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Effects of Dietary Protein and Lipid Levels on the Growth Performance and Serum Biochemical Indices of Juvenile Furong Crucian Carp

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Abstract: The impact of dietary protein and lipid levels on the growth performance, feed utilization, and serum biochemical indices of Furong crucian carp was examined. Five hundred and forty carp (2.35 ± 0.08 g) were randomly assigned to nine groups and fed diets with three different protein levels (30.0, 35.0, and 40.0%) and three different lipid levels (4.0, 7.0, and 10.0%) for 60 days. The current findings revealed that the interaction effect between dietary lipid and protein levels exhibited significance for the final average weight (FAW), weight gain rate (WGR), specific growth rate (SGR), feed efficiency (FE), energy deposition rate (EDR), whole-fish energy, ash, and fat content ($p < 0.05$). Specifically, there was a significant reduction in FAW, WGR, and SGR with increasing dietary fat supplementation. Conversely, FE, EDR, and protein efficiency ratios were significantly decreased with increasing dietary protein levels ($p < 0.05$). Furthermore, serum albumin and globulin levels exhibited significant increases in response to dietary lipid inclusion ($p < 0.05$). The findings collectively indicate that Furong crucian carp fed a diet comprising 4% lipid and 30% protein exhibited the optimal growth and feed utilization. Conversely, excessive protein and lipid supplementation were detrimental to growth and resulted in the aggravation of metabolic disorders.

Keywords: Furong crucian carp; growth performance; protein; lipid; blood biochemical index

Key Contribution: This study comprehensively evaluated various indices such as growth, feed utilization, and physiological indices, and investigated the main and interaction effects of dietary fat and protein levels on Furong crucian carp. This study aimed to identify the appropriate fat and protein levels to optimize the feed formulation for Furong crucian carp and to provide a theoretical basis for subsequent nutritional experiments related to Furong crucian carp.

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Citation: He, Z.; Tian, X.; Li, J.; Guo, J.; Cheng, X.; Wang, D. Effects of Dietary Protein and Lipid Levels on the Growth Performance and Serum Biochemical Indices of Juvenile Furong Crucian Carp. *Fishes* **2024**, *9*, 466. https://doi.org/10.3390/fishes9110466

Academic Editors: Houguo Xu and Qiang Ma

Received: 5 October 2024
Revised: 11 November 2024
Accepted: 13 November 2024
Published: 16 November 2024

1. Introduction

Protein is an essential ingredient of aquatic feeds given that it is a key source of metabolically active substances and amino acids needed for protein synthesis [1–3]. An essential metric for assessing the cost-effectiveness and quality of feed formulations is protein content [4]. Protein can be used as a partial source of energy when energy substances (fats and carbohydrates) are in short supply in the feed. It has been found that a lack of or insufficiency in protein in the feed can lead to stunted growth [5,6]. Yet, a great deal of protein in the diet can lead to excessive proteolytic metabolism for energy consumption, which can negatively impact fish health and the environment [7–9] and lead to the decrease in the protein efficiency rate [10]. For this reason, modern aquafeed companies typically lower the amount of protein in the diet to save costs and increase farming efficiency. Accordingly, the determination of the optimum protein requirement in feeds is of great importance for both fish nutritional studies and aquaculture interests.

It is widely recognized that dietary lipids, as macronutrients, are a main source of supplying the energy requirements of aquatic animals [11]. According to a previous study,

the availability of non-protein substances and the protein levels in the diet are related to the utilization of feed protein [12]. This implies that lipids are an important nutrient for providing fish with the energy they need to survive and for maintaining the normal physiological functions of fatty acids [13,14]. The digestion and absorption of feed fats require blood circulation for transportation to other tissues and organs, and the stored fats in the fish body need to be mobilized by the transport action of the blood. Consequently, the level of blood fat in fish can reflect, to some extent, the status of fat metabolism in the whole fish body [15]. Fish commonly suffer from metabolic disorders and reduced feed conversion rates caused by deficiencies or insufficiencies of fat in the feed [16]. Nevertheless, excessive fat content in feed can lead to oxidative deterioration of the feed, excessive fat deposition in the fish, and reduced immune resistance to disease [17]. This adversely affects fish health status and growth, making it imperative that the fat requirements in feeds receive attention.

The protein-sparing effect, which occurs when fat is used instead of protein as an energy source, can lower protein intake through catabolism [3,18]. In the case of insufficient fat within the supply, part of the protein in the feed is utilized to provide the energy needed for metabolism and tissue formation [19]. Nevertheless, high levels of fat in the feed may hinder fish feeding and growth, as evidenced by fat accumulation, growth decline, and reduced digestion and absorption capacity [20–22]. In general, an optimized protein and fat content in feeds can boost growth, lower nitrogen content, and simultaneously diminish feed costs [2,18,23]. Previous studies have identified some ideal protein and fat levels for aquatic animals, such as *Channa maculate* [18,24], *Misgurnus anguillicaudatus* [25], *Colossoma macropomum* [3], *Dentex dentex* [2], and *Macrobrachium americanum* [19], encouraging the critical role of comprehensive investigations into dietary protein and lipid inclusion levels. Therefore, the protein and fat levels in feeds are the most essential parameters in feed formulation development.

Furong crucian carp is a finfish species chosen and propagated by the Hunan Fisheries Science Institute. Furong crucian carp features the characteristics of fast growth, excellent meat tenderness, and high resistance to adversity [26]. Although previous studies have determined the appropriate levels of protein and fat for carp feed [16,27,28], excessive protein and lipid inclusion levels in aquafeed may contribute to high metabolic stress in fish, which in turn hinders rapid growth regeneration, nutrient uptake, and metabolic functions [7,29]. As is often the case, the optimal level of dietary protein and lipids depends on various variables such as aquatic species, feeding rate, protein source, age, maturity stage, and habitat. Furong crucian carp is a hybrid species, and its actual requirements for feed nutrients may be different from those of other carp species. Currently, research on major nutrients in Furong crucian carp feeds remains unknown, encouraging further study on the appropriate fat and protein levels and their interactions. Hence, this study was designed to assess the impacts of different fat and protein inclusion levels in Furong crucian carp feed on growth performance, whole-fish proximate composition, digestive capacities, and blood biochemical indices, which may be used to guide the selection of culture techniques in production.

2. Materials and Methods

2.1. Ethic Statement

Hunan Fisheries Science Institute approved all experiment procedures, which adhered to the ethical standards for the care and use of laboratory animals (grant number: HNFI20210322).

2.2. Experimental Diet and Feeding Management

A two-factor design of dietary protein and fat levels was used in this experiment, with three protein (30.0, 35.0, and 40.0%) and three lipid (4.0, 7.0, and 10.0%) levels each configured into nine experimental feeds (Table 1). The selection of dietary protein and fat level used in this study referred to the previous studies on carp [30–33]. The experimental feed was a granular feed prepared at the Hunan Fisheries Science Institute (Changsha City,

Hunan Province, China), of which the feed ingredients referenced those of the crucian carp feed [32]. Fish meal, rapeseed meal, and soybean meal were the main protein sources in the experimental diet. Soybean oil was the predominant source of dietary fat. The sugar source and adhesive were wheat middling and corn starch, respectively. The procedure for preparing feed is mentioned in the earlier literature [34]. Specifically, the ingredients were mixed using the gradual expansion method following being ground and then sieved through a 60-mesh sieve. Upon blending, the feed passed through a feed pelletizer to yield pellets with a particle size of around 1.2 mm. The pellets were then allowed to air dry in a cool environment before being refrigerated at −20 °C. The proximate nutritional composition of experimental diets was measured using the previously described methodology [35], the crude protein and crude fat contents of the experimental feeds were tested with reference to the Soxhlet extraction method (GB/T 6433-2006 [36]) and the Kjeldahl method (GB/T 6432-1994 [37]), respectively, which follow Chinese national standards.

Table 1. Feed formulation (dried mass, %).

Ingredients	30/10	30/7	30/4	35/10	35/7	35/4	40/10	40/7	40/4
Fish meal	12.00	12.00	12.00	14.00	14.00	14.00	17.00	17.00	17.00
Soybean meal	36.00	36.00	36.00	42.00	42.00	42.00	51.00	51.00	51.00
Rapeseed meal	12.00	12.00	12.00	14.00	14.00	14.00	17.00	17.00	17.00
Wheat middling	16.30	16.30	16.30	18.70	18.70	18.70	3.90	3.90	3.90
Corn starch	12.00	15.00	18.00	0.00	3.00	6.00	0.00	3.00	6.00
Soybean oil	7.70	4.70	1.70	7.30	4.30	1.30	7.10	4.10	1.10
Choline chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Premix *	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient analysis									
Crude protein	30.11	30.99	30.38	35.51	35.96	35.01	40.34	40.99	40.92
Crude lipid	10.21	7.11	4.19	10.09	7.21	4.17	10.17	7.16	4.20
Energy (MJ/kg)	18.10	17.41	16.71	18.12	17.42	16.73	18.34	17.64	16.95

* Premix: contained the following per kg of the diet. Provided vitamins: VA 375,000 IU; VB1 600 mg; VB2 1000 mg; VB6 850 mg; VB12 2 mg; VC 14,000 mg; VK3 400 mg; VE 4000 mg; VD3 150,000 IU; D-biotin 8 mg; folic acid 200 mg; niacin acid 10,000 mg; D-calcium pantothenate 2200 mg; inositol 12,000 mg. Provided minerals: Se 0.02 g; Fe 6 g; Co 0.007 g; Zn 4 g; Cu 0.3 g; I 0.07 g; Mn 1.5 g.

The experiment was carried out at the Hunan Fisheries Science Institute. The whole feeding trial was undertaken in an indoor glass tank (100 × 50 × 50 cm) recirculating water aquaculture system. No feed was given during the first 2 days of releasing the fish into tanks and the whole domestication lasted for 7 days. In total, 540 carp, with an initial body weight of 2.36 (±0.02) g by group weighing, were assigned into 9 groups and fed with experimental diets for 60 days. The whole feeding trial was carried out in 27 glass tanks, with each group consisting of 3 replicates (tanks) and housing 20 fish per tank. The pH (7.6 ± 0.6), temperature (28.4 ± 3.5 °C) (measured using a thermometer), ammonia ($\leq$0.2 mg·L^{-1}) (measured with a portable colorimeter, LH-M900, CHINCAN, Zhejiang, China), and dissolved oxygen (7.2 ± 0.3 mg·L^{-1}) in the culture water were all recorded. Throughout the feeding trial, the quality of the water did not change.

2.3. Sample Collection

At the final sampling, the carp were anesthetized with eugenol ($C_{10}H_{12}O_2$, 1:12,000) according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals at the end of the 60-day feeding trial. Specifically, eugenol and ethanol were mixed at a ratio of 1:8 to formulate an anesthetic agent for use. The mixture was splashed into the bucket in which the fish were placed, ensuring that the concentration of eugenol was around 50 mg/L. The anesthesia lasted for about 1 min, after which the anaesthetized fish

were dissected. Following the selection of six carp at random from each tank, samples of the midgut, liver, and viscera were taken. For the nutritional study of the entire body, another three carp per tank were further gathered using the previously described methodology [38]. A sterile syringe of 1 mL was utilized to extract blood from the tail vein of six carp in each group, with four 2 mL EP tubes collected from each group and stored at 4 °C overnight. The blood was then centrifuged for 10 min at 4000 r/min, and the supernatant was collected and kept at −80 °C.

The fish number, feed intake, and final weight (FW, g) of fish per tank were counted and recorded by group at the end of the feeding trial, and survival rate (SR, %), specific growth rate (SGR, %/d), weight gain rate (WGR, %), and feed efficiency (FE, %) were calculated according to the formulas of the previous report [38]. Furthermore, the protein efficiency rate (PER, %) and energy deposition rate (EDR, %) were figured out as described above [39,40]. Specifically, the formulas used to calculate growth-related parameters are listed below:

$$\text{Final average weight (FAW, g)} = W_2/N_2 \times 100;$$

$$\text{Weight gain rate (WGR, \%)} = (W_2 - W_1)/W_1 \times 100;$$

$$\text{Specific growth rate (SGR, \%/d)} = (\ln W_2 - \ln W_1)/60 \times 100;$$

$$\text{Feed efficiency (FE, \%)} = (W_2 - W_1)/W_f \times 100;$$

$$\text{Protein efficiency rate (PER, \%)} = (W_2 - W_1)/(W_f \times W_p);$$

$$\text{Energy deposition rate (EDR, \%)} = E_2/E_1 \times 100;$$

$$\text{Survival rate (SR, \%)} = N_2/N_1 \times 100;$$

$$\text{Hepatosomatic index (HSI, \%)} = W_3/W_t \times 100;$$

$$\text{Viscerosomatic index (VSI, \%)} = W_4/W_t \times 100;$$

$$\text{Condition factor (CF, g/cm}^3\text{)} = W_t/L^3 \times 100.$$

In the above equations, W_1 is the total weight of fish at the beginning of the experiment (g); W_2 is the total weight of fish at the end of the experiment (g); N_1 is the initial number of fish; N_2 is the final number of fish; W_3 is the liver weight of the selected individual fish (g); W_4 is the viscera weight of the selected individual fish (g); W_t is the weight of the selected individual fish (g); W_f is the weight of the feed intake (g); W_p is the crude protein content of the feed (g); E_2 is the energy of the selected individual fish (MJ/kg, air-dried basis); E_1 is the energy of the feed ingested by the selected individual fish (MJ/kg, air-dried basis); L is the body length (cm).

2.4. Metabolites and Enzymes Assays

Intestinal and hepatic enzyme activities, such as lipase (LPS, A054-2-1), amylase (AMS, C016-1-1), and trypsin (TPS, A080-2-2), were assayed. Serum total protein (TP, A045-4-2), albumin (ALB, A028-1-1), globulin (GLB, E025-1-1), total cholesterol (TC, A111-1-1), total triglyceride (TG, A110-1-1), low-density lipoprotein cholesterol (LDL-C, A113-1-1), high-density lipoprotein cholesterol (HDL-C, A112-1-1), and total bile acid (TBA, E003-2-1) contents, and alkaline phosphatase (AKP, A059-2-2), lactate dehydrogenase (LDH, A020-2-2), alanine aminotransferase (ALT, C009-2-1), and aspartate aminotransferase (AST, C010-2-1) activities were determined following the guidelines offered by Nanjing Jiancheng Bioengineering Institute and Zhejiang ERKN Biotech Co.

2.5. Statistical Analyses

All of the statistical analyses reported in the figures and tables were performed using microchat v0.1.0 (https://mineraltsai.shinyapps.io/shinymicrochat/, accessed on 22 August 2024). Data that satisfied normality (the Shapiro–Wilk normality test) and variance homogeneity (the Bartlett test of variances) were used to investigate the main and inter-

action effects of dietary protein and lipid inclusion levels on parameters using two-way analysis of variance (ANOVA). One-way ANOVA was employed to examine the simple effect of dietary protein inclusion level on parameters when maintaining the dietary lipid levels. Tukey's HSD multiple comparison test was used to compare differences across groups, with '$p < 0.05$' indicating significant differences. The Kruskal–Wallis rank sum test was employed to assess data that did not follow a normal distribution or possess homogeneous variances, along with Dunn's test to calculate pairwise multiple comparisons of the ranked data. Different letters in the same column represent significant differences in the main effects of all tables. For all tabulated simple effects, different letters in the same column indicate a significant effect of protein on the parameter at that level of fat fixed at a certain level.

3. Results

3.1. Growth Performance

Current findings revealed that the main effect of dietary lipid inclusion level and its interaction effect with dietary protein inclusion level exhibited significance for FAW, WGR, SGR, FE, and EDR ($p < 0.05$, Table 2), while dietary protein inclusion levels presented a considerable main effect on FE, PER, and EDR ($p < 0.05$). Specifically, FAW, WGR, and SGR decreased considerably with dietary lipid inclusion levels increasing ($p < 0.05$), whereas FE, PER, and EDR decreased significantly as dietary protein inclusion levels increased ($p < 0.05$). Of note, the best growth performance was observed in carp given the 4% lipid and 40% protein diet, as evidenced by higher FAW and WGR in carp given diets that included 30% protein and 35% protein levels at a 4% dietary lipid inclusion level ($p > 0.05$). Furthermore, feed utilization was observed in carp given the 4% lipid and 30% protein diet, as indicated by the highest FE.

Table 2. Growth performance subjected to the different protein and lipid levels.

Treatment		IAW, g	FAW, g	WGR, %	SGR, %/d	SR, %	FE, %	PER, %	EDR, %
Lipid, %	**Protein, %**								
4	30	2.38	13.92 ^{a}	485.44	2.94	98.33	53.94 ^{a}	2.00 ^{a}	82.24 ^{a}
4	35	2.41	12.93 ^{b}	436.34	2.8	100.00	41.67 ^{b}	1.32 ^{b}	60.60 ^{b}
4	40	2.34	13.97 ^{a}	496.52	2.97	95.00	49.97 ^{ab}	1.39 ^{ab}	69.37 ^{ab}
7	30	2.29	11.55 ^{ab}	405.51 ^{ab}	2.70 ^{ab}	100.00	42.71	1.58 ^{a}	63.73 ^{a}
7	35	2.34	13.01 ^{a}	455.55 ^{a}	2.86 ^{a}	98.33	44.09	1.40 ^{a}	58.83 ^{ab}
7	40	2.39	11.01 ^{b}	360.53 ^{b}	2.54 ^{b}	100.00	39.34	1.09 ^{b}	54.75 ^{b}
10	30	2.38	11.30	375.50	2.60	95.00	46.60 ^{a}	1.67 ^{a}	65.05 ^{a}
10	35	2.30	11.39	395.67	2.67	98.33	46.26 ^{a}	1.47 ^{a}	61.82 ^{a}
10	40	2.35	10.58	350.14	2.51	96.67	39.89 ^{b}	1.11 ^{b}	51.71 ^{b}
Pooled *s.e.*		0.05	0.34	16.88	0.06	1.76	1.76	0.06	2.33
Main effects									
Protein, %									
30		2.35	12.26	422.15	2.75	97.78	47.75 ^{a}	1.75 ^{a}	70.34 ^{a}
35		2.35	12.44	429.18	2.77	98.89	44.01 ^{b}	1.40 ^{b}	60.42 ^{b}
40		2.36	11.86	402.4	2.67	97.22	43.07 ^{b}	1.20 ^{b}	58.61 ^{b}
Lipid, %									
4		2.38	13.61 ^{a}	472.77 ^{a}	2.91 ^{a}	97.78	48.53 ^{a}	1.57	70.74 ^{a}
7		2.34	11.86 ^{b}	407.20 ^{b}	2.70 ^{b}	99.44	42.04 ^{b}	1.36	59.10 ^{b}
10		2.34	11.09 ^{c}	373.77 ^{b}	2.59 ^{b}	96.67	44.25 ^{b}	1.42	59.53 ^{b}

Table 2. *Cont.*

Treatment		IAW, g	FAW, g	WGR, %	SGR, %/d	SR, %	FE, %	PER, %	EDR, %
Lipid, %	Protein, %								
ANOVA, *p* value									
Protein		0.915	0.121	0.160	0.104	0.625	0.010	<0.001	0.012
Lipid		0.557	<0.001	<0.001	<0.001	0.148	0.001	0.647	0.020
Protein × Lipid		0.355	0.003	0.004	0.006	0.217	0.002	0.003	0.006

Note: The presented data in the simple effects and main effects represent the mean of three and nine replicates, respectively. Different letters in each column indicate statistically significant differences at a significance level of $p < 0.05$. Pooled *s.e.* = pooled *s.d.*/SQRT (3). Specifically, pooled *s.d.* = SQRT ($S_1^2 + S_2^2 + \ldots + S_9^2$)/9) is used with equal sample sizes, where S_1 represents the standard deviation of group 1 (4% lipid and 30% protein), S_2 represents the standard deviation of group 2 (4% lipid and 35% protein), S_9 represents the standard deviation of group 9 (10% lipid and 40% protein), 3 represents the number of biological replicates, and 9 represents the number of groups. Same below.

3.2. Morphological Parameters

The interaction effect of dietary fat and protein inclusion level exhibited no significant difference in CF, VSI, and HSI ($p > 0.05$, Table 3). Among these, dietary fat and protein inclusion levels exhibited a considerable main effect ($p < 0.05$) on VSI in carp, which decreased with increasing protein inclusion levels, followed by an increase in dietary fat supplementation.

Table 3. Morphological parameters subjected to different protein and lipid levels. Different letters in the same column represent significant differences.

Treatment		CF, g/cm^3	VSI, %	HSI, %
Lipid, %	Protein, %			
4	30	3.02	10.44	1.99
4	35	2.99	9.91	1.97
4	40	3.07	10.26	2.17
7	30	3.11	11.44 ^{a}	2.29
7	35	3.02	10.81 ^{ab}	2.37
7	40	2.91	10.22 ^{b}	2.13
10	30	3.10 ^{a}	11.49	2.45
10	35	3.11 ^{a}	11.56	2.53
10	40	3.01 ^{b}	10.65	2.24
Pooled *s.e.*		0.07	0.30	0.17
Main effects				
Protein, %				
30		3.08 ^{a}	11.12 ^{a}	2.24
35		3.04 ^{ab}	10.76 ^{ab}	2.29
40		3.00 ^{b}	10.38 ^{b}	2.18
Lipid, %				
4		3.02	10.20 ^{b}	2.04 ^{b}
7		3.01	10.82 ^{a}	2.26 ^{ab}
10		3.07	11.23 ^{a}	2.41 ^{a}
ANOVA, *p* value				
Protein		0.044	0.010	0.926
Lipid		0.632	<0.001	0.053
Protein × Lipid		0.061	0.171	0.278

3.3. Digestion Related Enzyme Activities

As presented in Table 4, liver TPS activity decreased considerably with dietary protein inclusion levels increasing ($p < 0.05$). Intestinal AMS activity showed a considerable

decrease as dietary lipid inclusion levels increased ($p < 0.05$). Intestinal LPS activity showed a decreasing and then increasing trend with dietary fat inclusion levels increasing, reaching a minimum at 7% fat and a maximum at 10% fat levels. In contrast, liver LPS activity showed an opposite trend. The interaction between feed protein and lipid levels presented significance for intestinal TPS and LPS activities ($p < 0.05$). At a 4% feed fat level, intestinal TPS activity was considerably higher in fish consuming 30% protein than that of the 35% protein group ($p < 0.05$). When the feed fat level was 10%, the intestinal TPS activity of fish consuming 30% and 35% protein level diets was considerably higher than that of the 40% protein group ($p < 0.05$).

Table 4. Digestion-related enzyme activities subjected to different protein and lipid levels. Different letters in the same column represent significant differences.

Treatment		Intestine			Liver		
Lipid, %	**Protein, %**	**TPS, U/mg**	**AMS, U/mg**	**LPS, U/g**	**TPS, U/mg**	**AMS, U/mg**	**LPS, U/g**
4	30	805.28 ^{a}	25.20	23.84 ^{ab}	406.17	30.52	148.17
4	35	601.85 ^{b}	25.00	31.09 ^{a}	450.82	25.72	153.15
4	40	770.45 ^{a}	21.33	19.68 ^{b}	340.61	25.93	198.82
7	30	649.77	25.20	18.85	402.66	35.30	208.12
7	35	544.54	18.49	17.73	320.83	27.56	177.08
7	40	589.96	12.73	23.54	342.94	30.45	228.37
10	30	731.73 ^{a}	13.15	38.32	528.03 ^{a}	20.12	178.56
10	35	753.85 ^{a}	10.81	28.95	482.83 ^{a}	20.12	179.12
10	40	523.88 ^{b}	20.30	21.42	275.25 ^{b}	31.80	166.36
Pooled *s.e.*		51.95	3.69	3.10	60.99	4.13	15.96
Main effects Protein, %							
30		728.93	21.18	27.00	445.62 ^{a}	28.65	178.28
35		633.41	18.10	25.92	418.16 ^{ab}	24.47	169.78
40		628.09	18.12	21.55	319.60 ^{b}	29.39	197.85
Lipid, %							
4		725.86 ^{a}	23.84 ^{a}	24.87 ^{ab}	399.20	27.39	166.71 ^{b}
7		594.75 ^{b}	18.81 ^{ab}	20.04 ^{b}	355.48	31.10	204.52 ^{a}
10		669.82 ^{ab}	14.75 ^{b}	29.56 ^{a}	428.70	24.02	174.68 ^{ab}
ANOVA, *p* value							
Protein		0.133	0.512	0.101	0.025	0.312	0.115
Lipid		0.065	0.025	0.005	0.459	0.138	0.023
Protein × Lipid		0.026	0.116	0.009	0.113	0.281	0.189

3.4. Proximate Nutrient Composition of Whole Fish

Dietary protein levels showed significance on energy and moisture and fat contents of whole fish ($p < 0.05$, Table 5). Energy and fat declined with increasing levels of protein, whereas protein, moisture, and ash showed an opposite trend. Dietary lipid levels showed significance on moisture in whole fish ($p < 0.05$). Whole fish moisture and ash decreased with an increase in dietary lipid inclusion levels. The interaction of feed protein and lipid levels showed significance on whole-fish energy ($p < 0.05$).

Table 5. Proximate nutrient composition of whole fish subjected to the different protein and lipid levels. Different letters in the same column represent significant differences.

Treatment		Moisture, %	Ash, %	Energy, kJ/g	Fat, %	Protein, %
Lipid, %	**Protein, %**					
4	30	71.10 ^{b}	2.63	26.43	10.60 ^{a}	15.01
4	35	72.85 ^{a}	2.73	26.04	8.40 ^{ab}	15.48
4	40	73.99 ^{a}	2.76	25.35	7.46 ^{b}	15.32
7	30	70.58 ^{b}	2.46	27.08 ^{a}	9.71	15.30
7	35	71.62 ^{ab}	2.64	25.06 ^{b}	8.97	15.53
7	40	72.37 ^{a}	2.68	26.00 ^{ab}	9.30	15.46
10	30	70.40 ^{b}	2.57	26.42 ^{a}	10.38 ^{a}	15.01
10	35	70.37 ^{b}	2.55	26.01 ^{ab}	8.64 ^{ab}	14.96
10	40	71.92 ^{a}	2.59	25.30 ^{b}	8.02 ^{b}	15.25
Pooled *s.e.*		0.29	0.07	0.28	0.61	0.19
Main effects Protein, %						
30		70.69 ^{c}	2.56	26.65 ^{a}	10.23 ^{a}	15.11
35		71.62 ^{b}	2.64	25.70 ^{b}	8.67 ^{b}	15.32
40		72.76 ^{a}	2.67	25.55 ^{b}	8.26 ^{b}	15.34
Lipid, %						
4		72.65 ^{a}	2.71	25.94	8.82	15.27
7		71.53 ^{b}	2.59	26.05	9.33	15.43
10		70.90 ^{c}	2.57	25.91	9.01	15.07
ANOVA, *p* value						
Protein		<0.001	0.120	<0.001	0.002	0.274
Lipid		<0.001	0.046	0.819	0.603	0.096
Protein × Lipid		0.051	0.623	0.019	0.303	0.670

Note: moisture, ash, fat, and protein are calculated based on fresh weight and energy is calculated based on dry matter.

3.5. Liver Health Indicators

Neither significant interactions nor main effects were presented on serum AST, ALT, or LDH activities in fish subjected to different dietary protein and lipid inclusion levels ($p > 0.05$, Table 6).

Table 6. Liver health indicators subjected to different protein and lipid levels.

Treatment		AST, U/L	ALT, U/L	LDH, U/L
Lipid, %	**Protein, %**			
4	30	222.93	32.13	308.83
4	35	254.90	46.37	375.47
4	40	253.30	43.70	456.57
7	30	251.07	33.70	450.30
7	35	255.27	32.40	557.83
7	40	300.63	39.80	469.93
10	30	286.87	38.97	437.20
10	35	287.07	34.77	643.67
10	40	289.23	42.50	451.60
Pooled *s.e.*		33.12	7.49	100.90

Table 6. *Cont.*

Treatment		AST, U/L	ALT, U/L	LDH, U/L
Lipid, %	**Protein, %**			
Main effects Protein, %				
30		253.62	34.93	398.78
35		265.74	37.84	525.66
40		281.06	42.00	459.37
Lipid, %				
4		243.71	40.73	380.29
7		268.99	35.30	492.69
10		287.72	38.74	510.82
ANOVA, *p* value				
Protein		0.546	0.622	0.526
Lipid		0.260	0.724	0.237
Protein × Lipid		0.738	0.919	0.647

3.6. Immunity-Related Indices

No significant interaction effect was presented on serum TP, ALB, GLB, AGR, or AKP levels in fish subjected to dietary protein and lipid inclusion levels ($p > 0.05$, Table 7). Notably, dietary lipid supplementation led to considerable increases in serum ALB and GLB levels in fish ($p < 0.05$), while other immunity-related indicators exhibited no significant difference subjected to dietary different lipid and protein inclusion levels ($p > 0.05$).

Table 7. Immunity-related indices subjected to the different protein and lipid levels. Different letters in the same column represent significant differences.

Treatment		TP, g/L	ALB, g/L	GLB, g/L	AGR	AKP, U/L
Lipid, %	**Protein, %**					
4	30	25.60	11.67	13.93	0.84	33.10
4	35	25.47	11.13	14.33	0.78	37.47
4	40	25.57	11.40	14.17	0.81	50.30
7	30	27.67	12.00	15.67	0.77	47.07
7	35	29.27	12.63	16.63	0.77	49.07
7	40	27.40	12.00	15.40	0.78	34.97
10	30	26.93	11.80	15.13	0.78	83.43
10	35	28.60	12.60	16.00	0.79	42.27
10	40	29.13	12.33	16.80	0.74	61.20
Pooled *s.e.*		1.77	0.57	1.24	0.03	9.68
Main effects Protein, %						
30		26.73	11.82	14.91	0.80	54.53
35		27.78	12.12	15.66	0.78	42.93
40		27.37	11.91	15.46	0.78	48.82
Lipid, %						
4		25.54	11.40 ^{b}	14.14 ^{b}	0.81	40.29
7		28.11	12.21 ^{ab}	15.90 ^{ab}	0.77	43.70
10		28.22	12.24 ^{a}	15.98 ^{a}	0.77	62.30
ANOVA, *p* value						
Protein		0.967	0.952	0.875	0.729	0.717
Lipid		0.248	0.043	0.041	0.251	0.184
Protein × Lipid		0.819	0.447	0.599	0.551	0.165

3.7. Lipid Metabolism-Related Indices

No significant interaction effect was presented on serum TBA, TG, TC, HDL-C, or LDL-C levels in fish subjected to dietary protein and lipid levels ($p > 0.05$, Table 8).

Table 8. Lipid metabolism-related indices subjected to the different protein and lipid levels. Different letters in the same column represent significant differences.

Treatment		TG, mmol/L	TC, mmol/L	HDL-C, mmol/L	LDL-C, mmol/L	TBA, μmol/L
Lipid, %	**Protein, %**					
4	30	2.45	5.32	1.04	3.78	0.40
4	35	1.88	5.23	0.89	3.96	0.60
4	40	2.10	4.78	0.86	3.49	1.57
7	30	2.60	5.40	0.96	3.91	0.70
7	35	2.46	5.25	0.94	3.81	0.33
7	40	2.50	4.91	0.75	3.73	0.41
10	30	2.63	5.27	0.89	3.85	0.60 ^{ab}
10	35	2.01	5.73	1.10	4.22	0.17 ^{b}
10	40	1.99	5.83	1.02	4.41	1.00 ^{a}
Pooled *s.e.*		0.24	0.30	0.07	0.24	0.43
Main effects Protein, %						
30		2.56	5.33	0.96	3.85	0.57
35		2.12	5.41	0.98	4.00	0.37
40		2.20	5.17	0.88	3.88	0.99
Lipid, %						
4		2.15	5.11	0.93	3.74	0.86
7		2.52	5.19	0.88	3.82	0.48
10		2.21	5.61	1.00	4.16	0.59
ANOVA, *p* value						
Protein		0.082	0.63	0.198	0.734	0.262
Lipid		0.153	0.121	0.149	0.105	0.546
Protein × Lipid		0.771	0.400	0.091	0.387	0.356

3.8. Correlation Profiling Analysis

As indicated in Figure 1A, 3 eigenvectors were determined and assigned to the main and interaction effects of dietary lipid and protein inclusion levels, while the matrices derived from the phenotypic data were grouped into 14 eigenvectors. In particular, Growth1 (eigenvectors related to the phenotype data matrices, consisting of IAW, WGR, SGR, and EDR) exhibited a greater negative correlation with dietary fat inclusion level ($p < 0.05$). This was also the opposite case for Immunity1 and Morpho1. Moreover, dietary protein supplementation levels contributed to considerable positive correlations with Composition1 and Composition2 ($p < 0.05$) and negatively correlated with Digest1 and Growth2 ($p < 0.05$). Of note, the interaction effect of dietary different lipids and proteins possessed a significant negative correlation with Digest2, Growth1, and Growth2 ($p < 0.05$) and a positive correlation with Composition2 ($p < 0.05$). Further data mining against all three eigenvectors identified that the interaction effect of dietary lipid and protein inclusion levels and the main effect of dietary protein inclusion levels were the strongest indirect drivers of changes in growth performance by reshaping intestinal and hepatic digestive enzyme activity patterns (Figure 1B).

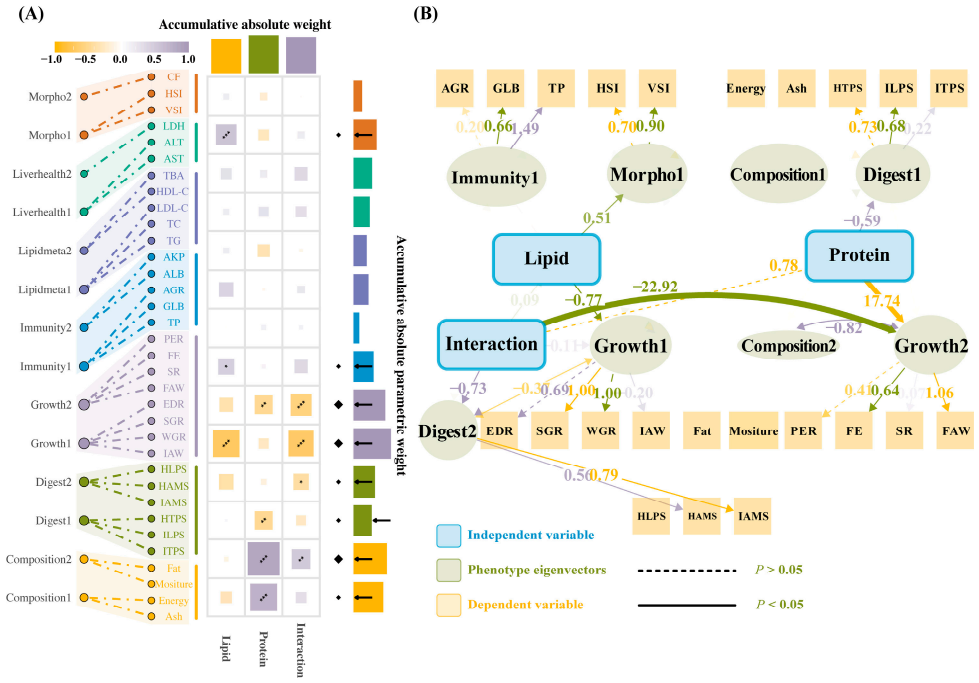

Figure 1. Potential regulatory patterns in Furong crucian carp. (**A**) Correlation analysis based on the Spearman coefficient was used for the interactions between the module eigengenes extracted from the matrix of the main and interaction effects of dietary lipid and protein inclusion levels and the secondary module eigenvectors extracted from the phenotype matrix. The presence of '*, **, ***' indicated the significant difference level at 0.05, 0.01, and 0.001, respectively. The different size of diamond represented that the number of significant pairwise comparison in each row of the heat map. The arrow stated that at least one significant pairwise comparison was observed in the corresponding row; (**B**) structural equation model. The structural equation model explains as much variance as possible in the variables in the model while understanding the covariance between the variables.

4. Discussion

As is often the case, dietary protein presently accounts for most aquaculture costs, from which the leftover feed and undigested proteins contribute to the degradation of aquaculture waters, encouraging studies to optimize the use of dietary proteins among aquatic animals. Protein and fat serve as the major components of aquatic feeds, playing the major parts in metabolically active substance generation. Fish take in more feed to meet specific nutritional requirements as an essential nutrient becomes deficient. Protein content in feed is a key determinant of fish growth performance and feed cost [41]. Generally, increasing protein content improves fish growth, especially in carnivorous fish [8,41]. In this study, FAW and WGR significantly increased and FCR significantly decreased in fish given 7% and 10% fat diets with increasing protein levels, which is consistent with the results of many species [19,23,42–44]. The significantly lower protein requirement in the 4% fat group was attributed to the optimal ratio of protein to fat in the feed and the fact that hibiscus crucian carp can make good use of the fat reserves to provide protein [43]. Earlier research reported that excess feed protein does not promote growth but increases feed costs [45]. This is similar to previous studies where the dietary protein was reduced from 36% to 30% and the fat requirement was increased from 4% to 6% [46,47]. Generally, changes in HSI reflect liver function or liver size, and high HSI values are often associated with poor growth and health status [48]. In this study, high-fat and high-protein diets significantly increased VSI and HSI, which may be due to the storage of fat in the mesentery and liver, suggesting that excessive fat and protein consumption contributed to liver damage. Similar responses were observed in *Nibea albiflora* [49] and hybrid snakeheads (*Channa maculata* ♀× *Channa argus* ♂) [24,41]. This was also confirmed by the higher LDH, AST, and ALT levels in the serum of Furong crucian carp given high-fat or high-protein diets. As is known to

all, blood LDH, AST, and ALT activities typically reflect liver metabolic status, with higher levels indicating reduced or impaired liver dysfunction [50,51].

Protein efficiency ratios (PERs) and energy deposition rates (EDRs) are commonly employed to assess the protein-sparing effect of fat in feeds since protein deposition is usually influenced by non-protein energy intake [52,53]. In the present study, PER and EDR appeared to decrease with dietary protein inclusion levels increasing, as described in other studies on other species [54,55]. Although it has been shown that high-fat diets do not increase PER and EDR, and the effect of high-fat diets on protein conservation is not significant, this may be due to either total or non-protein energy deficits in the diet. Protein is used for energy supply, whereas high-fat levels and total feed energy are used for energy supply [54]. With sufficient fat, protein is not only used for energy supply but also for protein synthesis, which makes the protein-sparing effect more pronounced at moderate-fat levels than at high-fat levels. In addition, high-fat diets are also detrimental to growth as they result in reduced food intake and nutrient inputs for growth [56,57]. The primary nutritional value of aquatic animals is mainly reflected in their nutrient composition [58]. In this experiment, dietary fat and protein levels significantly affected the crude fat, crude ash, and energy of Furong crucian carp, which were significantly lower at a 35% protein level than at a 30% level, indicating that excessively high-protein and high-fat levels are not only detrimental to growth but also aggravate the metabolic burden of the aquatic animals or lead to metabolic disorders, which can lead to a decrease in their fat content. However, in the study of *Hemibagrus wyckioides*, it was found that the fat level was positively proportional to the crude fat content of the fish [54]. In contrast, in the present experiment, the crude fat content of Furong crucian carp was not significantly affected by the fat level, reflecting the fat-sparing effect on protein, indicating that feeding diets containing a fat level of 4% and a protein level of 30% were appropriate for protein utilization in Furong crucian carp, which is in line with the results of a crab study at the lipid level [52], but the reason for the difference in protein levels may be related to the different utilization and conversion of feed nutrients by species, with crustaceans requiring higher levels of protein at the larval stage, which decreases as body weight increases.

Growth involves multiple processes that are strongly influenced by the physiology of digestion and absorption in the organism, affecting the underlying dynamics of nutrient utilization of ingested nutrients and controlling the degree of stress response in fish [18]. The ability of fish to utilize food efficiently may depend on digestive enzymes and their response to different dietary ingredients such as proteins and fats. Feed degradation in the digestive tract of fish is largely dependent on digestive enzymes to maintain nutrient effectiveness. Adaptive changes in digestive enzyme activities concerning feed fat levels were previously reported [59,60]. Feed protein and/or fat content influences hepatopancreatic digestive enzyme secretion [19]. In the present study, increased levels of feed protein/fat significantly decreased protease, lipase, and amylase activities in the gut and liver of carp given the 35%/7% group. Similar results were observed in other studies, where high protein/fat levels resulted in a low activity of digestive enzymes [41,60,61]. As information on the effects of dietary protein and fat effectiveness on foregut histomorphometry is limited, further studies are needed.

Serum TC and TG levels reflect the body's ability to utilize protein and fat. In addition, in high-protein fish diets, protein content exceeds requirements. Excess ammonia produced by metabolism may interfere with serum ion concentration balance and oxygen consumption, increasing the metabolic cost of excretion and reducing growth [62]. High-fat diets may also reduce growth because the body's ability to utilize high fats is limited. Excess fat is stored in the hepatopancreas or other organs, leading to metabolic imbalances, or the high-fat diets themselves are nutritionally unbalanced and do not meet the body's growth needs [63]. On the contrary, in the present study, changes in hematological parameters were observed concerning the protein and fat levels in the feeds, with significant differences in the hematological parameters due to the different fats in the feeds. The serum TC level increased as the feed fat level increased from 4% to 8%, and an increase in TG levels was

always accompanied by an increase in TC [64,65], while serum TC and TG levels decreased as the feed protein level increased. This suggests that endogenous fat consumption is active, which is similar to the studies on toothfish (*Paralichthys olivaceus*) [66] and yellow goby (*Nibea diacanthus*) [67]. Furthermore, dietary proteins play an important role in immune responses [34,35], as evidenced by the changes in serum ALB and GLB levels in fish given high-fat diets. AKP serves as an important regulatory factor in the innate immune system of animals [42], which can catalyze the hydrolysis of phosphate monoesters and the transfer reaction of phosphate groups and have an indispensable role in non-specific immunity. In this experiment, blood AKP was not significantly affected by protein levels, fat levels, or their interaction. The reason for the differences may be due to the interaction between fat and protein levels in this experiment, and differences in species and feed formulations. The mechanism of action of the interaction between protein and fat levels in crustaceans and fish also differs, which requires further research.

5. Conclusions

Taken above, the best growth performance was observed in carp given the 4% lipid and 40% protein diet, which was found to be only slightly higher than the 4% lipid and 30% protein group. Also, the highest feed utilization was shown in the 4% lipid and 30% protein group. To conclude, this study showed that Furong crucian carp given a 4% lipid and 30% protein diet obtained the best growth, feed utilization, and physiological status, given the feed cost in actual production. Excessive protein and lipid supplementation were detrimental to the growth of the carp, conversely aggravating metabolic disorders in Furong crucian carp.

Author Contributions: All authors contributed to the study conception and design. Conceptualization, Z.H., X.T., J.L. and X.C.; methodology, X.C.; software, J.G.; formal analysis, J.L.; resources, X.C.; data curation, J.G.; writing—original draft preparation, Z.H. and X.T.; writing—review and editing, Z.H. and X.T.; project administration, Z.H. and D.W.; funding acquisition, Z.H. and Dongwu Wang. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key R&D Program of China (Grant No. 2020YFD0900104) and the earmarked fund for the China Agriculture Research System (Grant No. CARS-48-39).

Institutional Review Board Statement: Hunan Fisheries Science Institute approved all experiment procedures, which adhered to the ethical standards for the care and use of laboratory animals (Grant number: HNFI20210322).

Data Availability Statement: The original data presented in the study are openly available in Github at https://github.com/mineraltsai/manuscript_FM.

Acknowledgments: Special thanks to Cai Minglang for his work on statistical analysis and visualization.

Conflicts of Interest: The authors declare no conflicts of interest.

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