Aquaculture 533 (2021) 736166

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effects of dietary protein and lipid levels on growth, body composition, enzymes activity, expression of IGF-1 and TOR of juvenile northern whiting, *Sillago sihama*

Hao Liu [a,b], Xiaohui Dong [a,b,*], Beiping Tan [a,b,*], Tao Du [c], Shuang Zhang [a,b], Yuanzhi Yang [a,b], Shuyan Chi [a,b], Qihui Yang [a,b], Hongyu Liu [a,b]

[a] Laboratory of Aquatic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang, Guangdong 524088, China
[b] Aquatic Animals Precision Nutrition and High-Efficiency Feed Engineering Research Centre of Guangdong Province, Zhanjiang, Guangdong 524088, China
[c] Guangdong Ocean University Marine Biology Research Base, Zhanjiang, Guangdong 524088, China

ARTICLE INFO

Keywords:
Protein
Lipid
Sillago sihama
Growth
Liver metabolic enzymes
Insulin-like growth factor-1
Target of rapamycin

ABSTRACT

The purpose of this study was to estimate the effect of dietary protein and lipid levels on growth performance, feed utilization, body composition, liver metabolic enzymes activity and expression of insulin-like growth factor-1 (IGF-1) and target of rapamycin (TOR) of the northern whiting, *Sillago sihama*. A 56-day feeding trial was a completely randomized 4 × 3 factorial design and twelve diets were formulated containing four different protein levels (400, 450, 500 and 550 g/kg) and three different lipid levels (80, 100 and 120 g/kg). 1080 healthy fish (average wt. 0.83 ±0.01 g) were equally distributed in twelve treatments in triplicates with 30 fish per tank. At the end of the experiment, fish fed higher-protein diets (50%) resulted in significantly higher weight gain rate (WG), specific growth rate (SGR), and protein efficiency ratio (PER) compared to those fed the lower-protein treatments (40%). However, when the protein level in diet was 55%, WG began to decrease. The WG of fish fed the 12% lipid diets was significantly higher than that of fish fed the 8% lipid diets ($P < 0.05$). Also, higher dietary protein levels significantly reduced body moisture content, improved morphometrical indices and increased body lipid ($P < 0.05$). High-energy diets led to a decrease in the daily feed intake (DFI) of *S. sihama*. Based on WG, the optimal dietary protein requirement for the quadratic regression models was estimated to be 48.42% of dry matter when the lipid level was 10% and 48.53% for a diet containing 12% lipid. The activities of glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) in liver increased with increasing dietary protein levels, and the activity of glutamate dehydrogenase (GDH) was significantly higher in the high-protein diets (50% and 55%) than that in the low-protein diets (40% and 45%) ($P < 0.05$). When lipid levels of the diet were higher than 10%, lipoprotein lipase (LPL) activities were significantly higher than that of the low-lipid groups (8% groups) ($P < 0.05$). Fatty acid synthase (FAS) activities were inhibited by the increasing lipid levels in diet. Dietary lipid levels significantly improved the expression level of insulin-like growth factor 1 (IGF-1) gene ($P < 0.05$). The relative expression of the target of rapamycin (TOR) gene in liver increased with dietary protein level at 45%–50%, however, it decreased at 55% of dietary protein levels and was significantly lower than that of the 50% group ($P < 0.05$). In conclusion, results of this study showed that the dietary protein requirements based on the growth of *S. sihama* were 48.42% (lipid level: 10%) and 48.53% (lipid level: 12%).

1. Introduction

The feed cost is the largest part of aquaculture costs and usually accounts for more than 50% of the total cost (Li et al., 2019). As a major component of feedstock costs, protein is also the most important factor affecting fish growth performance and feed costs (Lee and Kim, 2001; Wang et al., 2019). Carnivorous fish prefer proteins rather than lipids and carbohydrates as a source of energy (Watanabe, 1982). Excessive protein in diet increases the excretion of nitrogenous waste, which can damage fish growth (Zhang et al., 2017). Therefore, the suitable dietary protein levels can meet the nutritional needs of fish and provide the minimum essential amino acids needed for maximum growth at a low

* Corresponding authors at: Laboratory of Aquatic Animal Nutrition and Feed, College of Fisheries, Guangdong Ocean University, Zhanjiang 524088, China.
E-mail addresses: dongxiaohui2003@163.com (X. Dong), bptan@126.com (B. Tan).

https://doi.org/10.1016/j.aquaculture.2020.736166
Received 16 May 2020; Received in revised form 20 October 2020; Accepted 13 November 2020
Available online 21 November 2020

diet cost. Studies have shown that the use of lipids and carbohydrates in diets of fish can improve the efficiency of protein utilization (NRC, 2011). In this regard, however, carnivorous fish appear to make better use of lipids as an energy source rather than carbohydrates. (Kaushik and Médale, 1994; Rahimnejad et al., 2015). Also, lipids play an important role in fish nutrition as a source of essential fatty acids for fish (Rahimnejad et al., 2015). Appropriate amount of lipid in the diet can promote the growth of fish (Ghanawi et al., 2011). An excess of dietary lipids can lead to decrease in feed intake, which leads to growth retardation (Rahimnejad et al., 2015). Also, excess lipid in the diet can cause fatty liver (Shamsan and Ansari, 2010), body lipid deposition, and lower carcass quality (Chatzifotis et al., 2010). Therefore, it is important to develop diets that balance protein and lipid levels to maximize fish growth.

The northern whiting *Sillago sihama* (*S. sihama*) is mainly found in the coastal areas of China and the shallow waters of the western Indian Pacific (Guo et al., 2014; Huang et al., 2018). As a critical economic fish in coastal parts of China, it is deeply loved by people on its excellent meat quality and high economic and nutritional value. It plays an essential role in the fishing activities in coastal waters of China (Zhou et al., 2017b). In recent years, due to overfishing (Dian et al., 2010), the natural resources of *S. sihama* have been decreased, and the market price has continued to rise. In order to meet market demand, Guangdong Ocean University has successfully bred the first batch of *S. sihama* fry and added new marine aquaculture species. At present, related research on *S. sihama* mainly focuses on morphology (Cao et al., 2010; Wan, 1996; Zhang et al., 2010), reproductive biology and artificial breeding (Huang et al., 2013; Lee, 1981), genetics (Guo et al., 2014; Zhang et al., 2018), tissue physiology (Cao et al., 2010), biochemical composition (Shamsan and Ansari, 2010; Venkatesan and Nazeer, 2014), and ecology (Lee et al., 1981; Zhen et al., 2008).

Few studies have addressed nutrition in *S. sihama*, aside from our laboratory studies that investigated vitamin A, vitamin C and vitamin E requirement in the species (Huang et al., 2020; Huang et al., 2018; Huang et al., 2020b). In addition, the lack of exclusive forage production is due to the paucity of nutritional physiology research, leading to the use of shrimps or marine fish feed to cultivate the *S. sihama*. To expand the cultivation of *S. sihama*, further research on feed production is needed. Therefore, this study was to evaluate the effects of dietary protein and lipid levels on juvenile *S. sihama* growth performance, feed utilization, body composition, liver metabolic enzymes activity and expression of IGF-1 and TOR, to more accurately evaluate the optimal protein and lipid requirements of *S. sihama* and provide a theoretical basis for the production of *S. sihama* feed.

2. Materials and methods

2.1. Experimental design and diets preparation

Table 1 provides the formulation and proximate composition of the experimental diets. The dietary protein and lipid levels and their interactions on growth and body composition were studied using juvenile *S. sihama* as experimental subjects. A complete crossover experiment with four protein levels (40%, 45%, 50% and 55%) and three lipid levels (8%, 10% and 12%) was used to formulate a total of 12 diets with three replicates. White fishmeal (Icicle Seafoods, Inc., Washington), casein (Gansu Hualing Dairy Co., Ltd., Gansu), and vital wheat gluten (Yihai Kerry Syral Starch Technology Co., Ltd. Dongguan) were the main

Table 1
Formulation of the experimental diets.

Ingredients (%)	Diets											
	P40L8	P40L10	P40L12	P45L8	P45L10	P45L12	P50L8	P50L10	P50L12	P55L8	P55L10	P55L12
White fish meal[1]	29.80	29.80	29.80	33.50	33.50	33.50	37.20	37.20	37.20	40.95	40.95	40.95
Casein[2]	19.87	19.87	19.87	22.33	22.33	22.33	24.80	24.80	24.80	27.30	27.30	27.30
Vital wheat gluten[3]	2.98	2.98	2.98	3.35	3.35	3.35	3.72	3.72	3.72	4.10	4.10	4.10
Gelatinized corn starch[4]	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Corn starch[5]	22.00	20.00	18.00	16.00	14.00	12.00	10.00	8.00	6.00	10.57	8.57	6.57
Fish oil[6]	4.73	6.73	8.73	4.39	6.39	8.39	4.05	6.05	8.05	3.70	5.70	7.70
Soybean lecithin	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Mineral mixture[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin mixture[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin C	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.50	0.50	0.50	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	1.50	1.50	1.50
Attractante[c]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Microcrystalline cellulose	7.24	7.24	7.24	7.05	7.05	7.05	6.85	6.85	6.85			
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition												
Moisture	9.86	9.33	9.38	9.68	9.70	9.69	9.47	9.00	8.96	9.50	8.98	9.03
Crude protein	40.32	40.33	40.39	45.09	45.35	45.23	49.05	49.17	49.07	53.41	53.47	53.83
Crude lipid	7.81	10.16	11.96	8.33	10.26	12.14	8.05	10.01	12.12	7.96	10.19	12.13
Ash	9.19	9.30	9.16	10.20	10.21	9.95	11.03	11.21	10.79	12.00	11.85	11.63
Gross energy (kJ g^{-1})	17.77	18.35	18.74	18.07	18.55	18.92	17.87	18.33	18.79	18.96	19.51	20.02
P:E ratio (mg protein kJ^{-1})	22.70	21.97	21.56	24.95	24.44	23.91	27.46	26.83	26.11	28.17	27.41	26.89

[a] Mineral mixture (g/kg mixture): $CaCO_3$ 350 g; $NaH_2PO_4{\cdot}H_2O$ 200 g; KH_2PO_4 200 g; NaCl 12 g; $MgSO_4{\cdot}7H_2O$ 10 g; $FeSO_4{\cdot}7H_2O$ 2 g; $MnSO_4{\cdot}7H_2O$ 2 g; $AlCl_3{\cdot}6H_2O$ 1 g; $CuCl_2{\cdot}2H_2O$ 1 g; KF 1 g; $Na_2MoO_4.2H_2O$ 0.5 g; Na_2SeO_3 0.4 g; $CoCl_2{\cdot}6H_2O$ 0.1 g; KI 0.1 g; zeolite powder 219.9 g.

[b] Vitamin mixture (g/kg mixture): vitamin A 10 g; vitamin D_3 50 g; vitamin E 99 g; vitamin K 5.0 g; vitamin B_1 25.50 g; vitamin B_2 25 g; vitamin B_6 50 g; vitamin B_{12} 0.1 g; D-calcium pantothenate 61 g; nicotinic acid 101 g; biotin 25 g; inositol 153.06 g; folic acid 6.25 g; cellulose 389.09 g.

[c] Attractant composition: taurine: glycine: betaine = 1:3:3; they are obtained from Hangzhou King Techina Technology (Hangzhou, China).

[1] Provided by Icicle Seafoods, Inc. (Washington, USA).

[2] Purchased from Gansu Hualing Dairy Co., Ltd. (Gansu, China).

[3] Purchased from Yihai Kerry Syral Starch Technology Co., Ltd. (Dongguan, China).

[4] Purchased from Jiaxing Xinxin Food Technology Co., Ltd. (Xinjia, China).

[5] Purchased from Huailai Longchen Food Co., Ltd. (Zhangjiakou, China).

[6] Purchased from Shandong Yuwang Industrial Co., Ltd. (Shandong, China).

protein sources, and fish oil and soybean lecithin were the main lipid sources. P/E ratio of diets ranged from 21.56 to 28.17 mg protein kJ^{-1}. All raw materials were crushed and passed 60 mesh screens. Feedstuffs were weighed accurately according to the formula and thoroughly mixed in a Hobart-type mixer. After the addition of oils, choline chloride and water, the compound were made in pellets (1.5 mm) through the F-26 double-screw extruder (South China University of Technology, Guangzhou). The diets were air-dried and sealed in plastic Ziploc bags and stored in −20 °C refrigerator when the moisture was about 10%.

2.2. Experimental animals and breeding management

Juvenile *S. sihama* were obtained from artificial reproduction base at the Guangdong Ocean University. Before the trial, the juvenile fish were temporarily placed in a 4.5 m × 3.45 m × 1.8 m cement pond and fed commodity diets (Zhanjiang Aohua Feed Co., Ltd., Guangdong, China). The environment and feeding management of acclimation period for the two-week were like those of formal experiment. According to the experimental design, 1080 healthy fish (0.83 ± 0.01 g) which have been starved for 24 h were randomly divided into 36 fiberglass tanks ($1m^3$) and three repetitions in each group. The experiment was carried out in the indoor marine culture system of Marine Biology Research Base of Guangdong Ocean University. The experiment lasted 56 days and the salinity of the seawater was adjusted to 6–8 during the breeding cycle. Before changing the seawater, the seawater was disinfected with chlorine dioxide and aerated for 24 h. The daily water temperature was 27.4–33.5 °C; the pH was 7.5–8.0. Dissolved oxygen content ≥6 mg L^{-1}, ammonia nitrogen and nitrite concentration ≤ 0.5 mg L^{-1}. Fish fed twice daily (08: 00–09: 00, 16: 30–17: 30), and fed to apparent saturation. All animal experiments were conducted strictly based on the recommendations in the 'Guide for the Care and Use of Laboratory Animals' set by the National Institutes of Health. The animal protocols were approved by the Animal Ethics Committee of Guangdong Ocean University (Zhanjiang, China).

2.3. Sample collection and analysis

At the end of the trial, fish in each tank were counted and weighed (HZT-A500, Huazhi scientific instrument Co., Ltd., Fuzhou) after fasting 24 h at the last feeding to determine survival rate (SR) and weight gain rate (WG). After that, the livers of eight fish were randomly selected from each fiberglass tanks and stored at −80 °C for metabolic activity analysis. Three fish were randomly selected from each tank, weighed first, measured body length, then dissected the fish and weighed the viscera and liver to obtain hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF). Three fish were randomly selected and kept in the refrigerator at −20 °C for whole-body composition analysis.

Diet and body proximate analysis of dry matter (dried at 105 °C) (method 934.01), crude protein(by Kjeldahl apparatus, nitrogen × 6.25) (method 976.05), crude lipid(extraction with petroleum ether by Soxhlet apparatus) (method 920.29), ash content(incineration at 550 °C) (method 942.05), were determined according to the methods of Association of Official Analytical Chemists (AOAC, 1995).

Enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd) were used to determine liver metabolic enzymes (glutamic-oxalacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), glutamate dehydrogenase (GDH), lipoprotein lipase (LPL), fatty acid synthase (FAS), malate dehydrogenase (MDH)). The liver was first weighed (about 1 g), thawed and homogenized in a 9-volume frozen buffer (10 ml PBS, pH 7.4), then centrifuged at 4 °C for 20 min (2000–3000 rpm), and the supernatants were carefully collected where aliquots of samples were used in the testing process. The final tissue concentration was obtained by dividing the result by total protein (TP) in the liver tissue.

2.4. Real-time quantitative RT-PCR analysis of gene expression

Total RNA was extracted from the liver using a General RNA Extraction Kit and integrity was detected by electrophoresis on 1.2% denatured agarose gel. RNA integrity and quality were assessed using a NanoDrop 2000 (Thermo Scientific Inc.; Waltham, MA, USA) spectrophotometer. A PrimeScript™ RT-PCR Kit was used to perform first-strand cDNA synthesis in RT according to the manufacturer's instructions. The RNA was treated with gDNA Eraser and 1.0 μg was used for reverse transcription with a PrimeScript RT Reagent Kit. Real-time PCR assays were performed in a quantitative thermal cycler (LightCycler480, Roche Diagnostics, Switzerland) with a reaction volume of 10 μl containing 5 μl of SYBR@ Green Real-time PCR Master Mix, 1 μl of cDNA, 0.8 μM of primers, and 3.2 μl of sterilized double distilled water. Three copies of each sample in the reaction. Date acquisition occurs every 6 s; so, the thermal programmer consists of 30 s at 95 °C, 40 cycles of 5 s at 95 °C, and 34 s at 60 °C, as well as a gradual increase of 0.5 °C s^{-1} from 60 °C to 95 °C in melt curve steps. Based on our preliminary experimental results on the evaluation of internal control gene, the 60S ribosomal protein L38 was used as a reference gene to normalize the cDNA load. Gene expression results were analyzed using the $2^{-\Delta\Delta CT}$ method according to Livak and Schmittgen (2001). Normalised gene expression for the P40L8 diet group was set at 1. All primers were designed using PrimerQuest Tool and primer sequences are listed in Table 2.

2.5. Formula and statistical analysis

The following parameters and indices were calculated using a standard formula (NRC, 2011): growth performance parameters, including WG, specific growth rate (SGR) and SR; feed utilization indices, including feed conversion rate (FCR), protein efficiency ratio (PER) and daily feed intake (DFI); and morphology indices, including CF, HSI and VSI. Analyses of normality and homology were performed before applying ANOVA. Two-way ANOVA and Tukey's multiple range test were used to analyze the significance of mean differences between treatment groups using SPSS version 19 (SPSS, Michigan Avenue, Chicago, Illinois, USA). Statistical significance tests were tested at the $P < 0.05$ level. Data were analyzed by one-way ANOVA ($P < 0.05$) only when there was a significant interaction. Data are expressed as mean ± SD.

3. Result

3.1. Growth performance and feed utilization

The results concerning the growth performance and feed utilization of juvenile *S. sihama* were presented in Table 3. After 8 weeks of the experiment, the survival rates of fish from the 12 treatment groups ranged from 96.67% to 100.00%, with no significant differences among treatments. Based on two-way ANOVA, the Final body weight (FBW), WGR, PER FCR and DFI of juvenile *S. sihama* was significantly ($P < 0.05$) affected by dietary protein and lipid, but the effect of interaction was not significant ($P > 0.05$). Fish fed the diet with 45% protein and 12% lipid containing 23.91 mg kJ^{-1} P:E showed the best growth performance. Significant ($P < 0.05$) enhancements in WGR, FBW and SGR were observed by the increment of dietary lipid from 8% to 12% and protein from 40% to 50%, and the further increase of protein level to 55% resulted in reduced growth performance. FCR and DFI showed a downward trend with increasing dietary lipid and protein levels, both of which achieved minimum values at P55 and L12. Moreover, PER was negatively correlated with dietary protein levels and positively correlated with dietary lipid levels. When dietary lipid was 10%, the dietary protein requirement estimated by WG was 48.42% (Fig. 1) and when dietary lipid was 12%, the dietary protein requirement estimated by WG was 48.53% (Fig. 2).

Table 2
Primers used for determining gene expression of *S. sihama*.

Name	Primer type	Sequence	Genbank no.	function	Amplicon
60s	Sense	GACAGCCAGGAGGAAGGATG	ACN10033.1	Housekeeping gene	219
	Anti-sense	TGTCTGTGATGACCAGGGTG			
TOR	Sense	GCTGTACCAGGCACTTATGA	KY985002.1	TOR pathway genes	88
	Anti-sense	GCTGCTTGGAGGTGATGA			
IGF-1	Sense	GCCATAGCCTGGTTTACTGA	JQ794830.1		118
	Anti-sense	CCTGACTCCGACGGCAACA			

Note:60S: 60S ribosomal protein L38; TOR: target of rapamycin; IGF-1: insulin-like growth factor 1.

Table 3
Growth performance, survival and feed utilization of juvenile *S. sihama* fed diets containing various levels of protein and lipid ($n = 3$)[1].

Diets[2]	IBW (g)	FBW (g)	WG (%)	SGR (% day^{-1})	FCR	PER	DFI(%)	SR (%)
P40L8	0.84 ± 0.00^{a}	10.19 ± 0.13^{a}	1119.81 ± 16.46^{a}	3.99 ± 0.03^{a}	1.04 ± 0.01^{d}	2.39 ± 0.02^{ef}	3.15 ± 0.03^{e}	98.89 ± 1.92^{a}
P40L10	0.83 ± 0.00^{a}	10.01 ± 0.38^{a}	1105.04 ± 50.52^{a}	3.96 ± 0.07^{a}	1.05 ± 0.07^{d}	2.36 ± 0.14^{def}	3.16 ± 0.14^{e}	100.00 ± 0.00^{a}
P40L12	0.83 ± 0.01^{a}	10.12 ± 0.62^{a}	1122.97 ± 81.08^{a}	3.98 ± 0.12^{a}	1.01 ± 0.03^{bcd}	2.45 ± 0.08^{f}	3.03 ± 0.03^{bcde}	97.78 ± 3.85^{a}
P45L8	0.83 ± 0.01^{a}	10.26 ± 0.22^{a}	1139.00 ± 18.02^{a}	4.01 ± 0.04^{a}	1.05 ± 0.02^{d}	2.12 ± 0.04^{abc}	3.17 ± 0.05^{e}	100.00 ± 0.00^{a}
P45L10	0.83 ± 0.01^{a}	10.26 ± 0.15^{a}	1136.33 ± 12.34^{a}	4.02 ± 0.04^{a}	1.02 ± 0.01^{cd}	2.17 ± 0.01^{bcd}	3.13 ± 0.03^{de}	100.00 ± 0.00^{a}
P45L12	0.83 ± 0.01^{a}	11.04 ± 0.26^{a}	1226.72 ± 30.11^{a}	4.15 ± 0.04^{a}	0.98 ± 0.03^{abcd}	2.27 ± 0.07^{cdef}	2.97 ± 0.04^{abcde}	97.78 ± 3.85^{a}
P50L8	0.83 ± 0.01^{a}	10.24 ± 0.54^{a}	1138.48 ± 73.91^{a}	4.00 ± 0.11^{a}	1.02 ± 0.06^{cd}	2.00 ± 0.11^{ab}	3.10 ± 0.14^{de}	98.89 ± 1.92^{a}
P50L10	0.83 ± 0.01^{a}	10.97 ± 0.60^{a}	1220.80 ± 83.44^{a}	4.14 ± 0.09^{a}	1.00 ± 0.05^{bcd}	2.04 ± 0.11^{ab}	3.07 ± 0.15^{cde}	97.78 ± 3.85^{a}
P50L12	0.83 ± 0.00^{a}	11.03 ± 0.19^{a}	1221.34 ± 22.45^{a}	4.14 ± 0.03^{a}	0.93 ± 0.01^{abc}	2.19 ± 0.03^{bcde}	2.84 ± 0.03^{abc}	97.78 ± 1.92^{a}
P55L8	0.83 ± 0.01^{a}	10.13 ± 0.36^{a}	1122.51 ± 47.49^{a}	3.98 ± 0.06^{a}	0.96 ± 0.01^{abcd}	1.95 ± 0.03^{a}	2.89 ± 0.05^{abcd}	96.67 ± 3.33^{a}
P55L10	0.83 ± 0.01^{a}	10.16 ± 0.22^{a}	1118.27 ± 34.55^{a}	3.99 ± 0.04^{a}	0.91 ± 0.03^{ab}	2.04 ± 0.06^{ab}	2.80 ± 0.08^{ab}	100.00 ± 0.00^{a}
P55L12	0.83 ± 0.01^{a}	10.62 ± 0.09^{a}	1176.50 ± 18.31^{a}	4.07 ± 0.02^{a}	0.90 ± 0.02^{a}	2.08 ± 0.04^{abc}	2.73 ± 0.04^{a}	98.89 ± 1.92^{a}
Means of main effect								
Protein								
P40	0.83	$10.11^{\times}$	$1115.94^{\times}$	$3.98^{\times}$	1.03^{Z}	2.40^{Z}	3.11^{Z}	98.89
P45	0.83	10.52^{XY}	1167.35^{XY}	4.06^{XY}	1.01^{XY}	2.19^{Y}	3.09^{Y}	99.26
P50	0.83	10.74^{Y}	1193.54^{Y}	4.09^{Y}	0.98^{Y}	$2.08^{\times}$	3.00^{XY}	98.15
P55	0.83	10.31^{XY}	1139.09^{XY}	4.02^{XY}	$0.92^{\times}$	$2.02^{\times}$	$2.80^{\times}$	98.52
Lipid								
L8	0.83	10.20^{A}	1129.95^{A}	3.99^{A}	1.01^{B}	2.11^{A}	3.08^{B}	98.61
L10	0.83	10.35^{AB}	1145.11^{AB}	4.03^{AB}	1.00^{B}	2.15^{A}	3.04^{B}	99.44
L12	0.83	10.74^{B}	1186.88^{B}	4.09^{B}	0.95^{A}	2.25^{B}	2.89^{A}	98.06
ANOVA (*P*-value)								
Protein	0.90	0.007	0.027	0.005	0.000	0.000	0.000	0.789
Lipid	0.62	0.008	0.018	0.008	0.001	0.001	0.000	0.383
Protein × Lipid	0.32	0.165	0.858	0.176	0.627	0.646	0.589	0.638

Weight gain rate (WGR, %) = 100× (final body weight (g)-initial body weight (g))/initial body weight (g).
Specific growth rate (SGR, %) = 100× [ln final body weight (g)-ln initial body weight (g)]/days of trial.
Feed conversion ratio (FCR) = dry feed intake (g) / (final body weight (g)-initial body weight(g)).
Protein efficiency ratio (PER) = fish weight gain (g) / fish protein intake (g).
Survival rate (SR, %) = 100 × final fish number / initial fish number.
Condition factor (CF, %) = 100 × body wet weight (g) / body length (cm)^3.
Hepatosomatic indices (HSI, %) = 100 × (liver wet weight / body wet weight).
Viscerosomatic index (VSI, %) =100 × viscera wet weight (g) / body wet weight (g).
Daily feed intake (DFI, g 100 g fish^{-1} day^{-1}) = 100 × feed offered / average total weight / days.

[1] Values (Means±SD) are presented as means of three replications (n = 3). Means in the same column with different superscripts (A, B, C, etc. Or a, b, c, etc. Or X, Y, Z) are significantly different ($P < 0.05$). (Dietary protein = X, Y, Z etc.; Dietary lipid = A, B).

[2] Diets: P40/L8 = 400 g/kg crude protein, 80 g/kg crude lipid; P40/L10 = 400 g/kg crude protein, 100 g/kg crude lipid; P40/L12 = 400 g/kg crude protein, 120 g/kg crude lipid, etc.

3.2. Morphometric parameters

Morphometric parameters of juvenile *S. sihama* were presented in Table 4. According to two-way ANOVA, CF and VSI increased with increasing dietary lipid levels ($P < 0.05$), and HSI overall increased with increasing dietary protein levels from 40% to 50%, however, a further increase in dietary protein levels to 55% resulted in lower HSI. CF, HSI and VSI were not affected by the interaction ($P > 0.05$).

3.3. Whole-body composition

Proximate compositions of the whole-body of *S. sihama* fed diets with different dietary protein and lipid are presented in Table 5. Whole body moisture was significantly affected by dietary lipid levels ($P < 0.05$); however, dietary protein levels had no significant effect on whole body moisture ($P > 0.05$). Whole body crude lipid was significantly affected by dietary lipids ($P < 0.05$). However, whole body ash and crude protein were not significantly affected by dietary protein and lipid levels. Interaction of dietary protein and lipid didn't affect whole body compositon ($P > 0.05$).

3.4. Liver metabolic enzyme

The results about the effects of four protein levels (40%, 45%, 50% and 55%) and three lipid levels (8%, 10% and 12%) on liver metabolic enzymes of *S. sihama* were given in Table 6. The GOT activity was significantly affected by dietary protein levels ($P < 0.05$), which reached the maximum at 55%. GPT and GDH activity was significantly affected by dietary protein and lipid levels ($P < 0.05$) and was positively correlated with protein levels and negatively correlated with lipid levels. LPL

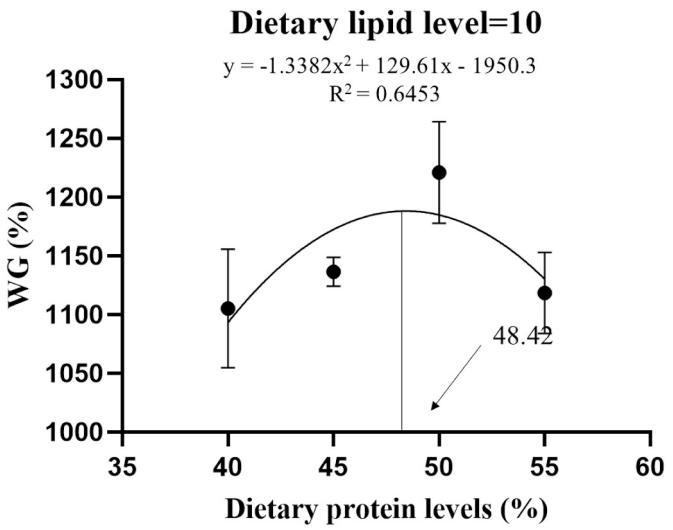

Fig. 1. Effects of dietary protein levels on WG of *S. sihama* fed experimental diets content 10% lipid. Data represent means of three tanks in each group; error bar indicates S. D.

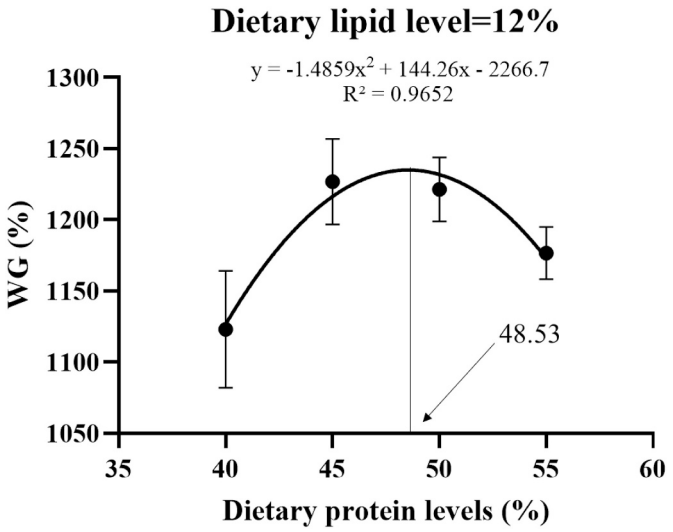

Fig. 2. Effects of dietary protein levels on WG of *S. sihama* fed experimental diets content 12% lipid. Data represent means of three tanks in each group; error bar indicates S. D.

activity was influenced not only by dietary protein levels but also by dietary lipid levels ($P < 0.05$), FAS activity was influenced only by dietary lipid levels ($P < 0.05$), while MDH activity was significantly influenced by dietary protein levels ($P < 0.05$) but not by dietary lipid levels ($P > 0.05$). There was significant lipid-protein interaction for GPT, GDH, LPL and MDH ($P < 0.05$).

3.5. Relative gene expression of TOR and IGF-1 in fish liver

The expression of the insulin-like growth factor-1 (IGF-1) and target of rapamycin (TOR) genes in the liver of *S. sihama*were shown in Figs. 3 & 4.

Dietary lipid levels significantly increased IGF-1 gene expression level ($P > 0.05$), with the lowest IGF-1 gene expression value in the P40L8 diet. In addition, IGF-1 gene expression is significantly affected by the interaction between dietary protein and lipid levels. However, different dietary protein levels did not cause significant differences ($P > 0.05$).

Table 4
Morphometrical parameters of juvenile *S. sihama* fed diets containing various levels of protein and lipid ($n = 3$)[1].

Diets[2]	CF (%)	HSI (%)	VSI (%)
P40L8	1.10 ± 0.06^{a}	0.96 ± 0.22^{a}	7.99 ± 0.80^{a}
P40L10	1.10 ± 0.05^{a}	1.06 ± 0.22^{a}	8.44 ± 1.09^{a}
P40L12	1.13 ± 0.04^{a}	1.32 ± 0.22^{a}	9.34 ± 1.69^{a}
P45L8	1.14 ± 0.05^{a}	1.03 ± 0.19^{a}	8.37 ± 1.94^{a}
P45L10	1.14 ± 0.05^{a}	1.22 ± 0.16^{a}	9.28 ± 1.31^{a}
P45L12	1.16 ± 0.04^{a}	1.51 ± 0.22^{a}	10.23 ± 1.62^{a}
P50L8	1.08 ± 0.04^{a}	1.18 ± 0.15^{a}	8.84 ± 1.18^{a}
P50L10	1.10 ± 0.05^{a}	1.32 ± 0.10^{a}	9.23 ± 1.93^{a}
P50L12	1.15 ± 0.07^{a}	1.48 ± 0.36^{a}	9.18 ± 0.91^{a}
P55L8	1.10 ± 0.06^{a}	1.12 ± 0.14^{a}	7.90 ± 1.12^{a}
P55L10	1.11 ± 0.05^{a}	1.19 ± 0.17^{a}	8.41 ± 1.20^{a}
P55L12	1.18 ± 0.07^{a}	1.25 ± 0.23^{a}	9.04 ± 0.78^{a}
Means of main effect			
Protein			
P40	1.11	1.16$^{\times}$	8.59
P45	1.14	1.25^{XY}	9.29
P50	1.11	1.33^{Y}	9.08
P55	1.13	1.19^{XY}	8.45
Lipid			
L8	1.10^{A}	1.07^{A}	8.27^{A}
L10	1.11^{A}	1.20^{A}	8.84^{AB}
L12	1.15^{B}	1.39^{B}	9.45^{B}
ANOVA (P-value)			
Protein	0.172	0.024	0.205
Lipid	0.003	0.000	0.015
Protein × Lipid	0.771	0.543	0.899

Abbreviations: CF: condition factor; HSI: hepatosomatic index; and VSI: viscerosomatic index.

[1] Values (Means±SD) are presented as means of three replications (n = 3). Means in the same column with different superscripts (A, B, C, etc. Or a, b, c, etc. Or X, Y, Z) are significantly different ($P < 0.05$). (Dietary protein = X, Y, Z etc.; Dietary lipid = A, B).

[2] Diets: P40/L8 = 400 g/kg crude protein, 80 g/kg crude lipid; P40/L10 = 400 g/kg crude protein, 100 g/kg crude lipid; P40/L12 = 400 g/kg crude protein, 120 g/kg crude lipid, etc.

The relative expression of the TOR gene in liver showed a tendency to increase and then decrease with the increase of dietary protein level, with the highest expression reaching 50% at the dietary protein level. The highest TOR activity was observed in fish fed the P50L10 diet. However, dietary lipid levels didn't result in significant differences in TOR expressions, neither the interaction between dietary protein and lipid.

4. Discussion

Because protein is one of the most important nutrients for growth, and the utilization of protein is affected by several dietary factors including protein content, amino acid balance and the amount of available energy (Zhang et al., 2017), it is important to precisely determine the dietary protein requirement for each species and to discuss the proper amount of energy to support adequate growth. In this study, *S. sihama* grew best when fed three diets containing 45% protein 12% lipid, 50% protein 10% lipid and 50% protein 12% lipid, with similar average levels of WG, FBW and SGR. Dietary protein requirements estimated based on WG were 48.42% (Fig. 1) and 48.53% (Fig. 2), respectively. These results were similar to those of other carnivorous fish such as grouper (48%–52%, Wang et al., 2017), hybrid snakehead (*Channa maculata* ♀ × *Channa argus* ♂) fingerlings (47.9%–50.5%, Zhang et al., 2017), snakehead (*Parachanna obscura*) (42.5%–53.5%, Kpogue et al., 2013). But estimates are much higher than for other carnivorous species, such as Eurasian perch (*Perca fluviatilis*) (43.6%, Fiogbé et al., 1996) and rockfish (*Sebastes schlegeli*) (42%, Lee et al., 2002). Inadequate P/E ratios in any diet will lead to the decrease of fish growth performance, protein and energy utilization (Huo et al.,

Table 5
Whole-body composition of juvenile *S. sihama* fed diets containing various levels of protein and lipid (n = 3)[1].

Diets[2]	Whole-body composition (% wet weight)			
	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
P40L8	73.60 ± 1.37^{c}	16.78 ± 0.61^{a}	7.70 ± 0.33^{a}	3.57 ± 0.39^{a}
P40L10	73.16 ± 0.51^{bc}	16.01 ± 0.36^{a}	7.93 ± 0.67^{a}	3.39 ± 0.32^{a}
P40L12	70.90 ± 1.47^{a}	16.71 ± 0.41^{a}	9.13 ± 0.90^{a}	3.67 ± 0.35^{a}
P45L8	72.76 ± 0.26^{abc}	16.23 ± 0.28^{a}	8.62 ± 0.77^{a}	3.86 ± 0.56^{a}
P45L10	72.44 ± 0.36^{abc}	16.08 ± 0.42^{a}	8.56 ± 0.59^{a}	3.78 ± 0.51^{a}
P45L12	71.03 ± 0.59^{ab}	16.33 ± 0.40^{a}	9.23 ± 0.41^{a}	3.80 ± 0.24^{a}
P50L8	72.03 ± 0.24^{abc}	16.32 ± 0.33^{a}	8.70 ± 0.14^{a}	4.12 ± 0.41^{a}
P50L10	72.09 ± 0.41^{abc}	16.53 ± 0.07^{a}	8.34 ± 0.23^{a}	3.95 ± 0.08^{a}
P50L12	71.69 ± 0.35^{abc}	16.26 ± 0.31^{a}	9.14 ± 0.19^{a}	3.73 ± 0.32^{a}
P55L8	72.76 ± 0.21^{abc}	16.47 ± 0.26^{a}	7.71 ± 0.89^{a}	3.89 ± 0.45^{a}
P55L10	72.28 ± 0.82^{abc}	16.23 ± 0.20^{a}	8.20 ± 0.89^{a}	3.50 ± 0.14^{a}
P55L12	71.44 ± 0.68^{ab}	16.41 ± 0.22^{a}	9.23 ± 0.43^{a}	3.74 ± 0.32^{a}
Means of main effects				
Protein				
40	72.55	16.50	8.25	3.54
45	72.08	16.21	8.80	3.82
50	71.94	16.37	8.73	3.93
55	72.16	16.40	8.38	3.71
Lipid				
8	72.79^{B}	16.47	8.18^{A}	3.86
10	72.49^{B}	16.23	8.25^{A}	3.66
12	71.26^{A}	16.41	9.18^{B}	3.74
ANOVA (P-value)				
Protein	0.342	0.386	0.183	0.168
Lipid	0.000	0.227	0.001	0.398
Protein × Lipid	0.201	0.241	0.657	0.860

[1] Values (Means±SD) are presented as means of three replications (n = 3). Means in the same column with different superscripts (A, B, C, etc. Or a, b, c, etc. Or X, Y, Z) are significantly different ($P < 0.05$). (Dietary protein = X, Y, Z etc.; Dietary lipid = A, B).
[2] Diets: P40/L8 = 400 g/kg crude protein, 80 g/kg crude lipid; P40/L10 = 400 g/kg crude protein, 100 g/kg crude lipid; P40/L12 = 400 g/kg crude protein, 120 g/kg crude lipid, etc.

Table 6
Liver metabolic enzymes of juvenile *S. sihama* fed diets containing various levels of protein and lipid (n = 3)[1].

Diets[2]	GOT (U/g prot)	GPT (U/g prot)	GDH (U/g prot)	LPL (U/g prot)	FAS (U/mg prot)	MDH (U/g prot)
P40L8	112.34 ± 14.94^{ab}	130.48 ± 11.49^{bcd}	7.79 ± 0.66^{bc}	191.75 ± 7.97^{a}	2.44 ± 0.22^{b}	85.16 ± 18.62^{ab}
P40L10	93.29 ± 27.23^{a}	103.05 ± 11.43^{bc}	6.47 ± 1.03^{abc}	226.47 ± 52.85^{a}	1.89 ± 0.54^{ab}	74.13 ± 10.83^{a}
P40L12	109.27 ± 26.51^{a}	34.67 ± 3.75^{a}	5.79 ± 0.61^{ab}	436.94 ± 16.76^{bc}	1.83 ± 0.23^{ab}	100.04 ± 15.02^{abcd}
P45L8	141.94 ± 30.54^{abcd}	153.73 ± 24.32^{cde}	5.96 ± 0.56^{abc}	348.33 ± 77.47^{b}	2.11 ± 0.17^{ab}	87.11 ± 8.95^{abc}
P45L10	137.22 ± 23.07^{abc}	191.48 ± 21.64^{defg}	7.04 ± 0.48^{abc}	373.96 ± 32.85^{b}	1.98 ± 0.55^{ab}	113.78 ± 13.60^{cd}
P45L12	135.23 ± 8.99^{abc}	155.16 ± 5.59^{cde}	5.04 ± 0.46^{a}	445.61 ± 12.73^{bc}	1.93 ± 0.10^{ab}	97.09 ± 9.12^{abcd}
P50L8	209.11 ± 35.15^{de}	234.74 ± 27.75^{g}	8.24 ± 0.74^{c}	418.18 ± 12.64^{bc}	1.84 ± 0.37^{a}	115.49 ± 10.38^{d}
P50L10	180.38 ± 13.11^{bcde}	122.94 ± 29.24^{bc}	6.68 ± 1.57^{abc}	523.35 ± 44.08^{cd}	1.66 ± 0.36^{ab}	104.17 ± 10.70^{bcd}
P50L12	156.51 ± 11.33^{abcde}	69.67 ± 16.52^{ab}	7.88 ± 1.62^{bc}	589.59 ± 64.62^{de}	1.69 ± 0.20^{ab}	119.07 ± 11.97^{d}
P55L8	211.13 ± 49.69^{e}	228.23 ± 15.76^{fg}	8.20 ± 0.67^{c}	499.04 ± 22.39^{cd}	2.08 ± 0.17^{ab}	108.03 ± 15.47^{bcd}
P55L10	200.32 ± 47.53^{cde}	199.38 ± 17.55^{efg}	6.95 ± 0.52^{abc}	498.43 ± 31.89^{cd}	1.92 ± 0.10^{ab}	96.82 ± 7.33^{abcd}
P55L12	140.25 ± 25.29^{abcd}	165.32 ± 52.10^{cdef}	6.73 ± 1.49^{abc}	651.64 ± 32.38^{e}	1.72 ± 0.27^{ab}	106.37 ± 27.97^{bcd}
Means of main effects[f]						
Protein						
40	104.97^{X}	89.41^{X}	6.68^{X}	285.05^{X}	2.06	86.44^{X}
45	138.13^{Y}	166.79^{Y}	6.02^{X}	389.30^{Y}	2.01	99.32^{Y}
50	182.00^{Z}	142.45^{Y}	7.60^{Y}	510.37^{Z}	1.73	112.91^{YZ}
55	183.90^{Z}	197.64^{Z}	7.29^{Y}	549.70^{Z}	1.91	103.74^{Z}
Lipid						
8	168.63^{B}	186.79^{C}	7.55^{B}	364.33^{A}	2.12^{B}	98.95
10	152.80^{A}	154.21^{B}	6.78^{AB}	405.55^{B}	1.86^{AB}	97.22
12	135.31^{A}	106.21^{A}	6.36^{A}	530.95^{C}	1.79^{A}	105.64
ANOVA (P-value)						
Protein	0.000	0.000	0.002	0.000	0.064	0.000
Lipid	0.007	0.000	0.790	0.000	0.014	0.109
Protein × Lipid	0.090	0.000	0.008	0.016	0.674	0.004

Abbreviations: GOT: glutamic-oxalacetic transaminase; GPT: glutamic-pyruvic transaminase; GDH: glutamate dehydrogenase; LPL: lipoprotein lipase; FAS: fatty acid synthase; MDH: malate dehydrogenase.
[1] Values (Means±SD) are presented as means of three replications (n = 3). Means in the same column with different superscripts (A, B, C, etc. Or a, b, c, etc. Or X, Y, Z) are significantly different ($P < 0.05$). (Dietary protein = X, Y, Z etc.; Dietary lipid = A, B, C).
[2] Diets: P40/L8 = 400 g/kg crude protein, 80 g/kg crude lipid; P40/L10 = 400 g/kg crude protein, 100 g/kg crude lipid; P40/L12 = 400 g/kg crude protein, 120 g/kg crude lipid, etc.

2014). In the present study, the optimal Protien/Energy ratio ranged from 23.91 mg kJ^{-1} to 26.83 mg kJ^{-1} and dietary protein content ranged from 48.42% to 48.53%, values similar to those obtained in brown trout (*Salmo trutta fario*) (24.56 mg kJ^{-1}, Wang et al., 2018) , Atlantic cod (*Gadus morhua* L.) (26.4 mg kJ^{-1} , Morais et al., 2001), hybrid grouper (*Epinephelus fuscoguttatus*×*E. lanceolatus*) (23.9 mg kJ^{-1}, Rahimnejad et al., 2015) and Japanese seabass (*Lateolabrax japonicus*) (25.9 mg kJ^{-1}, Ai et al., 2004). To some extent, the optimal P/E ratio varies among fish species, and the P/E ratio for the same fish varies in different studies. Previous studies in our laboratory suggested that the optimal P/E for *S. sihama* is 28.82 mg kJ^{-1} with 45% crude protein and 12% crude lipid in the diet (Huang et al., 2020a). These differences may be caused by two aspects. One is the experimental dietary P/E level. Differences in the design of dietary nutrient levels affect the estimation of nutrient requirements (Ai et al., 2004). Therefore, the estimate of the optimal dietary P/E ratio also varies depending on the design level. In previous studies, the use of five protein levels and three lipid levels produced 15 different dietary P/E ratios (from 23.36 to 37.71 mg kJ^{-1}) over a wider and higher range than the protein/energy ratios (from 23.91 to 26.83 mg kJ^{-1}) in the current study. The second is the difference in dietary ingredients. For better growth, dietary ingredients should be easily digestible and provide enough essential amino acids. However, different ingredients are different in terms of amino acid

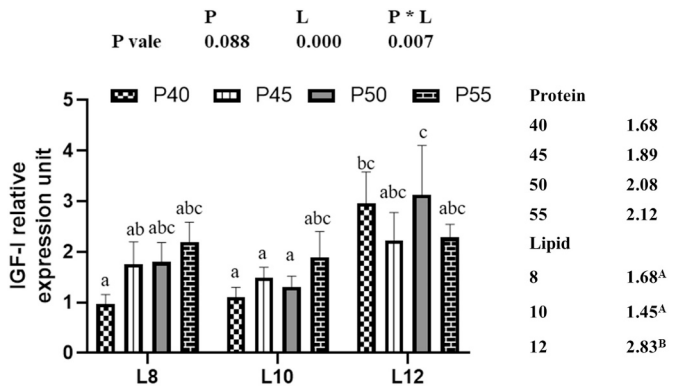

Fig. 3. Effect of dietary protein and lipid level on relative expressions of IGF-1 gene in liver of *S.sihama*. Data represent means of three fish in each group; error bar indicates S. D. Values had different letters are significantly different ($P < 0.05$). IGF-1: insulin-like growth factor 1.

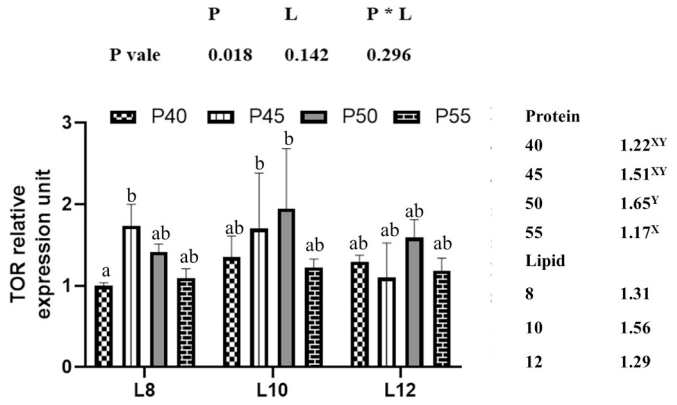

Fig. 4. Effect of dietary protein and lipid levels on relative expressions of TOR gene in liver of *S.sihama*. Data represent means of three fish in each group; error bar indicates S. D. Values had different letters are significantly different ($P < 0.05$). TOR: target of rapamycin.

composition and availability (NRC, 2011). Thus, for the same physiological fuel values, the optimal dietary protein/energy ratio is different (Catacutan and Coloso, 1995; Kpogue et al., 2013; Zhang et al., 2017), which probably affects the accuracy of optimal dietary P/E ratio. Hence, the digestible energy values of these components should be determined in the course of further studies of *S. sihama*.

PER is used to reflect the efficiency of the protein in the diet and to evaluate the quality of the protein (NRC, 2011). In general, the larger the PER value, the better the protein quality. In this study, PER was found to be negatively correlated with dietary protein levels, and it in *S. sihama* diets contain 40% protein were significantly higher than that of other diets containing higher protein levels. Some studies have indicated that excessive amounts of protein are metabolized and not used for growth and that low levels of dietary protein can be used effectively for protein synthesis (Hu et al., 2008). Also, in this study, PER increased as the level of dietary lipid increased. It shows a protein-sparing effect of dietary lipid in the *S. sihama* diet. The same result was reported in red-tailed catfish (*Hemibagrus wyckioides)* (Hung et al., 2017), catfish (*Mystus montanus)* (Raj et al., 2007), and Japanese seabass (*Lateolabrax japonicus)* (Ai et al., 2004). Besides, both FCR and DFI in this experiment decreased with the increase of dietary protein and lipid levels. The significant decrease in FCR indicates that the increase in dietary lipid and protein levels can improve the efficiency of diet utilization, which is similar to fish such as grouper (Wang et al., 2017), brown trout (*Salmo trutta fario*) (Wang et al., 2018). While the decrease in DFI indicates that high-energy diets will lead to a reduction in dietary intake of *S. sihama.* Fish seem to be able to adjust their feed intake to meet their energy needs (Pei et al., 2004). In the case of excess energy, fish feed and protein intake may be limited, resulting in a decrease in the growth rate of fish (Marais and Kissil, 1979). Rahimnejad et al. (2015) and Ellis and Reigh (1991) also reported reduced feeding and growth inhibition when fish were fed diets containing excessive energy. Consequently, a proper ratio of protein to non-protein energy is important to improve growth performance and feed utilization.

Morphological indicators, such as HSI, VSI and CF, are often used as a response to fish under different nutritional conditions (Chang et al., 2018; Luo et al., 2011). In the present study, whole-body lipid composition increased with dietary lipids and had a similar effect on HIS, VSI and CF in *S. sihama*. Although intra-peritoneal fat (IPF) was not measured in this study, increasing lipid levels in diets usually results in elevated IPF and affects VSI values. Same result were obtained in humpback grouper (Shapawi et al., 2011), and red-spotted grouper (Wang et al., 2017). In addition, high VSI values are caused by high HSI as well as high IPF values (Han et al., 2014). It was also observed in this experiment that HSI increased with increasing dietary lipid levels. The increase in dietary lipids in *S. sihama* induces an increase in lipid deposition and a decrease in moisture in the carcass. That agrees with catfish (Hung et al., 2017), masu salmon (*Oncorhynchus masou* Brevoort,1856), hybrid grouper (Rahimnejad et al., 2015) and blunt snout bream (*Megalobrama amblycephala*) (Li et al., 2010). The highest growth performance was achieved with the P45L12 diet, which also had the highest lipid content in the carcass. At the same time, increasing dietary protein did not change the ash, moisture, protein and lipid content of the carcass.

The activity of amino acid metabolizing enzymes is affected by dietary protein and lipid levels (Wang et al., 2018). GOT primarily transfers the amino of aspartic acid to a-ketone glutaric acid, producing oxaloacetic acid and glutamic acid; GPT primarily transfers the amino of alanine to a-ketoglutamic acid, producing pyruvate and glutamic acid (Lou et al., 2018). Oxaloacetic acid is mainly involved in the Krebs cycle (Wang, 2002) and provides the body with sufficient energy. Glutamate dehydrogenase (GDH) is involved in the breakdown of proteins by catalyzing the oxidative deamination of amino acids in the body (Wang et al., 2011). The combined deamination of GDH and transaminase is the main pathway of amino acid catabolism in the liver and pancreas. In the present study, we found that GPT and GOT activity increased with the increasing of dietary protein level, while GDH activity was significantly higher in the high-protein diets (50% and 55%) than that in the low-protein diets (40% and 45%), indicating that *S. sihama* were capable of adapting protein catabolism to protein input. Similar results were observed in brown trout (*Salmo trutta fario*) (Wang et al., 2018), sea bream (*Sparus aurata*, L.) (Fernández et al., 2007), *Pelteobagrus fulvidraco* (Lou et al., 2018). Decreased activity of GPT, GOT, and GDH indicates that the activity of proteolytic enzymes decreased with increasing dietary lipids. This may be due to a weakened role in driving amino acid gluconeogenesis to meet energy requirements. This implies that fish use lipids as an energy source, rather than natural proteins or carbohydrates (Wang et al., 2018). Furthermore, in the present study, reduced dietary protein and high dietary energy resulted in reduced protein catabolism. Similar observations were reported in rainbow trout (*Oncorhynchus mykiss*) (Kirchner et al., 2005), brown trout (*Salmo trutta fario*) (Wang et al., 2018) and tambaqui (*Colossoma macropomum*) (De Almeida et al., 2011).

LPL is a key enzyme in lipoprotein metabolism and is expressed mainly in tissues with high lipid oxidation or storage requirements (Preiss-Landl et al., 2002). Numerous studies have shown that improving dietary lipid level can significantly increase LPL mRNA expression levels and LPL activity in the liver of farmed fish (Li et al., 2013; Saera-Vila et al., 2005; Zhang et al., 2017). In the present study, we found that liver LPL activity also increased with increasing dietary lipid levels. When the lipid level is higher than 10%, the LPL activity was significantly higher than that in the low lipid group (8% group). This is

because high dietary lipids provide a large amount of TG to *S. sihama*, which requires a large amount of LPL to break down TG and then induce liver synthesis of LPL, resulting in a significant increase in LPL activity in the liver (Lou et al., 2018). Also, dietary protein levels did lead to a significant increase in LPL activity, which may suggest that additional energy, not just lipids, can affect LPL expression. There is little information on the relationship between LPL expression and dietary energy, although this hypothesis could be valuable for further research.

Fatty acid synthetase (FAS) is a complex system for fatty acid synthesis. Liver FAS plays an important role in animal body lipid production and deposition. It has been shown that the higher the diet lipid level, the lower the fatty acid synthase activity of large yellow croaker (*Larimichthys crocea*) (Yan et al., 2017) and hybrid clarias catfish (*Clarias macrocephalus*×*C.gariepinus*) (Jantrarotai et al., 1994). In this study, FAS activity was also significantly inhibited by dietary lipid levels. Besides, FAS activity was not significantly affected by dietary protein levels but showed a tendency to decrease before increasing. The increase in FAS activity at a dietary protein level of 55% indicates that excess available energy could be converted into lipids when the dietary protein level increases. It is unclear whether the excess amino acids in high-protein diets are used directly for energy and glycogen synthesis, or as a substrate for lipid synthesis. However, as previously suggested in the discussion of PER and FCR results, in *S. sihama*, protein appears to be used more effectively for energy purposes than carbohydrates when the protein level in the diet exceeds the required for maximum growth.

External stimuli and internal physiological conditions are processed in the brain and delivered through endocrine and hypothalamic control. Growth hormones released by the hypothalamus bind primarily to receptors in target organs in the liver and stimulate the synthesis and release of insulin-like growth factor (Kumar et al., 2018). Insulin-like growth factor is an important factor affecting growth-regulating factors, which are peptides that are essential for vertebrate growth (Duan, 1998). In many fish species, hepatic IGF-1 expression is influenced by the nutritional status of the specimen (Kumar et al., 2018). In vitro studies in rainbow trout muscle cells have shown that IGF-1 is also involved in regulating carbohydrate metabolism by stimulating intracellular glucose uptake (Castillo et al., 2004). IGF-1 mRNA levels in the liver of blunt snout bream significantly increased with increasing cottonseed meal replacement level (Zhou et al., 2017a). In this study, the mRNA expression of IGF-1 in *S. sihama* liver tissue correlates well with dietary lipid levels. Similarly, a positive correlation was found between IGF-1 in Senegalese sole (*Solea senegalensis*) with dietary lipid levels (Campos et al., 2010). The mRNA expression of IGF-1 in the liver tissue of *Labeo rohita* indicates a good correlation with the growth rates (Kumar et al., 2018). However, among the three groups with the best growth in this study (P45L12, P50L10, P50L12), only the IGF-1 expression level of P50L12 group was positively correlated with growth, while the IGF-1 expression levels of P45L12 and P50L10 did not reach higher levels. The possible reason is that liver IGF-1 expression level is not well related to growth. In the study of Nile tilapia (Campos et al., 2010), it was found that muscle IGF-1 expression level was positively correlated with growth and liver IGF-1 expression level was opposite to growth. In addition, it might be affected by the interaction between dietary protein and dietary lipid. Numerous studies have also shown that IGF-1 is also affected by dietary protein levels. Studies of gilthead seabream and salmon have found a positive correlation between IGF-1 expression levels and dietary protein levels. However, in this experiment, the dietary protein did not significantly affect the expression level of IGF-1. Studies on the effects of dietary protein and dietary lipid on liver IGF-1 expression are limited, and the underlying mechanism still needs further study.

Protein synthesis is a key process involved in animal growth reactions, and as far as we know, the TOR pathway plays an important role in this process (Zhang et al., 2017; Zhao et al., 2012). The restrictive step in protein synthesis is translation initiation, and translation initiation is regulated by the TOR signaling pathway (Zhou et al., 2017a). In this study, we found that the TOR expression level in liver of *S. sihama* received a significant effect from dietary protein levels, with a significant upward trend in protein levels at 40%–50%; however, TOR expression levels in liver were not affected by dietary lipid levels. The positive correlation between dietary protein levels and TOR mRNA levels in *S. sihama* liver suggests that appropriate dietary protein levels can reduce translation inhibition and increase TOR activity, thereby improving protein synthesis. Similar results were found on hybrid snakehead (*Channa maculata* ♀ × *Channa argus* ♂) (Zhang et al., 2017). In trout muscle cells, it was shown that amino acid levels could regulate TOR signaling by altering the phosphorylation status of TOR (Seiliez et al., 2008). Dietary amino acids such as threonine, isoleucine and leucine regulated TOR gene expression in the liver of blunt snout bream (Qian et al., 2014; Zhou et al., 2017a), Jian carp (Cai et al., 2012) and rainbow trout (Wacyk et al., 2012). Differences in protein levels in the diet lead directly to differences in dietary amino acid levels, which may also account for the 40%–50% increase in TOR expression levels. More studies are needed to elucidate more detailed models in which nutrients mediate the expression of the TOR gene in fish.

5. Conclusion

In conclusion, the present study suggested that the dietary protein requirements based on the growth of *S. sihama* were 48.42% (lipid level: 10%) and 48.53% (lipid level: 12%). Also, from the whole-body proximate compositions, *S. sihama* had a limited ability to utilize dietary lipids, with excess dietary lipids being deposited as body lipid rather than providing energy for improved protein utilization. Higher-lipid diets may inhibit proteolytic enzymes as judged by liver GOT, GPT and GDH activity. Further, the expression level of the TOR gene in the liver was significantly affected by dietary protein levels, while the expression level of the IGF-1 gene was more affected by dietary lipid levels. Finally, according to the economic benefits, the best diet combination was 48.42% protein level and 10% lipid level.

Funding information

National Key R&D Program of China (2019YFD0900200);
National Natural Science Foundation of China (31972808);
China Agriculture Research System (CARS-47);
"Chong yi liu (231419011)" Project of Guangdong Ocean University.

Declaration of Competing Interest

None.

References

Ai, Q., Mai, K., Li, H., Zhang, C., Zhang, L., Duan, Q., Tan, B., Xu, W., Ma, H., Zhang, W., Liufu, Z., 2004. Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, Lateolabrax japonicus. Aquaculture 230, 507–516. https://doi.org/10.1016/j.aquaculture.2003.09.040.

AOAC, 1995. Official Methods of Analysis of the Association of Analytical Chemistry, 16th ed. Association of Official Analytical Chemists, Aoac Official Method, Washington, DC.

Cai, C.-F., Wang, W.-J., Ye, Y.-T., Krogdahl, A., Wang, Y.-L., Xia, Y.-M., Yang, C.-G., 2012. Effect of soybean meal, raffinose and stachyose on the growth, body composition, intestinal morphology and intestinal microflora of juvenile allogynogenetic silver crucian carp (*Carassius auratus gibelio*♀×*Cyprinus carpio*♂). Aquac. Res. 43, 128–138. https://doi.org/10.1111/j.1365-2109.2011.02811.x.

Campos, C., Valente, L.M.P., Borges, P., Bizuayehu, T., Fernandes, J.M.O., 2010. Dietary lipid levels have a remarkable impact on the expression of growth-related genes in Senegalese sole (*Solea senegalensis* Kaup). J. Exp. Biol. 213, 200–209. https://doi.org/10.1242/jeb.033126.

Cao, J.X., Jain, J.C., Huang, Y., Du, T., 2010. Morphology and histology of the digestive system in silver sillago *Sillago sihama*(Forskál). Fish. Sci. 29, 339–343.

Castillo, J., Codina, M., Martínez, M.L., Navarro, I., Gutiérrez, J., 2004. Metabolic and mitogenic effects of IGF-I and insulin on muscle cells of rainbow trout. Am. J. Physiol. Integr. Comp. Physiol. 286, R935–R941. https://doi.org/10.1152/ajpregu.00459.2003.

Catacutan, M.R., Coloso, R.M., 1995. Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass, *Lates calcarifer*. Aquaculture 131, 125–133. https://doi.org/10.1016/0044-8486(94)00358-U.

Chang, J., Niu, H.X., Jia, Y.D., Li, S.G., Xu, G.F., 2018. Effects of dietary lipid levels on growth, feed utilization, digestive tract enzyme activity and lipid deposition of juvenile Manchurian trout, *Brachymystax lenok* (Pallas). Aquac. Nutr. 24, 694–701. https://doi.org/10.1111/anu.12598.

Chatzifotis, S., Panagiotidou, M., Papaioannou, N., Pavlidis, M., Nengas, I., Mylonas, C. C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. Aquaculture 307, 65–70. https://doi.org/10.1016/j.aquaculture.2010.07.002.

De Almeida, L.C., Avilez, I.M., Honorato, C.A., Hori, T.S.F., Moraes, G., 2011. Growth and metabolic responses of tambaqui (*Colossoma macropomum*) fed different levels of protein and lipid. Aquac. Nutr. 17 https://doi.org/10.1111/j.1365-2095.2010.00759.x.

Dian, L.J., Xin, Z.L., Lu, H.S., 2010. Estimation of growth and mortality parameters of the silver sillago (*Sillago sihama*) in beibu gulf. J. Zhejiang Ocean Univ. Sci. 1, 64–69. https://doi.org/10.3969/j.issn.1008-830X.2010.01.014.

Duan, C., 1998. Nutritional and developmental regulation of insulin-like growth factors in fish. J. Nutr. 128, 306S–314S. https://doi.org/10.1093/jn/128.2.306S.

Ellis, S.C., Reigh, R.C., 1991. Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 97, 383–394. https://doi.org/10.1016/0044-8486(91)90330-A.

Fernández, F., Miquel, A.G., Córdoba, M., Varas, M., Metón, I., Caseras, A., Baanante, I. V., 2007. Effects of diets with distinct protein-to-carbohydrate ratios on nutrient digestibility, growth performance, body composition and liver intermediary enzyme activities in gilthead sea bream (*Sparus aurata*, L.) fingerlings. J. Exp. Mar. Biol. Ecol. 343, 1–10. https://doi.org/10.1016/j.jembe.2006.10.057.

Fiogbé, E.D., Kestemont, P., Mélard, C., Micha, J.C., 1996. The effects of dietary crude protein on growth of the Eurasian perch *Perca fluviatilis*. Aquaculture 144, 239–249. https://doi.org/10.1016/S0044-8486(96)01293-8.

Ghanawi, J., Roy, L., Davis, D.A., Saoud, I.P., 2011. Effects of dietary lipid levels on growth performance of marbled spinefoot rabbitfish *Siganus rivulatus*. Aquaculture 310, 395–400. https://doi.org/10.1016/j.aquaculture.2010.11.012.

Guo, Y.S., Wang, Z.D., Yan, C.Z., Zhang, Y.L., Zheng, J.N., Xu, Y.M., Du, T., Liu, C.W., 2014. Isolation and characterization of microsatellite DNA loci from *Sillago sihama*. J. Genet. 93, 32–36. https://doi.org/10.1007/s12041-012-0133-y.

Han, T., Li, X., Wang, J., Hu, S., Jiang, Y., Zhong, X., 2014. Effect of dietary lipid level on growth, feed utilization and body composition of juvenile giant croaker *Nibea japonica*. Aquaculture 434, 145–150. https://doi.org/10.1016/j.aquaculture.2014.08.012.

Hu, Y., Tan, B., Mai, K., Ai, Q., Zheng, S., Cheng, K., 2008. Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. Aquac. Nutr. 14, 499–506. https://doi.org/10.1111/j.1365-2095.2007.00555.x.

Huang, Y., Du, T., Huang, H.L., 2013. A study on artificial breeding of *Sillago sihama* Forsskál. J. Guangdong Ocean Univ. 33, 18–24 (doi:CNKI:SUN:SHDX.0.2013-01-002).

Huang, Q., Kwaku, A., Du, T., Tan, B., Chi, S., Yang, Q., Zhang, S., Liu, H., Dong, X., Yang, Y., 2018. Dietary vitamin a requirement of *Sillago sihama* Forskál. Aquac. Res. 49, 2587–2596. https://doi.org/10.1111/are.13725.

Huang, Q., Zhang, S., Du, T., Yang, Q., Chi, S., Liu, H., Yang, Y., Dong, X., Tan, B., 2020. Modulation of growth, immunity and antioxidant-related gene expressions in the liver and intestine of juvenile *Sillago sihama* by dietary vitamin C. Aquac. Nutr. 26, 338–350. https://doi.org/10.1111/anu.12996.

Huang, Q.-C., Qin, D., Tan, B., Du, T., Yang, Y.-Z., Yang, Q., Chi, S., Dong, X., 2020a. The optimal dietary protein level of juvenile silver sillago *Sillago sihama* at three dietary lipid levels. Aquac. Res. 51, 816–827. https://doi.org/10.1111/are.14431.

Huang, Q.-C., Zhang, S., Du, T., Yang, Q.-H., Chi, S.-Y., Liu, H.-Y., Yang, Y.-Z., Dong, X.-H., Tan, B.-P., 2020b. Effects of dietary vitamin E on growth, immunity and oxidation resistance related to the Nrf2/Keap1 signalling pathway in juvenile *Sillago sihama*. Anim. Feed Sci. Technol. 262, 114403. https://doi.org/10.1016/j.anifeedsci.2020.114403.

Hung, L.T., Binh, V.T.T., Thanh Truc, N.T., Tham, L.H., Ngoc Tran, T., 2017. Effects of dietary protein and lipid levels on growth, feed utilization and body composition in red-tailed catfish juveniles (*Hemibagrus wyckioides*, Chaux & Fang 1949). Aquac. Nutr. 23, 367–374. https://doi.org/10.1111/anu.12401.

Huo, Y.-W., Jin, M., Zhou, P.-P., Li, M., Mai, K.-S., Zhou, Q.-C., 2014. Effects of dietary protein and lipid levels on growth, feed utilization and body composition of juvenile swimming crab, *Portunus trituberculatus*. Aquaculture 434, 151–158. https://doi.org/10.1016/j.aquaculture.2014.08.011.

Jantrarotai, W., Sitasit, P., Rajchapakdee, S., 1994. The optimum carbohydrate to lipid ratio in hybrid Clarias catfish (*Clarias macrocephalus*×*C. gariepinus*) diets containing raw broken rice. Aquaculture 127, 61–68. https://doi.org/10.1016/0044-8486(94)90192-9.

Kaushik, S.J., Médale, F., 1994. Energy requirements, utilization and dietary supply to salmonids. Aquaculture 124, 81–97. https://doi.org/10.1016/0044-8486(94)90364-6.

Kirchner, S., Seixas, P., Kaushik, S., Panserat, S., 2005. Effects of low protein intake on extra-hepatic gluconeogenic enzyme expression and peripheral glucose phosphorylation in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 140, 333–340. https://doi.org/10.1016/j.cbpc.2004.10.019.

Kpogue, D.N.S., Ayanou, G.A., Toko, I.I., Mensah, G.A., Fiogbe, E.D., 2013. International journal of fisheries and aquaculture influence of dietary protein levels on growth, feed utilization and carcass composition of snakehead, *Parachanna obscura* (Günther, 1861) fingerlings. Int. J. Fish. Aquac. 5, 71–77. https://doi.org/10.5897/IJFA12.074.

Kumar, S., Sahu, N.P., Ranjan, A., 2018. Feeding de-oiled rice bran (DORB) to Rohu, *Labeo rohita*: effect of varying dietary protein and lipid level on growth, body composition, and insulin like growth factor (IGF) expression. Aquaculture 492, 59–66. https://doi.org/10.1016/j.aquaculture.2018.04.001.

Lee, C.S., 1981. Factors affecting egg characteristics in the fish *Sillago sihama*. Mar. Ecol. Prog. Ser. 4, 361–363. https://doi.org/10.3354/meps004361.

Lee, S.M., Kim, K.D., 2001. Effects of dietary protein and energy levels on the growth, protein utilization and body composition of juvenile masu salmon (*Oncorhynchus masou* Brevoort). Aquac. Res. 32, 39–45. https://doi.org/10.1046/j.1355-557x.2001.00004.x.

Lee, C.S., Hu, F., Hirano, R., 1981. Salinity tolerance of fertilized eggs and larval survival in the flsh *Sillago sihama*. Mar. Ecol. Prog. Ser. 4, 169–174. https://doi.org/10.3354/meps004169.

Lee, S.M., Im, G.J., Jong, Y.L., 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211, 227–239. https://doi.org/10.1016/S0044-8486(01)00880-8.

Li, X., Liu, W., Jiang, Y., Zhu, H., Ge, X., 2010. Effects of dietary protein and lipid levels in practical diets on growth performance and body composition of blunt snout bream (*Megalobrama amblycephala*) fingerlings. Aquaculture 303, 65–70. https://doi.org/10.1016/j.aquaculture.2010.03.014.

Li, X.-F., Jiang, G.-Z., Qian, Y., Xu, W.-N., Liu, W.-B., 2013. Molecular characterization of lipoprotein lipase from blunt snout bream *Megalobrama amblycephala* and the regulation of its activity and expression by dietary lipid levels. Aquaculture 416–417, 23–32. https://doi.org/10.1016/j.aquaculture.2013.08.020.

Li, S., Li, Z., Chen, N., Jin, P., Zhang, J., 2019. Dietary lipid and carbohydrate interactions: implications on growth performance, feed utilization and non-specific immunity in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). Aquaculture 498, 568–577. https://doi.org/10.1016/j.aquaculture.2018.09.015.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Lou, Y., Zhang, J., Zhao, N., Sharawy, Z., Li, Y., Ma, J., 2018. Effects of dietary lipid and protein levels on growth and physiological metabolism of *Pelteobagrus fulvidraco* larvae under recirculating aquaculture system (RAS). Aquaculture 495, 458–464. https://doi.org/10.1016/j.aquaculture.2018.06.004.

Luo, Z., Tan, X.-Y., Liu, X.-J., Wen, H., 2011. Effect of dietary betaine levels on growth performance and hepatic intermediary metabolism of GIFT strain of Nile tilapia *Oreochromis niloticus* reared in freshwater. Aquac. Nutr. 17, 361–367. https://doi.org/10.1111/j.1365-2095.2010.00805.x.

Marais, J.F.K., Kissil, G.W., 1979. The influence of energy level on the feed intake, growth, food conversion and body composition of *Sparus aurata*. Aquaculture 17, 203–219. https://doi.org/10.1016/0044-8486(79)90124-8.

Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J., Morris, P.C., 2001. Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth, feed utilisation, muscle composition and liver histology. Aquaculture 203, 101–119. https://doi.org/10.1016/S0044-8486(01)00618-4.

NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, D.C. https://doi.org/10.17226/13039.

Pei, Z., Xie, S., Lei, W., Zhu, X., Yang, Y., 2004. Comparative study on the effect of dietary lipid level on growth and feed utilization for gibel carp (*Carassius auratus gibelio*) and Chinese longsnout catfish (*Leiocassis longirostris* Günther). Aquac. Nutr. 10, 209–216. https://doi.org/10.1111/j.1365-2095.2004.00291.x.

Preiss-Landl, K., Zimmermann, R., Hämmerle, G., Zechner, R., 2002. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. Curr. Opin. Lipidol. 13, 471–481. https://doi.org/10.1097/00041433-200210000-00002.

Qian, Y., Li, X.F., Sun, C.X., Jiang, G.Z., Xu, W.N., Wang, Y., Liu, W.B., 2014. Dietary biotin requirement of juvenile blunt snout bream, *Megalobrama amblycephala*. Aquac. Nutr. 20, 616–622. https://doi.org/10.1111/anu.12114.

Rahimnejad, S., Bang, I.C., Park, J.Y., Sade, A., Choi, J., Lee, S.M., 2015. Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile hybrid grouper, *Epinephelus fuscoguttatus*×*E. lanceolatus*. Aquaculture 446, 283–289. https://doi.org/10.1016/j.aquaculture.2015.05.019.

Raj, A.J.A., Haniffa, M.A., Seetharaman, S., Appelbaum, S., 2007. Effect of dietary lipid levels on survival and growth of the threatened freshwater catfish *Mystus montanus*. Su Ürünleri Derg. 24, 51–54.

Saera-Vila, A., Calduch-Giner, J.A., Gómez-Requeni, P., Médale, F., Kaushik, S., Pérez-Sánchez, J., 2005. Molecular characterization of gilthead sea bream (*Sparus aurata*) lipoprotein lipase. Transcriptional regulation by season and nutritional condition in skeletal muscle and fat storage tissues. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 142, 224–232. https://doi.org/10.1016/j.cbpb.2005.07.009.

Seiliez, I., Gabillard, J.-C., Skiba-Cassy, S., Garcia-Serrana, D., Gutiérrez, J., Kaushik, S., Panserat, S., Tesseraud, S., 2008. An in vivo and in vitro assessment of TOR signaling cascade in rainbow trout (*Oncorhynchus mykiss*). Am. J. Physiol. Integr. Comp. Physiol. 295, R329–R335. https://doi.org/10.1152/ajpregu.00146.2008.

Shamsan, E.F., Ansari, Z.A., 2010. Biochemical composition and caloric content in the sand whiting *Sillago sihama* (Forsskal), from Zuari estuary, Goa. Indian J. Fish. 57.

Shapawi, R., Mustafa, S., Ng, W.K., 2011. A comparison of the growth performance and body composition of the humpback grouper, *Cromileptes altivelis* fed on farm-made feeds, commercial feeds or trash fish. J. Fish. Aquat. Sci. 6, 523–534. https://doi.org/10.3923/jfas.2011.523.534.

Venkatesan, K., Nazeer, R.A., 2014. Antioxidant activity of purified protein hydrolysates from northern whiting fish (*Sillago sihama*) muscle. Int. J. Pept. Res. Ther. 20, 209–219. https://doi.org/10.1007/s10989-013-9384-6.

Wacyk, J., Powell, M., Rodnick, K., Overturf, K., Hill, R.A., Hardy, R., 2012. Dietary protein source significantly alters growth performance, plasma variables and hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) fed amino acid balanced diets. Aquaculture 356–357, 223–234. https://doi.org/10.1016/j.aquaculture.2012.05.013.

Wan, R.J., 1996. Morphology of early development of *Sillago sihama* Forsskál. Mar. Fish. Res 17, 35–41.

Wang, J., 2002. Biochemical. Higher Education Press, Beijing.

Wang, J., Hua, X., Xing, S., Han, J., Yu, N., Zhong, H., Zhu, Z., Huang, X., 2011. Effect of partial replacement of fish meal by corn gluten meal and nitrogen and phosphorus retention in muscle of *Carassius auratus gibelio* in fresh water cage culture. J. Shanghai Ocean Univ. 20, 741–747.

Wang, J.T., Han, T., Li, X.Y., Yang, Y.X., Yang, M., Hu, S.X., Jiang, Y.D., Harpaz, S., 2017. Effects of dietary protein and lipid levels with different protein-to-energy ratios on growth performance, feed utilization and body composition of juvenile red-spotted grouper, *Epinephelus akaara*. Aquac. Nutr. 23, 994–1002. https://doi.org/10.1111/anu.12467.

Wang, C., Hu, G., Sun, P., Gu, W., Wang, B., Xu, Q., Liu, H., 2018. Effects of dietary protein at two lipid levels on growth, gonadal development, body composition and liver metabolic enzymes of brown trout (*Salmo trutta fario*) broodstock. Aquac. Nutr. 24, 1587–1598. https://doi.org/10.1111/anu.12795.

Wang, L., Zhang, W., Gladstone, S., Ng, W., Zhang, J., Shao, Q., 2019. Effects of isoenergetic diets with varying protein and lipid levels on the growth, feed utilization, metabolic enzymes activities, antioxidative status and serum biochemical parameters of black sea bream (*Acanthopagrus schlegelii*). Aquaculture 513, 734397. https://doi.org/10.1016/j.aquaculture.2019.734397.

Watanabe, T., 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. Part B Comp. Biochem. 73, 3–15. https://doi.org/10.1016/0305-0491(82)90196-1.

Yan, J., Liao, K., Mai, K., Ai, Q., 2017. Dietary Lipid Levels Affect Lipoprotein Clearance, Fatty Acid Transport, Lipogenesis and Lipolysis at the Transcriptional Level in Muscle and Adipose Tissue of Large Yellow Croaker (*Larimichthys crocea*), pp. 3925–3934. https://doi.org/10.1111/are.13219.

Zhang, J.D., Ma, L., Cheng, G., 2010. Morphological variation analysis among three populations of *Sillago sihama* Forsskál. J. Anhui Agric. Sci. 38, 5678–5681.

Zhang, Y., Sun, Z., Wang, A., Ye, C., Zhu, X., 2017. Effects of dietary protein and lipid levels on growth, body and plasma biochemical composition and selective gene expression in liver of hybrid snakehead (*Channa maculata* ♀ × *Channa argus* ♂) fingerlings. Aquaculture 468, 1–9. https://doi.org/10.1016/j.aquaculture.2016.09.052.

Zhang, N., Du, W., Wang, Z., Huang, Y., Du, T., Dong, Z., 2018. Screening of reference genes for real-time PCR in different tissues from *Sillago sihama*. J. Guangdong Ocean Univ. 38, 8–14.

Zhao, J., Liu, Y., Jiang, J., Wu, P., Chen, G., Jiang, W., Li, S., Tang, L., Kuang, S., Feng, L., Zhou, X., 2012. Effects of dietary isoleucine on growth, the digestion and absorption capacity and gene expression in hepatopancreas and intestine of juvenile Jian carp (*Cyprinus carpio var*. Jian). Aquaculture 368–369, 117–128. https://doi.org/10.1016/j.aquaculture.2012.09.019.

Zhen, L.U., Xiao, E.N., Jian, D.U., 2008. The population dynamics and parameter of growth and mortality of *Sillago sihama* in the Minnan-Taiwan fishing grounds. Mar. Fish. Res. 29, 47–53.

Zhou, Q.-L., Habte-Tsion, H.-M., Ge, X., Liu, B., Xie, J., Ren, M., Miao, L., Pan, L., 2017a. Growth performance and TOR pathway gene expression of juvenile blunt snout bream, *Megalobrama amblycephala*, fed with diets replacing fish meal with cottonseed meal. Aquac. Res. 48, 3693–3704. https://doi.org/10.1111/are.13195.

Zhou, Y., Huang, H., Du, T., Huang, Y., 2017b. Study on adaptability of juvenile of *Sillago sihama* to salinity. J. Fish. Res. 39, 22–26. https://doi.org/10.14012/j.cnki.fjsc.2017.01.004.