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Growth, Survival, and Body Lipid Composition of the Prawn Larvae Receiving Several Dietary Phospholipids

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Abstract

Feeding trials using the prawn larvae *Penaeus japonicus* were conducted to examine the effects of several dietary phospholipids (PL) on the growth, survival, and body lipid composition. The deficiency in dietary PL caused a total mortality within 6-7 days. When the prawn larvae were fed carrageenan micro-bound diets with varying levels of supplemental soybean lecithin (SBL), the highest survival rates were obtained on diets with 3% SBL. Soybean phosphatidylcholine (PC) and soybean phosphatidylinositol (PI) showed a higher nutritive value than bonito-egg PC and soybean phosphatidylethanolamine (PE) at a 3% supplemental level. The deficiency in dietary PL resulted in a slight decrease in the concentrations of steryl esters, free sterols, PC, and PI in the bodies. The concentrations of PL such as PC seemed slightly higher in the prawn larvae receiving supplemental soybean PC than other supplemental PL such as soybean PI, soybean PE, and bonito-egg PC. However, the body lipid compositions, on the whole, were not variable notably with the kinds of supplemental PL examined. Also, the sum of 20 : 5 ω3 and 22 : 6 ω3 proportions of body PL was slightly higher in the prawn larvae receiving soybean PC rather than in those receiving other supplemental PL. The results of the present study offer additional evidence for the hypothesis that the PL requirements of prawn larvae are related to the efficient transport of dietary lipids such as cholesterol.

Previously, we have shown that the prawn *Penaeus japonicus* larvae necessitate dietary sources of phospholipids (PL) such as phosphatidylcholine (PC) and phosphatidylinositol (PI) for successful development and high survival.[1-4] Later, we also found the necessity of inclusion of some PL for the growth of *P. japonicus* juveniles.[5] On the other hand, the lobster *Homarus americanus* has also been demonstrated to require PC as an essential nutrient,[6,7] the absençe or deficiency in dietary PC resulting in a death due to incomplete ecdysis, so-called molt death syndrome.[8] Animals are generally capable of phospholipid synthesis from lower units such as diglycerides and fatty acids and do not require specific PL as indispensable nutrients for growth. Therefore, it is unusual that PL such as PC are required for normal growth and survival of crustaceans.

Previously, we have shown that dietary some PL probably contribute to the efficient transport of dietary lipids, especially cholesterol, in the juvenile prawn.[9-11] This work was

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conducted to understand better the nutritional role and metabolism of dietary PL in the prawn larvae. First, feeding trials were carried out in a small scale to reexamine the effects of dietary PL (soybean lecithin, SBL) levels on the growth and survival rates and also the nutritive value of several PL classes. Second, lipid analsis after feeding trials was performed to investigate the effects of dietary PL classes on the body lipid compositions of prawn larvae. This paper presents these results and discussion in the light of the mechanism whereby dietary PL exert enhancing effects of growth and survival in the prawns.

Materials and Methods

Feeding Trials and Test Diets

Mother prawns *P. japonicus* were obtained from Matsumoto-Suisan Co., in Miyazaki, and put into polycarbonate tanks (500 liters) for laying of eggs. Feeding trials were conducted by using $zoea_1$ larvae during the period of June to September. The larvae were reared with test diets (carrageenan micro-bound diet[12,13]: particle sizes, 50 μm for $zoea_1$ and $zoea_2$ larvae, and 125 μm for $zoea_3$ larvae) containing different PL or with a live food (control diet : *Chaetoceros gracilis*, 5-7 X 10^4 cells/m*l*, and *Artemia salina*, 500 individuals/larva) in the similar manner to that described previously.[13,14] Table 1 shows the compositions of test diets, which contained the same ingredients each other except for pollack liver oil

Table 1. Composition of test diets*[1]

Diet No.	Dietary lipid*[2]
1	Control : live food (*Chaetoceros* + *Artemia*)
2	10% PLO + 0.5% HUFA
3	1% SBL + 9% PLO + 0.5% HUFA
4	3% SBL + 7% PLO + 0.5% HUFA
5	5% SBL + 5% PLO + 0.5% HUFA
6	10% SBL + 0.5% HUFA
7	3% Sb-PE + 7% PLO + 0.5% HUFA
8	3% Sb-PI + 7% PLO + 0.5% HUFA
9	3% Sb-PC + 7% PLO + 0.5% HUFA
10	3% Bo-PC + 7% PLO + 0.5% HUFA

*[1] The basal ration of test diets was the same as reported previously and contained the following ingredients (g/100g): casein 50, glucose-sucrose-α-starch (5.5 : 10 : 4) 15, sodium citrate 0.3, sodium succinate 0.3, glucosamine HCl 0.8, vitamins 3.2, minerals 8.6, cholesterol 1, lipids 10, and α-cellulose 5.8. To this, 5% carrageenan was added as a binder.

*[2] PLO (pollack liver oil), SBL (soybean lecithin, commercial products), HUFA (a mixture of 20 : 5 ω3 and 22 : 6 ω3, about 3 : 2), and Bo-PC (phosphatidylcholine fraction isolated by a solvent-fractionation method from bonito-egg phospholipids). Sb-PE, Sb-PI, and Sb-PC are phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine fractions isolated from commercial SBL by column chromatography on Silica gel 60, respectively. See Table 2 for the phospholipid class and fatty acid compositions of Sb-PE, Sb-PI, Sb-PC, and Bo-PC.

(PLO) and PL sources. Test diets were prepared as reported previously.[13] Commercial SBL contained phosphatidylethanolamine (PE, 29.7% of total PL), phosphatidylserine (PS, 8.4%), PI (18.5%), PC (35.0%), and other PL classes (8.4%). Table 2 shows the purities and fatty acid compositions of the PL classes used in the present study as PL sources.

Table 2. Phospholipid class and fatty acid compositions of dietary lipids used in the feeding trials

Composition (%)*	Dietary lipid				
	Sb-PE	Sb-PI	Sb-PC	Bo-PC	PLO
Phospholipid (PL)					
PE	95	11		21	
PS	3	10	7	9	
PI		60	7	14	
PC		14	68	43	
SM			6	2	
LPC			5	6	
UK	2	5	7	5	
Fatty acid					
14 : 0	0.2			2.1	2.3
16 : 0	20.2	23.8	21.2	22.5	6.7
16 : 1				1.8	1.3
18 : 0	3.6	6.0	3.5	14.6	2.4
18 : 1ω9	8.8	11.5	12.0	5.5	12.8
18 : 2ω6	60.1	50.5	55.3	0.2	0.9
18 : 3ω3	7.3	8.2	6.2	0.5	0.4
20 : 1ω9				0.6	18.3
20 : 4ω3				5.3	
20 : 4ω6					1.1
20 : 5ω3			0.2	3.1	38.0
22 : 4ω6					2.2
22 : 5ω3				1.0	3.3
22 : 6ω3				32.9	7.4
Saturates	24.0	29.8	24.7	39.2	11.4
ω6-series	60.1	50.5	55.3	0.2	4.2
ω3-series	7.3	8.2	6.2	42.8	49.1

* Percentage of total phospholipids or fatty acids. PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; LPC, lysophosphatidylcholine; UK, unknown phospholipids.

In feeding trial-1, the zoea$_1$ larvae were divided into lots of 100 individuals into 1-liter beakers and reared with diets 1 to 10. The larvae were fed test diets at the feeding rate of 0.16 mg/larva/day (3 times a day) at 25-27℃ for 8 days by the same feeding and rearing techniques as described previously.[13,14] In feeding trial-2, the larvae were reared in a large

scale by using diets 2 (PL-deficient) and 4 (SBL-added) for lipid analysis of prawn bodies. The $zoea_1$ larvae were put into 3 polycarbonate tanks (500 liters) for respective diet groups and fed the diets at the feeding rate of 6.0 g/day/tank (twice a day ; 9.00 and 17:00 o'clocks). The half of sea water in tanks was renewed every other days. In feeding trial-3, the larvae were reared with diets containing 3% levels of different PL (diets 1, 7, 8, 9, and 10) in polycarbonate tanks (100 liters). In each diet group, the larvae were fed diets at the feeding rate of 3.0 g/tank/day (5 times a day).

Analysis of Body Lipid Compositions

Total lipids (TL) were extracted from the larvae by BLIGH and DYER method[15] and separated into neutral lipid (NL) and phospholipid (PL) fractions by column chromatography on Kieselgel 60. NL and PL fractions were further separated into lipid classes by thin-layer chroamtography (TLC) and quantified as described previously.[5] Fatty acid compositions (%) were determined by gas-liquid chromatography (GLC) on 5% Shinchrom E71 on Shimalite AW (column 3m x 3mm i.d., column temp. 217℃).[5]

Results

Results of Feeding Trials

Figs. 1, 2, and 3 show the results of feeding trial-1. All $zoea_1$ larvae died within 3-4 days when no food was given. The deficiency in dietary PL caused total mortality of the prawn larvae in about 7 days. Although the growth and survival rates of larvae were improved by the supplement of every level of SBL, the highest efficacy in improving the survival rates was obtained on the diet with 3% SBL supplements (Fig. 1). The prawn larvae showed better survival on the carrageenan micro-bound diet with 3% SBL (diet 4) than on the control diet (live food). The larvae on the PL-deficient diet (diet 2) died markedly prior to the metamorphosis from $zoea_1$ to $zoea_2$ (Fig. 2). This suggests that dietary PL are indispensable for successful metamorphosis. These results obtained in the present study confirmed the previous findings.[3,4]

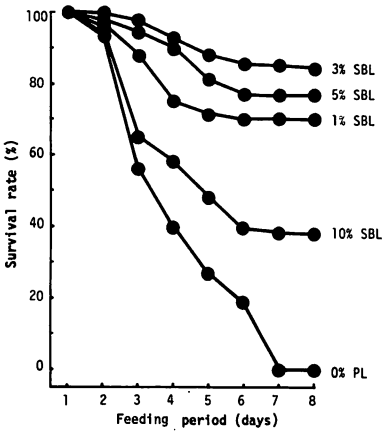

Fig. 1. Survival rates (%) of the prawn larvae receiving varying levels soybean lecithin (SBL) (diets 1, 3, 4, 5, and 6). 0% PL indicates a phospholipid-deficient diet (diet 2).

Fig. 3 shows the survival rates of the prawn larvae receiving diets with 3% level of various PL sources. High survival rates were obtained on the diets containing soybean PC (diet 9) and soybean PI (diet 8). Bonito-egg PC and soybean PE were also effective in improving the survival rates, but the survival rates on the diets

with these PL supplements were lower than that on the control diet. The previous study[3] showed that bonito-egg PC improved growth and survival of the prawn larvae than soybean PC when these PL classes were added to diets containing 7% PLO at a 1% level. Thus, there is apparent contradiction with the efficacy of supplemental bonito-egg PC between the present and previous[3] studies. However, our previous study[3] also showed that the survival rates of prawn larvae were reduced when 3% and 6% soybean PC were added to diets containing high levels (2%) of ω3-highly unsaturated fatty acids (HUFA). Therefore, it seems possible that the overdoses of some PL containing high levels of ω3-HUFA such as bonito-egg PC depress growth and survival due to unknown reasons; the adverse effects of excess ω3-HUFA as found in fish[16-18] and/or the increase in toxic oxidation products contaminating in PL sources. In both experimental and practical-type prawn diets, anyhow, it seems desirable to check the presence of oxidized lipids in PL supplements prior to use.

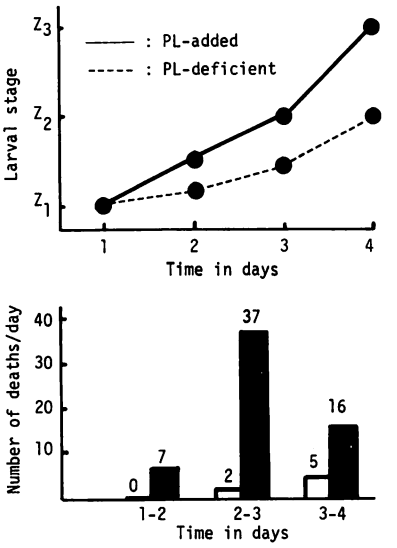

Fig. 2. Larval stages reached and number of daily deaths in the prawn larvae receiving phospholipid (PL)-added (diet 4) and PL-deficient (diet 2) diets. Open bar (□), PL-added diet (diet 4); closed bar (■), PL-deficient diet (diet 2).

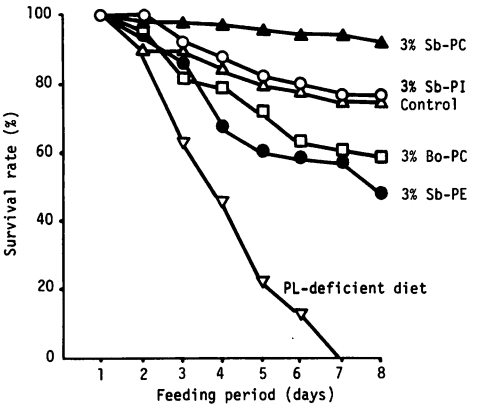

Fig. 3. Survival rates (%) of the prawn larvae receiving several soybean PL classes (Sb-PE, diet 7; Sb-PI, diet 8; Sb-PC, diet 9) and bonito-egg phosphatidylcholine (Bo-PC, diet 10) as supplemental PL sources.

Effects of Dietary PL on Body Lipid Compositions

Table 3 indicates the variation in lipid class concentrations (mg/g wet weight) of the prawn larvae receiving diets with 3% SBL or without supplemental PL during the larval development. The zoea$_3$ larvae receiving the PL-deficient diet had slightly lower concentrations of TL, NL, and PL than those receiving supplemental SBL (Table 3 and Fig. 4). Especially, steryl ester (SE), free sterol (FS), PC, PI, and PS were the prominent lipid classes, the concentrations of which were reduced on the feeding of PL-deficient diet. (Fig. 4 and 5). These results agree with those found in the prawn juveniles.[5]

Table 3. Variation in lipid class concentrations (mg/g wet wt.) of the prawn larvae receiving diets with 3% soybean lecithin (diet 4) and without supplemental phospholipid (diet 2)

Lipid class*	Nauplius	$Zoea_1$		$Zoea_2$		$Zoea_3$	
		Diet 4	Diet 2	Diet 4	Diet 2	Diet 4	Diet 2
TL	24.39	8.78	8.46	7.48	6.41	10.12	7.82
NL	16.22	6.10	5.73	5.82	4.79	7.68	6.54
PL	8.17	2.68	2.73	1.66	1.62	2.44	1.28
HC	2.68	0.78	0.58	0.82	0.64	0.89	0.95
SE	1.80	0.68	0.66	0.79	0.48	0.87	0.69
TG	6.23	1.34	0.58	1.02	1.10	1.48	1.38
FFA	1.44	0.88	0.58	0.68	0.73	1.04	0.96
DG	1.11	0.79	1.91	0.88	0.62	1.19	0.97
FS	1.95	0.89	0.68	0.82	0.54	1.06	0.75
MG	0.99	0.74	0.64	0.81	0.68	1.15	0.84
PE	1.75	0.76	0.89	0.32	0.50	0.50	0.38
PS	0.73	0.15	0.16	0.10	0.09	0.11	0.06
PI	1.02	0.35	0.32	0.16	0.14	0.16	0.07
PC	3.08	1.01	0.96	0.86	0.51	1.26	0.45
SM	0.49	0.13	0.13	0.07	0.14	0.08	0.10
LPC	0.20	0.06	0.06	0.02	0.09	0.10	0.09
UK	0.90	0.22	0.21	0.13	0.15	0.23	0.13

* TL, total lipid; NL, neutral lipid; PL, phospholipid; HC, hydrocarbon; SE, steryl ester; TG, triglyceride; FFA, free fatty acid; DG, diglyceride; FS, free sterol; MG, monoglyceride; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI; phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin; LPC, lysophosphatidylcholine; UK, unknown phospholipids.

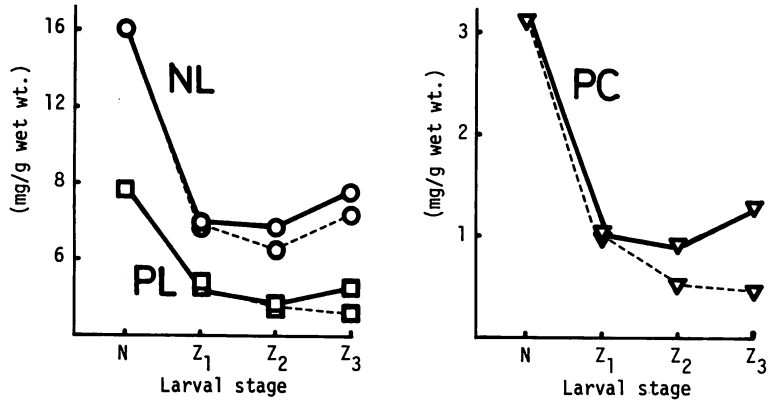

Fig. 4. Neutral lipid (NL), phospholipid (PL), and phosphatidylcholine (PC) concentrations (mg/g wet wt.) in the prawn larvae receivng PL-added and PL-deficient diets. Solid line (——), PL-added diet (diet 4); broken line (----), PL-deficient diet (diet 2).

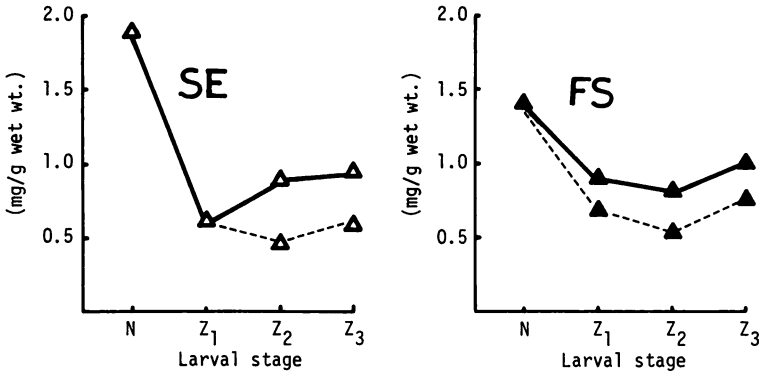

Fig. 5. Steryl ester(SE) and free sterol (FS) concentrations (mg/g wet wt.) of the prawn larvae receiving PL-added and PL-deficient diets. Solid line, PL-added diet; broken line, PL-deficient diet.

Table 4 shows the lipid class concentrations of the $zoea_2$ larvae receiving various kinds of 3% PL supplements. NL concentrations were almost similar each other among the diet groups irrespective of the kinds of supplemental PL examined. Whereas, the concentrations of PL such as PC seemed slightly higher in the larvae receiving soybean PC than in those receiving other PL classes. On the whole, however, lipid class concentrations were not markedly affected with the kind of supplemental PL.

The fatty acid composition of PL in the $zoea_2$ larvae were different with the kinds of dietary PL sources (Table 5). As shown in Table 2, soybean PE, soybean PI, and soybean PC contained linoleic acid (18 : 2ω6) (more than 50% of total fatty acids) and linolenic acid (18 : 3ω3, 6.2-8.2%) as the major essential fatty. acids. However, the feeding of

Table 4. Lipid class concentrations (mg/g wet wt.) of the prawn larvae ($zoea_2$ stage) receiving diets containing various phospholipids

Lipid class	Dietary phospholipid				
	Control	3% Sb-PE	3% Sb-PI	3% Sb-PC	3% Bo-PC
TL	21.0	18.9	19.3	22.8	23.8
NL	14.1	11.9	11.9	13.5	17.0
PL	6.9	7.0	7.4	9.3	6.8
PE	2.2	3.3	2.4	2.3	1.8
PS	0.4	0.1	0.5	0.5	0.4
PI	0.5	0.4	1.1	0.5	0.5
PC	2.8	2.0	2.6	3.6	3.1
SM	0.4	0.3	0.2	0.4	0.3
LPC	0.3	0.3	0.2	0.3	0.2
UK	0.3	0.4	0.7	1.7	0.5

Table 5. Fatty acid composition (%) of phospholipids in the prawn larvae ($zoea_2$ stage) receiving diets containing various phospholipids

Main fatty acid	Dietary phospholipid				
	Control	3% Sb-PE	3% Sb-PI	3% Sb-PC	3% Bo-PC
12 : 0	0.9	0.4	0.3	0.2	0.6
14 : 0	2.0	1.7	4.8	1.3	0.9
16 : 0	18.4	20.2	22.6	17.5	13.0
16 : 1	7.5	5.2	2.6	5.1	4.8
16 : 3ω3	2.4	1.2	1.4	1.5	1.0
18 : 1ω9	16.6	17.0	21.9	15.0	18.2
18 : 2ω6	2.6	4.8	3.0	2.0	5.8
18 : 3ω3	0.7	0.7	0.9	0.7	1.5
20 : 1ω9	3.6	2.9	2.3	3.8	1.3
20 : 2ω6	0.5	0.9	0.3	0.7	0.9
20 : 3ω9	0.3	3.0	0.5	1.3	4.8
20 : 4ω6	0.2	0.3	0.2	0.2	0.5
20 : 5ω3	8.9	17.0	9.1	14.4	17.1
22 : 1ω9	1.8	1.4	0.7	1.9	1.6
22 : 5ω3	1.5	0.5	1.2	1.7	1.3
22 : 6ω3	25.6	14.0	21.7	24.1	17.0

these supplemental PL at a 3% level did not result in the increase in ω6-HUFA proportions, suggesting no noticeable conversion of 18 : 2ω6 to ω6-HUFA such as 20 : 4ω6 in the prawn larvae. On the other hand, the proportions of palmitic acid (16 : 0) and ω3-HUFA such as icosapentaenoic acid (20 : 5ω3) and docosahexaenoic acid (22 : 6ω3) in the larvae varied with the kinds of supplemental PL. The sum of 20 : 5ω3 and 22 : 6ω3 proportions of body PL was the highest on the diet with soybean PC and the lowest on the diet with soybean PI. These results suggest that some dietary PL are necessary for the effective formation of specific PL classes in the prawn larvae.

Discussion

The present study confirmed the previous findings that the prawn larvae[3] require dietary sources of about 3% levels of some PL such as soybean PC and soybean PI for growth and survival as also found in the juveniles.[5] By tracer experiments using radioactive tripalmitin[10] and cholesterol,[11] we demonstrated that dietary PL such as SBL enhanced the transport of lipids, especially cholesterol, from the hepatopancreas to the extrahepatic tissues through the hemolymph. D'ABRAMO *et al.*[19] also revealed that the serum cholesterol and PL levels of purebred (*H. americanus*) or hybrid (*H. americanus* x *Homarus gammarus*) lobsters were decreased when they were fed a purified casein diet without supplemental PC. But, they have suggested that PC are required as dietary nutrients due to other functions except the efficient lipid emulsification and digestion.[19] Later, D'ABRAMO *et al.*[20] proved that

the transport rates of dietary [^{3}H] cholesterol out of the midgut gland (hepatopancreas) and into the hemolymph were reduced in the absence of PC in diets. The present study indicated that the feeding of PL-deficient diet resulted in the reduction of SE, FS, PC, and PI concentrations in the whole body of prawn larvae. This offers additional evidence for the hypothesis[11] that the PL requirement of prawns is related to the efficient transport of dietary lipids such as cholesterol.

On the other hand, HILTON *et al.*[21] showed by a 12 week feeding trial that the addition of soybean lecithin to a semi-purified diet containing casein-gelatin-wheat gluten (8 : 1 : 1) gave no effect on the weight gain and mortality rates of the freshwater prawn *Machrobrachium rosenbergii*. They have suggested that this prawn does not require dietary PL or necessitate extremely small amounts (less than 1% in diets) of PC for growth and survival even if there is a requirement for PC. Interestingly, KEAN *et al.*[22] pointed out that the growth and survival of juvenile lobsters *H. americanus* were not significantly affected with dietary PC at any level of dietary cholesterol (0.00, 0.25, 0.50, or 1.00%) when they were reared with diets containing 50% purified protein derived from a whole rock carb *Cancer irroratus*. But, they observed that the increase in dietary PC tended to enhance the weight gain in the case of diets containing 0.25% and 0.5% levels of cholesterol, in contrast to the case of 1.0% cholesterol-containing diets. This indicates the possibility of some interaction on a growth-enhancing effect between dietary cholesterol and PC levels. KEAN *et al.*[22] have suggested that the physiological effects of dietary PC in a casein diet may be related to sparing of some particular amino acid deficiency. These investigations[21,22] foresee the possibilities that the dietary PL requirements are variable with crustacean species and/or the kinds of dietary protein sources.

There are lines of evidence that the lipid transport in crustacean is operated in a different manner[23-28] to that in higher animals. In crustaceans, it is likely that lipid transport is principally carried out as a form of lipoproteins containing abundance of PL.[28] We assumes that the prawn does not synthesize sufficiently some specific PC-containing lipoproteins responsible for the transport of cholesterol and other lipids nevertheless crustaceans such as the lobster *H. americanus*,[29] the brine shrimp *A. salina*,[30] the crab *Carcinus maenas*,[31] and the prawn *P. japonicus*[24,32] have been shown to be capable *de novo* phospholipid synthesis. This assumption is required to be verified by more detailed biochemical studies. Obiously, further work is also necessary for the clarification of nutritional role of dietary PL in crustaceans.

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