Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 2485134, 11 pages
https://doi.org/10.1155/2022/2485134

Research Article

Estimation of the Optimum Dietary Protein to Lipid Ratio in Juvenile Pengze Crucian Carp (*Carassius auratus* Var. Pengze)

Liyun Ding,[1] **Wenjing Chen**,[1] **Huiyun Fu**,[1] **Jun Xiao**,[1] **Yilong Fu**,[1] **and Jingjing Ma**[2]

[1]*Jiangxi Fisheries Research Institute, Nanchang 330039, China*
[2]*Jiangsu Costal Area Institute of Agricultural Sciences, Yancheng 224002, China*

Correspondence should be addressed to Liyun Ding; dingliyun2008@163.com and Jingjing Ma; mjj-1981@163.com

Received 4 May 2022; Revised 20 July 2022; Accepted 23 July 2022; Published 6 August 2022

Academic Editor: Mansour Torfi Mozanzadeh

An 8-week feeding experiment was conducted to evaluate the effect of dietary protein to lipid ratio on fish growth performance, digestive enzyme activity, body composition, serum biochemistry, immunity, and antioxidant capacity of *Carassius auratus* var. Pengze juveniles. Nine experimental diets were formulated in a 3×3 factorial design to contain three protein levels (30%, 35%, and 40%) and three lipid levels (5%, 8%, and 11%), with protein-to-energy (P/E) ratios ranging 19.2–27.1 mg/kJ and named as Diet 1 (30/5), Diet 2 (30/8), Diet 3 (30/11), Diet 4 (35/5), Diet 5 (35/8), Diet 6 (35/11), Diet 7 (40/5), Diet 8 (40/8), and Diet 9 (40/11), respectively. Each diet was randomly assigned to triplicate groups of 20 fish per cylindrical fiberglass tank. The fish fed with Diet 4 had the highest specific growth rate (SGR) among the 9 groups. The whole-body lipid content increased significantly with increasing dietary lipid levels regardless of dietary protein content ($P < 0.05$), whereas moisture content showed an opposite trend ($P < 0.05$). The highest values of activities of serum lysozyme (LZM) and alkaline phosphatase (AKP) were detected in the fish fed Diet 4 (35/5), whereas malondialdehyde (MDA) was lowest in the fish fed Diet 4. In addition, serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were the lowest, while high-density lipoprotein (HDL-C) and high-density lipoprotein to total cholesterol ratio (HDL-C/TC) were the highest in the Diet 4 group. The results indicate that a combination of 35.1% protein and 5.1% lipid with dietary CP/GE (mg/kJ) ratio of 24.0 is optimal for the culture of *Carassius auratus* var. Pengze juveniles.

1. Introduction

Reducing the protein content in aquatic feeds is one of the strategies to reduce the feed cost and environmental pollution for sustainable aquaculture [1]. Protein is one of the most important nutrients in aquatic feeds. It is the main component of metabolic active substances in the body and a considerable factor affecting growth performance [2]. Generally, fish growth improves with increasing dietary protein up to the optimum level. However, excessive dietary protein content also increases the cost of feed production and affects profitability [3] as well as reduces the quality of culture water with higher ammonia levels [4, 5]. Considering this, much research has been conducted to decrease dietary protein incorporation, by increasing the lipid and/or carbohydrate content in fish diets [3]. Dietary lipid plays an important role in fish nutrition as a source of energy and essential fatty acids and could promote the absorption of fat-soluble vitamins. Within certain limits, increasing dietary lipid levels improves feed efficiency and growth performance [4–6]. Lipid and carbohydrate in fish diets also serve as energy sources for protein-sparing potential [7]. Therefore, the optimum ratio of dietary protein to lipid levels should be taken into consideration in formulated fish diets.

Pengze crucian carp (*Carassius auratus* var. Pengze) is a kind of native omnivorous freshwater fish in China. This species was originally produced in several lakes of Pengze County (Jiangxi, China). It has been regarded as one of the most economically valuable species over the past decades in China because of its palatability, fast growth, better flavor, and high disease resistance [8]. Many studies on the molecular biology and reproductive biology of Pengze crucian carp have been conducted [9–11], but only few of them have reported on its nutriology. Therefore, the objective of our

study was to evaluate the optimum protein to lipid ratio by analyzing the growth performance, digestive enzyme activity, body composition, blood lipid composition, antioxidant capacity, and immunity of juvenile Pengze crucian carp fed diets containing different protein and lipid levels.

2. Materials and Methods

2.1. Diet Preparation and Fish Culturing. A 3×3 factorial design with three replicates was used. Nine practical diets were formulated to contain three protein levels (30, 35, and 40%) and three lipid levels (5, 8, and 11%) to produce protein-to-energy (P/E) ratios in the range of 19.2–27.1 mg/kJ. Fish meal and fermented soybean meal were used as the main protein sources and fish oil and soybean oil (1 : 1) as the main lipid sources. The diets were designated as Diet 1 (30/5), Diet 2 (30/8), Diet 3 (30/11), Diet 4 (35/5), Diet 5 (35/8), Diet 6 (35/11), Diet 7 (40/5), Diet 8 (40/8), and Diet 9 (40/11), respectively. Formulation and proximate composition of the diets are presented in Table 1.

2.2. Experimental Fish and Feeding Trial. Carassius auratus var. Pengze juveniles were obtained from Jiangxi Fisheries Research Institute, Nanchang. Prior to the experiment, the fish were stocked into experimental tanks to adapt to the new rearing conditions for 15 days. After acclimatization, a total of 540 fish with initial weight of 31.58 ± 0.19 g were randomly distributed into 27 cylindrical fiberglass tanks (Φ800 mm $\times$ 650 mm), with 20 juveniles per tank. Each tank was then randomly assigned to one of three replicates of the nine experimental diets. The fish were cultured indoor with natural photoperiod. During the trial, the fish were fed to apparent satiation twice (09:00 and 17:00 h) daily for 56 days. The water temperature was 26 ± 1.5°C, pH remained 7.53 ± 0.12, dissolved oxygen > 7 mg/L, and ammonia nitrogen < 0.1 mg/L.

2.3. Sample Collection and Chemical Analysis. After the 56-day feeding trial, the total number and mean body weight of the fish in each tank were measured after 24 h starvation. Six fish per tank were randomly collected and stored (-20°C) for determination of proximate body composition. Another six fish from each tank were anesthetized with MS222 (100 mg/L); blood was collected from the caudal vein with a 1 mL syringe, left undisturbed for 4 h in the lab to clot (4°C), and centrifuged at 4000 g for 10 min at 4°C and immediately stored at -80°C until analysis. Samples of midintestine were dissected from six fish in each tank to determine digestive enzyme activity.

Standard methods of the Association of Official Analytical Chemists [12] were followed for the analysis of crude protein, crude lipids, moisture, and crude ash contents in fish body and diets. Briefly, moisture was determined by drying the samples to a constant weight at 105°C. The crude protein contents ($N \times 6.25$) were determined using the Dumas combustion method with a protein analyzer (FP-528, Leco, USA). Ash contents were determined using a muffle furnace run at 550°C for 8 h. Crude lipid contents were determined via the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Gross energy content in diets was measured using a bomb calorimeter (Parr 1351; Parr Instrument Co., Moline, IL, USA).

The levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in pooled serum were determined by an automatic Chemistry Analyzer (Hitachi 7600, Tokyo, Japan). Fish antioxidant capacity (total superoxide dismutase (TSOD, A001-3-2), catalase (CAT, A007-2-1), malondialdehyde (MDA, A003-1-2), and total antioxidant capacity (T-AOC, A015-1-2)), immunity (alkaline phosphatase (AKP, A059-2-2) and lysozyme (LZM, A050-1-1)), and intestinal digestive enzymes (trypsin (A080-2-2), lipase (LPS, A054-1-1), and amylase (AMS, C016-1-1)) and liver glycogen (LG, A043-1-1) were all determined using diagnostic reagent kits (Jiancheng Bioengineering Ltd., Nanjing, China) according to the instruction manuals of the manufacturer.

2.4. Statistical Analysis. All data were subjected to analysis of variance using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) for Windows. One-way ANOVA was used to determine whether there were significant differences between the treatments. Two-way ANOVA was used to determine the effect of dietary protein, dietary lipid, and their interaction. Differences among the means were tested by Tukey's multiple-range test. The level of significance was chosen at $P < 0.05$. The results are presented as means ± SD.

3. Results

3.1. Effect of Dietary Protein and Lipid Levels on Fish Growth Performance and Feed Utilization. Dietary protein and lipid combinations significantly affected the WG, SGR, HSI, and Fulton's condition factor (K) ($P > 0.05$), but did not influence FCR and VSI ($P < 0.05$) of *Carassius auratus* var. Pengze juveniles (Table 2). VSI and K were not significantly affected by dietary protein level, while WG, FCR, SGR, and HSI were not affected by dietary lipid level. Fish fed diet with 35% protein exhibited higher WG and SGR and lower FCR compared with those fed diets with 30% and 40% protein, whereas HSI was significantly depressed by dietary protein higher than 30%. VSI was elevated by dietary 8% and 11% lipid levels, while K of fish fed diets with 5% and 8% lipid levels was significantly lower than that of those fed diets with 11% lipid. Feed intake was lower in fish fed diets with 35% protein compared to fish fed diets with 30% and 40% protein, whereas feed intake was not influenced by dietary lipid levels. The fish fed diets with 35% protein showed lower FCR when compared with the groups fed 30% and 40% protein. According to one-way ANOVA, fish fed the diet with 35% protein and 5% lipid showed the highest WG and SGR, the lowest FCR, and lower VSI and HSI. Fish liver glycogen decreased from 36.16 mg/g to 25.09 mg/g among all dietary treatments and was significantly depressed by dietary protein levels at the same lipid level ($P < 0.05$).

3.2. Effect of Dietary Protein and Lipid Levels on Fish Whole-Body Proximate Composition. No interaction was found

Table 1: Formulation and proximate composition of the experimental diets (g/100 g dry matter).

Ingredient	Diet groups (protein/lipid)								
	30/5	30/8	30/11	35/5	35/8	35/11	40/5	40/8	40/11
Fermented soybean meal[1]	40	40	40	49	49	49	58	58	58
Fish meal[1]	10	10	10	10	10	10	10	10	10
Corn starch[1]	40	37	34	31	28	25	22	19	16
Fish oil	1.5	3	4.5	1.5	3	4.5	1.5	3	4.5
Soybean oil	1.5	3	4.5	1.5	3	4.5	1.5	3	4.5
Calcium biphosphate	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix[2]	1	1	1	1	1	1	1	1	1
Mineral premix[3]	2	2	2	2	2	2	2	2	2
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	2	2	2	2	2	2	2	2	2
Proximate composition									
Dry matter	90.8	89.2	89.8	90.1	89.9	89.3	90.2	91.5	91.3
Crude protein (CP)	30.3	30.7	30.5	35.1	35.3	35.4	39.8	40.7	40.8
Crude lipid	4.7	7.8	11.1	5.1	8	11.2	4.9	7.9	11.1
Ash	8.9	8.7	8.9	9.1	8.6	8.8	8.8	9.0	9.2
Gross energy (GE, kJ/g)	14.7	15.2	15.9	14.6	15.3	15.9	14.7	15.5	16.1
CP:GE (mg/kJ)	20.6	20.2	19.2	24.0	23.1	22.3	27.1	26.3	25.3

[1]Proximate composition as % dry-weight basis (fish meal (71.8% crude protein, 9.5% crude lipid); fermented soybean meal (54.2% crude protein, 2.7% crude lipid); corn starch (1.2% crude protein, 0% crude lipid)). [2]Vitamin premix (per kg diet): thiamin, 15 mg; riboflavin, 25 mg; pyridoxine, 15 mg; cyanocobalamine, 0.2 mg; folic acid, 5 mg; calcium pantothenate, 50 mg; inositol, 500 mg; niacin, 100 mg; biotin (2%), 1.2 mg; ascorbic acid, 100 mg; vitamin A, 12000 IU; vitamin D_3, 5000 IU; vitamin E, 55 mg; vitamin K_3, 5 mg. [3]Mineral premix (mg/kg diet): $MgSO_4 \cdot 7H_2O$: 450; $FeSO_4 \cdot 7H_2O$: 250; $CuSO_4 \cdot 5H_2O$: 10; $ZnSO_4 \cdot 7H_2O$: 108; $MnSO_4 \cdot 4H_2O$: 40; KI: 1.5; NaCl: 600; KH_2PO_4: 1350; $CoSO_4 \cdot 4H_2O$, 0.50.

between dietary protein and lipid levels for fish whole-body moisture, crude protein, and crude ash contents except for crude lipids (Table 3). Whole-body moisture, crude lipid, and crude ash contents were not significantly impacted by dietary protein levels, whereas crude protein and ash contents were not affected by dietary lipid levels to a significant extent ($P > 0.05$). Whole-body crude protein content increased with increasing dietary protein levels and reached the highest in the 40% protein group. Whole-body moisture content decreased, whiles whole-body crude lipid content increased, with increasing dietary lipid level.

3.3. Effect of Dietary Protein and Lipid Levels on Fish Digestive Enzyme Activities. Significant interactive effects were found between dietary protein and lipid levels on fish intestinal trypsin and lipase activities ($P < 0.05$), with no interaction detected in amylase activity ($P > 0.05$) (Table 4). Trypsin activity was not significantly affected by dietary lipid level, but its activity increased with increasing dietary protein level, with no significant difference between the 35% and 40% protein groups. Amylase activity decreased with increasing dietary protein level. Lipase activity was the highest in fish fed diet with 35% protein and then decreased, with no significant difference detected between that of fish fed diets with 30% and 40% protein. Amylase activity was depressed by dietary lipid levels higher than 5%, and lipase activity was significantly improved by 11% dietary lipid.

3.4. Effect of Dietary Protein and Lipid Levels on Fish Immunity and Antioxidant Capacity. Fish immunity and antioxidant capacity parameters determined in this study were significantly affected by dietary protein, dietary lipid, and their interactions ($P < 0.05$), excluding CAT, which was not influenced by dietary protein levels ($P > 0.05$) (Table 5). In the serum, both LZM and AKP were reduced by dietary lipids (8% and 11%) and dietary protein (40%). TSOD was elevated by dietary protein at 35% and dietary lipid levels higher than 5%. Liver TSOD and CAT were decreased by dietary lipid levels higher than 5%, while no significant differences in both parameters were found among fish from all the dietary protein groups. TAOC was highest in fish fed the diets with 35% protein, at 5% and 8% dietary lipid levels. MDA content generally reduced at 35% and 40% dietary protein levels and increased at 8% dietary lipid level. Fish fed Diet 4 had the lowest MDA content, the highest activities of the rest of the enzymes, and the highest TAOC content.

3.5. Effect of Dietary Protein and Lipid Levels on Fish Blood Lipid Metabolism. Except TG, all the serum lipid parameters were significantly affected by dietary treatments ($P < 0.05$) (Table 6). Interactions between dietary protein and lipid levels were detected for all indices but TG. Contents of TC and LDL-C were significantly improved by dietary lipid levels higher than 5%, irrespective of dietary protein contents. HDL-C and HDL-C/TC were all elevated by dietary protein at 35% and 40%, independent of dietary lipid levels. The HDL-C/LDL-C ratio was significantly enhanced by 35% dietary protein and 5% dietary lipid levels. Fish fed Diet 4 had the highest HDL-C, HDL-C/LDL-C, and HDL-C/TC, as well as the lowest TG, TC, and LDL-C.

Table 2: Growth response, feed utilization, and physical parameters of Pengze crucian carp (*Carassius auratus* var. Pengze) juveniles fed diets with different protein and lipid levels[1].

Diet no. (protein/lipid)	IBW[2]	WG[3] (%)	Feed intake[4] (g/100 g ABW[5]/d)	FCR[6]	SGR[7]	VSI[8] (%)	HSI[9] (%)	K[10]	Liver glycogen
Diet 1 (30/5)	31.58 ± 0.19	98.76 ± 1.68^{a}	1.67 ± 0.05^{b}	1.41 ± 0.06^{b}	1.23 ± 0.02^{a}	20.09 ± 0.77^{ab}	3.26 ± 0.34^{ab}	3.02 ± 0.00^{a}	36.16 ± 3.24^{b}
Diet 2 (30/8)	31.53 ± 0.15	100.94 ± 0.41^{ab}	1.59 ± 0.03^{ab}	1.36 ± 0.00^{ab}	1.25 ± 0.00^{ab}	20.90 ± 0.42^{ab}	4.02 ± 0.12^{c}	3.21 ± 0.05^{d}	35.72 ± 3.38^{b}
Diet 3 (30/11)	31.61 ± 0.04	101.35 ± 0.78^{ab}	1.62 ± 0.02^{ab}	1.34 ± 0.02^{ab}	1.25 ± 0.01^{ab}	20.71 ± 0.42^{ab}	3.61 ± 0.21^{bc}	3.22 ± 0.02^{d}	34.55 ± 3.48^{b}
Diet 4 (35/5)	31.60 ± 0.12	105.01 ± 1.63^{c}	1.63 ± 0.0^{ab}	1.29 ± 0.04^{a}	1.28 ± 0.01^{c}	20.42 ± 0.30^{ab}	2.99 ± 0.06^{a}	3.19 ± 0.02^{cd}	31.24 ± 1.23^{ab}
Diet 5 (35/8)	31.69 ± 0.34	102.95 ± 0.38^{bc}	1.59 ± 0.02^{ab}	1.31 ± 0.02^{a}	1.26 ± 0.01^{bc}	21.79 ± 0.38^{b}	3.16 ± 0.51^{ab}	3.05 ± 0.05^{ab}	30.59 ± 3.52^{ab}
Diet 6 (35/11)	31.64 ± 0.40	101.99 ± 1.28^{abc}	1.61 ± 0.02^{ab}	1.29 ± 0.03^{a}	1.26 ± 0.01^{abc}	21.51 ± 0.72^{ab}	3.26 ± 0.08^{ab}	3.17 ± 0.08^{cd}	29.70 ± 1.43^{ab}
Diet 7 (40/5)	31.73 ± 0.11	100.60 ± 0.72^{ab}	1.61 ± 0.02^{ab}	1.35 ± 0.02^{ab}	1.24 ± 0.01^{ab}	19.43 ± 2.19^{a}	3.04 ± 0.39^{a}	3.11 ± 0.05^{bc}	26.38 ± 0.61^{a}
Diet 8 (40/8)	31.74 ± 0.07	101.10 ± 0.95^{ab}	1.56 ± 0.06^{a}	1.34 ± 0.03^{ab}	1.25 ± 0.00^{ab}	20.99 ± 0.57^{ab}	2.82 ± 0.85^{a}	3.06 ± 0.06^{ab}	25.86 ± 2.06^{a}
Diet 9 (40/11)	31.68 ± 0.03	101.43 ± 1.24^{ab}	1.61 ± 0.02^{ab}	1.34 ± 0.03^{ab}	1.25 ± 0.01^{ab}	21.67 ± 2.05^{b}	3.03 ± 0.03^{a}	3.16 ± 0.00^{cd}	25.09 ± 2.90^{a}
Protein level (%)									
30		100.35 ± 1.53^{u}	1.64 ± 0.03^{v}	1.37 ± 0.04^{v}	1.24 ± 0.01^{u}	20.57 ± 0.61	3.63 ± 0.39^{v}	3.15 ± 0.10	35.48 ± 0.83^{w}
35		103.32 ± 1.85^{v}	1.58 ± 0.02^{u}	1.30 ± 0.03^{u}	1.27 ± 0.02^{v}	21.24 ± 0.76	3.14 ± 0.28^{u}	3.14 ± 0.08	30.51 ± 0.77^{v}
40		101.04 ± 0.93^{u}	1.61 ± 0.01^{uv}	1.34 ± 0.03^{v}	1.25 ± 0.00^{u}	20.70 ± 1.82	2.96 ± 0.23^{u}	3.11 ± 0.06	25.78 ± 0.65^{u}
Lipid level (%)									
5		101.46 ± 3.04	1.64 ± 0.04	1.35 ± 0.07	1.25 ± 0.03	19.98 ± 1.25^{x}	3.10 ± 0.29	3.11 ± 0.75^{x}	31.26 ± 4.89
8		101.67 ± 1.11	1.61 ± 0.02	1.33 ± 0.03	1.25 ± 0.01	21.23 ± 0.58^{y}	3.33 ± 0.60	3.11 ± 0.09^{x}	30.72 ± 4.93
11		101.59 ± 1.25	1.59 ± 0.03	1.32 ± 0.03	1.25 ± 0.01	21.30 ± 1.19^{y}	3.30 ± 0.28	3.18 ± 0.05^{y}	29.78 ± 4.73
Two-way ANOVA (P value)									
Protein		0.000	0.003	0.000	0.000	0.413	0.000	0.168	0.000
Lipid		0.935	0.102	0.208	0.923	0.035	0.136	0.003	0.498
Protein × lipid		0.010	0.610	0.244	0.010	0.765	0.047	0.000	1.000

[1]Data are expressed as mean ± SD ($n = 3$). Values in the same column with different superscripts are significantly different ($P < 0.05$). [uvw]Significant ($P < 0.05$) difference between protein levels within the same lipid level. [xy]Significant ($P < 0.05$) difference between lipid levels within the same protein level. [2]IBW: initial body weight (g). [3]WG: weight gain(%) = 100 × (final body weight (g) − initial body weight (g))/initial body weight (g). [4]Feed intake (g/100 g ABW/d) = feed consumption (g)/(ABW [g] × feeding days). [5]ABW: average body weight (g) = (final body weigh (g) + initial body weight (g))/2. [6]FCR: feed conversion ratio = dry feed fed (g)/weight gain (g). [7]SGR: specific growth rate (%/day) = 100 × (ln final body weight − ln initial body weight)/feeding days. [8]VSI: viscerosomatic index (%) = 100 × visceral weight (g)/whole body weight (g). [9]HSI: hepatosomatic index (%) = 100 × liver weight (g)/whole body weight (g). [10]K: K, Fulton's condition factor (g/cm^3) = 100 × final body weight (g)/final body length^3 (cm^3). [11]LG: liver glycogen (mg/g).

Table 3: Body composition (wet weight basis) of Pengze crucian carp (*Carassius auratus* var. Pengze) juveniles fed diets with different protein and lipid levels[1].

Diet no. (protein/lipid)	Moisture	Crude protein	Crude lipid	Ash
Diet 1 (30/5)	70.15 ± 0.87^{c}	16.89 ± 0.20	4.57 ± 0.41^{abc}	4.78 ± 0.06
Diet 2 (30/8)	67.99 ± 0.52^{abc}	16.73 ± 0.51	6.55 ± 0.36^{ef}	4.88 ± 0.30
Diet 3 (30/11)	67.91 ± 1.19^{abc}	16.76 ± 0.70	6.79 ± 0.42^{f}	4.93 ± 0.04
Diet 4 (35/5)	70.95 ± 0.93^{c}	16.73 ± 0.36	4.39 ± 0.13^{ab}	4.85 ± 0.04
Diet 5 (35/8)	68.96 ± 0.75^{abc}	16.89 ± 0.04	5.62 ± 0.21^{de}	4.79 ± 0.05
Diet 6 (35/11)	66.17 ± 2.33^{ab}	17.61 ± 0.05	5.48 ± 0.27^{cd}	5.07 ± 0.82
Diet 7 (40/5)	71.38 ± 1.66^{c}	17.83 ± 0.84	3.81 ± 0.33^{a}	4.97 ± 0.15
Diet 8 (40/8)	69.42 ± 0.38^{bc}	17.98 ± 0.48	5.28 ± 0.04^{bcd}	4.83 ± 0.05
Diet 9 (40/11)	65.47 ± 2.19^{a}	18.13 ± 1.05	6.07 ± 0.64^{def}	5.03 ± 0.10
Protein level (%)				
30	68.68 ± 1.35	16.80 ± 0.45^{u}	5.97 ± 1.11	4.86 ± 0.17
35	68.69 ± 2.45	17.08 ± 0.44^{u}	5.16 ± 0.61	4.90 ± 0.43
40	68.76 ± 2.95	17.98 ± 0.72^{v}	5.05 ± 1.06	4.94 ± 0.13
Lipid level (%)				
5	70.83 ± 1.18^{z}	17.15 ± 0.69	4.26 ± 0.44^{x}	4.87 ± 0.12
8	68.79 ± 0.80^{y}	17.20 ± 0.68	5.82 ± 0.61^{y}	4.84 ± 0.16
11	66.52 ± 2.02^{x}	17.50 ± 0.87	6.11 ± 0.70^{y}	4.50 ± 0.42
Two-way ANOVA (P value)				
Protein	0.992	0.001	0.000	0.846
Lipid	0.000	0.397	0.000	0.430
Protein × lipid	0.140	0.637	0.037	0.933

[1]Data are expressed as mean ± SD ($n = 3$). Values in the same column with different superscripts are significantly different ($P < 0.05$). [uv]Significant ($P < 0.05$) difference between protein levels within the same lipid level. [xyz]Significant ($P < 0.05$) difference between lipid levels within the same protein level.

4. Discussion

4.1. Estimation of Optimum Dietary Protein and Lipid Levels. The purpose of this study was to estimate the optimum protein and lipid combination for *Carassius auratus* var. Pengze juveniles based on growth performance, feed utilization, body composition, and blood biochemistry. In the present study, the best SGR, WG, and FCR occurred in fish fed the diet containing 35.1% protein and 5.1% lipid, suggesting these levels are optimal for Pengze crucian carp. The lower growth rate as well as higher feed conversion ratio beyond this protein level can be attributed to the inability of the fish to effectively utilize the dietary protein above the optimum level [13]. Our results are comparable to previous research on common carp, in which the optimum protein and lipid levels were reported to be 34.61% and 5.58%, respectively [14]. Moreover, the determined P/E ratio (24.0 mg protein/kJ) in our study is within the estimated range of 19.45 to 26.89 mg protein/kJ [2] and is similar to that reported in hybrid grouper *E. fuscoguttatus*×*E. lanceolatus* (23.9 mg protein/kJ) [15]. However, similar to other fish species consuming various lipid diets, such as *Sparus aurata* [16], *R. canadum* [17], and *S. rivulatus* [6], no protein-sparing effect of dietary lipid was found in our study. This reveals that it is necessary to carefully investigate the appropriate dietary lipid level for all cultured species [18].

4.2. Effect of Dietary Protein and Lipid Levels on Fish Physical Parameters. Physical indices such as hepatosomatic index (HSI), viscerosomatic index (VSI), and K indicate the body condition of fish [13]. VSI is an important index that directly affects fish yield [17, 19]. In the present study, fish fed diets with higher lipid levels (8% and 11%) had higher VSI irrespective of dietary protein levels, indicating the increment of VSI mainly resulted from increased lipid retention in viscera. This result is similar to the findings in yellow drum *Nibea albiflora* (Richardson) [5]. HSI often serves as an important parameter for assessing the nutritional status and physiological conditions of fish [20], and high HSI was suggested to be correlated with worse health condition along with poor growth performance [21]. In the present study, HSI decreased dramatically with increasing dietary protein levels ($y = 0.067x + 5.5883$, $R^2 = 0.9334$, y = HSI, x = dietary protein level), suggesting that the liver might not be the main organ for body lipid reserve in *Carassius auratus* var. Pengze. It is pertinent to note here that while formulating the diets in our study, dietary corn starch level was reduced with increasing dietary protein content. According to liver glycogen deposition data, we can deduce that the decline of HSI in our study is attributable to reduction in glycogen deposition caused by the decreasing corn starch inclusion in the diets, which is in agreement with previous studies [18, 22–24]. Interestingly, both the highest HSI and the

Table 4: Digestive enzyme activities of Pengze crucian carp (*Carassius auratus* var. Pengze) juveniles fed diets with different protein and lipid levels[1].

Diet no. (protein/lipid)	Trypsin activity (U/mg protein)	Amylase activity (U/mg protein)	Lipase activity (U/g protein)
Diet 1 (30/5)	3669.86 ± 155.86^{a}	27.76 ± 1.05^{c}	5.96 ± 0.70^{a}
Diet 2 (30/8)	3974.83 ± 6.19^{ab}	24.42 ± 1.03^{bc}	7.22 ± 0.23^{b}
Diet 3 (30/11)	4383.47 ± 305.37^{bc}	21.49 ± 0.83^{ab}	10.98 ± 0.41^{e}
Diet 4 (35/5)	4857.57 ± 92.66^{c}	21.25 ± 1.28^{ab}	9.23 ± 0.14^{c}
Diet 5 (35/8)	4532.42 ± 299.31^{bc}	20.79 ± 1.81^{ab}	10.48 ± 0.44^{de}
Diet 6 (35/11)	4786.46 ± 118.86^{c}	18.39 ± 4.15^{ab}	12.07 ± 0.09^{f}
Diet 7 (40/5)	4922.85 ± 186.67^{c}	19.81 ± 1.28^{ab}	8.69 ± 0.13^{c}
Diet 8 (40/8)	4847.20 ± 166.25^{c}	19.40 ± 1.49^{ab}	9.52 ± 0.34^{c}
Diet 9 (40/11)	4115.74 ± 258.97^{ab}	15.88 ± 3.52^{a}	8.78 ± 0.11^{c}
Protein level (%)			
30	4009.4 ± 354.33^{u}	24.56 ± 2.85^{v}	8.05 ± 2.30^{u}
35	4725.5 ± 223.58^{v}	20.14 ± 2.70^{u}	10.59 ± 1.25^{v}
40	4628.6 ± 462.95^{v}	18.36 ± 2.75^{u}	9.00 ± 0.44^{u}
Lipid level (%)			
5	4483.4 ± 624.53	22.94 ± 3.81^{y}	7.96 ± 1.56^{x}
8	4451.5 ± 419.16	21.54 ± 2.59^{xy}	9.07 ± 1.48^{x}
11	4428.6 ± 359.31	18.59 ± 3.68^{x}	10.61 ± 1.47^{y}
Two-way ANOVA (P value)			
Protein	0.000	0.000	0.000
Lipid	0.844	0.001	0.000
Protein × lipid	0.000	0.620	0.000

[1]Data are expressed as mean ± SD ($n = 3$). Values in the same column with different superscripts are significantly different ($P < 0.05$). ^{uv}Significant ($P < 0.05$) difference between protein levels within the same lipid level. ^{xy}Significant ($P < 0.05$) difference between lipid levels within the same protein level.

inferior growth parameters occurred in the insufficient protein level (30%), which may lead to metabolic disorders [25] and shows that high HSI correlates with worse health conditions and poor growth performance [21]. In this trial, the highest K was recorded in fish fed diets with the highest lipid levels (11%) and the highest gross energy (15.9 and 16.1 kJ/g). This is in agreement with previous studies, in which high body lipid or visceral lipid occurred in fish fed high-energy (lipid) diets [16, 26–29]. Fish whole-body lipid content in our study also correlated positively with dietary lipid level. All the above results confirmed the view that K performs better as a predictor of energy concentration per g body weight [30].

4.3. Effect of Dietary Protein and Lipid Levels on Fish Whole-Body Proximate Composition. As well documented, lipid deposition in fish body is closely associated with dietary lipid levels [31]; increasing dietary lipid levels generally results in an increase in whole-body lipid and the decline of whole-body moisture [17, 32–36]. In the present study, with elevated dietary lipid level (x), whole-body lipid content (y_1) increased and whole-body moisture content (y_2) declined linearly ($y_1 = 0.3083x + 2.93$, $R^2 = 0.8642$; $y_2 = -0.7183x + 74.46$, $R^2 = 0.9991$). Body protein content of the test fish tended to increase with the increase in the dietary protein level irrespective of dietary lipid content, which is consistent with the tendency of hybrid sunfish juveniles [33]. Whole-body lipid content was unaffected by dietary protein level in this study, suggesting that the juvenile *Carassius auratus* var. Pengze kept lipid content constant in the body when dietary protein level varied, which was also noted for juvenile *Nibea diacanthus* and *U. cirrosa* [37, 38].

4.4. Effect of Dietary Protein and Lipid Levels on Fish Digestive Enzyme Activities. Digestive enzymes play important roles in nutrient digestion, and their activities directly reflect the digestive capacity, nutritional status, and growth performance of aquatic animals [39–41]. Proteases can hydrolyze polypeptides into amino acids, which can be used to meet the growth and metabolism of fish [42]. In the present study, two-way ANOVA shows that trypsin activity was highest in fish fed diets with 35% protein and then plateaued indispensable of dietary lipid levels. From this aspect, we can explain why those fish fed the diet containing 35.1% protein and 5.1% lipid obtained the best growth performance among all dietary treatments. In agreement, researchers also detected that the proteases of *Litopenaeus setiferus* [43] and preadult red swamp crayfish [41] responded positively to dietary protein supplementation with the respective dietary energy of 13.5-16.1 kJ/g and 16.4 kJ/g. It is documented that lipase is an inducible enzyme influenced by dietary lipid levels [5, 44, 45]. This was shown in this study as intestinal lipase activity of the test fish positively correlated with dietary lipid level. Amylase activity has been shown to be

Table 5: Immunity and antioxidant capacity of Pengze crucian carp (*Carassius auratus* var. Pengze) juveniles fed diets with different protein and lipid levels[1].

Diet no. (protein/lipid)	Serum			Liver			
	LZM (U/ml)	TSOD (U/ml)	AKP (king unit/L)	TSOD (U/mgprot)	CAT (U/mgprot)	TAOC (mmol/gprot)	MDA (nmol/mgprot)
Diet 1 (30/5)	372.10 ± 17.26^{bc}	79.53 ± 14.54^{a}	74.85 ± 5.36^{abc}	1580.97 ± 154.53^{cde}	16.81 ± 0.67^{bcd}	0.22 ± 0.03^{a}	17.40 ± 0.63^{d}
Diet 2 (30/8)	274.19 ± 16.13^{a}	148.87 ± 13.39^{abcd}	66.41 ± 6.22^{abc}	1405.35 ± 45.80^{bc}	17.73 ± 2.04^{cd}	0.21 ± 0.01^{a}	15.90 ± 1.26^{bc}
Diet 3 (30/11)	387.10 ± 32.26^{c}	178.96 ± 42.97^{cd}	77.85 ± 10.98^{bc}	1637.03 ± 24.43^{def}	13.07 ± 2.11^{ab}	0.28 ± 0.02^{b}	11.64 ± 0.97^{b}
Diet 4 (35/5)	397.10 ± 22.26^{c}	188.99 ± 10.89^{cd}	102.50 ± 0.71^{d}	2034.76 ± 34.42^{g}	23.87 ± 2.01^{e}	0.33 ± 0.02^{c}	5.99 ± 0.10^{a}
Diet 5 (35/8)	354.84 ± 0.00^{abc}	222.93 ± 49.84^{d}	78.51 ± 4.73^{c}	1725.19 ± 20.61^{ef}	14.99 ± 0.29^{abc}	0.28 ± 0.01^{bc}	16.00 ± 1.97^{cd}
Diet 6 (35/11)	290.32 ± 64.52^{bc}	154.79 ± 23.42^{abcd}	62.00 ± 0.99^{a}	1186.96 ± 72.27^{a}	11.50 ± 0.12^{a}	0.20 ± 0.02^{a}	10.80 ± 0.50^{b}
Diet 7 (40/5)	338.71 ± 16.13^{abc}	86.95 ± 25.57^{ab}	74.00 ± 5.15^{abc}	1807.19 ± 37.08^{f}	17.93 ± 1.21^{cd}	0.23 ± 0.01^{a}	13.26 ± 2.51^{bc}
Diet 8 (40/8)	290.32 ± 32.26^{ab}	124.70 ± 34.25^{abc}	63.39 ± 3.36^{abc}	1483.76 ± 5.18^{bcd}	10.93 ± 1.58^{a}	0.21 ± 0.01^{a}	13.32 ± 0.20^{bc}
Diet 9 (40/11)	290.32 ± 32.26^{ab}	167.68 ± 11.83^{bcd}	62.59 ± 4.72^{ab}	1346.50 ± 31.95^{ab}	20.18 ± 2.23^{d}	0.22 ± 0.00^{a}	11.54 ± 0.57^{b}
Protein levels							
30	344.46 ± 56.74^{v}	135.79 ± 50.09^{u}	7.30 ± 0.86^{uv}	1541.1 ± 132.67	15.87 ± 2.61	0.24 ± 0.04^{uv}	14.98 ± 2.73^{v}
35	347.42 ± 57.73^{v}	188.91 ± 40.72^{v}	8.10 ± 1.78^{v}	1649.0 ± 373.82	16.78 ± 5.62	0.27 ± 0.06^{v}	10.93 ± 4.45^{u}
40	306.45 ± 34.21^{u}	126.44 ± 41.42^{u}	6.67 ± 0.67^{u}	1545.8 ± 206.32	16.34 ± 4.44	0.22 ± 0.01^{u}	12.71 ± 4.56^{uv}
Lipid levels							
5	369.30 ± 30.12^{y}	118.49 ± 55.25^{x}	8.38 ± 1.45^{y}	1807.6 ± 212.65^{y}	19.53 ± 3.50^{y}	0.26 ± 0.05	12.22 ± 5.17^{xy}
8	306.45 ± 41.12^{x}	165.50 ± 54.07^{y}	6.94 ± 0.81^{x}	1538.1 ± 146.56^{x}	14.55 ± 3.24^{x}	0.24 ± 0.04	15.07 ± 1.76^{y}
11	322.58 ± 62.47^{x}	167.14 ± 27.27^{y}	6.75 ± 0.98^{x}	1390.2 ± 201.90^{x}	14.92 ± 4.29^{x}	0.23 ± 0.04	11.33 ± 0.73^{x}
Two-way ANOVA (P value)							
Protein	0.020	0.000	0.000	0.003	0.482	0.000	0.000
Lipid	0.001	0.003	0.000	0.000	0.000	0.006	0.000
Protein × lipid	0.002	0.004	0.000	0.000	0.000	0.000	0.000

[1]Data are expressed as mean ± SD ($n = 3$). Values in the same column with different superscripts are significantly different ($P < 0.05$). ^{uv}Significant ($P < 0.05$) difference between protein levels within the same lipid level. ^{xy}Significant ($P < 0.05$) difference between lipid levels within the same protein level. LZM = lysozyme; TSOD = total superoxide dismutase; AKP = alkaline phosphatase.

Table 6: Blood lipid composition of Pengze crucian carp (*Carassius auratus* var. Pengze) juveniles fed diets with different protein and lipid levels[1].

Diet no. (protein/lipid)	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	HDL-C/LDL-C	HDL-C/TC
Diet 1 (30/5)	4.13 ± 0.52	6.91 ± 0.56^{ab}	1.96 ± 0.22^{ab}	2.79 ± 0.90^{ab}	0.73 ± 0.15^{bc}	0.47 ± 0.01^{ab}
Diet 2 (30/8)	4.41 ± 0.68	8.31 ± 0.62^{bc}	2.21 ± 0.28^{abc}	4.24 ± 0.78^{b}	0.52 ± 0.03^{ab}	0.50 ± 0.16^{ab}
Diet 3 (30/11)	4.28 ± 0.44	9.64 ± 0.17^{c}	1.55 ± 0.08^{a}	4.30 ± 0.39^{b}	0.36 ± 0.05^{a}	0.36 ± 0.02^{a}
Diet 4 (35/5)	3.30 ± 0.47	6.04 ± 0.44^{a}	2.81 ± 0.69^{c}	2.19 ± 0.22^{a}	1.27 ± 0.19^{d}	0.84 ± 0.09^{de}
Diet 5 (35/8)	3.31 ± 0.78	8.44 ± 0.28^{bc}	2.71 ± 0.03^{bc}	4.18 ± 0.09^{b}	0.65 ± 0.02^{bc}	0.85 ± 0.21^{e}
Diet 6 (35/11)	4.12 ± 0.08	8.48 ± 0.29^{bc}	2.53 ± 0.16^{bc}	4.00 ± 0.09^{b}	0.63 ± 0.02^{bc}	0.61 ± 0.05^{bc}
Diet 7 (40/5)	3.31 ± 0.47	7.52 ± 1.02^{ab}	2.34 ± 0.06^{bc}	3.33 ± 0.91^{ab}	0.74 ± 0.18^{bc}	0.72 ± 0.12^{cde}
Diet 8 (40/8)	4.27 ± 0.21	7.54 ± 0.65^{ab}	2.67 ± 0.11^{bc}	3.65 ± 0.67^{ab}	0.75 ± 0.11^{c}	0.63 ± 0.06^{bc}
Diet 9 (40/11)	3.38 ± 0.29	8.07 ± 0.65^{bc}	2.29 ± 0.06^{abc}	3.74 ± 0.26^{ab}	0.61 ± 0.03^{bc}	0.68 ± 0.07^{cd}
Protein levels						
30	4.27 ± 0.50^{v}	8.29 ± 1.26	1.90 ± 0.34^{u}	3.78 ± 0.97	0.54 ± 0.18^{u}	0.45 ± 0.07^{u}
35	3.58 ± 0.61^{u}	7.65 ± 1.25	2.68 ± 0.37^{v}	3.46 ± 0.96	0.85 ± 0.33^{w}	0.77 ± 0.17^{v}
40	3.65 ± 0.55^{u}	7.71 ± 0.74	2.43 ± 0.19^{v}	3.57 ± 0.61	0.70 ± 0.13^{v}	0.68 ± 0.08^{v}
Lipid levels						
5	3.58 ± 0.59	6.82 ± 0.90^{x}	2.37 ± 0.52	2.77 ± 0.81^{x}	0.91 ± 0.31^{y}	0.68 ± 0.18
8	3.99 ± 0.74	8.10 ± 0.63^{y}	2.53 ± 0.29	4.02 ± 0.59^{y}	0.64 ± 0.11^{x}	0.66 ± 0.19
11	3.93 ± 0.49	8.73 ± 0.80^{y}	2.12 ± 0.45	4.01 ± 0.34^{y}	0.54 ± 0.13^{x}	0.55 ± 0.15
Two-way ANOVA (P value)						
Protein	0.013	0.059	0.000	0.503	0.000	0.000
Lipid	0.183	0.000	0.015	0.000	0.000	0.000
Protein × lipid	0.090	0.007	0.451	0.146	0.001	0.020

[1]Data are expressed as mean ± SD ($n = 3$). Values in the same column with different superscripts are significantly different ($P < 0.05$). [uv]Significant ($P < 0.05$) difference between protein levels within the same lipid level. [xy]Significant ($P < 0.05$) difference between lipid levels within the same protein level. TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C/LDL-C = HDL-C to LDL-C ratio; HDL-C/TC = HDL-C to TC ratio.

adaptive to different nutrients in feed [42]. In this trial, amylase activity showed a decreasing trend with increasing dietary protein and lipid levels, which could also be as a result of the decreased corn starch levels with increased dietary protein and lipid content, as reported in red swamp crayfish (*Procambarus clarkii*) and *Apostichopus japonicus* [41, 46].

4.5. Effect of Dietary Protein and Lipid Levels on Fish Lipid Metabolism, Immunity, and Antioxidant Capacity. As reported, high-density lipoproteins (HDLs) are involved in the transport of cholesterol from plasma to the liver, acting as "cleaners" of body tissue cholesterol, and low-density lipoproteins (LDLs) play a role in the transport of cholesterol from liver to body tissues [47]; also, total cholesterol to high-density lipoprotein ratio (TC/HDL-C) is measured as an index for prediction of CHD risk [48]. In our study, fish fed with Diet 4 presented the highest HDL-C/LDL-C and relatively higher HDL-C/TC, suggesting that the diet with 35% protein and 5% lipid is good for fish health. In addition, all serum lipid metabolism parameters showed that the endogenous lipid transport was more active in fish fed diet with 35% protein and 5% lipid, and more cholesterol was transported from tissue cells back to the liver for metabolic transformation [49]. This may be another explanation for the best growth performance in fish fed Diet 4.

The antioxidant system is vital for fish health and protects fish from oxidative stress [50, 51]. Inhibition of antioxidant enzymes like TSOD and CAT as well as reduction in TAOC could accelerate lipid peroxidation and lead to the formation of MDA in cells eventually. In the present study, the highest TSOD, CAT, and TAOC together with lowest MDA were found in the serum and liver of fish fed Diet 4, which shows that optimal dietary protein to lipid ratio could improve the antioxidant capacity of juvenile Pengze crucian carp by enhancing antioxidant enzymes. AKP and LZM are considered as reliable indices in the assessment of immune status [52, 53]. The highest activities of LZM and AKP were recorded in the serum of fish fed Diet 4, indicating that the combination of 35% protein and 5% lipid could improve fish immunity and thus enhance their ability of disease resistance.

5. Conclusion

Our results suggest that a diet containing 35.1% protein and 5.1% lipid with CP/GE (mg/kJ) ratio of 24.0 could be the

optimum dietary protein and lipid levels based on WG and SGR as well as antioxidant and immunological parameters of *Carassius auratus* var. Pengze.

Data Availability

All data sets generated for this study are included in the article.

Conflicts of Interest

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

Acknowledgments

This research was supported by the Key Research and Development Program of Jiangxi Province, China (20181BBF60016 and 20203BBF63045), the earmarked fund for Jiangxi Agriculture Research System (JXARS-03), and the Natural Science Foundation of Jiangsu Province (BK20191215).

References

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

[2] National Research Council (NRC), *Nutrient requirements of fish and shrimp*, National Academies Press, Washington, DC, 2011.

[3] S. M. Lee, I. G. Jeon, and J. Y. Lee, "Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (Sebastes schlegeli)," *Aquaculture*, vol. 211, no. 1-4, pp. 227–239, 2002.

[4] K. N. Mohanta, S. Subramanian, and V. S. Korikanthimath, "Effect of dietary protein and lipid levels on growth, nutrient utilization and whole-body composition of blue gourami, Trichogaster trichopterusfingerlings," *Journal of animal physiology and animal nutrition*, vol. 97, no. 1, pp. 126–136, 2013.

[5] L. Wang, S. Hu, B. Lou et al., "Effect of different dietary protein and lipid levels on the growth, body composition, and intestinal digestive enzyme activities of juvenile Yellow drum Nibea albiflora (Richardson)," *Journal of Ocean University of China*, vol. 17, no. 5, pp. 1261–1267, 2018.

[6] J. Ghanawi, L. Roy, D. Davis, and I. Saoud, "Effects of dietary lipid levels on growth performance of marbled spinefoot rabbitfish Siganus rivulatus," *Aquaculture*, vol. 310, no. 3-4, pp. 395–400, 2011.

[7] T. Watanabe, "Strategies for further development of aquatic feeds," *Fisheries Science*, vol. 68, no. 2, pp. 242–252, 2002.

[8] G. Yang, R. Yu, H. Qiu et al., "Beneficial effects of emodin and curcumin supplementation on antioxidant defence response, inflammatory response and intestinal barrier of Pengze crucian carp (Carassius auratusvar. Pengze)," *Aquaculture Nutrition*, vol. 26, no. 6, pp. 1958–1969, 2020.

[9] H. L. Cheng, X. Wang, Y. X. Peng, X. P. Meng, S. P. Sun, and X. Y. Shi, "Molecular cloning and tissue distribution of lipoprotein lipase full-length cDNA from Pengze crucian carp (Carassius auratus var. Pengze)," *Comparative Biochemistry And Physiology B-biochemistry & Molecular Biology*, vol. 153, no. 1, pp. 109–115, 2009.

[10] M. Li, Y. Zheng, H. Liang et al., "Molecular cloning and characterization of cat, gpx1 and Cu/Zn-sod genes in pengze crucian carp (Carassius auratus var. Pengze) and antioxidant enzyme modulation induced by hexavalent chromium in juveniles," *Comparative Biochemistry And Physiology B-biochemistry & Molecular Biology*, vol. 157, no. 3, pp. 310–321, 2013.

[11] Y. Zheng, J. Chen, Y. Liu et al., "Molecular mechanism of endocrine system impairment by 17α-methyltestosterone in gynogenic Pengze crucian carp offspring," *Ecotoxicology and Environmental Safety*, vol. 128, pp. 143–152, 2016.

[12] Association of Official Analytical Chemist, *Official methods of analysis*, AOAC, Inc, Arlington, VA, 17th edition, 2003.

[13] T. K. Sivaramakrishnan, T. S. Ambasankar, K. P. Kumar et al., "Influence of dietary protein levels on growth, feed utilization, body indices and serum profile of silver moony Monodactylus argenteus," *Aquaculture*, vol. 549, article 737823, 2022.

[14] Z. Fan, J. Li, Y. Zhang, D. Wu, and L. Wang, "Excessive dietary lipid affecting growth performance, feed utilization, lipid deposition, and hepatopancreas lipometabolism of large-sized common carp (Cyprinus carpio)," *Frontiers in Nutrition*, vol. 8, article 694426, 2021.

[15] S. Rahimnejad, I. C. Bang, J. Y. Park, A. Sade, C. Jin, and S. M. Lee, "Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile hybrid grouper, Epinephelus fuscoguttatus × E. lanceolatus," *Aquaculture*, vol. 446, pp. 283–289, 2015.

[16] J. M. Vergara, G. Lopez-Calero, L. Robaina et al., "Growth, feed utilization and body lipid content of gilthead seabream (Sparus aurata) fed increasing lipid levels and fish meals of different quality," *Aquaculture*, vol. 179, no. 1-4, pp. 35–44, 1999.

[17] J. T. Wang, Y. J. Liu, L. X. Tian et al., "Effect of dietary lipid level on growth performance, lipid deposition, hepatic lipogenesis in juvenile cobia (Rachycentron canadum)," *Aquaculture*, vol. 249, no. 1-4, pp. 439–447, 2005.

[18] J. T. Wang, P. Liu, H. Zheng et al., "Effect of dietary lipid levels on growth performance, body composition, and feed utilization of juvenile spotted knifejaw Oplegnathus punctatus," *Aquaculture Reports*, vol. 21, article 100797, 2021.

[19] J. T. Wang, X. Y. Li, T. Han et al., "Effects of different dietary carbohydrate levels on growth, feed utilization and body composition of juvenile grouper Epinephelus akaara," *Aquaculture*, vol. 459, pp. 143–147, 2016.

[20] X. Y. Wu, S. Castillo, M. Rosales, A. Burns, M. Mendoza, and D. M. Gatlin, "Relative use of dietary carbohydrate, nonessential amino acids, and lipids for energy by hybrid striped bass, Morone chrysops ♀ × M. saxatilis ♂," *Aquaculture*, vol. 435, pp. 116–119, 2015.

[21] D.-F. Deng, Z. Y. Ju, W. Dominy, R. Murashige, and R. P. Wilson, "Optimal dietary protein levels for juvenile Pacific threadfin (Polydactylus sexfilis) fed diets with two levels of lipid," *Aquaculture*, vol. 316, no. 1-4, pp. 25–30, 2011.

[22] J. D. Kim and S. P. Lall, "Effects of dietary protein level on growth and utilization of protein and energy by juvenile haddock (Melanogrammus aeglefinus)," *Aquaculture*, vol. 195, no. 3-4, pp. 311–319, 2001.

[23] D. A. Nanton, A. Vegusdal, A. M. B. Rørå, B. Ruyter, G. Baeverfjord, and B. E. Torstensen, "Muscle lipid storage pattern, composition, and adipocyte distribution in different

parts of Atlantic salmon (Salmo salar) fed fish oil and vegetable oil," *Aquaculture*, vol. 265, no. 1-4, pp. 230–243, 2007.

[24] M. Yang, J. T. Wang, T. Han, Y. X. Yang, X. Y. Li, and Y. D. Jiang, "Dietary protein requirement of juvenile bluegill sunfish (Lepomis macrochirus)," *Aquaculture*, vol. 459, pp. 191–197, 2016.

[25] Y. L. Zhang, L. Song, R. P. Liu et al., "Effects of dietary protein and lipid levels on growth, body composition and flesh quality of juvenile topmouth culter,Culter alburnusBasilewsky," *Aquaculture Research*, vol. 47, no. 8, pp. 2633–2641, 2016.

[26] S. H. Cho, S. M. Lee, and J. H. Lee, "Effect of dietary protein and lipid levels on growth and body composition of juvenile turbot (Scophthalmus maximus L) reared under optimum salinity and temperature conditions," *Aquaculture Nutrition*, vol. 11, no. 4, pp. 235–240, 2005.

[27] R. Company, J. A. Calduch-Giner, S. Kaushik, and J. Perez-Sanchez, "Growth performance and adiposity in gilthead sea bream (Sparus aurata): risks and benefits of high energy diets," *Aquaculture*, vol. 171, no. 3-4, pp. 279–292, 1999.

[28] B. B. McGoogan and D. M. Gatlin, "Dietary manipulations affecting growth and nitrogenous waste production of red drum, Sciaenops ocellatus I. Effects of dietary protein and energy levels," *Aquaculture*, vol. 178, no. 3-4, pp. 333–348, 1999.

[29] J. M. Vergara, H. Fernandez-Palacios, L. Robaina, K. Jauncey, M. De La Higuera, and M. Izquierdo, "The effects of varying dietary protein level on the growth, feed efficiency, protein utilization and body composition of gilthead sea bream Fry," *Fisheries Science*, vol. 62, no. 4, pp. 620–623, 1996.

[30] S. Chellappa, F. A. Huntingford, R. Strang, and R. Y. Thomson, "Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback," *Journal of Fish Biology*, vol. 47, no. 5, pp. 775–787, 1995.

[31] X. Yi, F. Zhang, W. Xu, J. Li, W. Zhang, and K. S. Mai, "Effects of dietary lipid content on growth, body composition and pigmentation of large yellow croaker Larimichthys croceus," *Aquaculture*, vol. 434, pp. 355–361, 2014.

[32] K. Hua, W. Koppe, and R. Fontanillas, "Effects of dietary protein and lipid levels on growth, body composition and nutrient utilization of Channa striata," *Aquaculture*, vol. 501, pp. 368–373, 2019.

[33] H. Ma, K. Liu, X. Feng, H. Huang, and N. Xie, "The optimal dietary protein and lipid levels for juvenile hybrid sunfish (lepomiscyanellus♀ ×Lepomismacrochirus♂)," *Aquaculture Nutrition*, vol. 27, no. 3, pp. 841–849, 2021.

[34] K. N. Mohanta, S. N. Mohanty, and J. K. Jena, "Protein-sparing effect of carbohydrate in silver barb, Puntius gonionotus fry," *Aquaculture Nutrition*, vol. 13, no. 4, pp. 311–317, 2007.

[35] Q. D. Wang, G. Li, B. Lin, L. Chen, and J. Zhang, "Effects of dietary protein and lipid levels on growth performance, feed utilization and biochemical parameters of barbless carp (Cyprinus pellegrini)," *Israeli Journal of Aquaculture-Bamidgeh*, vol. 70, 2018.

[36] E. Welengane, R. Y. Sado, and Á. J. D. A. Bicudo, "Protein-sparing effect by dietary lipid increase in juveniles of the hybrid fish tambatinga (♀Colossoma macropomum × ♂Piaractus brachypomus)," *Aquaculture Nutrition*, vol. 25, no. 6, pp. 1272–1280, 2019.

[37] Z. Akpinar, H. Sevgili, T. Özgen, A. Demir, and Y. Emre, "Dietary protein requirement of juvenile shi drum, Umbrina cirrosa (l.)," *Aquaculture Research*, vol. 43, no. 3, pp. 421–429, 2012.

[38] W. Li, X. Wen, J. Zhao, S. Li, and D. Zhu, "Effects of dietary protein levels on growth, feed utilization, body composition and ammonia–nitrogen excretion in juvenile Nibea diacanthus," *Fisheries Science*, vol. 82, no. 1, pp. 137–146, 2016.

[39] M. Furne, M. C. Hidalgo, A. Lopez et al., "Digestive enzyme activities in Adriatic sturgeon Acipenser naccarii and rainbow trout Oncorhynchus mykiss. A comparative study," *Aquaculture*, vol. 250, no. 1-2, pp. 391–398, 2005.

[40] H. Lemieux, P. Blier, and J. D. Dutil, "Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (Gadusmorhua)?," *Fish physiology and biochemistry*, vol. 20, no. 4, pp. 293–303, 1999.

[41] X. Lu, D. Peng, X. Chen et al., "Effects of dietary protein levels on growth, muscle composition, digestive enzymes activities, hemolymph biochemical indices and ovary development of pre-adult red swamp crayfish (Procambarus clarkii)," *Aquaculture Reports*, vol. 18, article 100542, 2020.

[42] D. F. Campeche, D. H. Andrade, A. M. Souza, J. F. Melo, and R. D. Bezerra, "Dietary protein:lipid ratio changes growth, digestive enzyme activity, metabolic profile and haematological parameters in hybrid surubim (Pseudoplatystoma fasciatum × Leiarius marmoratus)," *Aquaculture Research*, vol. 49, no. 7, pp. 2486–2494, 2018.

[43] C. Guzman, G. Gaxiola, A. Torre-Blanco, and C. Rosa, "The effect of dietary protein and total energy content on digestive enzyme activities, growth and survival ofLitopenaeus setiferus(Linnaeus 1767) postlarvae," *Aquaculture Nutrition*, vol. 7, no. 2, pp. 113–122, 2001.

[44] M. Aliyu-Paiko, R. Hashim, and A. C. Shu-Chien, "Influence of dietary lipid/protein ratio on survival, growth, body indices and digestive lipase activity in Snakehead (Channa striatus, Bloch 1793) fry reared in re-circulating water system," *Aquaculture Nutrition*, vol. 16, no. 5, pp. 466–474, 2010.

[45] X. Ma, Y. Hu, X. Q. Wang et al., "Effects of practical dietary protein to lipid levels on growth, digestive enzyme activities and body composition of juvenile rice field eel (Monopterus albus)," *Aquaculture International*, vol. 22, no. 2, pp. 749–760, 2014.

[46] Y. H. Wu, Q. Y. Wang, Z. F. Feng, B. B. Li, and W. Zhu, "The effect of dietary protein on the enzymes and intestinal structure of Apostichopus japonicas," *Marine Science*, vol. 36, no. 1, pp. 36–41, 2012.

[47] L. Luo, M. Xue, C. Vachot, I. Geurden, and S. Kaushik, "Dietary medium chain fatty acids from coconut oil have little effects on postprandial plasma metabolite profiles in rainbow trout (Oncorhynchus mykiss)," *Aquaculture*, vol. 420-421, pp. 24–31, 2014.

[48] E. Davis, F. Huffman, and E. Onuoha, "Is the LDL/HDL - cholesterol ratio a better risk indicator for coronary heart disease than the TC/HDL - cholesterol ratio?," *The FASEB Journal*, vol. 29, no. S1, 2015.

[49] J. Y. Wang, S. G. Zhu, and C. F. Xu, *Biological chemistry*, Higher Education Press, Beijing, 2002.

[50] G. Atli and M. Canli, "Response of antioxidant system of freshwater fish Oreochromis niloticus to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures," *Ecotoxicology and Environmental Safety*, vol. 73, no. 8, pp. 1884–1889, 2010.

[51] R. M. Martínez-Alvarez, A. E. Morales, and A. Sanz, "Antioxidant defenses in fish: biotic and abiotic factors," *Reviews in Fish Biology and Fisheries*, vol. 15, no. 1-2, pp. 75–88, 2005.

[52] M. A. Dawood, M. S. Gewaily, and H. Sewilam, "The growth performance, antioxidative capacity, and histological features of intestines, gills, and livers of Nile tilapia reared in different water salinities and fed menthol essential oil," *Aquaculture*, vol. 554, no. 30, article 738122, 2022.

[53] E. Zhang, S. Dong, W. Fang, X. Tian, and Q. Gao, "Effects of l-tryptophan on the growth, intestinal enzyme activities and non-specific immune response of sea cucumber (Apostichopus japonicus Selenka) exposed to crowding stress," *Fish & Shellfish Immunology*, vol. 75, pp. 158–163, 2018.