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Research Article

Effects of Dietary Lysine Levels on Growth Performance and Glycolipid Metabolism via the AKT/FoxO1 Pathway in Juvenile Largemouth Bass, *Micropterus salmoides*

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A 56-day feeding experiment was conducted to determine the dietary lysine requirement of juvenile largemouth bass (*Micropterus salmoides*) and investigate the effects of dietary lysine on growth, whole-body composition, and hepatic gene expression related to glycolipid metabolism via the AKT/FoxO1 pathway. The juveniles (17.34 ± 0.02 g) were fed six graded lysine levels (2.11% (control), 2.56%, 2.92%, 3.33%, 3.68%, and 4.09%, dry diet). The results showed that the 3.33% dietary lysine level significantly increased the final body weight (FBW), weight gain rate (WGR), and specific growth rate (SGR) and improved the feed conversion ratio (FCR) compared with the control group. The whole-body composition was not significantly affected by dietary lysine levels, while lowest hepatic lipid contents were found in the 2.92% and 3.33% dietary lysine groups. Regarding glycolipid metabolism, compared with the control group, 3.33% dietary lysine improved the protein kinase B (AKT) and inhibited the forkhead box O1 (FoxO1), thus upregulated the pyruvate kinase (PK) mRNA levels to enhance glycolysis. Furthermore, sterol-regulatory element binding protein-1c (SREBP1c) and peroxisome proliferator-activated receptor-γ (PPAR-γ) were downregulated by 3.33% dietary lysine, which caused the downregulation of lipid synthesis-related genes acetyl-CoA carboxylase-1 (ACC) and stearyl-CoA desaturase (SCD) mRNA. In addition, 3.33% dietary lysine promoted the expression of the lipolysis-related genes peroxisome proliferator-activated receptor-α (PPAR-α) and carnitine palmitoyl transferase-1 (CPT1). According to the quadratic regression analysis based on the FCR and SGR values, the optimal dietary lysine levels were estimated to be 3.03% and 3.07% of the diet (6.39% and 6.48% of dietary protein), respectively.

1. Introduction

Due to the lack of the corresponding transaminase, many fish cannot synthesize lysine by themselves and must obtain it from food. Hence, lysine (Lys) is usually the first limiting essential amino acid that is insufficient in commonly used raw materials for fish feed, especially in plant protein sources [1]. In fish, lysine deficiency could decrease growth performance and reduce the feed conversion ratio [2]. This result has been verified in other reports by Forster and Ogata [3] and Ruchimat et al. [4] on juvenile red sea bream (*Pagrus major*) and yellowtail (*Seriola quinqueradiata*), respectively. Moreover, previous studies on other fish species observed that feeding excessive lysine reduced the growth rate [5, 6]. Berge et al. [7] explained that antagonistic reactions occur between amino acids when a single amino acid is in excess;

thus, the antagonistic effect of lysine and arginine is particularly apparent. Therefore, it is necessary to continue to evaluate the optimal addition amount of lysine in different fishes to obtain a more comprehensive aquatic feed. In previous studies, Gridale-Hellad et al. [8] reported that the lysine requirement of Atlantic cod (*Gadus morhua*) is 2.67% of the diet (5.34% of the dietary protein). Cao et al. [9] reported that the lysine requirement of juvenile yellow catfish (*Pelteobagrus fulvidraco*) was 3.31% of the diet or 8.32% of the dietary protein. However, for the same fish, different culture specifications and ages have different optimal requirements for lysine. Dai [10] found that the lysine requirements of tongue sole (*Cynoglossus semilaevis*) are 2.75%-2.85% of the diet (initial weight 3.66 g), 2.43%-2.49% of the diet (initial weight 113.0 g), and 2.46%-2.58% of the diet (initial weight 347.3 g). Thus, research on lysine requirements should also focus on the size and age of fish species to create a more accurate nutrition database.

As a growth amino acid, lysine has a variety of biological functions. It is necessary for maintaining the growth performance, development, and immunity of the body [11]. In addition, lysine also has many other biological functions. For example, lysine can be converted to carnitine in animals, and carnitine participates in energy metabolism. Early studies found that feeding low lysine and carnitine-free diets can cause fatty liver and growth retardation in rats [12]. However, another study showed that adding an appropriate amount of lysine at a 0.2% level to feed can promote the growth of rats, increase the content of carnitine in muscle tissues, and reduce the level of triglycerides in tissues [13]. Lysine can also be broken down into glucose and participate in glucose metabolism [14]. Similar findings were observed in grass carp (*Ctenopharyngodon idellus*), and an appropriate amount of dietary lysine (2.44% of diet) promoted glycolysis and lipolysis [15]. These processes are also regulated by the AKT pathway and FoxO1 pathway [16, 17]. AKT is one of the most important and multifunctional kinases. It takes part in maintaining the steady state of lipid metabolism and has a direct regulatory effect on multiple factors associated with glucose metabolism. FoxO1 also plays a role in mediating transcription in glucose and lipid metabolism diseases; it is involved in gluconeogenesis, glycogenolysis, and lipogenesis. To date, many studies have examined the effects of nutrients on glucose and lipid metabolism through the regulation of the AKT pathway in several fish species [18, 19]. Nevertheless, literature regarding the regulation of AKT/FoxO1 by dietary lysine treatments and its potential consequences on glycolipid metabolism in fish species is extremely scarce.

Largemouth bass, *Micropterus salmoides*, is a carnivorous warm-water fish species that was introduced to China in the early 1980s. Because of its fast growth and high economic value, it has been one of the most economically important fish species in China, accounting for the vast majority of fish (99%) of the Asian market [20]. In 2020, the annual output of largemouth bass in China reached 619,000 tons, an increase of 29% compared with 2019 [21]. With an increase in production each year, the nutritional requirements of largemouth bass have attracted more attention. To date, few studies have reported the optimal lysine requirement of largemouth bass. Dairiki et al. [22] suggested that the lysine requirement was 4.9% of dietary protein for largemouth bass fry (1.29 g). Moreover, it has been revealed that supplementation with lysine in feed could improve growth performance and regulate glycolipid metabolism in juvenile largemouth bass [23, 24]. As a carnivorous fish, largemouth bass can also have liver diseases caused by glycolipid metabolism disorders [25]. In addition, the dietary lysine level requirement for largemouth bass at the juvenile stage is still unknown, and few reports about the effects of dietary lysine levels on glycolipid metabolism have been published.

Overall, determining the optimal lysine requirement helps fish growth and is more conducive to the regulation of glucolipid metabolism, reducing the incidence of disease. Consequently, the objective of this study was to determine the optimal lysine requirement of juvenile largemouth bass and estimate the effects of dietary lysine levels on glycolipid metabolism through the AKT/FoxO1 signaling pathway.

2. Materials and Methods

2.1. Experimental Diets. Six practical diets were prepared containing six lysine levels (2.11%, 2.56%, 2.92%, 3.33%, 3.68%, and 4.09%, dry diet). The main protein sources were fish meal, rapeseed meal, and soybean meal. Fish oil was the main lipid source, and wheat flour and cassava starch were the main carbohydrate sources (Table 1). A combination of crystalline amino acids, excluding lysine, was supplemented to simulate the whole-body amino acid pattern of largemouth bass (Table 2). All ingredients were ground into powder through 60 mash-sieve and then thoroughly mixed with fish oil and water to produce stiff dough. The dough was forced through a pelletizer (F-26 (II), South China University of Technology, China) and dried in a ventilated oven [26]. After drying at 45°C for 24 h, the diets were stored at −20°C until use.

2.2. Experimental Design and Conditions. Experimental fish were obtained from the breeding farm of the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences and temporarily kept in floating cages (2 m × 2 m × 1 m) for two weeks, and the fish were fed with commercial feed (obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China, crude protein 47%, crude lipid 12%) to acclimate to the experimental conditions. Before the rearing trial, 540 juvenile largemouth bass (initial weight 17.34 ± 0.02 g) were divided into 18 floating cages (1 m × 1 m × 1 m). Three replicates were performed for each lysine supplementation group, and each cage contained 30 fish. During the experimental period, the juveniles were fed two times (7 : 00 and 17 : 00) daily at a feeding rate of 5% of their weight for 8 weeks. The fish were counted and weighed every two weeks to adjust the feeding amount. During this experiment, the water temperature was kept at 28 ± 2°C, the pH values were kept at 7–7.5, the dissolved oxygen levels were higher than 6.0 mg/L, and the photoperiod was the same as natural light.

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

Ingredients	Diet					
	1	2	3	4	5	6
Fish meal[1]	30.00	30.00	30.00	30.00	30.00	30.00
Rapeseed meal[1]	8.00	8.00	8.00	8.00	8.00	8.00
Soybean meal[1]	10.00	10.00	10.00	10.00	10.00	10.00
Wheat flour[1]	8.00	8.00	8.00	8.00	8.00	8.00
Cassava starch	8.00	8.00	8.00	8.00	8.00	8.00
Corn gluten meal[1]	8.00	8.00	8.00	8.00	8.00	8.00
Fish oil	5.00	5.00	5.00	5.00	5.00	5.00
Shrimp paste	2.00	2.00	2.00	2.00	2.00	2.00
Amino acid premix	8.58	8.58	8.58	8.58	8.58	8.58
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Calcium dihydrogen phosphate	2.50	2.50	2.50	2.50	2.50	2.50
Microcrystalline cellulose	0.81	0.81	0.81	0.81	0.81	0.81
Rice bran	5.00	5.00	5.00	5.00	5.00	5.00
Ethoxy quinoline	0.01	0.01	0.01	0.01	0.01	0.01
Glycine[4]	2.00	1.60	1.20	0.80	0.40	0.00
L-Lysine[5]	0.00	0.40	0.80	1.20	1.60	2.00
Bentonite	0.00	0.00	0.00	0.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed proximate composition						
Lysine (%)	2.11	2.56	2.92	3.33	3.68	4.09
Dry matter (%)	93.94	92.99	93.08	93.83	94.15	94.05
Crude protein (%)	47.48	47.32	47.40	47.49	47.42	47.51
Crude lipid (%)	10.73	10.56	10.03	10.77	11.30	11.19
Crude ash (%)	9.45	9.80	9.42	9.55	9.62	9.70
Crude fiber (%)	3.11	3.14	3.05	3.22	3.18	3.09
NFE (%)[6]	22.71	22.17	23.18	22.80	22.63	22.56
Gross energy (MJ/kg)	19.71	19.76	19.64	19.68	19.80	19.76

[1]Fish meal, obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China, crude protein 65.6%, crude lipid 9.5%; rapeseed meal obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China, crude protein 39.2%, crude lipid 6.1%; soybean meal obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China, crude protein 50.8%, crude lipid 4.3%; corn gluten meal obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China, crude protein 60.6%; wheat flour obtained from Wuxi Tongwei Feedstuffs Co., Ltd., Wuxi, China, crude protein 13.1%, crude lipid 4.0%. [2]Vitamin premix (IU or mg/kg of diet): vitamin A, 800,000 IU; vitamin D3, 150,000-250,000 IU; vitamin E, 4,500 IU; vitamin K3, 600 mg; thiamin, 800 mg; riboflavin, 800 mg; calcium pantothenate, 2,000 mg; pyridoxine HCl, 2,500 mg; cyanocobalamin, 8 mg; biotin, 16 mg; folic acid, 400 mg; niacin, 2800 mg; inositol, 10,000 mg; vitamin C, 10,000 mg. [3]Mineral mix (g/kg of diet): magnesium sulphate, 1.0-1.5%; ferrous sulphate, 15-30 g; zinc sulphate, 8-13.5 g; cupric sulphate, 0.35-0.8 g; manganese sulphate, 2-6 g; rice chaff and zeolite were used as a carrier. [4]Glycine, obtained from Feeer Co., Ltd. (Shanghai, China). [5]L-lysine, obtained from Feeer Co., Ltd. (Shanghai, China). [6]NFE (nitrogen free extract, %) = dry matter (%) − (crude protein (%) + crude lipid (%) + crude ash (%) + crude fiber (%)).

2.3. Sample Collection. After the 8-week feeding, all juvenile largemouth bass were fasted for 24 h. Then, all juvenile largemouth bass were quantified and weighed, and plasma and liver tissues were quickly collected from three fish per cage. Blood was collected from three of the sampled fish using caudal venipuncture and then immediately centrifuged at 3,000 rpm for 10 min at 4°C. Finally, the upper plasma was transferred into new tubes. In addition, liver tissues were dissected and removed. All plasma and liver tissues were stored at −80°C until analysis. Another two fish were collected from each cage and stored in a −20°C freezer for whole body composition analysis.

2.4. Chemical Analysis. The experimental diets, ingredients, whole-body composition, and liver lipid content were determined by established methods based on the AOAC [27]. The plasma biochemical parameters include triglycerides (TG), total cholesterol (TC), and glucose (GLU). Table 3 shows the testing parameters, kits, testing equipment, and main methods.

Total RNA was extracted from the liver tissues using the TRIzol method (Vazyme Biotech Co., Ltd., China) following the manufacturer's instructions. The quantity and quality of isolated RNA were measured using a NanoDrop 2000 spectrophotometer (Thermo, NanoDrop Technologies, USA),

Table 2: Amino acid composition of ingredients (% dry matter).

Amino acid	30% FM[1]	8% RM[1]	10% SM[1]	8% WF[1]	8% CGM[1]	5% RB[1]	CAPP[2]	Total	47% WBP[3]
EAA[4]									
Arginine	1.20	0.20	0.37	0.08	0.15	0.06	0.75	2.06	2.81
Histidine	0.45	0.09	0.13	0.04	0.10	0.02	0.33	0.83	1.17
Isoleucine	0.83	0.13	0.24	0.07	0.20	0.03	0.55	1.49	2.04
Leucine	1.45	0.23	0.39	0.14	0.80	0.05	0.43	3.07	3.50
Lysine	1.51	0.18	0.31	0.05	0.07	0.03	Variable	2.16	3.50
Methionine	0.55	0.07	0.07	0.03	0.13	0.01	0.25	0.86	1.11
Phenylalanine	0.79	0.13	0.27	0.10	0.30	0.03	0.33	1.62	1.95
Threonine	0.82	0.14	0.20	0.06	0.16	0.03	0.59	1.41	2.00
Valine	0.96	0.17	0.25	0.09	0.22	0.04	0.53	1.72	2.25
Tryptophan	0.21	0.05	0.07	0.02	0.02	0.01	0.12	0.38	0.50
NEAA[5]									
Aspartic acid	2.11	0.56	0.75	0.11	0.41	0.15	0.33	4.09	4.42
Serine	0.77	0.14	0.26	0.10	0.25	0.03	0.47	1.55	2.02
Glycine	1.99	0.36	0.41	0.03	0.23	0.04	0.85	3.06	3.90
Alanine	1.33	0.14	0.22	0.06	0.43	0.04	1.08	2.23	3.30
Cystine	0.18	0.08	0.07	0.05	0.08	0.02	0.00	0.48	0.27
Glutamic acid	3.25	0.67	1.08	0.73	1.22	0.13	1.42	7.08	8.50
Proline	0.91	0.21	0.26	0.23	0.44	0.03	0.56	2.08	2.64

[1]FM: fish meal; RM: rapeseed meal; SM: soybean meal; WF: wheat flour; CGM: corn gluten meal; RB: rice bran; [2]CAPP: crystalline amino acid premix; [3]WBP: whole-body protein; [4]EAA: essential amino acid; [5]NEAA: nonessential amino acid.

Table 3: The chemical analysis used in the experiment.

Items	Methods	Assay kits/testing equipment
Composition of diets/ingredients		
Moisture	Drying method	Electric blast drying oven (Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China)
Protein	Kjeldahl	Auto Kjeldahl apparatus: Hanon K1100 (Jinan Hanon Instruments Co., Ltd., Jinan, China)
Lipid	Soxhlet	Auto fat analy: Hanon SOX606 (Jinan Hanon Instruments Co., Ltd., Jinan, China)
Ash	Combustion	Muffle: XL-2A (Hangzhou Zhuochi Instrument Co., Ltd., Hangzhou, China)
Gross energy	Combustion	Oxygen bomb calorimeter: IKA C6000 (IKA Works Guangzhou, Guangzhou, China)
Amino acids (except tryptophan)	Acid hydrolysis	Amino acid analyzer: SYKAM S-433D (Sykam GmbH, Munich, Germany)
Tryptophan	Alkali hydrolysis	
Liver		
Lipid	Soxhlet	Auto fat analy: Hanon SOX606 (Jinan Hanon Instruments Co., Ltd., Jinan, China)
Plasma parameters		
TG[1]	International Federation of Clinical Chemistry recommended	Assay kits purchased from Mindray Medical International Ltd. (Shenzhen, China); Mindray BS-400 automatic biochemical analyzer (Mindray Medical International Ltd., Shenzhen, China)
TC[1]		
GLU[1]		

[1]TG: total triglyceride; TC: total cholesterol; GLU: glucose.

Table 4: Real-time PCR primer sequences used in the present study.

Target genes	Forward primer (5′-3′)	Reverse primer (5′-3′)	Length (bp)
AKT[1]	AGCGCACCTTCCATGTAGAC	GGCTATTTGCCACTTGCTGG	75
FoxO1[2]	ATAACCTGAACCTGCTGTC	TCATCCTCATCTGGCTGTA	168
SREBP1c[3]	TCACCTTCCTCTTCCTCTC	TCAGTAGCCACACCAGTAT	183
PPAR-α[4]	AGGCTTCATCACCAGAGA	TCCGCAGCAGATAATAGTAG	151
PPAR-γ[5]	GAGTTCTCAGTCAAGTTCAAC	AATGTAGCACCGTCTCCT	142
FAS[6]	AGTTGAAGGCTGCTGATG	GCTGTGGATGATGTTGGT	221
ACC[7]	TTACATCGCAGCCAACAG	CTCTCCACCTTCCTCTACA	190
SCD[8]	CGATGCTGCTTCTTCACT	GACACGGTTCTGCCATTA	271
CPT1[9]	TTACCGTATGGCTATGACTG	GGCTCCGATAACACCTCT	114
GK[10]	CCCTTGTGGGCAGGAGAAAA	ACAACTGAGTCCTCCTTGCG	142
PK[11]	CACGCAACACTGGCATCATC	TCGAAGCTCTCACATGCCTC	172
PEPCK[12]	GGCAAAACCTGGAAGCAAGG	ATAATGGCGTCGATGGGGAC	134
GAPDH[13]	ACTGTCACTCCTCCATCTT	CACGGTTGCTGTATCCAA	105

[1]AKT: protein kinase B; [2]FoxO1: forkhead box O1; [3]SREBP1c: sterol-regulatory element binding protein-1c; [4]PPAR-α: peroxisome proliferator-activated receptor-α; [5]PPAR-γ: peroxisome proliferator-activated receptor-γ; [6]FAS: fatty acid synthase; [7]ACC: acetyl-CoA carboxylase-1; [8]SCD; stearyl-CoA desaturase; [9]CPT1: carnitine palmitoyl transferase-1; [10]GK: glucokinase; [11]PK: pyruvate kinase; [12]PEPCK: phosphoenolpyruvate carboxykinase; [13]GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

and qRT–PCR was performed on a CFX96 real-time PCR detection system thermocycler (Bio-Rad). The primer pairs used in this experiment are presented in Table 4. GAPDH was selected as a reference, and there was no difference in its expression among the different treatments. Finally, Pfaffl's mathematical model [28] was selected to calculate the relative expression of the target genes in this experiment.

2.5. Statistical Analysis. The data were subjected to normality and homogeneity tests where necessary, and then, the experimental data (means ± SEM) were analyzed using the SPSS 24.0 statistical software for one-way analysis of variance (ANOVA). When the difference was significant ($P < 0.05$), Tukey's multiple comparisons were performed. After comparing the estimation coefficient (R^2) among the linear regression model (SGR, 0.015; FCR, 0.026), broken-linear regression model (SGR, 0.137; FCR, 0.185), and quadratic regression model (SGR, 0.836; FCR, 0.935), the quadratic regression model was selected to determine the optimum dietary lysine requirement of juvenile largemouth bass.

3. Results

3.1. Growth Performance. As shown in Table 5, the juvenile largemouth bass fed 3.33% dietary lysine gained the maximum FBW, WGR, and SGR ($P < 0.05$), and the FCR reached the lowest value ($P < 0.05$). In addition, no obvious changes were observed in FI and SR among all the dietary groups ($P > 0.05$). According to the quadratic regression analysis based on the FCR and SGR, the optimal dietary lysine levels were estimated to be 3.03% (Figure 1) and 3.07% (Figure 2) of the diet (6.39% and 6.48% of dietary protein), respectively.

3.2. Whole-Body Composition and Hepatic Lipid Content. The body composition data of juvenile largemouth bass fed the experimental diets are shown in Table 6. The moisture, lipid, protein, and ash contents were not significantly affected by lysine treatments ($P > 0.05$).

Figure 3 shows the hepatic lipid content of juvenile largemouth bass fed experimental diets. The liver lipid content decreased with increasing dietary lysine up to 3.33% and then gradually increased thereafter. The control group had the highest hepatic lipid content (2.11%) among all the groups ($P < 0.05$).

3.3. Plasma Total Triglycerides, Total Cholesterol, and Glucose Levels. The plasma TG, TC, and GLU levels of juvenile largemouth bass fed the experimental diets are shown in Figure 4. Plasma TG levels were significantly decreased in fish fed 2.56% dietary lysine ($P < 0.05$, Figure 4(a)). Compared with the control group, plasma TC levels were decreased in the groups fed a diet supplemented with lysine, but there were no significant changes among all dietary treatment groups ($P > 0.05$, Figure 4(b)). Figure 4(c) shows that the plasma GLU levels were not obviously affected by the lysine treatments ($P > 0.05$).

3.4. Relative mRNA Expression of AKT and FoxO1. The hepatic mRNA expression levels of AKT and FoxO1 are displayed in Figure 5. Figure 5 shows that AKT mRNA levels increased with increasing dietary lysine levels, and the maximum value was observed in the 4.09% dietary group ($P < 0.05$). In addition, the FoxO1 mRNA levels showed a decreasing trend with increasing levels of dietary lysine, and the control group (2.11% dietary lysine) demonstrated the highest expression levels of FoxO1 ($P < 0.05$).

3.5. Relative mRNA Expression of Glucose Metabolism-Related Genes. The expression levels of hepatic glucose metabolism-related genes are presented in Figure 6. The PK mRNA levels increased with increasing dietary lysine

Table 5: Growth performance of juvenile largemouth bass fed with the experimental diets.

Lysine levels (%)	IBW (g)[1]	FBW (g)[2]	WGR (%)[3]	SGR (% day^{-1})[4]	FCR[5]	FI (g fish^{-1} d^{-1})[6]	SR (%)[7]
2.11	17.26 ± 0.03	50.77 ± 0.41^{ab}	194.2 ± 2.70^{a}	1.93 ± 0.02^{a}	1.24 ± 0.01^{ab}	0.64 ± 0.02	100.0 ± 0.00
2.56	17.36 ± 0.06	52.42 ± 0.72^{abc}	202.0 ± 4.52^{ab}	1.97 ± 0.03^{ab}	1.19 ± 0.02^{ab}	0.64 ± 0.01	98.9 ± 1.11
2.92	17.37 ± 0.07	53.38 ± 0.15^{bc}	207.4 ± 1.28^{ab}	2.01 ± 0.01^{ab}	1.17 ± 0.00^{ab}	0.64 ± 0.00	98.9 ± 1.11
3.33	17.30 ± 0.10	54.63 ± 1.00^{c}	215.8 ± 7.29^{b}	2.05 ± 0.04^{b}	1.15 ± 0.04^{a}	0.64 ± 0.01	96.7 ± 1.92
3.68	17.36 ± 0.03	52.13 ± 0.25^{abc}	200.4 ± 0.91^{ab}	1.96 ± 0.01^{ab}	1.21 ± 0.01^{ab}	0.65 ± 0.01	100.0 ± 0.00
4.09	17.40 ± 0.04	50.51 ± 0.51^{a}	190.3 ± 2.29^{a}	1.90 ± 0.01^{a}	1.26 ± 0.02^{b}	0.66 ± 0.01	100.0 ± 0.00
Regressions							
$Y_{FBW} = -3.3653X^2 + 20.842X + 21.535$						$R^2 = 0.867$	$P < 0.01$
$Y_{WGR} = -19.217X^2 + 118.2X + 28.525$						$R^2 = 0.825$	$P < 0.01$
$Y_{SGR} = -0.1135X^2 + 0.698X + 0.9489$						$R^2 = 0.836$	$P < 0.01$
$Y_{FCR} = 0.089X^2 - 0.539X + 1.9348$						$R^2 = 0.935$	$P < 0.01$
$Y_{FI} = 0.0126X^2 - 0.0688X + 0.7302$						$R^2 = 0.890$	$P = 0.076$

All data are mean value of three replicates ± SEM. Means in the same column with different superscripts are significantly different ($P < 0.05$). [1]IBW: initial body weight. [2]FBW: final body weight. [3]Weight gain rate (WGR) (%) = 100 × (final body weight (g) − initial body weight (g))/initial body weight (g). [4]Specific growth rate (SGR) (%day^{-1}) = 100 × [(In (final body weight (g)) − In (initial body weight (g)))/days]. [5]Feed conversion ratio (FCR) = dry feed fed (g) / (final body weight (g) − initial body weight (g)). [6]Feed intake (FI) (g fish^{-1} d^{-1}) = dry feed fed (g)/(final body weight (g) + initial body weight (g)/2 × days). [7]Survival rate (SR) (%) = 100 × (survival fish number/total fish number).

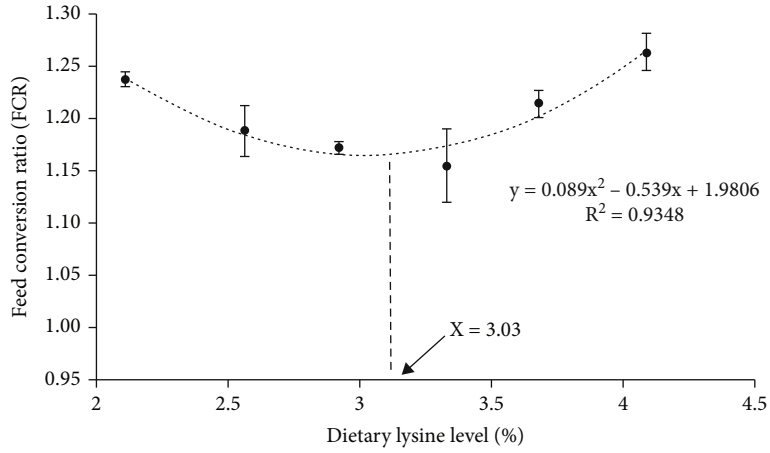

Figure 1: Quadratic regression analysis of feed conversion ratio (FCR) against graded different levels of dietary lysine.

up to 3.68% ($P < 0.05$) and then gradually decreased thereafter. The expression levels of GK showed a similar tendency to those of PK; however, there were no significant differences among all dietary treatments ($P > 0.05$). In addition, at the highest level of dietary lysine (4.09%), the PEPCK mRNA level was upregulated in comparison with that in the control group ($P < 0.05$).

3.6. Relative mRNA Expression of Lipid Metabolism-Related Genes. The mRNA expression levels of hepatic lipid metabolism-related genes are shown in Figures 7 and 8. As shown in Figure 7, the lipolysis-related genes PPAR-α and CPT1 were both regulated by dietary lysine levels. The mRNA expression levels of PPAR-α increased with increasing dietary lysine, and the highest level was observed in the 4.09% dietary lysine group ($P < 0.05$). The mRNA expression levels of CPT1 reached their maximum value when the fish were fed 2.92% and 3.33% dietary lysine ($P < 0.05$).

Figures 8(a) and 8(b) present the mRNA expression levels of hepatic lipid synthesis-related genes. The highest mRNA expression level of SREBP1c was observed in the control group (2.11% dietary lysine). When the fish were

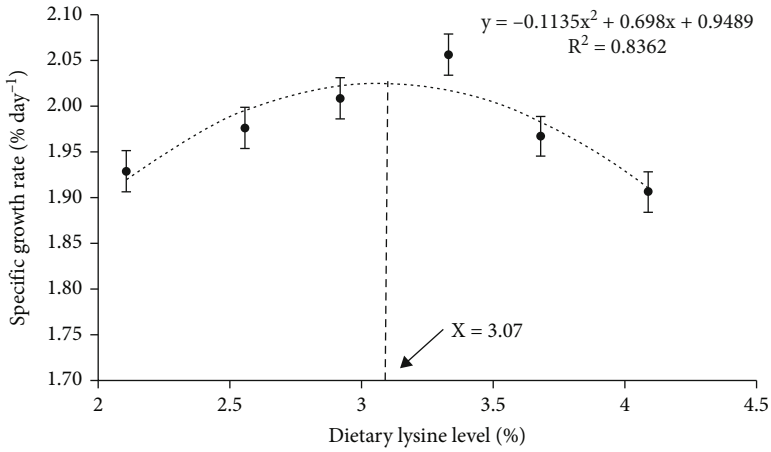

Figure 2: Quadratic regression analysis of specific growth rate (SGR, % day^{-1}) against graded different levels of dietary lysine.

Table 6: Whole body composition of juvenile largemouth bass fed with the experimental diets.

Lysine levels (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
2.11	70.45 ± 0.17	17.49 ± 0.49	7.93 ± 0.23	4.89 ± 0.12
2.56	70.03 ± 0.28	17.67 ± 0.37	7.85 ± 0.24	4.86 ± 0.31
2.92	70.38 ± 0.07	17.70 ± 0.29	7.87 ± 0.04	4.18 ± 0.38
3.33	69.91 ± 0.25	17.28 ± 0.11	7.87 ± 0.32	4.67 ± 0.20
3.68	70.61 ± 0.31	17.22 ± 0.38	7.02 ± 0.34	4.46 ± 0.25
4.09	70.14 ± 0.12	17.96 ± 0.20	7.49 ± 0.49	4.63 ± 0.26
Regressions				
$Y_{Moisture} = 0.1314X^2 - 0.8445X + 71.55$			$R^2 = 0.051$	$P = 0.846$
$Y_{Protein} = 0.2095X^2 - 1.2459X + 19.332$			$R^2 = 0.144$	$P = 0.608$
$Y_{Lipid} = -0.076\,X^2 + 0.1324X + 8.0303$			$R^2 = 0.487$	$P = 0.237$
$Y_{Ash} = 0.3144X^2 - 2.0961X + 7.9543$			$R^2 = 0.428$	$P = 0.376$

All data are mean value of three replicates ± SEM. Means in the same column with different superscripts are significantly different ($P < 0.05$).

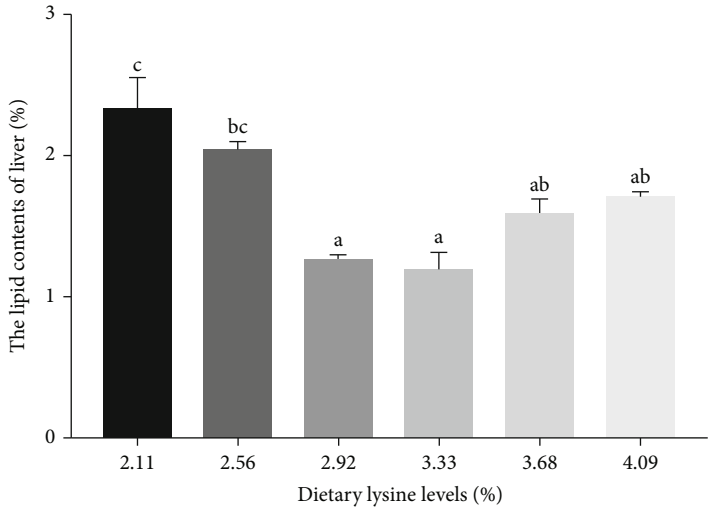

Figure 3: The lipid contents in the liver of juvenile largemouth bass fed with experimental diets.

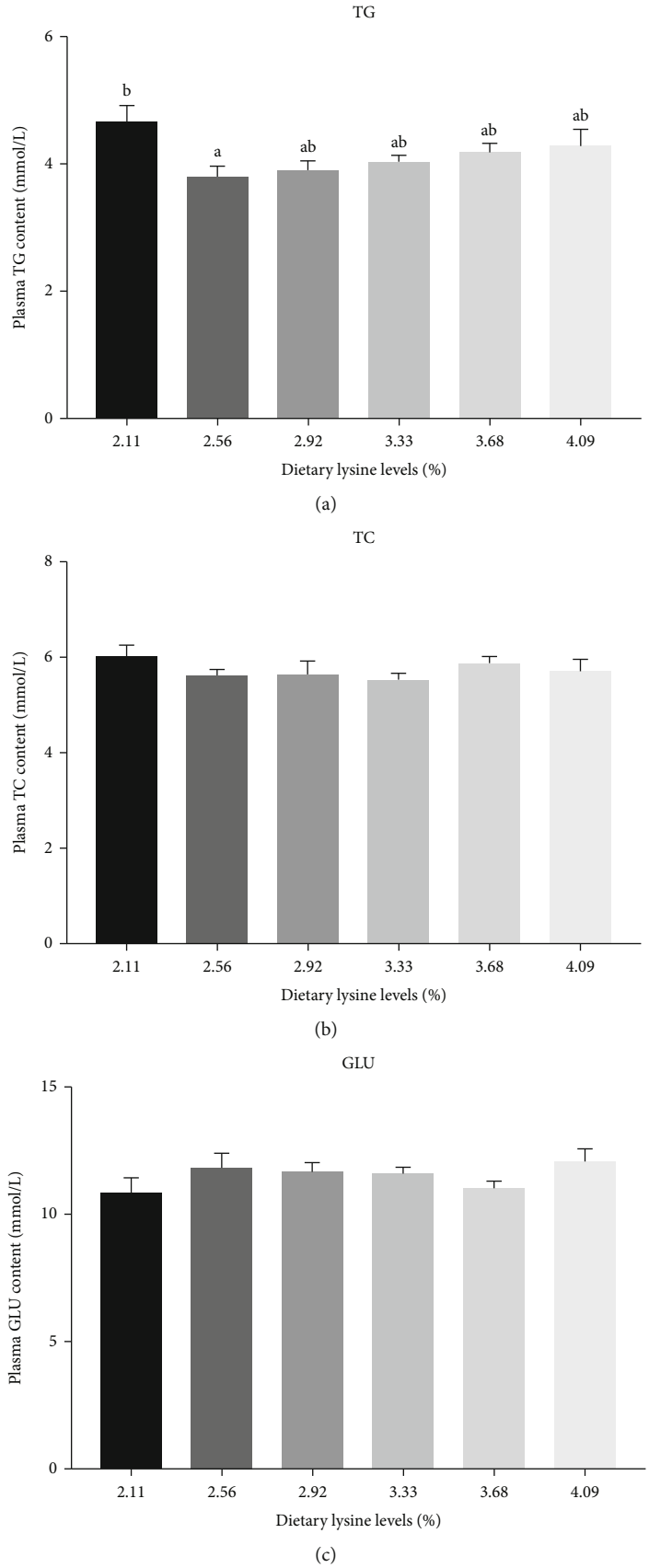

Figure 4: Plasma total triglycerides (TG) (a), total cholesterol (TC) (b), and glucose (GLU) (c) contents of juvenile largemouth bass fed with the experimental diets.

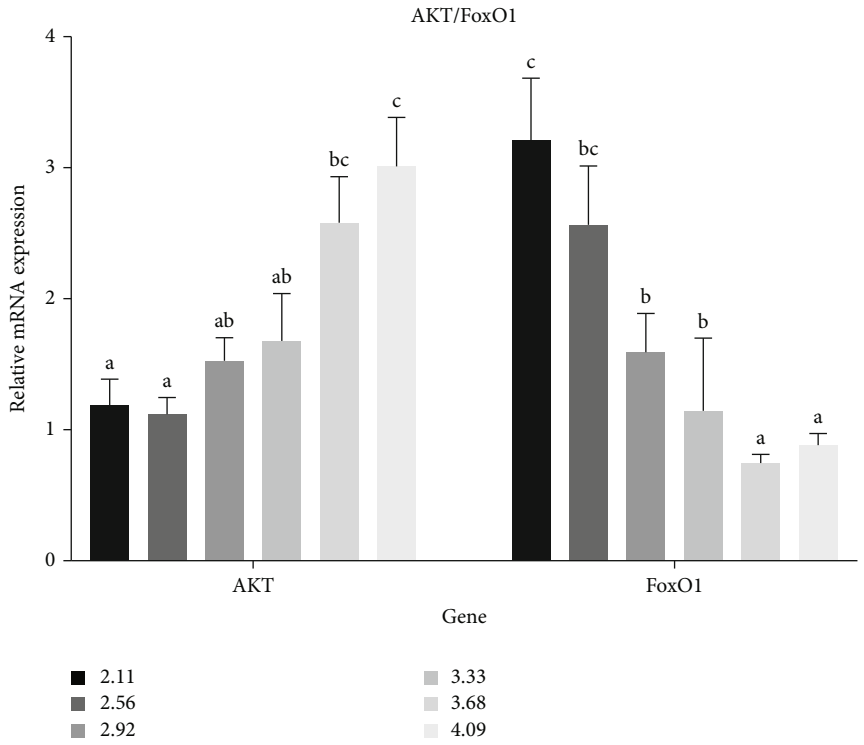

Figure 5: Relative mRNA expression of AKT/FoxO1 pathway in the liver of juvenile largemouth bass.

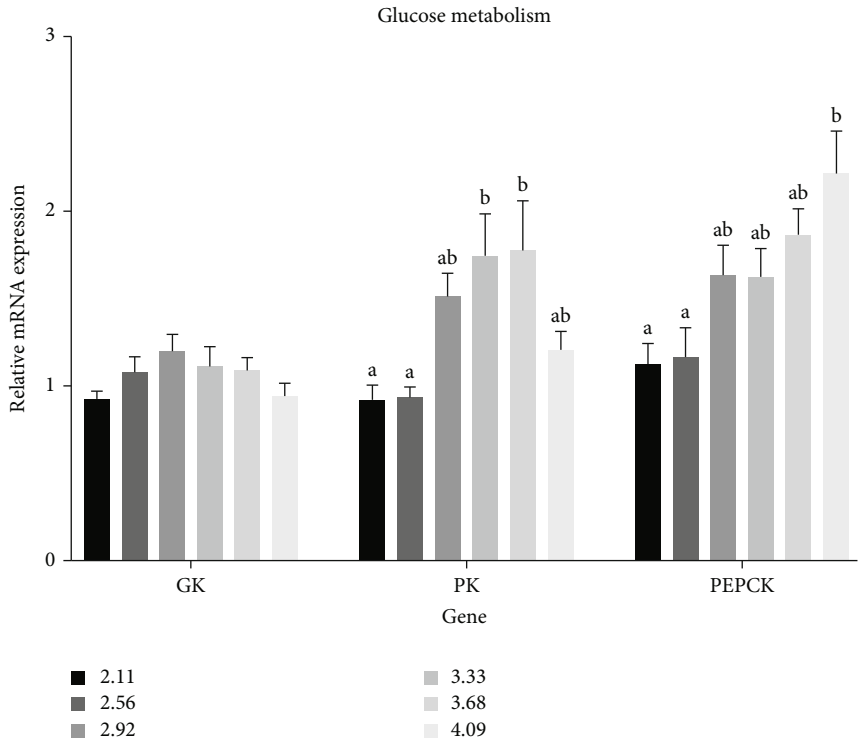

Figure 6: Relative mRNA expression of glucose metabolism in the liver of juvenile largemouth bass.

fed 3.33% dietary lysine, the mRNA expression levels of SREBP1c were significantly decreased ($P < 0.05$). Compared with the control group, the mRNA expression levels of PPAR-γ were markedly decreased in the 3.33% dietary lysine group ($P < 0.05$). The ACC and SCD mRNA expression levels also showed a similar phenomenon in that the relative

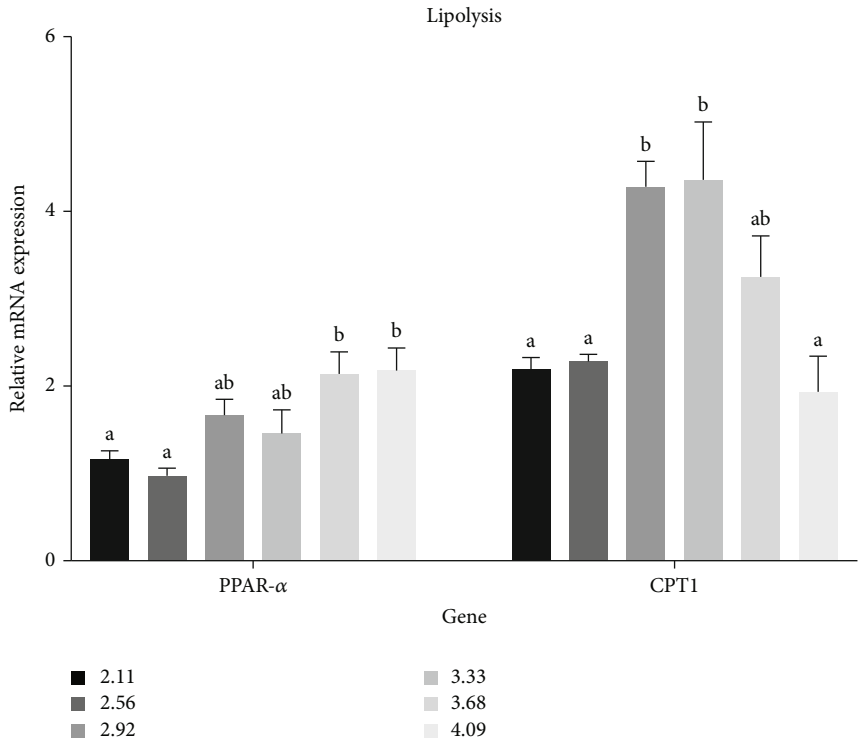

Figure 7: Relative mRNA expression of lipolysis in the liver of juvenile largemouth bass.

expression levels began to decrease as the amount of dietary lysine increased. The mRNA expression levels of ACC were lowest in the 3.33% dietary lysine group ($P < 0.05$), and the mRNA expression levels of SCD gradually decreased with increasing levels of dietary lysine in comparison with that in the control group ($P < 0.05$). Moreover, the FAS mRNA levels were not affected by the dietary lysine treatments ($P > 0.05$).

4. Discussion

4.1. Growth Performance of Largemouth Bass. In this experiment, the best growth performance was observed in the 3.33% dietary lysine group, as the FCR was the lowest in this group. In addition, the growth of largemouth bass was reduced in the low- and high-level lysine groups. Our experimental results indicate that appropriate lysine supplementation could promote the growth of juvenile largemouth bass and enhance feed utilization. Similar findings were also observed in juvenile totoaba (*Totoaba macdonaldi*) [29] and juvenile silver pompano (*Trachinotus blochii*) [30]. In addition, this study also demonstrated that high lysine levels reduced the growth performance of largemouth bass. Excessive lysine may cause arginine-lysine antagonism, or the increased lysine degradation consumes more energy in fish [31, 32]. According to the quadratic regression analysis of the FCR and SGR for dietary lysine, the optimal dietary lysine levels were estimated to be 3.03% (6.39% of dietary protein) and 3.07% (6.48% of dietary protein) of diet. However, these results are different from those reported in the previous study by Dairiki et al. [22] in largemouth bass fry (1.29 g, 4.9% of dietary protein). In their study, the largemouth bass fry were cultured in indoor tanks in a closed circulation system, and the main protein source was tilapia fillet. The different growth stages of aquatic animals could cause a wide variation in nutrient requirements. Moreover, dietary formulation, culture conditions, and dietary amino acid balance could also be potential causes for these observed differences [33].

4.2. Body Composition of Largemouth Bass. A previous study reported that the proximate body composition of cultured fish was influenced by numerous factors, including environmental conditions and diets [34]. Some authors reported that lysine supplementation could increase body protein contents and decrease body lipid contents in fish [35, 36]. Besides, it has also been found that lysine supplementation increases the protein and lipid contents [37]. However, in this study, the whole-body moisture, lipid, protein, and ash contents were not affected by the dietary lysine treatments. This result was the same as other studies reported by Prabu et al. [38] in GIFT tilapia (*Oreochromis niloticus*) and Li et al. [39] in juvenile grass carp (*Ctenopharyngodon idella*), where the whole-body composition was not notably affected by dietary lysine supplementation. The differences may be related to diet formulations, species, and farming conditions, and the specific mechanism needs further study.

4.3. AKT/FoxO1 Signaling Pathway. In recent years, research on glycolipid metabolism and the regulation of largemouth bass has become a hot spot in aquaculture. In some carnivorous fish, the liver has a low ability to regulate glycolysis

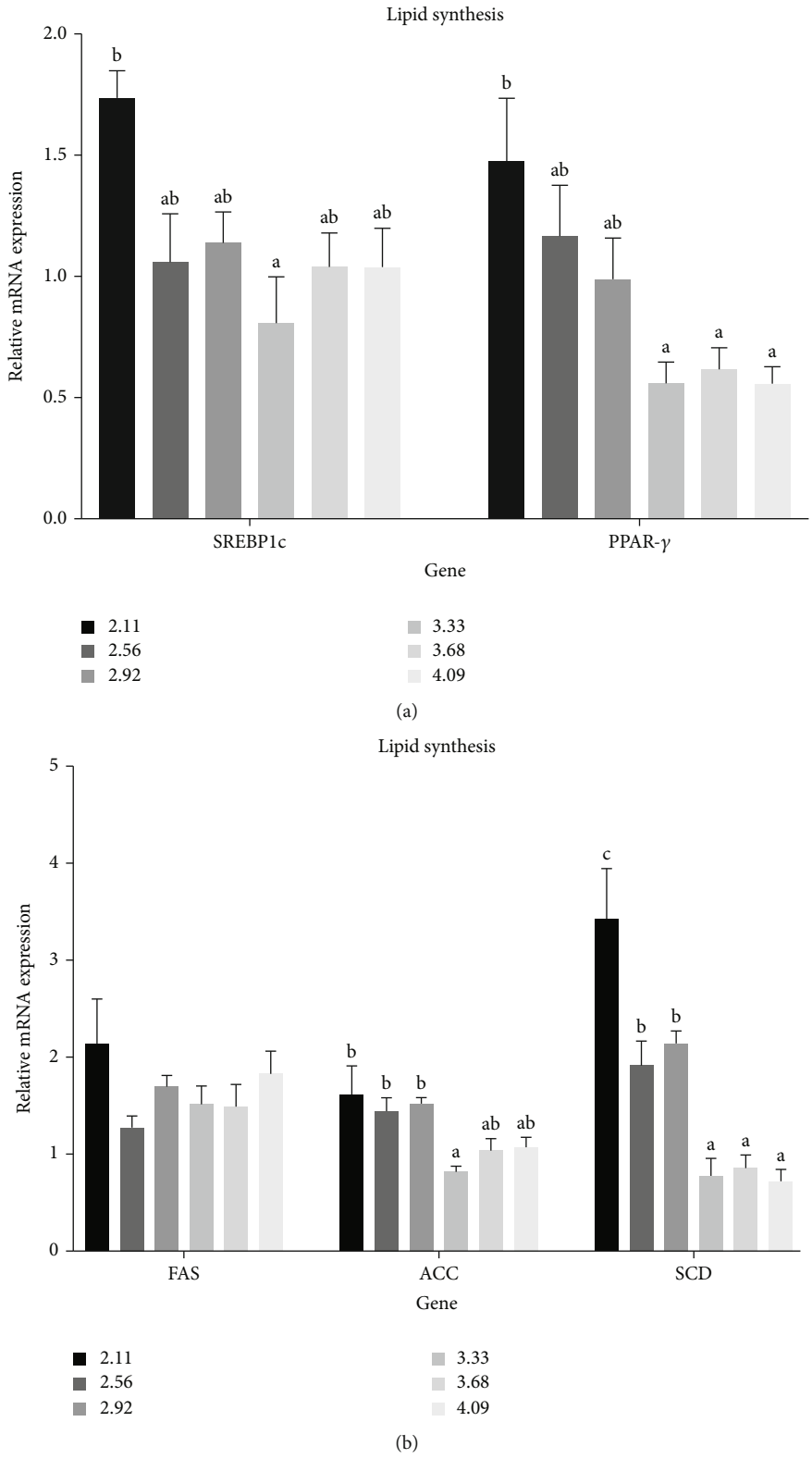

Figure 8: Relative mRNA expression of lipid synthesis in the liver of juvenile largemouth bass.

and gluconeogenesis, and this could result in serious metabolic disorders, including glycogenic liver disease and hepatic steatosis, in long-term culture [40]. In our experiment, the regulatory effect of dietary lysine on hepatic glycolipid metabolism in largemouth bass through the AKT/FoxO1 pathway was explored. The AKT signaling pathway is a key pathway by which insulin controls hepatic lipid metabolism [41], and AKT is also a kinase that is well-

known to be involved in improving glucose metabolism [42]. In addition, as a key molecule downstream of the AKT signaling pathway, FoxO1 also plays a transcriptional mediating role in glucose and lipid metabolism diseases, including gluconeogenesis, glycogenolysis, and lipogenesis [17]. Our experimental results showed that AKT mRNA expression increased with increasing lysine levels. The highest level was observed in the high-lysine group, while FoxO1 mRNA showed the opposite trend. Wang et al. [43] reported that compared to a low-lysine diet, lysine supplementation increased the mRNA expression levels of AKT, which was consistent with our findings. Furthermore, FoxO1 is regulated by the upstream signaling molecule AKT. After AKT phosphorylates FoxO1, FoxO1 is translocated from the nucleus. This translocation inhibits its transcriptional activity and decreases the expression of FoxO1 [44]. These results indicate that lysine supplementation could activate AKT, and the activation of AKT suppresses the mRNA expression of FoxO1, which then influences hepatic glucose and lipid metabolism in juvenile largemouth bass.

4.4. Glucose Metabolism. Glycolysis and gluconeogenesis are the decomposition and synthesis of sugar in their physiological functions [45]. Glycolysis is the only method of glucose metabolism in all organisms. Gluconeogenesis refers to the conversion of nonsugar substances into glucose, mainly in the liver [46]. In our experiment, the mRNA expression level of the glycolysis-related gene PK increased with increasing dietary lysine up to 3.68% and decreased thereafter. These results suggest that appropriate lysine supplementation could enhance the glycolytic ability of juvenile largemouth bass. A previous study on largemouth bass also showed that lysine supplementation could promote the expression of PK [24]. In addition, the mRNA levels of the gluconeogenesis-related gene PEPCK were upregulated with high dietary lysine levels (4.09% dietary lysine level); its expression was higher in this group than in all other groups. Moreover, a high plasma GLU content was observed in the high lysine supplementation group, and this finding is similar to the findings of previous studies on the essential amino acids arginine [47] and tryptophan [48] in blunt snout bream (*Megalobrama amblycephala*). Our previous study indicated that gluconeogenesis could be stimulated by high lysine levels in grass carp [15]. Furthermore, the FoxO1 transcription factor is thought to positively regulate the expression of PEPCK in the liver [49] and can also regulate the expression of key glycolysis enzymes [50]. Our results indicated that FoxO1 inhibition promoted glycolysis, while gluconeogenesis did not change significantly in the 3.33% or 3.68% dietary lysine group compared with the control group (2.11%). In contrast, the high lysine group (4.09%) promotes the expression of PEPCK mRNA. Normally, the inhibition of FoxO1 weakens the PEPCK activity; the differences may be caused by the lysine addition amount. A previous study in grass carp [15] found that high lysine levels could promote gluconeogenesis. Try to analyze the reasons, lysine is a cationic or basic amino acid with a long side chain capable of providing energy through the gluconeogenesis pathway [51], which might directly cause the high expression of the PEPCK mRNA in the high lysine treatment group. However, information regarding the regulation of FoxO1 on glucose homeostasis in fish is still scarce; thus, the regulatory mechanism of the AKT/FoxO1 pathway on hepatic glucose metabolism needs further verification.

4.5. Lipid Metabolism. Regarding lipid metabolism, FoxO1 can inhibit lipid synthesis by regulating the expression of SREBP1c [52]. The transcription factor SREBP1c participates in the synthesis of fatty acids, triglycerides, and cholesterol [53]. In this experiment, 3.33% dietary lysine inhibited FoxO1 expression and significantly suppressed the mRNA expression of SREBP1c. In addition, as the downstream transcription factors of SREBP1c, the expression levels of ACC and SCD mRNA were also downregulated significantly when juvenile largemouth bass were fed 3.33% dietary lysine. In mammals, appropriate lysine supplementation in feed can promote growth, increase the content of carnitine, and reduce triglyceride levels in tissues [13]. Previous studies in fish also found that optimal dietary lysine could reduce lipid accumulation in some fish species, including tambaqui (*Colossoma macropomum*) [54] and Nile tilapia [55]. These findings agree with our findings in juvenile largemouth bass. In addition, PPAR-γ can cooperate with SREBP1c to regulate lipid synthesis [56] and positively regulate downstream lipid synthesis-related transcription factors (FAS, ACC, and SCD) that are also regulated by FoxO1 [57]. In the present study, appropriate lysine supplementation (3.33%, 3.68%, and 4.09%) inhibited the mRNA expression of PPAR-γ and SREBP1c, which resulted in a decrease in hepatic lipid deposition.

Moreover, PPAR-α plays an important role in lipolysis and is expressed in many tissues [58]. In this study, as lysine supplementation increased, PPAR-α expression gradually increased. Additionally, the expression of the downstream transcription factor CPT1 was significantly increased when dietary lysine levels were increased to 2.92% and 3.33%. Our results indicate that appropriate dietary lysine could activate lipolysis-related genes to promote lipolysis, which might explain the low plasma TG and TC contents that were observed when the appropriate amount of lysine was supplemented in this study. Our results show that lysine supplementation could reduce the plasma TG content, but plasma TC contents were not influenced. As Zhou et al. [36] reported, lysine promotes the synthesis of carnitine, which can reduce the TG and TC contents and convert fatty acids into energy for the body. Thus, as a regulatory factor in the synthesis of carnitine, lysine indirectly regulates the plasma TG and TC contents. Furthermore, based on the hepatic lipid contents among all lysine treatments compared with the control group (Figure 3), the appropriate dietary lysine supplementation could reduce lipid accumulation in the liver of juvenile largemouth bass.

5. Conclusions

Overall, the optimal dietary lysine level for juvenile largemouth bass was determined to be 3.03% and 3.07% of the diet, according to quadratic regression analysis based on

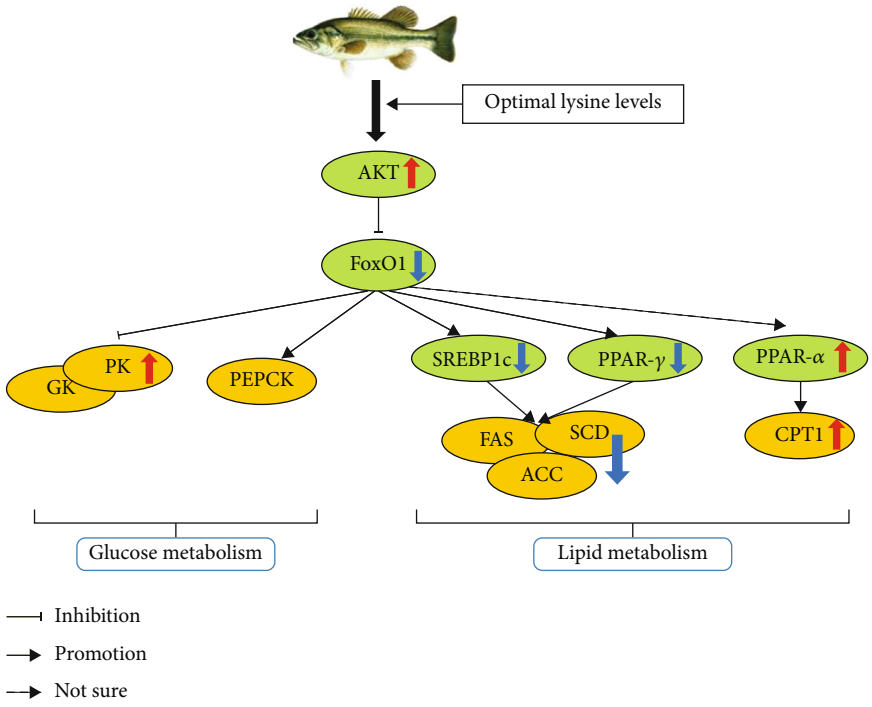

Figure 9: Scheme summarizing the mechanisms of optimal dietary lysine improved glycolipid metabolism via AKT/FoxO1 signaling pathway.

the FCR and SGR, respectively. Appropriate dietary lysine levels (3.33%) promoted glycolysis without affecting gluconeogenesis, inhibited lipid synthesis, and promoted lipolysis in the liver by regulating the AKT/FoxO1 pathway (Figure 9), resulting in decreased hepatic lipid content. Conversely, high lysine levels do not effectively regulate glycolipid levels and may lead to amino acid imbalances, causing arginine-lysine antagonism or increased lysine degradation, forcing the fish to consume more energy, and thus leading to reduced growth performance.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Mingchun Ren designed the study. Dongyu Huang carried out the experiments and wrote the manuscript. Hualiang Liang reviewed the manuscript. Jian Zhu, Songlin Li, Xiaoru Chen, and Yongli Wang provided technical assistance. Xianping Ge provided technical guidance. All authors contributed to the article and approved the submitted version.

Acknowledgments

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References

[1] R. P. Wilson, "Amino Acids and Proteins," in *Fish nutrition*, J. E. Halver and R. W. Hardy, Eds., pp. 144–175, CAAcademic Press, San Diego, 3rd ed edition, 2002.

[2] J. S. Anderson, S. P. Lall, D. M. Anderson, and M. A. McNiven, "Availability of amino acids from various fish meals fed to Atlantic salmon (*Salmo salar*)," *Aquaculture*, vol. 138, no. 1-4, pp. 291–301, 1995.

[3] I. Forster and H. Y. Ogata, "Lysine requirement of juvenile Japanese flounder P*aralichthys olivaceus* and juvenile red sea bream *Pagrus major*," *Aquaculture*, vol. 161, no. 1-4, pp. 131–142, 1998.

[4] T. Ruchimat, T. Masumoto, H. Hosokawa, Y. Itoh, and S. Shimeno, "Quantitative lysine requirement of yellowtail (*Seriola quinqueradiata*)," *Aquaculture*, vol. 158, no. 3-4, pp. 331–339, 1997.

[5] X. J. Li, X. Y. Wu, Y. Dong, Y. J. Gao, W. Yao, and Z. Y. Zhou, "Effects of dietary lysine levels on growth, feed utilization and related gene expression of juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂)," *Aquaculture*, vol. 502, pp. 153–161, 2019.

[6] S. D. Yang, L. Y. Liu, C. H. Liu, and C. H. Liou, "Assessment of dietary lysine requirement for silver perch (*Bidyanus bidyanus*) junveniles," *Aquaculture*, vol. 312, no. 1-4, pp. 102–108, 2011.

[7] G. E. Berge, H. Sveier, and E. Lied, "Nutrition of Atlantic salmon (*Salmo salar*); the requirement and metabolic effect of lysine," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 120, no. 3, pp. 477–485, 1998.

[8] B. Gridale-Helland, B. Hatlen, H. Mundheim, and S. J. Helland, "Dietary lysine requirement and efficiency of lysine utilization for growth of Atlantic cod," *Aquaculture*, vol. 315, no. 3-4, pp. 260–268, 2011.

[9] J. M. Cao, Y. Chen, X. Zhu et al., "A study on dietary L-lysine requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*," *Aquaculture Nutrition*, vol. 18, no. 1, pp. 35–45, 2012.

[10] W. W. Dai, *Lysine Requirement and Fish Meal Replacement in Diets of Tongue Sole*, Ocean University of China, Cynoglossus semilaevis Günther, 2011.

[11] N. Regmi, T. Wang, M. A. Crenshaw, B. J. Rude, and S. F. Liao, "Effects of dietary lysine levels on the concentrations of selected nutrient metabolites in blood plasma of late-stage finishing pigs," *Journal of Animal Physiology and Animal Nutrition*, vol. 102, no. 2, pp. 403–409, 2018.

[12] V. Tanphaichitr, M. S. Zaklama, and H. P. Broquist, "Dietary lysine and carnitine: relation to growth and fatty livers in rats," *The Journal of Nutrition*, vol. 106, no. 1, pp. 111–117, 1976.

[13] L. Khan and M. S. Banlji, "Tissue carnitine deficiency due to dietary lysine deficiency: triglyceride accumulation and concomitant impairment in fatty acid oxidation," *The Journal of Nutrition*, vol. 109, no. 1, pp. 24–31, 1979.

[14] B. C. Small and S. Jhjr, "Quantitative dietary lysine requirement of juvenile striped *bass Morone saxatilis*," *Aquaculture Nutrition*, vol. 6, no. 4, pp. 207–212, 2000.

[15] D. Y. Huang, H. L. Liang, M. C. Ren et al., "Effects of dietary lysine levels on growth performance, whole body composition and gene expression related to glycometabolism and lipid metabolism in grass carp, *Ctenopharyngodon idellus* fry," *Aquaculture*, vol. 530, article 735806, 2021.

[16] B. D. Manning and A. Toker, "AKT/PKB signaling: navigating the network," *Cell*, vol. 169, no. 3, pp. 381–405, 2017.

[17] S. M. Peng, W. Li, N. N. Hou, and N. Huang, "A review of FoxO1-regulated metabolic diseases and related drug discoveries," *Cell*, vol. 9, no. 1, article E184, 2020.

[18] Z. X. Gu, H. Mu, H. H. Shen et al., "High level of dietary soybean oil affects the glucose and lipid metabolism in large yellow croaker *Larimichthys crocea* through the insulin-mediated PI3K/AKT signaling pathway," *Comparative Biochemistry & Physiology Part B*, vol. 231, pp. 34–41, 2019.

[19] D. Y. Guan, H. W. Sun, J. T. Wang et al., "Rosiglitazone promotes glucose metabolism of GIFT tilapia based on the PI3K/Akt signaling pathway," *Physiological reports*, vol. 9, no. 5, article e14765, 2021.

[20] Fisheries and Aquaculture Software, *Fish Stat J-software for fishery and aquaculture statistical time series*, FAO Fisheries and Aquaculture Department [Online], Rome, 2020.

[21] Ministry of Agriculture and Rural Affairs of the People's Republic of China, "China fishery statistical yearbook," p. 25, 2021.

[22] J. K. Dairiki, C. T. D. S. Dias, and J. E. P. Cyrino, "Lysine requirements of largemouth bass, *Micropterus salmoides*: a comparison of methods of analysis of dose-response trials data," *Journal of Applied Aquaculture*, vol. 19, no. 4, pp. 1–27, 2007.

[23] S. D. Coyle, J. H. Tidwell, H. James, and C. D. Webster, "Response of largemouth bass *Micropterus salmoides* to dietary supplementation of lysine, methionine, and highly unsaturated fatty acids," *Journal of the World Aquaculture Society*, vol. 31, no. 1, pp. 89–95, 2000.

[24] P. Yang, W. Q. Wang, S. Y. Chi, K. S. Mai, F. Song, and L. Wang, "Effects of dietary lysine on regulating GH-IGF system, intermediate metabolism and immune response in largemouth bass (*Micropterus salmoides*)," *Aquaculture Reports*, vol. 17, article 100323, 2020.

[25] G. I. Hemre, T. P. Mommsen, and A. Krogdahl, "Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes," *Aquaculture Nutrition*, vol. 8, no. 3, pp. 175–194, 2002.

[26] M. C. Ren, Y. J. Liao, J. Xie et al., "Dietary arginine requirement of juvenile blunt snout bream, *Megalobrama amblycephala*," *Aquaculture*, vol. 414-415, pp. 229–234, 2013.

[27] AOAC (Association of Official Analytical Chemists), *Official Methods of Analysis of the Association of Official Analytical Chemists*, Washington, DC, USA, Association of Official Analytical Chemists, 15th ed. edition, 2003.

[28] M. W. Pfaffle, "A new mathematical model for relative quantification in real-time RT-PCR," *Nucleic Acids Research*, vol. 29, no. 9, pp. 45e–445, 2001.

[29] J. Madrid, C. Pohlenz, M. T. Viana, and J. P. Lazo, "Dietary lysine requirement for juvenile, *Totoaba macdonaldi*," *Aquaculture*, vol. 500, pp. 92–98, 2019.

[30] S. Ebeneezar, P. Vijayagopal, P. P. Srivastava et al., "Dietary lysine requirement of juvenile Silver pompano, *Trachinotus blochii* (Lacepede, 1801)," *Aquaculture*, vol. 511, article 734234, 2019.

[31] I. Ahmed and M. A. Khan, "Dietary lysine requirement of fingerling Indian major carp, *Cirrhinus mrigala* (Hamilton)," *Aquaculture*, vol. 235, no. 1-4, pp. 499–511, 2004.

[32] S. J. Kaushik and B. Fauconneau, "Effects of lysine administration on plasma arginine and on some nitrogenous catabolites in rainbow trout," *Comparative Biochemistry and Physiology Part A: Physiology*, vol. 79, no. 3, pp. 459–462, 1984.

[33] National Research Council, *Nutrient Requirements of Fish and Shrimp*, National Academy Press, Washington, DC, 2011.

[34] K. D. Shearer, "Factors affecting the proximate composition of cultured fishes with emphasis on salmonids," *Aquaculture*, vol. 119, no. 1, pp. 63–88, 1994.

[35] A. Z. Farhat and M. A. Khan, "Dietary L-lysine requirement of fingerling stinging catfish, *Heteropneustes fossilis* (Bloch) for optimizing growth, feed conversion, protein and lysine deposition," *Aquaculture Research*, vol. 44, no. 4, pp. 523–533, 2013.

[36] F. Zhou, J. Shao, R. Xu, J. Ma, and Z. Xu, "Quantitative L-lysine requirement of juvenile black sea bream (*Sparus macrocephalus*)," *Aquaculture Nutrition*, vol. 16, no. 2, pp. 194–204, 2010.

[37] P. A. Marcouli, M. N. Alexis, A. Andriopoulou, and J. Iliopoulou-Georgudaki, "Dietary lysine requirement of juvenile gilthead seabream *Sparus aurata* L," *Aquaculture Nutrition*, vol. 12, no. 1, pp. 25–33, 2006.

[38] E. Prabu, N. Felix, A. Uma, and J. Praveenraj, "Effects of dietary L-lysine supplementation on growth, body composition and muscle-growth-related gene expression with an estimation of lysine requirement of GIFT tilapia," *Aquaculture Nutrition*, vol. 26, no. 2, pp. 568–578, 2020.

[39] X. X. Li, L. L. Zhou, H. Y. Mo, Q. Pan, and L. Gan, "Interaction effects of dietary lipid and lysine on growth feed utilization and body composition of juvenile grass carp (*Ctenopharyngodon*

idella)," *Aquaculture International*, vol. 25, no. 4, pp. 1591–1606, 2017.

[40] X. Li, T. Han, S. Zheng, and G. Wu, "Hepatic Glucose Metabolism and Its Disorders in Fish," in *Recent Advances in Animal Nutrition and Metabolism*, pp. 207–236, Springer, Cham, 2022.

[41] M. Morales-Ruiz, A. Santel, J. Ribera, and W. Jiménez, *The role of Akt in chronic liver disease and liver regeneration*, vol. 37, no. 1, 2017Thieme Medical Publishers, 2017.

[42] M. Lu, M. Wan, K. F. Leavens et al., "Insulin regulates liver metabolism in vivo in the absence of hepatic Akt and Foxo 1," *Nature Medicine*, vol. 18, no. 3, pp. 388–395, 2012.

[43] W. Q. Wang, Y. J. Xu, S. Y. Chi, P. Yang, K. S. Mai, and F. Song, "Dietary lysine regulates body growth performance via the nutrient-sensing signaling pathways in largemouth bass (*Micropterus salmoides*)," *Frontiers in Marine Science*, vol. 7, 2020.

[44] D. N. Gross, M. Wan, and M. J. Bimbaum, "The role of FOXO in the regulation of metabolism," *Current diabetes reports*, vol. 9, no. 3, pp. 208–214, 2009.

[45] C. B. Cowey, D. Knox, M. J. Walton, and J. W. Adron, "The regulation of gluconeogenesis by diet and insulin in rainbow trout (*Salmo gairdneri*)," *British Journal of Nutrition*, vol. 38, no. 3, pp. 463–470, 1977.

[46] M. J. Walton and C. B. Cowey, "Aspects of intermediary metabolism in salmonid fish," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 73, no. 1, pp. 59–79, 1982.

[47] H. L. Liang, H. M. Habtetsion, X. P. Ge, M. C. Ren, J. Xie, and L. H. Miao, "Dietary arginine affects the insulin signaling pathway, glucose metabolism and lipogenesis in juvenile blunt snout bream *Megalobrama amblycephala*," *Scientific Reports*, vol. 7, no. 1, pp. 6874–7864, 2017.

[48] K. Ji, H. L. Liang, H. Chisomo-Kasiya et al., "Effects of dietary tryptophan levels on growth performance, whole body composition and gene expression levels related to glycometabolism for juvenile blunt snout bream, *Megalobrama amblycephala*," *Aquaculture Nutrition*, vol. 24, no. 5, pp. 1474–1483, 2018.

[49] A. Brunet, A. Bonni, M. J. Zigmond et al., "Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor," *Cell*, vol. 96, no. 6, pp. 857–868, 1999.

[50] K. E. Van der Vos and P. J. Coffer, "FOXO-binding partners: it takes two to tango," *Oncogene*, vol. 27, no. 16, pp. 2289–2299, 2008.

[51] S. F. Liao, T. Wang, and N. Regmi, "Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond," *Springer Plus*, vol. 4, no. 1, p. 147, 2015.

[52] W. W. Zhang, S. Patil, B. Chauhan et al., "Fox O1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression," *Journal of Biological Chemistry*, vol. 281, no. 15, pp. 10105–10117, 2006.

[53] J. D. Horton, J. L. Goldstein, and M. S. Brown, "SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver," *Journal of Clinical Investigation*, vol. 109, no. 9, pp. 1125–1131, 2002.

[54] S. O. Teixeira, Z. M. P. Nunes, A. S. P. Junior et al., "Optimal dietary lysine improves growth performance, increases protein deposition and reduces lipid accumulation in tambaqui (*Colossoma macropomum*) juveniles," *Aquaculture Research*, vol. 51, no. 12, pp. 5065–5073, 2020.

[55] R. de Souza Romaneli, T. M. do Nascimento, R. M. Gous et al., "Response of Nile tilapia (*Oreochromis niloticus*) to lysine: performance, body composition, maintenance and efficiency of utilization," *Aquaculture*, vol. 538, article 736522, 2021.

[56] J. Kerner and C. Hoppel, "Fatty acid import into mitochondria," *Biochimica et Biophysica Acta*, vol. 1486, no. 1, pp. 1–17, 2000.

[57] L. Ioannilli, F. Ciccarone, and M. R. Ciriolo, "Adipose tissue and FoxO1: bridging physiology and mechanisms," *Cell*, vol. 9, no. 4, article E849, 2020.

[58] T. Hashimoto, W. S. Cook, C. Qi, A. V. Yeldandi, J. K. Reddy, and M. S. Rao, "Defect in peroxisome proliferator-activated receptor α-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting," *Journal of Biological Chemistry*, vol. 275, no. 37, pp. 28918–28928, 2000.