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Research Article

Effects of Dietary Lipid Levels on Growth, Digestive Enzyme Activities, Antioxidant Capacity, and Lipid Metabolism in Turbot (*Scophthalmus maximus* L.) at Three Different Stages

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Three 56-day feeding trials were conducted to evaluate dietary lipid requirements and effects of dietary lipid levels on growth, digestive enzyme activities, antioxidant capacity, and lipid metabolism in turbot (*Scophthalmus maximus L.*) at three growth stages. The initial mean body weight of turbot was 9.13 ± 0.17 g, 50.10 ± 0.38 g, and 80.05 ± 0.76 g (small, medium, and large turbot), respectively. Five practical diets were formulated to contain 68.4, 93.9, 120.7, 147.8, and 171.2 g/kg lipid (L68.4, L93.9, L120.7, L147.8, and L171.2), respectively. Results of three trials showed that the weight gain rate and specific growth rate of turbot fed with L120.7, L147.8, and L171.2 diets were all significantly higher compared with turbot fed with L68.4 and L93.9 diets except for large turbot fed with the L147.8 diet. Activities of intestinal trypsin, lipase and hepatic catalase, superoxide dismutase, and total antioxidant capacity firstly increased and then decreased as dietary lipids increased. Meanwhile, malondialdehyde content decreased firstly but then increased with the elevation of dietary lipids in small and medium turbot, and it was significantly higher in the L171.2 group than the rest in large turbot. With increasing levels of dietary lipid, contents of whole-body lipid, liver lipid, serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol were markedly increased at three stages. In addition, serum high-density lipoprotein cholesterol contents increased firstly and then decreased over the L120.7 group. Transcriptional levels of lipolysis-related genes *lipin1* and *lpl* were significantly upregulated firstly and subsequently downregulated with increasing dietary lipid levels except *lipin1* in medium fish. Meanwhile, a significant increase in lipogenesis-related genes *lxr* and *pparγ* expressions was detected in all fish. Based on the specific growth rate, the dietary lipid level of 130.1, 120.1, and 107.7 g/kg was optimal for the growth performance of turbot cultured at three phases, respectively. Additionally, the results indicated that high lipid diets may lead to abnormal lipid deposition and affect the physiological health of turbot.

1. Introduction

Lipid is one of the essential nutrients as energy resources in fish diets [1]. Apart from its role as an energy resource, lipid also provides essential fatty acids and phospholipids and can be used as the carrier of lipid-solute vitamins and precursors of hormones [2, 3]. Appropriate dietary lipid content could improve feed utilization, survival, and growth performance of fish [4, 5]. Inadequate or deficient dietary lipid levels, however, may lead to essential fatty acid deficiencies and disturbance in fat metabolism [6, 7]. On the other hand, excessive lipid levels in diets may result in abnormal fat deposition and negative effects on growth performance in fish [8–10]. Furthermore, dietary lipid requirements may

vary across different developmental stages in aquatic animals [11, 12]. In general, smaller-sized fish have higher nutritional requirements than larger ones due to their rapid growth demand [13]. Hence, determining proper dietary lipid levels of different growth stages is essential for better growth performance of fish.

Previous studies have found that dietary lipid levels can affect growth performance, digestive enzyme activities, antioxidant capacity, and lipid metabolism in aquatic animals. Hamidoghli et al. [11] and Xu et al. [3] have revealed that the Pacific white shrimp (*Litopenaeus vannamei*) fed diets with appropriate lipid levels had higher weight gain and specific growth rate. Also, fish-fed diets with suitable lipid content exhibited higher growth performance [5, 7]. However, higher dietary lipid levels in triploid rainbow trout (*Oncorhynchus mykiss*) brought higher feed intake and specific growth rate, while swimming grabs (*Portunus trituberculatus*) fed with higher lipid diet showed lower survival and specific growth rate [2, 14]. Studies showed that lipase activities in the digestive tract of fish fed with appropriate lipid levels were higher than in other groups [4, 5]. Additionally, *Onychostoma macrolepis* fed with proper dietary lipid exhibited increases in activities of catalase (CAT) and superoxide dismutase (SOD) and decreases malondialdehyde (MDA) contents in the liver [6]. Furthermore, the research has demonstrated that dietary lipid contents had an impact on lipid metabolism of fish [15]. Higher dietary lipid levels increased both gene expressions and enzyme activity of lipoprotein lipase (LPL) in blunt snout bream (*Megalobrama amblycephala*) and enzyme activity of carnitine palmitoyltransferase (CPT1) in largemouth bass (*Micropterus salmoides*) [16, 17]. In addition, higher transcriptional levels of CPT1 were also obtained in *Onychostoma macrolepis* fed with higher dietary lipid levels ([6]), whereas the mRNA expression of LPL was higher in orange-spotted grouper (*Epinephelus coioides* H.) fed with proper lipid levels [5].

Turbot (*Scophthalmus maximus* L.), an economically important marine carnivorous flatfish species due to its rapid growth rate and high nutritional value, is extensively cultured in Asia and Europe [18, 19]. Dietary lipid requirements of turbot varied from 10.0% to 16.8% relying on experimental conditions and fish size in studies, and the studies on the lipid requirements of turbot were conducted for a single growth stage (initial body weights of 657 ± 6 g, 54.4 ± 0.2 g, and 39 ± 0.2 g, respectively) [20–22]. However, there is a lack of systematic studies on the lipid requirements in diets for turbot at different growth stages. Thus, turbot of three growth stages (initial weight 9.13 ± 0.17 g, 50.10 ± 0.38 g, and 80.05 ± 0.76 g, respectively), where fish gained weight faster or had higher intestinal digestive enzyme activity, were selected to evaluate dietary lipid requirements and the effects of dietary lipid levels on growth, digestive enzyme activities, antioxidant capacity, and lipid metabolism in turbot in the current study.

2. Materials and Methods

2.1. Fish and Experimental Procedure of the Feeding Trials. Experimental turbot in the same batch was obtained from a commercial farm (Weihai City, Shandong Province, China). Three stages of turbot were used in the trials. The mean initial body weights of turbot were 9.13 ± 0.17 g (small), 50.10 ± 0.38 g (medium), and 80.05 ± 0.76 g (large), respectively. In each stage, turbot were distributed randomly into 15 tanks (400 L). Each diet was randomly assigned to triplicate groups of 30, 20, and 15 fish per tank in three stages for 56 days. Prior to the start of the experiment, fish were acclimatized to conditions with a commercial feed (Great Seven Bio-Tech Co., Ltd., Qingdao, China) (crude protein 500 g/kg and crude lipid 130 g/kg dry matter) for 14 days and then fasted for 24 hours. Turbot were fed by hand with diets twice a day (07:00 and 17:00), and the uneaten feed left in the tank and feces were removed by the siphon immediately after feeding. During the experimental periods, sea water temperature ranged from 16.0 to 18.0°C, salinity ranged from 28.0‰ to 32.0‰, and dissolved oxygen content was above 7.0 mg/L.

Table 1: Ingredients and proximate composition of trial diets with different lipid contents (g/kg dry matter).

Ingredients	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Fish meal[a]	550.0	550.0	550.0	550.0	550.0
Soybean meal[a]	160.0	160.0	160.0	160.0	160.0
Wheat meal[a]	135.0	135.0	135.0	135.0	135.0
Soybean oil	0.0	12.5	25.0	37.5	50.0
Fish oil	0.0	12.5	25.0	37.5	50.0
Soybean lecithin	18.0	18.0	18.0	18.0	18.0
α-Starch	107.0	82.0	57.0	32.0	7.0
Vitamin premix[b]	10.0	10.0	10.0	10.0	10.0
Mineral premix[c]	10.0	10.0	10.0	10.0	10.0
Choline chloride	2.5	2.5	2.5	2.5	2.5
Sodium alginate	3.0	3.0	3.0	3.0	3.0
Calcium propionate acid	1.0	1.0	1.0	1.0	1.0
Ethoxyquin	0.5	0.5	0.5	0.5	0.5
Monocalcium phosphate	3.0	3.0	3.0	3.0	3.0
Proximate composition (%)					
Crude protein	502.8	502.6	502.4	502.2	502.0
Crude lipid	68.4	93.9	120.7	147.8	171.2
Ash	109.3	108.7	109.8	110.1	107.9

[a]Commercially available from Great Seven Bio-Tech Co., Ltd. (Qingdao, China); elementary composition: red fish meal (g/kg dry mater): protein 716.9, crude lipid 93.7; soybean meal (g/kg dry mater): crude protein 516.9, crude lipid 23.4; wheat meal (g/kg dry mater): crude protein 185.2, crude lipid 15.4. [b]Composition of vitamin premix (mg/kg premix): vitamin B_1, 25; vitamin B_6, 20; vitamin B_{12}, 0.01; vitamin K_3, 10; inositol, 800; folic acid, 20; pyridoxine-HCl, 4.0; niacin, 20.0; retinyl acetate, 32; inositol, 200.0; α-tocopherol, 240; folic acid, 1.5; 4-aminobenzoic acid, 5.0; ethoxyquin 3; ascorbic acid 2000; riboflavin, 45; niacin, 200; L-ascorgyl-2-monophosphate-Na (3%), 2000.0; biotin (2%), 60; cholecalciferol, 5; microcrystalline cellulose, 6470. [c]Mineral premix (mg/kg premix): $MnSO_4 \cdot H_2O$, 45; Na_2SeO_3 (1%), 20; $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 5H_2O$, 10; zeolite, 3485; $Ca(IO_3)_2$ (1%), 60; $CoCl_2 \cdot 6H_2O$ (1%), 50; $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 5.

2.2. Diet Formulation. Five isonitrogenous practical diets were formulated to contain 68.4, 93.9, 120.7, 147.8, and 171.2 g/kg

Table 2: The quantitative PCR primers used in this study.

Target genes	Forward primers (5′-3′)	Reverse primers (5′-3′)	References
β-Actin	GTAGGTGATGAAGCCCAGAGCA	CTGGGTCATCTTCTCCCTGT	Peng et al. [18]
pgc1α	ACGGATTGCCTTCGTTTGA	CATCTTGACGACGGCAGGT	Peng et al. [23]
lipin1	AGGACGCTGGTGGTTCTCG	CTGTCCGCTGAGGTCATAGTG	Peng et al. [23]
pparα	CGATCAGGTGACCCTGTTAA	TGGAACTTGGGCTCCATC	Peng et al. [18]
cpt1	GCCTTTCAGTTCACCATCACA	ATGCGGCTGACTCGTTTCTT	Peng et al. [18]
lpl	CTCCCACGAACGCTCTAT	GCGGACCTTGTTGATGTT	Peng et al. [18]
lxr	GCGTCATCAAGAGTGCCC	ATCTGATTTGCTCCTCCGAG	Peng et al. [18]
fas	GGCAACAACACGGATGGATAC	CTCGCTTTGATTGACAGAACAC	Peng et al. [18]
pparγ	AAGTGACGGAGTTCGCCAAGA	GTTCATCAGAGGTGCCATCA	Peng et al. [18]
srebp1	CGATCCGCACTCCAAGT	CCGCACTGCCCTGAAT	Peng et al. [18]
hnf4α	AGTGCGTGGTGGACAAAGAC	GAGTCGTACTGGCGGTCGTTG	Peng et al. [18]
apoB100	TCTCACCCTCGGTCTCGG	TTCAGGTTTCTCCTCACAACGA	Peng et al. [18]
mtp	CCAGCAAAGTCTTACGCCA	TACGCAGATGATGACCCAAC	Peng et al. [18]

Abbreviations: *pgc1α*: peroxisome proliferator-activated receptor-*γ* coactivator 1*α*; *pparα*: peroxisome proliferator-activated receptor *α*; *cpt1*: carnitine palmitoyl transferase 1; *lpl*: lipoprotein lipase; *lxr*: liver X receptor; *fas*: fatty acid synthase; *pparγ*: peroxisome proliferator-activated receptor *γ*; *srebp1*: sterol-regulatory element binding protein-1; *hnf4α*: hepatocyte nuclear factor 4*α*; *apoB100*: apolipoprotein B100; *mtp*: microsomal TAG transfer protein.

Table 3: Effects of dietary lipid level on growth performance of small turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
IBW (g)	9.13 ± 0.17	9.13 ± 0.17	9.13 ± 0.17	9.13 ± 0.17	9.13 ± 0.17
FBW (g)	40.32 ± 0.35^{b}	40.85 ± 0.23^{b}	43.05 ± 0.27^{a}	42.94 ± 0.14^{a}	43.38 ± 0.25^{a}
WGR (%)	341.57 ± 3.89^{b}	347.48 ± 2.59^{b}	371.58 ± 2.95^{a}	370.33 ± 1.51^{a}	375.15 ± 2.74^{a}
SGR (%/day)	2.65 ± 0.02^{b}	2.68 ± 0.01^{b}	2.77 ± 0.01^{a}	2.76 ± 0.01^{a}	2.78 ± 0.01^{a}
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
FER	1.22 ± 0.01^{a}	1.23 ± 0.02^{a}	1.29 ± 0.03^{a}	1.11 ± 0.03^{b}	1.26 ± 0.01^{a}
CF (%)	3.47 ± 0.05^{b}	3.65 ± 0.07^{ab}	3.61 ± 0.05^{ab}	3.68 ± 0.09^{ab}	3.83 ± 0.06^{a}

Abbreviations: SR: survival rate; IBW: initial body weight; SGR: specific growth rate; WGR: weight gain rate; FBW: final body weight; FER: feed efficiency ratio; CF: condition factor. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 4: Effects of dietary lipid level on growth performance of medium turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
IBW (g)	50.10 ± 0.38	50.10 ± 0.38	50.10 ± 0.38	50.10 ± 0.38	50.10 ± 0.38
FBW (g)	93.69 ± 0.54^{b}	94.00 ± 0.31^{b}	102.31 ± 0.17^{a}	101.42 ± 0.21^{a}	101.66 ± 0.58^{a}
WGR (%)	87.39 ± 1.07^{b}	88.01 ± 0.63^{b}	104.64 ± 0.33^{a}	102.84 ± 0.43^{a}	103.32 ± 1.16^{a}
SGR (%/day)	1.12 ± 0.01^{b}	1.13 ± 0.01^{b}	1.28 ± 0.00^{a}	1.27 ± 0.00^{a}	1.27 ± 0.01^{a}
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
FER	0.77 ± 0.02^{b}	0.77 ± 0.00^{b}	0.82 ± 0.01^{ab}	0.85 ± 0.01^{a}	0.84 ± 0.02^{a}
CF (%)	3.07 ± 0.02^{c}	3.08 ± 0.06^{c}	3.16 ± 0.01^{bc}	3.22 ± 0.02^{b}	3.37 ± 0.02^{a}

Abbreviations: SR: survival rate; IBW: initial body weight; SGR: specific growth rate; WGR: weight gain rate; FBW: final body weight; FER: feed efficiency ratio; CF: condition factor. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 5: Effects of dietary lipid level on growth performance of large turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
IBW (g)	80.05 ± 0.76	80.05 ± 0.76	80.05 ± 0.76	80.05 ± 0.76	80.05 ± 0.76
FBW (g)	166.24 ± 1.47^{c}	180.14 ± 3.66^{b}	192.49 ± 1.11^{a}	187.45 ± 2.73^{ab}	191.27 ± 1.68^{a}
WGR (%)	107.80 ± 1.84^{c}	125.17 ± 4.58^{b}	140.61 ± 1.39^{a}	134.32 ± 3.41^{ab}	139.09 ± 2.10^{a}
SGR (%/day)	1.31 ± 0.02^{c}	1.45 ± 0.03^{b}	1.57 ± 0.01^{a}	1.52 ± 0.03^{ab}	1.56 ± 0.02^{a}
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
FER	1.21 ± 0.03	1.35 ± 0.06	1.40 ± 0.06	1.39 ± 0.01	1.18 ± 0.07
CF (%)	3.44 ± 0.08^{c}	3.71 ± 0.06^{ab}	3.54 ± 0.04^{bc}	3.59 ± 0.04^{abc}	3.79 ± 0.04^{a}

Abbreviations: SR: survival rate; IBW: initial body weight; SGR: specific growth rate; WGR: weight gain rate; FBW: final body weight; FER: feed efficiency ratio; CF: condition factor. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

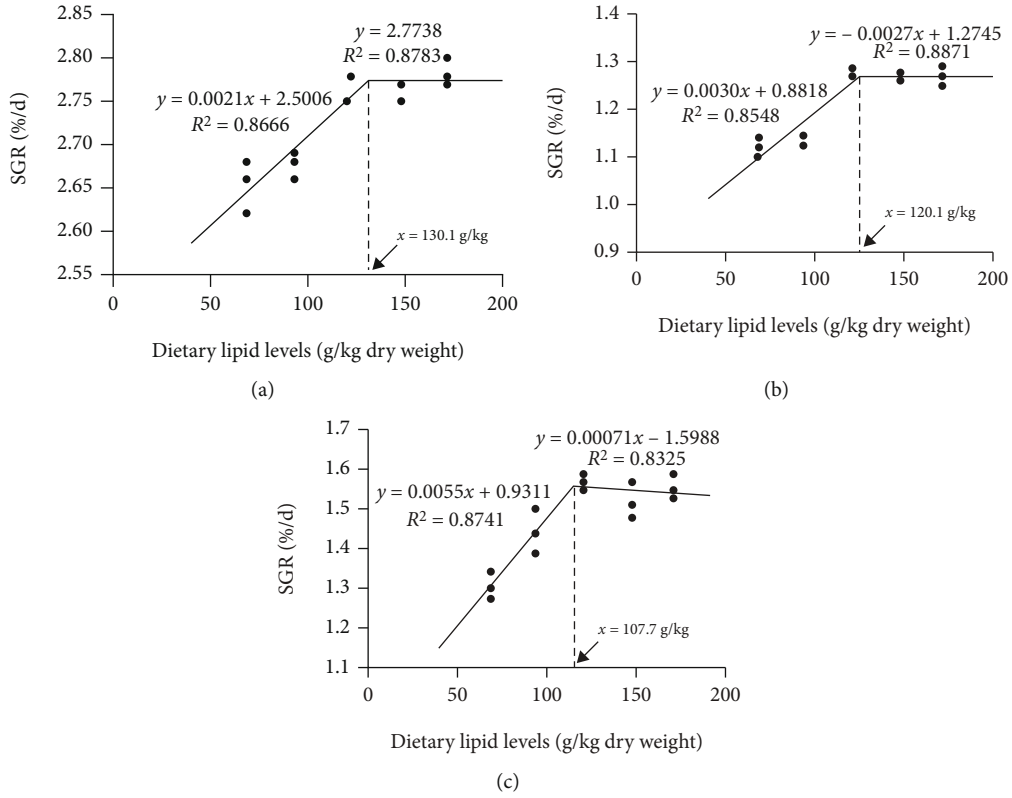

Figure 1: Relationship of specific growth rate (SGR, %/d) with dietary lipid levels of small (a), medium (b), and large (c) turbot fed the experiment diet.

lipid (dry matter) (L68.4, L93.9, L120.7, L147.8, and L171.2, respectively) (Table 1). All diets were similar in crude protein (502 g/kg dry matter), and each of them was fed to turbot in triplicate for 56 days. Fish meal, wheat meal, and soybean meal were used as the main sources of protein, and soybean oil and fish oil were used as the main sources of lipid. All ingredients were firstly ground through a 320 μm mesh before the diet preparation and then thoroughly blended with soybean oil, fish oil, and water to produce pellets. Pellets were dried for above 10 hours using a ventilated oven at 55°C. The sizes of pellets were 2 mm * 3 mm for small fish and 3 mm * 5 mm for medium and large fish. All experimental diets were stored at -20°C in the fridge until used.

2.3. Sample Collection. At the termination of the feeding trial, fish were fasted for 24 hours before harvest. The total number and final body weight of turbot in each tank were collectively measured. Four fish of each tank at the termination of experiments were collected and stored at -20°C to assess whole-body crude lipid. Another ten fish per tank were randomly selected, sacrificed, and recorded for individual weight and total length to assess the condition factor. The blood sample was pooled from the tail vein with a 1 mL syringe. The blood was centrifuged at 4000 rpm for 10 minutes after standing for 10 hours to isolate the serum, then frozen in liquid nitrogen and stored at -80°C for later analysis of serum biochemical indices. Livers were cut off

Table 6: Effects of dietary lipid level on intestinal digestive enzyme activities of small turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Trypsin (U/mgprot)	836.48 ± 82.91[b]	981.29 ± 48.90[b]	1406.39 ± 72.43[a]	1067.59 ± 16.42[b]	850.18 ± 61.07[b]
Amylase (U/mgprot)	0.16 ± 0.04	0.14 ± 0.03	0.18 ± 0.01	0.26 ± 0.03	0.21 ± 0.03
Lipase (U/gprot)	11.82 ± 0.51[c]	14.13 ± 0.39[c]	18.42 ± 0.60[b]	21.50 ± 0.91[a]	18.50 ± 0.47[b]

[a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 7: Effects of dietary lipid level on intestinal digestive enzyme activities of medium turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Trypsin (U/mgprot)	860.95 ± 32.15[bc]	1431.37 ± 76.99[a]	1269.47 ± 53.36[a]	1026.76 ± 47.94[b]	713.14 ± 32.89[c]
Amylase (U/mgprot)	0.21 ± 0.02	0.17 ± 0.01	0.20 ± 0.02	0.22 ± 0.03	0.19 ± 0.01
Lipase (U/gprot)	8.62 ± 0.71[b]	11.27 ± 0.58[b]	16.97 ± 1.11[a]	17.77 ± 1.03[a]	10.64 ± 1.20[b]

[a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 8: Effects of dietary lipid level on intestinal digestive enzyme activities of large turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Trypsin (U/mgprot)	751.56 ± 49.08[b]	1017.95 ± 94.10[ab]	1299.65 ± 104.99[a]	1330.12 ± 124.97[a]	809.78 ± 63.90[b]
Amylase (U/mgprot)	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.03	0.16 ± 0.02	0.24 ± 0.03
Lipase (U/gprot)	8.14 ± 0.47[c]	10.91 ± 1.15[bc]	11.96 ± 0.87[ab]	14.96 ± 0.34[a]	12.15 ± 0.70[ab]

[a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 9: Effects of dietary lipid level on liver antioxidant indices of small turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
CAT (U/mL)	12.85 ± 0.77[bc]	13.03 ± 0.90[bc]	18.41 ± 0.27[a]	15.23 ± 0.59[ab]	10.16 ± 1.17[c]
SOD (U/mL)	7.93 ± 0.93[b]	9.61 ± 0.54[ab]	11.39 ± 0.33[a]	12.16 ± 0.97[a]	8.00 ± 0.75[b]
T-AOC (U/mL)	1.82 ± 0.06[d]	2.34 ± 0.17[cd]	3.99 ± 0.12[a]	2.97 ± 0.08[b]	2.60 ± 0.16[bc]
MDA (nmol/mL)	8.17 ± 0.44[b]	6.02 ± 0.35[c]	1.42 ± 0.28[e]	3.34 ± 0.19[d]	10.42 ± 0.25[a]

Abbreviations: CAT: catalase; SOD: superoxide dismutase; T-AOC: total antioxidant capacity; MDA: malondialdehyde. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 10: Effects of dietary lipid level on liver antioxidant indices of medium turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
CAT (U/mL)	10.52 ± 0.63[c]	11.76 ± 0.44[bc]	16.18 ± 0.66[a]	13.79 ± 0.84[ab]	8.66 ± 0.71[c]
SOD (U/mL)	9.96 ± 0.51[c]	12.70 ± 0.47[a]	12.55 ± 1.01[ab]	12.24 ± 0.47[abc]	10.08 ± 0.23[bc]
T-AOC (U/mL)	2.61 ± 0.25	2.78 ± 0.40	3.21 ± 0.31	3.15 ± 0.32	2.73 ± 0.09
MDA (nmol/mL)	6.64 ± 0.25[ab]	5.84 ± 0.05[bc]	5.71 ± 0.10[c]	6.43 ± 0.14[abc]	6.86 ± 0.29[a]

Abbreviations: CAT: catalase; SOD: superoxide dismutase; T-AOC: total antioxidant capacity; MDA: malondialdehyde. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 11: Effects of dietary lipid level on liver antioxidant indices of large turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
CAT (U/mL)	10.74 ± 0.48^{d}	14.29 ± 0.57^{bc}	15.41 ± 0.46^{b}	19.27 ± 0.17^{a}	11.86 ± 0.93^{cd}
SOD (U/mL)	8.92 ± 0.53^{b}	11.52 ± 0.15^{a}	12.79 ± 0.29^{a}	11.77 ± 0.13^{a}	9.72 ± 0.57^{b}
T-AOC (U/mL)	3.33 ± 0.25^{abc}	2.79 ± 0.26^{c}	4.14 ± 0.23^{a}	3.93 ± 0.32^{ab}	3.03 ± 0.18^{bc}
MDA (nmol/mL)	3.68 ± 0.07^{b}	3.68 ± 0.08^{b}	4.06 ± 0.21^{b}	4.10 ± 0.18^{b}	4.76 ± 0.11^{a}

Abbreviations: CAT: catalase; SOD: superoxide dismutase; T-AOC: total antioxidant capacity; MDA: malondialdehyde. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 12: Effects of dietary lipid level on lipid deposition in whole body, muscle, and liver of small turbot (means ± S.E.M.)[a].

Crude lipid (g/kg wet weight)	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Whole body	25.2 ± 0.02^{e}	28.3 ± 0.01^{d}	35.8 ± 0.02^{c}	42.9 ± 0.02^{b}	56.5 ± 0.05^{a}
Muscle	9.4 ± 0.02	9.3 ± 0.01	9.4 ± 0.01	9.7 ± 0.03	9.6 ± 0.01
Liver	56.2 ± 0.03^{d}	68.0 ± 0.01^{c}	74.0 ± 0.02^{b}	74.5 ± 0.01^{b}	86.6 ± 0.02^{a}

[a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 13: Effects of dietary lipid level on lipid deposition in whole body, muscle, and liver of medium turbot (means ± S.E.M.)[a].

Crude lipid (g/kg wet weight)	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Whole body	25.4 ± 0.02^{d}	29.0 ± 0.05^{c}	30.7 ± 0.04^{c}	36.2 ± 0.04^{b}	46.6 ± 0.07^{a}
Muscle	10.4 ± 0.02	10.3 ± 0.03	10.2 ± 0.02	10.2 ± 0.03	10.1 ± 0.03
Liver	59.9 ± 0.06^{d}	68.7 ± 0.08^{c}	78.2 ± 0.12^{b}	81.4 ± 0.09^{b}	89.7 ± 0.09^{a}

[a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 14: Effects of dietary lipid level on lipid deposition in whole body, muscle, and liver of large turbot (means ± S.E.M.)[a].

Parameters (g/kg wet weight)	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
Whole body	25.1 ± 0.02^{d}	26.8 ± 0.02^{c}	27.6 ± 0.05^{c}	31.6 ± 0.05^{b}	41.7 ± 0.03^{a}
Muscle	9.1 ± 0.02	9.3 ± 0.03	9.3 ± 0.03	9.4 ± 0.03	9.4 ± 0.03
Liver	53.0 ± 0.05^{d}	63.4 ± 0.07^{c}	67.5 ± 0.12^{b}	71.2 ± 0.11^{b}	79.6 ± 0.04^{a}

[a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

followed by fixation in 4% paraformaldehyde for subsequent Oil Red O staining. Muscles and livers from four fish per tank were collected into 10 mL tubes, frozen in liquid nitrogen, and then stored at -80°C for the assay of crude lipid. Livers and intestines from four fish per tank were pooled into 1.5 mL tubes (RNase-Free, Axygen), frozen in liquid nitrogen, and then stored at -80°C for future analysis of antioxidant capacity, enzyme activities, and mRNA expression.

2.4. Biochemical Analysis

2.4.1. Crude Lipid of Whole Body, Muscle, and Liver. Crude lipid was assessed for feed and fish samples, which was determined by referring to the Soxhlet extraction method [23]. Total lipid of the muscle and liver was extracted by chloroform : methanol (2 : 1, *v*/*v*) according to the Folch method [24].

2.4.2. Serum Biochemical Index Assays. Contents of total triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in the serum were measured using commercial assay kits bought from Nanjing Jian Cheng Bioengineering Institute.

2.4.3. Hepatic Antioxidant Capacity and Intestinal Digestive Enzyme Activity Assays. For the enzyme activity analysis in the intestine, a total of 0.3 g frozen intestinal samples were minced in 0.9% NaCl solution at a ratio of 1 : 9 (weight/

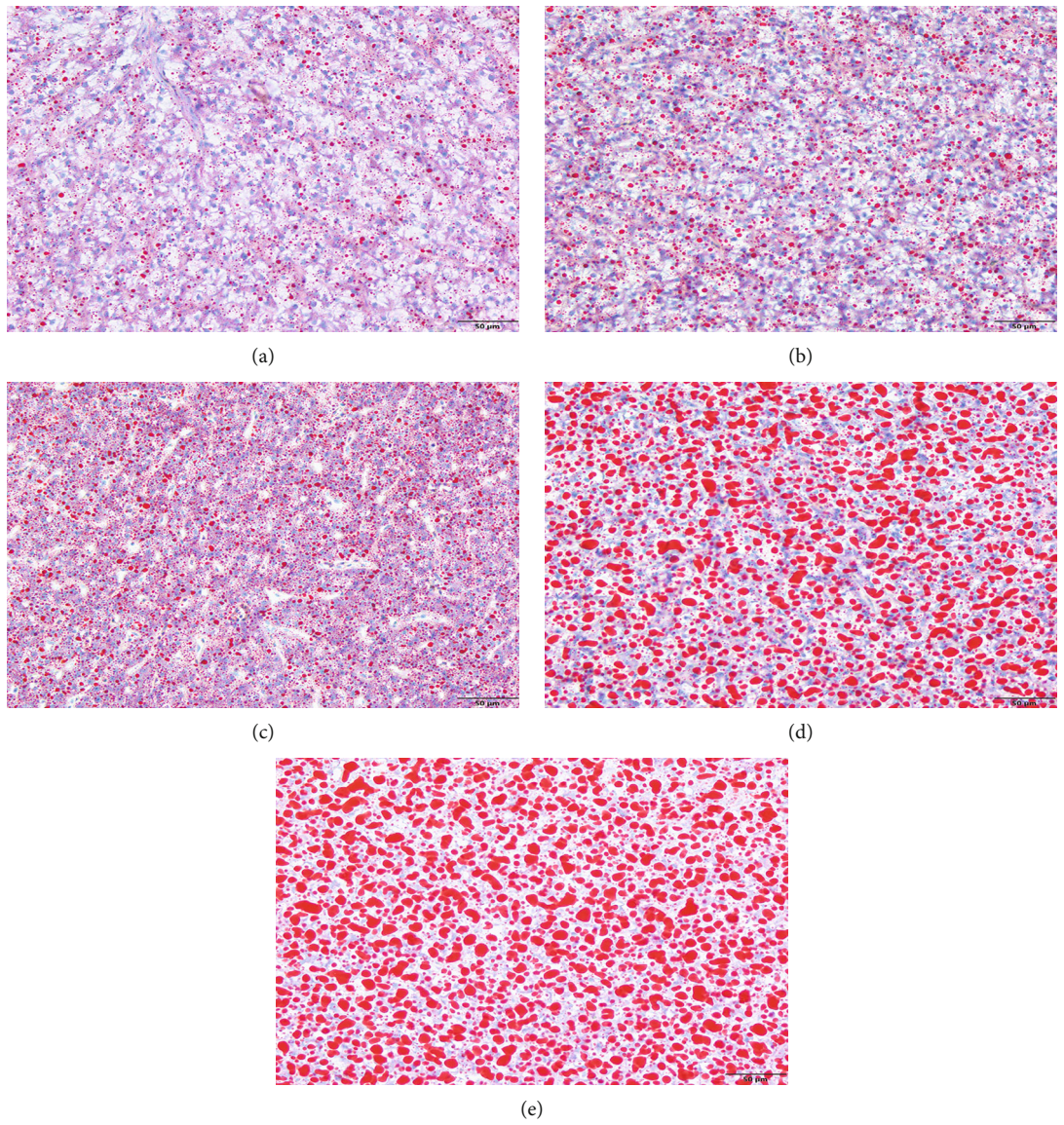

FIGURE 2: Effects of dietary lipid level on liver lipid deposition of small turbot (Oil Red O staining). Photomicrographs (×400) and scale bar (50 μm): (a) 68.4 g/kg lipid diet; (b) 93.9 g/kg lipid diet; (c) 120.7 g/kg lipid diet; (d) 147.8 g/kg lipid diet; (e) 171.2 g/kg lipid diet.

volume). Subsequently, the mixed solution was centrifuged at 4000 rpm for 20 minutes at 4°C, and the supernatant was transferred to a new 2.0 mL centrifuge tube. Liver samples were handled in the same manner. The activity of lipase, amylase, and trypsin in gut, catalase (CAT), superoxide dismutase (SOD), and total antioxidant capacity (T-AOC), and the content of malondialdehyde (MDA) in the liver were detected using commercial assay kits bought from Nanjing Jian Cheng Bioengineering Institute.

2.5. Total RNA Extraction, cDNA Synthesis, and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Total RNA was extracted from the liver of turbot using the TRIzol reagent (Takara, Japan), and the quality of extracted RNA was assessed using RNA electropherogram, and the concentration of isolated RNA was evaluated with a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). RNA samples were then reverse-transcribed to cDNA using the PrimeScript™ RT Reagent Kit (Takara, Japan) following the manufacturer's protocols. The RT-qPCR was performed in a PCR Thermal Cycler machine (CFX96™ Real-Time System, BIO-RAD, USA). The housekeeping gene used in the present study was *β-actin*. The RT-qPCR primer sequence of *β-actin*, peroxisome proliferator-activated receptor-*γ* coactivator 1*α* (*pgc1α*), *lipin1*, peroxisome proliferator-activated receptor *α* (*pparα*), carnitine palmitoyl transferase 1 (*cpt1*), lipoprotein lipase (*lpl*), liver X receptor (*lxr*), fatty acid synthase (*fas*), peroxisome proliferator-activated receptor *γ* (*pparγ*), sterol-regulatory element binding protein-1 (*srebp1*), hepatocyte nuclear factor 4*α* (*hnf4α*), apolipoprotein B100 (*apoB100*), and microsomal TAG transfer protein (*mtp*) are shown in Table 2 [18, 23]. The melting curve and standard curve analysis were carried out to verify the PCR product specificity and PCR amplification efficiency,

respectively. The amplification efficiency of *β-actin* and target genes ranged from 0.94 to 1.06. The $2^{-\Delta\Delta CT}$ method was used to assess the gene expression levels as described by Livak and Schmittgen [64].

2.6. Statistical Analysis and Calculation.

$$\begin{aligned}
\text{Weight gain rate (WGR, \%)} &= \frac{W_f - W_i}{W_i} \times 100,\\
\text{Survival rate (SR, \%)} &= \frac{N_f}{N_i} \times 100,\\
\text{Specific growth rate (SGR, \%/day)} &= \frac{\text{Ln } W_f - \text{Ln } W_i}{56\,\text{d} \times 100},\\
\text{Feed efficiency ratio (FER)} &= \frac{W_f - W_i}{\text{dry feed consumed}},\\
\text{Condition factor (CF, \%)} &= \frac{W_f}{L^3} \times 100,
\end{aligned} \tag{1}$$

where W_i and W_f were the initial and final wet weight of fish in the tank, respectively; N_i and N_f were the initial and final numbers of fish, respectively; 56 d was the duration of experimental days; and L was the final length of fish.

Statistical analysis was performed using SPSS version 26 statistical software. One-way ANOVA was used for data analysis followed by Tukey's multiple-range test. Results were presented as mean values along with standard error (means ± S.E.M.). All statistics performed with $P < 0.05$ were considered significant. In addition, figures were generated using GraphPad Prism version 8 for Windows (GraphPad Software).

3. Results

3.1. Survival, Growth, Feed Utilization, and Morphological Parameters. No significant difference in SR was found at the three stages. There was no mortality recorded among treatments during the three experiments (Tables 3–5). For three sizes of turbot, WGR and SGR were all significantly affected by dietary lipid levels ($P < 0.05$). Groups fed L120.7, L147.8, and L171.2 diets showed higher WGR and SGR than those of L68.4 and L93.9 diets (Tables 3–5). The broken-line analysis for SGR indicated that the maximum growth in the three different stages of turbot appeared in the diet containing 130.1, 120.1, and 107.7 g/kg lipid, respectively (Figure 1). For small turbot, FER increased with increasing dietary lipid levels up to L120.7 and then decreased in L147.8 and increased again in the L171.2 group ($P < 0.05$) (Table 3). For medium turbot, FER fed diets with L68.4 and L93.9 were significantly lower than those in the L120.7, L147.8, and L171.2 groups ($P < 0.05$) (Table 4). However, FER was independent of dietary lipids in large turbot ($P > 0.05$) (Table 5). In the three stages, there was a significant increasing trend in CF with increasing dietary lipid levels ($P < 0.05$) (Tables 3–5).

3.2. Intestinal Digestive Enzyme Activity Assays. The activities of trypsin and lipase in the intestine had a tendency to initially rise and then fall in turbot of three different sizes with the dietary lipid levels increasing ($P < 0.05$) (Tables 6–8). In three growth stages, however, the activity of amylase in the intestine did not reveal significant differences among dietary treatments ($P > 0.05$) (Tables 6–8).

3.3. Hepatic Antioxidant Capacity. In fish of three developmental stages, activities of CAT, SOD, and T-AOC showed a general trend of first increasing and then decreasing with the elevation of lipid levels; only T-AOC activity of medium turbot showed an insignificant difference ($P > 0.05$) (Tables 9–11). In small and medium fish, contents of MDA decreased at first but then increased with increasing lipid levels, and the lowest MDA was in the L120.7 group ($P < 0.05$) (Tables 9 and 10). In large fish, MDA of the L171.2 group was significantly higher than that of all other groups ($P < 0.05$) (Table 11).

3.4. Lipid Deposition in Whole Body, Muscle, and Liver. Crude lipid of fish and liver lipid contents increased in a stepwise fashion with increasing dietary lipid levels at three stages, and the lipid contents of fish and liver in the L171.2 group were markedly higher than those in the other four lower lipid groups ($P < 0.05$) (Tables 12–14). However, lipid contents in the muscle were not affected by the dietary lipid ($P > 0.05$) (Tables 12–14).

In three stages of turbot, the result of Oil Red O staining showed that the size or numbers of lipid droplets increased with the elevated dietary lipid levels (Figures 2–4). The result demonstrated that dietary lipid levels increased lipid droplets in the liver at microscopic levels.

3.5. Serum Biochemical Indices. For three stages of turbot, contents of TG, TC, and LDL-C in serum were significantly higher with an increase in dietary lipid levels. With the increasing dietary lipid levels, serum HDL-C content showed a trend of decrease after the increase at three stages, and the maximum was reached in the L120.7 group ($P < 0.05$) (Tables 15–17).

3.6. Expressions of Lipid Metabolism-Related Genes in Liver. For small turbot, there was a tendency to increase and then decrease for *pgc1α* gene expressions in the liver, but there was no significant difference among treatments ($P > 0.05$) (Figure 5(a)). For medium and large turbot, however, relative expression of *pgc1α* was significantly influenced by dietary lipid levels ($P < 0.05$) (Figures 6(a) and 7(a)). Specifically, mRNA expression of *pgc1α* was significantly lower in medium and large turbot fed L171.2 diets than those fed L120.7 diets ($P < 0.05$) (Figures 6(a) and 7(a)). Expressions of hepatic *lipin1* and *lpl* of turbot in three sizes were significantly upregulated firstly and subsequently downregulated except that no notable change in *lipin1* of medium fish was observed with increasing dietary lipid levels (Figures 5(a), 6(a), and 7(a)). For small and medium turbot, expressions of *pparα* and *cpt1* in the L171.2 group were significantly lower than that in other groups ($P < 0.05$) (Figures 5(a) and 6(a)). However, experimental diet had no significant effect on the expressions of *pparα* and *cpt1* in the large turbot ($P > 0.05$) (Figure 7(a)). With increasing

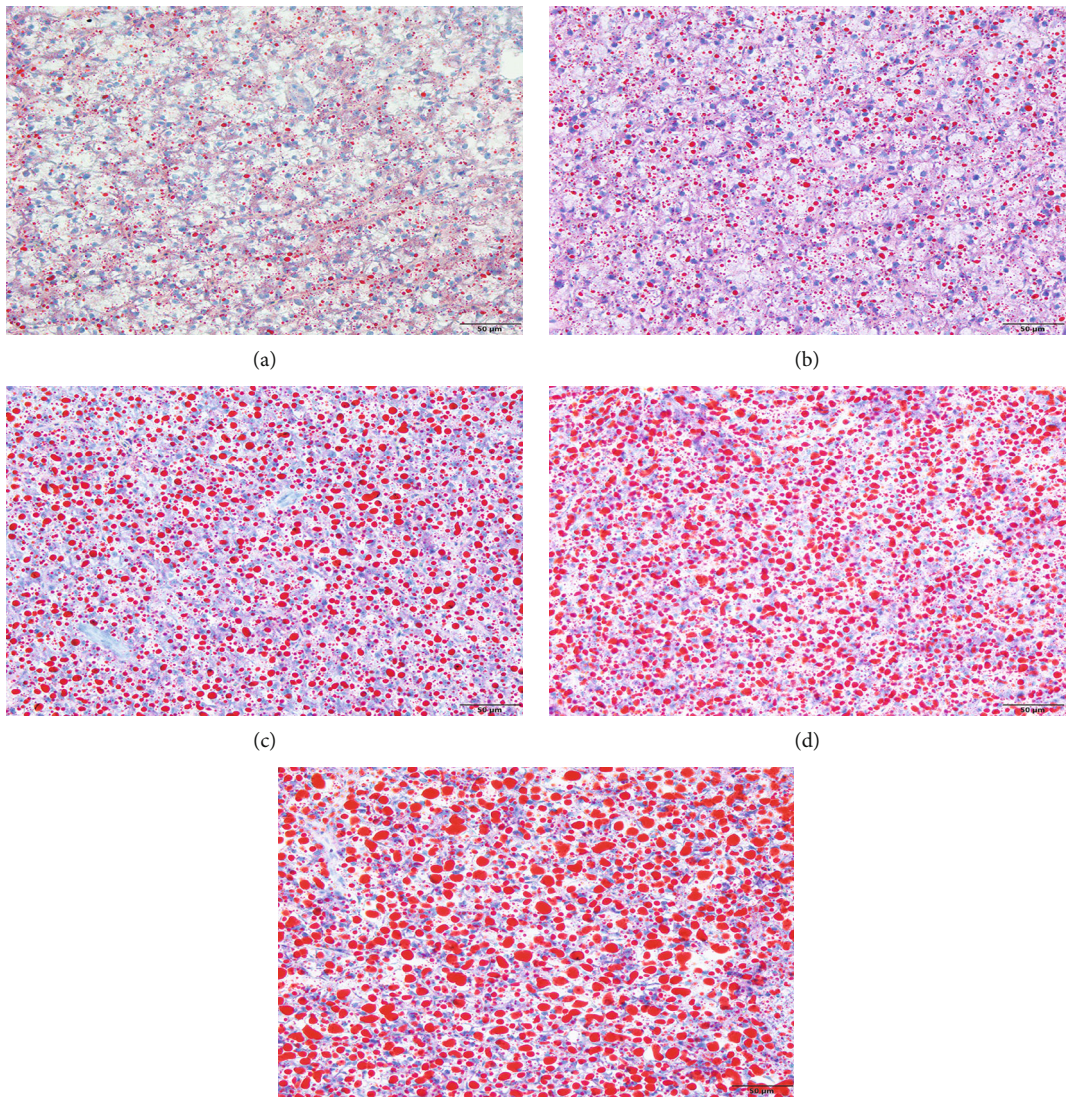

Figure 3: Effects of dietary lipid level on liver lipid deposition of medium turbot (Oil Red O staining). Photomicrographs (×400) and scale bar (50 μm): (a) 68.4 g/kg lipid diet; (b) 93.9 g/kg lipid diet; (c) 120.7 g/kg lipid diet; (d) 147.8 g/kg lipid diet; (e) 171.2 g/kg lipid diet.

levels of the dietary lipid, a significant increase in the expressions of *lxr* and *ppary* of the liver was detected at the three stages ($P < 0.05$), while the relative expression of *srebp1* was not altered by diets ($P > 0.05$) (Figures 5(b), 6(b), and 7(b)). The expression of *fas* was not affected by dietary lipid levels in the small and medium turbot ($P > 0.05$), although *fas* expression for the group of L171.2 was significantly higher than that for the groups of L93.9 and L120.7 in the large turbot ($P < 0.05$) (Figures 5(b), 6(b), and 7(b)). For all turbot, relative expression levels of *hnf4α*, *apoB100*, and *mtp* were not affected by dietary lipid levels ($P > 0.05$) (Figures 5(c), 6(c), and 7(c)).

4. Discussion

In the present study, turbot with three different growth stages (initial weights 9.13 g, 50.10 g, and 80.05 g, respectively) were researched. Dietary lipid levels did not affect the survival rate in three stages. For three stages of turbot, however, SGR and WGR were all significantly influenced by dietary lipid levels. Specifically, SGR and WGR increased remarkably initially and then tended to stabilize after the L120.7 group as dietary lipid levels increased. Previous studies also showed that relatively higher lipid levels in diets improved SGR and WGR of fish such as surubim (*Pseudoplatystoma corruscans*) [25], white seabass (*Atractoscion nobilis*) [26], large yellow croaker (*Larimichthys crocea*) [27], and yellow drum (*Nibea albiflora*) [28]. Nevertheless, lower SGR and WGR after excessive dietary lipids were observed in meagre (*Argyrosomus regius*) [29], giant croaker (*Nibea japonica*) [30], Gulf corvina (*Cynoscion othonopterus*) [31], and swimming crab [2], where the differences from this study might be due to different tolerances to high lipids in fish. The results in this study indicated that turbot could tolerate dietary lipid levels up to 171.2 g/kg without significant reduction in SGR and WGR. This study also

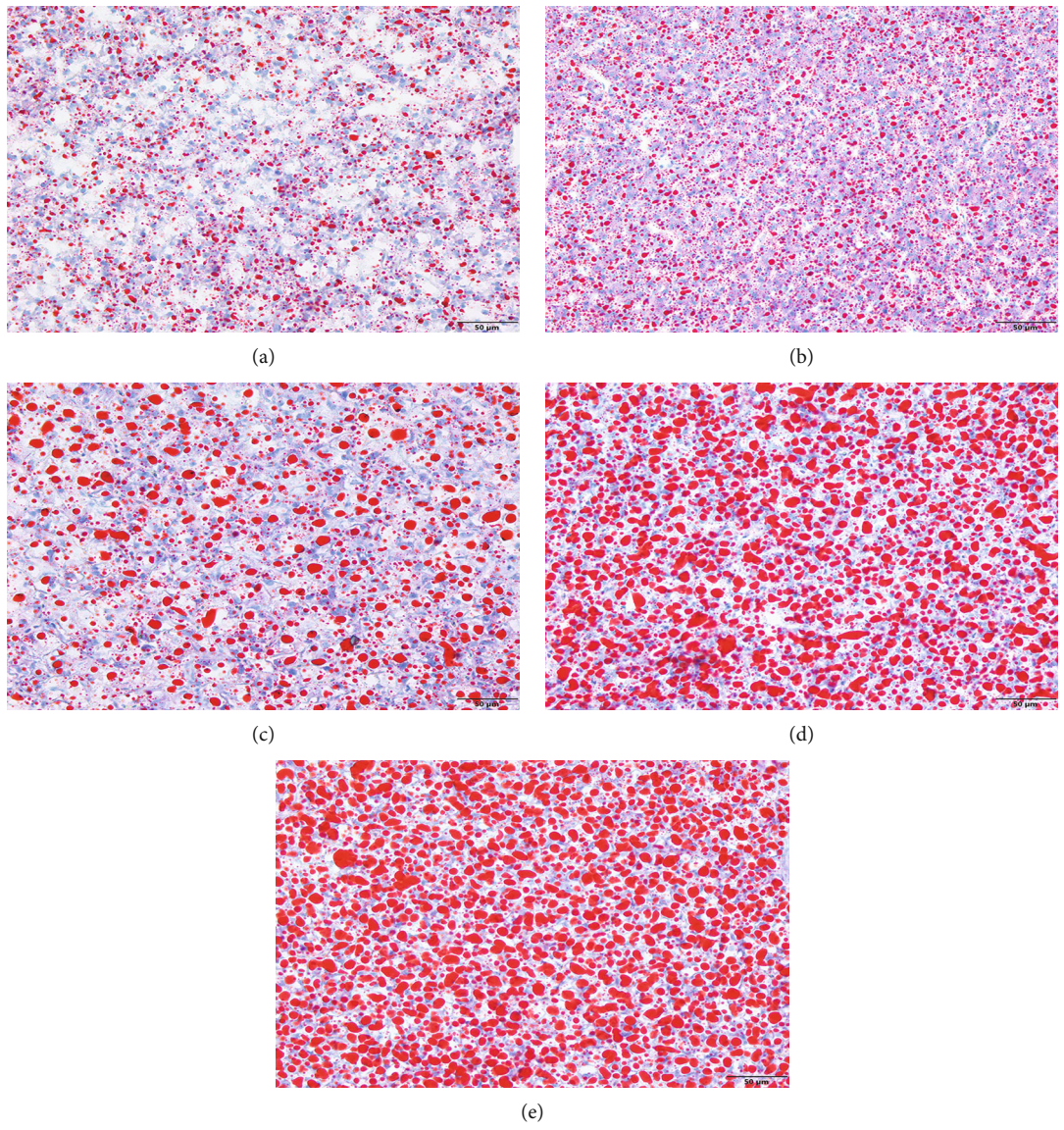

Figure 4: Effects of dietary lipid level on liver lipid deposition of large turbot (Oil Red O staining). Photomicrographs (×400) and scale bar (50 μm): (a) 68.4 g/kg lipid diet; (b) 93.9 g/kg lipid diet; (c) 120.7 g/kg lipid diet; (d) 147.8 g/kg lipid diet; (e) 171.2 g/kg lipid diet.

Table 15: Effects of dietary lipid level on serum biochemical indices of small turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
TG (mmol/L)	4.63 ± 0.25^{b}	5.53 ± 0.25^{b}	5.69 ± 0.30^{b}	8.46 ± 0.36^{a}	9.35 ± 0.50^{a}
TC (mmol/L)	2.17 ± 0.04^{d}	3.05 ± 0.08^{c}	3.10 ± 0.20^{c}	3.90 ± 0.15^{b}	4.78 ± 0.14^{a}
HDL-C (mmol/L)	2.85 ± 0.30^{c}	4.03 ± 0.15^{bc}	5.30 ± 0.17^{a}	4.42 ± 0.26^{ab}	4.50 ± 0.48^{ab}
LDL-C (mmol/L)	1.06 ± 0.13^{c}	1.68 ± 0.12^{bc}	1.92 ± 0.11^{b}	2.18 ± 0.16^{ab}	2.64 ± 0.27^{a}

Abbreviations: HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: total triglyceride; TC: total cholesterol. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 16: Effects of dietary lipid level on serum biochemical indices of medium turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
TG (mmol/L)	2.74 ± 0.29^{c}	2.99 ± 0.23^{c}	4.33 ± 0.22^{b}	4.45 ± 0.24^{b}	5.64 ± 0.25^{a}
TC (mmol/L)	2.23 ± 0.16^{b}	2.34 ± 0.17^{b}	3.40 ± 0.29^{a}	3.80 ± 0.20^{a}	4.00 ± 0.25^{a}
HDL-C (mmol/L)	3.73 ± 0.14^{b}	3.74 ± 0.19^{b}	5.12 ± 0.18^{a}	4.74 ± 0.20^{a}	4.66 ± 0.15^{a}
LDL-C (mmol/L)	1.46 ± 0.14^{b}	1.5 ± 0.17^{b}	2.14 ± 0.15^{ab}	2.97 ± 0.36^{a}	2.39 ± 0.30^{ab}

Abbreviations: HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: total triglyceride; TC: total cholesterol. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

Table 17: Effects of dietary lipid level on serum biochemical indices of large turbot (means ± S.E.M.)[a].

Parameters	Dietary lipid levels (g/kg)				
	68.4	93.9	120.7	147.8	171.2
TG (mmol/L)	2.43 ± 0.14^{b}	2.79 ± 0.12^{b}	2.76 ± 0.17^{b}	3.36 ± 0.30^{b}	4.80 ± 0.33^{a}
TC (mmol/L)	1.96 ± 0.23^{d}	2.01 ± 0.10^{cd}	2.6 ± 0.10^{bc}	2.72 ± 0.14^{b}	3.9 ± 0.16^{a}
HDL-C (mmol/L)	4.23 ± 0.39^{c}	4.59 ± 0.13^{c}	6.67 ± 0.41^{a}	5.90 ± 0.30^{ab}	5.32 ± 0.22^{bc}
LDL-C (mmol/L)	1.69 ± 0.34^{b}	2.34 ± 0.21^{ab}	2.76 ± 0.27^{ab}	2.93 ± 0.32^{a}	2.89 ± 0.13^{a}

Abbreviations: HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: total triglyceride; TC: total cholesterol. [a]Data are given as means ± S.E.M. Values in each row with the same superscript letter or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

demonstrated by the broken-line analysis for SGR that the optimal growth in the three stages of turbot appeared in the diet containing 130.1, 120.1, and 107.7 g/kg lipid, respectively. The smaller stage of fish had a more vigorous metabolism to supply growth, which might partly explain the decreased lipid requirement with the increased sizes of fish in this study [13]. In the present study, FER of the small turbot increased with increasing dietary lipids up to 120.7 g/kg and then decreased to 147.8 g/kg and increased again in the L171.2 group, which was different from the previous finding in adult Nile tilapia (*Oreochromis niloticus*) that dietary lipids markedly increased FER [32]. The possibility of the decrease of FER in small turbot fed the L147.8 diet may be due to weak digestion of excessive lipid intake [33]. For medium turbot, FER of fish fed L68.4 and L93.9 diets were significantly lower than those in the L120.7, L147.8, and L171.2 groups. However, FER was independent of dietary lipids in large turbot, which suggested that FER was not sensitive to the dietary lipids for the large turbot.

Growth is largely affected by intestinal digestion in fish, and digestion of feed relies heavily on the function of digestive enzymes [14, 34, 35]. In general, higher digestive enzyme activities may facilitate feed digestibility and utilization [36]. In the present study, the activity of amylase in the intestine of all turbot did not reveal significant differences among dietary treatments. The result may be due to the poor ability of carnivorous fish to utilize carbohydrate. However, activities of intestinal trypsin and lipase initially rose and then fell in all turbot as the dietary lipid levels increased, suggesting that too low or high dietary lipids could reduce activities of trypsin and lipase in the intestine. Similar findings were obtained in other studies [37, 38], which might partially explain the decreased growth performance in turbot fed the L68.4 diet.

In addition to growth performance, the physiological status has also been a focus of interest in fish nutrition research. One of the key indicators for the physiological status is the antioxidant capacity [39, 40]. T-AOC directly reflected the antioxidant capacity of fish, while CAT and SOD are major antioxidant enzymes, which play an important role in removing reactive oxygen species (ROS), and MDA could indirectly reflect severity of cell impairment and oxidative stresses in fish [40]. In this study, activities of hepatic CAT, SOD, and T-AOC for all fish showed a general trend of first increasing and then decreasing with the elevation of lipid levels; only T-AOC activity in medium turbot showed an insignificant difference. In the liver of small and medium fish, MDA contents decreased at first but then increased with increasing lipid levels, and the lowest MDA content was in the L120.7 group. Past research has shown that the appropriate dietary lipid may enhance the ability to cope with oxidative stress by increasing CAT and SOD activities and decreasing MDA contents in *Onychostoma macrolepis* [6] and Asian red-tailed catfish (*Hemibagrus wyckioides*) [41]. Besides, a study on grass carp (*Ctenopharyngodon idella*) suggested that low or excess dietary lipid levels may induce oxidative stress by producing excessive ROS [42]. These findings were consistent with the results observed in the present study. In addition, MDA content of the large turbot fed with L171.2 diet was significantly higher than that of all other groups. One explanation might be that larger turbot suffered less cell impairment from the low lipid diet than smaller fish. Anyway, according to the results above, excessive lipid levels (171.2 g/kg) could cause oxidative stress in turbot regardless of growth stages.

To further explore the impact of excessive dietary lipid levels (171.2 g/kg) on the physiological status of turbot, the current study detected lipid deposition in the whole body,

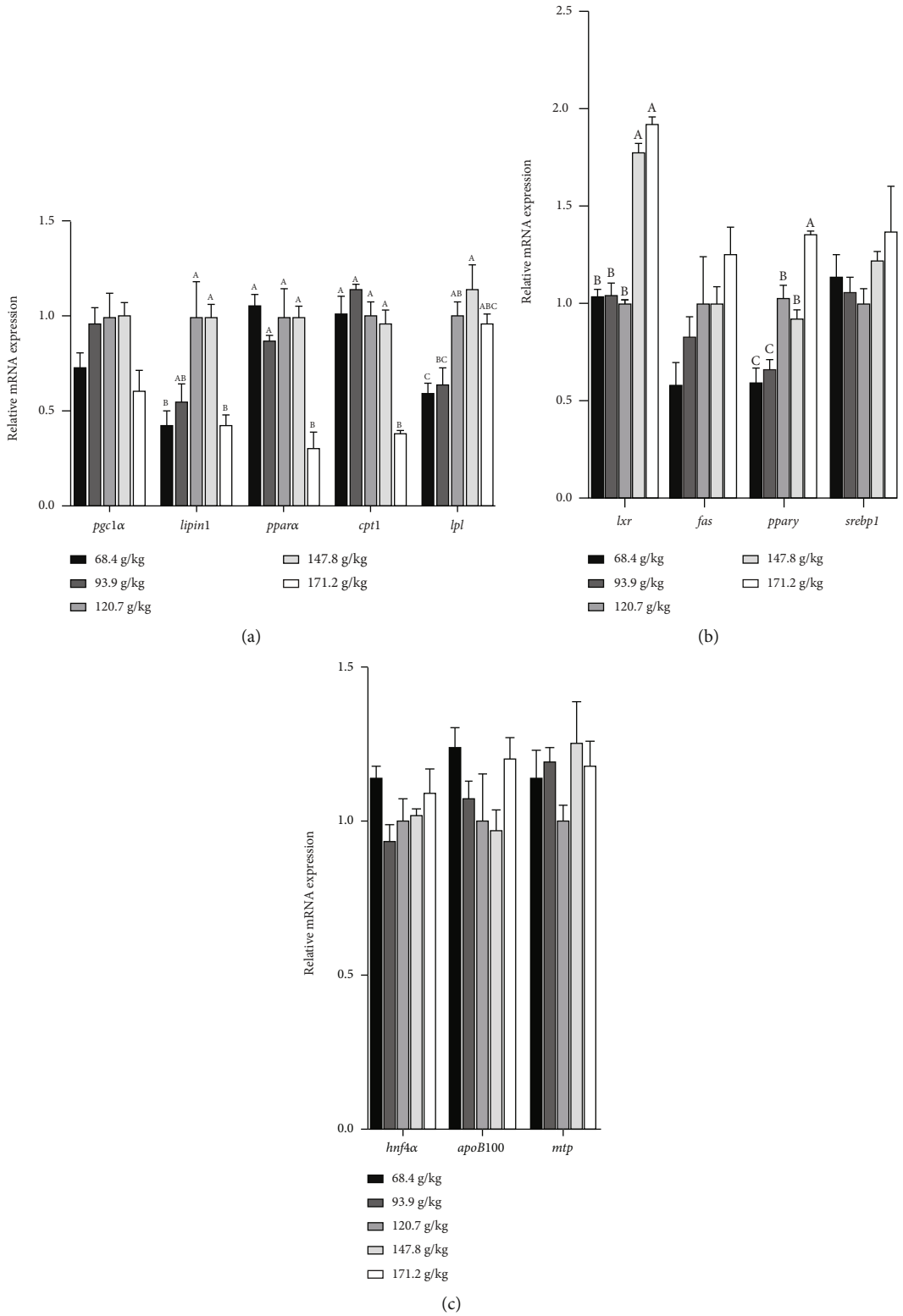

Figure 5: Effects of dietary lipid level on relative mRNA expression of lipolysis-related genes (*pgc1α*, *lipin1*, *pparα*, *cpt1*, and *lpl*) (a), lipogenesis-related genes (*lxr*, *fas*, *pparγ*, and *srebp1*) (b), and lipid transport-related genes (*hnf4α*, *apoB100*, and *mtp*) (c) in the liver of small turbot. *Pgc1α*: peroxisome proliferator-activated receptor-*γ* coactivator 1*α*; *pparα*: peroxisome proliferator-activated receptor *α*; *cpt1*: carnitine palmitoyl transferase 1; *lpl*: lipoprotein lipase; *lxr*: liver X receptor; *fas*: fatty acid synthase; *pparγ*: peroxisome proliferator-activated receptor *γ*; *srebp1*: sterol-regulatory element binding protein-1; *hnf4α*: hepatocyte nuclear factor 4*α*; *apoB100*: apolipoprotein B100; *mtp*: microsomal TAG transfer protein. Data are given as means ± S.E.M. Columns sharing the same letters or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

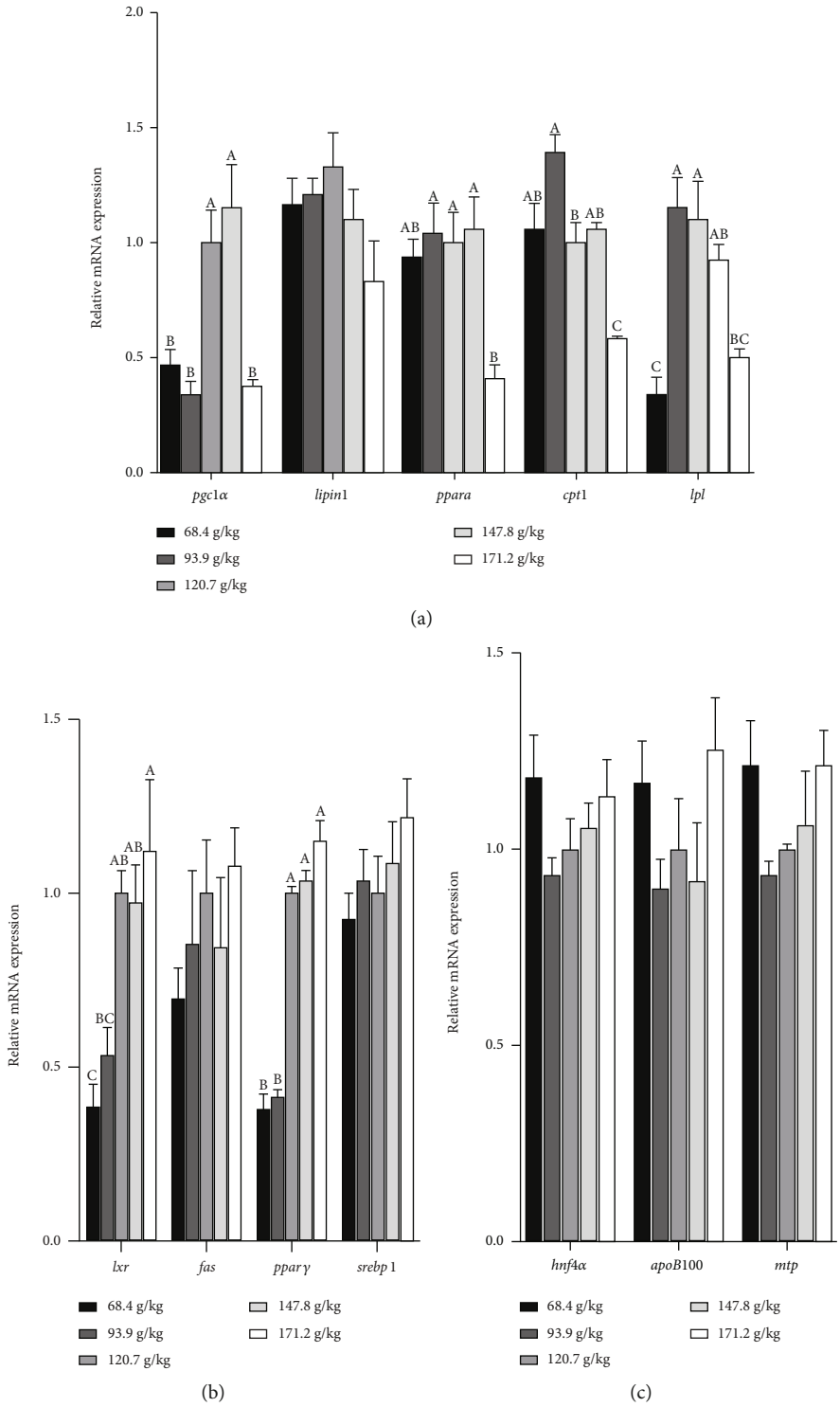

Figure 6: Effects of dietary lipid level on relative mRNA expression of lipolysis-related genes (*pgc1α*, *lipin1*, *pparα*, *cpt1*, and *lpl*) (a), lipogenesis-related genes (*lxr*, *fas*, *pparγ*, and *srebp1*) (b), and lipid transport-related genes (*hnf4α*, *apoB100*, and *mtp*) (c) in the liver of medium turbot. *Pgc1α*: peroxisome proliferator-activated receptor-*γ* coactivator 1*α*; *pparα*: peroxisome proliferator-activated receptor *α*; *cpt1*: carnitine palmitoyl transferase 1; *lpl*: lipoprotein lipase; *lxr*: liver X receptor; *fas*: fatty acid synthase; *pparγ*: peroxisome proliferator-activated receptor *γ*; *srebp1*: sterol-regulatory element binding protein-1; *hnf4α*: hepatocyte nuclear factor 4*α*; *apoB100*: apolipoprotein B100; *mtp*: microsomal TAG transfer protein. Data are given as means ± S.E.M. Columns sharing the same letters or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

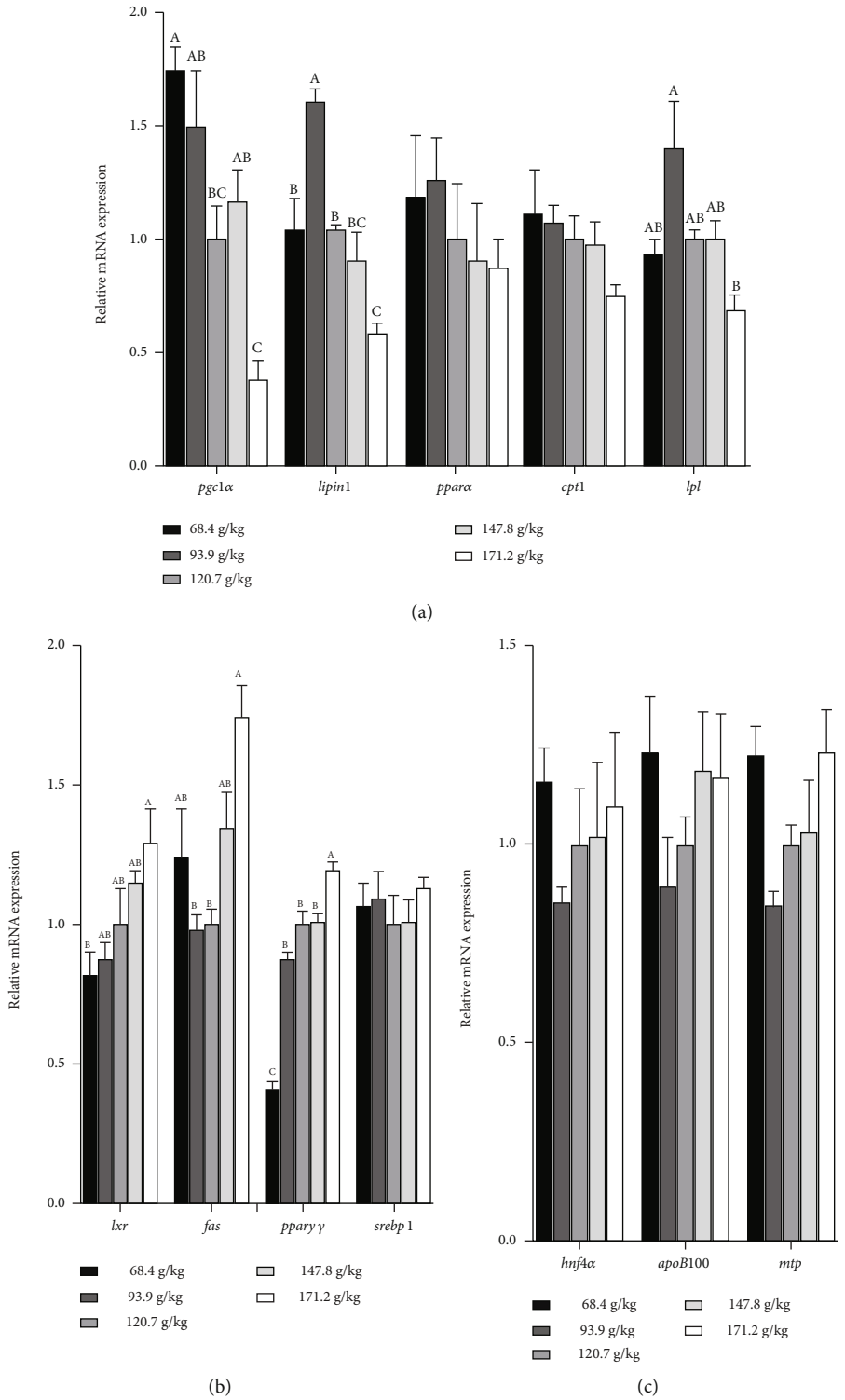

Figure 7: Effects of dietary lipid level on relative mRNA expression of lipolysis-related genes (*pgc1α*, *lipin1*, *pparα*, *cpt1*, and *lpl*) (a), lipogenesis-related genes (*lxr*, *fas*, *pparγ*, and *srebp1*) (b), and lipid transport-related genes (*hnf4α*, *apoB100*, and *mtp*) (c) in the liver of large turbot. *Pgc1α*: peroxisome proliferator-activated receptor-*γ* coactivator 1*α*; *pparα*: peroxisome proliferator-activated receptor *α*; *cpt1*: carnitine palmitoyl transferase 1; *lpl*: lipoprotein lipase; *lxr*: liver X receptor; *fas*: fatty acid synthase; *pparγ*: peroxisome proliferator-activated receptor *γ*; *srebp1*: sterol-regulatory element binding protein-1; *hnf4α*: hepatocyte nuclear factor 4*α*; *apoB100*: apolipoprotein B100; *mtp*: microsomal TAG transfer protein. Data are given as means ± S.E.M. Columns sharing the same letters or absence of superscript letters are not significantly different as determined by Tukey's test ($P > 0.05$). S.E.M.: standard error of means.

liver, and muscle. Lipid contents of whole fish and liver increased as dietary lipid levels increased at three stages, and the lipid contents in the L171.2 group were markedly higher than those in other groups, which was consistent with results reported in tiger puffer (*Takifugu rubripes*) [43], darkbarbel catfish (*Pelteobagrus vachelli*) [15], marbled spinefoot rabbitfish (*Siganus rivulatus*) [44], and common carp (*Cyprinus carpio*) [45]. Consistently, hepatic Oil Red O staining in all turbot showed that the size or number of lipid droplets increased with the increasing dietary lipid levels, demonstrating that dietary lipids increased lipid droplets in the liver at microscopic levels. As lipid droplets are the main form of triglyceride storage in hepatocytes, the finding suggested that excessive dietary lipids increased liver triglyceride content. However, the lipid contents in the muscle were not affected by the dietary lipid levels in this study, in line with a previous study on chu's croaker (*Nibea coibor*) [46]. The results may be because turbot is the "lean" fish species, the muscle lipid contents of which were only around 10 g/kg of wet weight [20, 47]. Therefore, dietary lipid levels had no significant effect on muscle lipid contents of turbot. In this study, excessive dietary lipids not only did not improve growth performance (SGR and WGR) of turbot but also might cause abnormal lipid deposition in the liver.

Serum biochemical parameters were useful to reflect physiological status and could be affected by alterations in dietary nutrition ingredients [6, 48]. Serum lipid indexes were detected to investigate further effects of dietary lipids on lipid deposition in turbot. For all turbot, increased TG, TC, and LDL-C contents in serum were obtained with the elevated dietary lipid levels, indicating increasing serum lipid levels in turbot fed with the higher lipids, which is consistent with results in grass carp [49], chu's croaker [46], snakehead (*Channa argus*×*Channa maculata*) [50], and largemouth bass [51]. However, results in all turbot suggested that serum HDL-C content firstly increased and then decreased with the increasing dietary lipid levels, and the maximum was reached in the L120.7 group, which agrees well with the finding in Nile tilapia [32]. HDL-C is primarily responsible for the reverse transport of cholesterol [52], and the result demonstrated an active lipid transport in turbot fed the L120.7 diet.

To gain additional insight into the regulation of lipid metabolism by dietary lipids in the liver, mRNA expression analyses related to lipid metabolism were conducted. *Pgc1α* has been shown to promote mitochondrial *β*-oxidation [39, 53]. In this study, relative expression of *pgc1α* was significantly lower in medium and large turbot fed diets with L171.2 than those fed L120.7 diets. In addition, there was a tendency of increasing and then decreasing in *pgc1α* expression in small fish, although this was not statistically significant. *Lpl* can mediate the uptake of mobilized fatty acids to the liver from extrahepatic tissues [23]. *Lipin1* activates mitochondrial lipid oxidation and reduces lipid levels in the liver [54]. In this study, expressions of hepatic *lipin1* and *lpl* in all turbot were significantly upregulated firstly and subsequently downregulated as dietary lipid levels increased except *lipin1* in medium fish. This trend was consistent with findings obtained in orange-spotted grouper and mammals [5, 55]. Past study showed that n-3 PUFA in fish oil increase *lpl* expression in adipose tissue of mammals [56], which might be part of the reason for the lower *lpl* expression in turbot fed the L171.2 diet, but further research is required. Studies have shown that activation of *pparα* could promote fatty acid *β*-oxidation by promoting the expression of *cpt1*, which is the rate-limiting enzyme for fatty acid oxidation [57, 58]. For small and medium turbot, expressions of *pparα* and *cpt1* in the L171.2 group were significantly lower than that in other groups, similar to a previous study in *Trachinotus ovatus*, demonstrating that excessive lipid intake might reduce hepatic lipolysis by the *pparα-cpt1* signal pathway [59]. Nevertheless, experimental diets had no significant effect on expressions of *pparα* and *cpt1* in large turbot, which might be due to the greater tolerance to high lipids of the large turbot.

In addition to lipolysis, lipogenesis also plays an important role in hepatic lipid deposition [23, 60]. A significant increase in expressions of *lxr* and *pparγ* in all turbot was detected as dietary lipid levels increased, which could be interpreted if excess free fatty acids from diets enter the liver. Lipogenic gene expression could be upregulated in the liver to maintain lipid homeostasis under this circumstance [61]. In this study, the expression of *srebp1* was not altered by diets in all turbot, which may be because the expression of *srebp1* was changed at the posttranslational level [23]. In addition, the expression of *fas* was not affected by dietary lipids in small and medium turbot, but *fas* expression for the L171.2 group was significantly higher than those in L93.9 and L120.7 groups in large turbot, consistent with the result of He et al. [62] that the fas expression level in Nile tilapia was increased by high lipid level diets. However, some studies have shown that lipogenesis genes including fas were downregulated as dietary lipid levels increased [2, 32, 63], which can be explained as an adaptive mechanism to maintain lipid homeostasis by regulating endogenous lipid synthesis [61]. The differences in these results may arise from the discrepancy in lipid tolerance in different fish species, which leads to the differences in the lipid metabolism of fish. Collectively, excessive dietary lipids may lead to lipid deposition in turbot by suppressing gene expressions for hepatic lipolysis while enhancing gene expressions for hepatic lipogenesis. However, further studies are needed to determine the specific signal pathways through which high lipid affects lipid deposition in turbot, in addition to whether there are differences in the mechanisms by which high lipids affect lipid deposition in turbot at different growth stages.

5. Conclusion

To conclude, the results of the current study suggested that based on SGR, the diet containing lipid levels of 130.1, 120.1, and 107.7 g/kg may be optimal for growth performance of turbot cultured at three growth phases, respectively. The low lipid diets may negatively affect the growth performance of turbot by reducing digestive enzyme activity and antioxidant capacity. The high lipid diets, on the other hand, would lead to abnormal lipid deposition and affect the physiological health of turbot, which might affect the growth performance of turbot in a long term.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

In the present study, all experimental procedures performed on turbot care were in strict accordance with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Mai Kangsen, Ai Qinghui, and Zhang Wencong designed the research; Zhang Wencong, Zhuang Yanwen, and Zheng Jichang conducted the research; Zhang Wencong and Dan Zhijie analyzed the data; Zhang Wencong wrote the manuscript; Gong Ye and Liu Yongtao provided experimental assistant and language help. All authors reviewed and approved the final manuscript.

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