クルマエビの栄養要求に関する研究II

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Nutritional Requirements of Prawn—II. Requirement for Sterols

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The requirement of prawn for sterols was examined by the feeding trials using the artificial diet devised by authors.

The prawn receiving the diet supplemented with cholesterol grew normally, and the survival rate and growth rate were 86–95% and 56–98%, respectively. However, the growth rate of prawn receiving the sterol-free diet was poor (22–64%).

The requirement for sterols of the prawn determined by 30- or 40-day feeding trials was about 0.5 g per 100 g of diet, under the experimental conditions used.

The survival rates of prawn fed the diet containing ergosterol, stigmasterol and β-sitosterol revealed a similar percentage to that of prawn fed the cholesterol-diet, but, the growth rates of these sterols were inferior to cholesterol.

From these results, it was suggested that the prawn requires the dietary sterol for normal growth similarly to insects.

It is recognized that insects generally require an exogenous source of sterols for normal growth and that this requirement is a manifestation of the lack of sterol biosynthesis.

Recently, in crustaceans, belonging to the same arthropods as insects, it has been demonstrated that they are incapable of biosynthesizing cholesterol from acetate as well as in insects. The crab, *Cancer pagurus*[1], crab, *Portunus trituberculatus*[2], crayfish, *Astacus astacus*[3–7], lobster, *Homarus gammarus*[8,9], lobster, *Panulirus japonica*[2], and prawn, *Penaeus japonicus*[2] are shown to be incapable of incoporating acetate-1-^{14}C and/or mevalonate-2-^{14}C into squalene or cholesterol. From these facts, it is assumed that these animals may require sterol as a diet.

In the present paper, the role of sterols in the nutrition of prawn was examined by the feeding trials using the artificial diet.

Experimental

Prawn. The prawn, *Penaeus japonicus*, weighing 0.5–1.5 g, spawned in The Subtropical Marine Biological Laboratory of Kagoshima, and The Fisheries Experimental Stations of Kumamoto and Kagoshima was used in this experiment.

Diet. The artificial diet devised by KANAZAWA *et al.*[10] for nutritional requirements of prawn was used as a basal diet. The composition of diet is given in Table 1. The

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Table 1. Composition of the diets used to test nutritive effect of sterols for the prawn

Substance	Dry diet in percentage	Substance	Dry diet in percentage
Glucose	5.5	Glycine	0.1
Sucrose	10.0	Citric acid	0.3
Starch	4.0	Succinic acid	0.3
Chitin	4.0	Soybean oil (Refined)	8.0
Glucosamine	1.5	Salt mixture*	7.7
Cellulose powder	4.0	Vitamin mixture**	2.6
Soybean casein (Lipid-free)	50.0	Morin	0.1
Methionine	1.0	Total	100.0
Tryptophan	0.2	Agar	5.0
Glutamic acid	0.2	Distilled water	100.0

* K_2HPO_4 2.310, KCl 0.723, $MgSO_4$ 1.149, $FeSO_4 \cdot 7H_2O$ 0.107, $Ca_3(PO_4)_2$ 2.109, $MnSO_4 \cdot 7H_2O$ 0.015, and $CaCO_3$ 1.293 g/100 g of dry diet.

** *p*-aminobenzoic acid 5, biotin 0.2, inositol 200, niacin 20, Ca-pantothenate 30, pyridoxine-HCl 6, riboflavin 4, thiamine-HCl 2, menadione 2, β-carotene 4.8, α-tocopherol 10, cyanocobalamin 0.04, calciferol 0.6, ascorbic acid 2000, folic acid 0.4, and choline-HCl 300 mg/100 g of dry diet.

soybean oil used was obtained from Nakarai Chemicals Ltd. (Japan) and contained 0.2% sterols (β-sitosterol and stigmasterol). The content may be negligible in the present study, as compared to the amount added to diets. The diet was sealed in a Kurehalon tube, and heated at 100°C for 30 minutes, and stored in a refrigerator until used. The details of the feeding methods for prawn have been described in the previous paper[10].

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

$$\frac{B-A}{C} \times 100 = (\%)$$

A: Average body weight of the prawn before feeding.

B: Average body weight of the prawn after feeding.

C: Body weight gain of the prawn fed on short-necked clam.

Sterols. Sterols used were cholesterol, ergosterol, stigmasterol, and β-sitosterol. Cholesterol, ergosterol and stigmasterol were obtained from Nakarai Chemicals Ltd. (Japan) and β-sitosterol was purchased from Nutritional Biochemicals Co. (U.S.A.). The purity of these sterols was analyzed by gas-liquid chromatography using 1.5% SE-30 column[11]. The results are as follows: cholesterol, 99.9%; ergosterol, 99.8%; stigmasterol, 99.9%; β-sitosterol, 94.4% β-sitosterol and 5.6% campesterol. Since it is difficult to separate β-sitosterol and campesterol, the mixture was used in this experiment.

Results

Requirement for cholesterol. In previous paper,[11] it was shown that sterol composi-

tion of prawn was cholesterol, 90%; 22-dehydrocholesterol, 3%; 24-methylenecholesterol, 7%. In this experiment, cholesterol, detected as a major sterol of prawn was firstly added to the basal diet. The results of the feeding trials of prawn maintained on the sterol-deficient diet and on the diet supplemented with 0.5% of cholesterol appear in Table 2.

Table 2. Results of feeding experimental on requirement for cholesterol

Experiment No.	Date of experimental	Experimental period (days)	Cholesterol added (g/100 g) of diet)	No. of prawn at start	Rate of survival (%)	No. of molting cycle	Average body weight		Rate of growth (%)
							Initial (g)	Final (g)	
1	Jan. 27, 1969 —Feb. 26, 1969	30	0	20	45	1.8	1.08	1.70	64
			0.5	20	95	2.0	1.57	2.28	72
2	Oct. 25, 1969 —Dec. 4, 1969	40	0	23	78		0.57	0.88	28
			0.5	23	86		0.51	1.59	98
3	July 20, 1970 —Aug. 19, 1970	30	0	24	63	1.8	1.49	1.73	22
			0.5	24	88	2.7	1.26	1.83	56

In experiments 1, 2, and 3, the prawn receiving the diet supplemented with cholesterol grew normally and the survival rate and growth rate of prawn were 86–95% and 56–98%, respectively. However, the growth rate of prawn receiving the sterol-free diet was poor (22–64%), although the detectable abnormality could not be seen among them during the feeding period on the length of antennae, the colour of body, and the furtive movement into the sand etc.

To decide the quantitative requirement, a similar experiment was carried out on the diets containing different quantities of the cholesterol, 0.05, 0.1, 0.5, 1.0, and 5.0 g per 100 g of diet. As shown in Table 3, 0.5–1.0 g addition was most effective. From this results, the requirement was tentatively estimated to be about 0.5 g per 100 g of diet under the experimental conditions adopted.

Table 3. Effect of cholesterol levels in the diet on growth and survival of prawn

Cholesterol added (g/100 g of diet)	Experimental period (days)	No. of prawn at start	Rate of survival (%)	Rate of growth (%)
0.05	30	24	82	45
0.1	30	24	88	57
0.5	30	24	88	84
1.0	30	24	92	84
5.0	30	24	83	42

Requirement for ergosterol, stigmasterol, and β-sitosterol. Phytoplanktons or yeast have been generally used as a diet of young prawn. To see the nutritive effect of sterols

Table 4. Effect of various sterols on growth and survival of prawn

Sterol added (0.5 g/100 g of diet)	Rate of survival (%)			Rate of growth (%)		
	Experiment			Experiment		
	1	2	3	1	2	3
Cholesterol	95	86	88	72	98	56
Ergosterol	94	87	92	51	79	48
Stigmasterol	96	83	88	62	67	56
β-sitosterol	89	83	92	56	29	50

Number and average weight of surviving prawn were determined at the end of 30- or 40-day feeding trials.

other than cholesterol, the mycosterol (C_{28}) such as ergosterol and the phytosterol (C_{29}) such as stigmasterol and β-sitosterol were used. The results are as on Table 4.

The survival rates of prawn fed the diet containing ergosterol, stigmasterol and β-sitosterol extended over 83–96%, revealing a similar percentage to that of prawn fed the cholesterol-diet. On the growth rate of prawn, ergosterol, stigmasterol and β-sitosterol were also effective, but inferior to cholesterol.

Discussion

In previous paper[2], the absence of sterol-synthesizing ability in prawn has been demonstrated. Furthermore, in present study, it was suggested that the prawn requires the dietary sterol for normal growth similarly to insects. The dietary concentration of sterol required for normal growth was estimated to be about 0.5 g per 100 g of diet, under the experimental conditions adopted. The value obtained in this experiment was generally agreeable with the requirement of insects[12–14].

The utilization of ergosterol, stigmasterol and β-sitosterol was inferior to cholesterol. It is obscure yet whether sterols received from a diet were digested or accumulated as an endogenous sterols in prawn. Recently, we demonstrated the bioconversion of the dietary ergosterol to cholesterol in *Artemia salina*[15].

In crustaceans, it is assumed that cholesterol may be a precursor of a vitamin D, steroid hormone, molting hormone, and brain hormone. The bioconversion of cholesterol to molting hormone has been demonstrated in experiments in which cholesterol-^{3}H was injected into pupae of *Calliphora erythrocephala* and labeled ecdysone was isolated[16]. Recently, HIKINO *et al.*[17] have proved that ponasterone A, an insect-molting substance, is biosynthesized in the plant, *Podocarpus macrophyllus* from cholesterol. As shown in Table 2, the increase of number of molting was also observed in the prawn fed the diet supplemented cholesterol, however, the effect of sterol to molting of prawn requires further examination.

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