Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 4160991, 18 pages
https://doi.org/10.1155/2022/4160991

Research Article

Evaluation of Necessity of Cholesterol Supplementation in Diets of Two Marine Teleosts, Turbot (*Scophthalmus maximus*) and Tiger Puffer (*Takifugu rubripes*): Effects on Growth and Lipid Metabolism

Xiaoxue Meng,[1,2] Qingzhu Bi,[1,2] Lin Cao,[3] Qiang Ma,[1] Yuliang Wei,[1,4] Mei Duan,[1] Mengqing Liang,[1,4] and Houguo Xu[1,4]

[1]*Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, 106 Nanjing Road, Qingdao 266071, China*
[2]*College of Fisheries and Life Sciences, Shanghai Ocean University, 999 Huchenghuan Road, Shanghai 201306, China*
[3]*Beijing Institute of Feed Control, 15 Beiyuan Road, Beijing 100107, China*
[4]*Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, 168 Wenhaizhong Road, Qingdao 266237, China*

Correspondence should be addressed to Houguo Xu; xuhg@ysfri.ac.cn

Received 11 April 2022; Revised 8 June 2022; Accepted 13 June 2022; Published 28 June 2022

Academic Editor: Ayşegül Kubilay

Two 70-day feeding trials were conducted to reevaluate the necessity of cholesterol supplementation in diets of two important marine fish species, turbot and tiger puffer. Graded levels (0, 0.5, 1.0, 2.0, and 4.0% of dry matter) of cholesterol were added into the basal diet (with 30% fishmeal but no fish oil) to make five experimental diets, which were designated as control, CHO-0.5, CHO-1.0, CHO-2.0, and CHO-4.0, respectively. The dietary cholesterol concentration was measured to be 0.11%, 0.65%, 1.10%, 2.32%, and 4.59%, respectively. Each group had triplicate tanks, and each tank was stocked with 30 fish. The results showed that compared to the control, dietary cholesterol supplementation had no significant effect on the growth of both turbot and tiger puffer. Nevertheless, excess dietary cholesterol (CHO-2.0 and CHO-4.0) led to significant lower weight gain of tiger puffer compared to CHO-1.0. The feed intake decreased with increasing levels of dietary cholesterol. In general, the cholesterol supplementation decreased the crude lipid content in the liver, whereas 0.5% dietary cholesterol supplementation tended to increase the crude lipid content in the muscle of both species. Dietary cholesterol supplementation significantly regulated the lipid-related biochemical parameters in serum, liver, and muscle, and compared to turbot, tiger puffer lipid compositions had a higher buffering capacity in response to changes in dietary cholesterol level. Dietary cholesterol supplementation increased the 20:4n-6 content in the liver. Dietary cholesterol supplementation significantly downregulated the gene expression of HMG-COAr, upregulated the CYP7A1 expression, and downregulated the expression of lipid absorption and biosynthesis genes, but had no significant effect on gene expression of CPT1 and lipid transport-related genes (ApoA1, ApoA4, ApoB100, and ApoEα) in the liver. In conclusion, under the present experimental condition, dietary cholesterol supplementation had no significant effect on the growth of turbot and tiger puffer. In both species, dietary cholesterol supplementation significantly regulated the lipid accumulation and metabolism.

1. Introduction

Cholesterol, as the most abundant steroid compound in animal body, is an important component of animal cell membranes [1] and an important precursor of steroid hormones, vitamin D_3, and bile acids [2, 3]. Cholesterol is commonly considered to be a non-essential nutrient in diets for fish because it can be synthesized in vertebrates [4]. However, cholesterol biosynthesis is an energy-consuming process. When the cholesterol in the diets is insufficient, fish need to mobilize considerable energy to synthesize cholesterol endogenously, which otherwise could support growth

alternatively [5]. Therefore, the necessity and importance of cholesterol supplementation in fish diets need to be reevaluated. In particular, the levels of fishmeal and fish oil, which is the main source of dietary cholesterol supply, are reducing due to the relative shortage in supply and consequently high price [6, 7].

In fact, when the necessity of cholesterol supplementation is evaluated, controversial results have been observed. Previous research has shown that the supplementation of cholesterol into diets with fishmeal as the main protein source could not significantly affect the growth of Atlantic salmon (Salmo salar) (fishmeal, 60%; the supplemented and measured cholesterol level, 1% and 1.38%, respectively) [8] and hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (fishmeal, 58.3%; the supplemented cholesterol level, 1%) [9]. In contrast, when fish were fed diets with high levels of plant protein, the growth performance and feed intake were enhanced with the supplementation of 0.9-2.0% cholesterol. This was observed in species such as channel catfish (Ictalurus punctatus) (fishmeal, 0%; the supplemented cholesterol level, 1%) [10], Japanese flounder (*Paralichthys olivaceus*) (fishmeal, 0%; the supplemented and measured cholesterol level, 1% and 1.2%, respectively) [11], turbot (*Scophthalmus maximus*) (fishmeal, 14.5%; the supplemented and measured cholesterol level, 1% and 1.25%, respectively) [12, 13], Nile tilapia (*Oreochromis niloticus*) (fishmeal, 16.5%; the supplemented cholesterol level, 2%) [14], and rainbow trout (*Oncorhynchus mykiss*) (fishmeal, 19-32%; the supplemented and measured cholesterol level, 0.9-1.2% and 1.30-1.68%, respectively) [15, 16]. The supplementation of 0.5-2% cholesterol (the measured level, 0.76-2.22%) to the defatted fishmeal-based diet (inclusion level, 65%) also promoted the growth of turbot [17]. Nevertheless, there were some exceptions. For example, the supplementation of 1% cholesterol (the measured level, 1.36%) to high plant protein-based diet (fishmeal, 27.5%; soybean meal, 42.9%) had no significant effect on the growth of the giant grouper (*Epinephelus lanceolatus*) [18]. In addition to the type of dietary protein source, the effect of cholesterol supplementation was also related to the processing of protein sources. The addition of 1-1.5% cholesterol (the measured level, 1.1-1.57%) to diets with soy protein concentrate as the main protein source had no significant effect on the growth of Atlantic salmon [19] and Japanese flounder [11], whereas the supplementation of 1% cholesterol (the measured level, 1.2%) into the diet with soy protein isolate as the main protein source enhanced the growth of Japanese flounder [11].

Both turbot and tiger puffer are important aquaculture species. They are also becoming good research models due to the detailed genome information and special lipid storage patterns [20]. The present study was aimed at reevaluating the necessity of cholesterol supplementation in diets of these two species, when a medium fishmeal level but no fish oil was used in the diets. Alongside the growth performance, the present study also evaluated the roles of dietary cholesterol supplementation in lipid metabolism, considering the important direct and indirect roles of cholesterol in lipid absorption, transport, and metabolism [21]. In addition, turbot and tiger puffer have different lipid storage patterns. They store lipid predominantly in subcutaneous adipose tissue around fin and liver, respectively. It is highly interesting but still elusive that whether the lipid storage pattern affects the cholesterol requirement and function. The comparison of results between these two species may provide basic data and part answer for this question.

2. Materials and Methods

2.1. Experimental Diets. A same basal diet with approximately 50% protein and 8% lipid, which were demonstrated to be suitable for both turbot and tiger puffer [22–25], was used for these two species. The basal diet (control) was made with a medium fishmeal level (30%, [26–29]) but no fish oil (Table 1). Other protein sources such as corn gluten meal, soybean meal, casein and brewer's yeast and lipid sources such as linseed oil, soybean oil, and rapeseed oil were used in the diet formulation. Graded levels (0.5, 1.0, 2.0, and 4.0% of dry matter) of cholesterol product (AR, purity >95%, Macklin) was added into the basal diet to make the four cholesterol-supplemented diets, which was designated as CHO-0.5, CHO-1.0, CHO-2.0, and CHO-4.0, respectively. The fatty acid compositions of the experimental diets are presented in Table 2. All the diets were made following the standard procedures in our laboratory and stored at -20°C before use [30].

2.2. Experimental Fish, Feeding Procedure and Sampling. The feeding trials with juvenile turbot (initial body weight 21 g) and juvenile tiger puffer (initial body weight 12 g) were conducted in Haiyang Huanghai Aquaculture Co., Ltd. Prior to the feeding trial, fish were reared in polyethylene tanks and fed a commercial diet (protein 50%; lipid 10%) for 2 weeks to acclimate to the experimental conditions. At the onset of the feeding trial, experimental fish were randomly distributed into 30 polyethylene tanks (200 L), including 15 tanks of turbot and 15 tanks of tiger puffer. Each dietary group had triplicate tanks, and each tank was stocked with 30 fish. Due to the difference in digestive characteristics, turbot were hand-fed to apparent satiation twice daily (7 : 00 and 19 : 00), whereas tiger puffer were fed three times a day to apparent satiation (6 : 30, 12 : 30, and 18 : 30). All the tanks were supplied with flow-through filtered seawater and cleaned daily by siphoning out residual feed and feces. The feeding trials lasted 70 days. During the experiment, the water temperature ranged from 19 to 21°C; salinity, from 28 to 30; pH, from 7.6 to 7.9; and dissolved oxygen > 8 mg L^{-1}.

At the end of the feeding trials, the fish were firstly fasted for 24 h. After being anesthetized with eugenol (1 eugenol: 10,000 water), the fish in each tank was bulk weighted, and the number of fish was recorded. After that, three randomly selected whole fish were collected from each tank for the analysis of proximate composition. Six more randomly selected fish per tank were dissected to collect the samples of serum, liver, and muscle for other assays. Blood was collected from the caudal vein, which was allowed to clot firstly at room temperature for 2 h and then at 4°C for 6 h. After that, centrifugation (4000 × *g*, 10 min, 4°C) was conducted to separate the straw-colored supernatants (serum samples). After dissection, before collection of tissue samples, weight of liver and viscera from 3 fish, as well as that of the corresponding whole fish, were recorded first to calculate the

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

Ingredients	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
Fishmeal	30	30	30	30	30
Corn gluten meal	10	10	10	10	10
Soybean meal	10	10	10	10	10
Casein	10	10	10	10	10
Wheat meal	16.68	16.68	16.68	16.68	16.68
Brewer's yeast	10	10	10	10	10
Mineral premix[a]	0.5	0.5	0.5	0.5	0.5
Vitamin premix[a]	1	1	1	1	1
Monocalcium phosphate	1	1	1	1	1
L-ascorbyl-2-polyphosphate	0.2	0.2	0.2	0.2	0.2
Choline chloride	0.2	0.2	0.2	0.2	0.2
Betaine	0.3	0.3	0.3	0.3	0.3
Ethoxyquin	0.02	0.02	0.02	0.02	0.02
Mold inhibitor[b]	0.1	0.1	0.1	0.1	0.1
Linseed oil	2	2	2	2	2
Soybean oil	1	1	1	1	1
Rapeseed oil	1	1	1	1	1
Soya lecithin	2	2	2	2	2
α-Starch	4	3.5	3	2	0
Cholesterol[c]	0	0.5	1	2	4
Proximate composition					
Crude protein	49.75	50.22	50.14	50.52	50.50
Crude lipid	8.34	8.92	9.47	10.56	12.73
Ash	8.02	8.12	8.07	8.07	7.98
Cholesterol	0.11	0.65	1.10	2.32	4.59

[a]Vitamin premix and mineral premix, designed for marine fish, were purchased from Qingdao Master Biotech Co., Ltd, Qingdao, China. [b]Contained 50% calcium propionic acid and 50% fumaric acid. [c]Cholesterol (AR, >95%) was purchased from Shanghai Macklin Biochemical Co., Ltd, Shanghai, China.

viscerosomatic index (VSI) and hepatosomatic index (HSI). VSI = wet viscera weight/fish body weight × 100; HSI = wet liver weight/fish body weight × 100. The body length of these three fish was also recorded for the calculation of condition factor (CF). CF = body weight/(body length^3) × 100. All the tissue samples were frozen with liquid nitrogen immediately and then stored at −76 °C before use. All sampling protocols, as well as all fish rearing practices, were approved by the Animal Care and Use Committee of Yellow Sea Fisheries Research Institute, and efforts have been taken to minimize the suffering of fish.

2.3. Analysis of the Proximate Composition and Fatty Acid Composition. The proximate composition analysis of experimental diets and whole fish (three individual fish per tank) was conducted according to the standard methods of the Association of Official Analytical Chemists (AOAC) [31]. The lipid content of the whole fish, liver, muscle, and subcutaneous adipose tissue around the fin was extracted and analyzed with the chloroform-methanol method according to Folch et al. [32].

The fatty acid compositions of diet and liver were analyzed with gas chromatograph (GC-2010 Pro, Shimadzu, Japan). Briefly, fatty acids in freeze-dried samples were reacted first with KOH-methanol and then with HCL-methanol on 75 °C water bath to obtain fatty acid methyl esters. Fatty acid methyl esters were extracted with hexane and then subjected to the gas chromatography equipped with a fused silica capillary column (SH-RT-2560, 100 m × 0.25 mm × 0.20 μm, Shimadzu, Japan; dicyano-propyl-polysiloxane as stationary phase) and a flame ionization detector. The column temperature increase was programmed: from 150 °C up to 200 °C at a rate of 15 °C min^{-1} and then from 200 °C to 250 °C at a rate of 2 °C min^{-1}. Both the injector and detector temperature were 250 °C. Results were expressed as percentage of each fatty acid with respect to total fatty acids (TFA).

2.4. Biochemical Parameters in Serum, Live and Muscle. The concentration of total cholesterol (TC), triglyceride (TG), free cholesterol (FC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and malondialdehyde (MDA) in the serum were measured using commercial kits. The FC kit was supplied by Applygen Technologies Inc., Beijing, China. The other kits were supplied by Nanjing Jiancheng Bioengineering Institute, Nanjing, China. The cholesteryl ester (CE) level was calculated by subtracting the FC from the TC value.

The tissue homogenates of liver and muscle were prepared in 10 volumes (w/v) of ice-cold anhydrous ethanol,

Table 2: Fatty acid compositions of the experimental diets (% total fatty acids).

Fatty acid	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
12 : 0	0.12	0.13	0.11	0.12	0.10
14 : 0	2.80	2.80	2.70	2.75	2.71
16 : 0	17.38	17.11	16.97	16.99	17.59
18 : 0	5.08	4.94	5.01	4.95	5.32
∑SFA	26.40	25.96	25.73	25.78	26.73
16:1n-7	3.22	3.22	3.25	3.20	3.26
18:1n-9	24.68	23.90	24.12	23.66	24.89
20:1n-9	0.62	0.54	0.60	0.55	0.62
22:1n-9	0.10	0.08	0.09	0.10	0.11
24:1n-9	0.16	0.17	0.19	0.18	0.21
∑MUFA	28.93	28.05	28.37	27.82	29.23
18:2n-6	23.27	23.41	23.39	23.42	23.23
18:3n-6	0.22	0.22	0.21	0.22	0.20
20:4n-6	0.45	0.45	0.45	0.45	0.51
∑n-6PUFA	23.94	24.12	24.05	24.10	23.94
18:3n-3	13.69	14.25	14.29	14.25	13.15
20:5n-3	4.32	4.63	4.66	4.87	4.27
22:5n-3	0.59	0.64	0.63	0.65	0.60
22:6n-3	2.13	2.31	2.28	2.52	2.09
∑n-3PUFA	20.73	21.87	21.85	22.31	20.10
n-3/n-6	0.87	0.91	0.91	0.93	0.84

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. In addition to the fatty acids listed in the table, total fatty acids include 13 : 0, 15 : 0, 17 : 0, 20 : 0, 21 : 0, 22 : 0, 14:1n-5, 15:1n-5, 17:1n-7, 20:2n-6, 20:3n-6, 22:2n-6, and 20:3n-3. Data in the same row not sharing a superscript were significantly ($P < 0.05$) different.

followed by centrifugation (2500 × g for 10 min at 4 °C), and the supernatant was sampled for the measurement of TC, TG, HDL-C, and LDL-C. When total bile acid (TBA) and MDA were measured, the homogenate is made from the normal saline. All the assay for liver and muscle samples was similar to the assay for serum samples.

2.5. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Analysis. Total RNA in liver was extracted using RNAiso Plus (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) and reverse transcribed with Evo M-MLV RT Mix Kit with gDNA Clean for qPCR (Accurate Biotechnology (Hunan) Co., Ltd., Hunan, China) according to the user manual.

Specific primers for target genes and the reference genes (β-actin and EF1α) were designed based on the sequences available in the GenBank database and synthesized by TsingKe Biological Technology Co., Ltd. (Qingdao, China) (Tables 3 and 4). The amplification efficiency for all primers, which was estimated by standard curves based on a 6-step 4-fold dilution series of target template, was within 95~105%, and the coefficients of linear regression (R^2) were >0.99. SYBR® Green Premix Pro Taq HS qPCR Kit II (Accurate Biotechnology (Hunan) Co., Ltd., Hunan, China) and a quantitative thermal cycler (Roche LightCycler 96, Basel, Switzerland) were used for the real-time qPCR. The reaction system consists of 2 μL cDNA template, 10 μL SYBR® Green Pro Taq HS Premix II, 0.8 μL forward primer (10 μM), 0.8 μL reverse primer (10 μM), and 6.4 μL sterilized water. The program was as follows: 95 °C for the 30s followed by 40 cycles of "95 °C for 5 s, 57°C for 30s, 72 °C for 30 s." Melting curve analysis (6.4 °C increment/min from 65 °C to 97 °C) was performed after the amplification phase for confirmation of the sole product. Each sample was run in triplicate. The mRNA expression levels were calculated with the qRT-PCR method: $2^{-\Delta\Delta Ct}$ [33].

2.6. Statistical Methods. All data were subjected to one-way analysis of variance (ANOVA) in SPSS 16.0 for Windows. Duncan's multiple range test was used to detect the significant differences between the means. The significance was accepted when $P < 0.05$. The results are presented as means of triplicate tanks ± standard error. Regression analysis with SPSS between dietary cholesterol and responding parameters was conducted where necessary.

3. Results

3.1. Growth Performances and Somatic Indices. For the turbot trial, no significant difference ($P > 0.05$) was observed among dietary groups in survival, final weight, and weight gain (Table 5). The survival of turbot was higher than 97% in all treatments. With increasing levels of cholesterol

Table 3: Sequences of the primers used in this work of turbot.

Primer	Sequence (5′-3′)	GenBank reference	Product length (bp)
FAS-F	GGCAACAACACGGATGGATAC	KC189927.1	205
FAS-R	CTCGCTTTGATTGACAGAACAC		
CPT1-F	GCCTTTCAGTTCACCATCACA	XM_035614266.1	113
CPT1-R	ATGCGGCTGACTCGTTTCTT		
GPAT-F	GTCGCAGCTAAAACCAAAC	XM_035607968.1	199
GPAT-R	TCCGCAGCAGGTAACTCAT		
DGAT-F	ATGTACTACTTTGTCTTTGCCC	KC189938.1	180
DGAT-R	GAATGGTTTCATTGAGTTCTGT		
ATGL-F	GCGAAAGAGGCAAGAAAGCGA	XM_035643755.1	263
ATGL-R	TGTAGTGCGGGAGGGATAAGG		
HSL-F	GTTGAGAGGGGCAGAATTTG	XM_035629599.1	226
HSL-R	GATGTGTGCTGACGGTGGAG		
DAGLα-F	CACAGATGGACAGCAGTTGGA	XM_035628256.1	147
DAGLα-R	GATGACAGAGGGAAGGAGGGT		
MGLL-F	CTGCTGCTCGTGAACTCTG	XM_035628286	84
MGLL-R	CGACTTCTTTGCTGCTAATGT		
PL-F	TCAGGGCTTTCTGGGATT	XM_035605303	289
PL-R	GAGCTGGAACTCGTTGGTG		
BSAL-F	CGCCGTCCTGACATTAGC	XM_035609042	289
BSAL -R	AGCCTTGCCCTTCTCCCT		
LPL-F	CTCCCACGAACGCTCTAT	KC189937.1	166
LPL-R	GCGGACCTTGTTGATGTT		
HL-F	GGGCTACGACATCAAGAAG	XM_035629475	197
HL-R	TGAAGGAGATATGGAGGTTT		
ApoA1-F	CAGCCTGGAGCAGAGTGT	XM_035637559	170
ApoA1-R	CCATTTGTTTCACCGAGTT		
ApoA4α-F	AGGATGCTTTCTGGGACTATGT	XM_035620874	101
ApoA4α-R	GAGGCTGTTCACTTCCTTTCC		
ApoB100-F	TCTCACCCTCGGTCTCGG	XM_035617338.1	158
ApoB100-R	TTCAGGTTTCTCCTCACAACGA		
ApoEα-F	GGCAGCAGATGGAGAAGT	XM_035620876	277
ApoEα-R	TTCAGCAGGTCGTTCAGG		
PPARα1-F	GCCTGGTGAATGTGGAGCA	JN253593.1	103
PPARα1-R	GGGAAGAGGAACGTGTCGT		
PPARα2-F	CCCTGATAACACCTTCCTCTTTCCC	JQ901838.1	109
PPARα2-R	TGTCTCGGTCGTCTTGATGTCCTG		
PPARβ-F	ACGGCAAAGGCTTCGTTACC	XM_035643796.1	147
PPARβ-R	CTAATGGCAGCAACAAACAGG		
PPARγ-F	AAGTGACGGAGTTCGCCAAGA	XM_035631101.1	121
PPARγ-R	GTTCATCAGAGGTGCCATCA		
SREBF1-F	CAGACCAGCAATGCGAAGAT	XM_035615397.1	224
SREBF1-R	CAGGAGCCGACAGGAAGGAG		
HMG-COAr-F	CCACGAGCAATGTTGTCCC	JN542428.1	206
HMG-COAr-R	TTAGGCATCGCTGGTCTTTT		
CYP7A1-F	TCAAATAGCCAGCGGCAAAC	XM_035635553.1	227
CYP7A1-R	CCATGACAGCTTCGACCCTC		
EF1α-F	TATTAACATCGTGGTCATTGG	KU057926.1	153
EF1α-R	CAGGCGTACTTGAAGGAG		

Table 3: Continued.

Primer	Sequence (5′-3′)	GenBank reference	Product length (bp)
β-Actin-F	GTAGGTGATGAAGCCCAGAGCA	MT023044.1	204
β-Actin-R	CTGGGTCATCTTCTCCCTGT		

FAS: fatty acid synthase; CPT1:carnitine O-palmitoyltransferase 1; GPAT: glycerol-3-phosphate acyltransferase; DGAT: diacylglycerol O-acyltransferase 1; ATGL: adipose triacylglyceride lipase; HSL: hormone-sensitive lipase-like; DAGLα:diacylglycerol lipase, alpha; MGLL: monoglyceride lipase; PL: pancreatic lipase; BSAL: bile salt-activated lipase; LPL: lipoprotein lipase; HL: hepatic lipase; Apo: apolipoprotein; PPAR: peroxisome proliferator-activated receptor; SREBF1: sterol regulatory element-binding factor 1; HMG-COAr: 3-hydroxy-3-methylglutaryl CoA reductase; CYP7A1: cholesterol 7-alpha-monooxygenase.

supplementation, the feed intake of turbot generally decreased (Figure 1), but the somatic indices of turbot (HSI, VSI, and CF) generally increased and the highest values were observed in group CHO-2.

For the tiger puffer trial, with increasing levels of dietary cholesterol, the weight gain firstly increased and then decreased. The highest and lowest value was observed in groups CHO-1 and CHO-4, respectively. Moreover, the weight gain in group CHO-1 was significantly ($P < 0.05$) higher than that in groups CHO-2 and CHO-4. However, 0.5-4% cholesterol supplementation had no significant ($P > 0.05$) effect on the growth performance of tiger puffer compared to the control group. Similar to the turbot trial, the feed intake of tiger puffer decreased with increasing levels of dietary cholesterol (Figure 1). No significant difference ($P > 0.05$) was observed among dietary treatments in VSI and CF of tiger puffer. The HSI of tiger puffer in group CHO-4 was lower than that in other groups.

3.2. Body Composition. For both species, there was no significant difference ($P > 0.05$) in whole-body protein content among dietary groups (Table 6), but dietary supplementation of 0.5% cholesterol significantly increased ($P < 0.05$) the crude lipid content in the muscle. The crude lipid content in the liver of both species gradually decreased with increasing levels of dietary cholesterol.

For the turbot trial, group CHO-1 had the highest lipid content in the whole fish body and the subcutaneous tissue around the fin. No significant difference ($P > 0.05$) was observed in the whole-body ash content of turbot. For the tiger puffer trial, compared to the control group, dietary cholesterol supplementation at 4% significantly ($P < 0.05$) reduced the whole-body ash content. No significant difference ($P > 0.05$) was observed in the crude lipid content of whole fish.

3.3. Fatty Acid Profiles in the Liver. The fatty acid profiles of turbot and tiger puffer liver in response to the experimental diets are presented in Table 7. For the turbot trial, in general, dietary cholesterol supplementation increased the 14 : 0 and 16:1n-7 content, but decreased the 18 : 0 content. Dietary cholesterol supplementation substantially ($P < 0.05$) increased the 20:4n-6 (ARA) content. In tiger puffer liver, the 20:4n-6 content was also significantly ($P > 0.05$) increased by dietary cholesterol supplementation, but the 22:5n-3 content was significantly ($P < 0.05$) decreased.

3.4. Biochemical Parameters in Serum, Live and Muscle. The dietary cholesterol supplementation significantly affected the biochemical parameters in serum including TC, FC, CE, TG, HDL-C, LDL-C, TBA, and MDA (Table 8, Figure 1). For the turbot trial, the concentration of all these parameters in the serum was increased by dietary cholesterol supplementation. However, for the tiger puffer serum, the changes were much milder. The concentration of only LDL-C and TBA was significantly ($P < 0.05$) increased by dietary cholesterol supplementation in tiger puffer serum. There was even no significant difference ($P > 0.05$) in the FC and HDL-C concentration among dietary groups of the tiger puffer trial.

In both species, dietary cholesterol inclusion did not significantly ($P > 0.05$) influenced the TG, HDL-C, and TBA contents in the liver, but significantly ($P < 0.05$) increased the TC and LDL-C contents (Table 9 and Figure 1).

For turbot muscle, no significant difference ($P > 0.05$) was observed in TC, HDL-C, and MDA among dietary groups (Table 10). Group CHO-4 had significantly higher LDL-C content than the control group and group CHO-2. Group CHO-0.5 had significantly higher TG content than group CHO-2. For tiger puffer muscle, group CHO-2 had significantly lower TC and TG contents than the control group, and other parameters such as HDL-C, LDL-C, and MDA also had decreasing trends with increasing levels of dietary cholesterol inclusion.

3.5. Lipid Metabolism–Related Gene Expression. The expression of key genes involved in lipid metabolism in fish liver is presented in Table 11 and Figures 2 and 3. In both species, dietary cholesterol supplementation substantially downregulated the 3-hydroxy-3-methylglutaryl CoA reductase (HMG-COAr) expression and linearly upregulated the cholesterol 7-alpha-monooxygenase (CYP7A1) expression (Figure 1). For turbot, the HMG-COAr expression in the control group was nearly 676-fold higher than that in group CHO-4, although the corresponding change in tiger puffer was milder. In both species, the expression of fatty acid synthase (FAS) in the control group was nearly two-fold that in other groups.

For the turbot trial, with increasing levels of dietary cholesterol supplementation, the transcription of monoglyceride lipase (MGLL) increased firstly and decreased afterward. The highest MGLL expression was observed in group CHO-2, which was significantly ($P < 0.05$) higher compared to the control group. The transcription of pancreatic lipase (PL) and bile salt-activated lipase (BSAL) in the control group was nearly two-fold that in group CHO-4. Group CHO-1 had higher expression of peroxisome proliferator-activated

Table 4: Sequences of the primers used in this work of tiger puffer.

Primer	Sequence (5′-3′)	GenBank reference	Product length (bp)
FAS-F	CTTTGCCGCTGTCATTCG	XM_011619859.1	78
FAS-R	TGTCTCAACCCATTTGTAGTCG		
CPT1-F	GGGGTTTGTGGTCAAGTTAGG	XM_011607269.1	186
CPT1-R	ATAGATCCGTGGCGCTCAT		
MOGAT-F	AAAGGCTTCATTAAATTGGC	XM_003978609.3	223
MOGAT-R	TGATGGCTTGTCTGTAGGG		
GPAT-F	CCCGTTCACAAATCCCACA	XM_011621885.1	235
GPAT-R	GGCACAACAACTCCTCCGTAT		
DGAT-F	TGGTTTGTGAGCCGTTTCC	XM_003969352.2	185
DGAT-R	CTGGCATTCGTTTGACTTCG		
ATGL-F	CCAACCTCTACAGGGTCTCA	XM_003967696.3	119
ATGL-R	GTTTAGCAGCCCGTTCTTC		
HSL-F	CTCTTGCTATCGGTCTTGTGG	XM_011621066.1	113
HSL-R	TTCTGGGTCAATGGCATACTT		
DAGLα-F	CTGTTGGTGGAGTTGGTGTATG	XM_011610175.1	72
DAGLα-R	ATCAGAGCACGGCTGGTAAT		
MGLL-F	CCATCCAGTCAAAGTGGGTCT	XM_003963030.2	110
MGLL-R	CATCAGCTGCATGCCGAA		
PL-F	CGTTTTCTCCTGTTCACCC	LOC101064949	97
PL-R	GACTCGTCCTCATCCCACT		
BSAL-F	TTGAAGATGACTGACCCCGA	XM_003978375.2	162
BSAL-R	GATGTCTGCTGCGTTGTGAA		
LPL-F	AGGGTCCACATCCGCAAA	NM_001305600.1	157
LPL-R	GTTTCTCCTTGCGGCTCAT		
HL-F	GCGGCTTCAACAGCAGTAA	XM_011610357.1	215
HL-R	GAGGTGCGCTATGTCTTTCC		
ApoA1-F	CGATGACGCCGAGTACAAA	AB183289.1	104
ApoA1-R	CGGTTATGGGAGAAACGCTA		
ApoA4-F	TGCTTTCTGGGACTATGTTGC	NM_001078591.1	124
ApoA4-R	GTTGACTTTGTCGGCACTCTC		
ApoB100-F	AGGGACATAGTCAAACCAAGGA	XM_011619944.1	127
ApoB100-R	AGAACACGAAGGCTGGACAC		
ApoEα-F	TATTCAGACCCGCACCTCA	NM_001078592.1	201
ApoEα-R	ATTTCCTCCATCTTGTCCTCC		
PPARα1-F	TCAGTAGTTTATGGGTTGGTGG	NM_001097630.1	119
PPARα1-R	GCGTGGACTCCGTAGTGGTA		
PPARα2-F	CCAGAAGAAGAACCGCAACA	NM_001097629.1	149
PPARα2-R	CCTCTTTCTCCACCATCTTGTT		
PPARβ-F	AGCTGGAATACGACCGATGT	AB275887.1	249
PPARβ-R	TCTTCAGGTAGGCGGAGTTG		
PPARγ-F	CGCTGTCCCGACATCTGTAT	NM_001097627.1	146
PPARγ-R	GAACTGCTCGCCTTCCATT		
SREBF1-F	TTTCAGCATCCCACCTTCC	XM_011603881.1	158
SREBF1-R	GGTGAACCGTGAGGACAACTA		
HMG-COAr-F	GCTGCTGGCAATCAAGTACAT	XM_003974466.2	143
HMG-COAr-R	AAACATACAACTCCTTCCTACAGC		
CYP7A1-F	CCTACCTGCTACCTTCTGGAGT	XM_003975521.2	237
CYP7A1-R	TCCTCTTTGGCAACACGAA		

Table 4: Continued.

Primer	Sequence (5′-3′)	GenBank reference	Product length (bp)
EF1α-F	TTGGAGGCATTGGAACTGT	NM_001037873.1	86
EF1α-R	GTTGACGGGAGCAAAGGT		
β-Actin-F	CCAGAAAGACAGCTACGTTGG	U37499.1	147
β-Actin-R	GCAACTCTCAGCTCGTTGTAG		

FAS: fatty acid synthase; CPT1: carnitine O-palmitoyltransferase-1; MOGAT: monoacylglycerol O-acyltransferase 2; GPAT: glycerol-3-phosphate acyltransferase; DGAT: diacylglycerol O-acyltransferase 1; ATGL: adipose triacylglyceride lipase; HSL: hormone-sensitive lipase-like; DAGLα:diacylglycerol lipase, alpha; MGLL: monoglyceride lipase; PL: pancreatic lipase; BSAL: bile salt activated lipase; LPL: lipoprotein lipase; HL: hepatic lipase; Apo: apolipoprotein; PPAR: peroxisome proliferator-activated receptor; SREBF1: sterol regulatory element-binding factor 1; HMG-COAr: 3-hydroxy-3-methylglutaryl CoA reductase; CYP7A1: cholesterol 7-alpha-monooxygenase.

Table 5: Growth performance of experimental fish during the feeding period (mean ± standard error).

Parameters	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
Initial weight g	20.66 ± 0.01	20.64 ± 0.01	20.64 ± 0.01	20.66 ± 0.01	20.66 ± 0.01
Final weight g	74.08 ± 1.08	72.11 ± 2.11	73.76 ± 3.06	75.33 ± 0.75	72.57 ± 1.11
Weight gain %	258.65 ± 5.30	249.30 ± 10.22	257.27 ± 14.94	264.69 ± 3.46	251.32 ± 5.53
Feed intake %	1.78 ± 0.03^{ab}	1.80 ± 0.03^{a}	1.79 ± 0.02^{ab}	1.74 ± 0.02^{ab}	1.72 ± 0.01^{b}
Feed efficiency ratio	1.00 ± 0.01^{ab}	0.97 ± 0.01^{b}	1.00 ± 0.02^{ab}	1.03 ± 0.01^{a}	1.03 ± 0.01^{a}
Survival %	97.78 ± 1.11	100.00 ± 0.00	100.00 ± 0.00	98.89 ± 1.11	100.00 ± 0.00
HSI %	1.66 ± 0.18	1.78 ± 0.19	2.03 ± 0.03	2.20 ± 0.36	1.67 ± 0.04
VSI %	4.26 ± 0.22^{b}	4.69 ± 0.23^{ab}	4.79 ± 0.03^{ab}	5.02 ± 0.36^{a}	4.53 ± 0.08^{ab}
CF g/cm^3	3.26 ± 0.14^{b}	3.43 ± 0.08^{ab}	3.41 ± 0.08^{ab}	3.64 ± 0.07^{a}	3.28 ± 0.08^{b}
			Tiger puffer		
Initial weight g	11.52 ± 0.01	11.51 ± 0.02	11.50 ± 0.00	11.49 ± 0.01	11.50 ± 0.00
Final weight g	43.27 ± 1.00^{ab}	43.16 ± 0.89^{ab}	45.69 ± 2.06^{a}	39.25 ± 1.71^{b}	39.35 ± 1.05^{b}
Weight gain %	275.50 ± 9.03^{ab}	274.91 ± 8.12^{ab}	297.26 ± 17.96^{a}	241.61 ± 14.58^{b}	242.18 ± 9.16^{b}
Feed intake %	1.55 ± 0.02^{a}	1.58 ± 0.06^{a}	1.43 ± 0.07^{ab}	1.28 ± 0.11^{bc}	1.14 ± 0.03^{c}
Feed efficiency ratio	0.95 ± 0.03^{ab}	0.95 ± 0.02^{ab}	0.99 ± 0.03^{a}	0.88 ± 0.05^{b}	0.87 ± 0.01^{b}
Survival %	62.22 ± 2.94^{ab}	70.00 ± 5.09^{a}	58.89 ± 6.19^{ab}	50.00 ± 6.94^{bc}	41.11 ± 1.11^{c}
HSI %	9.10 ± 0.32^{a}	7.75 ± 0.22^{ab}	8.87 ± 0.57^{a}	8.13 ± 0.45^{ab}	7.24 ± 0.38^{b}
VSI %	15.13 ± 0.61	14.90 ± 0.52	16.41 ± 0.32	15.51 ± 1.45	14.29 ± 0.23
CF g/cm^3	3.19 ± 0.12	3.18 ± 0.05	3.04 ± 0.10	3.22 ± 0.08	3.13 ± 0.12

Data in the same row not sharing a same superscript letter were significantly different ($P < 0.05$). VSI: viscerosomatic index; HSI: hepatosomatic index; CF: condition factor.

receptor γ (PPARγ) than the control group and group CHO-4. The expression of sterol regulatory element-binding factor 1 (SREBF1) was significantly ($P < 0.05$) upregulated by dietary cholesterol inclusion regardless of inclusion level.

For the tiger puffer trial, the glycerol-3-phosphate acyltransferase (GPAT) expression level in the control group was higher compared to the cholesterol-supplemented groups. The PPARα1 expression level in the cholesterol-supplemented groups was significantly ($P < 0.05$) lower compared to the control group. Group CHO-2 had the highest level of diacylglycerol lipase α (DAGLα) expression, while group CHO-1 had the lowest level of LPL expression.

A series of lipid metabolism–related genes in both turbot and tiger puffer showed no significant difference in expression among dietary groups ($P > 0.05$, Table 11).

4. Discussion

In the present study, when the basal diet contained 30% fishmeal but no fish oil, compared to the control, dietary cholesterol supplementation did not significantly affect the growth of both turbot and tiger puffer. Considering that the cholesterol in the basal diet was very low (0.11%), the present result indicates that turbot and tiger puffer may have a high capacity to biosynthesize cholesterol de novo. In other

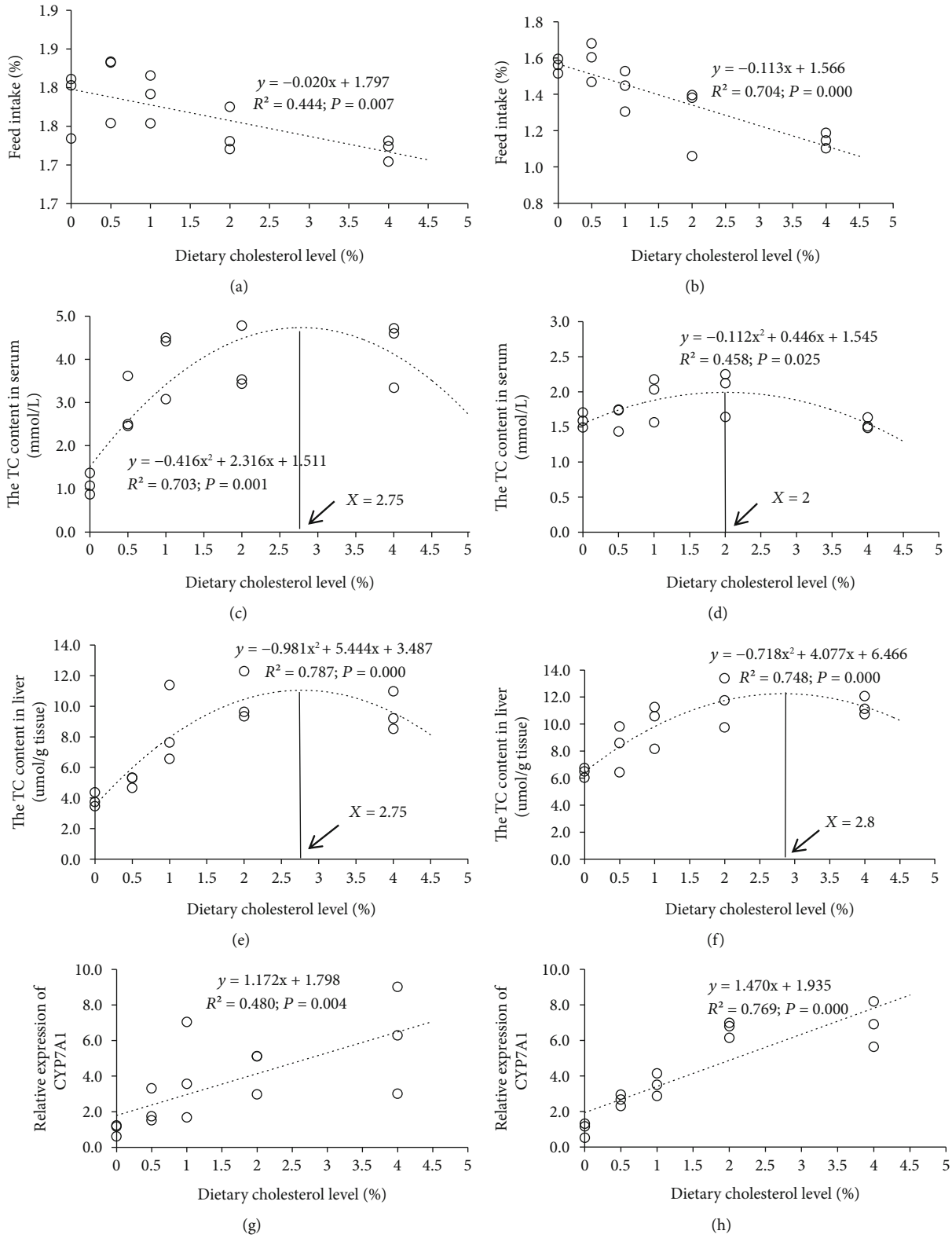

Figure 1: Regression analysis between dietary cholesterol supplementation (x) and feed intake, TC content in serum and liver, as well as the hepatic gene expression of CYP7A1 (y) in experimental turbot ((a), (c), (e), and (g), respectively) and tiger puffer ((b), (d), (f), and (h), respectively).

Table 6: Body composition of experimental fish fed with diets containing different cholesterol levels (% wet weight).

Parameters	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
Whole fish					
Moisture	76.19 ± 0.34^{ab}	76.88 ± 0.06^{a}	75.65 ± 0.32^{b}	76.17 ± 0.22^{ab}	76.43 ± 0.20^{ab}
Crude protein	15.41 ± 0.22	15.28 ± 0.05	15.54 ± 0.22	15.30 ± 0.12	15.61 ± 0.09
Crude lipid	3.73 ± 0.21^{ab}	3.06 ± 0.08^{b}	4.10 ± 0.35^{a}	3.75 ± 0.18^{ab}	3.31 ± 0.09^{b}
Ash	3.55 ± 0.01	3.62 ± 0.03	3.59 ± 0.11	3.55 ± 0.06	3.68 ± 0.08
Muscle					
Crude lipid	0.72 ± 0.02^{b}	0.88 ± 0.03^{a}	0.76 ± 0.02^{ab}	0.79 ± 0.05^{ab}	0.84 ± 0.05^{ab}
Liver					
Crude lipid	28.65 ± 1.26^{a}	25.44 ± 1.69^{ab}	27.82 ± 0.31^{ab}	25.31 ± 0.53^{ab}	24.57 ± 1.19^{b}
SAT					
Crude lipid	5.04 ± 0.44^{ab}	6.06 ± 0.26^{a}	6.34 ± 0.73^{a}	5.90 ± 0.49^{a}	3.92 ± 0.55^{b}
			Tiger puffer		
Whole fish					
Moisture	77.29 ± 0.21	78.11 ± 0.55	77.93 ± 0.71	78.15 ± 0.62	78.42 ± 0.28
Crude protein	15.34 ± 0.21	15.01 ± 0.23	14.90 ± 0.31	14.74 ± 0.38	14.73 ± 0.15
Crude lipid	4.37 ± 0.08	4.42 ± 0.41	4.54 ± 0.28	4.28 ± 0.07	4.63 ± 0.14
Ash	2.43 ± 0.02^{a}	2.35 ± 0.04^{ab}	2.32 ± 0.02^{ab}	2.38 ± 0.05^{ab}	2.30 ± 0.05^{b}
Muscle					
Crude lipid	0.83 ± 0.04^{ab}	0.93 ± 0.03^{a}	0.83 ± 0.02^{ab}	0.81 ± 0.02^{b}	0.91 ± 0.05^{ab}
Liver					
Crude lipid	53.64 ± 0.96	52.84 ± 1.21	50.51 ± 2.85	51.08 ± 0.67	51.93 ± 1.81

Data in the same row not sharing a same superscript letter were significantly different ($P < 0.05$). SAT: subcutaneous adipose tissue around the fin.

studies with turbot, when soybean meal-based diets (containing 14.5% fishmeal and 6% fish oil) were used, the supplementation of 1% cholesterol (the measured level, 1.25%) in the diet enhanced the growth performance [12, 13]. When turbot were fed diets with defatted fishmeal (the inclusion level, 65%), supplementation of 0.5-2% cholesterol (the measured level, 0.76-2.22%) in the diet also enhanced the growth performance [17]. Similar growth-enhancing effects of dietary cholesterol supplementation (1-2%) when plant protein-based diets were used were observed in other fish species, such as channel catfish (fishmeal, 0%; the supplemented cholesterol level, 1%) [10], Japanese flounder (fishmeal, 0%; the supplemented and measured cholesterol level, 1% and 1.2%, respectively) [11], Nile tilapia (fishmeal, 16.5%; the supplemented cholesterol level, 2%) [14], and rainbow trout (fishmeal, 19-32%; the supplemented and measured cholesterol level, 0.9-1.2% and 1.30-1.68%, respectively) [15, 16]. These studies indicate that if there are extremely low cholesterol levels in the diets, the dietary supplementation is necessary. The requirement of exogenous cholesterol should be considered when the fishmeal and fish oil levels are reduced to a certain low level.

Although compared to the control group dietary cholesterol supplementation also had no significant effect on the growth of tiger puffer, dietary cholesterol supplementation at 1% (the measured level, 1.10%) led to higher growth compared to groups with excess (2% and 4%) dietary cholesterol supplementation (the measured level, 2.32% and 4.59%). These results indicate the effects of dietary cholesterol supplementation could also be species-specific, namely, different between turbot and tiger puffer. In hybrid striped bass, despite that defatted fishmeal (58.3%) was used in the diet, the dietary cholesterol supplementation had no effect on fish growth [9]. Significant effects of dietary cholesterol supplementation (the supplemented and measured level, 1-1.5% and 1.38-1.57%, respectively) were also not observed in Atlantic salmon whether 60% fishmeal or 19% soy protein concentrate was used in the diet [8, 19].

The growth results in tiger puffer also suggested that excess dietary cholesterol supplementation may have adverse effects on fish. In both turbot and tiger puffer trials, dietary cholesterol supplementation linearly reduced the feed intake. However, other studies showed that when the diets contained high levels of soybean meal (42% for turbot, 55.5% for channel catfish), the dietary cholesterol supplementation at 1% increased the feed intake [10, 12], although very high levels of dietary cholesterol (fishmeal, 14.5%; the measured cholesterol level, 1.78%) reduced the feed intake [12]. Different from these studies, when 27.5% fishmeal and 42.9% soybean meal were used in the diets, 1% dietary cholesterol (the measured level, 1.36%) did not affect the feed intake of grouper [18]. The effects of dietary cholesterol supplementation on feed intake of fish could be closely related to basic dietary formulation and could also be related to fish species. Deng et al. [11] reported that dietary cholesterol supplementation at 1% (the

Table 7: Effects of dietary cholesterol level on liver fatty acid composition of experimental fish (% total fatty acids, mean ± standard error).

Fatty acid	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
14 : 0	2.81 ± 0.13^{b}	3.74 ± 0.28^{a}	3.28 ± 0.17^{ab}	3.38 ± 0.15^{ab}	2.99 ± 0.10^{b}
16 : 0	14.72 ± 0.22	15.18 ± 0.20	15.20 ± 0.28	14.79 ± 0.33	15.09 ± 0.25
18 : 0	3.48 ± 0.12^{a}	2.59 ± 0.28^{b}	2.48 ± 0.17^{b}	2.63 ± 0.06^{b}	2.69 ± 0.22^{b}
∑SFA	21.71 ± 0.22	22.08 ± 0.58	21.64 ± 0.43	21.38 ± 0.35	21.45 ± 0.09
16:1n-7	3.43 ± 0.07^{c}	4.24 ± 0.06^{a}	4.12 ± 0.12^{ab}	4.11 ± 0.10^{ab}	3.94 ± 0.04^{b}
18:1n-9	29.34 ± 0.97	28.84 ± 0.85	28.45 ± 0.35	29.43 ± 0.37	29.37 ± 1.12
20:1n-9	1.07 ± 0.14	0.94 ± 0.07	0.88 ± 0.02	1.03 ± 0.13	0.90 ± 0.06
∑MUFA	34.39 ± 1.12	34.53 ± 0.84	33.96 ± 0.26	35.11 ± 0.44	34.78 ± 1.15
18:2n-6	22.33 ± 0.72	21.74 ± 1.00	22.54 ± 0.28	21.51 ± 0.41	22.35 ± 0.65
20:2n-6	1.80 ± 0.23	1.51 ± 0.12	1.43 ± 0.06	1.70 ± 0.26	1.55 ± 0.13
20:4n-6	0.24 ± 0.01^{c}	0.35 ± 0.04^{bc}	0.45 ± 0.04^{ab}	0.47 ± 0.04^{ab}	0.54 ± 0.08^{a}
∑n-6PUFA	24.89 ± 0.47	24.10 ± 0.96	24.95 ± 0.28	24.24 ± 0.18	24.97 ± 0.66
18:3n-3	10.44 ± 0.72	11.38 ± 0.50	11.54 ± 0.30	10.82 ± 0.43	10.72 ± 0.32
20:5n-3	2.97 ± 0.28	2.83 ± 0.05	2.89 ± 0.13	2.89 ± 0.09	2.85 ± 0.26
22:5n-3	1.45 ± 0.07	1.24 ± 0.03	1.24 ± 0.03	1.37 ± 0.09	1.29 ± 0.08
22:6n-3	2.23 ± 0.10	1.99 ± 0.04	2.08 ± 0.06	2.15 ± 0.05	2.09 ± 0.16
∑n-3PUFA	19.02 ± 0.77	19.29 ± 0.44	19.45 ± 0.24	19.27 ± 0.09	18.80 ± 0.59
n-3/n-6	0.76 ± 0.02^{ab}	0.80 ± 0.01^{a}	0.78 ± 0.01^{ab}	0.80 ± 0.01^{a}	0.75 ± 0.01^{b}
			Tiger puffer		
14 : 0	1.70 ± 0.09	1.82 ± 0.03	1.80 ± 0.08	1.72 ± 0.03	1.73 ± 0.02
16 : 0	17.17 ± 0.49	17.76 ± 0.31	17.95 ± 0.58	17.51 ± 0.52	17.92 ± 0.68
18 : 0	9.57 ± 0.27	8.80 ± 0.54	8.64 ± 0.92	9.56 ± 0.30	8.87 ± 0.04
∑SFA	29.19 ± 0.74	29.26 ± 0.76	29.29 ± 0.46	29.71 ± 0.79	29.41 ± 0.58
16:1n-7	4.80 ± 0.12	4.87 ± 0.24	4.92 ± 0.32	4.88 ± 0.17	4.74 ± 0.25
18:1n-9	29.69 ± 0.35	30.05 ± 0.49	30.38 ± 0.28	29.87 ± 0.41	29.83 ± 0.18
20:1n-9	1.44 ± 0.10	1.35 ± 0.07	1.35 ± 0.06	1.53 ± 0.02	1.38 ± 0.04
∑MUFA	36.22 ± 0.26	36.56 ± 0.20	36.94 ± 0.15	36.6 ± 0.60	36.25 ± 0.26
18:2n-6	16.90 ± 0.46	16.74 ± 0.38	16.58 ± 0.27	16.57 ± 0.56	16.99 ± 0.41
20:2n-6	0.91 ± 0.06	0.84 ± 0.04	0.82 ± 0.02	0.88 ± 0.03	0.86 ± 0.02
20:4n-6	0.23 ± 0.01^{b}	0.25 ± 0.02^{ab}	0.27 ± 0.02^{ab}	0.30 ± 0.00^{a}	0.28 ± 0.01^{a}
∑n-6PUFA	18.48 ± 0.53	18.28 ± 0.44	18.11 ± 0.26	18.18 ± 0.58	18.6 ± 0.41
18:3n-3	9.33 ± 0.18	9.22 ± 0.20	9.26 ± 0.32	8.79 ± 0.50	9.01 ± 0.20
20:5n-3	1.84 ± 0.07	1.94 ± 0.09	1.83 ± 0.10	1.94 ± 0.14	1.92 ± 0.08
22:5n-3	2.16 ± 0.04^{a}	1.92 ± 0.07^{b}	1.85 ± 0.02^{b}	1.87 ± 0.07^{b}	1.95 ± 0.01^{b}
22:6n-3	2.03 ± 0.08	2.14 ± 0.13	2.03 ± 0.10	2.23 ± 0.07	2.18 ± 0.06
∑n-3PUFA	16.11 ± 0.39	15.90 ± 0.46	15.65 ± 0.33	15.51 ± 0.80	15.75 ± 0.33
n-3/n-6	0.87 ± 0.01	0.87 ± 0.00	0.86 ± 0.01	0.85 ± 0.02	0.85 ± 0.01

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. In addition to the fatty acids listed in the table, total fatty acids include 12 : 0, 13 : 0, 15 : 0, 17 : 0, 20 : 0, 21 : 0, 22 : 0, 14:1n-5, 15:1n-5, 17:1n-7, 221n-9, 18:3n-6, 20:3n-6, 22:2n-6, and 20:3n-3. Data in the same row not sharing a superscript were significantly ($P < 0.05$) different.

measured level, 1-1.2%) reduced the feed intake of Japanese flounder when the diet was based on fish protein concentrate, increased the feed intake in the soy protein isolate group (fishmeal, 0%), whereas did not affect the feed intake in the soy protein concentrate group (fishmeal, 0%).

The effects of dietary cholesterol supplementation on the proximate composition of fish whole body were also

Table 8: Serum biochemical parameters of experimental fish fed with diets containing different cholesterol levels.

Parameters	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
TC (mmol/L)	1.10 ± 0.14^{c}	2.85 ± 0.38^{b}	4.00 ± 0.46^{ab}	3.91 ± 0.43^{ab}	4.22 ± 0.44^{a}
FC (mmol/L)	0.61 ± 0.03^{c}	0.82 ± 0.06^{bc}	1.10 ± 0.18^{ab}	1.28 ± 0.08^{a}	1.23 ± 0.13^{a}
CE (mmol/L)	0.50 ± 0.11^{b}	2.04 ± 0.32^{a}	2.90 ± 0.29^{a}	2.64 ± 0.36^{a}	2.99 ± 0.47^{a}
TG (mmol/L)	0.38 ± 0.13^{b}	0.64 ± 0.11^{ab}	0.37 ± 0.08^{b}	0.71 ± 0.12^{ab}	0.83 ± 0.07^{a}
HDL-C (mmol/L)	0.20 ± 0.01^{c}	0.61 ± 0.01^{b}	0.85 ± 0.10^{ab}	1.01 ± 0.14^{a}	1.07 ± 0.19^{a}
LDL-C (mmol/L)	1.00 ± 0.08^{b}	2.04 ± 0.19^{b}	3.31 ± 0.33^{a}	4.24 ± 0.36^{a}	4.40 ± 0.59^{a}
TBA (umol/L)	1.68 ± 0.43	1.66 ± 0.05	2.11 ± 0.20	2.05 ± 0.48	2.45 ± 0.35
MDA (nmol/ml)	5.43 ± 1.40	7.13 ± 0.55	13.16 ± 3.04	14.12 ± 1.37	13.79 ± 4.02
			Tiger puffer		
TC (mmol/L)	1.59 ± 0.06^{ab}	1.64 ± 0.10^{ab}	1.92 ± 0.19^{ab}	2.00 ± 0.18^{a}	1.54 ± 0.05^{b}
FC (mmol/L)	0.42 ± 0.01	0.48 ± 0.05	0.47 ± 0.05	0.40 ± 0.05	0.49 ± 0.09
CE (mmol/L)	1.17 ± 0.07^{b}	1.16 ± 0.09^{b}	1.45 ± 0.13^{ab}	1.60 ± 0.22^{a}	1.05 ± 0.04^{b}
TG (mmol/L)	0.32 ± 0.02^{ab}	0.27 ± 0.01^{b}	0.41 ± 0.05^{a}	0.33 ± 0.03^{ab}	0.29 ± 0.03^{b}
HDL-C (mmol/L)	1.01 ± 0.24	0.92 ± 0.30	1.02 ± 0.21	1.37 ± 0.44	0.94 ± 0.35
LDL-C (mmol/L)	0.77 ± 0.20^{b}	1.08 ± 0.25^{ab}	0.96 ± 0.18^{ab}	1.08 ± 0.18^{ab}	1.49 ± 0.10^{a}
TBA (umol/L)	1.38 ± 0.07^{b}	1.76 ± 0.19^{ab}	1.70 ± 0.11^{ab}	2.05 ± 0.25^{a}	2.12 ± 0.11^{a}
MDA (nmol/ml)	5.54 ± 0.48^{ab}	5.61 ± 0.30^{ab}	4.74 ± 0.12^{b}	5.54 ± 0.23^{ab}	6.54 ± 0.46^{a}

Data in the same row not sharing a same superscript letter were significantly different ($P < 0.05$). TC: total cholesterol; FC: free cholesterol; CE: cholesterol esters; TG: triglyceride; TBA: total bile acid; MDA: malondialdehyde.

Table 9: Lipid-related biochemical parameters in liver of experimental fish fed with diets containing different cholesterol levels.

Parameters	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
TC (umol/g tissue)	3.85 ± 0.27^{b}	5.09 ± 0.22^{b}	8.51 ± 1.46^{a}	10.41 ± 0.94^{a}	9.56 ± 0.73^{a}
TG (mmol/g tissue)	0.26 ± 0.00	0.25 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.23 ± 0.01
HDL-C (umol/g tissue)	2.27 ± 0.60	2.74 ± 0.26	2.96 ± 0.93	3.14 ± 0.63	2.65 ± 0.19
LDL-C (umol/g tissue)	1.51 ± 0.34^{c}	2.22 ± 0.05^{bc}	4.72 ± 1.16^{ab}	5.46 ± 1.26^{a}	5.18 ± 0.43^{a}
TBA (mmol/L)	0.50 ± 0.04	0.79 ± 0.16	0.55 ± 0.23	0.50 ± 0.11	0.48 ± 0.09
MDA (nmol/mgprot)	8.87 ± 3.88	12.76 ± 4.77	12.08 ± 1.85	3.65 ± 1.12	7.01 ± 2.42
			Tiger puffer		
TC (umol/g tissue)	6.43 ± 0.21^{c}	8.28 ± 0.99^{bc}	10.0 ± 0.94^{ab}	11.63 ± 1.05^{a}	11.30 ± 0.40^{a}
TG (mmol/g tissue)	0.18 ± 0.02	0.15 ± 0.02	0.17 ± 0.00	0.18 ± 0.01	0.18 ± 0.02
HDL-C (umol/g tissue)	4.37 ± 1.16	5.52 ± 0.47	7.49 ± 1.50	6.23 ± 0.59	5.01 ± 0.47
LDL-C (umol/g tissue)	2.56 ± 0.09^{c}	3.66 ± 0.43^{bc}	4.64 ± 0.61^{ab}	5.86 ± 0.37^{a}	6.08 ± 0.60^{a}
TBA (mmol/L)	0.07 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.09 ± 0.05
MDA (nmol/mgprot)	60.25 ± 20.34	48.34 ± 10.54	35.45 ± 2.53	50.89 ± 12.88	41.62 ± 12.51

Data in the same row not sharing a same superscript letter were significantly different ($P < 0.05$). TC: total cholesterol; TG: triglyceride; TBA: total bile acid.

inconsistent among different studies. The effects on both turbot and tiger puffer were mild except that 4% dietary cholesterol (the measured level, 4.59%) significantly reduced the ash content in tiger puffer. In Nile tilapia, 1% or 2% dietary cholesterol supplementation (fishmeal, 30.2% or 16.5%) increased the crude protein content in the whole fish body [14]. This phenomenon was neither observed in the present study, nor observed in other studies with turbot or rainbow trout [13, 15–17].

In both turbot and tiger puffer, 0.5% dietary cholesterol supplementation (the measured level, 0.65%) increased the lipid content in the muscle. Similarly, dietary cholesterol supplementation at 0.6-1.5% (the measured level, 1.03-1.83%) increased the muscle lipid content in rainbow trout

Table 10: Lipid-related biochemical parameters in the muscle of experimental fish fed with diets containing different cholesterol levels.

Parameters	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
TC (umol/g tissue)	1.49 ± 0.26	1.71 ± 0.25	1.59 ± 0.06	1.63 ± 0.23	2.00 ± 0.20
TG (umol/g tissue)	1.88 ± 0.13^{ab}	2.36 ± 0.30^{a}	1.96 ± 0.09^{ab}	1.76 ± 0.11^{b}	1.95 ± 0.13^{ab}
HDL-C (umol/g tissue)	0.40 ± 0.05	0.45 ± 0.03	0.22 ± 0.12	0.41 ± 0.11	0.31 ± 0.09
LDL-C (umol/g tissue)	0.96 ± 0.02^{b}	1.07 ± 0.10^{ab}	1.09 ± 0.02^{ab}	0.98 ± 0.08^{b}	1.28 ± 0.09^{a}
MDA (nmol/mgprot)	1.06 ± 0.02	1.08 ± 0.06	1.17 ± 0.09	1.07 ± 0.10	1.19 ± 0.14
			Tiger puffer		
TC (umol/g tissue)	2.32 ± 0.24^{a}	2.03 ± 0.08^{ab}	1.93 ± 0.04^{ab}	1.84 ± 0.11^{b}	2.34 ± 0.09^{a}
TG (umol/g tissue)	3.14 ± 0.42^{a}	2.69 ± 0.16^{ab}	2.60 ± 0.13^{ab}	2.21 ± 0.15^{b}	2.46 ± 0.30^{ab}
HDL-C (umol/g tissue)	0.37 ± 0.06	0.58 ± 0.05	0.55 ± 0.16	0.30 ± 0.05	0.30 ± 0.09
LDL-C (umol/g tissue)	1.04 ± 0.16	1.01 ± 0.08	1.04 ± 0.10	0.77 ± 0.08	0.72 ± 0.09
MDA (nmol/mgprot)	1.01 ± 0.25	0.63 ± 0.12	0.65 ± 0.03	0.83 ± 0.17	1.02 ± 0.14

Data in the same row not sharing a same superscript letter were significantly different ($P < 0.05$). TC: total cholesterol; TG: triglyceride; MDA: malondialdehyde.

[15, 16]. In contrast to the muscle lipid content, the lipid content in the liver of both turbot and tiger puffer tended to be decreased by dietary cholesterol supplementation. The decrease of liver lipid content by 1% dietary cholesterol supplementation was also observed in Japanese flounder (fishmeal, 65%; the measured cholesterol level, 1.4%) and hybrid striped bass (defatted fishmeal, 58.3%) [9, 11]. However, in Japanese flounder or rainbow trout fed diets based on plant protein, the dietary cholesterol supplementation increased the liver lipid content [11, 15, 16].

It has been widely accepted that the fatty acid profile in fish closely reflected that of the diets [34]. Since the fatty acid composition was very similar among the present experimental diets, the change in fish fatty acid composition among groups could be mainly attributed to dietary cholesterol supplementation. In the present study, a most interesting finding was that the dietary cholesterol supplementation increased the hepatic ARA content in both species. Based on the present information, it was really difficult to explain this result. Other results, such as the increase of 14 : 0 and 16:1n-7 but decrease of 18 : 0 in turbot liver, as well as the decrease of 22:5n-3 in tiger puffer liver by dietary cholesterol supplementation were difficult to explain too. Studies on Atlantic salmon showed that dietary cholesterol supplementation (fishmeal, 60%; the supplemented and measured cholesterol level, 1% and 1.38%, respectively) increased the contents of 18:1n-9 and 20:1n-9 in the liver [8]. No study has investigated the mechanisms involved in the response of fish fatty acid composition to dietary cholesterol supplementation, and future studies are warranted.

The cholesterol-enriched diets resulted in remarkable changes in the lipid-related biochemical parameters in the serum of both species, which also has been observed in other studies [11–13, 15–18]. Nevertheless, this change was more drastic in turbot compared to tiger puffer. For example, there was a maximum 4-fold change in the serum TC concentration in turbot in response to dietary cholesterol supplementation, but the corresponding maximum change in tiger puffer was only 1.3 fold. Similar discrepancy was observed for the serum MDA concentration. In addition, it was noteworthy that the dietary cholesterol increased the MDA content in turbot serum, indicating that excess dietary cholesterol may exacerbate the lipid peroxidation in fish.

For the lipid-related biochemical parameters in the liver, unexpectedly only the concentration of TC and LDL-C was increased by dietary cholesterol supplementation, suggesting that other parameters such as TG, HDL-C, and TBA could have a self-regulating capacity, in order to prevent large fluctuations. The comparison between turbot and tiger puffer also indicates higher buffering capacity in tiger puffer compared to turbot. This could be due to that tiger puffer store lipid predominantly in the liver.

The changes of lipid-related biochemical parameters in the muscle were much milder than those in the liver. This could be due to that in both turbot and tiger puffer, the lipid content in the muscle is very low. In rainbow trout, dietary cholesterol supplementation (fishmeal, 19-32%; the supplemented and measured cholesterol level, 0.3-1.5% and 0.68-1.83%, respectively) increased the muscle TG concentration [15, 16], whereas in hybrid striped bass (fishmeal 58.3%; the supplemented cholesterol level, 1%), no such effect was found [9]. These discrepancies could be mainly related to the basal muscle lipid content. In tiger puffer, the lipid-related biochemical parameters even tended to decrease in response to dietary cholesterol supplementation. The muscle lipid content in tiger puffer was even lower compared to turbot [20]. The decrease of muscle lipid content in tiger puffer could be related to the inhibition of dietary cholesterol supplementation on basic lipid metabolism.

The liver plays a pivotal role in cholesterol homeostasis, as it is involved in the cholesterol biosynthesis pathway and degradation through conversion into bile acids [35]. That is why in the present study, the qRT-PCR studies were conducted with liver samples. It was widely demonstrated that

Table 11: Relative mRNA expression of genes related to lipid metabolism in the liver of experimental fish, which were not significantly affected by experimental diets (mean ± standard error).

Parameters	Control	CHO-0.5	CHO-1.0	CHO-2.0	CHO-4.0
			Turbot		
FAS	1.00 ± 0.25	0.50 ± 0.17	0.51 ± 0.12	0.52 ± 0.16	0.56 ± 0.04
CPT1	1.00 ± 0.10	1.28 ± 0.07	1.30 ± 0.14	1.13 ± 0.16	0.94 ± 0.14
GPAT	1.00 ± 0.16	1.26 ± 0.35	1.33 ± 0.09	1.52 ± 0.16	1.35 ± 0.13
DGAT	1.00 ± 0.13	1.15 ± 0.13	1.28 ± 0.16	1.18 ± 0.11	1.06 ± 0.16
ATGL	1.00 ± 0.19	0.25 ± 0.14	0.76 ± 0.42	0.90 ± 0.16	1.00 ± 0.27
DAGLα	1.00 ± 0.34	1.03 ± 0.04	1.12 ± 0.13	1.12 ± 0.23	1.17 ± 0.24
PL	1.00 ± 0.37	1.38 ± 0.41	0.37 ± 0.06	1.12 ± 0.39	0.36 ± 0.11
LPL	1.00 ± 0.15	0.81 ± 0.08	0.74 ± 0.18	0.69 ± 0.06	0.68 ± 0.13
HL	1.00 ± 0.09	0.81 ± 0.07	0.96 ± 0.09	0.89 ± 0.06	0.91 ± 0.06
ApoA1	1.00 ± 0.08	1.10 ± 0.15	1.54 ± 0.35	1.17 ± 0.06	1.16 ± 0.28
ApoA4	1.00 ± 0.39	0.48 ± 0.04	2.06 ± 1.20	0.35 ± 0.03	0.79 ± 0.42
ApoB100	1.00 ± 0.03	1.36 ± 0.17	1.34 ± 0.08	1.24 ± 0.07	1.21 ± 0.15
ApoEα	1.00 ± 0.25	0.87 ± 0.13	1.14 ± 0.57	0.78 ± 0.23	0.69 ± 0.36
PPARα1	1.00 ± 0.17	1.49 ± 0.34	1.94 ± 0.44	1.98 ± 0.41	1.17 ± 0.27
PPARβ	1.00 ± 0.12	0.86 ± 0.07	0.90 ± 0.03	0.98 ± 0.19	0.96 ± 0.14
			Tiger puffer		
FAS	1.00 ± 0.23	0.75 ± 0.09	0.56 ± 0.10	0.74 ± 0.07	0.58 ± 0.10
CPT1	1.00 ± 0.25	1.22 ± 0.13	1.02 ± 0.14	0.96 ± 0.06	0.97 ± 0.12
MOGAT	1.00 ± 0.10	0.79 ± 0.05	0.75 ± 0.14	0.71 ± 0.12	1.05 ± 0.10
DGAT	1.00 ± 0.12	1.01 ± 0.08	0.95 ± 0.06	1.05 ± 0.04	1.25 ± 0.22
ATGL	1.00 ± 0.23	0.96 ± 0.12	1.09 ± 0.17	0.98 ± 0.33	1.48 ± 0.41
HSL	1.00 ± 0.29	1.03 ± 0.26	0.75 ± 0.18	0.58 ± 0.07	1.35 ± 0.33
MGLL	1.00 ± 0.29	1.14 ± 0.05	1.25 ± 0.14	1.06 ± 0.35	1.31 ± 0.33
PL	1.00 ± 0.11	0.99 ± 0.15	1.16 ± 0.10	0.94 ± 0.16	1.17 ± 0.18
BSAL	1.00 ± 0.26	0.84 ± 0.12	0.83 ± 0.17	0.74 ± 0.13	1.05 ± 0.16
HL	1.00 ± 0.03	1.00 ± 0.07	0.92 ± 0.03	0.82 ± 0.15	1.06 ± 0.04
ApoA4	1.00 ± 0.16	0.84 ± 0.30	0.62 ± 0.10	0.59 ± 0.27	0.58 ± 0.18
ApoB100	1.00 ± 0.11	1.16 ± 0.06	1.00 ± 0.07	0.99 ± 0.14	1.04 ± 0.09
ApoEα	1.00 ± 0.20	1.25 ± 0.44	0.81 ± 0.21	0.80 ± 0.07	0.75 ± 0.15
PPARα2	1.00 ± 0.08	1.09 ± 0.06	0.92 ± 0.04	1.01 ± 0.04	0.92 ± 0.09
PPARβ	1.00 ± 0.06	1.31 ± 0.28	1.04 ± 0.21	0.87 ± 0.08	1.04 ± 0.24
PPARγ	1.00 ± 0.12	1.03 ± 0.21	1.09 ± 0.12	1.16 ± 0.07	0.85 ± 0.10
SREBF1	1.00 ± 0.10	0.92 ± 0.10	0.77 ± 0.09	0.95 ± 0.08	0.73 ± 0.10

dietary cholesterol could inhibit the cholesterol biosynthesis and enhance the bile acid biosynthesis [12, 16–18]. This was also evidenced by the present results. In both species, dietary cholesterol supplementation substantially downregulated the hepatic gene expression of HMG-COAr, which is a key enzyme mediating cholesterol biosynthesis [36], and substantially upregulated that of CYP7A1, which is the limiting enzyme in bile acid biosynthesis [37]. Interestingly, the changes in turbot were much more drastic compared to tiger puffer (maximum 676-fold change vs 16-fold change, respectively). This indicates again that the cholesterol homeostasis in tiger puffer has high buffering capacity compared to turbot.

Dietary cholesterol supplementation also downregulated the hepatic gene expression of PL and BSAL, which are important enzymes in intestinal lipid absorption [38, 39], in turbot. In tiger puffer, dietary cholesterol supplementation downregulated the hepatic gene expression of GPAT, which plays an

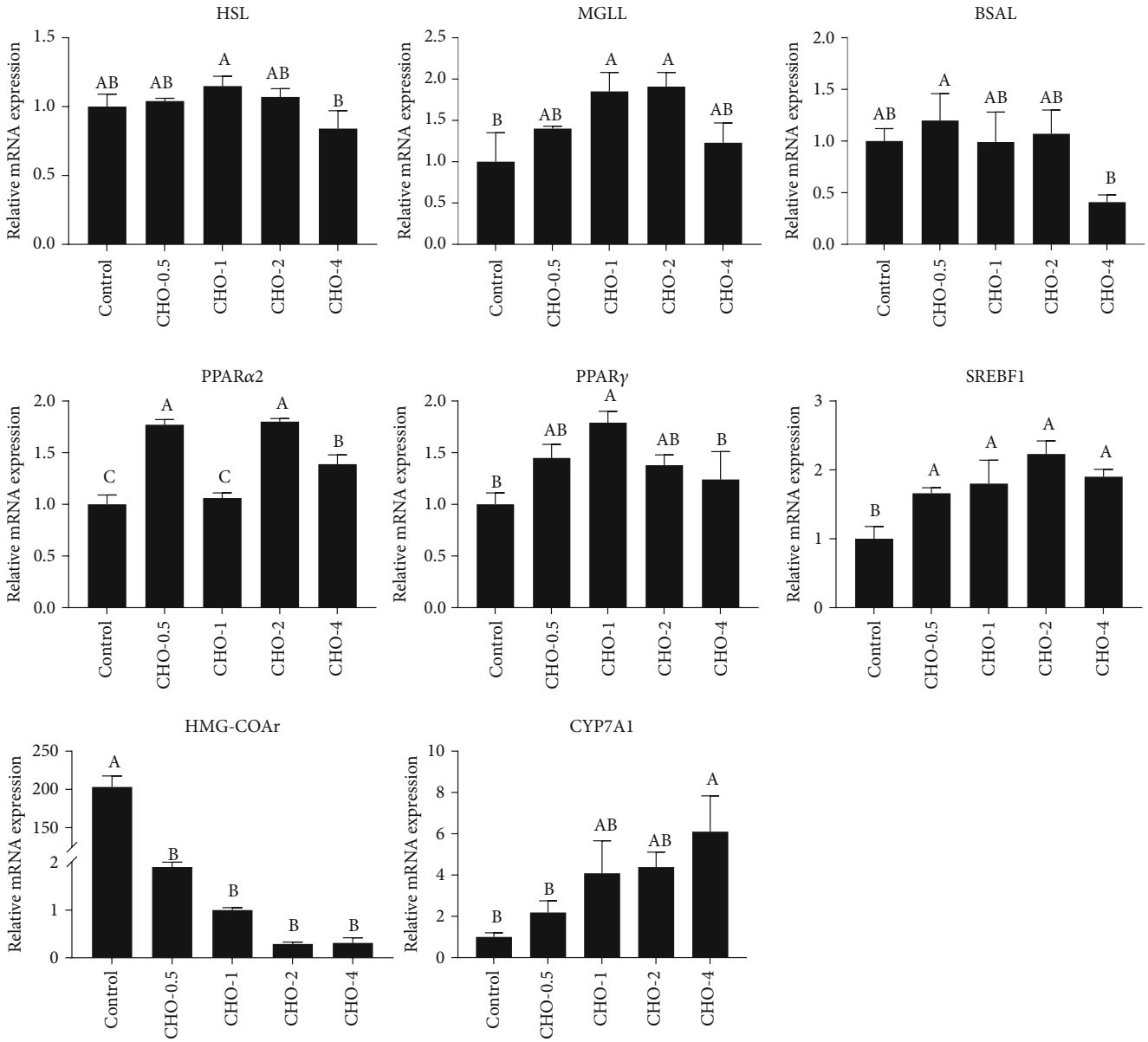

Figure 2: Effects of dietary cholesterol supplementation on the mRNA expression of genes related to lipid metabolism in the liver of experimental turbot (mean ± standard error). Data bars not sharing a same superscript letter were significantly different ($P < 0.05$).

important role in diacylglycerol (DAG) biosynthesis in intestine [40]. These results could partly explain the reduction of liver lipid content by dietary cholesterol supplementation. This reduction could also be explained by the downregulation of hepatic gene expression of FAS, which is a key enzyme in de novo biosynthesis of fatty acids [41, 42], as well as the upregulation of MGLL, which converts monoacylglycerides to free fatty acids and glycerol, for β-oxidation [43], by dietary cholesterol supplementation. The upregulation of hepatic gene expression of PPARα1, which is a transcription regulator closely related to lipogenesis [44], by dietary cholesterol supplementation was also in agreement with the reduction of liver lipid content. However, the upregulation of gene expression of PPAR-γ, which is a regulator of adipocyte differentiation, and SREBF-1, which is a regulator of fatty acid/lipid and cholesterol biosynthetic genes [45], by dietary cholesterol supplementation was difficult to explain. Besides, cholesterol is an important component of lipoproteins. Therefore, dietary cholesterol supplementation was expected to affect the gene expression of apolipoproteins. However, this was not observed in the present study. The precise mechanisms involved in the regulation of lipid metabolism by dietary cholesterol need to be elucidated by future studies.

5. Conclusions

In conclusion, when the diets contained 30% fishmeal and no fish oil, compared to the control group, dietary 0.5-4% cholesterol supplementation (the measured level, 0.65-4.59%) exerted no significant effects on the growth of both turbot and tiger puffer. Greater than 1% dietary cholesterol supplementation (the measured level, >1.10%) could reduce

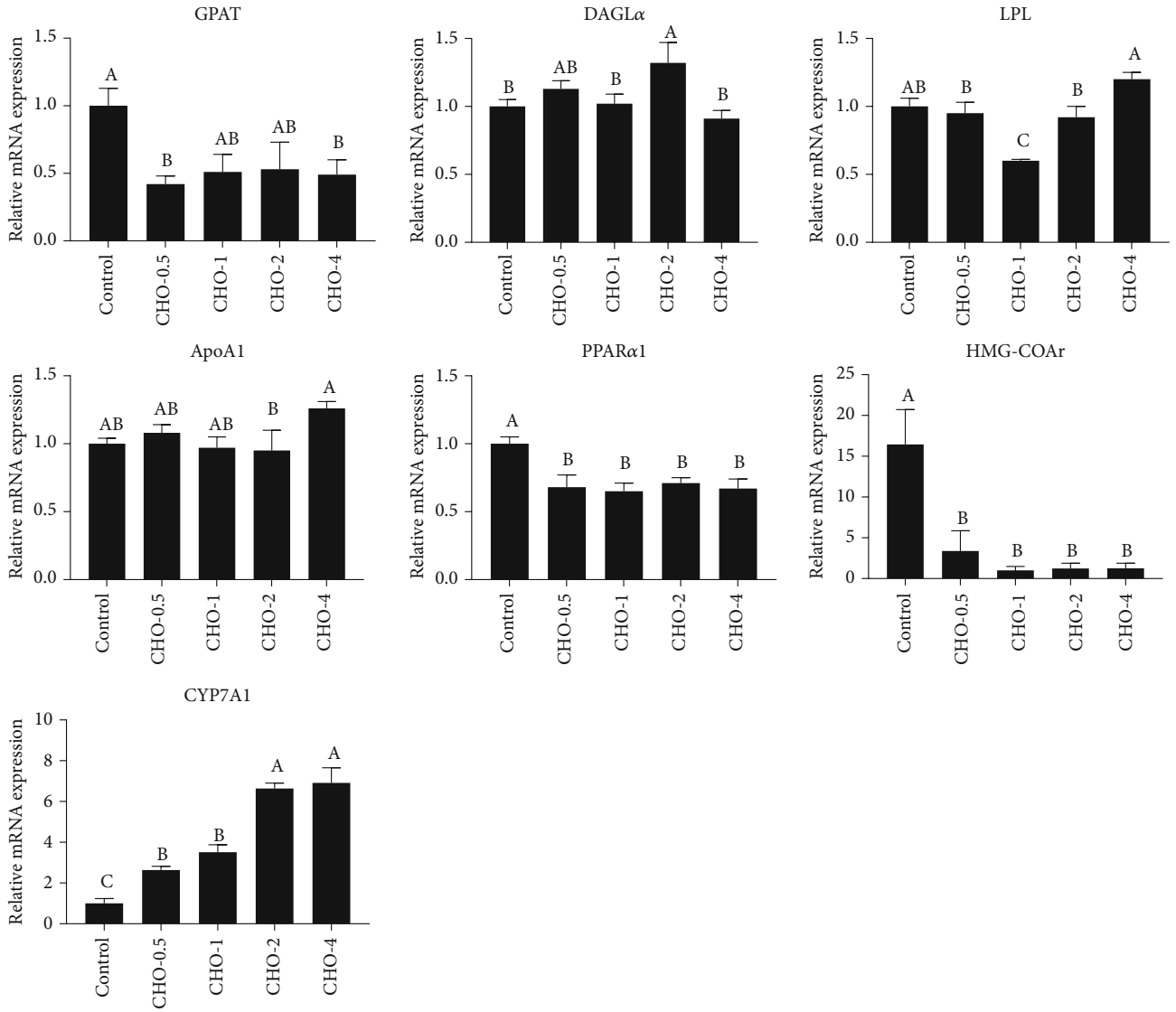

Figure 3: Effects of dietary cholesterol supplementation on the mRNA expression of genes related to lipid metabolism in the liver of experimental tiger puffer (mean ± standard error). Data bars not sharing a same superscript letter were significantly different ($P < 0.05$).

the feed intake in both species. Dietary cholesterol supplementation influenced the lipid contents and the lipid-related biochemical parameters in fish tissues and increased the hepatic 20:4n-6 content. Compared to turbot, tiger puffer lipid compositions had a higher buffering capacity in response to changes in dietary cholesterol level. Based on the lipid content and the expression of lipid metabolic genes in the liver, it appeared that increasing dietary cholesterol levels tended to decrease the lipid absorption and biosynthesis.

Data Availability

Raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Conceptualization, funding acquisition, and supervision were assigned to ML and HX. Formal analysis and data curation were assigned to QB, LC, and XM. Methodology and software were assigned to QM, YW, and MD. Conduction of feeding trial and writing-original draft was assigned to XM. Writing-review and editing was assigned to HX.

Acknowledgments

This research was funded by the Natural Science Foundation of Shandong Province-Outstanding Youth Foundation

(ZR2021YQ24), Central Public-interest Scientific Institution Basal Research Fund, YSFRI/CAFS (2020TD48, 20603022022004), and China Agriculture Research System of MOF and MARA (CARS-47).

References

[1] Z. J. Cheng and R. W. Hardy, "Protein and lipid sources affect cholesterol concentrations of juvenile Pacific white shrimp, Litopenaeus vannamei (Boone)1," *Journal of Animal Science*, vol. 82, no. 4, pp. 1136–1145, 2004.

[2] P. V. Hernández, M. A. Olvera-Novoa, and D. B. Rouse, "Effect of dietary cholesterol on growth and survival of juvenile redclaw crayfish Cherax quadricarinatus under laboratory conditions," *Aquaculture*, vol. 236, no. 1-4, pp. 405–411, 2004.

[3] M.-H. Holme, C. Zeng, and P. C. Southgate, "The effects of supplemental dietary cholesterol on growth, development and survival of mud crab, Scylla serrata, megalopa fed semi-purified diets," *Aquaculture*, vol. 261, no. 4, pp. 1328–1334, 2006.

[4] NRC, National Research Council (NRC), *Nutrient requirements of fish and shrimp*, The National Academies Press, Washington, D.C., 2011.

[5] F. Norambuena, M. Lewis, N. K. A. Hamid, K. Hermon, J. A. Donald, and G. M. Turchini, "Fish oil replacement in current aquaculture feed: is cholesterol a hidden treasure for fish nutrition?," *PLoS ONE*, vol. 8, no. 12, article e81705, 2013.

[6] S. J. Kaushik, D. Covès, G. Dutto, and D. Blanc, "Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, Dicentrarchus labrax," *Aquaculture*, vol. 230, no. 1-4, pp. 391–404, 2004.

[7] D. M. Gatlin III, F. T. Barrows, P. Brown et al., "Expanding the utilization of sustainable plant products in aquafeeds: a review," *Aquaculture Research*, vol. 38, no. 6, pp. 551–579, 2007.

[8] B. Bjerkeng, T. Storebakken, and E. Wathne, "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 Nutrition*, vol. 5, no. 3, pp. 181–191, 1999.

[9] W. M. Sealey, S. R. Craig, and D. M. Gatlin, "Dietary cholesterol and lecithin have limited effects on growth and body composition of hybrid striped bass (Morone chrysops × M. saxatilis)," *Aquaculture Nutrition*, vol. 7, no. 1, pp. 25–31, 2001.

[10] R. G. Twibell and R. P. Wilson, "Preliminary evidence that cholesterol improves growth and feed intake of soybean meal-based diets in aquaria studies with juvenile channel catfish, Ictalurus punctatus," *Aquaculture*, vol. 236, no. 1-4, pp. 539–546, 2004.

[11] J. Deng, K. Mai, Q. Ai et al., "Interactive effects of dietary cholesterol and protein sources on growth performance and cholesterol metabolism of Japanese flounder (Paralichthys olivaceus)," *Aquaculture Nutrition*, vol. 16, no. 4, pp. 419–429, 2010.

[12] B. Yun, K. Mai, W. Zhang, and W. Xu, "Effects of dietary cholesterol on growth performance, feed intake and cholesterol metabolism in juvenile turbot (Scophthalmus maximus L.) fed high plant protein diets," *Aquaculture*, vol. 319, no. 1-2, pp. 105–110, 2011.

[13] B. Yun, Q. Ai, K. Mai, W. Xu, G. Qi, and Y. Luo, "Synergistic effects of dietary cholesterol and taurine on growth performance and cholesterol metabolism in juvenile turbot (Scophthalmus maximus L.) fed high plant protein diets," *Aquaculture*, vol. 324-325, pp. 85–91, 2012.

[14] M. G. Rios-Duran, I. R. Valencia, L. G. Ross, and C. A. Martínez-Palacios, "Nutritional evaluation of autoclaved Salicornia bigelovii Torr. seed meal supplemented with varying levels of cholesterol on growth, nutrient utilization and survival of the Nile tilapia (Oreochromis niloticus)," *Aquaculture International*, vol. 21, no. 6, pp. 1355–1371, 2013.

[15] J. Deng, B. Bi, B. Kang, L. Kong, Q. Wang, and X. Zhang, "Improving the growth performance and cholesterol metabolism of rainbow trout (Oncorhynchus mykiss) fed soyabean meal-based diets using dietary cholesterol supplementation," *British Journal of Nutrition*, vol. 110, no. 1, pp. 29–39, 2013.

[16] J. Deng, X. Zhang, X. Long et al., "Effects of dietary cholesterol supplementation on growth and cholesterol metabolism of rainbow trout (Oncorhynchus mykiss) fed diets with cottonseed meal or rapeseed meal," *Fish Physiology and Biochemistry*, vol. 40, no. 6, pp. 1827–1838, 2014.

[17] T. Zhu, Q. Ai, K. Mai, W. Xu, H. Zhou, and Z. Liufu, "Feed intake, growth performance and cholesterol metabolism in juvenile turbot (Scophthalmus maximus L.) fed defatted fish meal diets with graded levels of cholesterol," *Aquaculture*, vol. 428-429, pp. 290–296, 2014.

[18] T.-M. Wu, J.-J. Jiang, R.-M. Lu, and Y.-H. Lin, "Effects of dietary inclusion of soybean meal and cholesterol on the growth, cholesterol status and metabolism of the giant grouper (Epinephelus lanceolatus)," *Aquaculture Nutrition*, vol. 26, no. 2, pp. 351–357, 2020.

[19] T. M. Kortner, I. Björkhem, A. Krasnov, G. Timmerhaus, and Å. Krogdahl, "Dietary cholesterol supplementation to a plant-based diet suppresses the complete pathway of cholesterol synthesis and induces bile acid production in Atlantic salmon (Salmo salar L.)," *British Journal of Nutrition*, vol. 111, no. 12, pp. 2089–2103, 2014.

[20] H. Xu, Q. Bi, E. Pribytkova et al., "Different lipid scenarios in three lean marine teleosts having different lipid storage patterns," *Aquaculture*, vol. 536, p. 736448, 2021.

[21] J. Hu, Z. Zhang, W.-J. Shen, and S. Azhar, "Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones," *Nutrition and Metabolism*, vol. 7, no. 1, p. 47, 2010.

[22] K. Takii, M. Ukawa, M. Nakamura, and H. Kumai, "Suitable lipid level in brown fish meal diet for tiger puffer," *Fisheries Science*, vol. 61, no. 5, pp. 841–844, 1995.

[23] J. K. Lee, S. H. Cho, S. U. Park, K.-D. Kim, and S.-M. Lee, "Dietary protein requirement for young turbot (Scophthalmus maximus L.)," *Aquaculture Nutrition*, vol. 9, no. 4, pp. 283–286, 2003.

[24] K. Kikuchi, T. Furuta, N. Iwata, K. Onuki, and T. Noguchi, "Effect of dietary lipid levels on the growth, feed utilization, body composition and blood characteristics of tiger puffer Takifugu rubripes," *Aquaculture*, vol. 298, no. 1-2, pp. 111–117, 2009.

[25] G. Yoo, H. Yun, and S. C. Bai, "Optimum dietary protein level in juvenile river puffer Takifugu obscurus," *Journal of Fisheries and Marine Sciences Education*, vol. 26, no. 4, pp. 915–922, 2014.

[26] T. Raggi, A. G. J. Tacon, and D. Lemos, "Feeding of juvenile cobia, Rachycentron canadum: evaluation of practical feeds,

comparison of commercial fishmeal replacers, and estimation of essential amino acid requirements," *Journal of the World Aquaculture Society*, vol. 50, no. 2, pp. 317–335, 2019.

[27] Y. Ma, M. Li, D. Xie et al., "Fishmeal can be replaced with a high proportion of terrestrial protein in the diet of the carnivorous marine teleost (Trachinotus ovatus)," *Aquaculture*, vol. 519, p. 734901, 2020.

[28] V. I. Fuchs, J. Schmidt, M. J. Slater, J. Zentek, B. H. Buck, and D. Steinhagen, "The effect of supplementation with polysaccharides, nucleotides, acidifiers and Bacillus strains in fish meal and soy bean based diets on gro J wth performance in juvenile turbot (Scophthalmus maximus)," *Aquaculture*, vol. 437, pp. 243–251, 2015.

[29] Y. Meng, R. Ma, J. Ma et al., "Dietary nucleotides improve the growth performance, antioxidative capacity and intestinal morphology of turbot (Scophthalmus maximus)," *Aquaculture Nutrition*, vol. 23, no. 3, pp. 585–593, 2017.

[30] H. Xu, Y. Mu, Y. Zhang et al., "Graded levels of fish protein hydrolysate in high plant diets for turbot (Scophthalmus maximus): effects on growth performance and lipid accumulation," *Aquaculture*, vol. 454, pp. 140–147, 2016.

[31] AOAC, *Official Methods of Analysis of the Association of Analytical Chemistry*, AOAC, Arlington, VA, USA, 15th edition, 2000.

[32] J. Folch, M. Lee, and G. H. Sloane-Stanley, "A simple method for the isolation and purification of total lipids from animal tissues," *Journal of Biological Chemistry*, vol. 226, no. 1, pp. 497–509, 1957.

[33] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real–time quantitative PCR and the 22DDCT method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[34] H. Xu, G. M. Turchini, D. S. Francis et al., "Are fish what they eat? A fatty acid's perspective," *Progress in Lipid Research*, vol. 80, p. 101064, 2020.

[35] Z. Liao, B. Sun, Q. Zhang et al., "Dietary bile acids regulate the hepatic lipid homeostasis in tiger puffer fed normal or high-lipid diets," *Aquaculture*, vol. 519, p. 734935, 2020.

[36] M. Maita, J. Maekawa, K. Satoh, K. Futami, and S. Satoh, "Disease resistance and hypocholesterolemia in yellowtail Seriola quinqueradiata fed a non-fishmeal diet," *Fisheries Science*, vol. 72, no. 3, pp. 513–519, 2006.

[37] T. Li and J. Y. L. Chiang, "Regulation of bile acid and cholesterol metabolism by PPARs," *PPAR Research*, vol. 2009, Article ID 501739, 15 pages, 2009.

[38] H. Tanaka, I. Mierau, and F. Ito, "Purification and characterization of bovine pancreatic bile salt-activated lipase," *The Journal of Biochemistry*, vol. 125, no. 5, pp. 883–890, 1999.

[39] M. L. González-Félix, E. B. Santana-Bejarano, M. Perez-Velazquez, and A. G. Villalba-Villalba, "Partial characterization, quantification and activity of pancreatic lipase in the gastrointestinal tract of Totoaba macdonaldi," *Archives of Biological Sciences*, vol. 70, no. 3, pp. 489–496, 2018.

[40] B. G. Bhat, P. Wang, J.-H. Kim et al., "Rat sn-glycerol-3-phosphate acyltransferase: molecular cloning and characterization of the cdna and expressed protein," *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, vol. 1439, no. 3, pp. 415–423, 1999.

[41] C. F. Semenkovich, "Regulation of fatty acid synthase (FAS)," *Progress in Lipid Research*, vol. 36, no. 1, pp. 43–53, 1997.

[42] K. Liu, H. Liu, S. Chi, X. Dong, Q. Yang, and B. Tan, "Effects of different dietary lipid sources on growth performance, body composition and lipid metabolism-related enzymes and genes of juvenile golden pompano, Trachinotus ovatus," *Aquaculture Research*, vol. 49, no. 2, pp. 717–725, 2018.

[43] J. R. Senior and K. J. Isselbacher, "Demonstration of an intestinal monoglyceride lipase: an enzyme with a possible role in the intracellular completion of fat digestion," *The Journal of Clinical Investigation*, vol. 42, no. 2, pp. 187–195, 1963.

[44] X. Dong, H. Xu, K. Mai, W. Xu, Y. Zhang, and Q. Ai, "Cloning and characterization of SREBP-1 and PPAR-α in Japanese seabass Lateolabrax japonicus, and their gene expressions in response to different dietary fatty acid profiles," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 180, pp. 48–56, 2015.

[45] J.-L. Zheng, Z. Luo, Q.-L. Zhu et al., "Molecular cloning and expression pattern of 11 genes involved in lipid metabolism in yellow catfish Pelteobagrus fulvidraco," *Gene*, vol. 531, no. 1, pp. 53–63, 2013.