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Microparticulate feeds for Penaeid larvae

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Abstract. — *The mass production of penaeid larvae still depends on live foods such as diatoms, **Chlorella** and **Artemia**. However, it requires much facilities, maintenance expenses, and labor to produce a desired amount of live foods safely and constantly. Also, the nutritive value of planktonic organisms is occasionally variable and this makes the use of live food for mass culture restrictive. Therefore, it is necessary to develop microparticulate diets as a substitute for live foods to further increase the productivity of seed for shrimp culture.*

Several types of microparticulate diets, micro-encapsulated diet, microbounded diet, and micro-coated diet, were prepared and their dietary values for the larvae of shrimp were evaluated. Micro-bound diet containing kappa-carrageenan as a binder supported the high growth and survival rates of shrimp larvae from Zoea 1 to post-larva 1.

Protein resources having a high nutritional value such as krill meal, squid meal, fish meal, scallop meal, short-necked clam extract, chicken egg, casein, soybean meal, and yeast are used for microparticulate diets of larval shrimp.

*Recently, the mass seed production of **Penaeus japonicus** was carried out by using microparticulate diets. As a result, 11,911,000 postlarvae (survival rate of 96 %) were produced in a 400 ton tank.*

MICROPARTICULATE DIETS

The mass production of penaeid larvae still depends on live foods such as diatoms, *Chlorella* and *Artemia*.

However, it requires much facilities, maintenance expenses, and labor to produce a desired amount of live foods safely and constantly. Also, the nutritive value of planktonic organisms is occasionally variable and this makes the use of live food for mass culture restrictive. Therefore, it is necessary to develop microparticulate diets as a substitute for live foods to further increase the productivity of healthy seed for shrimp culture.

The various types of microparticulate diets reported are categorized into three groups, micro-encapsulated diet, micro-bound diet and micro-coated (Kanazawa and Teshima, 1983; Kanazawa, 1985a; Kanazawa, 1986a; Kanazawa and Teshima, 1988). Micro-encapsulating a solution, colloid or suspension of diet ingredients with a membrane. Micro-coated diets are prepared by coating micro-bound diet with some materials such as zein or cholesterol-lecithin. The details of the procedures for preparation of microparticulate diets are described elsewhere (Teshima et *al.*, 1982; Kanazawa and Teshima, 1983; Kanazawa, 1985b).

Table 1. Composition of Carrageenan micro-bound diet.

INGREDIENT	(g/100g)
Skim milk	52.0
Chicken egg yolk (dry)	10.0
Egg albumin	20.0
Amino acid mixture	5.0
Pollack liver oil	5.5
Soybean lecithin	1.5
Mineral mixture	1.0
Vitamin mixture	5.0
TOTAL	100.0
Kappa-Carrageenan	5.0

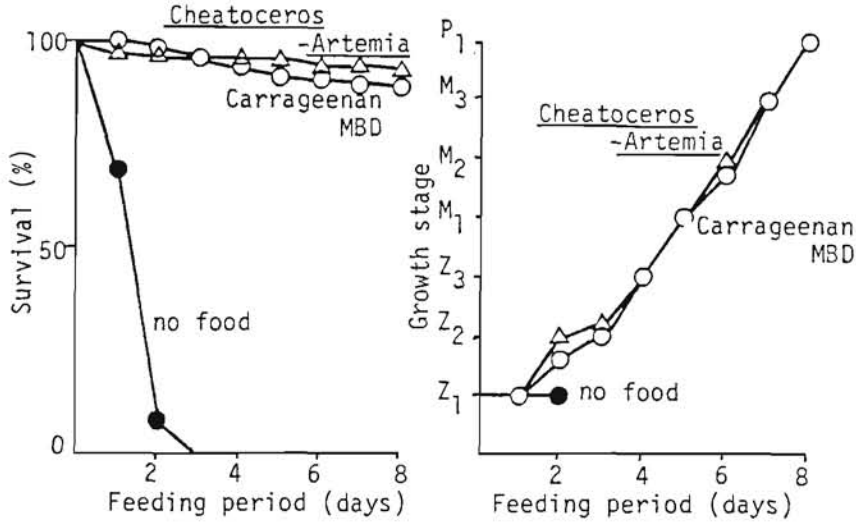

Fig. 1. — Survival and growth of larval prawn fed on micro-bound diet.

The nutritional components of the microparticulate diets for shrimp larvae should be determined on the basis of the requirements of the larval shrimp for protein, amino acid, lipid, carbohydrate, vitamin and minerals. However, as the requirements of the larval shrimp are still undefined, protein resources having a high nutritional value such as krill meal, squid

meal, scallop meal, short-necked clam, chicken-egg, skim milk, casein and fish meal are used (Kanazawa, 1985c). Micro-bound diet containing Kappacarrageenan as a binder supported the high growth and survival rates of prawn, *Penaeus Japonicus*, larvae from zoea$_1$ to postlarva$_1$ (Table 1 and Fig. 1) (Kanazawa, 1985b).

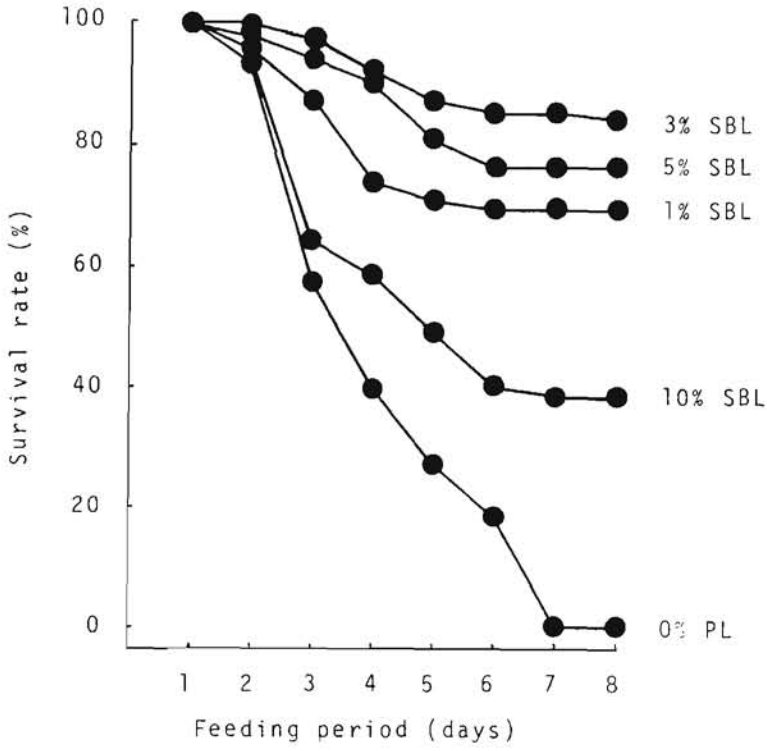

Fig. 2. — Survival rates (%) of the prawn larvae receiving varying levels of soybean lecithin (SBL). O % PL indicates a phospholipid-deficient diet.

PHOSPHOLIPID REQUIREMENTS

Some phospholipids in diets have also been demonstrated to be indispensable for su staining growth and survival of crustaceans such as the prawn (Kanazawa, 1983a, B; Kanazawa, 1984; Teshima, 1985; Teshima and Kanazawa, 1988) and the American lobster (Conklin et *al.*, 1980; D'Abramo et *al.*, 1981).

Feeding trials using the prawn, *P. Japonicus*, larvae were conducted to examine the effects of several dietary phospholipids on the growth, survival, and body lipid composition (Teshima et *al.*, 1986b). The deficiency in dietary phospholipids 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, the highest survival rates were obtained on diets with 3 % soybean lecithin (Fig. 2). Soybean

phosphatidylcholine and soybean phosphatidylinositol showed a higher nutritive value than bonito egg phosphatidylcholine and soybean phosphatidylethanolamine at a 3 % supplemental level (Fig. 3). The deficiency in dietary phospholipids resulted in a slight decrease in the concentrations of steryl esters, free sterols, phosphatidylcholine, and phosphatidylinositol in the bodies. The concentrations of phospholipids such as phosphatidylcholine seemed slightly higher in the prawn larvae receiving supplemental soybean phosphatidylcholine than other supplemental phospholipids such as soybean phosphatidylinositol, soybean phosphatidylethanolamine and bonito-egg phosphatidylcholine.

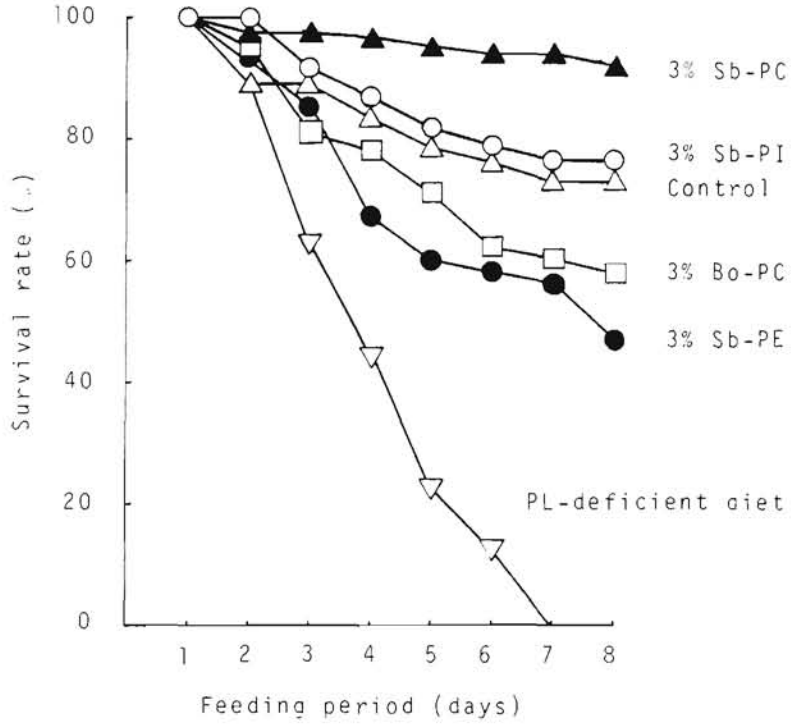

Fig. 3. — Survival rates (%) of the prawn larvae receiving several phospholipid sources Control : live feed (*Chactocero + Artemia*).

However, the body lipid compositions, on the whole, were not variable notably with the kinds of supplemental phospholipids examined. Also, the sum of 20 : 5W3 and 22 : 6W3 proportions of body phospholipids was slightly higher in the prawn larvae receiving soybean phosphatidylcholine rather than in those receiving other supplemental phospholipids.

The effects of dietary phospholipids on weight gain, retention of dietary lipids and body composition of the prawn juvenile were examined (Teshima et *al.*, 1986a).

The prawn *P. Japonicus* was reared with diets containing 3 % soybean lecithin (diet A) and no supplemental phospholipids (diet D) for 30 days. The deficiency of phospholipid in diets significantly reduced the weight

gain and feed conversion efficiency. The prawns receiving diet D without supplemental phospholipid contained a lower concentration of phospholipids such as phosphatidylcholine and phosphatidylinositol in the whole body than the ones receiving diet A with supplemental phospholipid. The retention (%) of dietary lipids, especially cholesterol, in the body was also significantly lower in the prawns receiving diet D than in those receiving diet A. These data indicate that the juvenile prawn requires dietary sources of phospholipid for good growth; suggesting that the dietaryphospholipid may be necessary for the effective utilization of lipids such as triglycerides and cholesterol in the diets by the prawn. However, it was not clear why dietary phospholipids exerted a growth-enhancing effect in the prawn *P. japonicus*. To confirm this, the postprandial variation in radioactive lipid classes was investigated after feeding of tripalmitin ^{-14}C and cholesterol ^{-14}C with or without dietary phospholipids.

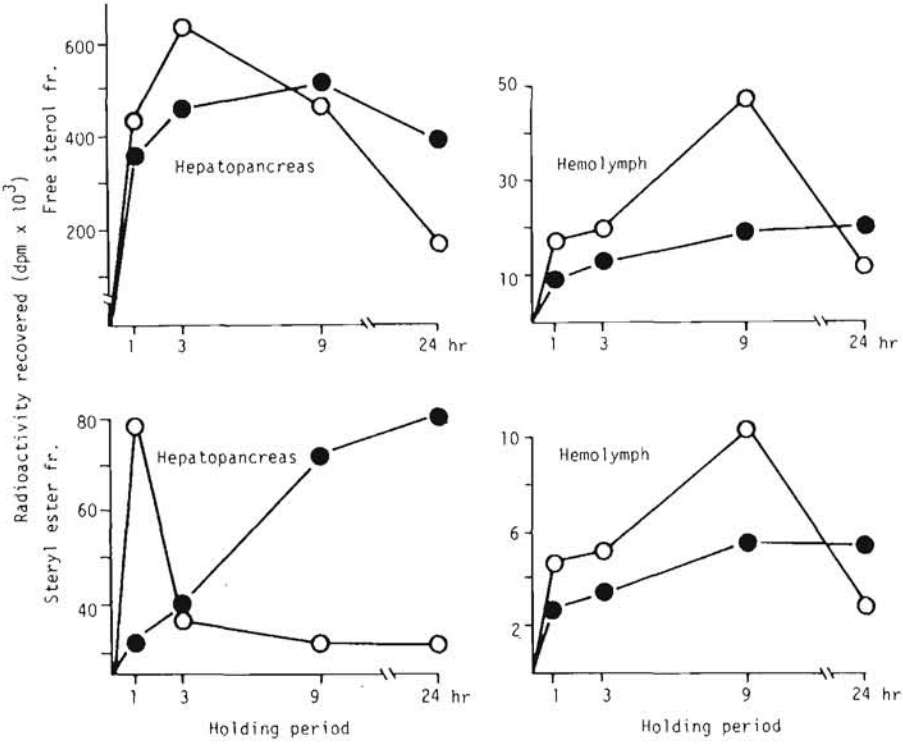

Fig. 4. — Postprandial incorporation of radioactivity into free sterol and esterified sterol fractions of the hepatopancreas and hemolymph in *P. japonicus* prawns after feeding of cholesterol-4-14 C in (0) phospholipid-added diets and (0) phospholipid-deficient diets.

The effects of supplemental phospholipids on the assimilation and transport of tripalmitin ^{-14}C in relation to the nutritional role of phospholipids in diets of the prawn *P. Japonicus* were examined (Teshima et *al.*, 1986c). The prawns were fed on the diets containing tripalmitin ^{-14}C with 3 % soybean lecithin (diet A) and without supplemental phospholipid (diet D) and then the incorporation of radioactivity into the organs and

tissues was examined after 1, 3 and 6 h of feeding. When the prawns received tripalmitin ^{-14}C, the inclusion of phospholipids (3 % soybean lecithin) in the diets resulted in an increase in radioactive phospholipids, especially phosphatidylcholine, in both the hepatopancreas and hemolymph. The supplementation of phospholipids to diets also brought on an increase in radioactive triglycerides in the hepatopancreas but not in the hemolymph.

Cholesterol ^{-14}C was orally administrated to the prawn to clarify the effects of dietary phospholipids on the mobilization of sterol in diets to various organs and tissues (Teshima et *al.*, 1986d). The prawns were fed on the test diets with 3 % soybean lecithin and without supplemental soybean lecithin and then dissected 1, 3, 9 and 24 h after feeding (Fig. 4). When fed the phospholipid added diet, the radioactivities of hepatopancreatic free and esterified sterols increased quickly and reached maximum levels 3 and 1 hours after feeding, respectively and then decreased, while those of free and esterified sterols in the hemolymph increased with the lapse of time and reached maximum level 9 hours after feeding. Thus, when fed the phospholipid-deficient diet, dietary cholesterol remained as a free sterol for a long time in the hepatopancreas and entered into the hemolymph slowly, and also the formation of cholesterol esters in the hepatopancreas proceeded at a slow rate. These results suggest that dietary phospholipids such as soybean lecithin contributes to the smooth mobilization of dietary cholesterol in the body especially from the hepatopancreas to the hemolymph. Growth of the prawns receiving the phospholipid-deficient diet was conceived to be retarded owing to the insufficient transport of dietary cholesterol rather than triglycerides in the body.

VITAMIN REQUIREMENTS

Kanazawa (1986b) have examined the requirements of larval *P. japonicus* for various vitamins by using microparticulate diets with carrageenan as a binder. As a result, the prawn larvae were found to require i-Carotene, thiamine, riboflavin, pyridoxine, nicotinic acid, folic acid, biotin, cyanocobalamin, choline, inositol, ascorbic acid, vitamin D and vitamin E. The shortage of one of these vitamins resulted in the cessation or retardation of metamorphosis and in high mortality during larval development.

The quantitative requirements of larval prawn for several vitamins have been done. The requirements of vitamin are as Table 2 : Thiamine-HCl. 4mg %; riboflavin, 8 mg %; pyridoxine-HCl, 12 mg %; nicotinic acid, 40 mg %; biotin, 0,2 mg %; choline chloride, 600 mg %; inositol, 200 mg %; Na-ascorbic acid, 1 000 mg %; vitamin E (tocopherol), 20 mg %. The requirements for some vitamins were apparently higher for *P. japonicus* larvae than for juvenile. It is conceivable, however, that some vitamins may have leached into the water before eating. This means that the vitamin requirements of larval prawn mentioned above should be regarded as « practical demand for rearing of the larvae ».

Table 2. — Vitamin requirements of larval prawn. *P. japonicus.*

VITAMIN	mg/100g of DRY DIET
Thiamine-HCl	4
Riboflavin	8
Pyridoxine-HCl	12
Nicotinic acid	40
Biotin	0.2
Choline chloride	600
Inositol	200
Na-Ascorbate	1 000
Tocopherol	20

MASS SEED PRODUCTION OF PRAWN WITH MICRO-BOUND DIET

We have attempted to rear larval prawn, *P. japonicus* with microparticulated diets. As a result, seed production of prawn was successfully achieved by partial or even complete substitution of microparticulate diets for live food.

Larval stage used	$Zoea_1$ stage
Number of larvae	410 000
Experimental period	16 days
Tank	15 ton
Water temperature	26.8 ± 1.5 °C
Feeding level	$Zoea_1$-$Zoea_3$: 0.16 mg/larva/day $Mysis_1$-$Mysis_3$: 0,20 mg/larva/day $Postlarva_1$-$Postlarva_3$: 0.24 mg/larva/day > $Postlarva_4$: 0.3 mg/larva/day
Feeding frequency	< $Postlarva_4$: 4 times/day > $Postlarva_5$: 5 times/day

Table 3. — Rearing and feeding methods of larval prawn.

Experiment I

In experiment I, 410,000 $zoea_1$ larvae were kept in a 15 ton tank. The feeding experiments were carried out under the conditions listed in table 3 (Kanazawa, 1985b). Kappa-Carrageenan micro-bound diet was used in this seed production. Control group of larvae was fed on diatom *Chaetoceros gracilis* until $mysis_2$, on *Artemia salina* until $postlarva_5$, and then on commercial diet until $postlarva_8$. From $zoea_1$ stage, the larval prawn reached $postlarva_8$ using only kappa-carrageenan micro-bound diet. As a result, 307, 500 postlarvae (survival rate of 75 %) were produced (Fig. 5 and 6). In the control group of live food, diatoms did not grow owing to the rain and the larvae almost died at $mysis_1$ stage.

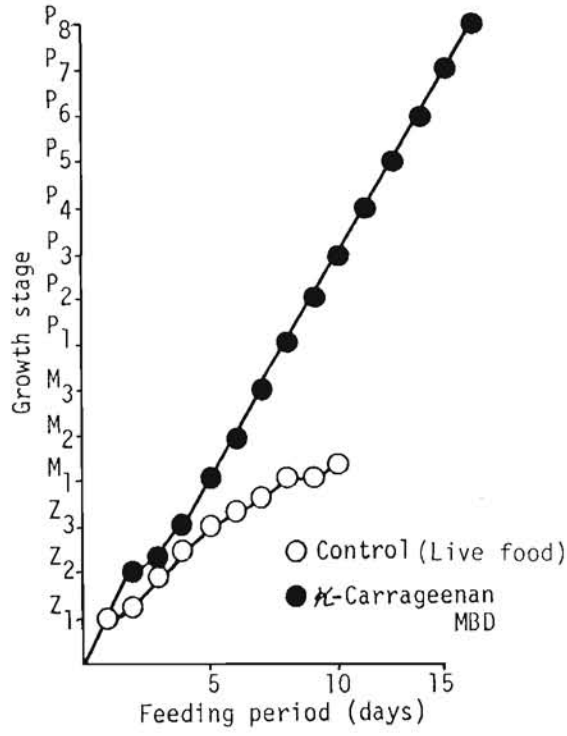

Fig. 5. — Growth stage of larval prawn fed on micro-bound diet.

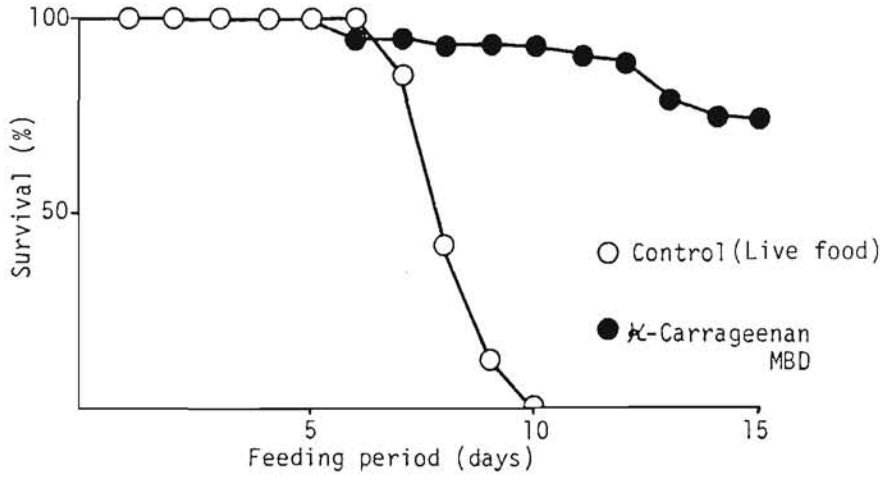

Fig. 6. — Survival rate of larval prawn fed on micro-bound diet.

Experiment II

12,400,000 larvae of *P. japonicus* were reared from zoea1 stage to postlarva10 stage with a mixture of microparticulate diet and live food in 400 ton tank. As a result, 11, 911,000 postlarvae (survival rate of 96 %) were produced.

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