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Research Article

Influence of Dietary Inositol Supplementation on Growth, Liver Histology, Lipid Metabolism, and Related Genes Expression on Juvenile Hybrid Grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatu*) Fed High-Lipid Diets

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An 8-week feeding trial was conducted to investigate the effects of dietary inositol on growth performance, lipid metabolism, and expression of lipid related genes of hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatu*) (initial body weight 6.76 ± 0.34 g). Six isonitrogenous and isolipidic (16.35%) diets were formulated to contain graded levels of inositol (0.17 g/kg (J1), 0.62 g/kg (J2), 1.03 g/kg (J3), 1.78 g/kg (J4), 3.43 g/kg (J5), and 6.59 g/kg (J6)). The results indicated that (a) weight gain rate appeared gradually increasing trend and significant increase in four inositol treatments (J3, J4, J5, and J6) ($P < 0.05$). Protein efficiency ratio was slightly improved in the J4 group ($P < 0.05$), but there was no significant difference exhibiting in survival rate between the inositol-treated groups and the control group ($P > 0.05$). (b) In serum, dietary inositol significantly reduced the contents of triglyceride (TG), total cholesterol (TC), low-density lipoprotein, and clearly increased the content of high-density lipoprotein ($P < 0.05$). In the liver, dietary inositol significantly decreased contents of TG, TC, and very low-density lipoprotein ($P < 0.05$). The activities of lipoprotein lipase, hepatic lipase, and adipose triglyceride lipase were slightly improved in the inositol-treated groups ($P < 0.05$). (c) With increasing of inositol supplement, decreasing of lipid droplet could be observed in hepatocyte. (d) In the liver, dietary inositol significantly downregulated the mRNA levels of fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), 6-phosphogluconate dehydrogenase (6PGD), and sterol regulatory element-binding protein-1 (SREBP1) ($P < 0.05$), while it remarkably upregulated the mRNA levels of lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), and carnitine palmitoyl transferase1 (CPT1) ($P < 0.05$). In summary, dietary inositol improved growth and reduced lipid composition in the liver of hybrid grouper through decreasing of fatty acid synthesis and increasing of lipid catabolism. Under the experimental conditions, broken-line regression analysis showed that 1.23~1.67 g/kg dietary inositol was recommended in juvenile grouper high-lipid diets.

1. Introduction

As an energy-dense macronutrient, lipid can provide energy and significant substances such as essential fatty acid (FA),

lipid-soluble vitamins, phospholipids, and sterols for maintaining life and cell normal structure and biological function [1, 2]. Recently, as the increasing cost and limited supplies of

fish meal, high-lipid diets are extensively used in numerous economic fish species to acquire protein-sparing effect and the higher growth performance of fish [3, 4]. However, superfluous lipid in diet has negative effects on fish on account of abnormal lipid accumulation in the liver, abdominal adipose tissues, and muscle [5, 6]. Furthermore, fish with a long term lipid excess intake appeared reactive oxygen species production, oxidative stress, and inflammation, and it is apparently harmful to the growth and health of fish [7–10].

Inositol, a natural occurring biologically active isomer, is widely distributed in plants and animals, and it participates in the structure of biological membranes in the form of phospholipid. Phosphatidylinositol plays important biochemical functions to regulate cellular response to external stimuli, neurotransmission, and enzyme activity through specific interactions with various proteins [11, 12]. Inositol is classified as a vitamin-like nutrient and supplemented to fish diets, because it can improve the efficiency of feed, promote lipid metabolism in the liver and other tissues, and accelerate the growth of fish [13–15]. Previous studies demonstrated that dietary inositol was required in most fish and shrimp, and there were pernicious symptoms including less protein deposition, poor growth, dark skin coloration, and high accumulation of lipid in the liver when inositol was deficient in diets [11, 16–18]. However, there are few studies of inositol application in the high-lipid diets to promote the normal lipid metabolism of fish, prevent the accumulation of lipid in tissues, and make fish in a good state of growth.

Hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatus*) is commercially significant cultivated in Chinese southeast coast because of its rapid growth, strong disease resistance, high nutritional value as well as economic value, and thus it has become one of the most important species in China aquacultural markets [19, 20]. Currently, numerous researches of hybrid grouper on the nutrition was mainly reported on requirement of dietary protein and lipid level, protein to energy ratio, amino acid patterns, requirement of arginine and isoleucine, addition amount of corn starch, etc. [21–26]. Moreover, there are also many studies reporting that the substitution by nonfish meal protein sources (soy protein, soybean meal, peanut meal, etc.) and oil sources (corn oil, sunflower oil, tea oil, etc.) for hybrid grouper [20, 27, 28]. However, as a shift from natural to artificial diets occurs, lipid deposition in grouper is a frequent finding. Lipid deposition affects disease resistance and the quality of harvest, especially in high-lipid diets [29]. The research on mitigating negative effects is limited. Therefore, this study aimed to investigate effect of dietary inositol levels on growth performance and lipid metabolism of hybrid grouper fed high-lipid diets.

2. Material and Methods

2.1. Experimental Diets.
Six isonitrogenous (50%) and isolipidic (16%) experimental diets with 0.17, 0.62, 1.03, 1.78, 3.43, and 6.59 g/kg inositol (named J1 (as control), J2, J3, J4, J5, and J6, respectively) were produced as shown in Table 1. All of crushed raw materials were sieved through a sixty mesh sieve and thoroughly mixed by the progressive enlargement method according to the way described by Ayisi and Zhao [30]. After adding oil and lecithin, it was ensuring to make them mixed thoroughly by rubbing the ingredients manually and putting them into V-type vertical mixer, and then distilled water (200–300 ml/kg) was added to them. The mixed raw materials were processed into 2.0 mm diameter pellets by a twin screw extruder (F–26, South China University of Technology, Guangdong Province, China). The pellets were air-dried to about 100 g/kg of moisture content at room temperature and were stored in a refrigerator at −20°C until being used.

2.2. Fish and Feeding Trial.
All animal experiments were conducted strictly based on the recommendations in the "Guide for the Care and Use of Laboratory Animals" set by the National Institutes of Health. The animal protocols were approved by the Animal Ethics Committee of Guangdong Ocean University (Zhanjiang, China). Hybrid groupers (♀ *Epinephelus fuscoguttatus* × ♂ *E. lanceolatus*) were provided by a local hatchery from Donghai Island (Zhanjiang, China) and domesticated to the experimental conditions at the Donghai Island Marine Biological Research Base of Guangdong Ocean University for one week. Before the form trial, fish were fed a commercial diet (Haida Aquatic Diet Co. Ltd., Zhanjiang, China). After fasting for 24 h, 540 fish (initial body weight = 6.76 ± 0.34 g) were randomly assigned to 18 tanks with 1.4 m deep and holding 1 m^3 seawater. Each diet was fed to triplicate tanks of fish and each tank has 30 fish. The fish were fed two times (08 : 30 and 16 : 30) daily to obvious satiation for eight weeks, and the amount of food ingestion was recorded daily. There was one piece of polyvinylchloride (PVC) pipe of 20.0 cm (diameter) ×30.0 cm (length) provided for each tank as shelter for the fish [31]. During the whole experiment, the temperature of the water was raged from 29 to 32°C, salinity was 28, dissolved oxygen was >7.1 mg/L, and total ammonia nitrogen was around 0.05 mg/L.

2.3. Sample Collection.
The fish fasted for 24 h at the end of 8 weeks period, and then the fish were counted and weighted collectively. After that, four fish from each tank were randomly selected for blood collection by 1 ml sterile syringes. Blood was placed in 1.5 ml microcentrifuge tubes and stored at 4°C for 12 h, and then it was freeze centrifuged at 3000 × g for 10 min to collect supernatant which should be immediately frozen and stored at −80°C before the analysis. Aimed to analyze enzyme activity, the liver of another three fish were sampled. At last, the livers from three of rest fish in each tank were immediately separated and collected in 2 ml enzyme-free centrifuge tubes with RNA later, then were stored at –80°C to analyze relative gene expression.

2.4. Methods of Analysis.
In serum, contents of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL); in the liver, activities of lipoprotein lipase (LPL), fatty acid synthetase (FAS), hepatic lipase (HL) and adipose triglyceride lipase (ATGL), and contents of very low-density lipoprotein (VLDL), total

TABLE 1: Composition of ingredient and nutrients levels of the test diets (g/kg).

Ingredients	Test diets					
	J1	J2	J3	J4	J5	J6
Fish meal	420.00	420.00	420.00	420.00	420.00	420.00
Wheat gluten	120.00	120.00	120.00	120.00	120.00	120.00
Casein	150.00	150.00	150.00	150.00	150.00	150.00
Wheat flour	135.00	135.00	135.00	135.00	135.00	135.00
Soybean lecithin	15.00	15.00	15.00	15.00	15.00	15.00
Fish oil	50.00	50.00	50.00	50.00	50.00	50.00
Corn oil	70.00	70.00	70.00	70.00	70.00	70.00
Starch	7.00	6.60	6.20	5.40	3.80	0.60
Compound premix*	10.00	10.00	10.00	10.00	10.00	10.00
Vitamin C	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride	5.00	5.00	5.00	5.00	5.00	5.00
Monocalcium phosphate	15.00	15.00	15.00	15.00	15.00	15.00
Antioxidant	1.00	1.00	1.00	1.00	1.00	1.00
Attractant	1.50	1.50	1.50	1.50	1.50	1.50
Inositol	0.00	0.40	0.80	1.60	3.20	6.40
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Proximate composition						
Crude protein	496.31	496.31	496.31	496.31	496.31	496.31
Crude lipid	163.55	163.55	163.55	163.55	163.55	163.55
Actual inositol content	0.17	0.62	1.03	1.78	3.43	6.59

*Compound premix (without inositol) provided by Qingdao Master Biotechnology Co: Ltd (Qingdao: China).

cholesterol (TC), and triglyceride (TG) were analyzed by commercial ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). The specific testing process strictly follows the steps of the instruction.

2.5. RNA Extraction and cDNA Synthesis. After grinding the samples thoroughly, 1 ml TransZol UP (TransGen Biotech, Beijing, China) of 1 ml was added to the tubes and according to the steps provided by manufacturer, total RNA was extracted and its concentration as well as quality were detected at 260 and 280 nm by a NanoDrop 2000 spectrophotometer (Gene Company Limited, Guangzhou, China). Then the RNA was also verified by electrophoresis in 1% agarose gel. Qualified RNA was used to synthesize the first-strand cDNA through PrimeScript™ RT Reagent Kits with cDNA Eraser (Takara, Japan). When it finished, the cDNA should be kept at −20°C.

2.6. Real-time Quantitative Polymerase Chain Reaction (RT-qPCR). The PCR primers including FAS, ACC, 6GPD, apolipoprotein B100 (ApoB100), LPL, HSL, CPT1, SREBP1, PPARα, and PPARβ were designed by Primer premier 5.0 (Table 2). The housekeeping gene β-actin was used as the reference gene. Standard curves were prepared using five different concentrations of cDNA and amplification efficiency of all primers was calculated by using the formula: $E = 10^{(-1/\text{slope})} -1$. Ensure a large 95% amplification efficiency for each primer. The mRNA expression levels of each sample were assayed by RT-qPCR under conditions at 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 34 s. Threshold

cycle (Ct) values were collected from each sample after finishing the process. The relative mRNA expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method [32].

2.7. Statistical Analysis. All the results were examined for homogeneity of variance using SPSS version 21.0 (SPSS Inc., USA) and expressed as means ± standard error. One-way ANOVA and Duncan's multiple range test were used to determine the significant differences among distinct treatments while probability values of $P < 0.05$ were deemed to be statistically significant.

3. Result

3.1. Growth Performance. During the 8-week feeding trial, the grouper appeared better growth performance, increasing from 6.75 g to 32.46~41.12 g as shown in Table 3. Weight gain rate (WGR) of the trial groups except for the J2 group was significantly higher than the control ($P < 0.05$), and the highest of it was shown in the J6 group. Based on WGR, broken-line regression (Figure 1) analysis showed that 1.67 g/kg dietary inositol was optimum level in high-lipid diets. The survival rate (SR) of grouper was not affected by the addition of inositol in high-lipid diets ($P > 0.05$). It decreased firstly increased with the increase of inositol level but increased in the J4 group and continued to reduce at last, and the highest SR appeared in the J4 group. Only protein efficiency ratio (PER) of the J4 group, the highest PER, was

TABLE 2: Primers used RT-qPCR.

Primer names	Forward and reverse primers sequence (5′ to 3′)	GenBank accession no.
FAS-F/R	GGCGGCATTGTAGGCATTA/CAATCAAAGTGTAGCCTCGGTAG	FJ196231.1
ACC-F/R	CTCAGCAAGACGACCAACGC/GGCAGCCATCCTGACAACCT	KX066238.1
6GPD-F/R	AGGCTGCATCATCCGAAGTGT/CAGCATACCGTGTCTGTAACCA	KX066237.1
ApoB100-F/R	ACCACATCCTCATTCCCTTCT/CATCTTTATCCTGGACAACACTCT	KM593126.1
LPL-F/R	CCACCTGTTCATCGACTCCC/TCGGACGGACCTTGTTGAT	EU683732.1
HSL-F/R	TGCTCCGAGATTTCCGACAG/AGGATGCCTCCGAAATGC	KF049203.1
CPT-1-F/R	TGCTCCACGGAAAGTGCTAC/GCAGTGACCCTCCTCAGTGTAT	HM037343.1
SREBP1-F/R	TGGAGTTTGGAGGACTGTTTG/AAGATGAGGGTGGAGTTGGA	KU179485.1
PPARα-F/R	AAAAGCACGGCTCATACTCAC/GCTCTGGCAGCAGTGGAA	FJ196235.1
PPARβ-F/R	GCTACAGAGCAGCACGACA/CTCCTCATCTTCGCTTTCC	DQ232867.1
β-Actin–F/R	ACTGCTGCCTCCTCTTCATC/ACCGCAAGACTCCATACCAA	KU746361.1

The amplification efficiency of all primers was higher than 95%. FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; 6GPD: 6-phosphogluconate dehydrogenase; Apo B100: apolipoprotein B100; LPL: lipoprotein lipase; HSL: hormone-sensitive lipase; CPT1: carnitine palmitoyl transferase; SREBP1: sterol regulatory element-binding protein-1; PPARα: peroxisome proliferator-activated receptor α; and PPARβ: peroxisome proliferator-activated receptor β.

TABLE 3: Effect of dietary inositol on growth performance of hybrid grouper fed high-lipid diets.

Diets	IBW(g)	FBW(g)	WGR (%)	SR (%)	PER (%)
J1	6.77 ± 0.04	32.46 ± 0.71^a	379.76 ± 10.42^a	96.00 ± 2.31	1.76 ± 0.02^{ab}
J2	6.76 ± 0.04	34.20 ± 0.53^{ab}	406.02 ± 7.86^{ab}	88.00 ± 4.00	1.73 ± 0.07^{ab}
J3	6.75 ± 0.06	36.81 ± 2.85^{abc}	445.48 ± 42.14^{bc}	82.00 ± 2.00	1.68 ± 0.23^a
J4	6.77 ± 0.04	35.73 ± 5.27^{abc}	494.97 ± 10.42^c	96.00 ± 4.00	2.03 ± 0.02^c
J5	6.76 ± 0.06	40.06 ± 0.34^{bc}	492.69 ± 5.12^c	84.00 ± 4.00	1.99 ± 0.10^{bc}
J6	6.75 ± 0.07	41.12 ± 1.96^c	509.13 ± 29.12^c	85.33 ± 10.67	1.83 ± 0.16^{abc}

The data of the table is expressed by mean average value ± standard error ($n = 3$). The significant difference is exhibited in the same column with different superscript letters ($P < 0.05$). IBW(g): initial mean body weight; FBW(g): final mean body weight; WGR (weight gain rate: %) = $100 \times$ (final weight-initial weight)/initial weight; SR (survival rate: %) = $100\% \times$ (total number of fish at termination/total number of fish stocked); PER (protein efficiency ratio: %) = $100 \times$ average weight gain/average protein intake.
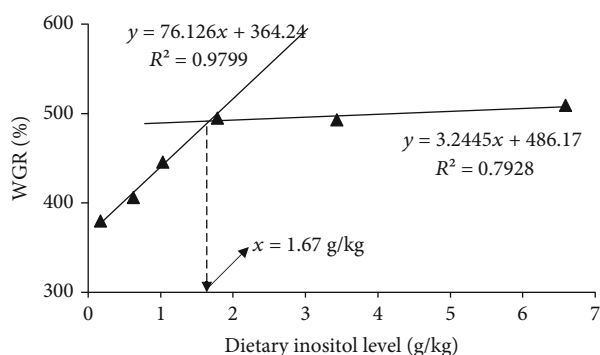

FIGURE 1: Broken-line analysis of the relationship between dietary inositol level and weight gain rate (WGR) of hybrid grouper. The appropriate dietary inositol level in high-lipid diets was 1.67 g/kg.

significantly higher than the control group ($P < 0.05$), while the other groups were not significantly difference with it.

3.2. Muscle and Whole Body Composition. In Table 4, it was obvious that moisture, lipid, and protein contents of muscle were no significant difference between the inositol-treated groups and control group ($P > 0.05$). In whole body, mois-ture, lipid, and protein contents of inositol-treated groups had no remarkable difference with the control group ($P > 0.05$).

3.3. Biochemical Indices in Serum. Shown in Table 5, TG content of the treat groups expect the J2 group was significantly lower than that in the control group ($P < 0.05$) and there was no significant difference in inositol-treated groups ($P > 0.05$). TC content showed a trend of first decrease and then increase, and the lowest value of it appeared in the J4 group, whose TC content was obviously lower than that of the control group ($P < 0.05$). TC content of the rest inositol-treated groups showed no significant difference with that of the control group ($P > 0.05$). The content of HDL increased first and then decreased, and HDL content of J4 group was significantly higher than that of the control group ($P < 0.05$) while the rest inositol-treated groups showed no difference with the control group ($P > 0.05$). The LDL content of J5 and J6 groups was obviously higher than that in others group ($P < 0.05$), and no significant difference was found among J1, J2, J3, and J4 ($P > 0.05$). Based on TC and HDL, broken-line regression (Figures 2 and 3) analysis

TABLE 4: Effect of dietary inositol on muscle and whole body composition (% wet weight) of hybrid grouper fed high-lipid diets.

Diets	Muscle			Whole body		
	Moisture (%)	Lipid (%)	Protein (%)	Moisture (%)	Lipid (%)	Protein (%)
J1	76.33 ± 0.44	1.67 ± 0.02^{ab}	19.79 ± 0.16^{ab}	72.23 ± 0.53	6.65 ± 0.21	16.92 ± 0.06
J2	76.92 ± 0.15	2.01 ± 0.00^{b}	19.26 ± 0.10^{a}	72.95 ± 0.45	6.63 ± 0.31	16.47 ± 0.13
J3	76.29 ± 0.46	1.99 ± 0.00^{b}	19.64 ± 0.16^{ab}	71.61 ± 0.72	7.14 ± 0.19	16.73 ± 0.31
J4	76.35 ± 0.31	1.70 ± 0.02^{ab}	19.94 ± 0.15^{ab}	73.09 ± 0.14	6.40 ± 0.06	16.61 ± 0.32
J5	76.25 ± 0.32	1.87 ± 0.02^{ab}	19.99 ± 0.38^{b}	72.00 ± 0.51	6.82 ± 0.36	16.69 ± 0.07
J6	77.86 ± 0.91	1.53 ± 0.15^{a}	19.42 ± 0.23^{ab}	71.59 ± 0.54	7.15 ± 0.19	17.07 ± 0.04

The data of the table is expressed by mean average value ± standard error ($n = 3$). The significant difference is exhibited in the same column with different superscript letters ($P < 0.05$).

TABLE 5: Effect of dietary inositol on biochemical indices in serum of hybrid grouper fed high-lipid diets.

Diets	TG (mmol/L)	TC (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
J1	0.25 ± 0.06^{b}	2.45 ± 0.32^{bc}	2.00 ± 0.02^{a}	0.38 ± 0.05^{a}
J2	0.20 ± 0.01^{ab}	1.94 ± 0.22^{ab}	2.31 ± 0.11^{ab}	0.33 ± 0.01^{a}
J3	0.18 ± 0.02^{a}	1.66 ± 0.31^{ab}	2.46 ± 0.09^{ab}	0.34 ± 0.04^{a}
J4	0.16 ± 0.03^{a}	1.36 ± 0.18^{a}	2.74 ± 0.25^{b}	0.29 ± 0.03^{a}
J5	0.18 ± 0.01^{a}	2.45 ± 0.08^{bc}	2.48 ± 0.14^{ab}	1.18 ± 0.06^{b}
J6	0.18 ± 0.00^{a}	2.89 ± 0.12^{c}	2.08 ± 0.20^{ab}	1.41 ± 0.11^{c}

The data of the table is expressed by mean average value ± standard error ($n = 3$). The significant difference is exhibited in the same column with different superscript letters ($P < 0.05$). TG: triglyceride; TC: total cholesterol; HDL: high-density lipoprotein; and LDL: low-density lipoprotein.

showed that optimal inositol levels were 1.23 g/kg and 1.54 g/kg, respectively.

3.4. Enzyme Activities and Biochemical Indices in the Liver. The results of lipid metabolism enzymes activities were shown in Table 6. It is obvious that the value of LPL of the trial groups except the J2 group was significantly higher than the control group ($P < 0.05$), and the highest value of it appeared in the J4 group. ATGL level with J4 group owing the highest value appeared the trend increasing firstly, then reducing at last, while the opposite trend was found in FAS activity which primarily induced and raised latterly. The former's level of the trial groups except the J2 group was prominently higher than the control ($P < 0.05$) and value of the latter among the J3, J4, J5, and J6 groups was significantly lower than the control ($P < 0.05$). Compared with the control, HL level of all the experimental groups was significantly higher ($P < 0.05$) while VLDL content of them was significantly lower ($P < 0.05$). The contents of TC and TG appeared significant decrease in the inositol-treated groups compared with the control group ($P < 0.05$).

3.5. Histological Structure of the Liver. In the J1 group without inositol supplement, extensive and dense large particles lipid droplet (LD) could be observed in hepatocyte of groupers fed high-lipid diets (Figure 4). Compared with the control group, there were relatively few small particles LD but still a lot of large particles of it in the liver of the J2 group. However, it's obvious that dramatically less LD appeared among J3, J4, J5, and J6 groups. In addition, when inositol level was over 1.78 g/kg (J4 group), the number of LD gradually increased but the particles of it was not obvious change.

3.6. Relative Genes Expression of Lipid Metabolism in the Liver. The expression of genes related lipid metabolism was shown in Figure 5. FAS and SREBP1 expression levels in inositol-treated groups showed significant downregulation compared with the control group ($P < 0.05$). ACC and 6GPD expression levels in the J3, J4, J5, and J6 groups were remarkably lower than that of the control group ($P < 0.05$), while CPT1 expression level in same groups was significantly higher than that of the control group ($P < 0.05$). ApoB100 expression level showed no difference in various groups ($P > 0.05$). Compared with the control group, the J3 and J4 groups showed distinct upregulation of LPL mRNA expression level ($P < 0.05$), but the rest inositol-treated groups were not significantly different with the control ($P < 0.05$). As shown in Figure 5, it was evident that HSL expression level in the control group was not significantly different with the inositol-treated groups expect the J4 group which appeared remarkably upregulated expression level of HSL ($P < 0.05$). Only PPARα and PPARβ mRNA expression levels of the J6 group were significantly higher than those in the control group ($P < 0.05$), and there was no significant difference in other groups ($P > 0.05$).

4. Discussion

The previous study reported that 7% dietary lipid at 45~55% dietary protein levels improved the grouper growth performance as it increased PER, but higher dietary lipid (10%, 13%) significantly reduced PER and appeared higher intraperitoneal fat ratio, and the results indicated ingestion of excess lipid induced a lipid deposition [22]. However, other studies demonstrated that 14% dietary lipid at 50% dietary protein level [33] or 16% dietary lipid at 44% dietary protein level [21] could keep higher PER and subsequently
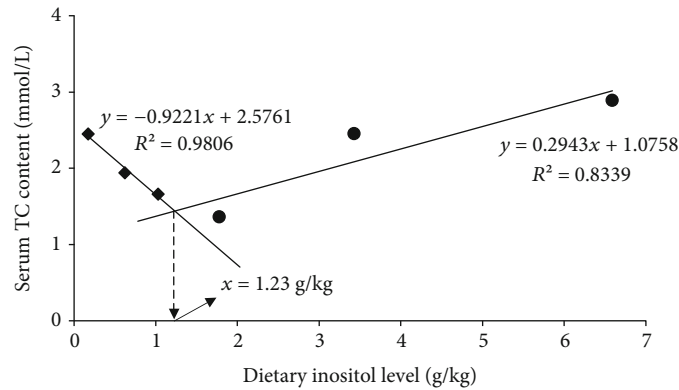
FIGURE 2: Broken-line analysis of the relationship between dietary inositol level and serum total cholesterol (TC) content of hybrid grouper. The appropriate dietary inositol level in high-lipid diets was 1.23 g/kg.
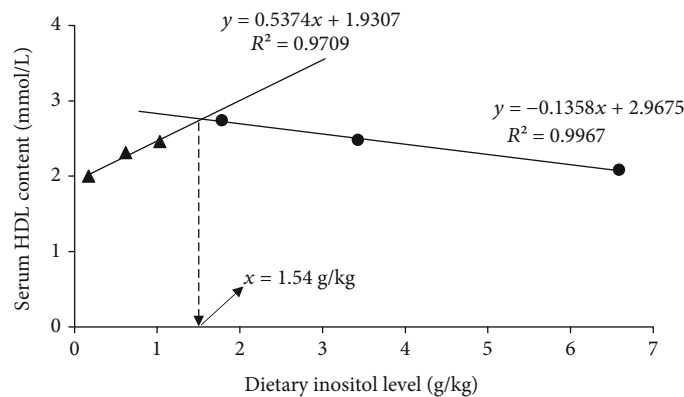

FIGURE 3: Broken-line analysis of the relationship between dietary inositol level and serum high-density lipoprotein (HDL) content of hybrid grouper. The appropriate dietary inositol level in high-lipid diets was 1.54 g/kg.

promoted the growth of the grouper. Lipids are more efficient energy source and readily metabolized by fish, especially by carnivorous fish [2], so that high lipid dietary can reduce the proportion of protein dietary as energy, and then increase the PER. In addition, it has been demonstrated that protein utilization efficiency could be improved by the utilization of lipids in fish diets [34, 35]. Hence, high-lipid diet (16%) was applied in this study to keep higher PER and WGR. Zhu et al. [36] found that 0.181 g/kg inositol supply was optimum requirement in the diets (11% dietary lipid, 52% dietary protein), and the inositol supply could improve growth performance and increase lipid metabolism of the grouper. Previous studies demonstrated that the vitamin E requirement of hybrid tilapia (Oreochromis niloticus × Oreochromis aureus) [37] and grouper (Epinephelus malabaricus) [38] increased as the dietary lipid level increased. Inositol belongs to a vitamin-like nutrient, and thus it is necessary to improve dietary inositol level (0.17~6.59 g/kg) in high-lipid diets in order to alleviate the negative symptoms and keep higher PER and WGR. The present result showed that dietary inositol had no effect on survival of grouper fed high-lipid diets, and the result was consistent with previous studies [13, 15, 16]. Several studies demonstrated that dietary inositol played an essential role to improve growth [14, 39]. WGR of grouper increased with dietary inositol increase, and the result agreed with reports of Jian carp (Cyprinus carpio var. Jian) [40] and hybrid tilapia [13]. However, there was no significant increase in inositol-treated groups when dietary inositol level was over 1.03 g/kg, and excessive inositol leaded to an increase in coats. Therefore, based on broken-line regression of WGR (Figure 1), 1.67 g/kg dietary inositol was considered to be optimum level in juvenile grouper high-lipid diets. Only PER of the J4 group was significantly higher than the control group while others were not. The result indicated that growth performance could not further improve when inositol supply reached a certain level. Some studies have shown dietary excessive inositol caused pathological damage to the tissue, for example, the liver appeared vacuolar degeneration and cell contour was blurred ([41, 42]). Similar results were found in previous study that excessive dietary inositol did not significantly improve growth performance and protein productive ratio in Amur sturgeon (Acipenser schrenckii) [43].

Fish blood indices are good indicators of the physiological status related to metabolism, nutrition status, and disease. Because the changes are primarily reflected by blood indices when fish is impacted by external factors and pathological or physiological changes [20]. Lipid content in blood is very active in metabolism [44] and lipid transportation of

TABLE 6: Effect of dietary inositol on lipid metabolism enzymes activities and biochemical indices in the liver of hybrid grouper fed high-lipid diets.

Diets	LPL (mU/mg.Pro)	HL (U/mg.Pro)	FAS (mU/mg.Pro)	ATGL (mIU/mg.Pro)	VLDL (μmol/mg.Pro)	TG (umol/mg.Pro)	TC (umol/mg.Pro)
J1	197.95 ± 26.65^a	37.72 ± 1.47^a	2785.12 ± 47.55^c	256.30 ± 7.18^a	18.41 ± 0.98^c	13.1 ± 0.01^a	12.18 ± 0.58^a
J2	245.85 ± 18.87^{ab}	44.67 ± 1.61^b	2365.17 ± 157.38^c	277.84 ± 3.72^a	14.42 ± 0.87^b	9.71 ± 0.78^b	9.18 ± 0.85^b
J3	347.82 ± 59.85^{bc}	47.76 ± 0.53^{bc}	1582.83 ± 119.61^{ab}	339.76 ± 12.81^b	13.41 ± 0.52^{ab}	9.71 ± 0.76^b	9.33 ± 0.14^b
J4	657.61 ± 38.81^d	46.77 ± 0.12^{bc}	1503.84 ± 17.26^a	360.47 ± 15.29^b	12.89 ± 0.24^{ab}	8.29 ± 0.52^b	8.71 ± 0.21^b
J5	577.04 ± 9.14^d	49.1 ± 0.26^c	1959.62 ± 89.19^b	432.30 ± 5.43^c	11.41 ± 0.50^a	10.57 ± 0.31^b	8.12 ± 0.48^b
J6	444.24 ± 12.39^c	45.53 ± 1.03^{bc}	1597.07 ± 17.39^{ab}	401.93 ± 39.66^{bc}	12.05 ± 0.12^{ab}	9.11 ± 0.34^b	8.04 ± 0.23^b

The data of the table is expressed by mean average value ± standard error ($n = 3$). The significant difference is exhibited in the same column with different superscript letters ($P < 0.05$). LPL: lipoprotein lipase; HL: hepatic lipase; FAS: fatty acid synthetase; ATGL: adipose triglyceride lipase; VLDL: very low-density lipoprotein; TG: triglyceride; and TC: total cholesterol.

fish is primarily dependent on plasma [45]. Therefore, whole-body lipid metabolism can be reflected by blood lipid levels. The previous studies have reported that inositol supplementation reduced TC and TG contents in serum [15, 46]. In present study, significantly reduced TG content of the inositol-treated groups (except the J2 group) was observed in serum. This result was consistent with a previous study of rats fed high-fat diets [47]. Simultaneously, it is obvious to discover that serum TC content of the J4 group was significantly lower than that of the control group. This result was consistent with study that dietary inositol decreased serum TC and TG levels of rats [46]. In addition, HDL and LDL are involved in the metabolic transport of lipids [48], while increase of HDL and decrease of LDL are vital factors to reduce risk of cardiovascular disease [49]. And increase of HDL was likely to represent a significant antiatherogenic factor [50]. HDL content of J4 group showed significantly higher value compared with the control ($P < 0.05$). Besides, no significant differences as well as a gradually reducing trend were found in LDL content of all groups except the J5 and J6 groups. These results showed that dietary inositol upregulated HDL content and promoted metabolism of TG and TC in serum. Disorders of lipid metabolism include increase of serum TC and TG contents, as well as decrease of HDL content [51]. A relatively low level of TC reflects better status of lipid metabolism, and a relatively high level of HDL is a feature of wellness of an organism [52]. Thus, TC and HDL contents of serum served as the basis for dietary inositol to promote lipid metabolism of the grouper. Broken-line analysis of serum TC and HDL contents (Figures 2 and 3) showed that optimum dietary inositol was 1.23 g/kg and 1.54 g/kg, separately.

The main pathways of lipid metabolism contain digestion and absorption, transport and production of lipid, and β-oxidation [2]. The liver is the main organ of FA β-oxidation and lipid synthesis in fish. In addition, dietary inositol could promote the metabolism of lipid in the liver and other tissues [13, 14] because it promoted the export of lipid in the liver [12, 53]. In this study, dietary inositol had significant effects on activities of LPL, HL, FAS and ATGL, and contents of VLDL, TC, and TG in the liver. Lipid metabolism enzymes include LPL, HL, and PL which play important roles in lipoprotein metabolism [54]. As a primary enzyme required for chylomicron (CM) and VLDL catabolism, LPL has been demonstrated to play vital role on the TG in plasm lipoproteins to release FAs which are transported to other organs or the adipose tissue for storage [55, 56]. In addition, the former study demonstrated that supply of Tibet kefir milk could increase LPL activity in the liver of rats fed high fat diets, and the change of LPL activity had positive role in the reduction of fat deposition [57]. TG content in serum may be broken down by HL, to produce free FA which crossed the hepatocyte membrane by a combination of facilitated transport and diffusion [58, 59]. Involved in different steps of lipoprotein metabolism, HL exerts both triglyceride lipase and phospholipase A1 activities and plays vital role to be regulated as a function of the cholesterol demand [60]. Activity of HL has been demonstrated to be inhibited by diets high in saturated fats or enriched in fish oil [60]. As one of the key enzyme in lipid metabolism, FAS not only participates in the de novo synthesis of long chain FAs in the cytoplasm but also catalyzes the condensation of acetyl-CoA, malonyl-CoA, and NADPH [61]. Additionally, a close relation was found that FAS affected FA synthesis and fat deposition in the body [62]. In teleost, FAS appears prevailingly tissue specific expression pattern, especially higher in the liver and adipose tissues [63]. There was a research showing that FAS level had increased in the liver of *salmon salar* which ingested suboptimal methionine and developed signs commonly described in the early stage of nonalcoholic fatty liver disease [64]. ATGL with crucial work in lipid catabolism is effective in limiting rate of hydrolysis of TC [65]. Besides, the research results of zebrafish corroborated that inhibition of ATGL highly expressing in the liver gave rise to severe fat accumulation, reduce FA β-oxidation, and accompanied by increase of oxidative stress and inflammation [66]. And another introduced that specific deletion of ATGL causes additional hepatic steatosis in mice [67]. An elevated hepatic lipid content reflects abnormal the production or the export of TC and TG and VLDL [68]. In natural conditions, fat is stored in the liver when provision of fatty exceeds the current demand, but it is secreted in the form of VLDL when it is essential to supply the energy for peripheral [69]. The raise of VLDL secretion usually
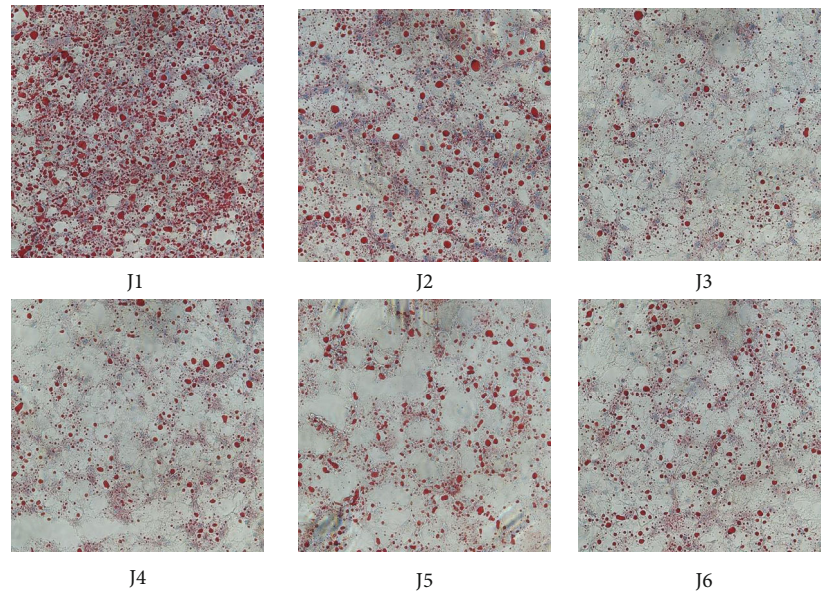
FIGURE 4: Effect of dietary inositol on liver histological sections of hybrid grouper fed high-lipid diets (Sliced oil red × 400).

appeared nonalcoholic fatty liver disease in mice fed high-fat diets [70]. In previous study, ingestion of high-lipid diets increased hepatic TG and TC levels [71]. It is easy to find that LPL and ATGL levels of J3, J4, and J5 significantly raised with inositol increase contrasting with the control group while VLDL content of them significantly lower than those of the control group. Upregulation of LPL and ATGL activities in the liver had the function to promote the hydrolysis of TG. Moreover, the change of LPL is a favor of storing lipids in extrahepatic tissues, and LPL can accelerate catabolism of VLDL. In inositol supply diets, significantly high activity of HL was observed in the liver of all the inositol-treatment groups. Along with inositol supply increase, FAS activity of the inositol-treated groups except the J2 group was significantly lower than that of the control group. The change of FAS activity was related to the lower content of TG caused by upregulation of LPL activity [72]. In the liver, the contents of TC and TG significantly reduced in the inositol-treated groups. This result indicated that inositol supply could decrease the high hepatic TC and TG levels caused by high-lipid diets [71]. The aforementioned results suggested that supply of inositol, appearing homologous functions of Tibet kefir milk [57], had great potentialities to increase adipose tissue lipolysis and FA oxidation, and thereby reduced lipid deposition caused by high-lipid diets.

Excess lipid accumulation in tissues is a key feature of many metabolic diseases. Therefore, techniques for imaging and quantifying lipids in various tissues are essential to understand and evaluate the overall metabolic status of a research subject. LD morphology can be stained by oil red O (ORO), and staining of ORO has been demonstrated to efficiently help to visualize the radical changes that occur in tissues as metabolic disease occurs and progresses [73]. LD accumulations in the liver of hybrid tilapia (*Oreochromis niloticus* ♀ × *O. aureus* ♂) and lipid deposition in the liver of zebrafish were evaluated by staining of ORO [74, 75]. In this study, LD in the liver was stained by ORO, and quantity and

particle size of LD exhibited remarkably downward trend with the increase of supply inositol. The phenomenon may be due to that supply inositol enhanced activity of AGTL which could promote LD catabolism in the liver and the subsequent oxidation of hydrolyzed FAs by lipophagy [76].

Lipid accumulation in animal results from the balance among the lipid absorption from the diets, de novo synthesis of FA, transport of lipoprotein and lipid catabolism. FAS, ACC, and 6GPD involve in fatty acid synthesis [77, 78]. FAS involved in the condensation of acetyl-CoA, malonyl-CoA, and NADPH catalyzes de novo fatty acid synthesis [62]. ACC is the first rate limiting enzyme in the de novo lipogenesis process, and simultaneously, its fatty acid synthesis and fatty acid oxidation [79]. As a key enzyme, 6GPD is related in NADPH production which is essential for fatty acid biosynthesis [63, 80]. FAS, ACC, and 6GPD expression levels in inositol-treated groups were significantly downregulated. The results indicated that dietary inositol supply could downregulate expression levels of FA synthesis related genes. ApoB100 is absolutely required for VLDL assembly and secretion [81]. In the liver, excessive accumulation of DHA and EPA caused by high-lipid diet would attack ApoB100 mRNA, and thus ApoB100 expression downregulated in the high-lipid diet [81]. But there was no difference between the inositol-treated groups and control group in this study. Lipid catabolism is carried out by β-oxidation, and there are numerous critical enzymes (such as LPL, HSL, and CPT1) and transcription factors (such as SREBP1, PPARα, and PPARβ) involved in the process. During the process of lipolysis, LPL plays an essential role to catalyze triacylglycerol into glycerol and nonesterified fatty acids [82]. According to plenty of studies, expression of LPL is demonstrated to be affected by several factors including nutrition, hormones, fasting, and temperature [62, 63]. As a crucial enzyme involved in lipid metabolism, HSL hydrolyzes the diglyceride from TG stored in adipose tissue and thus the release of free FAs [83]. The activity and
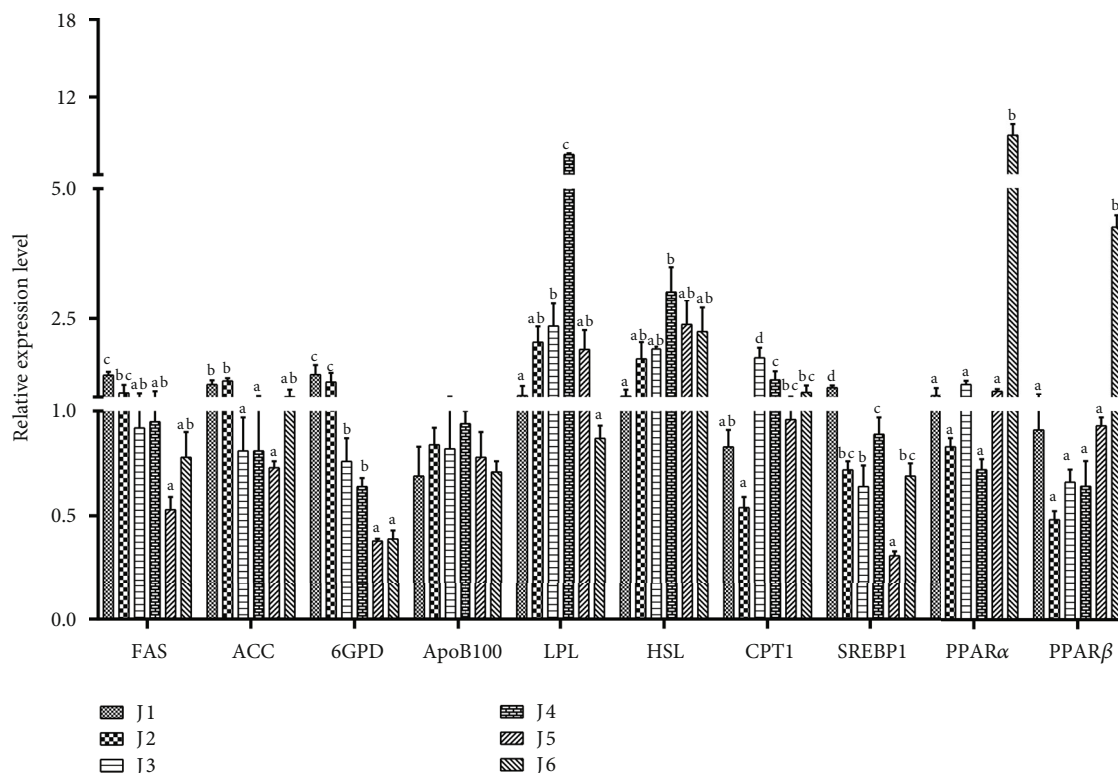
FIGURE 5: Effect of dietary inositol on lipid metabolism-related genes expression in the liver of hybrid grouper fed high-lipid diets. Values are means ± standard error ($n = 3$). Distinct letters assigned to the bars represent significant differences using Duncan's test ($P < 0.05$).FAS: fatty acid synthase; ACC: acetyl-CoA carboxylase; 6GPD: 6-phosphogluconate dehydrogenase; Apo B100: apolipoprotein B100; LPL: lipoprotein lipase; HSL: hormone-sensitive lipase; CPT1: carnitine palmitoyl transferase-1; SREBP1: sterol regulatory element-binding protein-1; PPARα: peroxisome proliferator-activated receptor α; and PPARβ: peroxisome proliferator-activated receptor β.

mRNA expression level of HSL are influenced by various hormones, dietary energy, fasting, and so on [56, 84]. During starvation of fish, LPL and HSL mRNA expressions levels were significantly upregulated in the liver so as to promote consuming the liver fat for sustaining normal energy supply [56, 62]. CPT1 is considered to be an essential enzyme in the FA β-oxidant pathway because it catalyzes the conversion of fatty acyl-CoAs into fatty acylcarnitine molecules for entry into the mitochondrial matrix [85, 86]. In present study, LPL, HSL, and CPT1 expression levels of the J4 group showed a significant upregulation. The results suggested that inositol (1.78 g/kg) could remarkably upregulate the expression levels of LPL, HSL, and CPT1 to promote lipolysis in the liver. As one of the key transcriptional factors, SREBP1 regulates lipid metabolism by targeting many genes related the synthesis of FA and TG (such as FAS and ACC) [87–89]. The expression of SREBP1 in the inositol-treated groups showed a significant downregulation. The results indicated that dietary inositol could downregulate SREBP1 expression to target FAS and ACC genes, and then reduced the synthesis of FA in the liver. Fatty acid metabolism is transcriptionally adjusted by nuclear factors and genes regulation under the control of either the liver X receptors or peroxisome proliferator-activated nuclear receptors (PPARs) [90]. PPARs including three isotypes termed PPARα, PPARβ and PPARγ, and ligand-dependent tran-

scription factors ([91]), play a crucial role in regulating expression of genes. PPARs are the key factors in the storage and mobilization of lipid, metabolism of glucose, and response of morphogenesis and inflammatory [92, 93]. It has been reported that PPARα and PPARβ have responsibility for regulating FA β-oxidation. PPARα promotes FA decomposition by upregulated expression of several key enzymes involved in FA oxidation, and PPARβ regulates the expression of target genes encoding enzymes related β-oxidation [92, 94]. PPARα and PPARβ are demonstrated to be activated by FAs, since they are natural receptors for FAs [95–97]. Otherwise, inositol promoted lipid decomposition in the liver, and then there were more producing of fatty acids [43]. In the J6 group, PPARα and PPRβ expressions levels were significantly activated owing to that inositol supply (6.59 g/kg) promoted more decomposition of lipid in the liver and produced sufficient fatty acids to activate PPARα and PPARβ expressions. However, the underlying mechanism needs further investigation.

5. Conclusion

The present investigation indicated that dietary inositol could improve growth performance and promoted lipid metabolism by decrease of FA synthesis and increase of lipid decomposition. And thus, dietary inositol reduced the liver

lipid deposition caused by high-lipid diets. Broken-line regression analysis of WGR, serum TC, and HDL contents against different doses of dietary inositol indicated that 1.23~1.67 g/kg dietary inositol supplementation was recommended in juvenile grouper high-lipid diets.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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