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Lipid nutrition of juvenile *Litopenaeus vannamei* II. Active components of soybean lecithin

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Abstract

Two experiments were conducted to investigate the active components of soybean lecithin for juvenile *Litopenaeus vannamei*. The first experiment was conducted to determine the dietary phosphatidylcholine (PC) requirement of juvenile *L. vannamei*, and to investigate whether other phospholipids (PL), mainly phosphatidylethanolamine (PE) and phosphatidylinositol (PI) were the active fractions of soybean lecithin. Seven levels of PC (0%, 0.35%, 0.7%, 1.4%, 2.1%, 2.8%, 4.2%) extracted from soybean lecithin (PC purity 93%) were used to determine the PC requirement; also, PE and PI (in a 25:22 proportion) were tested at 0.84% and 1.68% levels with PC levels controlled at 0.35% and 0.52% of diet to investigate the combined PE and PI effects. Results showed that no dietary PC requirement was evident based on shrimp growth and survival. Increasing purified PC in the diet decreased total lipid, free fatty acid and other PL levels in shrimp hepatopancreas (mid-gut gland) and increased PC level in shrimp muscle. However, other PL, mainly PE and PI, showed significant enhancing effects on shrimp growth when PC was provided at 0.35% or 0.52% of diet.

Another 4×2 factorial experiment was concluded to reevaluate the requirement of shrimp for PC by including purified PC at 0%, 0.7%, 1.4 % and 2.8% of diet with or without 0.1% cholesterol in the diet. A diet containing 1.4% PC provided by deoiled lecithin also was tested for comparison. Results showed no interaction between PC and cholesterol on shrimp growth, survival and feed conversion ratio (FCR). Compared with the apparent growth-enhancing effect of dietary cholesterol, the effect of purified PC was negligible. With PC at 1.4% of diet, the presence of other PL from lecithin or 0.1% cholesterol significantly enhanced shrimp growth and FCR.

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In summary, purified soybean PC showed different effects from deoiled lecithin on shrimp growth, lipid composition, and relationship with dietary cholesterol. Beneficial effects of soybean lecithin on growth of *L. vannamei* could be attributed to the presence of PL other than PC in the diet under the experimental conditions of this study.

Keywords: Litopenaeus vannamei; Lipid nutrition; Phospholipids; Phosphatidylcholine; Cholesterol

1. Introduction

Dietary phospholipids (PL) are required by shrimp for normal growth and survival. These PL can be obtained from a variety of sources and may vary in composition. Previous studies have demonstrated that the effects of various PL on shrimp growth and survival might be different. For example, Kanazawa et al. (1985) compared a control diet containing 8% pollack liver oil, with eight test diets containing 7% pollack liver oil and either 1% bonito egg phosphatidylcholine (PC), soy lecithin phosphatidylinositol (PI), soy lecithin PC, ovine brain phosphatidylethanolamine (PE), ovine brain phosphatidylserine (PS), bonito egg PE, chicken egg PC, or bovine brain sphingomyelin. The results showed that soy lecithin PI, soy lecithin PC and bonito egg PC markedly improved both survival and growth of larval *Marsupenaeus japonicus*, while the other sources of PL showed only little or no effect. Also, a study with *Penaeus monodon* showed that soybean lecithin gave better growth and survival of shrimp than egg lecithin (Piedad-Pascual, 1985).

Soybean lecithins have been widely used for dietary supplementation of PL in shrimp studies because of their industrial importance, as well as their beneficial effects on shrimp performance. Based on a recent review, recommended levels of dietary PL from soybean sources range from 1.25% to 6.5%, depending on shrimp species, developmental stage, as well as purity of the lecithin (Coutteau et al., 1997). Generally, PL from soybean lecithins are mixtures of PC, PE, PI and phosphatidic acid, which may vary greatly in regard to purity and composition. Most importantly, the active components of soybean lecithin remain unclear, though PC and PI have been suggested to be the main growth-promoting fractions (Coutteau et al., 1997). Thus, it is difficult to draw conclusions in regard to optimal levels and types of dietary PL.

Identification of active components of soybean lecithin also is very important to better understand the role of PL in shrimp nutrition and lipid metabolism. Compared with other individual PL, PC has been investigated more because it is the most abundant PL in animal tissues, accounting for 20% to 75% of the total PL in cell membranes (Sheard and Zeisel, 1989). By using highly purified soybean PC, several studies have evaluated its effect on different penaeid species of various developmental stages. For example, juvenile *Fenneropenaeus penicillatus* (Chen and Jenn, 1991), *P. monodon* (Chen, 1993), larval and postlarval *M. japonicus* (Camara et al., 1997), and postlarval *Litopenaeus vannamei* (Coutteau et al., 1996) require different levels of PC for optimal performance. Unlike PC, other individual PL have yet to be studied in shrimp. This is due in part to the high cost of purifying these individual PL and the relatively large quantities required for dietary supplementation.

The essentiality of dietary PL and cholesterol, as well as the interaction between PL and cholesterol on juvenile *L. vannamei* growth were addressed in the study of Gong et al. (2000). As a part of soybean PL, PC has been assumed to be the major active component in lecithin, which contributes to beneficial effects, such as enhanced shrimp growth. Based on the results of studies concerning the essentiality of PL and the interaction between dietary PL and cholesterol on growth of *L. vannamei*, it was postulated that if PC is the major active component in lecithin, it should be required in the diet of *L. vannamei* and interact with dietary cholesterol.

Therefore, this study aimed to evaluate other PL (mainly PE and PI), as well as PC on growth, survival and hepatopancreas and muscle lipid fractions of juvenile *L. vannamei*, and to determine if there was interaction between dietary PC and cholesterol and to evaluate the effect of purified PC on growth of juvenile *L. vannamei* in comparison to regular lecithin.

2. Materials and methods

2.1. Experiment one

2.1.1. Diets

A total of 12 semipurified experimental diets were prepared without cholesterol supplementation. Ingredients of the basal diet were the same as previously described (Gong et al., 2000). The basal diet and diets containing six levels of PC (0.35%, 0.7%, 1.4%, 2.1%, 2.8% and 4.2% of diet) provided by Phospholipon90 (American Lecithin, Oxford, CT) were used to determine effects of dietary PC on shrimp performance. Phospholipon90 is a product refined from deoiled soybean lecithin, which is waxy in consistency and light beige in color. Chemical analyses by the manufacturer indicated that Phospholipon90 contained 93% PC and 3% lysophosphatidylcholine. It took about 40 min at 45°C to 50°C to disperse Phospholipon90 into small amounts of water before adding it to the well-mixed dry ingredients. In addition, PE and PI enriched lecithin (Riceland Foods, Stuttgart, AR) processed from deoiled soybean lecithin was combined with Phospholipon90 to provide diets with three levels of PE and PI in combination (0%, 0.84% or 1.68%) at 0.35% PC and three levels of PE and PI in combination (0.84%, 1.68% or 2.53%) at 0.52% PC. The PE and PI enriched lecithin contained 9.3% PC, 25.0% PE and 21.8% PI, according to the manufacturer's analyses. All diets were designed to be isolipidic by reducing soybean oil levels as supplemental PL increased. Diets were prepared by cold extrusion with a Hobart A-200 extruder (Hobart, Troy, OH) and were dried at 45°C overnight. Pellets were ground to desired sizes for juvenile shrimp and stored at −20°C until used.

2.1.2. Experimental system

A 50-day feeding trial was conducted in a recirculating water system with approximately 8% new water exchange daily. A total of 102 tanks (bottom area 0.33 m^2) were used. A completely random design was employed. The water exchange rate in each tank was 4 l/min or 2880% water recirculation/day. A photoperiod of 12-h light and 12-h

dark was used. Temperature and salinity during the feeding trial were controlled at 30 ± 1°C and 25 ± 1‰, respectively. Dissolved oxygen was maintained in the range of 5.3 to 6.2 mg/l. Ammonia and nitrite were monitored weekly, and their concentrations were 0.06 ± 0.01 mg ammonia-N/l and 0.018 ± 0.002 mg nitrite-N/l, respectively.

2.1.3. Experimental shrimp, feeding and maintenance

Specific-pathogen-free *L. vannamei* postlarvae, obtained from the Oceanic Institute in Hawaii, were maintained at the Nutrition Laboratory of the Texas A&M University System Shrimp Mariculture Project (Port Aransas, TX). Shrimps were acclimated to the experimental conditions and fed a commercial postlarval diet (Rangen, Buhl, ID) supplemented with live *Artemia* nauplii twice daily until 1 week before the experiment was started. Then shrimps were fed the basal diet (without PL and cholesterol supplementation) for 1 week. Shrimps of similar size were selected, and groups of 10 shrimps were blotted dry and weighed before being stocked into individual tanks. Either eight or nine replicate tanks (with 10 shrimps initially in each tank) were used for each dietary treatment. Group weights averaged 6.03 ± 0.13 g (SD) with no significant difference observed among dietary treatments. At the termination of the feeding trial, all shrimps from each tank also were weighed as a group. Initial and final weights were calculated by dividing the group weight by the number of shrimp. Shrimps were fed the experimental diets 15 times daily via automatic feeders. Feeding rate was adjusted so that juvenile shrimps were fed to slight excess. Uneaten feed, fecal waste and molt exuviae were removed daily before placing the next daily ration into the automatic feeders. From a subpopulation of shrimp obtained immediately before initiation of the feeding trial and from pooled shrimp from the various dietary treatments immediately after termination of the feeding trial, shrimps were also randomly sampled and stored at -80°C for analysis. Prior to lipid analysis, shrimps were thawed and samples of hepatopancreas (mid-gut gland) and tail muscle were removed.

2.1.4. Lipid analysis

Tissue samples from 10 shrimps (before the feeding trial) were pooled together for one whole-body sample. Duplicate pooled samples of hepatopancreas and muscle were obtained from 10 shrimps as well. Hepatopancreas and tail muscle from three to five shrimps (after the feeding trial) were pooled as one sample and three replicates from each dietary treatment were taken for lipid analysis. Duplicate samples of each diet also were analyzed for lipid. The method of Folch et al. (1957) was applied for the lipid extraction of diet and shrimp tissue samples. An aliquot of extraction solution was dried under nitrogen and quantified gravimetrically for total lipid content. Total lipid content was expressed as percent of wet tissue or diet. Lipid fractions were separated and quantified utilizing an Iatroscan MK-5 TLC/FID analyzer according to the method of Fraser et al. (1985) with minor modification (Gong et al., 2000). Levels of lipid fractions were expressed as mg per g wet tissue or mg per g diet.

2.1.5. Statistical analysis

Survival, instantaneous growth rate (IGR) and feed conversion ratio (FCR) were used as the indices of shrimp performance. The IGR was calculated from the following

equation (Cushing, 1968): IGR = 100 × [ln (final weight/initial weight)]/duration of feeding trial in days.

Survival was transformed by arcsine square root before being submitted to further analysis. One-way ANOVA was applied for detecting the effects of purified PC on shrimp growth, survival and FCR. Two-way ANOVA was used for analyzing the effects of dietary PC (0.35% and 0.52%) and dietary PE and PI (0.84% and 1.68%) on shrimp performance. One-way ANOVA was used to evaluate the PC effect on shrimp growth at certain PE and PI levels, as well as PE and PI effects on shrimp growth at certain PC levels. Student–Newman–Keuls' (SNK) multiple range test was used to determine differences among means ($P < 0.05$).

Effects of dietary PC and PE and PI on total lipid content and lipid fractions of shrimp hepatopancreas and muscle were analyzed by multiple regression models and ANOVA followed by SNK multiple range test. All statistical analyses were performed using the SAS microcomputer software package (SAS Institute, 1995).

2.2. Experiment two

2.2.1. Diets

The composition of the basal diet was the same as in experiment one. Eight diets with combinations of PC (0%, 0.7%, 1.4% and 2.8% of diet) and cholesterol (0% and 0.1% of diet) were formulated to reevaluate the PC requirement, as well as investigate effects of dietary cholesterol on that requirement. PC, provided by Phospholipon90 (American Lecithin), consisted of 93% PC and 3% lysophosphatidylcholine. Also, a diet containing 5.38% deoiled lecithin (26% PC, Riceland Foods) was used to compare the effects of other PL at 1.4% PC, with or without dietary cholesterol. All diets were maintained isolipidic by reducing the level of soybean oil in the basal diet as PC (or PL) levels increased.

2.2.2. Experimental system

A 6-week growth trial was conducted in two systems with a total of 67 rectangular tanks (bottom area 0.33 m^2) with approximately 8% new water exchange daily. The water flow rate into each tank was 4 l/min or 2880% water recirculation/day/tank. A randomized block design was employed, with each system as a block. There were either seven or eight replicate tanks for each dietary treatment. Temperature and salinity were 32 ± 1°C and 25 ± 1‰, respectively. Dissolved oxygen was maintained above 5.4 mg/l. Ammonia and nitrite were monitored weekly and maintained within the recommended ranges for commercial shrimp production (Samocha et al., 1993). A photoperiod of 12-h light and 12-h dark was used.

2.2.3. Shrimp

Specific-pathogen-free *L. vannamei* postlarvae, obtained from the Harlingen Shrimp Farms (Los Fresnos, TX) were maintained at the Nutrition Laboratory of the Texas A&M University System Shrimp Mariculture Project at 32 ± 2°C and 25 ± 1‰ salinity.

Shrimps were fed commercial postlarval feed and supplemented with live *Artemia* nauplii twice daily until 1 week before the experiments were started. Then shrimps were fed a basal diet without PL and cholesterol supplementation for 7 days. Groups of eight shrimps initially averaging ($\pm$SD) 7.22 ($\pm$0.17) g were stocked into each tank. All shrimps from each tank were weighed as a group at the end of the feeding trial. Initial and final weights were calculated by dividing the group weight by the number of shrimp weighed. No significant difference in initial shrimp weight was observed among dietary treatments. Shrimps were fed experimental diets 15 times daily by using automatic feeders. Shrimps were fed to slight excess. Uneaten feed, fecal waste and molt exuviae were removed daily before placing the next daily ration into the automatic feeders.

2.2.4. Lipid analysis

Lipids from the two types of lecithin, deoiled lecithin and Phospholipon90, were extracted by the Folch method (Folch et al., 1957). An aliquot of the extract was used for determination of total lipid and the remaining lipid extract for fatty acid and lipid fraction analysis. Fatty acids in lipid extracts were subjected to methylation and dried with nitrogen, then dissolved into 300 μl hexane. About 0.2 μl of fatty acid methyl esters were injected by auto sampler and analyzed by a Varian 3400 gas chromatograph equipped with a 30 m $\times$ 1.53 mm Supelcowax™ fused silica capillary column employing a flame-ionization detector. Helium was used as the carrier gas. Fatty acids were identified by comparison of retention times to those of known standards and expressed as percentage of total fatty acids. Lipid fractions were analyzed using a TLC/FID analyzer according to the method of Fraser et al. (1985) with modification (Gong et al., 2000). Total lipid content was expressed as percent of diet. The lipid fractions were expressed as mg per g lecithin. Duplicate samples of each soybean lecithin were analyzed.

2.2.5. Statistical analysis

Shrimp IGR, survival and FCR were subjected to three-way ANOVA for analyzing the effects of dietary PC and cholesterol with system as a block factor. Two-way ANOVA was utilized to analyze the effects of other PL and cholesterol on shrimp performance when 1.4% PC was also supplemented in the diets, with system as a block factor. SNK multiple range test was used to determine differences among means. Linear regression models also were applied to analyze the effects of dietary cholesterol and PC on shrimp IGR. All the statistical analyses were performed using the SAS microcomputer software package (SAS Institute, 1995).

3. Results

3.1. Experimental one

3.1.1. Lipid analysis

Dietary lipid analyses (Table 1) showed that lipid content of diets was relatively constant. Among the dietary lipid fractions, only triglycerides and PL were detected.

Table 1
Total lipid, triglycerides, phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylcholine (PC) of diets supplemented with different levels (percent of diet) of PC, PE and PI[a]

Dietary PC/PE and PI	Lipid (%)	Triglycerides (mg/g)	PE (mg/g)	PI (mg/g)	PC (mg/g)
0/0	9.00	77.12	–[b]	–	–
0.35/0	8.94	71.61	–	–	3.85
1.4/0	9.99	66.03	–	–	9.20
2.1/0	9.79	49.79	–	–	22.92
2.8/0	9.76	48.66	–	–	27.59
0.35/0.84	9.20	60.42	4.00	3.50	4.00
0.35/1.68	8.78	31.26	8.13	7.08	3.70
0.52/0.84	9.47	54.81	4.84	3.48	5.90
0.52/1.68	8.96	40.80	9.08	7.91	5.33
0.52/2.52	9.16	17.15	12.01	10.46	6.07

[a]Values represent means of duplicate samples.
[b]Indicates not detected.

Surprisingly, not only the PC fraction, but also PE and PI fractions in the diets, were well separated. Analyzed levels of the various lipid fractions in the diets, especially the PL fractions, were very close to the calculated levels.

3.1.2. Biological performance

Initial weight and final weight, survival, IGR and FCR values of *L. vannamei* during the 50-day feeding trial are shown in Table 2. Overall survival in the experiment was 98.4% with no significant ($P > 0.05$) difference observed among dietary treatments.

Table 2
Initial and final weights, survival, instantaneous growth rate (IGR) and feed conversion ratio (FCR) of *L. vannamei* fed experimental diets containing graded levels (percent of diet) of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) for 50 days[a]

Dietary PC/PE and PI	Initial weight (g)	Final weight (g)	Survival (%)	IGR (%/day)	FCR
0/0	0.60 ± 0.02	4.84 ± 0.25	98.9 ± 3.3	4.19 ± 0.14	3.73 ± 0.36
0.35/0	0.60 ± 0.01	5.20 ± 0.40	100.0 ± 0.0	4.30 ± 0.14	3.41 ± 0.30
0.7/0	0.61 ± 0.01	4.98 ± 0.31	97.8 ± 6.7	4.20 ± 0.15	3.59 ± 0.33
1.4/0	0.61 ± 0.01	4.96 ± 0.22	97.8 ± 6.7	4.20 ± 0.07	3.74 ± 0.48
2.1/0	0.61 ± 0.02	5.06 ± 0.34	98.9 ± 3.3	4.24 ± 0.17	3.52 ± 0.38
2.8/0	0.61 ± 0.02	5.12 ± 0.22	100.0 ± 0.0	4.26 ± 0.08	3.55 ± 0.23
4.2/0[b]	0.60 ± 0.01	5.09 ± 0.23	97.5 ± 4.6	4.26 ± 0.08	3.59 ± 0.15
0.35/0.84[b]	0.61 ± 0.02	5.79 ± 0.29	98.8 ± 3.5	4.51 ± 0.13	2.98 ± 0.23
0.35/1.68[b]	0.60 ± 0.01	6.49 ± 0.13	100.0 ± 0.0	4.75 ± 0.06	2.69 ± 0.16
0.52/0.84[b]	0.60 ± 0.01	5.91 ± 0.34	98.8 ± 3.5	4.57 ± 0.12	2.95 ± 0.21
0.52/1.68[b]	0.60 ± 0.02	6.30 ± 0.30	95.1 ± 7.5	4.70 ± 0.12	2.88 ± 0.28
0.52/2.52[b]	0.60 ± 0.01	7.18 ± 0.61	97.36 ± 4.90	4.96 ± 0.15	2.45 ± 0.46

[a]Values represent means of nine replicates ± SD.
[b]Values represent means of eight replicates ± SD.

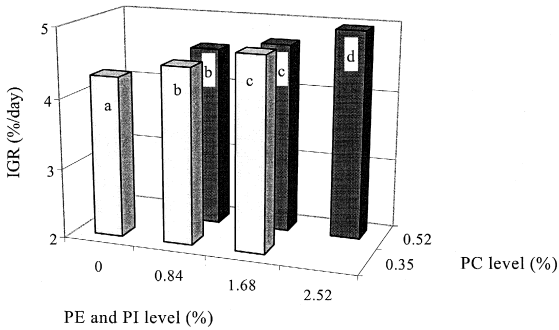

Fig. 1. Effect of dietary phosphatidylethanolamine (PE) and phosphatidylinositol (PI) combined with phosphatidylcholine (PC) on shrimp instantaneous growth rate (IGR) (means of $n = 9$ for the treatment without PE and PI supplementation, $n = 8$ for the other treatments). Significant differences are indicated with different letters (SNK $P < 0.05$).

One-way ANOVA showed that there was no effect of dietary PC on shrimp growth ($P = 0.4045$) or FCR ($P = 0.8231$) as PC level increased from 0% up to 4.2%.

Two-way ANOVA showed that there was no interaction between dietary PC (0.35%, 0.52%) and PE and PI (0.84%, 1.68%) on shrimp IGR ($P = 0.2430$) or FCR ($P = 0.2191$). Dietary PE and PI significantly increased shrimp IGR ($P = 0.0001$) and decreased FCR ($P = 0.0405$). However, no effect of dietary PC at 0.35% or 0.52% was detected on shrimp IGR ($P = 0.7753$) or FCR ($P = 0.3798$) at the same PE and PI inclusion level.

As shown in Fig. 1, dietary PE and PI significantly increased IGR and decreased FCR when provided at 1.68% of diet compared to 0% or 0.84% at 0.35% supplemental PC. At 0.52% supplemental PC, increasing dietary PE and PI (0.84%, 1.68% and 2.53%) also resulted in significantly improved shrimp growth and FCR.

Shrimp samples collected before the feeding trial was started but after shrimps had been fed the basal diet for 1 week were analyzed and data are presented in Table 3. Because the PL fractions in shrimp tissues are more complex than in diets, other PL of

Table 3
Total lipid, cholesterol ester (CE), triglycerides (TG), free fatty acids (FFA), cholesterol (Chol), other phospholipids (Other PL) and phosphatidylcholine (PC) in shrimp hepatopancreas, muscle and whole body before the feeding trial but after being fed the basal diet for 1 week[a]

Tissue	Lipid (%)	CE (mg/g)	TG (mg/g)	FFA (mg/g)	Chol (mg/g)	Other PL (mg/g)	PC (mg/g)
Hepatopancreas 1	6.07	1.03	40.64	–[b]	2.02	4.04	–
Hepatopancreas 2	5.37	2.53	35.37	–	1.97	3.72	2.24
Muscle 1	1.60	–	–	1.51	2.80	3.36	5.20
Muscle 2	1.60	–	–	1.08	2.50	3.38	4.82
Whole body	1.70	–	–	2.75	1.80	2.77	4.41

[a] Values represent means of duplicate samples.
[b] Indicates not detected.

Table 4
Total lipid, triglycerides (TG), free fatty acids (FFA), cholesterol (Chol) and other phospholipids (Other PL) in hepatopancreas of juvenile *L. vannamei* fed diets supplemented with different levels (percent of diet) of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) for 50 days[a]

Dietary PC/PE and PI	Lipid (%)	TG (mg/g)	FFA (mg/g)	Chol (mg/g)	Other PL[b] (mg/g)
0/0	21.23±0.61	15.36±3.00	67.55±6.11	14.39±1.55	25.45±3.30
0.35/0	21.03±0.52	35.73±19.48	67.46±4.75	18.57±4.18	21.31±1.75
1.4/0	15.32±1.93	16.68±7.80	39.69±12.42	11.71±4.43	15.16±1.36
2.1/0	15.46±1.85	27.00±8.73	44.12±8.09	13.20±2.85	13.99±1.76
4.2/0	12.61±0.27	15.69±1.57	34.93±3.07	8.67±0.56	10.55±0.67
0.35/0.84	17.24±1.16	53.24±9.10	40.32±10.41	12.73±4.14	12.07±1.61
0.35/1.68	19.15±1.48	54.27±12.84	40.80±9.93	16.16±4.05	14.66±2.43
0.52/0.84	15.08±0.88	27.56±5.32	34.40±2.38	11.75±0.95	12.27±0.90
0.52/1.68	14.81±1.59	38.52±9.16	31.23±2.31	12.89±0.38	11.57±1.13
0.52/2.52	19.38±0.54	44.22±7.18	33.93±2.72	13.26±1.17	13.92±0.87

[a]Values represent means of three composite samples of three to five shrimp ± SD.
[b]Other PL refers to phospholipids other than PC.

tissues, besides PC, were categorized as one group. Triglycerides in hepatopancreas were very high compared with other lipid fractions in the same tissue. Concentrations of cholesterol and other PL were similar in both hepatopancreas and muscle; however, PC was even lower in hepatopancreas than in muscle, and FFA was not detected in hepatopancreas. Lipid fractions in the whole body of shrimps were more similar to those in muscle than to those in hepatopancreas.

The results of lipid analyses of shrimp hepatopancreas and muscle tissues after the 50-day feeding trial are presented in Tables 4 and 5, respectively. Compared with tissue composition of shrimp before the feeding trial, total lipid in hepatopancreas was higher in shrimp after the feeding trial; while, total lipid in muscle did not change much.

Table 5
Total lipid, free fatty acids (FFA), cholesterol, phosphatidylcholine (PC) and other phospholipids (Other PL) in muscle of juvenile *L. vannamei* fed diets supplemented with different levels (percent of diet) of PC, phosphatidylethanolamine (PE) and phosphatidylinositol (PI) for 50 days[a]

Dietary PC/PE and PI	Lipid (%)	FFA (mg/g)	Cholesterol (mg/g)	Other PL[b] (mg/g)	PC (mg/g)
0/0	1.26±0.03	0.62±0.01	1.35±0.04	2.87±0.13	4.23±0.23
0.35/0	1.26±0.02	0.71±0.08	1.49±0.06	2.97±0.60	4.38±0.46
1.4/0	1.25±0.06	0.55±0.03	1.19±0.07	2.86±0.19	4.15±0.15
2.1/0	1.30±0.03	0.69±0.08	1.87±0.29	3.43±0.72	5.79±1.12
4.2/0	1.32±0.04	0.67±0.03	1.93±0.17	3.70±0.31	6.50±0.48
0.52/0.84	1.26±0.02	0.67±0.04	1.37±0.22	2.92±0.33	4.43±0.05
0.52/1.68	1.27±0.04	0.62±0.05	1.76±0.09	2.83±0.39	4.29±0.22
0.35/0.84	1.32±0.03	0.70±0.15	1.24±0.15	2.64±0.06	3.83±0.16
0.35/1.68	1.26±0.05	0.54±0.01	1.53±0.10	2.49±0.10	3.98±0.16
0.52/2.52	1.37±0.05	0.59±0.17	2.00±0.16	2.91±0.21	4.50±0.32

[a]Values represent means of three composite samples of three to five shrimp ± SD.
[b]Other PL refers to phospholipids other than PC.

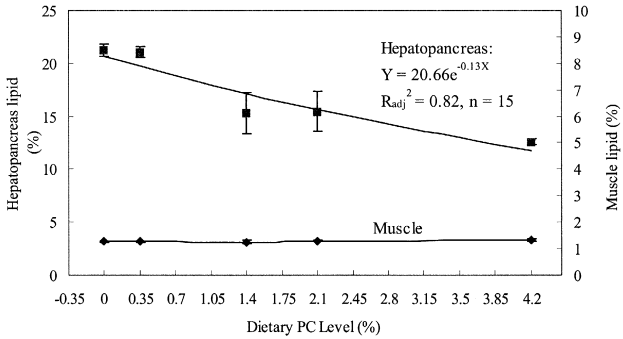

Fig. 2. Effect of dietary phosphatidylcholine (PC) on shrimp tissue lipid contents (mean ± SD, $n = 3$).

Without PE and PI inclusion, increments of dietary PC were associated with decreases in total lipid ($P = 0.0001$), free fatty acids ($P = 0.0006$) and other PL ($P = 0.0001$) in hepatopancreas (Figs. 2 and 3). Total lipid of shrimp muscle was not affected by dietary PC. Nevertheless, dietary PC significantly affected PC ($P = 0.0018$) and cholesterol levels ($P = 0.0006$) in shrimp muscle. Cholesterol and PC levels were higher in muscle of shrimps fed 2.1% and 4.2% dietary PC treatment compared to those fed lower PC levels.

Two-way ANOVA showed that there was no interaction between dietary PC levels (0.35%, 0.52%) and PE and PI levels (0.84%, 1.68%) on lipid content or any tissue lipid fraction. Elevated dietary PE and PI levels caused an increase in muscle cholesterol ($P = 0.0037$). When dietary PC increased from 0.35% to 0.52%, total lipid of hepatopancreas significantly ($P = 0.0026$) dropped from 18.2% to 14.9%. The regression model showed that total lipid of hepatopancreas (Hlipid) had a negative linear relationship with dietary PC level and a quadratic relationship with the PE and PI levels, which was expressed as: $\text{Hlipid} = 18.41 - 5.225X_1 - 5.217X_2 + 6.302X_2^2$ ($R^2 = 0.8295$, $R^2_{\text{adj}} = 0.7930$, $n = 15$), where X_1 represented dietary PC level, and X_2 represented dietary

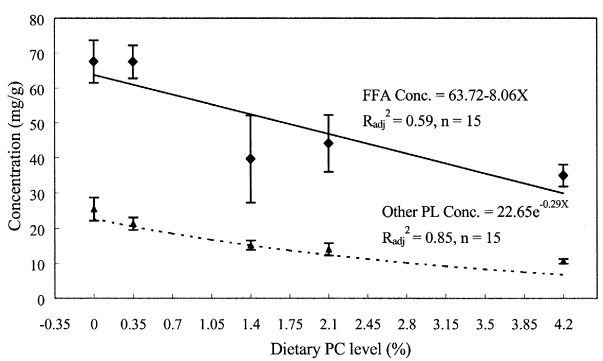

Fig. 3. Effects of dietary phosphatidylcholine (PC) on free fatty acids (FFA) and other phospholipids (Other PL) in hepatopancreas of *L. vannamei* (mean ± SD, $n = 3$).

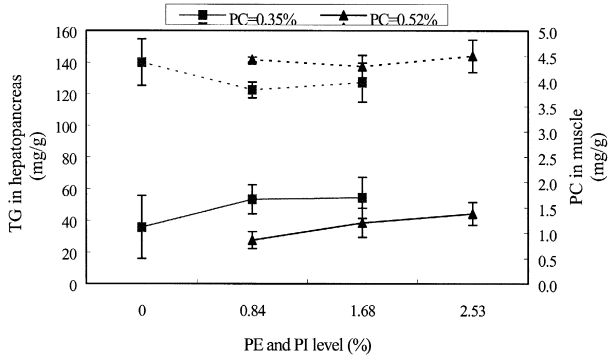

Fig. 4. Effects of dietary phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) on triglycerides (TG) (solid line) in hepatopancreas and PC (dotted line) in muscle of *L. vannamei* juveniles (mean $\pm$ SD, $n = 3$).

PE and PI levels. Dietary PC had somewhat different effects on tissue lipid fractions in combination with PE and PI supplementation. As presented in Fig. 4, triglyceride level significantly decreased from 53.8 to 33.0 mg/g hepatopancreas ($P = 0.0054$) and PC level increased from 3.9 to 4.4 mg/g muscle ($P = 0.0105$) when dietary PC level increased from 0.35% to 0.52% when either 0.84% or 1.68% PE and PI were included in the diet.

3.2. Experiment two

Analyzed fatty acid and lipid compositions of the two lecithin products are shown in Tables 6 and 7, respectively. Among the fatty acids, the analyzed values of major fatty acids were very close to values provided by the manufacturers. The major fatty acid profiles were similar in the two kinds of lecithin. The polyunsaturated fatty acids in deoiled lecithin were even lower than those in Phospholipon90. Slightly higher levels of $n-3$ fatty acids were in deoiled lecithin compared with Phospholipon90, especially 20:5$n-3$ (eicosapentaenoic acid or EPA). However, with less than 6% deoiled lecithin included in the diets, the total amount of 20:5$n-3$ may not be a main contributor to growth enhancement of shrimp. All fatty acids in the products were incorporated in PL as no free fatty acid was detected. Phospholipon90 consisted of a high level of PC without other PL detected, while deoiled lecithin consisted of other PL (mainly PE and PI) besides PC.

Initial and final weights, IGR, survival, and FCR of juvenile *L. vannamei* are presented in Table 8. Three-way ANOVA showed that there was neither a significant block (system) effect ($P = 0.0649$) nor interaction between dietary PC and cholesterol ($P = 0.464$) on shrimp IGR, but significant effects due to dietary PC ($P = 0.0351$) and cholesterol ($P = 0.0001$) were observed. The effects of dietary cholesterol and PC on shrimp IGR are shown in Fig. 5. Increasing dietary cholesterol from 0% to 0.1% at all PC levels significantly increased shrimp IGR from a mean of 3.96% to 4.55% per day.

Table 6
Fatty acid composition (percent of total fatty acids) of the two lecithins[a,b]

Fatty acid	Regular lecithin	Phospholipon 90
14:0	0.1	0.1
16:0	18.7 (20)	12.3 (12 ± 2)
16:1	0.2	0.2
16:2	0.2	0.1
16:3	–[c]	0.1
18:0	3.9 (5)	4.3 (3 ± 1)
18:1	6.8 (9)	9.2 (10 ± 3)
18:2$n-6$	59.5 (59)	65.9 (66 ± 5)
18:3$n-3$	8.6 (7)	7.0 (5 ± 2)
20:5$n-3$	0.7	0.3
22:1	0.2	0.3
22:5$n-3$	0.4	–
Totals		
Saturates	22.7	16.8
Monoenoics	7.2	9.8
PUFA[d]	70.1	73.4
$n-3$	9.9	7.2
$n-6$	59.5	65.9

[a] Values represent means of duplicate samples.
[b] Values in parentheses indicte data obtained from the manufacturer.
[c] Not detected.
[d] Polyunsaturated fatty acids.

However, there were no differences ($P > 0.05$) among the graded PC levels, even though shrimp fed diets containing 1.4% and 2.8% PC grew slightly faster than those fed 0% and 0.7% PC. The obvious growth-enhancing effect of dietary cholesterol, and the negligible contribution of purified PC to shrimp growth was described by the regression model: IGR = 3.884 + 0.058 × PC level + 5.964 × Cholesterol level ($R^2_{adj} = 0.7288$, $n = 60$). The FCR values were significantly affected by dietary cholesterol level ($P = 0.0001$), but not by block ($P = 0.5457$), dietary PC level ($P = 0.6076$) or interaction between PC and cholesterol ($P = 0.3332$). Dietary cholesterol level increasing from 0% to 0.1% significantly reduced FCR values.

Table 7
Lipid content and fractions (%) of the two soybean lecithins[a]

Lecithin	Lipid content	PC	Other PL[b]
Phospholipon90	100.0	93.9	–[c]
Deoiled lecithin	96.2	26.2	64.5

[a] Values are means of duplicate samples.
[b] The separation of other phospholipids (phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine, etc.) was not distinct except for PC, therefore these PL were categorized as ''Other PL''.
[c] Not detected.

Table 8
Initial weight, final weight, survival, instantaneous growth rate (IGR) and feed conversion ratio (FCR) of *L. vannamei* fed the experimental diets containing graded levels (percent of diet) of phosphatidylcholine (PC) and cholesterol (Chol) for 6 weeks[a]

Dietary PC/Chol	Initial weight (g)	Final weight (g)	Survival (%)	IGR (%/day)	FCR
0/0	0.91 ± 0.03	4.77 ± 0.53	100.00 ± 0.00	3.92 ± 0.24	3.87 ± 0.69
0.7/0	0.90 ± 0.02	4.51 ± 0.40	98.21 ± 5.05	3.83 ± 0.22	4.28 ± 0.63
1.4/0	0.90 ± 0.02	4.96 ± 0.36	98.44 ± 4.42	4.06 ± 0.18	3.87 ± 0.45
2.8/0	0.90 ± 0.01	4.84 ± 0.35	98.44 ± 4.42	4.01 ± 0.16	4.15 ± 0.46
0/0.1[b]	0.90 ± 0.02	5.83 ± 0.29	100.00 ± 0.00	4.44 ± 0.12	2.91 ± 0.16
0.7/0.1[b]	0.90 ± 0.02	6.05 ± 0.19	100.00 ± 0.00	4.54 ± 0.10	2.78 ± 0.11
1.4/0.1[b]	0.91 ± 0.03	6.24 ± 0.33	100.00 ± 0.00	4.59 ± 0.17	2.75 ± 0.20
2.8/0.1[b]	0.90 ± 0.02	6.32 ± 0.54	100.00 ± 0.00	4.64 ± 0.24	2.73 ± 0.44
1.4[c]/0[b]	0.91 ± 0.02	6.87 ± 0.52	98.21 ± 4.72	4.82 ± 0.23	2.46 ± 0.22

[a] Values represent means of eight replicates ± SD.
[b] Values represent means of seven replicates ± SD.
[c] 1.4% PC in this group was provided by 5.38% deoiled lecithin.

Another two-way ANOVA comparing the three diets containing 1.4% PC showed that there was a significant diet effect ($P = 0.0001$) and block (system) effect ($P = 0.0003$) on shrimp growth, but no interaction between diet and system ($P = 0.5711$). The diet containing 1.4% PC provided by deoiled lecithin had the best growth-promoting effect on shrimp, followed by the diet included with the same level of PC provided by Phospholipon90 combined with 0.1% cholesterol, and finally the diet without cholesterol inclusion (Fig. 6). As for FCR, a significant diet effect was found ($P = 0.0001$), with either PL or cholesterol reducing FCR significantly (Fig. 6). Survival

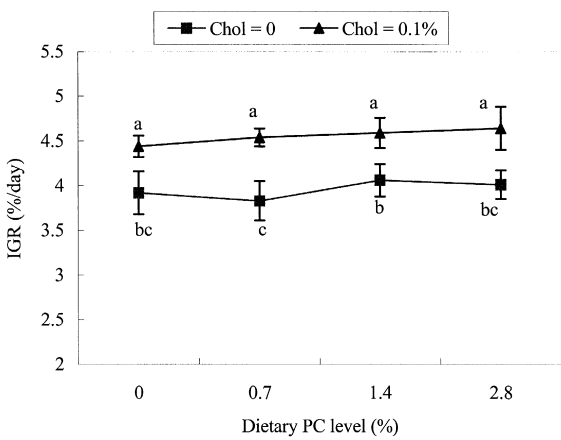

Fig. 5. Effect of dietary phosphatidylcholine (PC) on shrimp instantaneous growth rate (IGR) with and without 1% dietary cholesterol (Chol) (mean ± SD, $n = 8$ for treatments without cholesterol supplementation, $n = 7$ for the other treatments with 0.1% cholesterol). Significant differences are indicated with different letters (SNK $P < 0.05$).

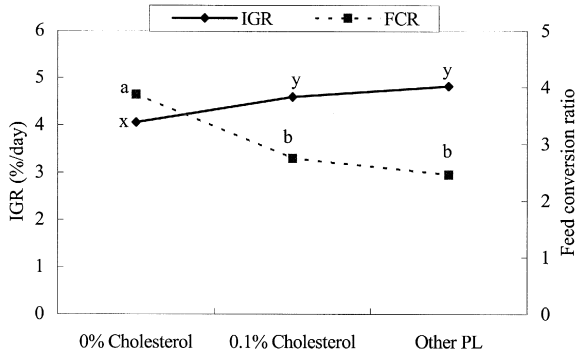

Fig. 6. Comparison of dietary cholesterol and other phospholipids (Other PL) on shrimp growth and feed conversion ratio at 1.4% PC (means of $n = 32$ in 0% cholesterol group, $n = 28$ in 0.1% cholesterol group, $n = 7$ in Other PL group). Significant differences are indicated with different letters (SNK $P < 0.05$).

values in all dietary treatments were high and no significant difference was found due to any dietary factor.

4. Discussion

All the parameters of water quality in the present feeding trials were very stable and within the recommended ranges for commercial shrimp production (Samocha et al., 1993). This may be a primary reason for high survival during the 50-day and 6-week feeding trials. D'Abramo et al. (1981) demonstrated that neither ovine PE, soy PI nor hydrolysis products of soy PC could effectively substitute for the refined soy PC in reducing mortality of *H. americanus*. However, survival values in the present study were very high and not affected by the levels or compositions of PL. This result is in line with results from other studies (Chen and Jenn, 1991; Chen, 1993; Coutteau et al., 1996).

In experiment one, PC, other PL and cholesterol in hepatopancreas of shrimps were reduced by feeding shrimp the basal diet without PL and cholesterol supplementation for 1 week as compared with their levels in hepatopancreas of shrimp from our previous study (Gong et al., 2000). This could make the shrimp more sensitive to dietary PL supplementation. In addition, the total lipid, especially TG level, was dramatically higher in hepatopancreas of shrimp from the present study than those from the previous study. These differences are more diet-oriented than shrimp size-related since the weights of analyzed shrimps were similar.

Although the beneficial effect of soybean lecithin on shrimp growth has been widely accepted, active components in soybean lecithin have remained unclear. A few studies have used highly purified soy PC, such as 80% PC with *F. penicillatus* juveniles (Chen and Jenn, 1991), 80% PC with *P. monodon* juveniles (Chen, 1993), 95% PC with *L. vannamei* postlarvae (Coutteau et al., 1996), and 95% PC with *M. japonicus* larvae and postlarvae (Camara et al., 1997). These studies suggested that PC was the major if not

the only active component in lecithin; however, these studies did not pinpoint the PC requirement. The present feeding trials using PC at 93% purity from 0% to 4.2% of diet showed that there was no effect of PC on growth and survival of *L. vannamei* juveniles when no dietary cholesterol was supplemented. Also, at certain levels of PE and PI, dietary PC did not affect shrimp growth.

PI also is considered as active PL component besides PC. Kanazawa et al. (1985) indicated that soybean PC and PI were more effective in improving growth and survival of larval *M. japonicus* than chicken egg PC, ovine brain PE, bonito egg PE, bovine brain sphingomyelin or ovine brain PS. In the present study, soybean PI together with PE significantly increased *L. vannamei* growth at certain PC levels. Teshima (1997) studied the effectiveness of several fractions derived from soybean PL with juvenile *M. japonicus*, and found 0.35% PI gave higher shrimp growth than 3% soybean lecithin. These results seem opposite to those from previous studies with postlarval *L. vannamei* (Coutteau et al., 1996) and *M. japonicus* (Camara et al., 1997), which showed PE and PI in soybean lecithin were not required for shrimp during postlarval stage. This obvious contradiction may be explained in that the relative importance of PC and PI may not necessarily be the same for different species (Coutteau et al., 1996) and developmental stages. PI is not only a component of cell membranes, but also an intracellular messenger, which is important for normal growth and proliferation of cells. The cellular message-transmitting function of PI may be more significant in the juvenile stage than earlier stages of development. Although there is a growing body of evidence that choline PL, such as PC and their metabolites, also are important mediators and modulators of transmembrane signaling (Zeisel, 1993), most studies have been mainly focused on mammals, and little is known about crustaceans in this regard.

Synthesis of PI is via the cytidine diphosphate diacylglycerol pathway, which is different from de novo synthesis of nitrogen-containing PL through hydrolysis of phosphatidic acid to sn-1,2-diacylglycerol (Longmuir, 1993). An alternative route along which PC can be synthesized involves the stepwise methylation of PE (Bremer and Greenberg, 1961). Although PE could be a precursor of PC, as well as a membrane component, its function in enhancing shrimp growth may be subsidiary.

Few studies have investigated the effect of dietary PC on shrimp tissue lipid fractions, and the results have varied from species to species. Increasing dietary PC was reported to decrease PC and increase free fatty acid levels in muscle of *P. monodon* (Chen, 1993), and to increase muscle lipid content in *F. penicillatus* (Chen and Jenn, 1991). Comparing the results from the present and previous studies (Gong et al., 2000), dietary purified PC and PL showed different effects on lipid fractions of shrimp tissues. Diets supplemented with PL resulted in higher total lipid in hepatopancreas and lower total lipid in muscle of shrimp than diets without supplemental PL (Gong et al., 2000). In the present study, in response to increasing dietary PC levels alone, total lipid content, free fatty acids and other PL in hepatopancreas decreased, whereas muscle PC increased. According to results from the present study, dietary PC may facilitate lipid mobilization and transport from the hepatopancreas through hemolymph to muscles for utilization and resynthesis in *L. vannamei*.

In addition, this study showed that with 0.84% or 1.68% dietary PE plus PI, total lipid and triglyceride levels in hepatopancreas decreased and PC levels in muscle

increased as dietary PC level increased from 0.35% to 0.52%. Even though with such a slight increment of dietary PC, its function of mobilizing neutral lipid in hepatopancreas and increasing PC in muscle was revealed in the presence of dietary PE and PI.

In *M. japonicus*, PL were demonstrated to be the principal lipid moiety released from the hepatopancreas into the hemolymph, and it was suggested that lipoproteins accelerated their release (Teshima and Kanazawa, 1978). Lack of a dietary source of PL apparently resulted in a PC deficiency in lobster hemolymph that restricted cholesterol transport from the hepatopancreas to the hemolymph; therefore, interaction between dietary PL and cholesterol was assumed (D'Abramo et al., 1982). Dietary PL and cholesterol have been shown to have beneficial effects and a highly significant interaction on the growth of *L. vannamei* juveniles (Gong et al., 2000). Based on the results from the study of Gong et al. (2000), cholesterol at 0.1% of diet was chosen in this study because that level should be enough to show a cholesterol effect, but not mask effects of PL, with PC generally considered to be the most active of all PL. However, no interaction between dietary PC and cholesterol was determined in the present study. Inclusion of dietary cholesterol did significantly enhance shrimp growth. Purified PC showed little effect on shrimp growth regardless of cholesterol supplementation. Likewise, interactions between dietary PC and cholesterol were not significant on growth of *F. penicillatus* (Chen and Jenn, 1991) and *P. monodon* (Chen, 1993). Thus, the function of PC is different from that of dietary PL or cholesterol based on shrimp growth.

Studies with postlarval *L. vannamei* (Coutteau et al., 1996) and larval and postlarval *M. japonicus* (Camara et al., 1997) indicated that PL in lecithin other than PC could not compensate for a PC deficiency when 0.5% cholesterol was included in the diets. However, the superior effect of deoiled lecithin over purified PC on shrimp growth was obvious from the experiment two, in which weight gain of shrimp fed 5.38% deoiled lecithin (equivalent to 1.4% dietary PC) was more than 50% higher than that of shrimp fed Phospholipon90 at any inclusion level (0.7%, 1.4% and 2.8%). This result supports those from experiment one, which demonstrated that other PL (most likely PI) was the active component of soybean lecithin, which contributed to the growth-promoting effect of soybean PL on juvenile *L. vannamei*. The discrepancies between the present study and other reports may arise from difference in species and developmental stages or experimental conditions.

Another factor contributing to the beneficial effect of deoiled soybean lecithin on shrimp growth compared with PC from Phospholipon90 may be related to the fatty acid classes in the PL. Shrimp, like other crustaceans, lack the ability to synthesize $n-6$ and $n-3$ fatty acids de novo (Kayama et al., 1980). Dietary fatty acids are generally presumed to be absorbed mainly in the region of the hepatopancreas and mid-gut. The absorbed fatty acids are resynthesized to PL in the hepatopancreas and hind-gut, and then released into the hemolymph in that form (Teshima and Kanazawa, 1979). Fatty acid analysis of the two types of lecithin used in the present study showed that profiles of fatty acids were similar with around 60% as linoleic acid. Because both lecithin products were refined from soybean, any difference in fatty acid location in the glycerol carbon backbone should be minor. Fatty acids of the $n-3$ family have been observed to have the greatest nutritional value to some marine shrimp compared with fatty acids of the $n-6$ family (Kanazawa et al., 1977; Xu et al., 1993, 1994). In the present study,

1.78% menhaden fish oil, which is a good source of highly unsaturated fatty acids of the $n-3$ family, was included in all diets to presumably satisfy the shrimp's requirements for essential fatty acids. Thus, fatty acid composition of the PL should not be considered a major reason for the differences in shrimp growth detected in the present study.

In summary, purified PC showed no favorable effect on growth of *L. vannamei* juveniles, but reduced lipid content, FFA and other PL of hepatopancreas and increased PC in muscle. At certain PC levels (0.35% or 0.52%), increment of dietary PI- and PE-enhanced shrimp growth increased the beneficial effects of dietary PC on lipid mobilization, utilization and resynthesis. No interaction between purified PC and cholesterol was detected on growth and FCR of *L. vannamei* juveniles. Purified PC alone was nutritionally inferior to deoiled lecithin in improving shrimp growth under the experimental conditions of this study.

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