Effects of Cholesterol and Taurine Supplementation in Soybean Meal Replacing Fish Meal on Growth Performance, Hepatopancreas and Serum Cholesterol Content, and Body Composition of Pacific White Shrimp (Litopenaeus vannamei) Postprint

Authors: Luo Yuan

In academic contexts, Chinese names are typically romanized using pinyin. "骆源" is rendered as "Luo Yuan" (surname first) or occasionally "Yuan Luo" (given name first) depending on publication style guidelines., Song Kai, Zhang Chunxiao, Ling Wang, Huang Fei

Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to investigate the effects of dietary cholesterol and taurine supplementation in fish meal-replaced soybean meal diets on growth performance, hepatopancreatic and serum cholesterol content, and body composition of Litopenaeus vannamei. Six iso-nitrogenous and iso-energetic experimental diets were formulated: one high-fish meal diet containing 30% fish meal (FM group), and five low-fish meal, high-soybean meal diets containing 12% fish meal (SBM1-5 groups). The SBM1 group contained no added cholesterol or taurine, SBM2 and SBM3 groups were supplemented with 0.3% and 0.6% cholesterol, respectively, SBM4 group was supplemented with 0.3% cholesterol and 0.2% taurine, and SBM5 group was supplemented with 0.6% cholesterol and 0.2% taurine. A total of 540 juvenile L. vannamei with initial body weight of (0.35±0.01) g were randomly divided into 6 groups with 3 replicates per group and 30 shrimp per replicate, and subjected to an 8-week growth trial. The results showed that the weight gain rate (WGR), specific growth rate (SGR), and survival rate (SR) of shrimp in FM, SBM3, SBM4, and SBM5 groups were significantly higher than those in SBM1 group ($P<0.05$), while feed conversion ratio (FCR) was significantly lower ($P<0.05$). Among these, FM group exhibited the highest WGR, SGR, and SR, and the lowest FCR, but showed no significant differences compared with SBM3, SBM4, and SBM5 groups ($P>0.05$). The

serum and hepatopancreatic total cholesterol (TC) contents in SBM3, SBM4, and SBM5 groups were significantly higher than those in SBM1 group (P<0.05), but showed no significant differences from FM group (P>0.05). Additionally, the serum low-density lipoprotein cholesterol (LDL-C) content in SBM1 group was significantly lower than that in FM, SBM2, SBM3, SBM4, and SBM5 groups (P<0.05), and serum high-density lipoprotein cholesterol (HDL-C) content was significantly lower than that in SBM3 and SBM5 groups (P<0.05). No significant differences were observed in whole-body moisture and crude ash contents among all groups (P>0.05). The whole-body crude protein and crude lipid contents in SBM1 group were significantly lower than those in FM, SBM3, and SBM5 groups (P<0.05), but showed no significant differences compared with SBM2 and SBM4 groups (P>0.05). Based on the results of this experiment, supplementation with 0.6% cholesterol or combined supplementation with 0.3% cholesterol and 0.2% taurine in low-fish meal, high-soybean meal diets could effectively improve the growth performance and feed efficiency of L. vannamei.

Full Text

Replacement of Fish Meal by Soybean Meal to Supplement Cholesterol and Taurine in Diet for *Litopenaeus vannamei*: Effects on Growth Performance, Hepatopancreas and Serum Cholesterol Content and Body Composition

LUO Yuan, SONG Kai, ZHANG Chunxiao*, WANG Ling, HUANG Fei

(Key Laboratory of Healthy Mariculture for the East China Sea, Ministry of Agriculture; Key Laboratory for Feed Quality Testing and Safety Evaluation, Fisheries College, Jimei University, Xiamen 361021, China)

Abstract: This study investigated the effects of supplementing cholesterol and taurine in soybean meal-based diets replacing fish meal on growth performance, hepatopancreas and serum cholesterol content, and body composition of juvenile Pacific white shrimp (*Litopenaeus vannamei*). Six isoenergetic and isonitrogenous experimental diets were formulated: one high-fish-meal diet containing 30% fish meal (FM group) and five low-fish-meal/high-soybean-meal diets (SBM1–5 groups) containing 12% fish meal. The SBM1 diet contained no supplemental cholesterol or taurine, SBM2 and SBM3 were supplemented with 0.3% and 0.6% cholesterol, respectively, SBM4 contained 0.3% cholesterol plus 0.2% taurine, and SBM5 contained 0.6% cholesterol plus 0.2% taurine. A total of 540 juvenile shrimp with an initial body weight of (0.35±0.01) g were randomly allocated into six groups with three replicates each (30 shrimp per replicate) and fed the experimental diets for eight weeks. The results showed that shrimp in the FM, SBM3, SBM4, and SBM5 groups exhibited significantly higher weight gain rate (WGR), specific growth rate (SGR), and survival rate (SR), and significantly lower feed conversion ratio (FCR) compared to those in the SBM1 group (P<0.05). Shrimp in the FM group achieved the highest

WGR, SGR, and SR and the lowest FCR, though these values did not differ significantly from the SBM3, SBM4, and SBM5 groups (P>0.05). The serum and hepatopancreas total cholesterol (TC) contents in the SBM3, SBM4, and SBM5 groups were significantly higher than in the SBM1 group (P<0.05) but showed no significant difference from the FM group (P>0.05). Additionally, the serum low-density lipoprotein cholesterol (LDL-C) content in the SBM1 group was significantly lower than in the FM, SBM2, SBM3, SBM4, and SBM5 groups (P<0.05), while serum high-density lipoprotein cholesterol (HDL-C) content was significantly lower than in the SBM3 and SBM5 groups (P<0.05). No significant differences were observed among groups in whole-body moisture or crude ash content (P>0.05). The whole-body crude protein and crude lipid contents in the SBM1 group were significantly lower than those in the FM, SBM3, and SBM5 groups (P<0.05) but did not differ significantly from the SBM2 and SBM4 groups (P>0.05). These results indicate that supplementation with 0.6% cholesterol or combined supplementation with 0.3% cholesterol and 0.2% taurine in low-fish-meal/high-soybean-meal diets can effectively improve the growth performance and feed efficiency of *L. vannamei*.

Keywords: soybean meal; cholesterol; taurine; growth performance; *Litopenaeus vannamei*

Introduction

Pacific white shrimp (*Litopenaeus vannamei*), commonly known as South American white shrimp, is one of the three most commercially important cultured shrimp species worldwide [1]. Fish meal has been widely used as a high-quality protein source in aquafeeds [2], providing not only protein and essential amino acids but also other essential nutrients such as essential fatty acids, vitamins, minerals, cholesterol, taurine, and nucleotides [3-4]. However, with the rapid development of aquaculture, fish meal consumption has increased continuously while global fish meal supply remains stagnant, inevitably driving up prices and keeping feed costs high. Consequently, finding alternative protein sources to replace fish meal has become crucial for reducing production costs [5]. Previous studies have demonstrated the feasibility of using plant protein sources to replace fish meal [6], but it is particularly noteworthy that the insufficient content of certain essential nutrients—such as cholesterol and taurine—limits the effectiveness of such replacement.

Cholesterol is an essential nutrient for crustacean growth and serves as a precursor for molting hormone synthesis [7-8]. Although crustacean tissues contain abundant sterols, these animals lack the ability to synthesize sterols endogenously [9]. Dietary cholesterol supplementation has been reported to maintain normal growth, development, and survival of crustaceans, while cholesterol deficiency can cause molting death syndrome [10-12]. Fish meal contains higher cholesterol levels than most plant protein sources, and studies have shown that supplementing high-plant-protein diets with appropriate cholesterol levels can improve growth and survival of aquatic animals [4]. Taurine, an amino acid

derivative originally isolated from bovine bile [13], plays important physiological roles in mammals and fish, including cell membrane composition, antioxidant detoxification, and osmoregulation [14-15]. Although aquatic animals can synthesize some taurine, this amount cannot meet their growth requirements, and most plant proteins are deficient in taurine [16]. Therefore, the lack of cholesterol and taurine in plant protein sources may be a key factor limiting their effectiveness as fish meal replacements.

Currently, few studies have investigated the effects of cholesterol or taurine supplementation in high-plant-protein diets on *L. vannamei* performance. Therefore, this study aimed to examine the effects of dietary cholesterol supplementation alone or in combination with taurine in soybean meal-based diets replacing fish meal on growth performance, hepatopancreas and serum cholesterol content, and body composition of *L. vannamei*, providing a reference for formulating low-fish-meal diets for this species.

1.1 Experimental Diets

Six isoenergetic and isonitrogenous experimental diets were formulated using fish meal, soybean meal, and wheat gluten meal as protein sources, and fish oil, soybean oil, and soybean lecithin as lipid sources. The composition and nutrient levels are shown in Table 1 . Among the six diets, one was a high-fish-meal diet containing 30% fish meal (FM group), while the other five were low-fish-meal/high-soybean-meal diets (SBM1–5 groups) in which soybean meal replaced 60% of the fish meal protein. The SBM1 diet contained no supplemental cholesterol or taurine, SBM2 and SBM3 were supplemented with 0.3% and 0.6% cholesterol, respectively, SBM4 contained 0.3% cholesterol plus 0.2% taurine, and SBM5 contained 0.6% cholesterol plus 0.2% taurine. Feed ingredients were ground and passed through an 80-mesh sieve, then mixed thoroughly according to the formulation ratios. Oil and water were added, and the mixture was processed into 1.0 mm diameter pellets using a twin-screw extruder (CD4×1TS multifunctional catalytic molding machine, developed by South China University of Technology). The pellets were air-dried and stored at -20 °C until use.

Table 1 Composition and nutrient levels of experimental diets (dry matter basis) %

Note: The table content is preserved as in the original format.

Footnotes: 1) Mineral premix for FM group provided per kg of diet: Mg-Gly 7.31 g, Mn-Met 0.366 g, Cu-Met 0.138 g. Mineral premix for SBM groups provided per kg of diet: Mg-Gly 4.85 g, Mn-Met 0.366 g, Cu-Met 0.138 g, Fe-Met 1.09 g, CaCl 9.79 g, Ca(H PO) 10.78 g, NaCl 5.45 g. 2) Vitamin premix provided per kg of diet: VA 10 mg, VD 10 mg, VC 1,000 mg, VK 40 mg, VE 500 mg, VB 60 mg, VB 70 mg, VB 80 mg, VB 0.4 mg, nicotinic acid 200 mg, calcium pantothenate 200 mg, biotin 2 mg, inositol 500 mg, folic acid 8 mg, crystalline cellulose 17,229.6 mg. 3) Amino acid mixture provided per kg of diet: Met 2.81 g, His 0.107 g, Lys 0.110 g, Gly 0.779 g, Ala 1.23 g. 4) Obtained from

Geneary Biotech Co., Ltd., Shanghai, China (grade: ultra purity). 5) Obtained from Huikangyuan Biotech Co., Ltd., Beijing, China (purity: 99%).

1.2 Experimental Animals and Husbandry

Juvenile *L. vannamei* shrimp were purchased from a hatchery in Haicang District, Xiamen City, Fujian Province. The shrimp were acclimated at the Jimei University aquaculture facility to adapt to the experimental diets and culture conditions. A total of 540 healthy, uniformly sized juvenile shrimp with an initial average body weight of (0.35±0.01) g were selected and disinfected with 10 mg/L povidone-iodine for 10 minutes before being randomly stocked into 18 circular experimental tanks (150 L) in a recirculating aquaculture system. Each tank contained 30 shrimp, with six dietary groups and three replicates per group. The experiment lasted for eight weeks.

The water source was natural seawater treated with UV disinfection and sand filtration. Shrimp were fed three times daily at 08:00, 14:00, and 20:00 at 6–10% of body weight. To minimize nutrient leaching, feed was delivered in 3–4 portions at each feeding time until apparent satiation. Uneaten feed was collected to calculate feed intake. After siphoning waste, one-third of the water volume was exchanged daily. The experiment was conducted under natural light (12 h light:12 h dark). Water temperature was maintained at 22–24 °C, salinity at 26–28, ammonia nitrogen below 0.2 mg/L, dissolved oxygen above 6.5 mg/L, pH at 8.0–8.2, and nitrite below 0.02 mg/L. Feeding, molting, and mortality were observed and recorded daily.

1.3 Sample Collection

At the end of the feeding trial, shrimp were fasted for 24 hours. The total weight and number of shrimp in each tank were recorded. Ten shrimp were randomly selected from each tank, pooled, minced, dried at 70 °C for 6 hours, then at 105 °C to constant weight. The dried samples were ground and stored in sealed bags at -20 °C for whole-body composition analysis. Additionally, eight shrimp from each tank were randomly selected, and hemolymph was collected from the pericardial cavity using a 1 mL sterile syringe. The hemolymph was allowed to clot at 4 °C for 12 hours, then centrifuged at 3,500 r/min for 10 minutes at 4 °C [17] to collect serum, which was stored at -80 °C for analysis. The hepatopancreas was dissected from the sampled shrimp and stored at -80 °C for biochemical analysis.

1.4 Analytical Methods

Proximate composition analysis: Feed proximate composition was determined according to AOAC (2005) methods [18]. Moisture content was measured by oven drying at 105 °C under atmospheric pressure. Crude protein content was determined by the Kjeldahl method (N×6.25) using an automatic Kjeldahl analyzer (FOSS Kjeltec 8400, Switzerland). Crude lipid content was

measured by Soxhlet extraction using ether as the solvent. Crude ash content was determined by incineration in a muffle furnace at 550 °C.

Total cholesterol measurement: Total cholesterol (TC) content in feed and hepatopancreas was determined by colorimetric enzymatic methods. Approximately 500 mg of feed or hepatopancreas sample was extracted with a chloroform:methanol mixture (2:1, v/v) at a 1:9 mass-to-volume ratio for 24 hours, then centrifuged at 4,000×g for 5 minutes. The supernatant (0.5 mL) was evaporated under high-purity nitrogen, and the residue was redissolved in 1 mL isopropanol containing 100 g/L Triton X-100 [19]. The samples were analyzed using a commercial kit (No. A111-1) from Nanjing Jiancheng Bioengineering Institute.

Taurine measurement: Dietary taurine content was determined according to the method described in reference [20]. Briefly, 4% sulfosalicylic acid was added to feed samples, homogenized by ultrasonication, and centrifuged at 15,000 r/min. The supernatant was diluted with 0.002 mol/L HCl, filtered through a 0.45 m membrane, and analyzed using a Hitachi L-8900 amino acid analyzer.

Serum biochemical indices: Serum total cholesterol was measured by the copper reagent method, while high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by direct dual-reagent methods using kits from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions.

1.5 Calculation Formulas

Growth indices were calculated using the following formulas at the end of the trial:

Weight gain rate (WGR, %) = 100 × (W -W)/W
Specific growth rate (SGR, %/d) = 100 × (lnW -lnW)/t
Feed conversion ratio (FCR) = FC/(W -W)
Survival rate (SR, %) = 100 × N /N

Where: W = initial average body weight (IBW), g/shrimp; W = final average body weight (FBW), g/shrimp; t = feeding duration, days; FC = average total feed intake per shrimp (dry weight), g; N = initial number of shrimp; N = final number of shrimp.

1.6 Statistical Analysis

Experimental data were initially processed using Excel 2010 and then analyzed by one-way ANOVA and two-way ANOVA using SPSS 19.0 statistical software. When significant differences among groups were detected ($P < 0.05$), multiple comparisons were performed using Student-Newman-Keuls test. All data are presented as mean ± standard error (mean ± SE).

Results

2.1 Effects of Cholesterol and Taurine Supplementation on Growth Performance

As shown in Table 2 , the WGR, SGR, and SR of shrimp in the SBM1 group were significantly lower than those in the FM, SBM3, SBM4, and SBM5 groups ($P < 0.05$), but showed no significant difference compared to the SBM2 group ($P > 0.05$). Conversely, the FCR of shrimp in the SBM1 group was significantly higher than that in the FM, SBM3, SBM4, and SBM5 groups ($P < 0.05$), but did not differ significantly from the SBM2 group ($P > 0.05$). Supplementation with 0.3% cholesterol in the soybean meal-based diets did not significantly affect any growth indices ($P > 0.05$), whereas supplementation with 0.6% cholesterol significantly increased WGR, SGR, and SR ($P < 0.05$) and significantly decreased FCR ($P < 0.05$). Compared to supplementation with 0.3% cholesterol alone, the combined addition of 0.3% cholesterol and 0.2% taurine significantly improved WGR, SGR, and SR ($P < 0.05$). However, compared to supplementation with 0.6% cholesterol alone, the combined addition of 0.6% cholesterol and 0.2% taurine did not significantly alter any growth indices ($P > 0.05$). Taurine supplementation in diets containing 0.3% or 0.6% cholesterol had no significant effect on any growth parameters ($P > 0.05$). No significant interaction between cholesterol and taurine was observed for FCR or SR ($P > 0.05$), but a significant interaction was detected for FBW, WGR, and SGR ($P < 0.05$).

Table 2 Growth performance of *Litopenaeus vannamei* for each group

Note: The table content is preserved as in the original format. Values in the same column with different small letter superscripts indicate significant difference ($P < 0.05$).

2.2 Effects of Cholesterol and Taurine Supplementation on Hepatopancreas and Serum Cholesterol Content

As shown in Table 3 , the serum and hepatopancreas TC contents in the SBM1 group were significantly lower than those in the FM, SBM3, SBM4, and SBM5 groups ($P < 0.05$), but showed no significant difference from the SBM2 group ($P > 0.05$). In the soybean meal-based diets, serum and hepatopancreas TC contents increased with increasing dietary cholesterol levels. Notably, the SBM3 group (0.6% cholesterol alone) did not differ significantly from the SBM5 group (0.6% cholesterol + 0.2% taurine) ($P > 0.05$). Serum LDL-C content in the SBM1 group was significantly lower than that in the FM, SBM2, SBM3, SBM4, and SBM5 groups ($P < 0.05$). Additionally, serum HDL-C content in the SBM1 group was significantly lower than that in the SBM3 and SBM5 groups ($P < 0.05$), with no significant differences observed among other groups ($P > 0.05$).

Table 3 Cholesterol content in hepatopancreas and serum of *Litopenaeus vannamei* for each group

Note: The table content is preserved as in the original format. Values in the

same row with different small letter superscripts indicate significant difference ($P < 0.05$). The same applies to Table 4.

2.3 Effects of Cholesterol and Taurine Supplementation on Body Composition

As shown in Table 4 , neither cholesterol alone nor combined cholesterol and taurine supplementation significantly affected whole-body moisture or crude ash content ($P > 0.05$). However, whole-body crude protein and crude lipid contents increased with increasing dietary cholesterol levels. The SBM1 group exhibited significantly lower crude protein and crude lipid contents compared to the FM, SBM3, and SBM5 groups ($P < 0.05$), but showed no significant difference from the SBM2 and SBM4 groups ($P > 0.05$).

Table 4 Body composition of *Litopenaeus vannamei* for each group

Note: The table content is preserved as in the original format.

Discussion

Cholesterol is a precursor for the synthesis of sex hormones, adrenal corticosteroids, molting hormones, and vitamin D in crustaceans and is essential for maintaining optimal growth performance and survival [21]. Crustaceans such as shrimp and crabs lack the ability to synthesize cholesterol endogenously and therefore must obtain it from dietary sources. Insufficient dietary cholesterol adversely affects crustacean growth, development, and survival [22]. Compared with fish meal, most plant protein sources contain very low cholesterol levels [23]. The present study confirmed that high-level replacement of fish meal with plant protein sources significantly reduced dietary cholesterol content, and this deficiency severely impaired the growth and survival of *L. vannamei*. Dietary cholesterol content is directly correlated with crustacean survival rate. D' Abramo et al. [24] reported that crayfish fed sterol-free diets developed severe molting death syndrome, while studies on giant river prawn [*Macrobrachium rosenbergii*] [10], kuruma shrimp [*Penaeus japonicus*] [25], and black tiger shrimp [*Penaeus monodon*] [26] demonstrated that cholesterol-free diets significantly reduced growth and survival. Paibulkichakul et al. [21] found that dietary supplementation with 1% cholesterol significantly improved the survival of *P. monodon*, and Duerr et al. [27] reported that dietary cholesterol supplementation at various levels significantly enhanced the survival of *L. vannamei*. Our results showed that supplementation with 0.6% cholesterol in soybean meal-based diets significantly improved WGR, SGR, and SR, indicating that high-level plant protein replacement requires cholesterol supplementation to maintain normal growth, development, and survival of *L. vannamei*.

In this study, supplementation with 0.2% taurine in addition to 0.3% cholesterol in high-soybean-meal diets significantly improved WGR, SGR, and SR while reducing FCR. Previous research found that taurine supplementation had no significant effect on the growth of *P. monodon* when diets contained fish

meal, squid meal, and shrimp meal as protein sources [28], whereas taurine significantly improved WGR, feed conversion ratio, and protein efficiency when diets contained casein as the protein source. This discrepancy likely relates to the inability of casein-based diets to meet the taurine requirements for *P. monodon* growth. Studies have reported that fish meal contains 0.5–0.7 g taurine per 100 g dry matter, whereas plant protein sources are virtually devoid of taurine [29]. Therefore, high-level replacement of fish meal with soybean meal drastically reduces dietary taurine content. Our results demonstrate that high soybean meal replacement leads to taurine deficiency in diets for *L. vannamei*, suggesting that taurine supplementation is recommended in high-plant-protein diets for this species. Additionally, the WGR and SR in the SBM2 group were significantly lower than in the SBM3, SBM4, and SBM5 groups, indicating that 0.3% cholesterol supplementation alone was insufficient in diets where soybean meal replaced 60% of fish meal. However, the addition of 0.2% taurine enhanced cholesterol utilization efficiency, demonstrating a cholesterol-sparing effect. This may be attributed to the ability of crustacean hepatopancreas to synthesize taurine, which is released into the intestine to solubilize cholesterol and facilitate its digestion and absorption [30]. Research has shown that taurine is a component of the emulsifying agent in the gastric fluid of crabs and participates in cholesterol digestion and absorption [31]. Shiau et al. [28] also found that taurine affects the cholesterol requirement of *P. monodon* by influencing cholesterol digestion and absorption. The significant interaction between cholesterol and taurine on WGR and SGR observed in this study may be realized through taurine' s promotion of cholesterol digestion and absorption.

In the present study, when soybean meal replaced 60% of fish meal, dietary cholesterol supplementation at 0.3% and 0.6% increased serum TC, HDL-C, and LDL-C contents in *L. vannamei*, particularly significantly increasing serum LDL-C content, though these values did not differ significantly from shrimp fed the fish meal diet. Previous studies have shown that when fish were fed diets containing plant protein sources such as soybean meal, wheat gluten meal, and soy protein concentrate as primary protein sources, plasma cholesterol levels were significantly lower than in fish fed fish meal-based diets, and cholesterol supplementation could elevate plasma cholesterol content [32-34]. Chen [35] reported that plasma TC, HDL-C, and LDL-C contents were significantly correlated with dietary cholesterol levels in Japanese flounder [*Paralichthys olivaceus*]. Although the mechanism by which plant protein replacement reduces plasma cholesterol remains unclear, the inability of crustaceans to synthesize cholesterol suggests that the absence of cholesterol in plant protein sources is the primary reason for low serum cholesterol levels in *L. vannamei*.

Our results also demonstrated that cholesterol supplementation in soybean meal-based diets significantly increased whole-body crude lipid content in *L. vannamei* in a dose-dependent manner. Thongrod and Boonyaratpalin [36] reported that dietary cholesterol supplementation significantly increased crude lipid content in banana shrimp [*Penaeus merguiensis*], and Gong et al. [37] found that cholesterol increased lipid storage in *L. vannamei* and *P. japonicus*. These find-

ings suggest that cholesterol supplementation in high-plant-protein diets may enhance lipid utilization and storage in shrimp. In conclusion, supplementation with 0.6% cholesterol alone or combined supplementation with 0.3% cholesterol and 0.2% taurine in soybean meal-based diets effectively improved the growth performance and feed efficiency of *L. vannamei*, with cholesterol and taurine exhibiting significant synergistic growth-promoting effects.

References

[1] Ministry of Agriculture of China. 2005 China Fisheries Yearbook [M]. Beijing: Ocean Press, 2005.
[2] TACON A G J, METIAN M. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects [J]. *Aquaculture*, 2008, 285(1/2/3/4): 146-158.
[3] TAKAGI S, MURATA H, GOTO T, et al. Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate [J]. *Aquaculture*, 2008, 280(1/2/3/4): 198-205.
[4] CHENG Z J, HARDY R W. Protein and lipid sources affect cholesterol concentrations of juvenile Pacific white shrimp, *Litopenaeus vannamei* (Boone) [J]. *Journal of Animal Science*, 2004, 82(4): 1136-1145.
[5] TAN R K H, DOMINY W G. Commercial pelleting of crustacean feeds [M]//D' ABRAMO L R, CONKLIN D E, AKIYAMA D M. *Advances in World Aquaculture. Volume 6, Crustacean Nutrition*. US: World Aquaculture Society, 1997: 520-549.
[6] MORRIS T C, SAMOCHA T M, DAVIS D A, et al. Cholesterol supplements for *Litopenaeus vannamei* reared on plant based diets in the presence of natural productivity [J]. *Aquaculture*, 2011, 314(1/2/3/4): 140-144.
[7] HOLME M H, ZENG C S, SOUTHGATE P C. The effects of supplemental dietary cholesterol on growth, development survival of mud crab, *Scylla serrata*, megalopa semi-purified diets [J]. *Aquaculture*, 2006, 261(4): 1328-1334.
[8] TESHIMA S. Phospholipids and sterols [M]//D' ABRAMO L R, CONKLIN D E, AKIYAMA D M. *Crustacean Nutrition*. Baton Rouge: World Aquaculture Society, 1997.
[9] TESHIMA S I, KANAZAWA A. Biosynthesis of sterols in the lobster, *Panulirus japonica*, the prawn, *Penaeus japonicus*, and the crab, *Portunus trituberculatus* [J]. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, 1971, 38(3): 597-602.
[10] BRIGGS M R P, JAUNCEY K, BROWN J H. The cholesterol and lecithin requirements of juvenile prawn (*Macrobrachium rosenbergii*) fed semi-purified diets [J]. *Aquaculture*, 1988, 70(1/2): 121-129.
[11] SHUDO K, NAKAMURA K, ISHIKAWA S, et al. Studies on formula feed for Kuruma prawn . On the growth-promoting effects of both squid liver oil and cholesterol [J]. *Bulletin of the Japanese Society of Scientific Fisheries*, 1971, 65: 129-137.
[12] CONKLIN D E, D' ABRAMO L R, BORDNER C E, et al. A successful purified diet for the culture of juvenile lobsters: the effect of lecithin [J].

Aquaculture, 1980, 21(3): 243-249.
[13] PARK T, LEE K. Dietary taurine supplementation reduces plasma and liver cholesterol and triglyceride levels in rats fed a high-cholesterol or a cholesterol-free diet [M]//SCHAFFER S, LOMBARDINI J B, HUXTABLE R J. *Taurine 3: Cellular and Regulatory Mechanisms*. New York: Springer, 1998: 319-325.
[14] WRIGHT C E, TALLAN H H, LIN Y Y, et al. Taurine: biological update [J]. *Annual Review of Biochemistry*, 1986, 55: 427-453.
[15] THURSTON J H, HAUHART R E, DIRGO J A. Taurine: a role in osmotic regulation of mammalian brain and possible clinical significance [J]. *Life Sciences*, 1980, 26(19): 1561-1568.
[16] YAMAMOTO T, AKIMOTO A, KISHI S, et al. Apparent and true availabilities of amino acids from several protein sources fingerling rainbow trout, common carp, and bream [J]. *Fisheries Science*, 1998, 64(3): 448-458.
[17] CHENG K M, HU C Q, LIU Y N, et al. Dietary magnesium requirement and physiological responses of marine shrimp *Litopenaeus vannamei* reared in low salinity water [J]. *Aquaculture Nutrition*, 2005, 11(5): 385-393.
[18] AOAC. Official methods of analysis of AOAC international [S]. 18th ed. Gaithersburg, MD: Association of Official Analytical Chemists International, 2005.
[19] YUN B. Effects of cholesterol, taurine and soyasaponin supplementation in high plant protein diets on growth performance and cholesterol metabolism of Japanese flounder (*Paralichthys olivaceus*) [D]. Ph.D. thesis. Qingdao: Ocean University of China, 2012.
[20] ZHOU M W, WANG H W, YE J D. Effects of dietary taurine on growth, body composition and tissue free amino acid content of Nile tilapia (*Oreochromis niloticus*) [J]. *Journal of Fisheries of China*, 2015, 39(2): 213-223.
[21] PAIBULKICHAKUL C, PIYATIRATITIVORAKUL S, KITTAKOOP P, et al. Optimal dietary levels of lecithin cholesterol black tiger prawn *Penaeus monodon* larvae postlarvae [J]. *Aquaculture*, 1998, 167(3/4): 273-281.
[22] HARRISON K E. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review [J]. *Journal of Shellfish Research*, 1990, 9(1): 1-28.
[23] DENG J, MAI K, AI Q, et al. Interactive effects of dietary cholesterol and protein sources on growth performance and cholesterol metabolism of Japanese flounder (*Paralichthys olivaceus*) [J]. *Aquaculture Nutrition*, 2010, 16(4): 419-429.
[24] D' ABRAMO L R, WRIGHT J S, WRIGHT K H, et al. Sterol requirement of cultured juvenile crayfish, *Pacifastacus leniusculus* [J]. *Aquaculture*, 1985, 49(3/4): 245-255.
[25] TESHIMA S I, KANAZAWA A, SASADA H. Nutritional value of dietary cholesterol and other sterols to larval prawn, *Penaeus japonicus* Bate [J]. *Aquaculture*, 1983, 31(2/3/4): 159-167.
[26] SHEEN S S, LIU P C, CHEN S N, et al. Cholesterol requirement of juvenile tiger shrimp (*Penaeus monodon*) [J]. *Aquaculture*, 1994, 125(1/2): 131-137.

[27] DUERR E O, WALSH W A. Evaluation of cholesterol additions to a soyabean meal-based diet for juvenile Pacific white shrimp, *Penaeus vannamei* (Boone), in outdoor growth trial [J]. *Aquaculture Nutrition*, 1996, 2(2): 111-116.
[28] SHIAU S Y, CHOU B S. Grass shrimp, *Penaeus monodon*, growth as influenced by dietary taurine supplementation [J]. *Comparative Biochemistry and Physiology Part A: Physiology*, 1994, 108(1): 137-142.
[29] YAMAMOTO T, SUZUKI N, FURUITA H, et al. Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout *Oncorhynchus mykiss* [J]. *Fisheries Science*, 2007, 73(1): 123-131.
[30] VAN DEN OORD A, DANIELSSON H, RYHAGE R. On the structure of the emulsifiers in gastric juice from the crab, *Cancer pagurus* L [J]. *Journal of Biological Chemistry*, 1965, 240: 2242-2247.
[31] LESTER R, CAREY M C, LITTLE J M, et al. Crustacean intestinal detergent promotes sterol solubilization [J]. *Science*, 1975, 189(4208): 1098-1100.
[32] REGOST C, ARZEL J, KAUSHIK S J. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*) [J]. *Aquaculture*, 1999, 180(1/2): 99-117.
[33] VENOU B, ALEXIS M N, FOUNTOULAKI E, et al. Effects of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (*Sparus aurata*) [J]. *Aquaculture*, 2006, 261(1): 343-356.
[34] LIM S J, LEE K J. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus* [J]. *Aquaculture*, 2009, 290(3/4): 283-289.
[35] CHEN J H. Effects of microbial fermentation, exogenous enzymes and feeding stimulants on the utilization of soybean meal protein by Japanese flounder (*Paralichthys olivaceus*) [D]. Ph.D. thesis. Qingdao: Ocean University of China, 2006.
[36] THONGROD S, BOONYARATPALIN M. Cholesterol and lecithin requirement of juvenile banana shrimp, *Penaeus merguiensis* [J]. *Aquaculture*, 1998, 161(1/2/3/4): 315-321.
[37] GONG H, LAWRENCE A L, JIANG D H, et al. Lipid nutrition of juvenile *Litopenaeus vannamei*: . Dietary cholesterol and de-oiled soy lecithin requirements and their interaction [J]. *Aquaculture*, 2000, 190(3/4): 305-324.

Note: Figure translations are in progress. See original paper for figures.