Culture of the Prawn Larvae with Micro-Particulate Diets

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Several types of micro-particulate diets were prepared and their dietary values for the larvae of a prawn *Penaeus japonicus* were evaluated. Some microencapsulated diets with nylon-protein membranes (Nylon-MED) and with zein-membranes (Zein-MED) supported the growth of prawn larvae from zoea₁ to post-larvae₁. In terms of both survival and growth rates, however, the powdered diets with zein as a coating (Zein-MCD) gave better results than the Nylon-MED and Zein-MED. The dietary value of the Zein-MCD containing a defined diet was almost comparable to those of live feeds, *Chaetoceros gracilis* and *Artemia salina*; the prawn which received the Zein-MCD grew up from zoea₁ to post-larvae₁ in 8 days with a survival rate of 94%.

The seedling production of the prawn *Penaeus japonicus* has been depending on a live food such as the diatoms and *Artemia salina*.¹⁻⁴⁾ The mass culture of planktonic organisms not only requires lots of manual help and expensive equipments but also fluctuates according to weather conditions, etc. In the previous studies, therefore, we have attempted to rear the larval prawns with micro-particulate diets (MD).^{5,6)} In the present study, several types of MD were further prepared and their dietary values for the larval prawns were examined by the feeding trials. This paper deals with these results, indicating the success in rearing the prawn larvae from zoea₁ to postlarvae (P₁) with the MD comprising all chemically known compounds.

Materials and Methods

Preparation of Diets

Table 1 shows the composition of diets examined in this study. All ingredients or compounded diets were powdered into fine particles (<10-20 μ m diameters) prior to the preparation of MD. Diets 1, 2 and 3 are the diets encapsulated with a nylon-protein membrane (Nylon-MED) which were prepared by the interfacial polymerisation procedure of CHANG *et al.*⁷⁾ as described in the previous papers.⁵⁾ The composition of artificial diet, diet A, was the same as reported previously⁸⁾ and contained the following

ingredients (g/100 g dry diet): glucose 5.5, sucrose 10, α -starch 4.0, glucosamine-HCl 0.8, casein 50, pollack liver oil 6.0, cholesterol 1.0, sodium citrate 0.3, sodium succinate 0.3, minerals 8.52, vitamins 3.2, cellulose powder 7.36, and agar 3.0. Mysid extract was supplied from a certain company in Japan. To 2 g of diet A or mysid extract, chicken egg (1.34 g in dry weight) was added as the homogenate (20 ml) of whole chicken eggs-distilled water (1:1, v/v) and encapsulated.

Diets 4, 5, 6, 7, 8, and 9 are the diets encapsulated with a zein membrane (Zein-MED) by using sodium hydroxide (NaOH-method) or ethanol (EtOH-method) as follows. Zein-MED (NaOH-method): The powdered diet (7 g) was suspended in the NaOH solution (pH 12, 70 ml) containing zein (1.4 g) with continuous stirring by means of a magnetic stirrer. The suspension was diluted with distilled water (120 ml) and acidified with acetic acid to a pH value of 4.5 to form the Zein-MED. Zein-MED (EtOH-method): The powdered diet (6 g) was mixed with zein (1.2 g) dissolved in 76% ethanol (74 ml) with continuous stirring, and then distilled water (150 ml) was added drop by drop to this suspension to form the Zein-MED. In both methods, the Zein-MED was collected by centrifugation (1,500 rpm, 15 min) at 0°C.

Diets 10 and 11 are the powdered diet with a zein-coating, which we classified as micro-coating diets (MCD). The Zein-MCD was prepared as follows. The formulated diets (pellet or semi-

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Table 1. Diets used in feeding experiments with the larvae of *P. japonicus*

Group (Diet No.)	Type of diet	Ingredient	Size (μ m)	Feeding concentration (mg/larva/day)
1	Nylon-MED	Mysid extract + Chicken eggs	50–100	0.44–0.60* ³
2	Nylon-MED	Diet A* ¹ + Chicken egg	50–100	0.44–0.60
3	Nylon-MED	Diet A free of lipids + Chicken eggs	50–100	0.44–0.60
4	Zein-MED (EtOH-method)	Mysid extract	100–150	0.33
5	Zein-MED (EtOH-method)	Diet A	100–150	0.33
6	Zein-MED (NaOH-method)	Diet A	100–150	0.33
7	Zein-MED (EtOH-method)	Diet A free of lipids	100–150	0.33
8	Zein-MED (EtOH-method)	Diet A free of cholesterol	100–150	0.33
9	Zein-MED (NaOH-method)	Diet B* ²	100–150	0.33
10	Zein-MCD	Diet A	10–50	0.20
11	Zein-MCD	Diet B	10–50	0.20
12	Live food	<i>Chaetoceros</i> , <i>Artemia</i>		
13	No food			

*¹ Artificial diet for the prawn *P. japonicus*, designed by KANAZAWA *et al.*⁸⁾*² Commercial diet for the prawn *P. japonicus*, (Evian, Kyowa-hakko Kogyo Co. Ltd.).*³ 500–700 capsules/ml water were supplied.

solid diet) were dried, crushed, and sieved through a mesh. To the powdered diets (8 g), zein (5 g) dissolved in 60% ethanol (25 ml) was added, mixed thoroughly, and then heated in an oven at 40°C for 24–48 hr. The dried diets were powdered into particles (10–50 μ m).

The diatom *Chaetoceros gracilis* was cultured as described previously.⁶⁾

Prawn Larvae and Feeding Experiments

Eggs of the prawn *P. japonicus* were hatched in laboratory tanks. The feeding experiments on the test diets (Table 1) were carried out during the period of August 9 to August 16, 1979, by using the larvae of zoea₁ stage which were came from the same spawner. In each experimental group, 100 zoea₁ larvae were put into a 1-l-beaker containing 800 ml of the filtered sea water and reared with the test diet. Control groups of larvae (diet 12) were fed on *C. gracilis* (50–70,000 cells/ml) until mysis stage and then on *Artemia salina* (15 nauplii/ml). The apparatus and feeding methods were the almost same as those mentioned in the previous paper.⁶⁾ All the larvae in each beaker were counted and staged at 1-day interval. The dietary values of diets were evaluated in terms of survival rates and developmental stages of larvae as described in the previous paper.⁶⁾

Results

In the present study, 11 kinds of the MD were prepared and the dietary values for the larvae of *P. japonicus* were examined by the feeding trials.

Table 2. Growth and survival of *P. japonicus* fed on the various types of diets

Diet No.	Survival (%) at P ₁	Developmental stage reached* ¹
1	78	P ₁ (8 days)* ²
2	70	P ₁ (8 days)
3	48	P ₁ (8 days)
4	42	P ₁ (8 days)
5	0	M ₁ (5 days)
6	0	M ₂ (5 days)
7	0	Z ₃ (5 days)
8	0	M ₁ (5 days)
9	67	P ₁ (8 days)
10	94	P ₁ (8 days)
11	75	P ₁ (8 days)
12	95	P ₁ (7 days)
13 (starved)	0	Z ₂ (3 days)

*¹ Developmental stages were abbreviated as follows: Zeoa₂ (Z₂), zoea₃ (Z₃), mysis₁ (M₁), mysis₂ (M₂), mysis₃ (M₃), and post-larvae₁ (P₁).*² Zoea₁ (Z₁) grew up to P₁ in 8 days.

The results are shown in Table 2 and Figs. 1, 2, and 3.

Fig. 1 shows the survival and growth of the prawn larvae fed on the Nylon-MED. In all 3 diets, the prawn larvae grew from zoea₁ to post-larvae (P₁) in 8 days. Also, a high survival rate was attained on the diets 1 and 2. However, the retrenchment of lipids from the diet 2 resulted in the decrease of survival rate, indicating the necessity of lipids for the larval prawns. JONES *et al.*⁶⁾ have reported the success in rearing the prawn larvae from zoea₁ to P₁ with the Nylon-MED containing a mixture of short necked clam and

Fig. 1. Growth and survival rates of the prawn larvae fed on the Nylon-MED. Numerals indicate the diet No. (see Table 1).

Fig. 2. Growth and survival rates of the prawn larvae fed on the Zein-MED. Numerals indicate the diet No. (see Table 1).

Fig. 3. Growth and survival rates of the prawn larvae fed on the Zein-MCD and live feeds. Numerals indicate the diet No. (see Table 1).

whole chicken egg (1:1) in 12 days, with the survival rate of 50%. As compared with their results, the Nylon-MED containing diet A and chicken egg seemed to be a superior diet in terms of both the survival and growth rates. The results may predict the possibility of rearing the

prawn larvae by using the Nylon-MED containing a defined diet.

Fig. 2 shows the survival and growth of the prawn larvae fed on the Zein-MED. Two the Zein-MED containing mysid extract (diet 4) and commercial diet (diet 9) supported the growth of

larvae from zoea₁ to P₁. The survival rates on the diets 4 and 9 after 8 days, when the zoea grew up to P₁, were 42% and 67%, respectively. All other Zein-MED only supported the growth of prawn larvae until mysis₁-mysis₂ stages, and all the larvae died in 6 days. Thus, the Zein-MED containing chemically defined diets were revealed to be imperfect as the diet for the prawn larvae. This seemed to be due not always to the insufficiency of nutrients but to other factors such as the stability of the diets in water, etc. GATESOUBE and LUQUET⁹⁾ have studied the water stability of the diets prepared by the several methods, and revealed that the water stability of the unprotected diet was very low; only 3% of the diets were recovered by filtration after 4 hr in water. Furthermore, they have shown that the water-standing capacity was higher in the particles encapsulated with natural polymers such as gelatin and zein (stability, 74–79%) than in the particles encapsulated with nylon (stability, 56–66%). About the Zein-MED containing defined diets, it was thought advisable to check the leaking of nutrients during the encapsulation process and the completeness of encapsulation in coming investigation.

Fig. 3 shows the survival and growth of the prawn larvae fed on the Zein-MCD. Two the Zein-MCD containing diet A (diet 10) and commercial diet (diet 11) sustained the growth of prawn larvae from zoea₁ to P₁. The survival rates on the diets 10 and 11 after 8 days, when the zoea grew up to P₁, were 94% and 75%, respectively. Except for an 1-day tardiness of growth to P₁, the Zein-MCD containing diet A was almost comparable to the live feeds (diet 12) in respect to the dietary values. Since the diet 10 was composed of all chemically known compounds, we believe that this type of particulate diets will provide an effective method for determination of nutritional requirements in the larval prawns.

Discussion

There are several attempts to improve the techniques for rearing the larvae of *P. japonicus* by using baker's yeast and fermented soybean cake as supplements to planktonic feeds such as diatoms and *Artemia*.^{10–12)} Crustacean larvae have been revealed to accept artificial food particles.^{13–15)} In fact, a compounded diet has been demonstrated to support the growth and survival of larval prawn *P. japonicus* to some

extent when it was powdered and supplied as acceptable particles.⁶⁾ However, unprotected diets are generally susceptible to disintegration and bacterial attack.¹⁶⁾ To resolve these problems, hence, JONES and coworkers have prepared the Nylon-MED and reared several species of crustacean larvae.^{16–20)} For the prawns and *Artemia*, some Nylon-MED have been shown to give a high dietary value.^{5, 17)}

The results of the present study also showed that the Nylon-MED containing a whole chicken egg and either mysid extract or artificial diet sustained the growth of prawn larvae to post-larval stage (P₁) with considerably high survival rates. The attempts to rear the larval prawns with the Zein-MED containing a defined diet were unsuccessful. Whereas, the Zein-MCD containing a defined diet sustained the growth and survival to post-larval stage (P₁) as observed on natural feeds, *Chaetoceros* and *Artemia*. As far as we know, this paper is the first one showing the success in rearing the prawn larvae from zoea₁ to post-larva (P₁) with the particulate diet containing all chemically defined ingredients.

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