CrossRef]

58. Aguilar, E.C.; Leonel, A.J.; Teixeira, L.G.; Silva, A.R.; Silva, J.F.; Pelaez, J.M.N.; Capettini, L.S.A.; Lemos, V.S.; Santos, R.A.S.; Alvarez-Leite, J.I. Butyrate impairs atherogenesis by reducing plaque inflammation and vulnerability and decreasing NFκB activation. *Nutr. Metab. Cardiovasc. Dis.* **2014**, *24*, 606–613. [CrossRef]
59. 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. [CrossRef]
60. Wu, P.; Tian, L.; Zhou, X.Q.; Jiang, W.D.; Liu, Y.; Jiang, J.; Xie, F.; Kuang, S.Y.; Tang, L.; Tang, W.N.; et al. 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*, 121–132. [CrossRef]
61. Song, P.; Li, M.; Zhang, M.; Jiang, H.; Shao, J.; Wang, R.; Qian, Y.; Feng, D. Inhibition of argininosuccinate synthase (ASS) affected ammonia excretion in yellow catfish *Pelteobagrus fulvidraco* during acute ammonia poisoning. *Aquac. Rep.* **2022**, *22*, 100931. [CrossRef]
62. Li, H.; Zhang, M.; Jiang, H.; Fan, Y.; Li, X.; Wang, R.; Qian, Y.; Li, M. Arginase plays an important role in ammonia detoxification of yellow catfish *Pelteobagrus fulvidraco*. *Fish Shellfish Immunol.* **2021**, *115*, 171–178. [CrossRef] [PubMed]
63. Nakamura, H.; Itoh, K.; Kawabuchi, M. NADPH-diaphorase and cytosolic urea cycle enzymes in the rat accessory olfactory bulb. *J. Chem. Neuroanat.* **1999**, *17*, 109–117. [CrossRef] [PubMed]
64. Lepiller, S.; Franche, N.; Solary, E.; Chluba, J.; Laurens, V. Comparative analysis of zebrafish nos2a and nos2b genes. *Gene* **2009**, *445*, 58–65. [CrossRef] [PubMed]
65. Achike, F.I. The l-arginine–nitric oxide pathway: A potential therapeutic target in dengue haemorhagic fever. *Clin. Exp. Pharmacol. Physiol.* **2008**, *35*, 1135–1136. [CrossRef]
66. Ayata, C.; Ayata, G.; Hara, H.; Matthews, R.T.; Beal, M.F.; Ferrante, R.J.; Endres, M.; Kim, A.; Christie, R.H.; Waeber, C.; et al. Mechanisms of Reduced Striatal NMDA Excitotoxicity in Type I Nitric Oxide Synthase Knock-Out Mice. *J. Neurosci.* **1997**, *17*, 6908–6917. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.