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Requirements of the Larval Prawn, *Penaeus japonicus*, for Cholesterol and Soybean Phospholipids

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Abstract

The present study is planned to examine the requirements of cholesterol for the prawn, *Penaeus japonicus*, larvae in relation to the dietary levels of soybean phospholipids (PL). The experimental groups were designed as 2-way layout experiment (4 x 4 type): factor A, cholesterol content (%) in diets; factor B, soybean PL content (%) in diets. The zoeal larvae of *P. japonicus* were reared with 16 defined diets containing carrageenan as a binder for 7 days. The effects of cholesterol in improving growth and survival of *P. japonicus* larvae seemed not to be affected by the dietary levels of soybean PL. The optimum levels of cholesterol and soybean PL for *P. japonicus* larvae were estimated to be 1.0% and 3.0%, respectively, when pollack liver oil was used as basal lipid sources.

Although much information is available for the nutritional requirements of juvenile crustaceans (NEW, 1976, 1980; KANAZAWA, 1980, 1982), the knowledge of nutritional requirements of larval prawns and shrimps is scanty presumably due to the difficulty in rearing them with artificial, defined diets. In the previous studies, we have successfully reared the larval prawn, *Penaeus japonicus*, from zoeal stages to post-larvae with the purified diets containing carrageenan as a binder (carrageenan MBD) (TESHIMA *et al.*, 1982a), indicating the necessity of cholesterol or other sterols in diets for growth and survival (TESHIMA *et al.*, 1982b).

On the other hand, KANAZAWA *et al.* (1979) have shown that growth of *P. japonicus* juveniles were enhanced by the addition of phospholipids from the short-necked clam, *Tapes philippinarum*, suggesting certain effects of the molecular form of phospholipids. The inclusion of phospholipids in the diets has also been demonstrated to be essential for survival of the American lobster, *Homarus americanus*, juveniles (CONKLIN *et al.*, 1980; D'ABRAMO *et al.*, 1981). However, little is known with the mechanism by which dietary phospholipids play an important role as essential nutrients for crustaceans. LESTER *et al.* (1975) have shown that cholesterol solubilization was increased by the adition of lecithin to N-(N-dodecanoylsarcosyl) taurine, which is a representative emulsifier in the gastric juice of crabs (VAN DEN OORD *et al.*, 1964, 1965). The above information suspects that the optimum cholesterol levels in diets for crustaceans may be variable

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with dietary phospholipids levels, because micellar solubilization is possibly essential for effective assimilation of dietary cholesterol. The lack of sufficient amounts of phospholipids in diets may impare the growth-promoting effect of cholesterol on crustaceans. In the present study, therefore, we intend to examine the requirements of cholesterol for *P. japonicus* larvae in connection with dietary phospholipid levels. This paper deals with these results and discussion.

Materials and Methods

P. japonicus nauplii were obtained from 6 egg-bearing females in October, 1981, and raised to zoea$_1$ stage in a round polycarbonate tank (500 liters) at 27-28℃. Zoea$_1$ larvae were divided into lots of 100 larvae in 1-liter beakers containing 800 m*l* of the sea water (specific gravity, 1.027) filtered through a column of cotton for feeding trials using the test diets. The experimental groups were designed for 2-way layout experiment (4 x 4 type) of the following factors. Factor A: cholesterol content (%) in diets. Levels: A_1, cholesterol-free; A_2, 0.5% cholesterol; A_3, 1.0% cholesterol; A_4, 5.0% cholesterol. Factor B: soybean phosphatidylcholine (PC) content (%) in diets. Levels: B_1, phospholipid-free; B_2, 1.0% soybean PC; B_3, 3.0% soybean PC; B_4, 6.0% soybean PC. Table 1 shows experimental groups and the test diets used in this study. All test

Table 1. Composition of the test diets for *P. japonicus* larvae

Group (Diet*1)	Ingredient (%)				
	Cholesterol	Soybean PC*2	PLO*3	Cellulose	Other ingredients*4
1	0	0	9	8.4	82.6
2	0	1	8	8.4	82.6
3	0	3	6	8.4	82.6
4	0	6	3	8.4	82.6
5	0.5	0	9	7.9	82.6
6	0.5	1	8	7.9	82.6
7	0.5	3	6	7.9	82.6
8	0.5	6	3	7.9	82.6
9	1.0	0	9	7.4	82.6
10	1.0	1	8	7.4	82.6
11	1.0	3	6	7.4	82.6
12	1.0	6	3	7.4	82.6
13	5.0	0	9	3.4	82.6
14	5.0	1	8	3.4	82.6
15	5.0	3	6	3.4	82.6
16	5.0	6	3	3.4	82.6

*1 All diets are carrageenan MBD: 5.0% carrageenan was added to 100g of dry diets.
*2 Soybean phosphatidylcholine (Nakarai Chemicals Co., Japan) was the mixture of phosphatidylcholine (23.6%), phosphatidylethanolamine (30.4%), phosphatidylinositol (18.1%), and other polar lipids.
*3 Pollack liver oil (Riken Vitamin Co., Japan).
*4 Other ingredients (% of dry diet): casein 50.0, glucose 5.5, sucrose 10.0, α-starch 4.0, glucosamine HCl 0.8, sodium succinate 0.3, vitamins 3.2, minerals 8.5.

diets were carrageenan MBD (TESHIMA, *et al.*, 1982a) with particulate sizes of 50 μm (for zoeal larvae) and 50-125 μm (for mysis larvae). The composition of the basal ration was essentially the same as that used in the previous study (KANAZAWA *et al.*, 1982).

The feeding and rearing methods for *P. japonicus* larvae were similar to those described previously (JONES *et al.*, 1979; KANAZAWA *et al.*, 1982). The prawn larvae were fed the test diets at the concentration of 0.08 mg/larva twice per day (HIRATA *et al.*, 1975). The developmental stages of larvae (HUDINAGA, 1942) were determined microscopically on 10 random samples every day as a parameter of growth. The growth index adopted in this paper is as follows: 1, zoea$_1$ (Z_1); 2, zoea$_2$ (Z_2); 3, zoea$_3$ (Z_3); 4, mysis$_1$ (M_1); 5, mysis$_2$ (M_2); 6, mysis$_3$ (M_3); 7, post-larva$_1$ (P_1). Survival rates were also measured daily in a similar manner to that of growth indexes. All alive larvae were counted and staged at the end of the feeding trials when one of the experimental groups of larvae reached P_1 stage. Statistical analysis of the data on growth index and survival rate was performed according to ISHIKAWA *et al.* (1967).

Results

Tables 2 and 3 show the survival rates and growth indexes of *P. japonicus* larvae fed the test diets containing varying levels of cholesterol (0, 0.5, 1.0, and 5.0% in diets) and soybean PC (0, 1.0, 3.0, and 6.0% in diets). The prawn larvae had low survival rates when fed the test diets free

Table 2. Survival rate (%) of the prawn larvae reared with the test diets for 7 days

Factor B: soybean PC (%) in diets	Survival rate (%)*1			
	Factor A: cholesterol (%) in diets			
	A_1 (0%)	A_2 (0.5%)	A_3 (1.0%)	A_4 (5.0%)
B_1 (0%)	13	21	25	35
B_2 (1.0%)	25	32	69	42
B_3 (3.0%)	32	64	72	55
B_4 (6.0%)	26	59	80	68

*1 Zoea$_1$ larvae were reared with the test diets for 7 days.

from cholesterol and/or soybean PC as observed in the previous studies (KANAZAWA *et al.*, 1982; TESHIMA *et al.*, 1982b). The analysis of variance of the data on survival rates (Table 4) showed that both factors A and B are significant ($P<0.01$), indicating that the survival rates of *P. japonicus* larvae are affected by the dietary levels of cholesterol and soybean PC. Although the present experiment does not give a statistically reliable answer for the interaction between factors A and B, the effect of dietary cholesterol on the survival of *P. japonicus* larvae seemed not to vary with the soybean PC levels in the diets (Table 2). Hence, the population means of survival rates were infered providing that there is no interaction between factors A and B. Fig. 1 shows the

estimated population means and confidence limits (P=0.95) of survival rates at 4 levels of factors A and B. The population means of survival rates were significantly ($P<0.01$ or 0.01) different between the pairs of following levels: A_1-A_2, A_1-A_3, A_1-A_4, A_2-A_3, B_1-B_2, B_1-B_3, B_1-B_4, and B_2-B_4. No significant difference ($P>0.05$) was observed between the levels A_2-A_4, A_3-A_4, B_2-B_3, and B_2-B_4. As shown in Fig. 1, the survival rates of *P. japonicus* larvae were increased with increasing levels of cholesterol from 0 to 1.0% and soybean PC from 0 to 3.0% in the diets, but the supplement of large amounts of cholesterol (5.0%) or soybean PC (6.0%) did not result in further improvement of survival rates. In terms of survival rates, therefore, the optimum levels of cholesterol and soybean PC for *P. japonicus* larvae were estimated to be about 1.0% and 3.0% in the diets, respectively; the population mean and confidence limits of the survival rate were infered to be 79 ± 18% (P=0.95) when the prawn larvae fed the diet containing 1.0% cholesterol, 3.0% soybean PC, and 6.0% PLO as lipid sources.

Table 3. Growth index of the prawn larvae reared with the test diets for 7 days

Factor B: soybean PC (%) in diets	Growth index			
	Factor A: cholesterol (%) in diets			
	A_1 (0%)	A_2 (0.5%)	A_3 (1.0%)	A_4 (5.0%)
B_1 (0%)	4.0	4.5	5.0	5.0
B_2 (1.0%)	5.0	6.7	7.0	6.2
B_3 (3.0%)	5.5	7.0	7.0	6.5
B_4 (6.0%)	5.5	6.9	6.9	6.3

Table 4. Analysis of variance on the data of survival rates and growth indexes

Data	Factor	Sum of squares		df	V	F_0
Survival rate	A	S_A	2956.8	3	985.6	9.66**
	B	S_B	3049.3	3	1016.4	9.96**
	Error	S_E	917.8	9	102.0	
Growth index	A	S_A	5.14	3	1.71	15.5**
	B	S_B	9.34	3	3.11	28.2**
	Error	S_E	0.98	9	0.11	

Asterisks (**): $P<0.01$

Regarding growth indexes, factors A and B were also highly significant ($P<0.01$) by the analysis of variance. Fig. 1 shows the estimated population means and confidence limits of growth indexes at 4 levels of factors A and B. The population means of growth indexes were significantly different between the levels of A_1-A_2 ($P<0.01$) and B_1-B_2 ($P<0.01$), but not between the pairs of other levels. Thus, growth of the prawn larvae was shown to be improved by the

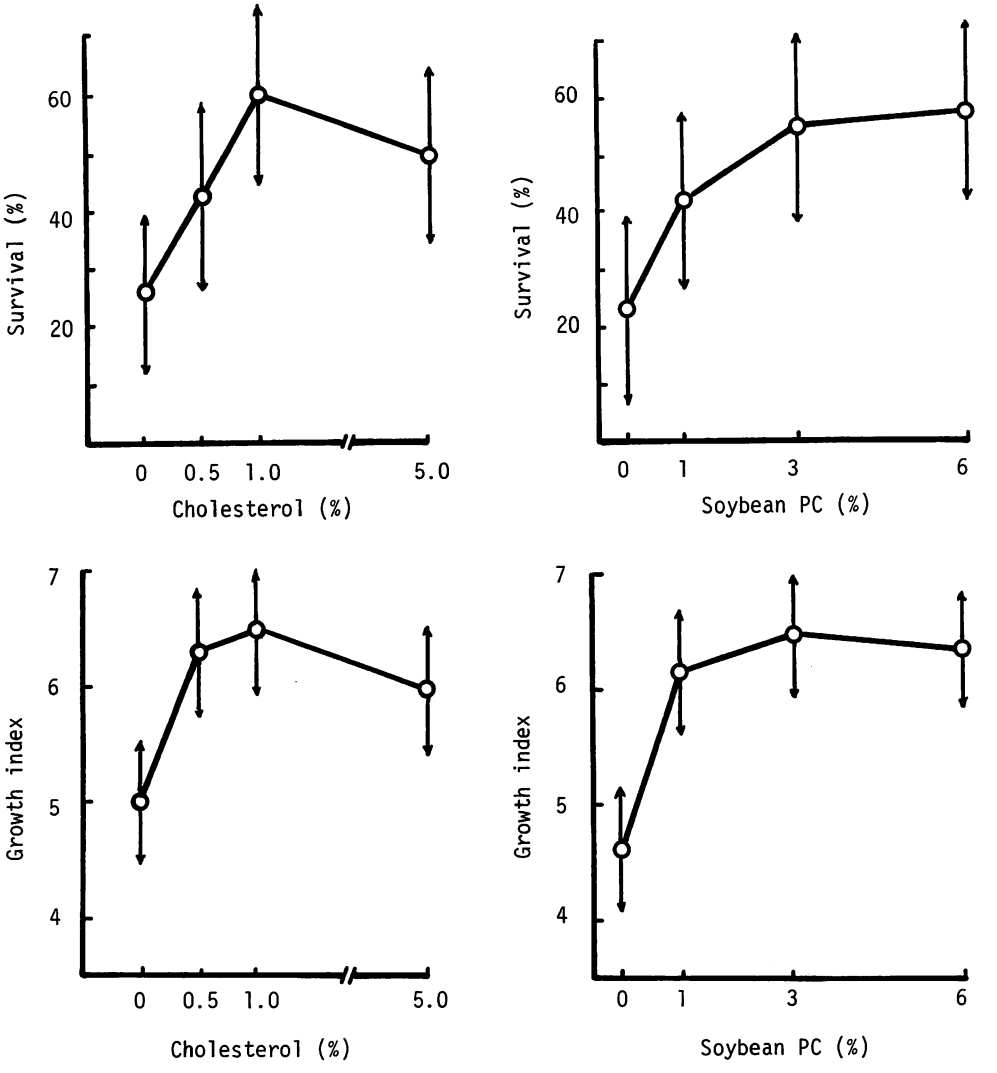

Fig. 1. Effects of cholesterol and soybean PC levels in the diets on growth index and survival rate of *P. japonicus* larvae. Growth indexes 4, 5, 6, and 7 correspond M_1, M_2, M_3, and P_1 stages, respectively.
←→: confidence limits (P=0.95).

addition of cholesterol and soybean PC. But, the increase of cholesterol from 0.5 to 5.0% in the diets did not result in further improvement of growth. The increaease of of soybean PC levels from 1.0 to 6.0% did not enhance further growth of *P. japonicus* larvae.

In terms of both growth and survival, *P. japonicus* larvae were shown to require 1.0% cholesterol and 3.0% soybean PC when the diet contained 6.0% PLO as other lipid sources.

Discussion

In the previous study (TESHIMA *et al.*, 1982b), we have shown that *P. japonicus* larvae require sterols for growth and survival as well as the juveniles (KANAZAWA *et al.*, 1971). As for the juvenile crustaceans, the optimum cholesterol levels have been reported to be 0.2% (SHUDO *et al.*, 1971), 0.5% (KANAZAWA *et al.*, 1971), and 2.0% (DESHIMARU and KUROKI, 1974) for *P. japonicus* and 0.5% for *H. americanus* (CASTELL *et al.*, 1975). However, the results of SHUDO *et al.* (1971) should be critisized somewhat because the diet ingredients such as a squid meal probably contained cholesterol. Recently, phospholipids have also been demonstrated to be indispensable diet ingredients for *P. japonicus* larvae (KANAZAWA, 1983; KANAZAWA *et al.*, 1982) and *H. americanus* juveniles (CONKLIN *et al.*, 1980; D'ABRAMO *et al.*, 1981). These informations assume that cholesterol and phospholipids may interact in improving growth and survival of the above crustaceans, because phospholipids possibly play an important role in the intestinal absorption of cholesterol (LESTER *et al.*, 1975) and also cholesterol transport in the hemolymph (TESHIMA and KANAZAWA, 1980a, 1980b, 1980c).

The results of the present study suggest that the effectiveness of cholesterol in improving growth and survival of *P. japonicus* larvae is unlikely to be affected by the dietary levels of phospholipids such as soybean PC. The effects of soybean PC on the prawn larvae also seemed not to vary with the dietary cholesterol levels. Under the experimental conditions adopted in this study, the optimum levels of cholesterol and soybean PC were determined to be 1.0% and 3.0% in the diets, respectively. The information available has shown that the efficacy of phospholipids in improving growth and/or survival of *P. japonicus* larvae (KANAZAWA *et al.*, 1982) and *H. americanus* juveniles (D'ABRAMO *et al.*, 1981) was variable with the kinds and sources of phospholipids, suggesting the high efficacy of phosphatidylcholine and/or phosphatidylinositol which contain $\omega 3^-$ and $\omega 6^-$ fatty acids as constituting fatty acids. The soybean PC used in the present study was the mixture of phosphatidylcholine (23.6%), phosphatidylethanolamine (30.4%), phosphatidylinositol (18.1%), and other polar lipids.

Considering the above facts, we think that the optimum phospholipid levels in diets for *P. japonicus* and other crustacean larvae should be evaluated in connection with the kinds of phospholipids used and coexistent other lipids. The feeding experiments using pure phospholipids containing limited fatty acids will give a clear answer for the requirements of dietary phospholipids.

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