Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 2012958, 12 pages
https://doi.org/10.1155/2022/2012958

Research Article

Effect of Dietary Cholesterol on Growth Performance, Cholesterol Deposition, and Lipid Metabolism in Adult Chinese Mitten Crab (*Eriocheir sinensis*)

Hui-xing Guo, Guang-zhen Jiang, Yong-jun Dai, Yang-yang Huang, Xi Wang, Kang Xiao, Mang-mang Wang, Kenneth Abasubong, Yun Xu, Ding-dong Zhang, and Wen-bin Liu

Key Laboratory of Aquatic Nutrition and Feed Science of Jiangsu Province, College of Animal Science and Technology, Nanjing Agricultural University, No. 1 Weigang Road, Nanjing 210095, China

Correspondence should be addressed to Wen-bin Liu; wbliu@njau.edu.cn

Received 14 March 2022; Revised 10 April 2022; Accepted 5 May 2022; Published 20 May 2022

Academic Editor: Jianguang Qin

This research investigated the effects of dietary cholesterol (CHO) on growth, molting performance, CHO deposition, and lipid metabolism of Chinese mitten crab (*Eriocheir sinensis*). Six diets were formulated to contain 0% (control), 0.1%, 0.2%, 0.4%, 0.8%, and 1.6% CHO and were administered to *E. sinensis* ($43.35 \pm 0.05\,g$), for 16 weeks. From our results, crabs fed with 0.2% and 0.4% CHO diet, the final body weight (FBW), and weight gain ratio (WGR) increased significantly and the feed conversion ratio (FCR) decreased significantly compared with that of the control group. Furthermore, the molting performance of *E. sinensis* was significantly improved in crabs fed 0.2% CHO. Meanwhile, 0.2% CHO tends to upregulate molt-promoting genes and downregulate the expression of molt-inhibiting genes. It indicated that CHO might improve the molting performance of crabs by regulating ecdysteroid signal pathway transduction. In addition, dietary CHO can significantly increase the content of CHO in the hepatopancreas, muscle, and hemolymph. Moreover, dietary CHO increases the content of triglyceride (TG) in the hepatopancreas and hemolymph. The gene-related lipid metabolism shows that CHO may increase the lipid content by promoting lipid synthesis and inhibiting lipolysis. According to the results of this study, the dietary CHO requirement of *E. sinensis* is 0.27% for growth requirement and 0.45% for tissue CHO deposition based on the broken-line model.

1. Introduction

Cholesterol (CHO), as a kind of lipid, widely exists in various animal tissues [1], especially in the brain and nervous system. Plants generally contain limited or no CHO content [2]. The particularity structure of CHO makes it significant to all kinds of creatures. CHO is a key component of the cell membrane, which has great significance in maintaining cell membrane stability and fluidity [3]. CHO plays a significant role in lipid metabolism by synthesizing apolipoprotein [4]. In addition, CHO is a precursor for synthesizing a variety of active substances in organisms, including vitamin D and various steroid hormones [5]. Vertebrates can synthesize CHO with acetyl-CoA, usually without CHO deficiency [6, 7]. Due to the lack of some key enzymes in CHO synthesis, crustaceans cannot synthesize CHO. Therefore, crustaceans mainly obtain CHO through animal components in feed (e.g., fish meal and fish oil) [7, 8]. In recent years, with the replacement of animal components by plant components in the feed, the utilization of CHO is further limited [9]. Therefore, it is necessary to investigate the nutritional requirements of CHO in order to develop a more comprehensive formula feed.

It has been reported that adding CHO in the feed of *Scylla serrata* is critical to maintain their survival [10]. The addition of CHO in the diet of *Macrobrachium rosenbergii* had no effects on the growth rate, but the addition of CHO enabled *Macrobrachium rosenbergii* to achieve optimal growth performance [11]. Meanwhile, as an important component of lipoprotein, CHO has a significant influence on lipid metabolism [12]. For instance, several studies have shown that adding CHO to rainbow trout (*Oncorhynchus mykiss*) feed can inhibit β-oxidation and increase the body's lipid content [13]. In crustaceans, CHO is used to synthesize ecdysone, while the addition of 0.5% CHO in the diet of *Procambarus clarkii* was reported to promote molting [14, 15]. The lack of CHO in the diet can cause prolonged molting cycles in shrimp and crabs [16]. According to date from previous studies, the CHO requirement is 0.1%~0.2% for *Penaeus japonicus*, 0.14% for *Litopenaeus vannamei*, 0.5% for *Homarus americanus*, and 0.2% for *Penaeus monodon* [2, 17–19]. Previous studies have shown that there are great differences in CHO requirement of crustaceans at different developmental stages, but at present, the research on CHO requirement in Chinese mitten crab (*Eriocheir sinensis*) is focused on juvenile crabs [8, 20, 21], while the CHO requirement in adult *Eriocheir sinensis* has not been reported.

In China, *E. sinensis* is a unique economic aquatic species, which plays a significant role in the Chinese aquaculture system in alleviating poverty and improving economic growth in many regions [22, 23]. In 2020, the output of *E. sinensis* has reached 775,000 tons, which has become an important part of people's diet (Chinese fishery statistical yearbook, 2020). At present, juvenile *E. sinensis* can be fed with formula feed in the whole process, but for adult crab, the whole process of feeding formula feed is prone to problems such as unsuccessful molting and slow maturation. As an essential nutrient of crab, CHO plays an important role in growth and development. The deficiency of CHO in the feed may be one of the reasons for these problems [8, 24]. Given its great economic value, the research on the nutritional requirements of *E. sinensis* has been more in depth in recent years. However, there has been no report on the CHO requirement of adult *E. sinensis*. This study is aimed at evaluating the effects of dietary CHO on the growth, molting, CHO deposition, and lipid metabolism of adult *E. sinensis*. In addition, the optimum CHO content requirement in the diet will be assessed.

2. Materials and Methods

All experimental operations and feeding schemes in this study were approved by the Animal Care and Use Committee of Nanjing Agricultural University (Nanjing, China) (permit number: SYXK (Su) 2011–0036).

2.1. Diets. Six isonitrogenous and isolipid diets were formulated containing CHO 0% (control), 0.1%, 0.2%, 0.4%, 0.8%, and 1.6% (actually 0.06%, 0.14%, 0.23%, 0.44%, 0.82%, and 1.64%) at the cost of soybean oil. The fundamental diet formula of this experiment is shown in Table 1.

All the experimental diets were made in laboratory conditions. First of all, all ingredients were processed through a 60 mm mesh; then, weigh all kinds of ingredients, and mix thoroughly with lipid sources. Thereafter, about 30% distilled water was continually added to the ingredient and was mixed manually to homogenize the mixture. Subsequently, the homogeneous mixture was processed through a 2.5 mm diameter die of a single-screw meat grinder extruder and later ventilated to dry at room temperature for 24 hours. After drying, the feed was ground into an appropriate size. Finally, all diets were stored at −20°C in a sealed plastic bag.

2.2. Experiment Crab and Feeding Trial. *E. sinensis* were purchased from a local farm in Pukou, Jiangsu province, China. A total of 240 healthy crabs $(43.35 \pm 0.05\,g)$ were randomly selected and divided into 6 groups, with four replicates (5 male crabs and 5 female crabs/replicate), and each replicate was fed in one cement pool, 24 cement pools in total $(1.0 \times 1.0 \times 0.8\,m, L : W : H)$, with each cement pool having 12 pipes (20 cm long and 15 cm diameter) as shelters. Before the experiment, the crabs were fed for a week to adapt to the experimental condition. After the conditioning period, all crabs were hand fed with one of six experiment diets once daily (18 : 00) with 4%–6% body weight till obvious satiation for 16 weeks. During the experiment, clean up the residual feed and change 1/3 of the water every day, the number of death and molting crabs were recorded daily, the water temperature was at 22–28°C, dissolved oxygen was at 5.0–7.0 mg/L, pH was at 7.3–8.4, and ammonia was under 0.05 mg/L.

2.3. Sample Collection. Before sampling, all crabs were starved for 24 hours. Subsequently, all the crabs of each cement pool were quickly anesthetized on ice, then weighed, and counted. Four individuals from each cement pool were randomly selected to collect hemolymph with 1.0 mL syringes on the leg joints and mixed with precooled anticoagulant (EDTA, 15 mmol/L; citric acid, 30 mmol/L; citrate, 26 mmol/L; NaCl, 450 mmol/L; glucose, 100 mmol/L; and $pH = 7.2$) 1 : 1, then centrifuged at 4500 rpm, 4°C for 15 min, and stored at −20°C for subsequent analysis. Concurrently, the hepatopancreas and muscle samples were quickly collected, frozen in liquid nitrogen immediately, and then stored in the refrigerator at −20°C for gene expression and biochemical index analysis.

2.4. The Proximate Composition of Diet and Cholesterol Deposition. The total lipid, crude protein, moisture, and crude ash content in the diet was measured according to the standard method [25]. The specific procedures of determining crude protein, total lipid, crude ash, and moisture were the same as those in Cheng et al. [26]. The content of CHO in feed, hepatopancreas, and muscle samples were detected by high-performance liquid chromatography (HPLC). In summary, 1 gram of the sample was placed in a 50 mL centrifuge tube, and 15 mL ethanol absolute and 5 mL 60% KOH were added to it and then saponified in the water bath at 90°C for 1 hour. Subsequently, centrifuge

Table 1: Ingredient formulation and proximate composition (% dry matter) in this feeding trial.

	Cholesterol supplementation (%)					
	0	0.1	0.2	0.4	0.8	1.6
Ingredients (%)						
Defatted fish meal[a]	18.50	18.50	18.50	18.50	18.50	18.50
Soybean meal	15.00	15.00	15.00	15.00	15.00	15.00
Rapeseed meal	2.50	2.50	2.50	2.50	2.50	2.50
Cottonseed meal	3.00	3.00	3.00	3.00	3.00	3.00
Peanut meal	28.50	28.50	28.50	28.50	28.50	28.50
α-Starch	19.00	19.00	19.00	19.00	19.00	19.00
EPA oil: DHA oil (1 : 1)[b]	1.20	1.20	1.20	1.20	1.20	1.20
Soybean oil	5.20	5.10	5.00	4.80	4.40	3.60
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2{\cdot}H_2O$	2.20	2.20	2.20	2.20	2.20	2.20
Cholesterol (purity 99%)[c]	0.00	0.10	0.20	0.40	0.80	1.60
Lecithin	0.20	0.20	0.20	0.20	0.20	0.20
Zeolite	0.40	0.40	0.40	0.40	0.40	0.40
Premix[d]	1.00	1.00	1.00	1.00	1.00	1.00
Mixture[e]	2.30	2.30	2.30	2.30	2.30	2.30
Total	100.00	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)						
Crude protein	36.21	36.15	36.01	36.31	35.96	36.24
Crude lipid	7.95	8.08	8.05	7.98	8.05	7.96
Crude ash	7.15	7.21	7.16	7.07	7.10	7.16

[a]Fishmeal had been skimmed from 0.38% to 0.11% cholesterol. [b]DHA oil and EPA oil (DHA content, 70% of oil; EPA content, 70% of oil) were purchased from Shanxi Pioneer Biotech Co. Ltd., Xian, Shanxi, China. [c]Cholesterol (purity 99%) was purchased from Shanxi Pioneer Biotech Co. Ltd., Xian, Shanxi, China. [d]Premix supplied the following minerals (g/kg) and vitamins (IU or mg/kg): $CuSO_4{\cdot}5H_2O$, 2 g; $FeSO_4{\cdot}7H_2O$, 25 g; $ZnSO_4{\cdot}7H_2O$, 22 g; $MnSO_4{\cdot}4H_2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2{\cdot}6H_2O$, 0.1 g; vitamin A, 900000 IU; vitamin D, 200000 IU; vitamin E, 4500 mg; vitamin K3, 220 mg; vitamin B1, 320 mg; vitamin B2, 1090 mg; vitamin B5, 2000 mg; vitamin B6, 500 mg; vitamin B12, 1.6 mg; vitamin C, 10000 mg; pantothenate, 1000 mg; folic acid, 165 mg; choline, 60000 mg; Biotin, 100 mg; and myoinositol, 15000 mg. [e]Mixture includes the following ingredients (%): choline chloride 4.75%; antioxidants 1.72%; mildew-proof agent 2.35%; salt 22.06%; Lvkangyuan 59.30%; and biostimep 9.51%.

at 5,000 g for 5 minutes; 1 mL supernatant was taken into 10 mL with ethanol absolute for HPLC detection (Agilent ZORBAX Eclipse Plus, column C18 5 μm $4.6 \times 150\,mm$). 100% methanol was used as the mobile phase with a flow rate at 1 mL/min.

2.5. The Biochemical Analysis of Hepatopancreas, Hemolymph, and Muscle. The triglyceride (TG) (cat. no. A110-1-1) and nonesterified fatty acid (NEFA) (cat. no. A042-2-1) contents in hemolymph, hepatopancreas, and muscle and total cholesterol (TC) (cat. no. A111-1-1), high-density lipoprotein cholesterol (HDL-C) (cat. no. A112-1-1), and low-density lipoprotein cholesterol (LDL-C) (cat. no. A113-1-1) contents in the hemolymph were measured. The abovementioned parameters were measured by the commercial kit (Jiancheng Bioengineering Co., Nanjing, China) according to the instructions.

2.6. Gene Expression. Total RNA was extracted from hepatopancreas using RNAiso Plus (TaKaRa, Dalian, China); then, the purity and concentration of RNA were measured by spectrophotometry on the basis of OD 260/280 using a NanoDrop ND-1000 UV Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Subsequently, 1 μg RNA was reverse transcribed into cDNA immediately by HiScript® III RT SuperMix for qPCR (Vazyme, Nanjing, China) according to the manufacturer's instructions and then stored at −20°C for real-time qPCR. After the reverse transcription, the expression level of mRNA was detected by real-time qPCR. The specific primer sequences of genes in this experiment are shown in Table 2. Briefly, the reaction system of RT-qPCR including 10 μL 2×ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China), 2 μL cDNA template, and 7.2 μL DEPC-water and the primers of forward and reverse were 0.4 μL (10 μM). The PCR procedure included 95°C for 30 s and then at 95°C for 10 s and 60°C for 28 s for 40 cycles. The melting curve step was from 95°C to 60°C for 15 s and 60°C for 1 min; then, the temperature was raised to 95°C at a rate of 1.6°C per second for 15 s. At the end of the reaction, the specificity of the PCR product was determined by the melting curve. The β-actin gene was used as the internal reference and the target gene expression of crabs in the group with 0% CHO as the correct factor. The gene expression level was calculated by the $2^{-\Delta\Delta CT}$ [27] method.

Table 2: Primer pair sequences and product size of the genes used for real-time PCR (qPCR).

Gene	Position	Primer sequence	Length	Product size (bp)	Reference
srebp-1	Forward	TCTTCACACCCTCTGGACGC	20	162	[59]
	Reverse	CCAAGGTTGTAATGGCACGC	20		
cpt-1a	Forward	CATCTGGACACCCACCTCCA	20	183	[22]
	Reverse	ATCTCCTCACCCGGCACTCT	20		
fas	Forward	GTCCCTTCTTCTACGCCATCC	21	127	[59]
	Reverse	CGCTCTCCAGGTCAATCTTCAC	22		
mttp	Forward	TAGGACAAGCAGGACTTTCCTCA	24	138	[59]
	Reverse	CCACATCCACAAACACATCAACA	23		
ecr	Forward	CTCCCGGGTGCCATATTACC	20	212	KF469223.1
	Reverse	TGCTACACGGCACATTCACT	20		
rxr	Forward	ACCCTGTGCTAACCCTCTGA	20	74	MK604180.1
	Reverse	TGCTCACCACATCCTGCTTT	20		
mih	Forward	TTTAGCTCCGTTCACGCCTT	20	102	DQ341280.1
	Reverse	TGGAGAACCCAGGAAAGCAC	20		
β-Actin	Forward	TCGTGCGAGACATCAAGGAAA	21	178	KM244725.1
	Reverse	AGGAAGGAAGGCTGGAAGAGTG	22		

srebp-1: sterol-regulatory element-binding protein 1; *cpt-1a*: carnitine palmitoyl transterase-1a; *fas*: fatty acid synthase; *mttp*: microsomal triglyceride transfer protein; *ecr*: ecdysteroid receptor; *rxr*: retinoid X receptor; *mih*: molt-inhibiting hormone.

2.7. Calculations. The related parameters were calculated as follows:

$$\begin{aligned}
\text{FBW (g)} &= \frac{\text{total final weight}}{\text{total crab number}},\\
\text{SR(\%)} &= 100 \times \frac{\text{final number of crabs}}{\text{initial number of crabs}},\\
\text{WGR(\%)} &= 100 \times \frac{\text{final body weight} - \text{inital body weight}}{\text{inital body weight}},\\
\text{FI (g)} &= \frac{\text{total feed intake of each tank}}{\text{total number of crab in each tank}},\\
\text{FCR} &= \frac{\text{dry feed intake}}{\text{final body weight} - \text{inital body weight} + \text{dead crab weight gain}},\\
\text{MY (\%)} &= \frac{\text{total muscle weight}}{\text{final body weight}},\\
\text{MF} &= \frac{\text{total molting times}}{(\text{initial number of crabs} + \text{final number of crabs})/2},
\end{aligned} \tag{1}$$

where *FBW* is the final body weight, SR is the survival rate, WGR is the weight gain ratio, FI is the feed intake, FCR is the feed conversion ratio, MY is the meat yield, and MF is the molting frequency.

2.8. Statistical Analysis. The statistical analysis of the data in this experiment was implemented using the software SPSS23.0 (Chicago, IL, USA). The data were shown as means and pooled SEM (pooled standard error of means). Before analysis, test the normality and homogeneity of variance. Subsequently, a one-way analysis of variance was performed, and then, Duncan's multiple comparison test to estimate the significant differences ($P < 0.05$) among mean values was performed. Furthermore, orthogonal polynomial contrasts were used to determine whether the data effect was linear or quadratic.

3. Results

3.1. Growth Performance. As shown in Table 3, the FBW, SR, WGR, and FCR of crabs were markedly influenced by the supplementation level of CHO at quadratic ($P < 0.05$). Compared to those of the control group, CHO supplementation levels at 0.2% and 0.4%, FBW, SR, and WGR were significantly increased ($P < 0.05$) and FCR decreased significantly ($P < 0.05$). When the crabs were fed a 0.4% CHO diet, the FBW, WGR, and FCR reached the highest. However, the addition of CHO in the diet has no significant effects on MY and FI of crabs. In addition, through the broken-line model ($y = 48.13x + 99.89$, $R^2 = 0.8885$; $y = -6.28x + 114.55$, $R^2 = 0.9036$) (Figure 1) based on the relationship between the FBW and the dietary CHO levels, it was observed that the FBW of the crabs increased significantly ($P < 0.05$) with the addition of CHO from 0% to 0.4% and then decreased; when the CHO supplementation level was 0.27%, the crabs reached the highest FBW.

As shown in Figure 2(b), the total molting times were increased significantly as the addition levels of CHO increased from 0% to 0.2% and then decreased. We observed that from the sixth week to the sixteenth week, the total molting times of crabs in the group with 0.2% CHO were always higher than those in other groups. As shown in Figure 2(a), the molting frequency (MF) was affected by dietary CHO supplementation, and it reached the highest when the CHO supplementation level is at 0.2%. Additionally, compared to the control group, no significant difference was observed among the groups with 0.1%, 0.4%, 0.8%, and 1.6% CHO.

Table 3: Effect of dietary cholesterol level on the growth performance of *E. sinensis*.

Dietary CHO level	FBW (g)	SR (%)	WGR (%)	MY (%)	FI (g)	FCR
0	102.40^{a}	57.50^{a}	140.10^{a}	20.24	136.00	2.28^{a}
0.1	102.70^{a}	65.00^{abc}	141.70^{a}	21.06	135.90	2.26^{a}
0.2	110.50^{bc}	75.00^{c}	161.30^{b}	19.78	136.70	2.01^{bc}
0.4	113.00^{c}	70.00^{bc}	163.30^{b}	20.15	136.60	1.96^{c}
0.8	104.80^{ab}	62.50^{ab}	144.60^{a}	19.32	136.90	2.23^{ab}
1.6	106.60^{abc}	62.50^{ab}	148.50^{ab}	19.37	136.40	2.16^{abc}
SEM	0.01	3.28	2.09	0.68	0.60	0.08
P value	0.04	0.03	0.04	0.60	0.85	0.04
Regression analysis						
Linear						
Adj. R^2	0.03	−0.04	−0.01	0.03	−0.00	0.00
P value	0.20	0.73	0.33	0.15	0.34	0.31
Quadratic						
Adj. R^2	0.23	0.29	0.20	0.01	−0.03	0.20
P value	0.03	0.01	0.04	0.33	0.50	0.04

FBW: final body weight; SR: survival rate; WGR: weight gain ratio; MY: meat yield; FI: feed intake; FCR: feed conversion ratio; SEM: pooled standard error of: ($n = 4$); Adj. R^2: adjusted R square. Values in the same column with different superscripts are significantly different at $P < 0.05$.

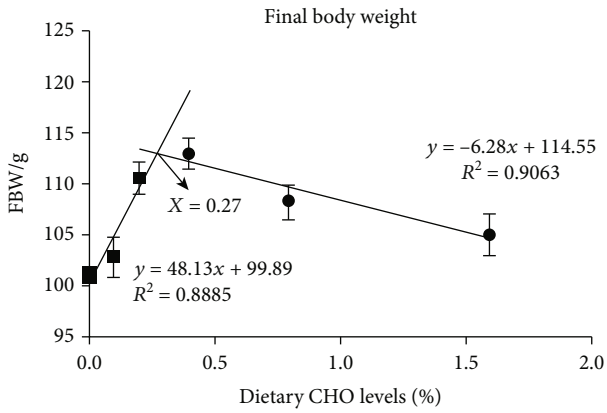


Figure 1: Broken-line relationship of the dietary CHO level to FBW for *E. sinensis*. Data appeared as *means* ± *SEM* ($n = 4$). Values in the same curve with different superscripts are significantly different ($P < 0.05$).

3.2. The Molting-Related Gene Expression. The molting-related gene mRNA expression was markedly affected ($P < 0.05$) by the dietary CHO supplementation as shown in Figures 2(c)–2(e). Compared with the group of CHO content at 0%, the mRNA expression of *ecr* (ecdysteroid receptor) and *rxr* (retinoid X receptor) was increased among the CHO addition groups and the significant differences were observed when the dietary CHO level is at 0.2% ($P < 0.05$) (Figures 2(c) and 2(d)). Compared to the control group, the *mih* (molt-inhibiting hormone) mRNA expression was decreased among the 0.1%, 0.2%, 0.4%, 0.8%, and 1.6% CHO groups. When the addition of CHO reached 0.8% and 1.6%, the expression of *mih* was significantly lower than that of the 0% CHO group ($P < 0.05$) (Figure 2(e)).

3.3. Analysis of Cholesterol Deposition. The content of CHO in hepatopancreas, muscle, and hemolymph of crab was significantly elevated with the increase of dietary CHO levels ($P < 0.05$) (Figures 3(a) and 3(c)). And the CHO deposition in hepatopancreas was much higher than that in muscle and hemolymph. The broken-line model ($y = 1.877x + 1.144$, $R^2 = 0.9392$; $y = 0.3393x + 1.837$, $R^2 = 0.9854$) (Figure 3(g)) of CHO deposition in hepatopancreas against the dietary CHO addition demonstrated that when the CHO content was lower than 0.45%, the CHO deposition efficiency in hepatopancreas was higher than that in the groups with 0.8% and 1.6% CHO. Meanwhile, the contents of HDL-C and LDL-C in hemolymph of crabs increased with the increase of the CHO level, and when the addition level exceeds 0.4%, significant differences were observed ($P < 0.05$) (Figures 3(d) and 3(e)). As shown in Figure 3(f), when the CHO level was less than 0.4%, the ratio of HDL-C/LDL-C could be significantly improved ($P < 0.05$) with the increase of CHO, while when the CHO content exceeded 0.4%, the ratio of HDL-C/LDL-C tends to decrease.

3.4. Parameters of Lipid Metabolism. The content of TG in the hepatopancreas and hemolymph was significantly affected by the CHO addition levels when linear and quadratic. However, there is no significant difference in the content of TG in muscle (Table 4). In addition, with the increase of CHO in the diet, the content of TG in hepatopancreas and hemolymph also increased significantly ($P < 0.05$), and when the amount of CHO was 1.6%, the content of TG reached the highest. Among the CHO addition groups, the contents of NEFA in the hepatopancreas, muscle, and hemolymph had no significant differences.

3.5. Related Gene mRNA Expression of Lipid Metabolism. The addition of CHO in the diet had a significant effect on the mRNA expression of *cpt-1a*, *srebp-1*, *fas*, and *mttp* in hepatopancreas, and compared to the groups without CHO supplementation, the expression of *cpt-1a* was markedly

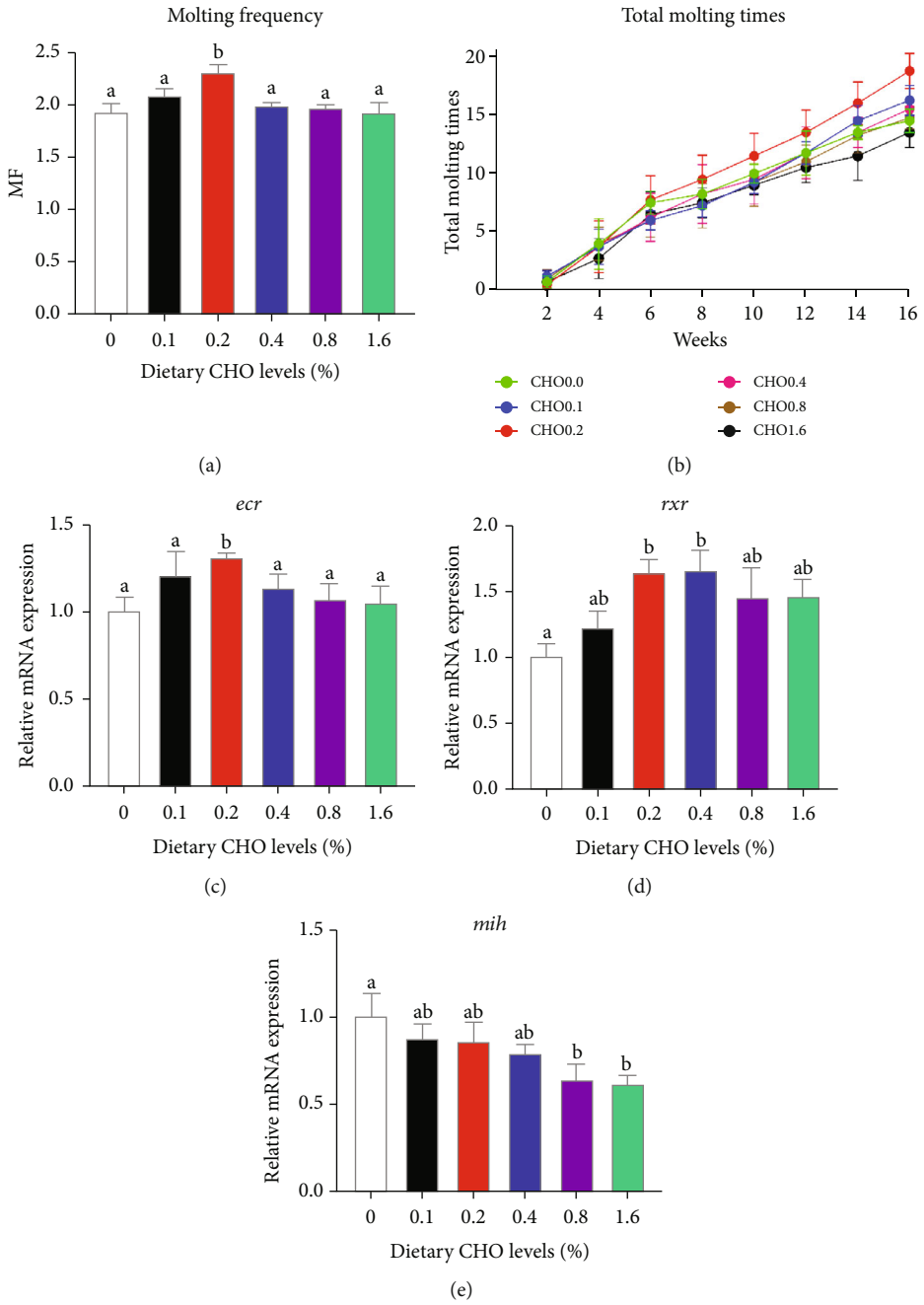


Figure 2: Effects of dietary CHO on (a) molting frequency (MF); (b) total molting times; (c) ecdysteroid receptor (*ecr*); (d) retinoid X receptor (*rxr*); and (e) molting-inhibiting hormone (*mih*). The values are the *means* ± *SEM* ($n = 4$). Different letters indicate significant differences ($P < 0.05$).

decreased among the groups with CHO addition ($P < 0.05$) (Figures 4(a)–4(d)). On the contrary, the expression of *mttp*, *srebp-1*, and *fas* showed an upward trend after adding CHO. With the increase of the addition of CHO in diet, the expression of *srebp-1* also increased significantly ($P < 0.05$). When the addition of CHO is below 0.2%, the expression of *mttp* does not change significantly. However, with the further addition of CHO, the expression of *mttp* increases significantly ($P < 0.05$). In addition, when the amount of CHO was less than 0.4%, the mRNA expression of *fas* tends to increase. In comparison, when the supplementation of CHO exceeds 0.4%, the expression of *fas* decreases significantly ($P < 0.05$).

4. Discussion

In this research, the result is that appropriate CHO in the diet could improve the SR, WGR, and FCR of *E. sinensis*, which was consistent with the results of previous studies [1, 14, 28–30]. According to a previous report, CHO deficiency caused poor growth performance of *Procambarus clarkia* [14]. Similar results were found in this experiment,

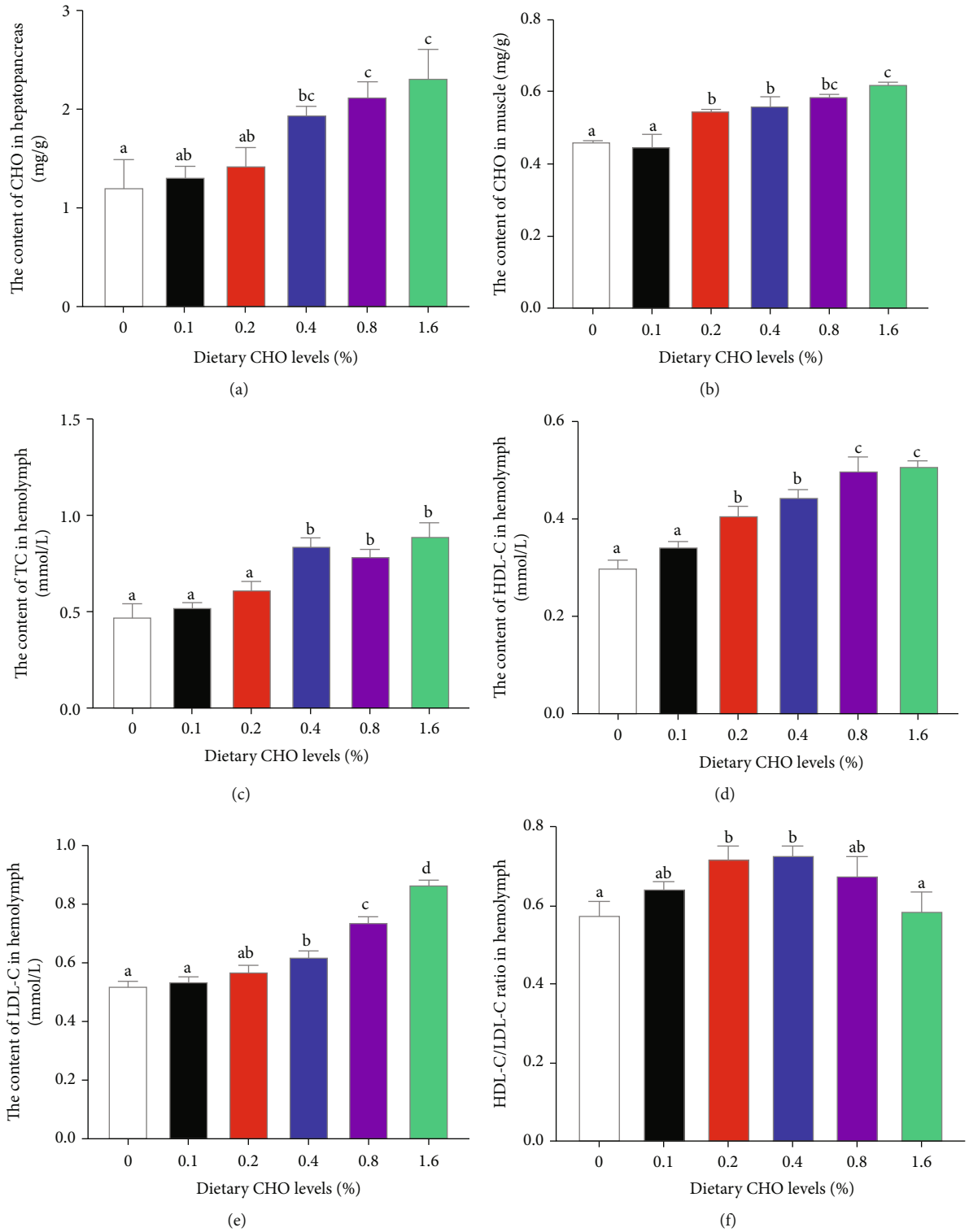
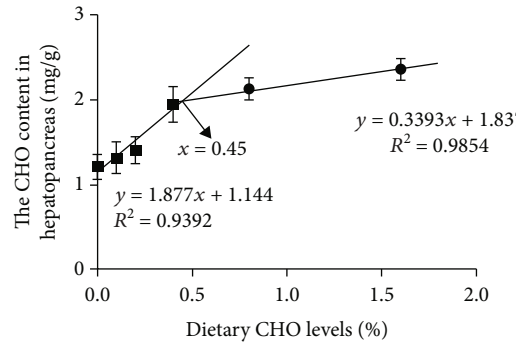


Figure 3: Continued.



(g)

Figure 3: Effects of dietary CHO on (a) the content of CHO in hepatopancreas; (b) the content of CHO in muscle; (c) the content of total CHO in hemolymph; (d) the content of high-density lipoprotein cholesterol in hemolymph (HDL-C); (e) the content of low-density lipoprotein cholesterol in hemolymph (LDL-C); and (f) the ratio of HDL-C/LDL-C in hemolymph. (g) Broken-line relationship of the dietary CHO level to the content of CHO deposition in hepatopancreas. The values are the *means* ± *SEM* ($n = 4$). Different letters indicate significant differences ($P < 0.05$).

Table 4: Effect of dietary CHO level on lipid metabolism related indices of *E. sinensis*.

Dietary CHO level	Hepatopancreas		Muscle		Hemolymph	
	TG (mmol/gprot)	NEFA (mmol/gprot)	TG (mmol/gprot)	NEFA (mmol/gprot)	TG (mmol/L)	NEFA (mmol/L)
0	0.45[a]	0.40	0.17	0.12	0.73[a]	0.78
0.1	0.50[ab]	0.38	0.18	0.11	0.73[a]	0.82
0.2	0.58[bc]	0.39	0.18	0.11	0.79[ab]	0.85
0.4	0.61[bc]	0.40	0.18	0.10	0.86[b]	0.84
0.8	0.70[cd]	0.41	0.18	0.10	0.91[b]	0.88
1.6	0.77[d]	0.40	0.19	0.10	1.08[c]	0.86
SEM	0.04	0.04	0.01	0.01	0.04	0.03
P value	0.00	0.90	0.93	0.50	0.00	0.47
Regression analysis						
Linear						
Adj. R^2	0.57	−0.04	0.01	0.07	0.57	0.11
P value	0.00	0.68	0.25	0.12	0.00	0.07
Quadratic						
Adj. R^2	0.60	−0.08	0.03	0.08	0.61	0.10
P value	0.00	0.85	0.23	0.16	0.00	0.13

Effect of the dietary CHO level on lipid metabolism-related indices of *E. sinensis*. SEM: pooled standard error of means ($n = 4$); Adj. R^2: adjusted R square. Values in the same column with different superscripts are significantly different ($P < 0.05$). TG: triglyceride; NEFA: nonesterified fatty acid.

and the worst growth performance was observed when the crabs were fed the diet with 0% CHO. In addition, this study found that excessive CHO will also have adverse effects on the growth performance and previous studies have shown that dietary CHO may accumulate in the body and increase the metabolic burden on the body, resulting in poor growth performance [28, 31, 32].

As an important nutrient, CHO is necessary for the growth of many aquatic animals. At present, the requirement of CHO for some aquatic animals has been reported, such as 0.51% for *Scylla serrata* [32], 0.2% for *Penaeus monodon* [1], and 0.2%~0.5% for *Litopenaeus vannamei* [19]. However, the data on CHO requirement of *E. sinensis* are limited. In this experiment, according to the regression curve based on the FBW and dietary CHO levels, we inferred that the optimal CHO level of *E. sinensis* feed was 0.27%. This result is consistent with the finding in *Penaeus monodon* (0.2%; [1]), but lower than *Scylla serrata* (0.51%; [32]). The difference may be due to the differences in species, growth stages, and experimental conditions [18]. In addition, at present, the content of fish meal in commercial feed is about 30% and the CHO content of fish meal is about 0.4%; therefore, formula feed can only provide 0.12% CHO, which may not meet the needs.

Molting is a necessary physiological process for the growth and development of crustaceans [8, 33]. In crustaceans, ecdysone and molt-inhibiting hormone (MIH) are two hormones that regulate the process of molting. Ecdysone is synthesized in the Y organ with CHO as a raw material, and then, ecdysone enters the nucleus to activate the

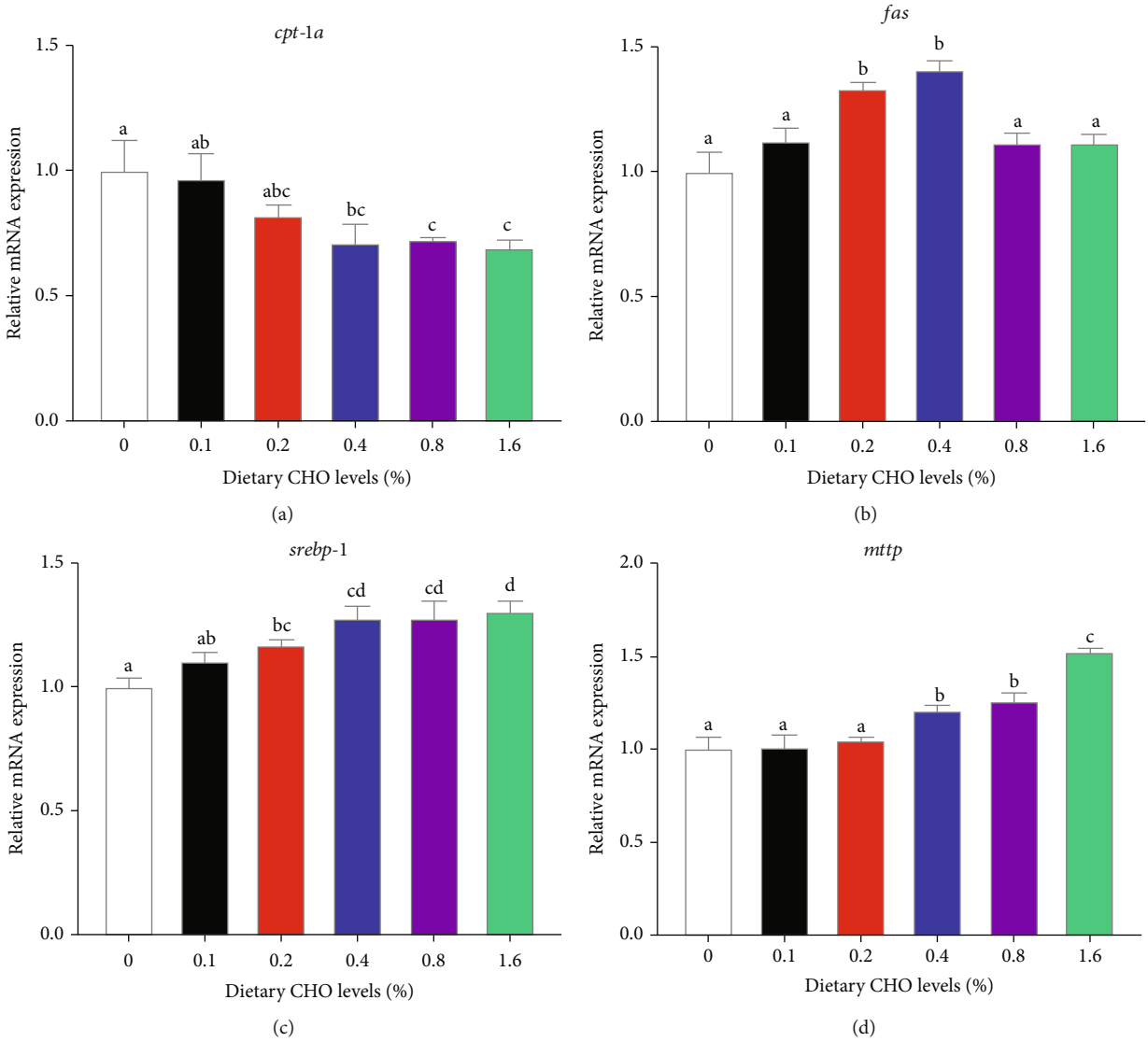


FIGURE 4: Effects of dietary CHO on (a) carnitine palmitoyl transterase-1a (*cpt-1a*); (b) fatty acid synthase (*fas*); (c) sterol-regulatory element binding protein 1 (*srebp-1*); and (d) microsomal triglyceride transfer protein (*mttp*). The values are the *means* ± *SEM* ($n = 4$). Different letters indicate significant differences ($P < 0.05$).

heterodimer formed by the ecdysone receptor (EcR) and retinoid X receptor (RXR), so as to regulate the transduction of the ecdysone signal pathway [34–37]. Previous studies have reported that an appropriate amount of CHO can promote the activation of the ecdysone signal pathway and improve molting performance, while excessive CHO will inhibit the activation of the ecdysone signal pathway, resulting in the decline of the molting performance. Similar results were also obtained in this experiment, and 0.2% CHO could significantly improve the molting performance of the *E. sinensis*, while no obvious changes were observed in those supplemented with 0.4%, 0.8%, and 1.6%. [14, 38, 39]. In order to further investigate the effects of CHO on the ecdysone signal pathway, we measured the expression levels of molting-related genes in the ecdysone signal pathway. The results showed that the expression levels of *ecr* and *rxr* among the CHO-added group were higher than those in the control group and reached the highest in the 0.2% CHO group. This result is lower than the study of Tian et al. [14]. Tian et al. [14] reported that 0.5% CHO could significantly improve the expression level of *ecr* and *rxr*. This may be caused by species or different sampling times. The MIH can inhibit molting of crustaceans by inhibiting the secretion of ecdysone by the Y organ; after removing the eyestalk of *Uca pugilator*, its molting is advanced [40–42]. In addition, the secretion of MIH is negatively regulated by the molting hormone, so it is reasonable to add CHO and decrease the expression of *mih* in this experiment. [14, 43].

In the present study, the content of CHO in the hepatopancreas, hemolymph, and muscle was positively correlated with the amount of CHO in the diet, which is similar to the results of previous studies on *Penaeus japonicus* [44]. As the most important organ for CHO storage and metabolism, the hepatopancreas is suitable for evaluating the deposition of

CHO in tissues [8]. According to a previous study, when a nutrient in the environment will not be able to meet the demand of the animal growth, the animal will store this nutrient quickly to meet the needs of subsequent growth and development, and when the nutrient content is sufficient, the deposition efficiency will be greatly decreased [5, 45, 46]. This can also explain that the CHO content in the hepatopancreas increases rapidly when the content of dietary CHO is lower than 0.45% but increases slowly with further supplementation. Therefore, according to the results of CHO deposition in hepatopancreas, we concluded that the appropriate deposition amount of CHO in tissues of *E. sinensis* was 0.45%. The maturation of the *E. sinensis* can be divided into physical maturity and physiological maturity, and the nutrient requirements for these two physiological processes are different [47, 48]. At the stage of physical maturation, CHO is mainly used to maintain their basic growth requirement, and there is little demand for CHO [5, 47]. But when the crabs complete their pubertal molt, the gonad begins to enter a rapid development period and the demand for CHO also increases. In this process, the hepatopancreas must store sufficient CHO to meet the needs of physiological maturity [8, 10, 49]. As we all know, LDL-C and HDL-C are the transporters of CHO in the body. LDL-C is responsible for transporting CHO from the hepatopancreas to extrahepatic tissues, while HDL-C is responsible for transporting peripheral CHO back to the hepatopancreas [50, 51]. In this study, with the addition of CHO ranging from 0% to 1.6%, the contents of HDL-C and LDL-C in hemolymph markedly increased, similar to the results in *Litopenaeus vannamei* [52]. The ratio of HDL-C/LDL-C is an important indicator of CHO transport [52, 53]. According to our results, when the addition of CHO was from 0% to 0.4%, the ratio of HDL-C/LDL-C significantly improved, indicating that CHO was accumulated in the hepatopancreas, while when the addition of CHO was more than 0.4%, the ratio tends to decrease, which indicates that CHO was mainly transferred to peripheral tissue [5, 54, 55]. This further proves that when the content of CHO is lower than the requirement, it accumulates rapidly in the hepatopancreas, while when the demand is reached, it flows to the extrahepatic tissue.

Lipid, as one of the most important energy substances, can provide enough energy for the development of the crabs. The TG and NEFA as the energy currency in the body can reflect the status of lipid metabolism to some extent [4, 56]. Liu et al. [36] reported that the level of CHO in the diet was positively correlated with the content of TG in hemolymph of *Litopenaeus vannamei*. Similarly, in this study, the content of TG also increased with CHO added. NEFA is decomposed by TG under the action of hormone-sensitive lipase. There is no significant difference in the content of NEFA in this experiment, which may be caused by the activity of hormone-sensitive lipase. However, the specific mechanism of cholesterol affecting the contents of TG and NEFA in crustaceans is still unclear ([52, 57]). This study speculated that it may be as follows: first of all, CHO can participate in the synthesis of lipoprotein, thus accelerating lipid transfer to promote lipid accumulation in the body; secondly, CHO may increase the lipid content of the body by promoting lipid synthesis and inhibiting lipid oxidation [5, 58]. Subsequently, we detected the expression of key genes involved in lipid synthesis and oxidation. The *srebp-1* is an important transcription factor, which can activate the transcription of downstream lipid synthesis genes, such as *fas*, one of the key genes of fatty acid synthesis [59]. In this study, the expression of *srebp-1* and *fas* increased significantly after crabs were fed the diet with CHO supplemented, which could be one of the reasons leading to the increase of TG; similar results have also been reported in other aquatic animals [52, 60, 61]. In addition, *cpt-1a* is one of the key genes in the process of β-oxidation, and its expression decreases significantly with the addition of CHO, indicating that the supplementation of CHO inhibits the β-oxidation of fatty acids [13]. The *mttp* is closely related to the assembly of VLDL. Furthermore, VLDL is mainly responsible for transporting TG from hepatopancreas to extrahepatic tissues. This can be interpreted as when there are excessive amounts of TG in the hepatopancreas, the expression of *mttp* will be upregulated to reduce the fat content in the hepatopancreas [59].

5. Conclusion

In conclusion, the results of this study show that adding 0.2% CHO to the diet can improve the molting performance of *E. sinensis*. And an appropriate amount of CHO can promote the deposition of lipids in the hepatopancreas, which is beneficial to accumulating sufficient nutrients in the growth and development of *E. sinensis*. In addition, based on the regression analysis of the FBW, we found that the growth requirement of *E. sinensis* for CHO was 0.27%. Through the regression analysis of CHO deposition in the hepatopancreas, we concluded that the tissue CHO deposition of *E. sinensis* for CHO was 0.45%. Therefore, according to the results of the present investigation, we suggest that 0.27%–0.45% CHO should be added to the diet to promote the growth and development of *E. sinensis*.

Data Availability

The data of this research can be obtained from the corresponding author under reasonable requirements.

Conflicts of Interest

We declare that we have no financial interest relationship with any individual and organization that may affect this article.

Acknowledgments

This research was supported by the Development Plan Project in Key Areas of Guangdong Province (Project no: 2020B0202010001) and Agriculture Technical System of Jiangsu, China (JATS [2021]474).

References

[1] S.-S. Sheen, P.-C. Liu, S.-N. Chen, and J.-C. Chen, "Cholesterol requirement of juvenile tiger shrimp (*Penaeus monodon*)," *Aquaculture*, vol. 125, no. 1-2, pp. 131–137, 1994.

[2] S.-I. Teshima, A. Kanazawa, and H. Sasada, "Nutritional value of dietary cholesterol and other sterols to larval prawn, *Penaeus japonicus* Bate," *Aquaculture*, vol. 31, no. 2-4, pp. 159–167, 1983.

[3] I. Hanukoglu, "Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 43, no. 8, pp. 779–804, 1992.

[4] Q. Huang, X. Wang, X. Bu et al., "Dietary vitamin A affects growth performance, immunity, antioxidant capacity, and lipid metabolism of juvenile Chinese mitten crab *Eriocheir sinensis*," *Aquaculture*, vol. 548, article 737556, 2022.

[5] V. Kumar, A. K. Sinha, N. Romano et al., "Metabolism and nutritive role of cholesterol in the growth, gonadal development, and reproduction of crustaceans," *Reviews in Fisheries Science & Aquaculture*, vol. 26, no. 2, pp. 254–273, 2018.

[6] S.-I. Teshima, "Bioconversion of β-sitosterol and 24-methylcholesterol to cholesterol in marine crustacea," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 39, no. 4, pp. 815–822, 1971.

[7] T. Zhang, D. Yuan, J. Xie et al., "Evolution of the cholesterol biosynthesis pathway in animals," *Molecular Biology and Evolution*, vol. 36, no. 11, pp. 2548–2556, 2019.

[8] X. Tao, C. Wang, H. Wei, Z. Ren, X. Ma, and W. Lu, "Effects of dietary cholesterol levels on moulting performance, lipid accumulation, ecdysteroid concentration and immune enzymes activities of juvenile Chinese mitten crab *Eriocheir sinensis*," *Aquaculture Nutrition*, vol. 20, no. 5, pp. 467–476, 2014.

[9] D. J. Trider and J. D. Castell, "Effect of dietary lipids on growth, tissue composition and metabolism of the oyster (*Crassostrea virginica*)," *The Journal of Nutrition*, vol. 110, no. 7, pp. 1303–1309, 1980.

[10] S. Teshima, M. Ishikawa, S. Koshio, and A. Kanazawa, "Assessment of cholesterol requirements in the prawn, *Penaeus japonicus*," *Aquaculture Nutrition*, vol. 3, no. 4, pp. 247–253, 1997.

[11] M. R. P. Briggs, K. Jauncey, and J. H. Brown, "The cholesterol and lecithin requirements of juvenile prawn (*Macrobrachium rosenbergii*) fed semi-purified diets," *Aquaculture*, vol. 70, no. 1-2, pp. 121–129, 1988.

[12] S. I. Teshima, A. Kanazawa, and A. Kanazawa, "Digestibility of dietary lipids in the prawn," *Bulletin of the Japanese Society of Scientific Fisheries*, vol. 49, pp. 963–966, 1983.

[13] F. Norambuena, M. Lewis, N. K. A. Hamid, K. Hermon, J. A. Donald, and G. M. Turchini, "Fish oil replacement in current aquaculture feed: is cholesterol a hidden treasure for fish nutrition?," *PLoS One*, vol. 8, no. 12, article e81705, 2013.

[14] H. Tian, C. Yang, Y. Yu et al., "Dietary cholesterol level affects growth, molting performance and ecdysteroid signal transduction in *Procambarus clarkii*," *Aquaculture*, vol. 523, p. 735198, 2020.

[15] Y. Zhang, Y. Sun, Y. Liu et al., "Molt-inhibiting hormone from Chinese mitten crab (*Eriocheir sinensis*): cloning, tissue expression and effects of recombinant peptide on ecdysteroid secretion of YOs," *General and Comparative Endocrinology*, vol. 173, no. 3, pp. 467–474, 2011.

[16] X. Li, Z. Xu, G. Zhou et al., "Molecular characterization and expression analysis of five chitinases associated with molting in the Chinese mitten crab, *Eriocheir sinensis*," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 187, pp. 110–120, 2015.

[17] J. D. Castell, E. G. Mason, and J. F. Covey, "Cholesterol requirements of juvenile American lobster (*Homarus americanus*)," *Journal of the Fisheries Board of Canada*, vol. 32, no. 8, pp. 1431–1435, 1975.

[18] H. Chen, "Requirements of marine shrimp, *Penaeus monodon* , juveniles for phosphatidylcholine and cholesterol," *Aquaculture*, vol. 109, pp. 165–176, 1993.

[19] H. Gong, A. L. Lawrence, D.-H. Jiang, F. L. Castille, and D. M. Gatlin III, "Lipid nutrition of juvenile *Litopenaeus vannamei* : I. Dietary cholesterol and de-oiled soy lecithin requirements and their interaction," *Aquaculture*, vol. 190, no. 3-4, pp. 305–324, 2000.

[20] L. Wang, H. Li, W. Hu, and H. Jiang, "Effects of dietary cholesterol level on the growth and feed efficiency of juvenile Eriocheir sinensis," *Journal of Freshwater Fishery*, vol. 34, pp. 13–15, 2004, http://www.cqvip.com/qk/93836x/200401/9043609.html.

[21] Y. Zhu, J. Wang, G. Zhang, and Z. He, "Effects of dietary cholesterol and protein content on growth and precocious puberty of juvenile Eriocheir sinensis," *Journal of Fishery Modernization*, vol. 3, pp. 3–6, 1999, http://qikan.cqvip.com/Qikan/Article/Detail?id=3606387.

[22] Y. Lei, Y. Sun, X. Wang et al., "Effect of dietary phosphorus on growth performance, body composition, antioxidant activities and lipid metabolism of juvenile Chinese mitten crab (*Eriocheir sinensis*)," *Aquaculture*, vol. 531, article 735856, 2021.

[23] X. Zheng, L. Zhang, W. Jiang et al., "Effects of dietary icariin supplementation on the ovary development-related transcriptome of Chinese mitten crab (*Eriocheir sinensis*)," *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, vol. 37, article 100756, 2021.

[24] L. I. Gilbert, R. Rybczynski, and J. T. Warren, "Control and biochemical nature of the ecdysteroidogenic pathway," *Annual Review of Entomology*, vol. 47, no. 1, pp. 883–916, 2002.

[25] AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists*, ACOC, Arlington, VA, USA, 16th edition, 1995.

[26] H. Cheng, W. Liu, X. Yuan, M. Liu, D. Zhang, and G. Jiang, "Effects of dietary cottonseed meal protein hydrolysate on growth, antioxidants and immunity of Chinese mitten crab *Eriocheir sinensis*," *Journal of Oceanology and Limnology*, vol. 38, no. 3, pp. 869–882, 2020.

[27] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta\_C\_{T}}$ method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[28] J. Guo, A. S. Hussain, A. G. J. Tacon et al., "Cholesterol requirement and phytosterols efficiency in semi-purified diets of juvenile Pacific white shrimp *Litopenaeus vannamei*," *Aquaculture Nutrition*, vol. 26, no. 4, pp. 1231–1243, 2020.

[29] M. H. Holme, P. C. Southgate, and C. Zeng, "Assessment of dietary lecithin and cholesterol requirements of mud crab, *Scylla serrata*, megalopa using semi-purified microbound diets," *Aquaculture Nutrition*, vol. 13, no. 6, pp. 413–423, 2007.

[30] T. C. Morris, T. M. Samocha, D. A. Davis, and J. M. Fox, "Cholesterol supplements for *Litopenaeus vannamei* reared on plant based diets in the presence of natural productivity," *Aquaculture*, vol. 314, no. 1-4, pp. 140–144, 2011.

[31] P. V. Hernández, M. A. Olvera-Novoa, and D. B. Rouse, "Effect of dietary cholesterol on growth and survival of juvenile redclaw crayfish *Cherax quadricarinatus* under laboratory conditions," *Aquaculture*, vol. 236, no. 1-4, pp. 405–411, 2004.

[32] S.-S. Sheen, "Dietary cholesterol requirement of juvenile mud crab *Scylla serrata*," *Aquaculture*, vol. 189, no. 3-4, pp. 277–285, 2000.

[33] E. S. Chang and D. L. Mykles, "Regulation of crustacean molting: a review and our perspectives," *General and Comparative Endocrinology*, vol. 172, no. 3, pp. 323–330, 2011.

[34] Y. Chan, K. Chu, and K. Chan, "Ecdysteroid-mimicking compounds act as both agonists and antagonists to the crustacean ecdysone receptor," *Chemosphere*, vol. 237, article 124551, 2019.

[35] S. Huang, Q. Yi, X. Lian et al., "The involvement of ecdysone and ecdysone receptor in regulating the expression of antimicrobial peptides in Chinese mitten crab, *Eriocheir sinensis*," *Eriocheir sinensis. Developmental & Comparative Immunology*, vol. 111, article 103757, 2020.

[36] T. Liu, H. Xu, T. Han, J. Wang, F. Yin, and C. Wang, "Effect of dietary egg yolk lecithin levels on survival, growth, lipid metabolism, and antioxidant capacity of early juvenile green mud crab *Scylla paramamosain*," *Aquaculture*, vol. 540, article 736706, 2021.

[37] K. Yu, C. Shi, X. Liu et al., "Tank bottom area influences the growth, molting, stress response, and antioxidant capacity of juvenile mud crab *Scylla paramamosain*," *Aquaculture*, vol. 548, article 737705, 2022.

[38] L. R. D'Abramo, C. E. Bordner, D. E. Conklin, and N. A. Baum, "Sterol requirement of juvenile lobsters, *Homarus* sp," *Aquaculture*, vol. 42, no. 1, pp. 13–25, 1984.

[39] W. Dall, B. J. Hill, P. C. Rothlisberg, and D. J. Staples, "The biology of the Penaeidae," *Advances in Marine Biology*, vol. 27, pp. 441–461, 1990.

[40] E. S. Chang and M. J. Bruce, "Ecdysteroid titers of juvenile lobsters following molt induction," *Journal of Experimental Zoology*, vol. 214, no. 2, pp. 157–160, 1980.

[41] P. M. Hopkins, "Patterns of serum ecdysteroids during induced and uninduced proecdysis in the fiddler crab, *Uca pugilator*," *General and Comparative Endocrinology*, vol. 52, no. 3, pp. 350–356, 1983.

[42] X. Zhang, D. Huang, X. Jia, Z. Zou, Y. Wang, and Z. Zhang, "Functional analysis of the promoter of the molt-inhibiting hormone (*mih*) gene in mud crab *Scylla paramamosain*," *General and Comparative Endocrinology*, vol. 259, pp. 131–140, 2018.

[43] M. R. Abdullah-Zawawi, N. Afiqah-Aleng, M. Ikhwanuddin et al., "Recent development in ecdysone receptor of crustaceans: current knowledge and future applications in crustacean aquaculture," *Reviews in Aquaculture*, vol. 13, no. 4, pp. 1938–1957, 2021.

[44] S. I. Teshima and A. Kanazawa, "Sterol Compositions of Marine Crustaceans," *Bulletin of the Japanese Society for the Science of Fish*, vol. 37, pp. 63–67, 1971.

[45] G. Jiang, M. Wang, W. Liu, G. Li, and Y. Qian, "Dietary choline requirement for juvenile blunt snout bream, *Megalobrama amblycephala*," *Aquaculture Nutrition*, vol. 19, no. 4, pp. 499–505, 2013.

[46] M. Sun, X. Li, Y. Ge, L. Zhang, B. Liu, and W. Liu, "Dietary thiamine requirement and its effects on glycolipid metabolism in oriental river prawn (*Macrobrachium nipponense*)," *Aquaculture*, vol. 550, article 737824, 2022.

[47] X. Jiang, H. Wang, Y. Cheng, and X. Wu, "Growth performance, gonad development and nutritional composition of Chinese mitten crab *Eriocheir sinensis* selected for growth and different maturity time," *Aquaculture*, vol. 523, article 735194, 2020.

[48] X. Wu, M. Liu, J. Pan, H. Chen, C. Zeng, and Y. Cheng, "The ovarian development pattern of pond-reared Chinese mitten crab, *Eriocheir sinensis*. 1853," *Crustaceana*, vol. 90, pp. 449–470, 2017.

[49] X. Wu, S. Zhu, H. Zhang et al., "Fattening culture improves the gonadal development and nutritional quality of male Chinese mitten crab *Eriocheir sinensis*," *Aquaculture*, vol. 518, article 734865, 2020.

[50] J. Chen, J. Chen, and J. Wu, "Molecular cloning and functional analysis of zebrafish high-density lipoprotein-binding protein," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 136, no. 1, pp. 117–130, 2003.

[51] N. Myant, *Cholesterol metabolism, LDL, and the LDL receptor*, Elsevier, 2012.

[52] C. Su, J. Li, Y. Lu et al., "Interactive effects of dietary cholesterol and bile acids on the growth, lipid metabolism, immune response and intestinal microbiota of *Litopenaeus vannamei*: sparing effect of bile acids on cholesterol in shrimp diets," *Aquaculture*, vol. 547, article 737412, 2022.

[53] P. E. Fielding and C. J. Fielding, "Chapter 15 dynamics of lipoprotein transport in the circulatory system," *New Comprehensive Biochemistry*, vol. 20, pp. 427–459, 1991.

[54] F. R. Kapourchali, G. Surendiran, A. Goulet, and M. H. Moghadasian, "The role of dietary cholesterol in lipoprotein metabolism and related metabolic abnormalities: a mini-review," *Critical Reviews in Food Science and Nutrition*, vol. 56, no. 14, pp. 2408–2415, 2016.

[55] J. Svoboda, M. Thompson, W. Robbins, and J. Kaplanis, "Insect steroid metabolism," *Lipids*, vol. 13, no. 10, pp. 742–753, 1978.

[56] S. Hirako, H. Ju Kim, T. Arai, H. Chiba, and A. Matsumoto, "Effect of concomitantly used fish oil and cholesterol on lipid metabolism," *The Journal of Nutritional Biochemistry*, vol. 21, no. 7, pp. 573–579, 2010.

[57] E. Recazens, E. Mouisel, and D. Langin, "Hormone-sensitive lipase: sixty years later," *Progress in Lipid Research*, vol. 82, article 101084, 2020.

[58] G. Yepiz-Plascencia, F. Vargas-Albores, and I. Higuera-Ciapara, "Penaeid shrimp hemolymph lipoproteins," *Aquaculture*, vol. 191, no. 1-3, pp. 177–189, 2000.

[59] Z. Lin, F. Han, J. Lu et al., "Influence of dietary phospholipid on growth performance, body composition, antioxidant capacity and lipid metabolism of Chinese mitten crab, *Eriocheir sinensis*," *Aquaculture*, vol. 516, article 734653, 2020.

[60] D. Eberlé, B. Hegarty, P. Bossard, P. Ferré, and F. Foufelle, "SREBP transcription factors: master regulators of lipid homeostasis," *Biochimie*, vol. 86, no. 11, pp. 839–848, 2004.

[61] N. Fukuda and J. A. Ontko, "Interactions between fatty acid synthesis, oxidation, and esterification in the production of triglyceride-rich lipoproteins by the liver," *Journal of Lipid Research*, vol. 25, no. 8, pp. 831–842, 1984.

[62] X. Yu, L. Xu, and F. Wu, *Chinese Fishery Statistical Yearbook*, China Agriculture Press, Beijing, Fisheries Agency of China Agriculture Ministry, 2017, https://cafs.ac.cn/info/1397/37342.htm.