Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 8690138, 14 pages
https://doi.org/10.1155/2022/8690138

Research Article

Berberine Chloride Supplementation Ameliorates Excessive Hepatic Lipid Deposition and Proinflammatory Gene Upregulation in the Soybean-Oil-Based Diet of Juvenile Yellow Drum (*Nibea albiflora*)

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Received 17 March 2022; Revised 7 June 2022; Accepted 22 June 2022; Published 14 July 2022

Academic Editor: Zhen-Yu Du

In this study, we fed yellow drum juveniles (initial body weigh 5.57 ± 0.03 g) a fish-oil-based, soybean-oil-based (SO), or berberine chloride (BBR 50 mg kg^{-1} in a SO-based diet) diet with three replicates of each treatment for 56 days. Dietary BBR supplementation significantly increased growth performance but decreased SO-induced viscerosomatic index and condition factor increases. Hepatic tissue Oil Red O staining indicated that BBR supplementation completely compensated for the SO-induced excessive lipid deposition. BBR supplementation resulted in a significant decrease in saturated fatty acids but a significant increase in the proportion of n-6 polyunsaturated fatty acids in hepatic tissue. RNA sequencing revealed that amelioration of hepatic lipid deposition by BBR supplementation could be attributed to an increase in fatty acid β-oxidation. Dietary BBR also decreased the SO-induced proinflammatory gene expression, characterized by the suppression of cytokine–cytokine receptor interaction pathway. Collectively, our findings demonstrated the beneficial effects of dietary BBR on growth performance, hepatic lipid deposition, and proinflammatory gene expression. The amelioration of hepatic lipid deposition by BBR is likely caused by an increase in fatty acid β-oxidation, which promotes energy production for body growth.

1. Introduction

Static capture fishery production and the rapid development of the aqua-feed industry have led to increased costs, unreliability, and competition with other fish oil (FO)-consuming sectors [1]. Soybean oil (SO) is a promising alternative to FO, owing to its abundant unsaturated fatty acid quantity (particularly linolenic acid), widespread sources, and relatively low cost. At an appropriate level, dietary FO can be replaced by SO without affecting growth performance or health status; however, the increasing concern is that excessive SO inclusion could lead to growth retardation [2, 3], excessive hepatic lipid deposition [4], and proinflammatory responses [5–8]. Research towards solving this problem has increased, and certain functional feed additives were found to ameliorate liver dysfunction, such as bile acid [9], choline [10], fenofibrate [11], L-carnitine [12], and short-chain fatty acids [13].

The feasibility of herbs and their extracts as supplements in aquatic feed has gradually been recognized, primarily because of their high safety and environmentally friendly advantages, in addition to their excellent performance in affecting growth, immunity, and metabolism [14]. As a traditional Chinese medicine, berberine has long been used to treat fatty liver, hypertension, obesity, and type 2 diabetes, primarily because of its strong anti-inflammatory and antimicrobial activities, along with its lipid-lowering properties [15]. The mechanisms through which berberine alleviates hepatic lipid accumulation in fish are similar to those observed in other animals: mainly by increasing lipid oxidation and reducing lipogenesis. Berberine downregulates the expression of hepatic lipogenesis and fatty acid

synthesis gene in black sea bream (*Acanthopagrus schlegelii*) [16] but upregulates the expression of hepatic lipid β-oxidation gene when blunt snout bream (*Megalobrama amblycephala*) is fed a high-fat (HF) and high-carbohydrate (HC) diet [17–19]. Dietary berberine supplementation may increase mitochondrial density and repair the mitochondrial respiratory chain in hepatocytes, thereby improving liver energy expenditure [18, 20]. Berberine administration can directly downregulate the expression of several proinflammatory cytokines [21]; furthermore, dietary berberine supplementation increases nonspecific immunity parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio*) (Zhou et al., 2016). Similarly, in blunt snout bream, berberine reduced intestinal tumor necrosis factor-a (*tnfa*) and interleukin 6 (*il6*) gene expressions, which were upregulated by HF and HC diets [22]. Therefore, berberine has immense potential as a novel additive to the fish diet.

In Chinese Fisheries Pharmacopoeia, 30 mg/kg of berberine is the highest level for controlling bacterial diseases in fish (Pan et al., 2019). A study in blunt snout bream reported that supplementation of 50 mg/kg berberine in a high-fat diet (15% fat) increased growth performance significantly, while 100 mg/kg berberine did not influence the growth of fish, which might be because 100 mg/kg berberine reduced the palatability of the diet since feed intake was significantly reduced; thus, 50 mg/kg berberine was recommended as a suitable level in fish feed [19]. Therefore, 50 mg/kg berberine was widely applied in the studies on blunt snout bream [17, 22–24] and black sea bream [16, 20]. Chemical synthesis has been developed as a highly productive method of obtaining berberine, and berberine hydrochloride salt and chloride are commonly used in clinics because they are relatively more soluble than berberine [25]. Berberine displays beneficial effects that have been extensively studied across species; however, whether berberine can ameliorate hepatic lipid deposition and proinflammatory responses in a vegetable-oil-based diet is largely unknown. In addition, a mechanistic investigation and systematic understanding of how berberine affects hepatic metabolism and immunity in fish are still in their infancy. Unique molecular identifiers (UMIs) have been used to accurately qualify PCR duplicates in high-throughput sequencing [26, 27]. By incorporating a UMI into the same location in each fragment during library preparation, mRNA duplicates can be accurately qualified because they have identical alignment coordinates and identical UMI sequences [28]. *Nibea albiflora* (Richardson), which is widely distributed in the coastal waters of China, Korea, Japan, and Southeast Asian countries, is an important fish species used as a commercial seafood resource. As such, in this study, we aimed to elucidate the effects of berberine chloride supplementation on the growth performance, hepatic metabolism, and immunity of yellow drums fed a SO-based diet.

2. Materials and Methods

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China. The study was approved by the Animal Experiments Ethics Committee of Zhejiang Ocean University. We performed this study in compliance with the ethical principles and standards of aquaculture. We anesthetized juveniles with 20 mg L^{-1} tricaine methanesulphonate (Sigma, USA) until the respiratory opercula stopped moving before sampling.

2.1. Experimental Diet Formulation. We purchased berberine chloride (BBR) from MedChemExpress (purity: 99.84%; Sweden). Casein, defatted fishmeal (crude lipid <10 g kg^{-1}), and soy protein concentrate served as the main protein source; fish oil (FO) or soybean oil (SO) served as the main lipid source. We formulated three equal-protein-content (500 g kg^{-1}) and equal-lipid (120 g kg^{-1}) experimental diets: FO diet, SO diet with 100% FO replaced by SO, and BBR diet with 50 mg kg^{-1} berberine chloride supplemented in the SO-based diet. We analyzed the approximate compositions of the experimental diets (Table 1). We formulated the diets as previously described [13].

2.2. Fish Husbandry. Juvenile *Nibea albiflora* were obtained from the Xixuan Fishery Science and Technology Island Farm in Zhoushan City, Zhejiang Province, China, and the experiment was conducted in a flowing water aquaculture system. Before the feeding trial, we conditioned the experimental fish for an additional two weeks to acclimatize them to the experimental conditions. After fasting for 24 h, we randomly distributed fish of similar size (5.57 ± 0.03 g) into nine tanks (volume 500 L, seawater 400 L) with 25 fish per tank. We randomly assigned each experimental diet to one of three tanks. We fed fish to apparent satiation twice daily (at 7 : 00 and 16 : 00). We siphoned, collected, dried, and weighed the leftover pellets. We refreshed sand-filtered water twice per day throughout the experimental period and provided additional aeration through a single air stone in each tank. During the experimental period, we maintained the following conditions: temperature, 27.5 ± 1.5°C; pH, 8.0-8.4; salinity, 27.0 ± 1.00 g L^{-1}; unionized ammonia nitrogen, <0.05 mg L^{-1}; dissolved oxygen, >6.0 mg L^{-1}. We cleaned the tanks twice per week with sponges.

2.3. Sample Collection. At the end of the 56-day feeding trial, we fasted the fish in each tank for 24 h before sample collection. We anesthetized the fish with tricaine methanesulphonate (MS-222) then individually weighed them and recorded the values. We randomly collected four individuals per tank and stored them at -20°C for whole body composition analysis. To collect the serum samples, five fish were sacrificed to collect caudal vein blood. We stored blood samples at 4°C for 6 h to separate the serum and blood cells. We further centrifuged the samples to collect serum and stored them at −80°C until biochemical analysis. We measured the full length and body length and weighed visceral mass and total liver tissue from the five individuals to calculate the hepatosomatic index (HSI), viscerosomatic index (VSI), and condition factor (CF). We dissected another five fish from each tank to obtain hepatic tissues, which we stored at -20°C for fatty acid composition analysis. We randomly collected the hepatic tissues of another three bloodless fish from each

Table 1: Ingredients and nutrient contents of the experimental diets (g kg^{-1}, dry weight).

Ingredient	Experimental diets (g kg^{-1}, dry weight)		
	FO [1]	SO [2]	BBR [3]
Casein[4]	120	120	120
Defatted fish meal [5]	360	360	360
Soy protein concentrate [6]	130	130	130
Wheat meal	100	100	100
Fish oil [7]	100	0	0
Soybean oil [8]	0	100	100
Soybean lecithin [9]	20	20	20
Berberine chloride [10]	0	0	0.05
$Ca(H_2PO_4)_2$	5	5	5
Chloride choline	3	3	3
Vitamin premix [11]	5	5	5
Mineral premix [12]	10	10	10
Microcrystalline cellulose	115.5	115.5	115.45
Attractant [13]	5	5	5
Mould inhibitor [14]	1	1	1
Y_2O_3	5	5	5
Sodium alginate	20	20	20
Tert-butylhydroquinone	0.5	0.5	0.5
Total	1000	1000	1000
Proximate analysis			
Gross energy (KJ g^{-1})	22.07	23.46	23.12
Crude protein (g kg^{-1}, dry weight)	510.4	506.8	505.7
Crude lipid (g kg^{-1}, dry weight)	122.4	123.8	124.6

[1-3] FO: control diet; SO: soybean oil diet with fish oil replaced by soybean oil; BBR: 50 mg kg^{-1} berberine chloride supplemented in the SO-based diet. [4-6] Casein (g kg^{-1}, dry matter): crude protein 894.4, crude lipid 14.6 from Alfa Aesar, Avocado Research Chemicals Co., Ltd., U.K.; fatted fish meal (g kg^{-1}, dry matter): crude protein 751.1, crude lipid <10; soy protein concentrate (g kg^{-1}, dry matter): crude protein 668.0, crude lipid 11.0. [7-9] Fish oil: Rongcheng Ayers Ocean Biotechnology Co., Ltd., China; soybean oil: Fortune brand soybean oil from COFCO Group Co., Ltd., China; Soybean lecithin: Cargill Co., Ltd., Germany, EPIKURON 100G, containing approximately 630 g phospholipids kg $^{-1}$. [10] Berberine chloride: MedChemExpress, Sweden, purity 99.84%. [11] Vitamin premix (mg or g kg^{-1} diet): carotene 0.10 g, vitamin D 0.05 g, tocopherol 0.38 g, vitamin B_1 0.06 g, vitaminB_2 0.19 g, vitamin B_6 0.05 g, cyanocobalamin 0.1 mg, biotin 0.01 g, inositol 3.85 g, niacin acid 0.77 g, pantothenic acid 0.27 g, folic acid 0.01 g, chloride choline 7.87 g, and cellulose 1.92 g. [12] Mineral premix (mg or g kg^{-1} diet): NaF 2 mg, KI 0.8 mg, $CoCl_2{\cdot}6H_2O$ 50 mg, $CuSO_4{\cdot}5H_2O$ 10 mg, $FeSO_4{\cdot}H_2O$ 80 mg, $ZnSO_4{\cdot}H_2O$ 50 mg, $MnSO_4{\cdot}H_2O$ 60 mg, $MgSO_4{\cdot}7H_2O$ 1200 mg, Ca $(H_2PO_4)_2{\cdot}H_2O$ 3000 mg, NaCl 100 mg, and zoelite powder 15.45 g. [13-14] Attractant: glycine: betaine = 1: 3; mould inhibitor: fumaric acid: calcium propionate = 1 : 1.

tank, which were stored in 1.5 mL microtubes (Biotech, China) with RNA later (Thermo Fisher Scientific, USA), and stored at -20°C before RNA sequencing analysis and gene expression analysis. After washing with phosphate-buffered saline, we transferred hepatic tissue tips from three fish per tank to a 4% paraformaldehyde-fixed solution for morphological structure analysis.

2.4. Body Composition Analysis. We determined the crude protein, lipid, moisture, and ash contents according to the methods of the Association of Official Analytical Chemists (AOAC, 2003). We obtained moisture content by drying the comminuted pieces of fish and ground diets in an oven at 105°C to constant weight for 17 and 5 h, respectively. We determined crude protein content using the Kjeldahl method, following acid digestion with an auto-digester (KjelFlex K-360, BUCHI, Switzerland). We used ether extraction to determine crude lipids using a Soxtec System HT (Soxtec 2055; FOSS Tecator, Sweden). We determined ash content by burning off the organic matter from the samples in a muffle furnace at 550°C for 6 h, leaving only the inorganic residue for further evaluation of nutrients. We determined the fatty acid composition of the diet and hepatic tissues as described in our previous study [29].

2.5. Oil Red O Staining Section Analysis. We fixed the hepatic tip tissues of the yellow drum with paraformaldehyde solution, followed by freezing and slicing to a 10 μm thickness. We used an Oil Red O staining kit (Sun Yat-sen University, China) for staining. We differentiated samples using 50% ethanol that we washed with water. We counterstained the nuclei with haematoxylin, and sealed the samples with glycerine–gelatine. We imaged the sections using a Pannoramic 250 (3DHISTECH, Hungary). For a total of 27 slices, 9 slices for each treatment, we scaled and tagged the deep-red areas to calculate the proportions using ImageJ software (National Institutes of Health, USA).

Table 2: Primer sequences used for RNA-seq validation by qRT-PCR.

Gene	Primer sequence		Product size (bp)	Amplification efficiency (%)	Tm (°C)
	Forward primer (5'-3)	Reverse primer (5'-3')			
acsl4a	TGGTTCCCAACCAGAAAAAG	ACGGCTCTGGACTCAGATGT	186	99	60
ccr9	GGGGCAAACTCAAAGCAGTA	GAGCAGCTTGGTGACAATGA	156	101	60
csf1r	TTTACGGGCAAGGTTACGTC	ATGTAGGCTGGAGGTTGGTG	202	99	60
cxcr4	GACCTACCAGGAGCCATGTG	CGGAGAGATGGAGCGTACTT	177	98	61
egfr	GAAACTGCTTCCTCCGTCAG	ATGGCTGAAGCTGCAAAGTT	215	98	61
emg1	CAGGCCTGTTGCAGGTTTAT	GGTCAGACACCGGGTTTTTA	189	97	60
fabp3	CCAAGCCCACCACTATCATC	CTTTGCCATCCCACTTCTGT	195	97	60
il20ra	CCAAGCAGCAAAAACACTCA	CCTTTGCTTCGTCTTTCTGG	237	99	60
igf1	AACGGGATCCCCAAGTTTAC	TGGTGACCACCAGAAGATCA	168	98	59
me3	GGAGCTGGACGTCTCTTCAC	GTCACTGGACCGAATGGACT	177	99	59
nop56	TCACGCATCGACTGTTTCTC	TTGATCTCAGCTGCAACACC	182	102	60
ppara	GCAGTTGGACCAGGAAATGT	CAGTGAGTCTGATGGCAGGA	217	101	60
tnfrsf11b	ATCAATGCTTGGATCGCTTC	GTCCACCTCGTTGGTCAGAT	159	100	60
tlr1	GTGCTTCTTTCCCTGACAGC	GTCCTCCAGGAGTGGTGTGT	230	100	60
18S rRNA	CTGCACGGACAGAAACTCAA	CTCTTTAGCCCCCTCTGCTT	167	96	60
α-tubulin	AGGTGGGCATCAACTACCAG	TGAGAACTCTCCCTCCTCCA	203	99	60

Abbreviations: *acsl4a*: acyl-CoA synthetase long-chain family member 4a; *ccr9*: C-C chemokine receptor type 9; *csf1r*: macrophage colony-stimulating factor 1 receptor; *cxcr4*: C-X-C chemokine receptor type 4; *egfr*: epidermal growth factor receptor; *emg1*: ribosome biogenesis protein nep1; *fabp3*: fatty acid-binding protein 3; *il20ra*: interleukin-20 receptor subunit a; *igf1*: insulin-like growth factor 1; *me3*: NADP-dependent malic enzyme, mitochondrial; *nop56*: ribosome biosynthesis protein sik1; *ppara*: peroxisome proliferator-activated receptor a; *tnfrsf11b*: tumor necrosis factor receptor superfamily member 11b; *tlr1*: toll-like receptor 1.

2.6. UMI-RNA-Seq and Bioinformatics Analysis. We extracted the total RNA of hepatic tissue from fish fed FO, SO, and BBR diets using TRIzol reagent (Invitrogen, USA). We monitored DNA contamination and RNA degradation on 1% agarose gel and measured RNA purity using a NanoPhotometer® spectrophotometer (Implen, USA). We determined RNA concentration using a Qubit® RNA Assay Kit with a Qubit® 2.0 Fluorometer (Life Technologies). We measured RNA integrity using an RNA Nano 6000 Assay Kit on an Agilent Bioanalyzer 2100 system (Agilent Technologies, USA). We performed clustering and sequencing in the Experimental Department of Novogene Co., Ltd. (Beijing, China). The library preparation for RNA-seq, sequence quality assessment and assembly, annotation, and differential expression gene (DEG) analyses were described in detail in our previous publication, which we performed with some modifications [30]. In the synthesis of second-strand cDNA, we added a unique molecular identifier (UMI) label and sequencing adaptor to cDNA duplicates. We sequenced all libraries on an Illumina HiSeq 2500 sequencing platform (Illumina, USA). We set the DEG cutoff as an adjusted P (q) value <0.05 with fragments per kilobase per million-fold change >1.5.

2.7. DEG Validation by Real-Time Quantitative PCR. Experimental procedures for total RNA extraction, quality control, quantification, reverse transcription, and real-time quantitative PCR (qRT-PCR) were performed as previously described [31]. We selected the *Nibea albiflora* housekeeping genes *β-actin* and 18S rRNA as internal reference genes. All the specific primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) (Table 2). We analyzed relative gene expression using the $2^{-\Delta\Delta CT}$ method [32].

2.8. Calculations and Statistics. The following variables were calculated:

Survival rate (SR, %) = Nt × 100/N0,

Weight gain rate (WGR, %) = 100 × (Wt − W0)/W0,

Feed efficiency ratio (FER) = wet weight gain (g) /dry feed intake (g),

Table 3: Experimental fish growth performance, body indices, and body composition in response to experimental diets.

Parameters	Experimental diets		
	FO	SO	BBR
Initial body weight, g	5.57 ± 0.03	5.56 ± 0.03	5.57 ± 0.04
Final body weight, g	52.06 ± 1.20[a]	35.67 ± 1.33[c]	40.61 ± 1.43[b]
Survival rate, %	100 ± 0	100 ± 0	100 ± 0
Weight gain rate, %	934.65 ± 21.56[a]	641.01 ± 23.89[c]	729.07 ± 25.62[b]
Feed intake, % day^{-1}	2.71 ± 0.02	2.79 ± 0.07	2.73 ± 0.13
Feed efficiency ratio	1.09 ± 0.01[a]	0.92 ± 0.02[b]	0.95 ± 0.02[b]
Hepatosomatic index, %	1.44 ± 0.17	1.63 ± 0.11	1.45 ± 0.15
Viscerosomatic index, %	3.80 ± 0.23[b]	4.47 ± 0.05[a]	4.29 ± 0.11[ab]
Condition factor	1.74 ± 0.02[b]	1.93 ± 0.05[a]	1.84 ± 0.04[ab]
Ash, g kg^{-1}	47 ± 3	51 ± 4	52 ± 2
Moisture, g kg^{-1}	753 ± 24	764 ± 12	757 ± 16
Crude protein, g kg^{-1}	167 ± 3	156 ± 3	161 ± 3
Crude lipid, g kg^{-1}	33 ± 1	29 ± 2	30 ± 1

Data are presented as the mean ± S.E.M. ($n = 3$). Values in the same row without a superscript or with the same superscript are not significantly different ($P > 0.05$).

Feed intake (FI, %/d) = 100 × total feed intake (g) /(W0 + Wt)/2)/t,

Hepatosomatic index (HSI%) = 100 × (hepatic tissue weight/Wt),

Viscerosomatic index (VSI, %) = 100 × (visceral weight/wt),

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

where Nt and N0 are the final and initial fish in each cage, respectively; Wt and W0 are the final and initial fish weights, respectively; and t is the experimental duration in days.

2.9. Statistical Analysis. All data were processed using GraphPad version 9.0.0 for Windows (USA, https://www.graphpad.com/) and are presented as mean ± standard error of the mean (SEM). We confirmed the normality and homogeneity of variances of the data using Levene's test. We performed the Kruskal–Wallis test when the data were not homogeneous. Otherwise, we used one-way ANOVA with the Tukey honest significant difference (HSD) test to assess statistically significant differences among treatments at a significance level of $P < 0.05$. We generated the volcanic and heat maps for RNA-seq using the R package ggplot2 [33].

3. Results

3.1. Growth Performance, Body Indices, and Body Composition Analysis. We observed no significant difference in the survival rate or feed intake among the fish fed different experimental diets ($P > 0.05$; Table 3). The replacement of dietary FO with SO significantly decreased the weight gain rate (WGR) and feed efficiency ratio (FER; $P < 0.05$; Table 3). Conversely, supplementation with BBR in the SO-based diet significantly increased WGR ($P < 0.05$) and FER to some extent ($P > 0.05$). Feeding different experimental diets did not significantly affect the hepatosomatic index (HSI) ($P > 0.05$; Table 3). Replacement of FO with SO significantly increased the viscerosomatic index (VSI) and condition factor (CF; $P < 0.05$; Table 3). BBR supplementation reversed SO-induced VSI and CF increases, reaching values comparable to those of the FO group ($P > 0.05$) (Table 3). We found no significant differences in ash, moisture, crude protein, or crude lipid contents among the groups ($P > 0.05$; Table 3).

3.2. Hepatic Tissue Fatty Acid Composition Analysis. The fatty acid composition of hepatic tissues mainly reflects the fatty acid composition of the dietary lipid sources (FO and SO). We noted a significant difference in the fatty acid composition between the SO and BBR groups ($P < 0.05$; Table 4). BBR supplementation in the SO-based diet significantly decreased the proportion of SFAs but significantly increased the proportion of n-6 PUFAs (%, detected fatty acids). We mainly attributed the decreased SFAs proportion with BBR supplementation to the decreased C18:0 proportion, whereas the increased n-6 PUFAs proportion to the increased C18:2 n-6 proportion. The BBR in the SO-based diet also decreased the proportion of MUFAs, but the changes did not reach a significant level.

3.3. Hepatic Morphology Structure and Serum Biological Analysis. The statistical results from hepatic tissue histological sections stained with Oil Red O indicated that the SO diet significantly increased the proportion of lipid droplets (%, total area) compared to those from FO ($P < 0.05$; Figure 1). Supplementation with BBR significantly decreased the proportion of lipid droplets compared to that in the SO group, with no significant difference between the BBR and FO groups ($P > 0.05$; Figure 1).

We found no significant differences in serum alanine aminotransferase (ALT) activity, high-density lipoprotein content, or low-density lipoprotein content among the groups ($P > 0.05$; Table 5). Compared to the FO group, we observed

Table 4: Fatty acid composition analysis of experimental diets and hepatic tissue of fish fed experimental diets.

Fatty acid	Experimental diets		Hepatic tissue		
	FO	SO	FO	SO	BBR
C14:0	6.77	0.69	4.26 ± 0.07^{a}	0.72 ± 0.12^{b}	0.60 ± 0.03^{b}
C15:0	1.07	0.07	0.96 ± 0.02^{a}	0.12 ± 0.00^{b}	0.14 ± 0.01^{b}
C16:0	21.79	12.34	26.22 ± 0.43^{a}	17.07 ± 0.62^{b}	15.25 ± 0.52^{b}
C17:0	0.9	0.08	0.91 ± 0.02	0.08 ± 0.00	0.07 ± 0.01
C18:0	4.47	4.21	5.06 ± 0.24^{ab}	6.45 ± 0.39^{a}	4.44 ± 0.45^{b}
C20:0	0.71	ND	0.06 ± 0.00^{b}	0.28 ± 0.01^{a}	0.29 ± 0.01^{a}
C22:0	0.24	0.02	0.11 ± 0.01^{a}	0.01 ± 0.00^{b}	0.03 ± 0.01^{b}
C24:0	0.13	0.16	ND	0.03 ± 0.02	0.06 ± 0.01
∑SFAs	36.09	17.57	37.60 ± 0.42^{a}	24.76 ± 0.40^{b}	20.88 ± 0.25^{c}
C16:1	6.18	0.64	10.31 ± 0.29^{a}	2.47 ± 0.42^{b}	1.78 ± 0.14^{b}
C17:1	0.03	0.06	0.33 ± 0.01^{a}	0.04 ± 0.00^{b}	0.10 ± 0.03^{b}
C18:1n-9 t	0.12	0.01	0.17 ± 0.03	0.12 ± 0.00	0.13 ± 0.03
C18:1n-9c	16.44	24.51	20.49 ± 0.23^{b}	24.51 ± 0.55^{a}	23.45 ± 0.23^{a}
C20:1n-9	4.09	0.35	0.30 ± 0.01^{a}	0.20 ± 0.01^{b}	0.20 ± 0.01^{b}
C22:1n-9	0.18	0.02	0.15 ± 0.01	0.15 ± 0.03	0.15 ± 0.01
C24:1n-9	0.68	0.05	0.20 ± 0.01^{a}	0.02 ± 0.01^{b}	0.05 ± 0.01^{b}
∑MUFAs	27.71	25.62	31.96 ± 0.38^{a}	27.51 ± 1.02^{b}	25.84 ± 0.35^{b}
C18:2 n-6t	0.09	0.01	0.09 ± 0.02^{b}	0.27 ± 0.01^{a}	0.29 ± 0.01^{a}
C18:2 n-6c	8.43	48.92	10.13 ± 0.04^{c}	41.95 ± 0.60^{b}	47.48 ± 0.55^{a}
C18:3 n-6	0.2	0.47	0.42 ± 0.01	0.75 ± 0.14	0.43 ± 0.10
C20:3 n-6	0.11	0.09	0.34 ± 0.02^{a}	0.12 ± 0.04^{b}	0.06 ± 0.01^{b}
C20:4 n-6	0.95	0.06	0.69 ± 0.02^{a}	0.07 ± 0.01^{b}	0.08 ± 0.00^{b}
∑n-6 PUFAs	9.78	49.56	11.66 ± 0.03^{c}	43.16 ± 0.51^{b}	48.34 ± 0.57^{a}
C18:3 n-3	2.36	4.88	1.81 ± 0.07^{b}	3.10 ± 0.06^{a}	3.29 ± 0.07^{a}
C20:3 n-3	0.21	0.06	0.16 ± 0.00	0.07 ± 0.04	0.10 ± 0.01
EPA	8.66	1.1	4.65 ± 0.13^{a}	0.49 ± 0.03^{b}	0.53 ± 0.01^{b}
DHA	13.24	0.71	9.05 ± 0.22^{a}	0.47 ± 0.00^{b}	0.60 ± 0.03^{b}
DPA	1.02	0.16	1.18 ± 0.02^{a}	0.18 ± 0.01^{b}	0.20 ± 0.00^{b}
∑n-3 PUFAs	25.49	6.91	16.85 ± 0.40^{a}	4.31 ± 0.06^{b}	4.72 ± 0.03^{b}
∑DHA+EPA	21.9	1.81	13.86 ± 0.34^{a}	1.03 ± 0.02^{b}	1.24 ± 0.04^{b}
∑n-3 LC-PUFAs	23.14	2.03	13.69 ± 0.34^{a}	0.96 ± 0.03^{b}	1.14 ± 0.04^{b}
∑PUFAs	35.27	56.47	28.51 ± 0.39^{c}	47.48 ± 0.56^{b}	53.06 ± 0.58^{a}

Values (mean ± S.E.M., $n = 3$) within a row with the same superscript letter or without a superscript are not significantly different from the other experimental treatments ($P > 0.05$). [1] SFAs: saturated fatty acids; [2] t: trans-fatty acids; [3] c: cis-fatty acids; [4] MUFAs: monounsaturated fatty acids; [5] n-6 PUFAs: n-6 polyunsaturated fatty acids; [6] n-3 PUFAs: n-3 polyunsaturated fatty acids; [7] n-3 LC-PUFAs: n-3 long-chain polyunsaturated fatty acids; [8] ND: not detected.

significantly higher serum aspartate aminotransferase (AST) activity and free fatty acid (FFAs) content but significantly lower glucose, triglyceride, and cholesterol contents in the SO group ($P < 0.05$; Table 5). BBR supplementation decreased serum AST activity and FFA content, reaching values comparable to those of the FO group ($P > 0.05$; Table 5). Moreover, BBR supplementation increased serum glucose content, but we found no significant differences between the FO and BBR groups or between the SO and BBR groups ($P > 0.05$; Table 5).

3.4. RNA Sequencing and Bioinformatics Analysis. We obtained a total of 58.47 GB of raw data with 389,873,032 raw reads after sequencing. After removing redundancies, we obtained 57.48 GB of clean data, with 383,214,286 clean reads. The UMI reads for clean reads were >90% for each sample. The error rate was 0.2–0.3%, and Q30 was >93.5% among the groups. Most UMI reads mapped to the reference genome sequence, and the rates were all >96% among the groups. The basic information for RNA-seq was provided in Table S1.

Based on the FPKM method, we detected 626 and 595 significantly differentially expressed genes (DEGs) between the FO and SO groups, and SO and BBR groups, respectively (Figure 2(a)). The results of the top 10 Gene Ontology (GO) analysis showed strong enrichment of DEGs in three main categories: biological process, cellular component, and molecular function (Figure 2(b)). Among these, the oxidation-reduction process, carbon–carbon lyase activity, and tetrapyrrole binding were the most enriched GO pathways in the FO versus SO group. The small-molecule biosynthetic process, lipid biosynthetic process, and GTP binding were the most enriched

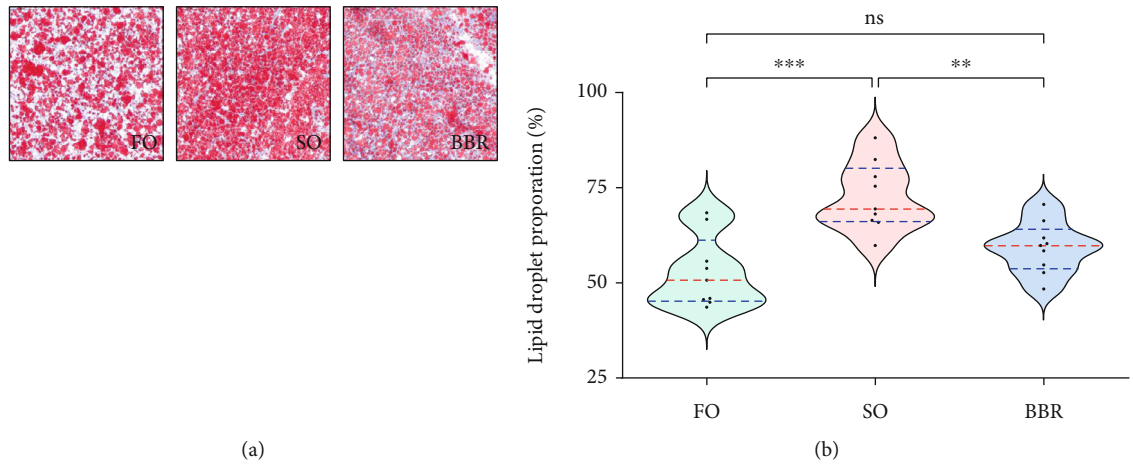


Figure 1: Hepatic tissue histological sections stained with Oil Red O (30x). Representative histomorphological images from Oil Red O stained hepatic tissue sections from fish-fed FO, SO, and BBR (a) diets. Lipid droplet proportions between treatments were significantly different at $**P < 0.01$ and $*** P < 0.001$, as determined with the Wilcoxon/Kruskal–Wallis test followed by the post hoc Wilcoxon test (b).

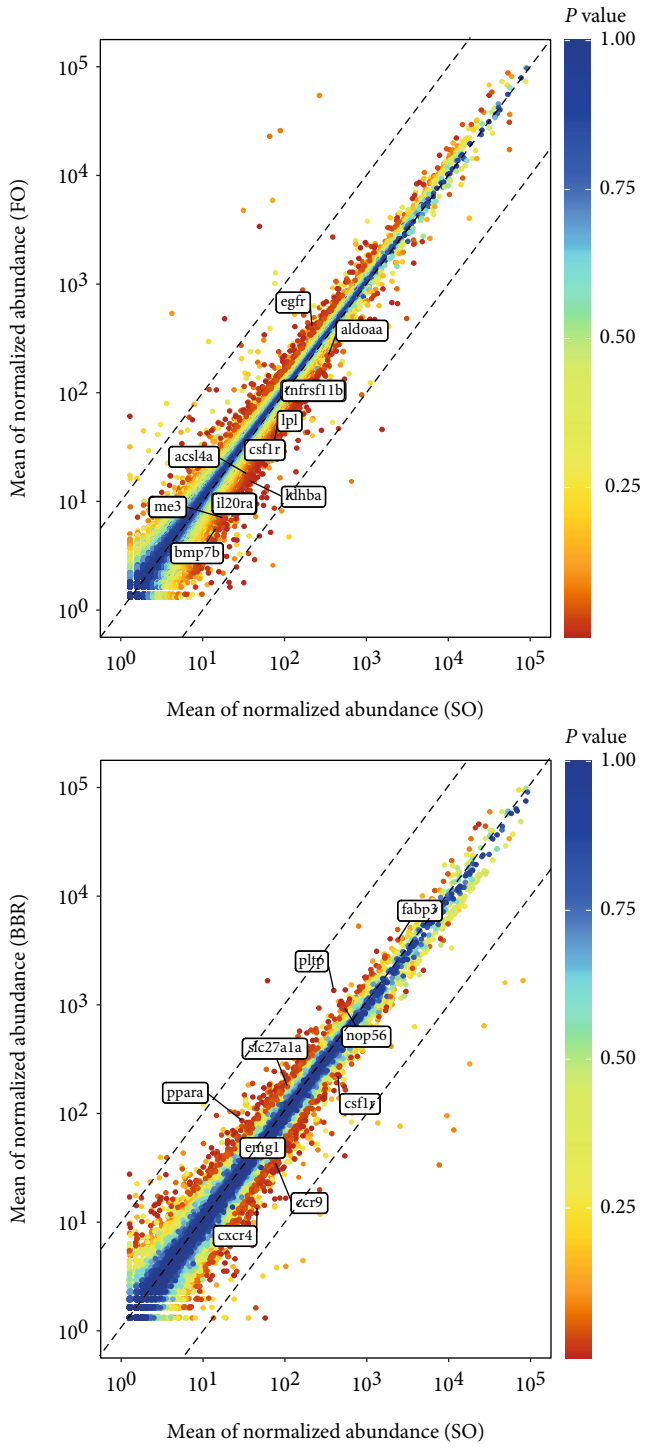
Table 5: Experimental fish blood sample biochemical parameters.

Parameter	FO	SO	BBR
Alanine aminotransferase (U L^{-1})	8.87 ± 4.53	9.16 ± 1.11	8.40 ± 1.13
Aspartate aminotransferase (U L^{-1})	32.51 ± 11.20^{a}	69.44 ± 28.64^{b}	50.11 ± 18.30^{ab}
Glucose (mmol L^{-1})	11.55 ± 2.99^{b}	6.99 ± 2.84^{a}	7.61 ± 1.26^{ab}
Triglyceride (mmol L^{-1})	2.92 ± 1.22^{b}	1.45 ± 0.33^{a}	1.52 ± 0.21^{a}
Cholesterol (mmol L^{-1})	3.55 ± 0.39^{b}	2.26 ± 0.08^{a}	2.14 ± 0.22^{a}
Free fatty acids (mmol L^{-1})	0.71 ± 0.06^{a}	1.08 ± 0.11^{b}	0.82 ± 0.16^{ab}
High-density lipoprotein (mmol L^{-1})	1.35 ± 0.16	1.58 ± 0.18	1.37 ± 0.23
Low density lipoprotein (mmol L^{-1})	0.48 ± 0.11	0.36 ± 0.14	0.30 ± 0.13

Values in the same row without superscripts are not significantly different, as determined by one-way ANOVA ($P > 0.05$).

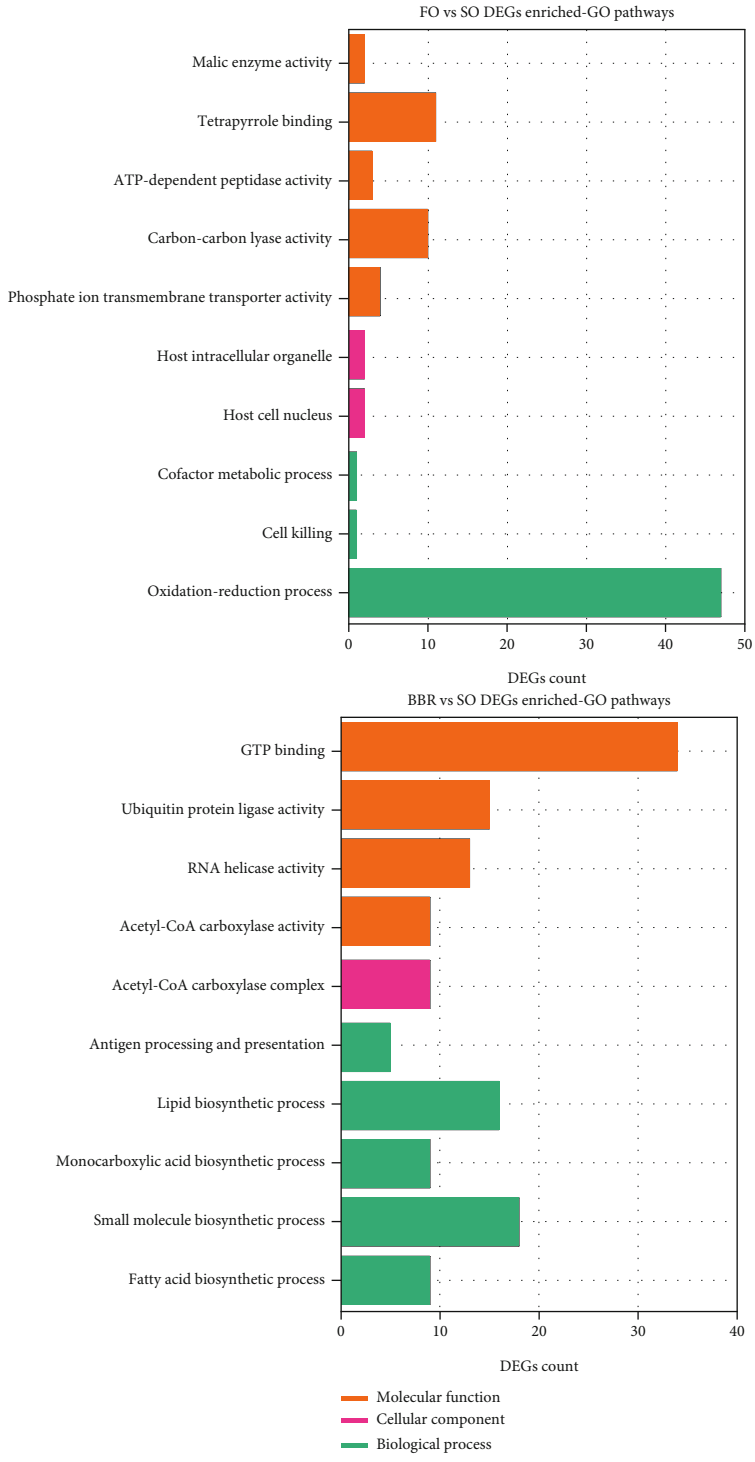
GO pathways in the BBR versus SO group. Notably, both FO versus SO and BBR versus SO DEGs were enriched in immunity-related GO pathways, such as host cell structure, cell killing, and antigen processing. The top 10 Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways are presented in Figure 2(c). Carbon metabolism, cytokine–cytokine receptor interaction, and cell adhesion molecules were the KEGG pathways with the highest number of DEGs in the FO versus SO group. Ribosome biogenesis in eukaryotes, adhesion molecules, and endocytosis was the KEGG pathways with the highest number of DEGs in the BBR versus SO groups. We also found that some metabolism-related pathways, such as PPAR and pentose phosphate pathways, were significantly enriched. Some important DEGs are shown in Figure 2(d) in the form of a heat map. Among the DEGs in the FO versus SO group, we found significantly higher cytokine-related genes, such as *toll-like receptor 1* (*tlr1*), *interleukin-20 receptor subunit a* (*il20ra*), and *macrophage colony-stimulating factor 1 receptor* (*csf1r*). We observed significantly lower *epidermal growth factor receptor* (*egfr*) and *insulin-like growth factor 1* (*igf1*) gene expressions in the SO group compared to those of the FO group ($P < 0.05$). The supplementation of BBR in a SO-based diet significantly increased ribosome-biogenesis-related gene expression, such as *ribosome biosynthesis protein sik1* (*nop56*) and *ribosome biogenesis protein nep1* (*emg1*) ($P < 0.05$). Moreover, BBR significantly increased the expression of lipid-metabolism-related genes such as *peroxisome proliferator-activated receptor a* (*ppara*) and *fatty acid-binding protein 3* (*fabp3*) ($P < 0.05$). Compared to the SO group, BBR significantly decreased the expression of proinflammatory genes, such as *C-C chemokine receptor type 9* (*ccr9*), *C-X-C chemokine receptor type 4* (*cxcr4*), and *csf1r* ($P < 0.05$). In summary, replacement of FO with SO decreased growth-related gene expression but induced proinflammatory gene expression. Supplementation with BBR in a SO-based diet significantly increased ribosome-biogenesis-related gene expression as well as fatty acid β-oxidation-related gene expression. Moreover, BBR supplementation significantly decreased SO-induced proinflammatory gene expression. The DEGs list for SO versus FO was provided in Table S2 and the DEGs list for BBR versus SO was provided in Table S3.

3.5. RNA-Seq DEGs Validation. To validate the DEGs identified by RNA-Seq analysis, we performed qRT-PCR on 14 selected genes (Figure 3). qRT-PCR analysis revealed that all the genes shared the same expression tendencies as those from the transcriptome data ($\Delta\Delta$Ct versus $\log_2$ fold change). Pearson's correction analysis indicated that the *R*-values were



(a)

Figure 2: Continued.



(b)

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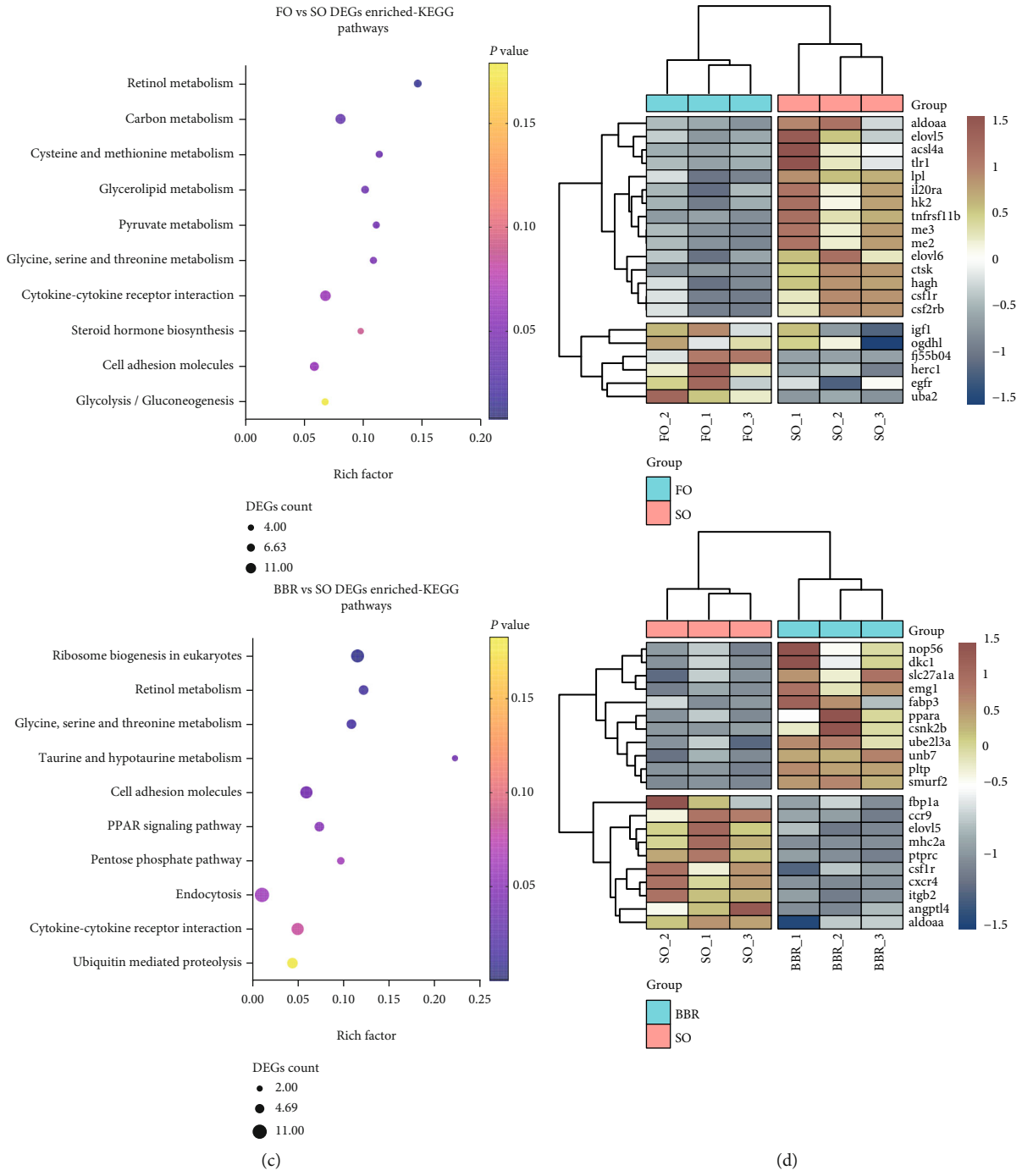

Figure 2: Transcriptome analysis of hepatic tissue of fish subject to experimental diets. (a) Volcanic map of detected genes expression levels between groups. We detected 626 differentially expressed genes (DEGs) in the FO v. SO group (250 up- and 376 downregulated) and 595 DEGs in the SO vs. BBR groups (281 up- and 314 downregulated). (b) DEGs-enriched GO pathways between groups. (c) DEGs-enriched KEGG pathways between groups. (d) Heap map for some DEGs.

0.8924 and 0.9844, respectively. The qRT-PCR results confirmed the credibility of the UMI-RNA-seq data obtained in this study.

4. Discussion

In this study, dietary BBR (50 mg kg^{-1}) supplementation significantly increased growth performance but decreased viscerosomatic index and condition factor as well as excessive lipid deposition. The amelioration of hepatic lipid deposition by BBR supplementation could be attributed to an increase in fatty acid β-oxidation. Dietary BBR also decreased the SO-induced proinflammatory gene expression. We discussed these results from (4.1) growth performance and body indices; (4.2) hepatic tissue lipid metabolism; and (4.3) proinflammatory gene expression.

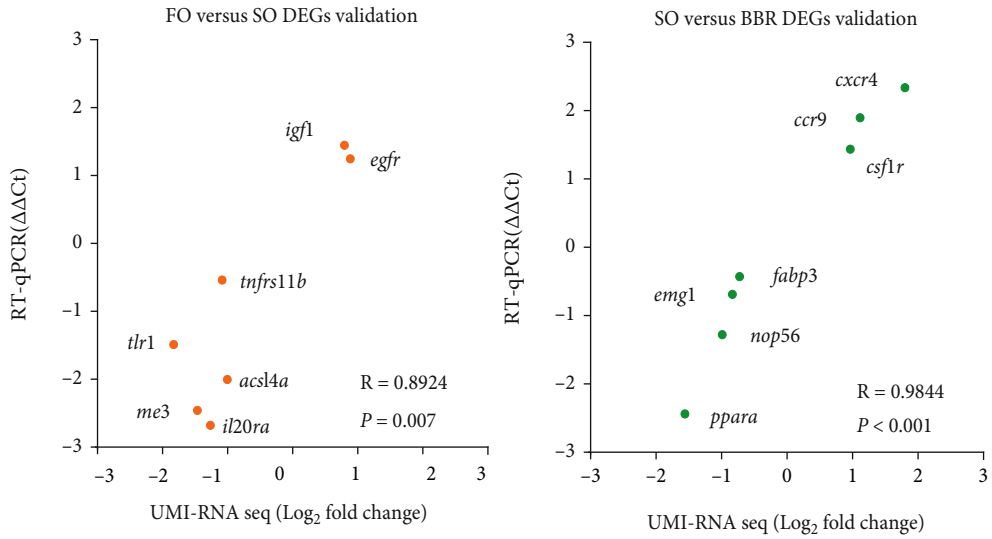

Figure 3: UMI-RNA seq DEG validation by qRT-PCR. The qRT-PCR analysis revealed that all the genes shared the same expression tendencies as those from the transcriptome data ($\Delta\Delta$Ct versus Log2 fold change). Pearson's correction analysis indicated the R-value was 0.8924 and 0.9844 for FO vs. SO and SO vs. BBR, respectively.

4.1. Growth Performance and Body Indices. The replacement of dietary FO by SO significantly decreased the weight gain rate (WGR) and feed efficiency ratio (FER). We mainly attributed these results to the lack of n-3 LC-PUFAs in the SO-based diet because the n-3 LC-PUFA content was far below its optimal content for best growth performance and feed conversion (9.0–9.5 g kg^{-1} diet) in this species [34]. At the molecular regulation level, the GH/IGF-1 growth axis tightly regulates fish growth, and the significantly decreased *efg1* and *igf1* expressions in the hepatic tissue of fish fed the SO diet might have accounted for growth retardation. Supplementation with BBR in a SO-based diet significantly increased the WGR. Studies have reported the growth promotion effect of feeding 50 mg kg^{-1} berberine in blunt snout bream [19, 22, 23] and 1–9 g kg^{-1} in Nile tilapia (*Oreochromis niloticus*) [35]. Conversely, no significant differences in growth performance were observed in largemouth bass (*Micropterus salmoides*) fed 100 or 400 mg kg^{-1} berberine hydrochloride [36] or 100 mg kg^{-1} berberine hydrochloride in grass carp (*Ctenopharyngodon idella*) [37]. We hypothesized that the growth promotion effects of BBR supplementation in this study is strongly related to ribosome production, as ribosome biogenesis in eukaryotes was the most significantly enriched-KEGG pathway in the BBR versus SO group. A ribosome is an essential unit of all living organisms that commands protein synthesis and therefore fuels cell growth. Individual growth, more precisely protein retention, primarily takes place in the ribosome. The ribosome is the essential unit of all living organisms that executes protein synthesis and therefore fuels cell growth and cell proliferation. The biogenesis of ribosomes requires abundant nutrients and ATP; therefore, the synthesis of ribosomes is tightly controlled. Dietary BBR supplementation significantly increased ribosome protein synthesis-related gene expression, such as those of *nop56* and *emg1*, and therefore increased step-forward protein production. BBR promoted growth in the SO-based diet of the juvenile yellow drum, and the mechanism might have involved the increased synthesis of proteins, predominantly RPs. BBR supplementation did not significantly affect the body nutrient composition but partially improved the SO-induced viscerosomatic index (VSI) and condition factor (CF). Feeding BBR seems to slim the fish, which agrees with findings in mammalians [38–40].

4.2. Hepatic Tissue Lipid Metabolism. The growth-promoting effect of berberine can also be attributed to its hepatoprotective effects, similar to those of other Chinese herbs [41]. Berberine can alleviate excessive hepatic lipid accumulation and pathological changes caused by HF and HC diets in black sea bream [16, 20], blunt snout bream [17–19], and zebrafish (*Danio rerio*) [2]. Dietary berberine suppresses hepatic tissue lipid accumulation by activating the farnesoid X receptor (FXR) in grass carp [37]. In the present study, hepatic tissue histological sections stained with Oil Red O indicated that the SO diet significantly increased the proportion of lipid droplets, whereas supplementation with BBR significantly decreased the proportion of lipid droplets compared to the SO group, and we found no significant differences between the BBR and FO groups. Berberine alleviates hepatic excessive lipid accumulation by increasing lipid β-oxidation, as we detected significantly increased *ppara* expression by RNA sequencing. PPARα is a master regulator conserved from fish to mammals that transcriptionally regulates the expression of genes involved in lipid metabolism [42]. Berberine upregulates the expression of hepatic lipid β-oxidation genes, such as carnitine palmitoyltransferase-1A (*cpt1a*) and hormone-sensitive lipase (*hsl*) in black sea bream [16], *cpt1* in blunt snout bream [17], and *cpt1* and *ppara* in blunt snout bream [24] along with peroxisome proliferator-activated receptor-gamma coactivator-1α (*pgc1a*) and *ppara* in blunt snout bream [19]. Findings in Nile tilapia indicated that the activation of *ppara* decreased the SFA and MUFA contents but did

not significantly affect the EPA + DHA content in hepatic tissue [43]. In this study, the changes in hepatic lipid metabolism were reflected by the significantly decreased hepatic tissue SFA proportion, but significantly increased proportion of PUFAs in BBR supplementation. Teleost studies demonstrated that MUFAs and SFAs are the primary targets of β-oxidation, followed by PUFAs [44]. We speculated that the SFA proportion in the hepatic tissue of fish fed the BBR diet decreased owing to the use of these fatty acids, so they used less protein (protein-sparing effect) and increased protein retention for growth.

BBR supplementation decreased free fatty acid content in the serum, which could also be attributed to the promotion of fatty acid oxidation [45]. BBR supplementation increased serum glucose content, which could be attributed to *para*-activation-induced energy homeostasis remodeling. Knocking out *ppara-b* increased glycogen consumption in zebrafish [42]. They hypothesized that PPARα activation causes more fatty acids to be catabolized to provide more energy, which reduces the energy supply from glycogen.

4.3. Proinflammatory Gene Expression. Excessive lipid deposition in the hepatic tissue can lead to hepatic injury and a proinflammatory response [46, 47]. Hepatic injury normally correlates with increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Our findings indicated that BBR supplementation in a SO-based diet ameliorated hepatic injury, characterized by a decrease in AST activity to a value comparable to that produced by the FO diet. The hepatoprotective effects of dietary berberine supplementation have also been reported in blunt snout bream fed an HF diet [18] and in black sea bream fed a high-starch diet [20].

Our previous results showed that vegetable oil could induce proinflammatory response through the nuclear factor-kappa B (NF-κB) pathway [47, 48]. In agreement, the SO diet significantly increased the expression of proinflammatory genes, such as the *csf1r*, *tlr1*, *tnfrs11b*, and *il20ra*. According to KEGG pathway analysis, these proinflammatory genes were enriched in the cytokine–cytokine receptor interaction pathway. The BBR diet significantly decreased cytokine–cytokine receptor interaction pathway-related gene expressions of *ccr4*, *ccr9*, *csf1r*, etc. In aquatic animals, Zhou et al. (2016) reported that common carp fed a 0.78 g kg^{-1} berberine-supplemented diet showed downregulated *il10* expression but upregulated the *tnfa* and *il1b* expressions in the head kidney. Additionally, Yu et al. [22] reported that berberine reduced intestinal *tnfa* and *il6* expressions that were upregulated by an HF and HC diet in blunt snout bream. These findings demonstrated that berberine supplementation decreased the proinflammatory-related gene expressions that were induced by HF, HC, and SO diets. Mammalian studies showed that berberine can directly downregulate the expressions of numerous proinflammatory cytokines, such as IL6, TNFα, and IL1β, as well as indirectly affect the expressions of activator protein 1 (AP-1) and the NF-κB signaling pathway to decrease inflammation response [21, 49]. Recently, the negative regulation of NF-κB contributed to berberine-mediated protection of crucian carp (*Carassius auratus gibelio*) from virus infection via simultaneously attenuating viral gene expression and the host inflammatory response [50]. Further studies are warranted to elucidate how dietary BBR decreases proinflammatory responses in aquatic animals.

5. Conclusions

In this study, feeding trials, biochemical analysis, and subsequent transcriptome analysis revealed the effects of dietary berberine chloride supplementation on the growth, body composition, hepatic fatty acid composition, hepatic histological sections, and serum biochemical indices, and hepatic tissue transcriptome analysis in yellow drum juveniles. Our conclusions are summarized as follows:

Dietary berberine chloride supplementation partly reversed SO-induced growth retardation, viscerosomatic index, and condition factor increases.

Dietary berberine chloride supplementation in a SO-based diet ameliorated excessive hepatic lipid deposition, probably by increasing SFA β-oxidation.

Berberine chloride supplementation decreases the SO-induced pro-inflammatory gene expression, characterized by the suppression of the cytokine–cytokine receptor interaction pathway.

Data Availability

Raw data were generated at the Marine Fisheries Research Institute of Zhejiang Province, China. Derived data supporting the findings of this study are available from Dr. Peng Tan or the corresponding author on request.

Conflicts of Interest

We have no conflicts of interest to declare.

Authors' Contributions

Peng Tan and Dongdong Xu designed the study. Peng Tan, Ruiyi Chen, and Ligai Wang performed the study. Peng Tan analyzed the data. Peng Tan and Dongdong Xu wrote the paper. All authors read and approved the final manuscript.

Acknowledgments

We thank Pian Zhang and Lei Zhang from Zhejiang Ocean University for providing support with the feeding trials and sampling. This work was supported by the National Natural Science Foundation of China (grant number: 41806206) and the Key Research and Development Project of Zhejiang Province (grant number: 2020C02015).

Supplementary Materials

Supplementary 1. Table S1. Basic information for the RNA sequencing data and mapping.

Supplementary 2. Table S2. DEGs list for SO versus FO.

Supplementary 3. Table S3. DEGs list for BBR versus SO.

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