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Dietary supplementation with sodium propionate and tributyrin alleviated hepatic lipid deposition and improved the antioxidant capacity and hypoxic stress resistance of spotted seabass (*Lateolabrax maculatus*)

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We investigated the effects of dietary supplementation with sodium propionate (SP) and tributyrin (TB) on hepatic lipid deposition and antioxidant capacity of spotted seabass (*Lateolabrax maculatus*) via an 8-week feeding experiment and a hypoxia stress experiment. The fish were fed five experimental diets: a control diet (CON), a diet supplemented with 2 g/kg SP (SP-0.2%), 4 g/kg SP (SP-0.4%), 2 g/kg TB (TB-0.2%), or 4 g/kg TB (TB-0.4%). No significant difference in growth performance was presented among the groups ($P > 0.05$). The SP-0.4% and TB-0.2% groups presented significantly lower hepatosomatic and viscerasomatic indexes compared with the CON group. Then, the SP-0.4% and TB-0.2% groups presented stronger resistance to hypoxic stress than the other groups and were analyzed further. The hepatic histology and triglyceride levels revealed that SP-0.4% and TB-0.2% reduced hepatic lipid deposition. Similarly, the downregulation of malondialdehyde and the upregulation of total antioxidant capacity, superoxide dismutase, and catalase activities and the related gene expression levels revealed that SP-0.4% and TB-0.2% improved the antioxidant capacity. Additionally, the RNA sequencing demonstrated that SP-0.4% and TB-0.2% regulated gene expression to a similar extent. Among the 117 differentially expressed genes, 67 genes were enriched in the same pattern, and involved the FoxO signaling, PI3K-Akt signaling, and insulin-related pathways. In conclusion, supplementing SP-0.4% and TB-0.2% as feed additives effectively improved hepatic lipid metabolism, antioxidant capacity, and hypoxic stress resistance of spotted seabass.

KEYWORDS

sodium propionate, tributyrin, hepatic lipid deposition, antioxidant capacity, hypoxic stress

1 Introduction

With the rapid development of the global aquaculture industry, the occurrence of fish diseases is increasing, which has become an important factor affecting the sustainable and healthy development of aquaculture (Abdelsalam et al., 2023). Antibiotic misuse has led to the emergence of drug-resistant pathogens and drug residues, which seriously affects sustainable aquaculture development (Okocha et al., 2018; Shao et al., 2021). Therefore, efficient, green, and safe alternative antibiotic additives are increasingly attracting research attention.

Nutritional modulation of the immune system is a potentially powerful tool for improving fish health, and includes amino acids, fatty acids, vitamins, and minerals (Oliva-Teles, 2012; Pohlenz and Gatlin, 2014). Given their wide range of physiological functions and high safety, short-chain fatty acids (SCFAs) have important application potential for improving fish health, and have been identified as a potential alternative to traditional antibiotic treatments (Hoseinifar et al., 2017; Ng and Koh, 2017; Palma et al., 2023). SCFAs generally refer to organic acids with a carbon atomic number < 6, which are produced mainly by intestinal flora fermentation of fiber in food, among which the main functional products are acetic acid, propionic acid, and butyric acid (Tran et al., 2020; Rauf et al., 2022). Sodium propionate (SP) and tributyrin (TB) are stable and efficient forms of propionic acid and butyric acid, respectively (Miyoshi et al., 2011; Silva et al., 2016). Solid-form SCFAs are a practical addition to animal feed formulations due to their better handling and storage properties (Silva et al., 2016), and SP is a high-stability, high-safety chelated form of propionic acid that affects lipid metabolism and regulates the immune system. In a recent study, dietary supplementation with SP improved growth performance, hepatic lipid deposition, and health status in rainbow trout (*Oncorhynchus mykiss*) (Yousefi et al., 2024). TB is a butyric acid derivative formed by the esterification of three butyric acid molecules and one glycerol molecule (Gerunova et al., 2024), which is a naturally stable compound compared with butyrate and can effectively pass through the stomach and be broken down in the intestine, releasing butyric acid to function (Miyoshi et al., 2011; Hou et al., 2014). In addition, TB has fewer palatability issues than butyrate (Palma et al., 2023). In aquatic animals, TB also play a regulatory role in fish growth, metabolism and health. The growth-promoting effects of TB have been extensively reported, such as in snakehead fish (*Channa argus*) (Hou et al., 2019), blunt snout bream (*Megalobrama amblycephala*) (Liang et al., 2021), and common carp (*Cyprinus carpio*) (Xie et al., 2021), and the improvement of digestive enzyme activities may be an important reason. In addition, TB reduced triglycerides content and cholesterol levels in large yellow croaker (*Larimichthys crocea*) (Xu et al., 2021) and hybrid grouper *(Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂) (Yin et al., 2021), in which the hepatic lipid metabolism was involved. Moreover, TB could also modulate the antioxidant capacities of fish (Hou et al., 2019; Liang et al., 2021). Therefore, in addition to promoting growth, SP and TB modulated lipid metabolism and immune responses, presenting a stable and safe option for inclusion in animal feed formulations (Silva et al., 2016; Palma et al., 2023). However, few studies have focused on both SP and TB.

Spotted seabass (*Lateolabrax maculatus*) is a popular farmed fish in China because of its delicious meat, rapid growth, and high economic value, with a production of 246,918 tons in 2023 (Cai et al., 2020; Xing et al., 2023). High stocking density, excess feed, and exogenous pathogens endanger the health of spotted seabass (Dong et al., 2022). Abnormal hepatic lipid deposition and hypoxic stress are two major problems in fish farming, weakening immune function and disease resistance and even leading to death, which seriously damages economic interests (Hou et al., 2023). Therefore, the objectives of the present study were to investigate the effects of SP and TB on the hepatic lipid deposition and health status of spotted seabass, which will provide references for aquatic feed and improve our understanding of the functional mechanism of SCFAs in fish.

2 Materials and methods

2.1 Diet formulation

Five experimental diets were formulated: a control diet (CON), a diet supplemented with 2 g/kg SP (SP-0.2%), 4 g/kg SP (SP-0.4%), 2 g/kg TB (TB-0.2%), or 4 g/kg TB (TB-0.4%) (Table 1), the dosage of the additives used was referred to the previous studies (Safari et al., 2016; Safari et al., 2017; Cheng et al., 2021; Xu et al., 2021). The basis for the formulation of the control diet was referred to those of commercial feeds. White fish meal, poultry byproduct meal, soybean meal, corn gluten meal, and cottonseed protein concentrate were used as the source of protein. Fish oil and soybean oil were used as the source of lipid. Wheat flour was used as the main source of carbohydrate. The crude protein and crude lipid contents of the experimental diets were ~48% and ~16%, respectively. The SP (99.0%, #S100122) and TB (98%, #G106350) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (China), and the other ingredients were obtained from Guangdong Haid Group Co., Ltd. (China). The ingredients were crushed to a fine powder and mixed thoroughly to create pellets. The experimental diets were prepared and stored following previous study procedures (Li et al., 2019). According to a previous study (Li et al., 2023), the proximate composition of experimental diets was analyzed by the standard procedures of AOAC.

2.2 Experimental procedure and sampling

Spotted seabass of similar size (mean weight: ~58 g) were randomly assigned to the five groups. Each group contained four replicates, and each replicate contained 30 fish (in an indoor aquarium with a diameter of 2 meters). The fish were reared for 8 weeks. The fish were fed twice a day at 06:00 and 18:00. During the feeding experiment, the water temperature was maintained at 28 ± 1°, the dissolved oxygen was maintained at 7–7.5 mg/L, and the ammonia nitrogen was maintained at < 0.2 mg/L. After the feeding experiment, the fish were anesthetized using MS222 (1:10,000; Sigma–Aldrich, USA). All fish in each aquarium were weighed, the physical indices were measured, and the fish livers were dissected for analysis.

The feeding experiment was followed by a hypoxic stress experiment. For each group, three fish from four aquariums were

TABLE 1 Formulation and chemical proximate analysis of the experimental diets (%).

Ingredient [a]	CON	SP-0.2%	SP-0.4%	TB-0.2%	TB-0.4%
White fish meal	36	36	36	36	36
Poultry byproduct meal	10	10	10	10	10
Soybean meal	8	8	8	8	8
Corn gluten meal	10	10	10	10	10
Cottonseed protein concentrate	6	6	6	6	6
Wheat flour	13	13	13	13	13
Fish oil	10	10	10	10	10
Soybean oil	2	2	2	2	2
Vitamin premix	2	2	2	2	2
Mineral premix	2	2	2	2	2
SP	0	0.2	0.4	0	0
TB	0	0	0	0.2	0.4
Microcrystalline cellulose	1	0.8	0.6	0.8	0.6
Proximate analysis					
Dry matter	93.22	93.40	93.35	93.53	93.58
Protein	47.94	48.19	47.87	47.95	48.16
Lipid	15.92	16.21	16.16	16.04	15.93
Ash	8.23	8.32	8.28	8.31	8.26

Sodium propionate (SP) and tributyrin (TB) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (China), and the other ingredients were obtained from Guangdong Haid Group Co., Ltd. (China).

gathered in one aquarium. Finally, 12 fish were included in each group. The experimental fish were acclimated to the new environment for one day, and the dissolved oxygen concentration was maintained consistently (the dissolved oxygen was maintained at 7–7.5 mg/L). After a day of acclimatization, the oxygen supply was halted in all aquariums simultaneously. Subsequently, the number of dead fish was counted every 10 min to evaluate the hypoxic stress.

The experimental procedures were performed in strict accordance with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017).

2.3 Oil red O staining and triglyceride measurement

Oil red O staining was carried out as described previously (Li et al., 2021). Briefly, the fish liver (5 mm × 5 mm pieces) was stored in 4% paraformaldehyde before staining. The samples were sectioned into 6-µm sections using a cryostat microtome, fixed in cold 10% buffered formalin, and stained with oil red O. The TG content of the fish liver was measured using a commercial kit (#A110, Nanjing Jiancheng Bioengineering Institute, China) and the glycerol-3 phosphate oxidase phenol-4-chlorophenol aminophenazone (GPO-PAP) method.

2.4 Antioxidant capacity

Malondialdehyde (MDA, #BC0025), total antioxidant capacity (T-AOC, #BC1310), superoxide dismutase (SOD, #BC0170), and catalase (CAT, #BC1190) were measured using specific commercial kits (Solarbio, China). The reagent preparation, liver tissue homogenization, and operation were performed in strict accordance with the manufacturer's instructions.

2.5 Real-time quantitative PCR

RT-qPCR was carried out according to a previous study (Cui et al., 2020). Briefly, total RNA was extracted from liver tissue using TRIzol according to the manufacturer's instructions (Takara, Japan). The RNA was reverse-transcribed into complementary DNA (cDNA) using a PrimeScript™ Reverse RT Reagent Kit (Takara). PCR amplification was performed containing 1 µl cDNA, 1 µl each primer, 12.5 µl PrimeSTAR® Max DNA Polymerase (Takara), and 9.5 µl RNase-free water. The PCR cycling conditions were 10 s at 98°, 15 s at 58°, and 20 s at 72° for 35 cycles. A single PCR product was confirmed by a melting curve analysis. The RT-qPCR primers referenced that of a previous study (Tan et al., 2017) (Table 2). The housekeeping gene was *β-actin*. The gene expression levels were calculated using the comparative threshold (CT) cycle ($2^{-\Delta\Delta CT}$) method (Livak and Schmittgen, 2001).

TABLE 2 The RT-qPCR primers used in this study.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product length
β-actin	CAACTGGGATGACATGGAGAAG	TTGGCTTTGGGGTTCAGG	114
nrf2	AGAAGGAGCGTCTGTTGAGTGA	GGAAGATGCTGCCGTTAGTTGA	174
sod	AGAATCATGCCGGTCCTAATG	CGGTGATGTCTATCTTGGCTAC	96
cat	TGTGGGACTTCTGGAGCCTGAG	TGTGAGAGCCGTAGCCGTTCAT	111

nrf2, nuclear erythroid 2-related factor 2; sod, superoxide dismutase; cat, catalase.

2.6 RNA sequencing

The RNA extraction, cDNA library construction, and sequencing were conducted by Majorbio (China). The gene expression levels were calculated using transcripts per million reads (TPM) in RSEM (http://deweylab.biostat.wisc.edu/rsem/), and the threshold for significance was a *P*-adjusted value of 0.05. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg) was used for pathway enrichment, and the significance threshold in the KEGG pathway enrichment was a *P*-adjusted value of 0.05. The heatmap analysis was conducted using fastcluster, which used $log_2 10$ gene expression, and the subcluster was calculated according to the relative gene distance. The raw data were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (PRJNA1150789).

2.7 Calculations and statistical analysis

Survival rate (SR, %) = (final fish number/initial fish number) × 100;

Weight gain (WG, %) = (final body weight − initial body weight) × 100/initial body weight;

Feed conversion ratio (FCR) = feed consumption/body weight gain;

Condition factor (CF, g/cm^3) = (final body weight/final body length^3) × 100;

Hepatosomatic index (HSI, %) = (liver weight/body weight) × 100;

Viscerosomatic index (VSI, %) = (viscera weight/body weight) × 100.

All statistical analyses were conducted using SPSS 25.0 (IBM, USA). All data are reported as the mean ± standard error of the mean (SEM). All data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple range test or independent sample *t*-test. Differences were considered statistically significant when $P < 0.05$.

3 Results

3.1 SP and TB supplementation on survival and growth

The survival rates of the five groups ranged from 97-99%, and there were no significant differences among the groups ($P > 0.05$). Compared with those of the CON group, the growth performance, including final body weight, WG rate, and FCR, was not significantly different among the groups, although the SP-0.2% group presented a slightly higher WG rate and slightly lower FCR ($P > 0.05$) (Table 3).

3.2 SP and TB supplementation on physical indices

The CF was not significantly different among the groups. Notably, the HSI and VSI were different among the groups, and the SP-0.4% and TB-0.2% groups had a significantly lower HSI and VSI ($P < 0.05$) (Table 4), revealing a potentially lower hepatic lipid deposition.

3.3 SP and TB supplementation on hypoxic stress

Hypoxic stress causes severe losses in farmed spotted seabass, thus the 8-week feeding experiment was followed by a hypoxic stress experiment. The CON fish began to die after 6 h after the oxygen supply was halted (the dissolved oxygen in each group reduced to approximately 1 mg/L), and all CON fish died after 7 h after oxygen was halted, demonstrating the weakest hypoxia resistance in all groups (Figure 1). Notably, all the SP and TB fish began to die later than that of the CON fish. The SP-0.4% and TB-0.2% fish began to die last, and the total death time in the SP-0.4% and TB-0.2% groups was also the longest, as it was ~2 h longer than that of the CON group, indicating stronger hypoxia stress resistance.

3.4 SP and TB supplementation on oil red O staining and TG levels

Considering that the SP-0.4% and TB-0.2% groups had lower HSI and VSI than the CON group (Table 4), oil red O staining was conducted to determine the fish hepatic TG levels (Figure 2). The results revealed that the SP-0.4% and TB-0.2% groups had smaller lipid droplets and significantly downregulated liver TG levels, indicating that SP-0.4% and TB-0.2% dietary supplementation reduced hepatic lipid deposition.

3.5 SP and TB supplementation on antioxidant capacity

The results of antioxidant capacity revealed that the SP-0.4% and TB-0.2% groups had significantly lower MDA contents (*P* <

TABLE 3 Effects of dietary sodium propionate (SP) and tributyrin (TB) on spotted seabass survival and growth.

Parameter	CON	SP-0.2%	SP-0.4%	TB-0.2%	TB-0.4%
SR/%	98.00 ± 1.15	99.00 ± 1.00	98.00 ± 2.00	97.00 ± 3.00	99.00 ± 1.00
IBW/g	58.36 ± 0.15	58.32 ± 0.09	58.12 ± 0.08	58.21 ± 0.08	58.38 ± 0.05
FBW/g	158.26 ± 1.46	160.16 ± 1.37	158.62 ± 0.47	157.73 ± 2.20	158.02 ± 1.23
WG/%	171.54 ± 1.91	174.75 ± 2.53	172.92 ± 0.93	170.97 ± 3.92	170.68 ± 2.23
FCR	1.02 ± 0.01	0.99 ± 0.01	1.03 ± 0.01	1.04 ± 0.02	1.04 ± 0.01

No significant differences were presented among survival and growth parameters ($P > 0.05$).
SR, survival rate; IBW, initial body weight; FBW, final body weight; WG, weight gain; FCR, feed conversion ratio.

0.05) and significantly upregulated T-AOC, SOD, and CAT, and the expression levels of the antioxidant capacity-related genes: *nrf2* (nuclear erythroid 2-related factor 2), *cat*, and *sod* ($P < 0.05$) (Figure 3). The results suggested that the SP-0.4% and TB-0.2% groups had improved antioxidant capacity.

3.6 SP and TB supplementation on RNA-seq

The SP-0.4% and TB-0.2% regulation of hepatic gene expression were analyzed using RNA-seq (Figure 4). Compared with the CON group, SP upregulated 57 genes and downregulated 28 genes, while TB upregulated 43 genes and downregulated 5 genes. Additionally, TB upregulated only 7 genes and downregulated 9 genes compared with SP, revealing that SP and TB had similar effects on gene expression levels (Figure 4A). The Venn diagram and heatmap analyses supported these results (Figures 4B, E). The subcluster analysis revealed that 67 genes from 117 differentially expressed genes were enriched in the same cluster (Figure 4F). KEGG enrichment analysis revealed that the FoxO signaling, PI3K-Akt signaling, and insulin-related pathways were key in SP and TB functions (Figures 4C, D, G).

4 Discussion

In the present study, the survival rate and growth performance were not significantly different among the groups. Previous studies reported that SCFAs had no significant growth-promoting effects on Atlantic salmon (*Salmo salar*) (Bjerkeng et al., 1999), rainbow trout (*Oncorhynchus mykiss*) (Gao et al., 2011), gilthead sea bream (*Sparus aurata*) (Benedito-Palos et al., 2016), and red hybrid tilapia (*Oreochromis* sp.) (Ebrahimi et al., 2017). However, SCFAs promoted the growth performance of sea bream (*S. aurata*) (Robles et al., 2013), grass carp (*Ctenopharyngodon idellus*) (Liu et al., 2017), Nile tilapia (*Oreochromis niloticus*) (Hu et al., 2018), turbot (*Scophthalmus maximus*) (Liu et al., 2019), golden pompano (*Trachinotus ovatus*) (Zhou et al., 2019), Barramundi (*Lates calcarifer*) (Aalamifar et al., 2020), and yellow drum (*Nibea albiflora*) (Wu et al., 2020). There are hundreds of fish species farmed around the world, and they have different feeding habits and different living environments. Therefore, the different results might primarily be due to the fish species and the experimental conditions. Then, the differences in basal feed formula and supplementation levels can also lead to different results.

The liver is key to maintaining normal fish growth and health, and excessive hepatic lipid deposition can severely affect liver function. In mammals, SCFAs play a role in energy homeostasis and metabolism (den Besten et al., 2013; Sahuri-Arisoylu et al., 2016), and SCFAs alleviate lipid deposition in the liver (Morrison and Preston, 2016). In the present study, both SP-0.4% and TB-0.2% downregulated the fish HSI, VSI, and TG, reducing hepatic lipid deposition. The downregulation of HSI was consistent with that reported in previous fish studies. In juvenile Pengze crucian carp (*Carassius auratus* Pengze), the HSI and lipid were significantly decreased in supplementing SB groups compared with that of the control group, and the antioxidant capacity, intestinal histomorphology, and immune response were all improved (Fang et al., 2021). In juvenile largemouth bass (*Micropterus salmoides*), dietary supplementation with 2.0 g/kg SB had significantly lower HSI, and the antioxidant activities, inflammatory response, and resistance to hypoxic stress were all improved (Hou et al., 2023). Therefore, the improvement of

TABLE 4 Effects of dietary sodium propionate (SP) and tributyrin (TB) on spotted seabass physical indices.

Parameter	CON	SP-0.2%	SP-0.4%	TB-0.2%	TB-0.4%
CF/(g/cm^3)	1.94 ± 0.01	1.95 ± 0.04	1.89 ± 0.03	1.91 ± 0.06	1.93 ± 0.04
HSI/%	0.85 ± 0.03^a	0.82 ± 0.06^{ab}	0.77 ± 0.05^b	0.71 ± 0.02^b	0.89 ± 0.04^a
VSI/%	12.04 ± 0.24^a	12.31 ± 0.44^a	10.83 ± 0.36^b	10.80 ± 0.62^b	11.32 ± 0.42^{ab}

Values (mean ± SEM, n = 4) in the same row with different superscript letters are significantly different ($P < 0.05$).
CF, condition factor; HSI, hepatosomatic index; VSI, viscerosomatic index.

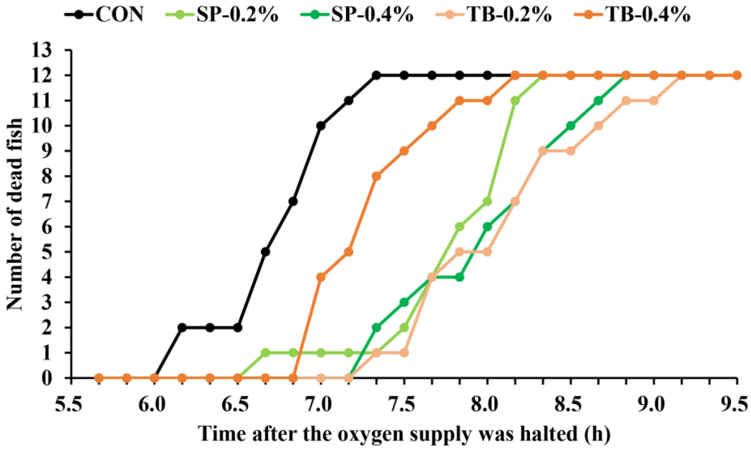

FIGURE 1
Number of dead fish after the oxygen supply was halted. After the feeding experiment and sampling, the oxygen supply was halted simultaneously, and the number of dead fish in each group was counted every 10 min.

physical indices by SCFAs is often accompanied by the promotion of health. The factors affecting lipid deposition include absorption, lipogenesis, and lipid oxidation. Under certain conditions of absorption, lipogenesis and lipid oxidation determine the level of lipid deposition. In juvenile large yellow croaker (*Larimichthys crocea*), replacement of fish oil with soybean oil in diets significantly increased the lipid deposition in the liver, and SCFAs alleviated the abnormal lipid deposition by decreasing the expression of lipogenesis-related genes and increasing the expression of lipid oxidation-related genes (Xu et al., 2021). Therefore, SCFAs could be useful for reducing hepatic lipid deposition in fish, which could regulate lipogenesis and lipid oxidation at the same time.

Unlike terrestrial animals, fish basically obtain their oxygen requirements from water, and hypoxic stress has received more attention. Hypoxic stress is an important factor affecting the

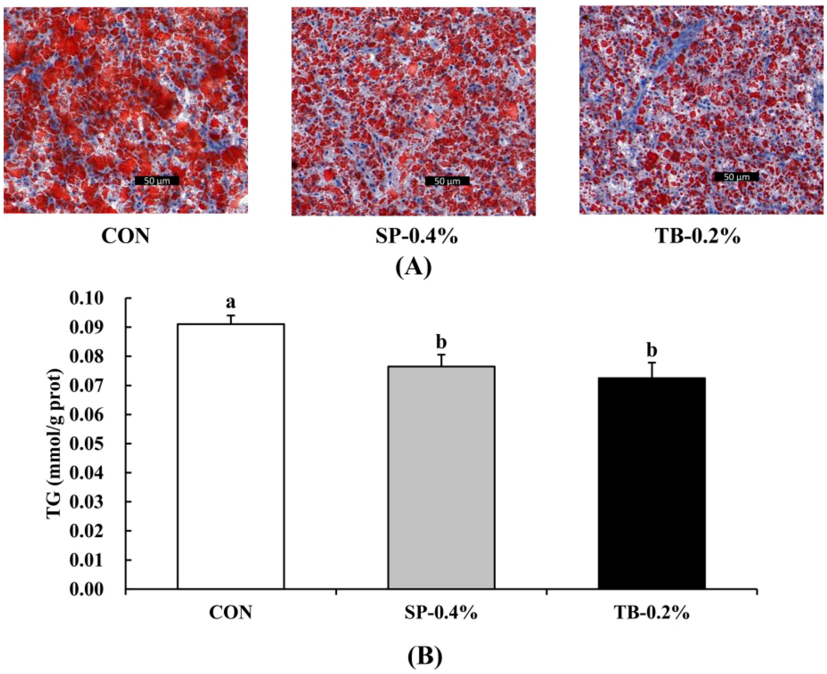

FIGURE 2
Effects of SP and TB dietary supplementation on hepatic lipid deposition. **(A)** Liver histochemical characteristics (oil red O staining). Scale bars = 50 µm. **(B)** Liver TG levels. Mean values (mean ± SEM, n = 4) with different letters are significantly different (*P* < 0.05).

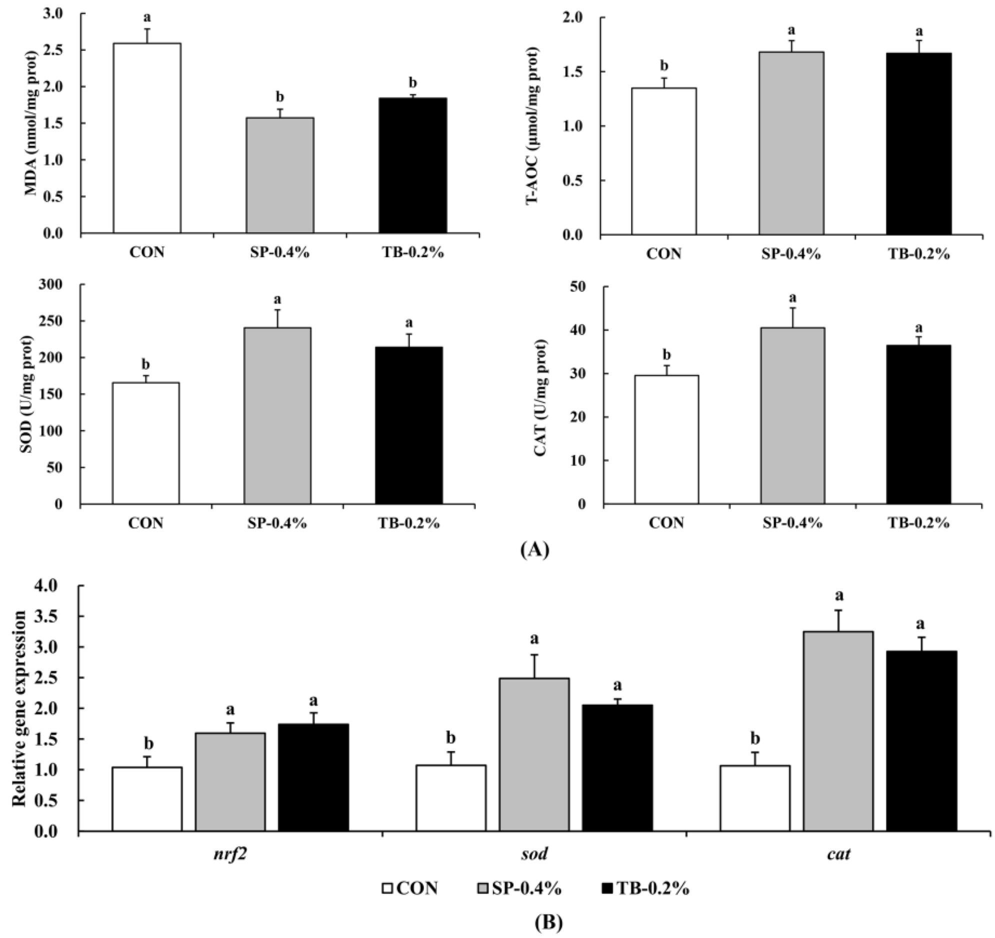

FIGURE 3
Effects of SP and TB dietary supplementation on antioxidant capacity. **(A)** MDA, T-AOC, SOD, and CAT levels. **(B)** Relative gene expression levels of *nrf2*, *sod*, and *cat*. Mean values (mean ± SEM, n = 4) with different letters are significantly different ($P < 0.05$).

survival rate of spotted bass in aquaculture. In the present study, both SP-0.4% and TB-0.2% improved the antioxidant capacity and hypoxic stress resistance of spotted seabass. In aquaculture, water eutrophication and increased culture density can decrease dissolved oxygen concentrations, increasing the risk of hypoxic stress (Hou et al., 2023; Jia et al., 2023), especially for spotted seabass, which has a relatively high oxygen demand. In fish, hypoxia stress leads to excessive accumulation of reactive oxygen species, leading to protein denaturation, lipid peroxidation, and even cell damage (Martínez-Álvarez et al., 2005; Peng et al., 2022), which can cause many diseases and even death (Zhao et al., 2020). The antioxidant system, which mainly consists of the antioxidant enzyme, can prevent the production of reactive oxygen species and avoid cell damage (Hughes, 1999). In previous studies, SCFAs improved the antioxidant enzymes activities, including SOD, CAT, and T-AOC, and reduced the MDA in zebrafish (*Danio rerio*) (Safari et al., 2016), carp (*Cyprinus carpio* L.) (Safari et al., 2017), Nile tilapia (*O. niloticus*) (Dawood et al., 2020), black sea bream (*Acanthopagrus schlegelii*) (Volatiana et al., 2020), yellow catfish (*Pelteobagrus fulvidraco*) (Zhao et al., 2021), and grass carp (*C. idella*) (Cheng et al., 2021), thereby improving the fish health. Furthermore, the improvement of antioxidant capacity improved the stress resistance in fish. SCFAs improved hypoxia stress resistance in largemouth bass (*M. salmoides*) (Hou et al., 2023) and improved ammonia stress resistance in yellow catfish (*P. fulvidraco*) (Zhao et al., 2021). Therefore, SCFAs could be used to improve antioxidant capacity and hypoxic stress resistance in aquaculture, thereby improving the survival rate of fish in aquaculture.

RNA-seq was conducted to analyze the global regulation of gene expression and explore the potential functional pathways of SP and TB. The results revealed that SP-0.4% and TB-0.2% had similar effects on the gene expression levels, and the FoxO signaling, PI3K-Akt signaling, and insulin-related pathways might be key pathways involved in their functions. The three signaling pathways all played key roles in lipid metabolism and health (Sell et al., 2012; Martini et al., 2014; Farhan et al., 2017), which improved the understanding of the functional mechanism of SCFAs in fish. There are a few studies on the mechanism of SCFA function in aquatic animals. In grass carp (*C. idella*), sodium butyrate improved the intestinal immune function associated with the NF-κB and p38-MAPK

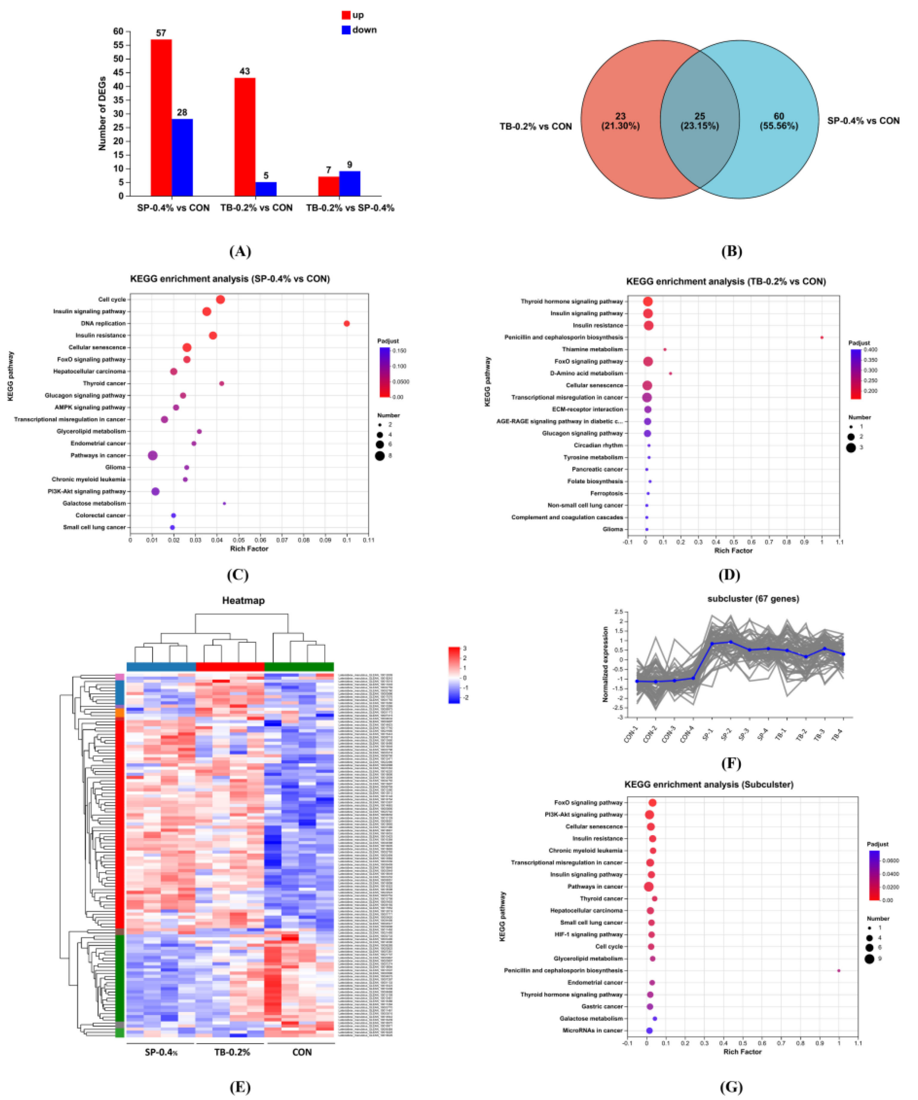

FIGURE 4
RNA-seq of SP and TB dietary supplementation. **(A)** The number of differentially expressed genes. **(B)** Venn diagram of the differentially expressed genes. **(C)** KEGG enrichment analysis of SP-regulated genes. **(D)** KEGG enrichment analysis of TB-regulated genes. **(E)** Heatmap analysis of differentially expressed genes. **(F)** Subcluster of 67 genes. **(G)** KEGG enrichment analysis of 67 genes.

signaling pathways (Tian et al., 2017) and enhanced the physical barrier function of the Nrf2, JNK, and MLCK signaling pathways (Wu et al., 2018). Furthermore, SCFAs improved largemouth bass (*M. salmoides*) immunity through the TLR22-MyD88-NF-κB signaling pathway (Hou et al., 2023). In addition, mammalian studies provide more references. For example, SP activated the small intestinal gluconeogenesis pathway through the GPR41 gut-brain axis, improving metabolism (De Vadder et al., 2014). Moreover, SP improved the abnormal lipid deposition induced by a high-fat diet through the PPARγ-UCP2-AMPK pathway (den Besten et al., 2015). Additionally, SCFAs inhibited glycogen synthase kinase 3β and increased Nrf2 activity, increasing the antioxidant capacity (Xing et al., 2016). Therefore, the in-depth functional mechanism of SP, TB, and other SCFAs in fish has many research gaps and should be studied in further studies.

5 Conclusion

Dietary supplementation with 0.4% sodium propionate and 0.2% tributyrin were feasible strategies for improving the hepatic lipid deposition, antioxidant capacity, and hypoxic stress resistance of spotted seabass, which provides references for aquatic feed. The sodium propionate and tributyrin-regulated genes presented similar expression patterns and involved the FoxO signaling, PI3K-Akt signaling, and insulin-related pathways, which could improve our understanding of the functional mechanism of SCFAs in fish. The in-depth functional mechanisms of certain SCFAs should be studied. Subsequently, considering the similar functions and gene regulation of sodium propionate and tributyrin, the synergistic effects of sodium propionate, tributyrin, and other SCFAs should also be examined.

Data availability statement

The raw data were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) (PRJNA1150789).

Ethics statement

The experimental procedures were performed in strict accordance with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

KC: Writing – original draft, Methodology, Data curation, Conceptualization. HZ: Writing – review & editing, Methodology, Data curation, Conceptualization. BY: Writing – review & editing, Methodology, Data curation, Conceptualization. JW: Writing – review & editing, Methodology, Data curation, Conceptualization. XQ: Writing – review & editing, Validation, Methodology, Conceptualization. MX: Writing – review & editing, Validation, Methodology, Conceptualization.

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Conflict of interest

Authors KC, HZ, BY, JW, and XQ were employed by the company Guangdong Haid Group Co., Ltd.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aalamifar, H., Soltanian, S., Vazirzadeh, A., Akhlaghi, M., Morshedi, V., Gholamhosseini, A., et al. (2020). Dietary butyric acid improved growth, digestive enzyme activities and humoral immune parameters in Barramundi (*Lates calcarifer*). *Aquaculture Nutr.* 26, 156–164. doi: 10.1111/anu.12977

Abdelsalam, M., Elgendy, M. Y., Elfadadny, M. R., Ali, S. S., Sherif, A. H., and Abolghait, S. K. (2023). A review of molecular diagnoses of bacterial fish diseases. *Aquaculture Int.* 31, 417–434. doi: 10.1007/s10499-022-00983-8

Benedito-Palos, L., Ballester-Lozano, G. F., Simó, P., Karalazos, V., Ortiz, A., Calduch-Giner, J., et al. (2016). Lasting effects of butyrate and low FM/FO diets on growth performance, blood haematology/biochemistry and molecular growth-related markers in gilthead sea bream (*Sparus aurata*). *Aquaculture* 454, 8–18. doi: 10.1016/j.aquaculture.2015.12.008

Bjerkeng, B., Storebakken, T., and Wathne, E. (1999). 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. *Aquaculture Nutr.* 5, 181–191. doi: 10.1046/j.1365-2095.1999.00103.x

Cai, L. S., Wang, L., Song, K., Lu, K. L., Zhang, C. X., and Rahimnejad, S. (2020). Evaluation of protein requirement of spotted seabass (Lateolabrax maculatus) under two temperatures, and the liver transcriptome response to thermal stress. *Aquaculture* 516, 734615. doi: 10.1016/j.aquaculture.2019.734615

Cheng, Z., Yang, H., Xu, Z., Li, X., and Leng, X. (2021). Dietary supplementation of tributyrin improved the growth, feed utilization and intestinal histology of grass carp (*Ctenopharyngodon idella*). *Aquaculture Nutr.* 27, 2007–2018. doi: 10.1111/anu.13336

Cui, K., Li, X., Chen, Q., Li, Q., Gao, S., Tan, P., et al. (2020). Effect of replacement of dietary fish oil with four vegetable oils on prostaglandin E2 synthetic pathway and expression of inflammatory genes in marine fish Larimichthys crocea. *Fish Shellfish Immunol.* 107, 529–536. doi: 10.1016/j.fsi.2020.09.038

Dawood, M. A., Eweedah, N. M., Elbialy, Z. I., and Abdelhamid, A. I. (2020). Dietary sodium butyrate ameliorated the blood stress biomarkers, heat shock proteins, and immune response of Nile tilapia (*Oreochromis niloticus*) exposed to heat stress. *J. Thermal Biol.* 88, 102500. doi: 10.1016/j.jtherbio.2019.102500

den Besten, G., Bleeker, A., Gerding, A., van Eunen, K., Havinga, R., van Dijk, T. H., et al. (2015). Short-chain fatty acids protect against high-fat diet–induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. *Diabetes* 64, 2398–2408. doi: 10.2337/db14-1213

den Besten, G., Lange, K., Havinga, R., van Dijk, T. H., Gerding, A., van Eunen, K., et al. (2013). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am. J. Physiology-Gastrointestinal Liver Physiol.* 305, G900–G910. doi: 10.1152/ajpgi.00265.2013

De Vadder, F., Kovatcheva-Datchary, P., Goncalves, D., Vinera, J., Zitoun, C., Duchampt, A., et al. (2014). Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 156, 84–96. doi: 10.1016/j.cell.2013.12.016

Dong, Y., Li, L., Xia, T., Wang, L., Xiao, L., Ding, N., et al. (2022). Oxidative stress can be attenuated by 4-PBA caused by high-fat or ammonia nitrogen in cultured spotted seabass: the mechanism is related to endoplasmic reticulum stress. *Antioxidants* 11, 1276. doi: 10.3390/antiox11071276

Ebrahimi, M., Daeman, N. H., Chong, C. M., Karami, A., Kumar, V., Hoseinifar, S. H., et al. (2017). Comparing the effects of different dietary organic acids on the growth, intestinal short-chain fatty acids, and liver histopathology of red hybrid tilapia (*Oreochromis* sp.) and potential use of these as preservatives. *Fish Physiol. Biochem.* 43, 1195–1207. doi: 10.1007/s10695-017-0365-0

Fang, L., Wang, Q., Guo, X., Pan, X., and Li, X. (2021). Effects of dietary sodium butyrate on growth performance, antioxidant capacity, intestinal histomorphology and immune response in juvenile Pengze crucian carp (*Carassius auratus* Pengze). *Aquaculture Rep.* 21, 100828. doi: 10.1016/j.aqrep.2021.100828

Farhan, M., Wang, H., Gaur, U., Little, P. J., Xu, J., and Zheng, W. (2017). FOXO signaling pathways as therapeutic targets in cancer. *Int. J. Biol. Sci.* 13, 815. doi: 10.7150/ijbs.20052

Gao, Y., Storebakken, T., Shearer, K. D., Penn, M., and Øverland, M. (2011). Supplementation of fishmeal and plant protein-based diets for rainbow trout with a mixture of sodium formate and butyrate. *Aquaculture* 311, 233–240. doi: 10.1016/j.aquaculture.2010.11.048

Gerunova, L. K., Gerunov, T. V., P'yanova, L. G., Lavrenov, A. V., Sedanova, A. V., Delyagina, M. S., et al. (2024). Butyric acid and prospects for creation of new medicines based on its derivatives: a literature review. *J. Veterinary Sci.* 25. doi: 10.4142/jvs.23230

Hoseinifar, S. H., Sun, Y. Z., and Caipang, C. M. (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: An updated view. *Aquaculture Res.* 48, 1380–1391. doi: 10.1111/are.13239

Hou, Y., Hou, Y., Yao, L., Chen, S., Fan, J., and Qian, L. (2019). Effects of chromium yeast, tributyrin and bile acid on growth performance, digestion and metabolism of Channa argus. *Aquaculture Res.* 50, 836–846. doi: 10.1111/are.2019.50.issue-3

Hou, D., Li, M., Li, P., Chen, B., Huang, W., Guo, H., et al. (2023). Effects of sodium butyrate on growth performance, antioxidant status, inflammatory response and resistance to hypoxic stress in juvenile largemouth bass (*Micropterus salmoides*). *Front. Immunol.* 14, 1265963. doi: 10.3389/fimmu.2023.1265963

Hou, Y., Wang, L., Yi, D., Ding, B., Chen, X., Wang, Q., et al. (2014). Dietary supplementation with tributyrin alleviates intestinal injury in piglets challenged with intrarectal administration of acetic acid. *Br. J. Nutr.* 111, 1748–1758. doi: 10.1017/S0007114514000038

Hu, E. D., Chen, D. Z., Wu, J. L., Lu, F. B., Chen, L., Zheng, M. H., et al. (2018). High fiber dietary and sodium butyrate attenuate experimental autoimmune hepatitis through regulation of immune regulatory cells and intestinal barrier. *Cell. Immunol.* 328, 24–32. doi: 10.1016/j.cellimm.2018.03.003

Hughes, D. A. (1999). Effects of dietary antioxidants on the immune function of middle-aged adults. *Proc. Nutr. Soc.* 58, 79–84. doi: 10.1079/PNS19990012

Jia, Y., Wang, F., Gao, Y., Qin, H., and Guan, C. (2023). Hypoxia stress induces hepatic antioxidant activity and apoptosis, but stimulates immune response and immune-related gene expression in black rockfish Sebastes schlegelii. *Aquat. Toxicol.* 258, 106502. doi: 10.1016/j.aquatox.2023.106502

Li, X., Cui, K., Fang, W., Chen, Q., Xu, D., Mai, K., et al. (2019). High level of dietary olive oil decreased growth, increased liver lipid deposition and induced inflammation by activating the p38 MAPK and JNK pathways in large yellow croaker (*Larimichthys crocea*). *Fish Shellfish Immunol.* 94, 157–165. doi: 10.1016/j.fsi.2019.08.062

Li, X., Sun, J., Wang, L., Song, K., Lu, K., Zhang, L., et al. (2023). Effects of dietary vitamin E levels on growth, antioxidant capacity and immune response of spotted seabass (*Lateolabrax maculatus*) reared at different water temperatures. *Aquaculture* 565, 739141. doi: 10.1016/j.aquaculture.2022.739141

Li, J., Xu, W., Lai, W., Kong, A., Zhang, Z., Pang, Y., et al. (2021). Effect of dietary methionine on growth performance, lipid metabolism and antioxidant capacity of large yellow croaker (*Larimichthys crocea*) fed with high lipid diets. *Aquaculture* 536, 736388. doi: 10.1016/j.aquaculture.2021.736388

Liang, H., Ji, K., Ge, X., Xi, B., Ren, M., Zhang, L., et al. (2021). Tributyrin plays an important role in regulating the growth and health status of juvenile blunt snout bream (*Megalobrama amblycephala*), as evidenced by pathological examination. *Front. Immunol.* 12, 652294. doi: 10.3389/fimmu.2021.652294

Liu, Y., Chen, Z., Dai, J., Yang, P., Xu, W., Ai, Q., et al. (2019). 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.* 88, 65–75. doi: 10.1016/j.fsi.2019.02.064

Liu, M., Guo, W., Wu, F., Qu, Q., Tan, Q., and Gong, W. (2017). Dietary supplementation of sodium butyrate may benefit growth performance and intestinal function in juvenile grass carp (*Ctenopharyngodon idellus*). *Aquaculture Res.* 48, 4102–4111. doi: 10.1111/are.2017.48.issue-8

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Martínez-Álvarez, R. M., Morales, A. E., and Sanz, A. (2005). Antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish Biol. Fisheries* 15, 75–88. doi: 10.1007/s11160-005-7846-4

Martini, M., De Santis, M. C., Braccini, L., Gulluni, F., and Hirsch, E. (2014). PI3K/AKT signaling pathway and cancer: an updated review. *Ann. Med.* 46, 372–383. doi: 10.3109/07853890.2014.912836

Miyoshi, M., Sakaki, H., Usami, M., Iizuka, N., Shuno, K., Aoyama, M., et al. (2011). Oral administration of tributyrin increases concentration of butyrate in the portal vein and prevents lipopolysaccharide-induced liver injury in rats. *Clin. Nutr.* 30, 252–258. doi: 10.1016/j.clnu.2010.09.012

Morrison, D. J., and Preston, T. (2016). Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* 7, 189–200. doi: 10.1080/19490976.2015.1134082

Ng, W. K., and Koh, C. B. (2017). The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. *Rev. Aquaculture* 9, 342–368. doi: 10.1111/raq.2017.9.issue-4

Okocha, R. C., Olatoye, I. O., and Adedeji, O. B. (2018). Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Rev.* 39, 1–22. doi: 10.1186/s40985-018-0099-2

Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *J. Fish Dis.* 35, 83–108. doi: 10.1111/j.1365-2761.2011.01333.x

Palma, M., Magnoni, L. J., Morais, S., and Viegas, I. (2023). Tributyrin supplementation in fish and crustacean nutrition: a review. *Rev. Aquaculture* 15, 785–800. doi: 10.1111/raq.12759

Peng, K., Lv, X., Zhao, H., Chen, B., Chen, X., and Huang, W. (2022). Antioxidant and intestinal recovery function of condensed tannins in *Lateolabrax maculatus* responded to in *vivo* and in *vitro* oxidative stress. *Aquaculture* 547, 737399. doi: 10.1016/j.aquaculture.2021.737399

Pohlenz, C., and Gatlin, D. M. III (2014). Interrelationships between fish nutrition and health. *Aquaculture* 431, 111–117. doi: 10.1016/j.aquaculture.2014.02.008

Rauf, A., Khalil, A. A., Rahman, U. U., Khalid, A., Naz, S., Shariati, M. A., et al. (2022). Recent advances in the therapeutic application of short-chain fatty acids (SCFAs): An updated review. *Crit. Rev. Food Sci. Nutr.* 62, 6034–6054. doi: 10.1080/10408398.2021.1895064

Robles, R., Lozano, A. B., Sevilla, A., Márquez, L., Nuez-Ortín, W., and Moyano, F. J. (2013). Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*). *Fish Physiol. Biochem.* 39, 1567–1580. doi: 10.1007/s10695-013-9809-3

Safari, R., Hoseinifar, S. H., and Kavandi, M. (2016). Modulation of antioxidant defense and immune response in zebra fish (*Danio rerio*) using dietary sodium propionate. *Fish Physiol. Biochem.* 42, 1733–1739. doi: 10.1007/s10695-016-0253-z

Safari, R., Hoseinifar, S. H., Nejadmoghadam, S., and Khalili, M. (2017). Non-specific immune parameters, immune, antioxidant and growth-related genes expression of common carp (*Cyprinus carpio* L. *) fed sodium propionate. Aquaculture Res.* 48, 4470–4478. doi: 10.1111/are.2017.48.issue-8

Sahuri-Arisoylu, M., Brody, L. P., Parkinson, J. R., Parkes, H., Navaratnam, N., Miller, A. D., et al. (2016). Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int. J. Obes.* 40, 955–963. doi: 10.1038/ijo.2016.23

Sell, H., Habich, C., and Eckel, J. (2012). Adaptive immunity in obesity and insulin resistance. *Nat. Rev. Endocrinol.* 8, 709–716. doi: 10.1038/nrendo.2012.114

Shao, Y., Wang, Y., Yuan, Y., and Xie, Y. (2021). A systematic review on antibiotics misuse in livestock and aquaculture and regulation implications in China. *Sci. Total Environ.* 798, 149205. doi: 10.1016/j.scitotenv.2021.149205

Silva, B. C., Nolasco-Soria, H., Magallón-Barajas, F., Civera-Cerecedo, R., Casillas-Hernández, R., and Seiffert, W. (2016). Improved digestion and initial performance of whiteleg shrimp using organic salt supplements. *Aquaculture Nutr.* 22, 997–1005. doi: 10.1111/anu.2016.22.issue-5

Tan, P., Dong, X., Xu, H., Mai, K., and Ai, Q. (2017). Dietary vegetable oil suppressed non-specific immunity and liver antioxidant capacity but induced inflammatory response in Japanese sea bass (*Lateolabrax japonicus*). *Fish Shellfish Immunol.* 63, 139–146. doi: 10.1016/j.fsi.2017.02.006

Tian, L., Zhou, X. Q., Jiang, W. D., Liu, Y., Wu, P., Jiang, J., et al. (2017). Sodium butyrate improved intestinal immune function associated with NF-κB and p38MAPK signalling pathways in young grass carp (*Ctenopharyngodon idella*). *Fish Shellfish Immunol.* 66, 548–563. doi: 10.1016/j.fsi.2017.05.049

Tran, N. T., Li, Z., Wang, S., Zheng, H., Aweya, J. J., Wen, X., et al. (2020). Progress and perspectives of short-chain fatty acids in aquaculture. *Rev. Aquaculture* 12, 283–298. doi: 10.1111/raq.12317

Volatiana, J. A., Wang, L., Gray, N., Tong, S., Zhang, G., and Shao, Q. (2020). Tributyrin-supplemented high-soya bean meal diets of juvenile black sea bream, *Acanthopagrus schlegelii*: Study on growth performance and intestinal morphology and structure. *Aquaculture Res.* 51, 135–146. doi: 10.1111/are.14355

Wu, P., Tian, L., Zhou, X. Q., Jiang, W. D., Liu, Y., Jiang, J., et al. (2018). 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.* 73, 121–132. doi: 10.1016/j.fsi.2017.12.009

Wu, X., Wang, L., Xie, Q., and Tan, P. (2020). Effects of dietary sodium butyrate on growth, diet conversion, body chemical compositions and distal intestinal health in yellow drum (*Nibea albiflora*, Richardson). *Aquaculture Res.* 51, 69–79. doi: 10.1111/are.14348

Xie, D., Dai, Q., Xu, C., and Li, Y. (2021). Dietary tributyrin modifies intestinal function by altering morphology, gene expression and microbiota profile in common carp (*Cyprinus carpio*) fed all-plant diets. *Aquaculture Nutr.* 27, 439–453. doi: 10.1111/anu.13197

Xing, X., Jiang, Z., Tang, X., Wang, P., Li, Y., Sun, Y., et al. (2016). Sodium butyrate protects against oxidative stress in HepG2 cells through modulating Nrf2 pathway and mitochondrial function. *J. Physiol. Biochem.* 73, 405–414. doi: 10.1007/s13105-017-0568-y

Xing, S., Liang, X., Wang, H., Xie, X., Wierenga, P. A., Schrama, J. W., et al. (2023). The impacts of physical properties of extruded feed on the digestion kinetics, gastrointestinal emptying and stomach water fluxes of spotted seabass (*Lateolabrax maculatus*). *Aquaculture* 570, 739442. doi: 10.1016/j.aquaculture.2023.739442

Xu, N., Ding, T., Liu, Y., Zheng, W., Liu, Q., Yin, Z., et al. (2021). Effects of dietary tributyrin on growth performance, body composition, serum biochemical indexes and lipid metabolism-related genes expression of juvenile large yellow croaker (*Larimichthys crocea*) fed with high level soybean oil diets. *Aquaculture Nutr.* 27, 395–406. doi: 10.1111/anu.13192

Yin, B., Liu, H., Tan, B., Dong, X., Chi, S., Yang, Q., et al. (2021). Supplementing tributyrin to cottonseed protein concentrate-based diets can improve growth performance, lipid metabolism and distal intestinal immunity in hybrid grouper (*Epinephelus fuscoguttatus*♀× *Epinephelus lanceolatus*♂). *Aquaculture Nutr.* 27, 2378–2391. doi: 10.1111/anu.13370

Yousefi, M., Hoseini, S. M., Kulikov, E. V., Kharlitskaya, E. V., Petukhov, N. V., and Khomenets, N. G. (2024). Dietary propionate administration improves growth performance, hepatic lipid deposition, and intestinal activity of digestive enzymes, inflammation, bacterial population, and antioxidant capacity in rainbow trout, Oncorhynchus mykiss. *Aquaculture* 578, 740099. doi: 10.1016/j.aquaculture.2023.740099

Zhao, H., Peng, K., Wang, G., Mo, W., Huang, Y., and Cao, J. (2021). Metabolic changes, antioxidant status, immune response and resistance to ammonia stress in juvenile yellow catfish (*Pelteobagrus fulvidraco*) fed diet supplemented with sodium butyrate. *Aquaculture* 536, 736441. doi: 10.1016/j.aquaculture.2021.736441

Zhao, L., Yuan, B. D., Zhao, J. L., Jiang, N., Zhang, A. Z., Wang, G. Q., et al. (2020). Amelioration of hexavalent chromium-induced bioaccumulation, oxidative stress, tight junction proteins and immune-related signaling factors by *Allium mongolicum Regel* flavonoids in Ctenopharyngodon idella. *Fish Shellfish Immunol.* 106, 993–1003. doi: 10.1016/j.fsi.2020.09.005

Zhou, C., Lin, H., Huang, Z., Wang, J., Wang, Y., and Yu, W. (2019). Effect of dietary sodium butyrate on growth performance, enzyme activities and intestinal proliferation-related gene expression of juvenile golden pompano Trachinotus ovatus. *Aquaculture Nutr.* 25, 1261–1271. doi: 10.1111/anu.12940