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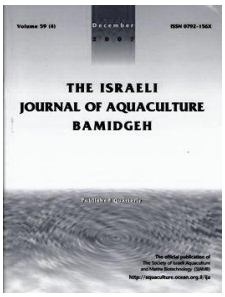

Effect of Dietary Soybean Lecithin and Cholesterol on Growth, Antioxidant Status and Fatty Acid Composition of Juvenile Swimming Crab, *Portunus trituberculatus*

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Key words:*Portunus trituberculatus*, soybean lecithin, cholesterol, growth performance, enzyme activities, fatty acids

Abstract

An 8-week feeding trial was conducted to evaluate the effects of dietary soybean lecithin and cholesterol levels on the growth, antioxidant status and fatty acid composition in the tissues of swimming crab *(Portunus trituberculatus)*. Eight experimental diets were formulated to contain four levels of soybean lecithin (0, 10, 20 and 40 g kg^{-1} of diet) and two cholesterol levels (0 and 6 g kg^{-1} of diet). Each diet was randomly assigned to triplicate groups of 60 swimming crabs (approximately 3.70 $\pm$ 0.03 g). The highest weight gain (WG), specific growth rate (SGR) and molting frequency (MF) were observed with crabs fed the diet supplemented with 40 g kg^{-1} lecithin and 6 g kg^{-1} cholesterol. Crabs fed the diet without lecithin and cholesterol had a lower WG and SGR as compared to those fed the other diets. Cholesterol, triglyceride and glucose concentrations in the serum were significantly influenced by soybean lecithin and cholesterol levels. Crabs fed the diets containing 40 g kg^{-1} lecithin with or without cholesterol supplementation had lower malondialdehyde (MDA) in the hepatopancreas as compared to those fed the other diets. Moreover, glutathione peroxidase (GPx) and lysozyme activities in the hepatopancreas were significantly affected by the dietary soybean lecithin and cholesterol levels. Highly unsaturated fatty acids (HUFAs) in the muscles were not significantly influenced by the dietary soybean lecithin and cholesterol levels. The concentrations of total HUFA in hepatopancreas were significantly influenced by the dietary soybean lecithin and cholesterol levels. In conclusion, interaction between dietary soybean lecithin and cholesterol affects growth performance, feed utilization and fatty acids in the hepatopancreas, dietary soybean lecithin and cholesterol supplementation enhance lipid transportation and metabolism.

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Introduction

Cholesterol and phospholipids (PLs) are bio-membrane components that are essential for maintaining cellular structure and function. They are important nutrients that enhance growth and survival of marine crustaceans (Holme *et al*., 2007). Cholesterol is also an essential component of all animal cell membranes with important structural roles, reducing both fluidity and permeability of the plasma membrane to protons and sodium ions (Lange and Steck, 2008). Cholesterol is the precursor of several other functional molecules in different tissues, including adrenal corticoids, bile acids, sex hormones and molting hormones in crustaceans (Sheen *et al*., 1994; Holme *et al*., 2007). It is also a precursor of synthesized ecdysone, which is closely linked to the process of molting in crustaceans (Sheen, 2000; Tao *et al*., 2014). However, many crustaceans are unable to perform de novo synthesis of cholesterol from acetate, a process performed by higher animals (Tocher *et al*., 2008). Cholesterol requirement of crustaceans have been reported to range from 4 to 20 g kg^{-1} (National Research Council, 2011).

PLs are associated with the molecular constitution of cells, especially membranes; PLs are precursors for highly biologically active mediators of metabolism and physiology, including diacylglycerol, eicosanoids etc. (Tocher *et al*., 2008). PLs have been reported to enhance lipid absorption in the body by acting as an emulsifier (Coutteau *et al*., 1997). PLs are also essential nutrients to marine crustaceans, and have beneficial roles in survival (Niu *et al*., 2008), growth (Li *et al*., 2014; Wang *et al*., 2016) and immunity (Li *et al*., 2016). Most crustaceans can synthesize PLs, but the de novo synthesis of PLs is unable to meet the metabolic requirements of crustaceans (Tocher *et al*., 2008). Dietary PLs requirement range from 5 to 65 g kg^{-1} for some crustaceans (Gong *et al*., 2000; Thongrod and Boonyaratpalin, 1998; Wang *et al*., 2016). A previous study demonstrated that 10 g kg^{-1} soybean lecithin in diet (supplemented with 6 g/kg cholesterol) was required for *Potunus trituberculatus* (Li *et al*., 2014). However, another study found that the best growth performance of *P. trituberculatus* was obtained in 40 g kg^{-1} soybean lecithin diet (without cholesterol supplementation) (Hou *et al*., 2016).

The swimming crab, *P. trituberculatus*, is widely distributed along the coasts of China, Korea and Japan (Hamasaki *et al*., 2006). It has become one of the most important aquaculture crustaceans in China. In recent decades, much progress has occurred in the hatching, growing-out methods and accelerated growth of this species. Therefore, the nutritional requirements of swimming crab need to be investigated to develop commercial diets. Until now, a few studies have focused on lipid nutrients requirements of *P. trituberculatus* (Han *et al*., 2013; Li *et al*., 2014, 2016; Wang *et al*., 2016; Han *et al*., 2017). However, information about the influence of dietary PLs and cholesterol on growth, antioxidant status as well as fatty acids composition in tissue is still lacking. Thus, the objective of the present study was to evaluate the effects of dietary cholesterol and soybean lecithin on growth performance, antioxidant capability in hepatopancreas, hematological characteristics and fatty acid composition in the tissues of juvenile swimming crab and compare the different effects of the supplemental cholesterol diet and the non-supplemental cholesterol diet on *P. trituberculatus*.

Materials and Methods

A 2×4 factorial experiment was formulated to contain four lecithin levels (0, 10, 20 and 40 g kg^{-1} of the dietary dry weight) and two cholesterol levels (0 and 6 g kg^{-1} of the dry weight) (Table 1).

The ingredients were purchased from Ningbo Tech-Bank Feed Co. Ltd (Ningbo, China). Fish meal, krill meal, soybean meal and wheat gluten meal were used as protein sources; fish oil and soybean oil were used as lipid sources; and wheat flour was used as carbohydrate source. The de-oiled soybean lecithin is a light yellow powder containing 500 g kg^{-1} phosphatidylcholine (PC) with 950 g kg^{-1} acetone insolubility (Siwei Company, Zhengzhou, China). Cholesterol is analytical reagent grade (Kayon Biological Technology, Shanghai, China). All diets were rendered iso-lipidic by adjusting the levels of soybean oil as soybean lecithin levels increased. All ingredients were ground into fine powder with a particle size less than 177 microns. Then, the micro-components such as vitamins and minerals pre-mixtures were mixed thoroughly. Finally, lipids and distilled water (400 g kg^{-1} by w/w) were added to the premixed ingredients and mixed until homogenous with a Hobart-type mixer. Cold-extruded pellets were produced, and the pellet strands were broken into two uniform pellet sizes (a) 3.0 mm in diameter and 5.0 mm in length and (b) 5.0 mm in diameter and 7.0 mm in length) with a granulating machine (G-250, machine factory of South China University of Technology, Guangzhou, China). They were then steamed for 30 min at 90 °C, and then air-dried to approximately 10% moisture. The diets were stored frozen in sealed plastic bags and stored at -20 °C until used in the feeding trial.

Table 1. Formulation and proximate composition of experimental diets.

Ingredients (g kg^{-1})	*Dietary Cholesterol (%)/Soybean lecithin (%)*							
	0/0	*0/1*	*0/2*	*0/4*	*0.6/0*	*0.6/1*	*0.6/2*	*0.6/4*
Fish meal	280	280	280	280	280	280	280	280
Krill meal	50	50	50	50	50	50	50	50
Soybean meal	210	210	210	210	210	210	210	210
Wheat gluten meal	90	90	90	90	90	90	90	90
Wheat flour	207	207	207	207	207	207	207	207
Fish oil	20	20	20	20	20	20	20	20
Soybean oil	80	70	60	40	74	64	54	34
Soybean lecithin	0	10	20	40	0	10	20	40
Cholesterol	0	0	0	0	6	6	6	6
Vitamin mix[1]	10	10	10	10	10	10	10	10
Mineral mix[2]	15	15	15	15	15	15	15	15
Choline chloride	3	3	3	3	3	3	3	3
$Ca(H_2PO_4)_2$	15	15	15	15	15	15	15	15
Sodium alginate	20	20	20	20	20	20	20	20
Proximate composition (dry matter g kg-1								
Dry matter	910.4	905.5	907	918	919	905	907.4	912
Crude protein	452.3	454.1	452	453	454	452	454.2	452
Crude lipid	135.1	135.3	134	135	134	135	133.2	135

The juvenile *P. trituberculatus* were obtained from the Jing-Ye nursery farm (Ningbo, China). Prior to the start of the feeding trial, the juvenile crabs were acclimated and fed a commercial diet (450 g kg^{-1} dietary protein, 80 g kg^{-1} crude lipid, Ningbo Tech-Bank Corp., Ningbo, China) for 2 weeks. Before the trial, 480 juvenile swimming crabs with an approximate initial weight of 3.69 ± 0.03 g were randomly sorted into 240 rectangular plastic baskets (80 cm × 40 cm × 40 cm) in six cement pools (8.0 m × 4.0 m × 2.0 m; length × width × depth) and fed twice daily at 06:00 h and 18:00 h. Sixty juvenile swimming crabs were used for each diet were distributed in 60 plastic baskets. Each test diet was randomly assigned to three replicates, twenty plastic baskets in each replicate, with one crab in each basket. Each basket was divided into two equal parts; one partitioned area was filled with sand and served as the crab habitat, and the other area was filled with seawater and served as the feeding area, as shown in Fig. 1. All groups of crabs were fed at the same rate, and the amount of feed was 6-8% of wet body weight; the daily feeding amount was adjusted every two weeks on the basis of the weight of the crab in each basket to maintain a level approaching apparent satiation.

Fig.1. Swimming crab rearing and experimental conditions.

All plastic baskets were placed in a cement pool (7.0m × 5.0m × 1.8m, length × width × depth). The seawater of the pools was provided with continuous aeration through many air tubes to retain high levels of dissolved oxygen levels. During the experimental period, water temperature in the pool was 26-30 °C, pH was 7.7-8.3, salinity was 26-28 ppt, ammonia nitrogen was lower than 0.05

mg l^{-1}, and dissolved oxygen was 6.7-7.1 mg l^{-1}. The pH, salinity, ammonia nitrogen and dissolved oxygen were measured using the YSI Proplus instrument (YSI, Yellow Springs, OH, USA). The feeding trial lasted for 8 weeks.

At the termination of the feeding trial, the crabs in each plastic basket were weighed and sampled for analysis. In each treatment, hemolymph samples from six crabs were taken immediately from the pericardial cavity with a 1-ml syringe, sorted into1.5 ml Eppendorf tubes. All hemolymph samples were placed in 4 °C for 24 h, followed by centrifugation (6000 rpm, 10 min, 4 °C). Then, the supernatant was stored at -80 °C until analysis. Hepatopancreas and muscle samples were taken from 6 crabs in each replicate after blood was drawn, and stored at -80 °C until analysis of fatty acids composition. Hepatopancreas samples were taken 4 crabs from each replicate and stored at -80 °C until analysis of the antioxidant enzyme activities.

The crude protein, crude lipid and moisture contents in the diets were analyzed by standard methods. Crude protein (N × 6.25) was determined using the Dumas combustion methods with an auto-protein analyser (FP-528, Leco, USA). The crude lipid was determined by the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Hoganas, Sweden), moisture content was determined by drying the samples to a constant weight at 105 °C. The total protein, cholesterol, triglyceride and glucose contents in serum were determined with an automatic chemistry analyzer (Hitachi 7170-110, Tokyo, Japan).

The concentration of malondialdehyde (MDA) and the total antioxidative capacity (T-AOC), glutathione peroxidase (GPx), lysozyme (LZM) and superoxide dismutase (SOD) in the hepatopancreas were assayed by commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), according to the manufacturer's instructions and analyzed by Multiskan Spectrum (Thermo, USA).

The fatty acid profile of diets and hepatopancreas and muscle were determined as follow. The freeze-dried samples (every hepatopancreas sample ~80mg and muscle sample ~120mg) were added into a 12 ml volumetric glass lidded screwed tube. Then 3 ml potassium hydroxide methanol (1 N) was added and heated in a 72°C water bath for 20 min. and then cooled down. 3 ml HCL–methanol (2 N) was added and the mixture was heated on 72°C water bath for another 20 min. Previous tests were conducted to make sure that all fatty acids can be esterified following the procedures above. Finally, 1 ml hexane was added into the mixture above, shaken vigorously for 1 min, and then allowed to separate into two layers. Fatty acid methyl esters were separated, and measured by GC-MS machine (Agilent technologies 7890B -5977A, USA). Results were showed as percentage of total fatty acids.

The interaction between cholesterol and lecithin was analyzed using two-way ANOVA. Significant differences between the treatments were determined using Turkey's multiple comparison test at the 0.05 level of significance. In the absence of interactions, the data was analyzed using a one-way ANOVA and expressed as the means ± S.E.M. of the replicates using Turkey’s multiple comparison test. $P < 0.05$ was regarded as statistically significant. All statistics were calculated using the SPSS 17.0 (SPSS, IL, USA).

Results

Effect of dietary soybean lecithin and cholesterol on growth performance, feed utilization and survival of swimming crab are presented (Table 2). Survival ranged from 71.67% to 88.33%, and there were no significant differences among all treatments ($P > 0.05$). There were highly significant interactions between soybean lecithin and cholesterol on weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and molting frequency (MF) ($P < 0.05$). The crabs fed diets supplemented with cholesterol exhibited significantly higher WG, SGR and MF than those fed diets without cholesterol supplementation. The crabs fed diet containing 40 g kg^{-1} soybean lecithin and 6 g kg^{-1} cholesterol showed a significantly higher FW, WG and SGR than those fed the diets containing 6 g kg^{-1} cholesterol without soybean lecithin supplementation and containing 40 g kg^{-1} soybean lecithin without cholesterol supplementation. However, no significant differences in WG, SGR and MF were found between the crabs fed a diet containing 40 g kg^{-1} soybean lecithin without cholesterol supplementation and those fed a diet containing 6 g kg^{-1} cholesterol without soybean lecithin supplementation. The highest feed conversion ratio (FCR) was observed at crab fed the diet without soybean lecithin and cholesterol supplementation, and the lowest FCR was occurred at crab fed the diet containing 40 g kg^{-1} soybean lecithin with 6 g kg^{-1} cholesterol supplementation. Protein efficiency ratio (PER) were not significantly different among the treatments ($P > 0.05$).

Table 2. Growth performance, feed utilization, survival and molting frequency of juvenile swimming crab fed diets containing different soybean lecithin and cholesterol levels.

Dietary Chol (%)/PLs(%)	*Initial weight (g)*	*Final weight (g)*	*Weight gain (%)* [1]	*SGR(%day^{-1})* [2]	*FCR* [3]	*Survival (%)* [4]	*PER* [5]	*Molting frequency* [6]
Diet1 (0/0)	3.70±0.04	38.39±1.53^{a}	937.62±51.48^{a}	4.41±0.09^{a}	1.78±0.14^{d}	71.67±9.28	1.26±0.11	2.76±0.22^{a}
Diet2 (0/1)	3.70±0.06	42.31±1.61^{ab}	1045.02±56.83^{ab}	4.60±0.09^{ab}	1.61±0.09^{c}	76.67±1.67	1.38±0.09	2.82±0.03^{a}
Diet3 (0/2)	3.67±0.06	41.93±1.50^{ab}	1041.84±22.00^{ab}	4.60±0.035^{ab}	1.59±0.20^{c}	75.00±2.89	1.45±0.21	3.04±0.07^{b}
Diet4 (0/4)	3.69±0.06	44.48±0.96^{bc}	1105.58±42.58^{bc}	4.70±0.07^{bc}	1.28±0.07^{a}	88.33±4.41	1.75±0.10	3.29±0.10^{c}
Diet5 (0.6/0)	3.74±0.05	44.01±3.64^{bc}	1077.41±108.02^{bc}	4.64±0.17^{ab}	1.48±0.21^{b}	76.67±4.41	1.54±0.19	2.90±0.20^{ab}
Diet6 (0.6/1)	3.68±0.08	47.20±1.13^{cd}	1183.99±19.49^{cd}	4.82±0.03^{cd}	1.41±0.25^{b}	71.67±6.67	1.68±0.32	3.37±0.10^{cd}
Diet7 (0.6/2)	3.64±0.05	48.93±1.25^{cd}	1243.81±15.78^{cd}	4.90±0.02^{cd}	1.26±0.17^{a}	78.33±4.41	1.80±0.22	3.53±0.06^{d}
Diet8 (0.6/4)	3.69±0.10	52.22±1.48^{d}	1316.09±47.62^{d}	5.00±0.07^{d}	1.22±0.13^{a}	80.00±5.77	1.86±0.19	3.57±0.19^{d}
Two-way ANOVA *Pr > F*								
Cholesterol	0.944	0	0	0	0.042	0.748	0.069	0.002
PLs	0.784	0.01	0.012	0.01	0.045	0.244	0.252	0.003
Chol × PLs	0.95	0.007	0.005	0.001	0.029	0.56	0.937	0.008

Data are expressed as the mean ± S.E.M. of three replicates. Values in the same line with different superscripts are significantly different (P < 0.05).
1. Weight gain (WG, %) =100× (final body weight - initial body weight)/ initial body weight
2. Specific growth rate (SGR, % day-1) =100 × (Ln (final body weight) - Ln (initial body weight)) / days
3. Feed conversion ratio (FCR) = feed consumed (g, dry weight)/ weight gain (g, wet weight)
4. Survival (%) =100 × (final number of crabs) / (initial number of crabs)
5. Protein efficiency ratio (PER) = weight gain (g, wet weight) / protein intake (g, dry weight)
6. Molting frequency = 2 × molting / (initial crabs + final crabs).

The hemolymph biochemical compositions of the juvenile swimming crabs are shown (Table 3). The total protein in serum was not significantly different among all the treatments (P > 0.05). Cholesterol and glucose concentration in serum significantly increased with dietary soybean lecithin increasing from 0 to 40 g kg^{-1} without cholesterol supplementation (P < 0.05). However, the cholesterol and glucose concentration in serum did not show an obvious tendency among the cholesterol-supplementation treatments. No significant differences were observed in the triglyceride concentration in serum among the treatments without dietary cholesterol supplementation (diet 1, 2, 3 and 4; P > 0.05). Triglyceride concentrations significantly decreased when dietary soybean lecithin levels increased from 0 to 40 g kg^{-1} at 6 g kg^{-1} cholesterol supplementation (P < 0.05).

Table 3**.** Hematological characteristics of juvenile swimming crab fed diets containing different soybean lecithin and cholesterol levels.

Dietary Chol (%)/PLs (%)	*Total protein (g L^{-1})*	*Cholesterol (mmol L^{-1})*	*Triglyceride (mmol L^{-1})*	*Glucose (mmol L^{-1})*
Diet1(0/0)	39.87±3.09	0.20±0.03^{ab}	0.07±0.01^{ab}	1.29±0.09^{a}
Diet2(0/1)	37.83±2.72	0.21±0.01^{ab}	0.09±0.01^{b}	1.53±0.10^{ab}
Diet3(0/2)	40.13±3.67	0.43±0.03^{d}	0.10±0.01^{b}	1.76±0.13^{b}
Diet4(0/4)	34.60±3.34	0.37±0.03^{d}	0.08±0.01^{b}	1.88±0.14^{bc}
Diet5(0.6/0)	37.13±2.86	0.20±0.02^{ab}	0.09±0.01^{b}	2.27±0.15^{c}
Diet6(0.6/1)	34.77±4.45	0.29±0.02^{c}	0.07±0.01^{ab}	1.94±0.14^{bc}
Diet7(0.6/2)	40.07±2.48	0.26±0.03^{bc}	0.05±0.01^{a}	2.23±0.12^{c}
Diet8(0.6/4)	33.07±3.08	0.17±0.02^{a}	0.05±0.01^{a}	1.71±0.15^{b}
Two-way ANOVA *Pr > F*				
Cholesterol	0.434	0.001	0.02	0
PLs	0.281	0	0.504	0.238
Chol × PLs	0.966	0	0.007	0.004

Data are expressed as the mean ± S.E.M. of three replicates. Values in the same line with different superscripts are significantly different (P < 0.05).

The results of antioxidant enzyme activities and lipid peroxidation in hepatopancreas of swimming crabs are presented (Table 4). T-AOC activity in hepatopancreas was not significantly influenced by the dietary soybean lecithin and cholesterol levels (P > 0.05). Crabs fed the diets without soybean lecithin supplementation had lower SOD activity than those fed the other diets. Meanwhile, crabs fed the diet containing 40 g kg^{-1} soybean lecithin without cholesterol supplementation had the highest hepatopancreas GPx activity among all treatments (P < 0.05).

MDA concentration in hepatopancreas significantly decreased with dietary soybean lecithin levels increasing from 0 to 40 g kg^{-1} when dietary cholesterol level was 0 or 6 g kg^{-1} ($P < 0.05$); the lower MDA concentrations were observed in diets containing 40 g kg^{-1} soybean lecithin with or without cholesterol supplementation. Crab fed the diets containing 20 g kg^{-1} and 40 g kg^{-1} soybean lecithin with 6 g kg^{-1} cholesterol supplementation had higher lysozyme activity in hepatopancreas than those fed the other diets.

Table 4. Antioxidant enzyme activity in hepatopancreas of juvenile swimming crab fed diets containing different soybean lecithin and cholesterol levels

Dietary Chol (%)/PLs (%)	*SOD (U mgprot^{-1})*	*T-AOC (U mgprot^{-1})*	*GPx (U mgprot^{-1})*	*MDA(nmol mgprot^{-1})*	*LZM (ug mgprot^{-1})*
Diet1(0/0)	77.41±8.59^{a}	6.08±0.78	237.10±11.63^{a}	13.71±1.07^{c}	33.08±1.89^{a}
Diet2(0/1)	101.89±9.97^{bc}	6.73±0.81	225.95±19.26^{a}	13.19±0.86^{c}	37.12±1.65^{a}
Diet3(0/2)	94.69±9.54^{b}	7.44±0.78	278.95±20.72^{ab}	11.02±1.07^{b}	34.93±3.16^{a}
Diet4(0/4)	96.46±6.57^{b}	6.44±0.70	328.14±14.04^{b}	10.28±0.31^{ab}	38.21±2.26^{ab}
Diet5(0.6/0)	72.13±7.68^{a}	7.23±1.19	231.82±15.75^{a}	12.81±1.29^{bc}	34.71±1.64^{a}
Diet6(0.6/1)	91.35±7.71^{b}	8.20±1.15	250.30±13.54^{a}	11.43±0.87^{b}	36.57±2.11^{a}
Diet7(0.6/2)	95.93±10.99^{b}	6.16±0.82	259.86±16.47^{a}	10.02±0.95^{ab}	44.69±2.69^{b}
Diet8(0.6/4)	113.38±7.22^{c}	7.86±0.53	268.90±18.39^{a}	9.12±1.05^{a}	45.28±1.60^{b}
Two-way ANOVA *Pr > F*					
Cholesterol	0.925	0.278	0.222	0.099	0.011
PLs	0.02	0.789	0.004	0.008	0.013
Chol × PLs	0.441	0.355	0.125	0.971	0.109

Data are expressed as the mean ± S.E.M. of three replicates. Values in the same line with different superscripts are significantly different ($P < 0.05$).

The fatty acid composition (% total fatty acids) of the muscle and hepatopancreas of swimming crab are presented in Table 5 and Table 6. The concentrations of total SFA, MUFA, PUFA and HUFA in muscle were not significantly influenced by dietary soybean lecithin and cholesterol levels ($P > 0.05$). Moreover, the crabs fed the diets without cholesterol supplementation had higher 18: 3n-3 level in muscle than those fed the diets supplemented with cholesterol.

Ingredients (g kg^{-1})	*Dietary Cholesterol (%)/Soybean lecithin (%)*							
	0/0	*0/1*	*0/2*	*0/4*	*0.6/0*	*0.6/1*	*0.6/2*	*0.6/4*
Fish meal	280	280	280	280	280	280	280	280
Krill meal	50	50	50	50	50	50	50	50
Soybean meal	210	210	210	210	210	210	210	210
Wheat gluten meal	90	90	90	90	90	90	90	90
Wheat flour	207	207	207	207	207	207	207	207
Fish oil	20	20	20	20	20	20	20	20
Soybean oil	80	70	60	40	74	64	54	34
Soybean lecithin	0	10	20	40	0	10	20	40
Cholesterol	0	0	0	0	6	6	6	6
Vitamin mix[1]	10	10	10	10	10	10	10	10
Mineral mix[2]	15	15	15	15	15	15	15	15
Choline chloride	3	3	3	3	3	3	3	3
$Ca(H_2PO_4)_2$	15	15	15	15	15	15	15	15
Sodium alginate	20	20	20	20	20	20	20	20
Proximate composition (dry matter g kg^{-1})								
Dry matter	910.4	905.5	907.4	917.5	919	905	907	912
Crude protein	452.3	454.1	451.5	452.6	454	452	454	452
Crude lipid	135.1	135.3	133.5	134.7	134	135	133	135

Table 5**.** Fatty acid composition of the muscle of juvenile swimming crabs fed diets containing different soybean lecithin and cholesterol levels.

Data are expressed as the mean ± S.E.M. of three replicates. Values in the same line with different superscripts are significantly different ($P < 0.05$).
1 ∑SFA, saturated fatty acids: C14:0, C16:0, C18:0.
2 ∑MUFA, monounsaturated fatty acids: C18:1n-9, C20:1n-9.
3 ∑PUFA, polyunsaturated fatty acids: C18:2n-6, C18:3n-3.
4 ∑HUFA, highly unsaturated fatty acids: C20:4n-6, C20:5n-3, C22:6n-3.
5 ∑n-3PUFA: C18:3n-3, C20:5n-3, C22:6n-3.
6 ∑n-6PUFA: C18:2n-6, C20:4n-6.

Table 6. Fatty acid composition of the hepatopancreas of juvenile swimming crab fed diets containing different soybean lecithin and cholesterol levels.

	Dietary Chol (%)/PL (%)								*Two-way ANOVA Pr > F*		
Fatty acid	*Diet1(0/0)*	*Diet2(0/1)*	*Diet3(0/2)*	*Diet4(0/4)*	*Diet5(0.6/0)*	*Diet6(0.6/1)*	*Diet7(0.6/2)*	*Diet8(0.6/4)*	*Chol*	*PLs*	*Chol × PLs*
C14:0	1.16±0.01^{b}	1.24±0.04^{b}	1.36±0.06^{bc}	0.73±0.09^{a}	1.39±0.15^{bc}	1.55±0.08^{c}	1.42±0.11^{bc}	1.29±0.01^{bc}	0	0.002	0.009
C16:0	14.82±0.25^{a}	16.01±0.29^{b}	16.33±0.24^{b}	15.17±1.05^{ab}	17.59±0.26^{c}	17.17±0.28^{bc}	17.74±0.19^{c}	18.05±0.06^{c}	0	0.437	0.102
C18:0	6.70±0.19^{b}	6.22±0.01^{ab}	5.69±0.42^{a}	8.90±0.21^{d}	5.96±0.52^{ab}	6.10±0.38^{ab}	6.57±0.13^{ab}	7.62±0.01^{c}	0.151	0	0
∑SFA[1]	23.45±0.09 ^{a}	24.24±0.22^{ab}	24.21±0.20^{ab}	25.70±1.14^{bc}	25.92±0.56^{c}	25.69±0.54^{bc}	26.67±0.12^{cd}	27.94±0.09^{d}	0	0.379	0.005
C16:1n-7	1.17±0.03^{b}	1.23±0.03^{b}	1.37±0.09^{bc}	0.82±0.16^{a}	1.41±0.15^{bc}	1.63±0.10^{c}	1.38±0.10^{bc}	1.37±0.01^{bc}	0.001	0.026	0.055
C18:1n-9	30.97±0.15^{c}	29.03±1.28^{bc}	27.72±0.75^{ab}	30.25±1.88^{bc}	25.96±0.42^{a}	25.64±0.33^{a}	25.89±0.27^{a}	25.89±0.23^{a}	0	0.276	0.321
C20:1n-9	1.46±0.05^{ab}	1.29±0.10^{a}	1.41±0.06^{ab}	1.75±0.01^{c}	1.64±0.13^{bc}	1.44±0.04^{ab}	1.33±0.12^{a}	1.72±0.00^{c}	0.311	0.001	0.078
∑MUFA[2]	34.62±0.27^{c}	32.58±1.40^{ab}	31.67±0.73^{ab}	33.78±1.79^{bc}	30.38±0.31^{a}	29.97±0.35^{a}	29.93±0.19^{a}	30.17±0.16^{a}	0	0.274	0.47
C18:3n-3	1.91±0.04^{bc}	1.86±0.02^{bc}	2.32±0.17^{c}	0.95±0.29^{a}	1.75±0.21^{b}	2.19±0.11^{bc}	1.84±0.17^{bc}	1.69±0.00^{b}	0.339	0.001	0.004
C20:5n-3	2.67±0.03^{ab}	2.33±0.04^{a}	2.85±0.01^{ab}	3.74±0.63^{c}	2.91±0.09^{ab}	2.90±0.05^{ab}	3.06±0.19^{ab}	3.35±0.07^{bc}	0.352	0.063	0.023
C22:6n-3	5.71±0.12^{a}	5.70±0.03^{a}	5.84±0.05^{a}	6.39±0.17^{b}	5.99±0.12^{a}	5.95±0.10^{a}	5.65±0.12^{a}	6.35±0.02^{b}	0.304	0.002	0.001
∑n-3PUFA	10.28±0.10^{ab}	9.88±0.09^{a}	11.01±0.14^{cd}	11.07±0.52^{cd}	10.65±0.06^{bc}	11.05±0.21^{cd}	10.54±0.17^{b}	11.40±0.09^{d}	0.039	0.277	0.001
C18:2n-6	28.34±0.36^{bc}	30.12±1.02^{c}	30.29±0.91^{c}	24.08±0.55^{a}	29.59±0.88^{c}	30.44±0.94^{c}	29.62±0.19^{c}	26.59±0.13^{b}	0.108	0	0.001
C20:4n-6	0.37±0.01^{a}	0.38±0.01^{a}	0.39±0.01^{a}	0.95±0.33^{b}	0.44±0.05^{a}	0.47±0.05^{a}	0.46±0.09^{a}	0.48±0.01^{a}	0.498	0.105	0.059
∑n-6PUFA	31.65±0.46^{ab}	33.30±1.09^{b}	33.12±1.07^{b}	29.78±0.28^{a}	33.05±0.33^{b}	33.29±0.80^{b}	32.86±0.08^{b}	30.49±0.13^{a}	0.332	0.007	0.019
∑n-3[5]/∑n-6[6]	0.32±0.01^{a}	0.30±0.01^{a}	0.33±0.01^{a}	0.37±0.02^{b}	0.32±0.00^{a}	0.33±0.01^{a}	0.32±0.01^{a}	0.38±0.00^{b}	0.422	0.009	0
DHA/EPA	2.14±0.02^{b}	2.45±0.03^{c}	2.05±0.03^{ab}	1.79±0.38^{a}	2.06±0.03^{ab}	2.05±0.04^{ab}	1.86±0.13^{ab}	1.90±0.04^{ab}	0.051	0.012	0.015
∑PUFA[3]	33.19±0.51^{bc}	34.79±1.09^{c}	35.05±0.89^{c}	29.78±0.67^{a}	34.36±0.54^{c}	35.01±0.88^{c}	34.25±0.30^{c}	31.70±0.12^{ab}	0.218	0.001	0.002
∑HUFA[4]	8.75±0.15^{a}	8.40±0.08^{a}	9.08±0.04^{ab}	11.08±1.12^{c}	9.34±0.26^{ab}	9.33±0.07^{ab}	9.16±0.29^{ab}	10.19±0.09^{bc}	0.557	0.031	0.009

Data are expressed as the mean ± S.E.M. of three replicates. Values in the same line with different superscripts are significantly different ($P < 0.05$).
1 ∑SFA, saturated fatty acids: C14:0, C16:0, C18:0.
2 ∑MUFA, monounsaturated fatty acids: C18:1n-9, C20:1n-9.
3 ∑PUFA, polyunsaturated fatty acids: C18:2n-6, C18:3n-3.
4 ∑HUFA, highly unsaturated fatty acids: C20:4n-6, C20:5n-3, C22:6n-3.
5 ∑n-3PUFA: C18:3n-3, C20:5n-3, C22:6n-3.
6 ∑n-6PUFA: C18:2n-6, C20:4n-6.

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The concentration of total SFA, MUFA, PUFA and HUFA in hepatopancreas were significantly influenced by the dietary soybean lecithin and cholesterol levels. Total SFA, PUFA and HUFA in hepatopancreas significantly increased with dietary soybean lecithin levels increasing from 0 to 40 g kg^{-1} with or without cholesterol supplementation. Crab fed the diets containing 40 g kg^{-1} soybean lecithin with or without cholesterol supplementation had higher EPA and DHA in hepatopancreas than those fed the other diets. However, crab fed the diets containing soybean lecithin with 6 g kg^{-1} cholesterol supplementation had lower total MUFA than those fed the diet without soybean lecithin and cholesterol supplementation.

Discussion

The interaction between PLs and cholesterol has been of great interest in crustacean nutrition, although PLs are suggested to facilitate the transport of cholesterol, which implies an interaction between them, most growth trials have not demonstrated this interaction (Paibulkichakul *et al*., 1998; Han *et al*., 2017). The results of the present study showed a significant interaction between soybean lecithin and cholesterol on growth and feed utilization of juvenile swimming crabs. Crabs fed the diet containing 40 g kg^{-1} PLs grew faster whether with or without cholesterol supplementation. Crabs fed the diet containing 40 g kg^{-1} soybean lecithin but without cholesterol had similar weight gain and specific growth rate as those fed the diets containing soybean lecithin levels ranging from 0 to 20 g kg^{-1} with 6 g kg^{-1} cholesterol supplementation. Similar results were observed in *P. monodon* (Paibulkichakul *et al*., 1998). It was reported that a significant interaction existed between dietary cholesterol and de-oiled soybean lecithin on the growth performance of juvenile *L. vannamei* and *S. serrata* (Gong *et al*., 2000; Holme *et al*., 2007). However, a previous study showed that no interaction exists between dietary lecithin and cholesterol on growth performance of swimming crab (Han *et al*., 2017). The different results are most probably due to the different dietary protein sources in these experiments. Swimming crabs fed diets based on casein and defatted fish meal had lower growth performance as compared to commercial ingredients, such as fish meal, soybean meal (Coutteau *et al*., 1997). Lobsters fed diets based on casein displayed lower survival than fed with crab protein (Conklin *et al*., 1980).The results of the present study indicated that dietary PLs and cholesterol did not significantly influence the survival of swimming crab. Our findings are in agreement with previous studies for other crustaceans' species, such as Pacific white shrimp (Gong *et al*., 2000) and mud crab (Holme *et al*., 2007). Moreover, the discrepancies may be also due to the differences in crustaceans' species, life stage, dietary status, composition and purity of PLs used, and culture environmental conditions (Gong et al., 2000). Further studies should be needed to evaluate the effects of different PLs as supplementation in crab diets and to identify the active components in PLs.

Some studies have suggested that dietary PLs can improve growth performances and feed utilization of mud crab (Holme *et al*., 2007), swimming crabs (Li *et al*., 2014, Wang *et al*.,2016) and Pacific white shrimp (Gong *et al*., 2000; González-Félix *et al*., 2002). PLs requirement for crustacean range from 5 to 65 g kg^{-1} (National Research Council, 2011). In the present study, the crabs fed the diet containing 40 g kg^{-1} soybean lecithin had significantly higher FW, WG and SGR than those fed the other diets at the same cholesterol level. Moreover, crab fed the diet containing 40 g kg^{-1} soybean lecithin had lower FCR than those fed the diet without soybean lecithin supplementation at the same cholesterol level. The survival of *P. trituberculatus* and *Fenneropenaeus merguiensis* were not significantly influenced by different soybean lecithin levels (Thongrod and Boonyaratpulin, 1998; Li *et al*., 2014).

Cholesterol is a major dietary sterol and nutritionally superior to other sterols for many crustacean species, it is an essential component of all animal cell membranes with important structural roles, reducing both fluidity and the permeability of the plasma membrane to protons and sodium ions (Teshima, 1997; Lange and Steck, 2008). Reported dietary cholesterol requirements are approximately 5 g kg^{-1} for *P. japonicus* (Teshima et al., 1997), 4.1 g kg^{-1} for *L. vannamei* (Gong et al., 2000); 6 g kg^{-1} for *P. trituberculatus* (Han et al., 2013); 5.1 g kg^{-1} for *S. serrata*; 2 g kg^{-1} for juvenile *P. monodon* (Sheen et al., 1994) and 10 g kg^{-1} for larval or post larval *P. monodon* (Paibulkichakul et al., 1998). In the present study, supplementation of cholesterol to the diet resulted in significant benefits to the FW, WG, SGR and MF of *P. trituberculatus*. Previous study demonstrated that 6 g kg^{-1} cholesterol supplementation in the diet supported faster growth

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than those without cholesterol supplementation for *P. trituberculatus* (Li *et al*., 2014).

Some studies have demonstrated the favorable effect of supplementing PLs in the diets of crustaceans, generally contributing to the transport of dietary lipids such as cholesterol and triglycerides in the organism, improving lipid deposition and energy availability for growth (D'Abramo *et al*., 1982; Coutteau *et al*., 1997). Lack of dietary PLs resulted in a PLs deficiency in the hemolymph and limited the effective transport of cholesterol from the hepatopancreas to the hemolymph of lobster (D'Abramo *et al*., 1982). Cholesterol in the serum was enhanced with dietary PLs supplementation in *L. vannamei* (Hu *et al*., 2011). Total cholesterol in serum of *P. trituberculatus* significantly increased with the increasing dietary PLs level (Han *et al*., 2017). It was also reported that dietary PLs can improve lipid mobilization and transport from the hepatopancreas through the hemolymph to the muscle in *L. vannamei* (Gong *et al*., 2000). The results of present study indicated that cholesterol concentration of the serum increased with dietary soybean lecithin increasing from 0 to 20 g kg^{-1} without cholesterol supplementation; moreover, the serum cholesterol concentration increased with lecithin supplementation from 0 to 10 g kg^{-1} when the diet contained 6 g kg^{-1} cholesterol. The cholesterol level in serum also increased after soybean lecithin supplementation from 0 to 10 g kg^{-1} when the diet contained 6 g kg^{-1} cholesterol (Li *et al*., 2014). However, the cholesterol level in the serum was not different with PC supplementation from 5 g kg^{-1} to 20 g kg^{-1} when the diet contained 6 g kg^{-1} cholesterol (Wang *et al*., 2016). In addition, the PLs can increase the dietary lipid emulsification and transport of lipids absorbed into the hemolymph (Coutteau *et al*., 1997). In the present study, the triglyceride level in serum showed a significant decrease when soybean lecithin was supplemented in the diets with 6 g kg^{-1} cholesterol. However, no differences between triglyceride and glucose concentration in serum have been observed for the swimming crab (Li *et al*., 2014; Wang *et al*., 2016).

The antioxidant capacities of an organism, such as the SOD, T-AOC and GPx, are closely related to health. These enzymes can maintain the complex immune system of crustaceans, effectively clearing free radicals of their superoxide anions, and protect cells from damage (National Research Council, 2011). The results of the present study showed that the crabs fed the diet containing 40 g kg^{-1} lecithin with 6 g kg^{-1} cholesterol supplementation had significantly higher SOD activity than those fed the other diets. Furthermore, GPx activity significantly increased with dietary soybean lecithin levels increasing from 0 to 40 g kg^{-1} without cholesterol supplementation. Crabs fed the diet containing 40 g kg^{-1} soybean lecithin showed significantly lower MDA than those fed the diets that containing 0 and 10 g kg^{-1} soybean lecithin levels at the same cholesterol, these results demonstrated that dietary soybean lecithin could enhance antioxidant status and reduce damage of hepatopancreas for juvenile swimming crab. Dietary lecithin supplementation can promote sterol solubilization, making dietary cholesterol more available (Lester *et al*., 1975). This may be because dietary cholesterol becomes more available when supplemental dietary lecithin is supplied, reducing the requirement for dietary cholesterol.

HUFA (C20:4n-6, C20:5n-3 and C22:6n-3), which is not synthesized from PUFA, is the essential fatty acid for most marine crustaceans, and they need more HUFA than PUFA (C18:3n-3 and C18:2n-6) (Lim *et al*., 1997). The evaluation of the fatty acid composition of tissue is essential to the utilization of fatty acids in diets (Sánchez *et al*., 2014). The results of present study indicated that dietary soybean lecithin and cholesterol could affect fatty acid compositions in the hepatopancreas. Meanwhile, crabs fed the diets containing higher soybean lecithin had a significant increase of EPA, DHA, total n-3 PUFA, HUFA and n-3/n-6 in the hepatopancreas. These results demonstrated that dietary soybean lecithin could improve the efficiency of diet HUFA accumulation in the hepatopancreas of juvenile swimming crab. Our findings were agreement with previous studies, such as L. vannamei (González-Félix et al., 2002; Hu et al., 2011; Sánchez et al., 2014) and P. trituberculatus (Li et al., 2014; Li et al., 2016; Wang et al., 2016). Compared with hepatopancreas, the muscle of swimming crab had higher HUFA level (especially for C20:5n-3 and C22:6n-3) and lower MUFA levels. These findings agreed with the results of swimming crab (Li et al., 2014, 2016; Han *et al*., 2017) and *L. vannamei* (González-Félix *et al*., 2002; Hu *et al*., 2011; Sánchez *et al*., 2014). Moreover, sparing or accumulation of HUFA at the cost of MUFA consumption has been demonstrated in other studies (Hu *et al*., 2011).

In summary, dietary soybean lecithin and cholesterol interact to improve growth as well as affect fatty acids composition in hepatopancreas and muscle of juvenile swimming crab. The study further indicated a significant interaction between dietary soybean lecithin and cholesterol on growth performance, feed utilization with supplemental cholesterol being beneficial only when an insufficient level of dietary soybean lecithin is present. Dietary soybean lecithin and cholesterol enhanced lipid transportation and improve the efficiency of dietary HUFA absorption and deposition. The results indicated that supplemental dietary cholesterol may not be essential for juvenile

swimming crab when fed diets supplemented sufficient levels of phospholipids. The results of present study are an important step towards the development of a nutritionally balanced and cost-effective formulated diet for *P. trituberculatus*.

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