クルマエビの栄養要求に関する研究IV

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Nutritional Requirements of Prawn—IV. The Dietary Effect of Ecdysones

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The dietary effect of three different ecdysones isolated from plants on molting in the prawn was studied.

All three ecdysones were effective in inducing molting at the concentrations used, but the growth rate of prawn fed on diet supplemented with ecdysones revealed a lower percentage as compared with that of prawn fed on an ecdysone-free diet.

Several ecdysones, molting hormones, have been found in the insects[1–5] and the crustaceans[6–10]. Since NAKANISHI *et al.*[11] and TAKEMOTO *et al.*[12] demonstrated the presence of ecdysones in the plants, a variety of ecdysones have been isolated from several different plants. The occurrence of ecdysones in plants is very interesting for both the existence and action of molting hormone in the natural kingdom. Recently, it has been shown that these ecdysones induce molting in the crustaceans[13–18]. However, there is no report on the dietary effect of ecdysones in crustaceans.

The present paper describes the effective result to stimulate molting in prawn with the basal diet containing ecdysones.

Experimental

Prawn and feeding methods. The prawn, *penaeus japonicus*, about 3 g body weight, was supplied from the Mariculture Center of Seto-Inland Sea, Shibushi Biological Station, and maintained in the troughs, equipped with circulating systems by filtration through a sand bed. The troughs were supplied at the rate of 2 liters per min. with the sea water previously adjusted at 25±1°C, pH 8–9, and specific gravity 1.024. Further, the sea water was replaced every week. For each experiment, 20–25 animals per one trough were maintained for 30 days.

Diet and ecdysones. The artificial diet devised by KANAZAWA *et al.*[19,20] for the feeding trials as to the nutritional requirements of prawn was used as a basal diet. The ecdysones used in this experiment were inokosterone, cyasterone, and ecdysterone, supplied by Professor T. TAKEMOTO, Tohoku University, Japan and Dr. S. OGAWA, Rohto Pharmaceutical Co., Ltd., Japan. Two phytoecdysones, inokosterone[12] and cyasterone[21]

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were isolated from *achyranthis radix* and *Cyathula capitata,* respectively. Hitherto, ecdysterone has been demonstrated in the crustacean[6] and the insects[2-4]. The ecdysterone used in the present experimental was isolated from a plant source, and identical to that obtained from crustacean and insects. To the basal diet, the ecdysones dissolved in a small amount of ethanol were added, and mixed thoroughly with water. The diets were poured into a kurehalon tube, and both sides of the tube were tied tightly. And then, the diets were heated at 100°C for 20 min., and stored in a refrigerator until used. An excess amount of each diet was fed before sunset; and next morning, the remaining diet and the feces were removed. The ecdysone content in the diets was 12.5, 2.5, 0.5, 0.1 mg% of inokosterone, 2.5, 0.5 mg% of cyasterone, and 2.5, 0.5 mg% of ecdysterone. The ecdysone-free diet was used as control. The effect of ecdysones was detected by the number of times molted during the 30 day period, survival rate, and growth rate.

The growth rate of prawn is given by the following formula.

$$\text{Growth rate}(\%) = \frac{\text{A}}{\text{B}} \times 100$$

A: Body weight gain (g) of prawn fed on the test diets

B: Body weight gain (g) of prawn fed on the clam, *Tapes philippinarum*

The number of times molted during the 30 day period is given by the following formula.

$$\text{Number of times molted during the } n \text{ day period} = \sum_{i=1}^{n} \frac{y_i}{x_i}$$

x_i: Number of survival in i day

y_i: Number of molting in i day

n: Experimental period (30 days)

Results

Effect of inokosterone on molting, survival, and growth. The number of times molted, survival rate, and growth rate of prawn fed on each diet containing 12.5, 2.5, 0.5, 0.1 mg% of inokosterone are shown in Table 1.

In the prawn fed on the inokosterone-containing diets, the number of times molted during the 30 day period increased remarkably as compared with that of prawn fed on the inokosterone-free diet. On the survival rate, a definite difference was not observed among each experimental group, however, the low growth rate was found to cause when the diet contained inokosterone.

Effect of cyasterone on molting, survival, and growth. The molting, survival, and growth of prawn fed on the diet supplemerted with cyasterone are shown in Table 2.

Table 1. Effect of inokosterone on molting, survival, and growth.

Inokosterone added to diet	Number of times molted during the 30 day period	Survival rate (%)	Growth rate (%)
None	0.9	85	70
0.1 mg% inokosterone	0.9	80	47
0.5 mg% inokosterone	1.5	81	32
2.5 mg% inokosterone	1.4	80	37
12.5 mg% inokosterone	1.6	51	22
Short-necked clam diet	2.7	90	100

Table 2. Effect of cyasterone on molting, survival, and growth.

Cyasterone added to diet	Number of times molted during the 30 day period	Survival rate (%)	Growth rate (%)
None	0.9	85	70
0.5 mg% cyasterone	1.2	80	24
2.5 mg% cyasterone	2.5	40	24

Table 3. Effect of ecdysterone on molting, survival, and growth.

Ecdysterone added to diet	Number of times molted during the 30 day period	Survival rate (%)	Growth rate (%)
None	0.9	85	70
0.5 mg% ecdysterone	1.5	84	45
2.5 mg% ecdysterone	1.1	70	40

The molting times of prawn receiving the 2.5 mg% cyasterone diet were 2.5 times during the 30 day period, but the survival rate and growth rate were 40% and 24%, respectively, revealing a lower percentage as compared with that of prawn receiving the cyasterone-free diet.

Effect of ecdysterone on molting, survival, and growth. In the case of prawn fed on the ecdysterone-containing diets, as shown in Table 3, the concentration of 0.5 mg% ecdysterone caused a marked acceleration of molting.

Effect of short-necked clam diet on molting. In the case of prawn fed on the clam (Table 1), the molting of prawn was more effective. It is supposed that the basal diet used in this experiment is nutritiously sufficient, though the problem as to attractants, feeding factor etc. for prawn is remained. Therefore, the substance responsible for molting of prawn may be contained in the clam.

Discussion

When the prawn was maintained on the artificial diet containing inokosterone,

cyasterone, and ecdysterone, respectively, the remarkable effect of ecdysones to molting of prawn was perceived. These results suggest that ecdysones added to the diet are thoroughly absorbed through the intestines, and that dietary ecdysones cause molting similarly to injected ecdysones.

In the previous papers, the authors reported that the prawn lacks the sterol-synthesizing ability from acetate[22], though cholesterol was contained as a major sterol in this animal[23]. Further, it was also found by a feeding trial that the prawn requires a dietary source of sterol for normal growth[20,24]. The artificial diet used in the present experiment contains 0.5% of cholesterol. However, it is obscure yet whether cholesterol serves as a precursor for molting hormone in the prawn. The bioconversion of cholesterol to molting hormone in crustaceans should be confirmed by a radioisotopic method.

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