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著者	KANAZAWA Akio, TESHIMA Shin-ichi, SASAKI Mitsuru
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Requirements of the Juvenile Prawn for Calcium, Phosphorus, Magnesium, Potassium, Copper, Manganese, and Iron*1

AKIO KANAZAWA*2, Shin-ichi TESHIMA*2 and Mitsuru SASAKI*3

Abstract

The feeding experiments were conducted to examine the requirements of the juvenile prawn *Penaeus japonicus* BATE for the minerals such as Ca, P, Mg, K, Cu, Mn, and Fe. The dietary value of these minerals for the prawns was evaluated in terms of weight gain, feed conversion, survival rate, and molting frequency. Growth of the prawns was improved by the supplement of 1~2% P to the diets, however such an effect of P was affected by dietary Ca levels. The best growth of the prawns was found with the diets supplemented with 1~2% levels of Ca and P at the Ca/P ratio of 1:1. Growth of the prawns was also improved by the supplements of Mg (0.1~0.5%) and K (0.9 and 1.8%), but not by those of Cu (0.06 and 0.12%), Mn (0.01~0.1%), and Fe (0.006~0.012%). These results show that *P. japonicus* juveniles require dietary sources of P (1~2%), Ca (1~2%), Mg (0.3%), and K (0.9%), but possibly not Fe, Cu, and Mn for normal growth.

KANAZAWA *et al.*¹⁾ have introduced a refined diet for studying the nutritional requirements of the prawn *Penaeus japonicus* Bate. After that, much information has been accumulated for the nutritional requirements of the prawn and other crustacean species.^{2,3)} However, the knowledge of mineral requirements with prawns is still scanty. The composition of minerals used in our earlier studies⁴⁾ was provisionally designed in consideration of the mineral mixtures of diets for fish and insects. In the present study, therefore, we intend to clarify the mineral requirements of *P. japonicus* juveniles in order to make up a well-balanced mineral mixture. This paper presents the requirements of the prawn for P, Ca, Mg, K, Cu, Mn, and Fe, indicating the necessity of inclusion of P, Ca, Mg, and K in the purified diet for good growth.

Materials and Methods

Prawns and feeding methods

The prawns *P. japonicus*, weighing each about 0.2 g, were obtained from Mitsui-Nohrin Kaiyosangyo Co. (Kagoshima, Japan), maintained on a commercial diet (Evian; Kyowa-Hakko Kogyo Co., Japan) until they grew up to 0.5~1.0 g in body weight, and then divided into lots of 15 prawns in an aquarium (30 liter-capacity) for feeding trials. The prawn juveniles were given the test diets at the 15% level of their body weight daily at 25°C for 30 or 60 days. The basal diet was similar to that used in the previous study¹⁾, but slightly modified as follows. Soybean oil was substituted with pollack liver oil and the pH value of diets was adjusted to 6.8 with NaOH

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*2 Faculty of Fisheries, Kagoshima University, 50-20 Shimoarata-4, Kagoshima 890, Japan.

*3 Present address: Kibun Co., Ltd., Ha-41 Kanmushi, Ninomiya-cho, Kozu City, Shimane 695, Japan.

solution. The test diets used in this study contained the same levels of ingredients each other, except for minerals and cellulose equal to 100%. The concentrations of P, Ca, K, Mg, Fe, Mn, and Cu in the diets were adjusted with the following salts (see Tables 1, 4, 6, and 8); $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, K_2HPO_4 , $\text{Ca}_3(\text{PO}_4)_2$, CaCO_3 , CH_3COOK , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, and CuSO_4 . The methods for preparation of diets and rearing of the prawn were similar to those described previously¹⁾.

Experimental designs and feeding experiments

Five feeding experiments were conducted in this study. Experiments I, II, and III were designed as one-way layout experiments in order to check preliminarily the requirements of *P. japonicus* juveniles for P, Ca, Mg, and Fe. The dietary value of test diets was evaluated in terms of weight gain, feed conversion, survival rate, and molting frequency. In experiment I, the requirements of prawns for Ca and P were examined with the diets 1 to 9 (Table 1) containing various levels of Ca (0.53 to 2.11% in diets) and P (0 to 4.2%). In experiments II and III, the requirements of prawns for Mg and Fe were investigated with the diets 10 to 13 containing Mg (0, 0.036, 0.072, and 0.154%) (Table 4), and the diets 14 to 17 containing Fe (0, 0.007, 0.014, and 0.027%) (Table 6), respectively.

The results of experiments I, II, and III suggested that the prawn juveniles necessitate dietary sources of Ca, P, and Mg but not Fe for growth. In experiments IV and V, therefore, the requirements of prawns for Ca, P, and Mg were reexamined to obtain more reliable evidence. In experiments IV and V, the experimental groups were designed by using an orthogonal array (L_8)²⁾, and the requirements of prawns for K, Mn, Fe, and Cu in addition to Ca, P, and Mg were checked by assigning the kinds and quantities (% in diets) of the minerals as factors and levels, respectively, as follows: Factors A (Ca: $A_1=1.06\%$, $A_2=2.11\%$), B (P: $B_1=1.06\%$, $B_2=2.11\%$), C (Mg: $C_1=0.1\%$, $C_2=0.3\%$, $C_3=0.5\%$), D (K: $D_1=0.9\%$, $D_2=1.8\%$), E (Fe: $E_1=0$, $E_2=0.003\%$), F (Cu: $F_1=0$, $F_2=0.003\%$), and G (Mn: $G_1=0$, $G_2=0.001\%$, $G_3=0.01\%$). The factors and levels were allotted to the following files in the orthogonal array. Experiment IV: file numbers 1 (A), 2 (B), 3 (A × B), 4 (C_1C_2), 5 (D), 6 (E + F), and 7 (G_2G_3). Experiment V:

Table 1. Composition of the mineral mixtures used in experiment-I

Mineral	Content (mg/g diet) and diet No.*								
	1	2	3	4	5	6	7	8	9
K_2HPO_4	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
$\text{Ca}_3(\text{PO}_4)_2$	54.4	54.4	27.2	35.0	27.2	13.6	8.6	8.6	0
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	75.5	17.6	30.7	0	7.9	21.6	0	0	0
CaCO_3	0	0	0	18.0	0	0	44.4	4.9	52.7
CH_3COOK	0	0	0	0	0	0	0	0	22.8
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	28.9	28.9	28.9	28.9	28.9	28.9	28.9	28.9	28.9
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
$\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Total	240.1	138.5	137.4	105.8	87.1	87.1	105.0	65.5	107.5

* The test diets (pH 6.8-6.9) contained the following ingredients (g/100 g of dry diet) besides minerals: glucose 5.5, sucrose 10.0, α -starch 4.0, glucosamine HCl 0.8, casein 50.0, sodium citrate 0.3, sodium succinate 0.3, cholesterol 0.5, pollack liver oil 8.0, vitamins 2.7, agar 3.0, and cellulose equal to 100.

file numbers 1 (A), 2 (B), 3 (A×B), 4 (C₂C₃), 5 (D), 6 (G₁G₂), and 7 (e, part of errors). Table 8 shows the diets used in experiments IV and V. The data obtained from experiments IV and V (Table 9) were put together and analysed synthetically (Table 10) to evaluate the effects of factors and levels on the dietary value (Table 11).

Mineral concentrations of the prawn bodies after the feeding trials

At the end of feeding trials, the concentrations of Ca, P, Mg, and Fe were determined with the whole body and/or hepatopancreas of the prawns after ashing with nitric acid and perchloric acid. Ca and P concentrations were determined by orthocresolphthalein complexone⁶⁾ and molybden blue⁷⁾ methods, respectively. Fe concentration was estimated by the orthophenanthroline method⁸⁾. Mg concentration was determined by the EDTA method⁹⁾.

Results

Experiments I, II, and III

Tables 2 and 3 show the results of experiment I. The prawn *P. japonicus* gave the highest values for weight gain, feed conversion, and survival rate when fed the diet 5 supplemented with 1.06% Ca plus 1.06% P and the diet 8 supplemented with 2.11% Ca plus 2.11% P (Table 2). The diet 7 supplemented with 1.06% Ca plus 2.11% P also gave a high weight gain, but the survival rate of prawns with this diet was lower than that of prawns with the diets 5 and 8. These results suggested that the dietary value of supplemental Ca and Mg was variable with the ratio of Ca/P. The molting frequency of prawns seemed not to vary with the dietary Ca and P levels, although the prawns fed the diets 3, 4 and 6 gave a slightly lower values for molting frequency than the other groups. Table 3 shows the Ca and P concentrations of the whole bodies of prawns after the feeding trials. Both Ca and P concentrations of the prawns bodies remained almost constant regardless the dietary Ca and P levels. The results of experiment I indicate that *P. japonicus* juveniles require supplemental 1.06~2.11% levels of Ca and P at the Ca/P ratio of 1.0 in the purified diets.

Table 2. Effects of Ca and P levels in diets on the weight gain, feed conversion, survival rate, and molting frequency of *P. japonicus* juveniles

Diet No.	Content in diets (mg/g)			Weight gain (%) ^{*1}	Feed conversion ^{*2} (%)	Survival rate (%)	Molting frequency ^{*3}
	P	Ca	Ca/P				
1	0	2.11		42	8	70	2.3
2	0.53	0.53	1.0	25	4	90	2.0
3	0.53	2.11	4.0	40	8	90	1.5
4	1.06	0.53	0.5	41	9	100	1.6
5	1.06	1.06	1.0	79	17	100	1.9
6	1.06	2.11	2.0	22	4	60	1.3
7	2.11	1.06	0.5	85	16	80	1.8
8	2.11	2.11	1.0	80	17	90	2.1
9	4.22	2.11	0.5	19	3	70	1.8

*1 Fifteen prawns, weighing 0.36-0.46 g in initial body weight, were reared for 30 days.

*2 The quantities of food inteked may be not always determined correctly because the prawn nibbles the diets.

*3 Molting frequency = $\sum_{i=1}^n \left(\frac{b}{a} \right)_i$, a, number of alive prawns; b, number of prawns molted during a day; i, date

Table 3. Effects of dietary Ca and P levels on the Ca and P contents of the prawn bodies

Diet No.	Dietary level (%)		Content of the prawns (mg/g fresh wt.)*		
	P	Ca	P	Ca	Ca/P
1	0	2.11	2.70	11.9	4.4
2	0.53	0.53	2.71	13.1	4.8
3	0.53	2.11	2.22	13.8	6.2
4	1.06	0.53	2.15	11.4	5.3
5	1.06	1.06	2.73	13.0	4.8
6	1.06	2.11	2.44	13.1	5.4
7	2.11	1.06	2.70	12.5	4.6
8	2.11	2.11	2.77	12.7	4.6
9	4.22	2.11	2.68	11.2	4.2

* The Ca and P contents were determined with the whole bodies of the prawns fed the test diets for 30 days.

Table 4 shows the results of experiment II. The weight gain, feed conversion, and survival rate of the prawns were improved with the increasing levels of Mg in diets, indicating that *P. japonicus* juveniles probably require dietary sources of Mg for growth. The highest values for weight gain, feed conversion, and survival rate were observed with the diet 13 supplemented with 0.154% Mg. But, the molting frequency of prawns was not variable with the dietary Mg levels at the levels of 0.036~0.154% in diets. Table 5 shows the Mg and Ca concentrations of the hepatopancreas and remains of prawns after the feeding trials. The Mg and Ca concentrations of the hepatopancreas and remains did not vary markedly with the dietary Mg levels.

Table 4. Effects of dietary Mg levels on the weight gain, feed conversion, survival rate, and molting frequency of *P. japonicus* juveniles

Diet No.	Mg content (% of diets)* ¹	Weight gain (%) ^{*2}	Feed conversion (%)	Survival rate (%)	Molting frequency
10	0	155	13.4	90	1.7
11	0.036	259	19.8	100	1.9
12	0.077	280	22.3	100	2.0
13	0.154	357	25.0	100	1.9

*¹ Mg content was adjusted with MgSO₄·7H₂O. The diets 10 to 13 contained the following minerals (mg/g diet) besides Mg; K₂HPO₄, 20.0; Ca₃(PO₄), 27.2; Na₂HPO₄, 7.9; FeSO₄·7H₂O, 2.7; MnSO₄·5H₂O, 0.4.

*² Fifteen prawns, weighing 0.30-0.33 g in initial body weight, were reared for 60 days.

Table 5. Effects of dietary Mg levels on the Mg and Ca contents of the prawn tissues*

Diet No.	Dietary Mg level (% of diets)	Mg content (mg/g fresh wt.)		Ca content (mg/g fresh wt.)	
		Hepatopancreas	Remains	Hepatopancreas	Remains
10	0	37.7	2.2	3.9	16.7
11	0.036	37.3	1.6	4.3	17.9
12	0.077	37.3	2.0	4.0	17.7
13	0.154	35.4	1.0	4.4	17.2

* The Mg and Ca contents of the hepatopancreas and remained tissues were determined with the prawns fed the test diets for 60 days.

Table 6 shows the results of experiment III. The weight gain and feed conversion of prawns decreased with the increasing levels of Fe in diets, although the survival rate and molting frequency remained roughly constant regardless the dietary Fe levels. These results indicated that growth of the prawn was suppressed on the diets supplemented with more than 0.007% Fe. Table 7 shows the Fe and Ca concentrations of the hepatopancreas and remains of prawns after the feeding trials. As the dietary Fe levels were increased, the Fe concentration of the hepatopancreas decreased, whereas that of the remains increased slightly. With the increasing Fe levels in diets, the Ca concentration of the hepatopancreas also decreased slightly, but that of the remains remained almost constant.

Table 6. Effects of dietary Fe levels on the weight gain, feed conversion, survival rate, and molting frequency of *P. japonicus* juveniles

Diet No.	Fe content (% of diets)* ¹	Weight gain (%) ^{*2}	Feed conversion (%)	Survival rate (%)	Molting frequency
14	0	455	43.3	60	1.7
15	0.007	395	45.0	70	1.9
16	0.014	138	15.9	70	2.0
17	0.027	126	7.9	60	1.9

*¹ Fe content was adjusted with FeSO₄·7H₂O. The diets 14 to 17 contained the following minerals (mg/g diet) besides Fe: K₂HPO₄, 20.0; Ca₃(PO₄), 27.2; Na₂HPO₄, 7.9; MgSO₄·7H₂O, 28.9; MnSO₄·5H₂O, 0.4.

*² Fifteen prawns, weighing 0.25-0.28 g in initial body weight, were reared for 60 days.

Table 7. Effects of dietary Fe levels on the Fe and Ca contents of the prawn tissues*

Diet No.	Dietary Fe level (% of diets)	Fe content (mg/fresh wt.)		Ca content (mg/fresh wt.)	
		Hepatopancreas	Remains	Hepatopancreas	Remains
14	0	6.60	0.95	5.34	15.5
15	0.007	5.53	0.99	4.41	15.4
16	0.014	4.38	1.52	4.81	15.7
17	0.027	4.30	1.13	4.60	15.4

* The Fe and Ca contents of the hepatopancreas and remained tissues were determined with the prawns fed the test diets for 60 days.

Experiments IV and V

As mentioned above, the results of experiments I, II, and III indicated that the prawn probably requires the supplemental Ca, P, and Mg but not Fe for growth. In experiments IV and V, hence, the dietary value of Ca, P, and Mg was reexamined by the feeding experiments which were designed as factorial experiments using an orthogonal array L₈ (Table 8). In addition, the dietary value of supplemental Mn and a mixture of Fe-Cu (1:1) was investigated in experiment IV. The results of the feeding trials are given in Table 8. Table 9 shows the results of the analysis of variance with the weight gain, feed conversion, survival rate, and molting frequency data.

As for the weight gain, a significant difference (P < 0.10) was observed in the following factors: Ca × P (interaction between Ca and P), Mg (0.3%, 0.5%), K (0.9%, 1.8%), and R (variability of data between experiments IV and V). But, no significant difference (P < 0.01) was found in

Table 8. Experimental groups, the mineral composition of diets, and the results of feeding trials in experiments IV and V

Experiment*	Group	Diet No.	Mineral supplemented (mg/g dry diet)							Weight gain (%)	Feed conversion (%)	Survival rate (%)	Molting frequency
			Ca	P	Mg	K	Mn	Fe	Cu				
IV	1	18	10.6	10.6	1.0	9.0	0.01	0	0	46.1	3.9	63	1.9
	2	19	10.6	10.6	3.0	18.8	0.1	0.03	0.03	61.8	6.6	50	2.3
	3	20	10.6	21.1	1.0	9.0	0.1	0.03	0.03	21.2	2.0	38	2.0
	4	21	10.6	21.1	3.0	18.0	0.01	0	0	57.4	5.1	63	1.8
	5	22	21.1	10.6	1.0	18.0	0.1	0	0	11.0	0.9	50	1.7
	6	23	21.1	10.6	3.0	9.0	0.1	0.03	0.03	36.4	3.7	38	1.8
	7	24	21.1	21.1	1.0	18.0	0.01	0.03	0.03	42.2	3.5	50	1.6
	8	25	21.1	21.1	3.0	9.0	0.1	0	0	39.6	3.5	50	1.4
V	1	26	10.6	10.6	3.0	9.0	0	0	0	121.5	23.5	100	0.9
	2	27	10.6	10.6	5.0	18.0	0.01	0	0	30.7	5.3	78	0.5
	3	28	10.6	21.1	3.0	9.0	0.01	0	0	90.5	17.4	100	0.3
	4	29	10.6	21.1	5.0	18.0	0	0	0	18.6	3.6	78	1.0
	5	30	21.1	10.6	3.0	18.0	0	0	0	52.2	8.7	67	0.9
	6	31	21.1	10.6	5.0	9.0	0.01	0	0	58.2	12.2	100	0.8
	7	32	21.1	21.1	3.0	18.0	0.01	0	0	75.1	15.5	100	0.3
	8	33	21.1	21.1	5.0	9.0	0	0	0	108.4	21.6	89	0.7

* Experimental groups were designed using an orthogonal array L₈. The test diets contained a fixed level of K₂HPO₄ (20.0 mg/g diet) and Ca₃(PO)₄ (27.2 mg/g diet) and varying levels of other minerals. The Ca, P, Mg, K, Fe, and Cu in diets were adjusted by the quantities of CaCO₃, NaH₂PO₄·2H₂O, MgSO₄·7H₂O, CH₃COOK, MnSO₄·5H₂O, FeSO₄·7H₂O, and CuSO₄, respectively.

Table 9. Analysis of variance with the data from experiments IV and V

Factor and level*	Weight gain (%)		Feed conversion (%)		Survival rate (%)		Molting frequency	
	V	F ₀	V	F ₀	V	F ₀	V	F ₀
R	3603	7.1	390	19.7	5259	74.9	5142	179.2
Ca (1.06%, 2.11%)	46	—	1	—	42	—	146	5.1
P (1.06%, 2.11%)	77	—	3	—	30	—	150	5.2
Ca × P	2048	4.0	68	3.5	42	—	39	1.4
Mg (0.1%, 0.3%)	703	1.4	10	—	0	—	3	—
Mg (0.3%, 0.5%)	1922	3.8	72	3.6	61	—	39	1.4
K (0.9%, 1.8%)	1661	3.3	95	4.8	240	3.4	4	—
Mn (0%, 0.001%)	265	—	13	—	242	3.5	281	9.8
Mn (0.001%, 0.01%)	276	—	1	—	32	—	14	—
Fe-Cu (0%, 0.006%)	6	—	1	—	113	1.6	128	4.5
Pooled error (df=11)	509	—	19.5	—	70.2	—	28.7	—

* R: variability of the data between experiments IV and V. Ca × P indicates the interaction between the factors, Ca and P.

the factors such as Ca (1.06%, 2.11%), P (1.06%, 2.11%), Mg (0.1%, 0.3%), Mn (0%, 0.001%), Mn (0.001%, 0.01%), and Fe-Cu (0%, 0.006%). The factors such as Ca × P, Mg (0.3%, 0.5%), and K (0.9%, 1.8%) were also significant ($P < 0.10$) with the feed conversion. The survival rates were significantly ($P < 0.10$) different with the factors, K (0.9%, 1.8%) and Mn (0%, 0.001%).

The molting frequency was significantly different with the factors, Ca (1.06%, 2.11%; $P < 0.05$), P (1.06%, 2.11%; $P < 0.05$), Mn (0%, 0.001%; $P < 0.01$), and Fe-Cu (0%, 0.0006%; $P < 0.10$). Table 10 shows the population means and confidence limit ($P = 0.95$) of the weight gain, feed conversion, survival rate, and molting frequency of the prawn juveniles which were fed the diets with varying levels of Ca, P, Mg, K, Mn, and Fe-Cu. The results of experiments IV and V (Tables 9 and 10) supported the results of the dietary value of supplemental Ca, P, and Mg in experiments I and II.

Table 10. Population means and confidence intervals of the weight gain, feed conversion, survival rate, and molting frequency of *P. japonicus* juveniles

Mineral	Content (% of diets)	Weight gain (%)	Feed conversion (%)	Survival rate (%)	Molting frequency
Ca, P	1.06, 1.06	65.3 ± 24.8*	10.3 ± 4.9*	73 ± 9*	1.4 ± 0.2*
	1.06, 2.11	47.0 ± 24.8	7.0 ± 4.9	72 ± 9	1.4 ± 0.2
	2.11, 1.06	39.2 ± 24.8	6.5 ± 4.9	66 ± 9	1.3 ± 0.2
	2.11, 2.11	66.3 ± 24.8	11.5 ± 4.9	72 ± 9	1.0 ± 0.2
Mg	0.1	48.1 ± 24.8	8.6 ± 4.9	72 ± 9	1.2 ± 0.2
	0.3	66.9 ± 24.8	10.9 ± 4.9	72 ± 9	1.2 ± 0.2
	0.5	35.9 ± 24.8	4.9 ± 4.9	67 ± 9	1.2 ± 0.2
K	0.9	65.3 ± 17.5	11.3 ± 3.5	75 ± 9	1.2 ± 0.2
	1.8	44.9 ± 17.5	6.4 ± 3.5	67 ± 9	1.3 ± 0.2
Mn	0	66.0 ± 24.8	10.9 ± 4.9	63 ± 9	1.5 ± 0.2
	0.001	54.5 ± 24.8	8.4 ± 4.9	74 ± 9	1.1 ± 0.2
	0.01	42.9 ± 24.8	7.6 ± 4.9	70 ± 9	1.2 ± 0.2
Fe-Cu (1:1)	0	38.5 ± 24.8	3.5 ± 4.9	51 ± 9	1.9 ± 0.2
	0.006	40.2 ± 24.8	4.3 ± 4.9	57 ± 9	1.7 ± 0.2

* Confidence limits ($P = 0.95$)

Discussion

Considering the results of experiments I to V, we concluded the following. (1) The supplements of 1.06~2.11% levels of Ca and P to the purified diets were indispensable for growth of *P. japonicus* juveniles. But, the Ca/P ratio in diets affected the dietary value of supplemental Ca and P, the favorable ratio of Ca/P being about 1.0. (2) The additions of 0.3% Mg and 0.9% K to the diets were also effective in improving growth. (3) The supplemental Mn, Fe, and Cu had no effect on the improvement of dietary value of diets. Contrarily, the addition of excessive Fe (more than 0.014% in diets) suppressed growth of the prawn.

By the feeding experiments designed as one-way layout experiments, DESHIMARU and YONE¹⁰ have shown that the prawn juveniles require supplemental 2.0% P, 1.0% K, and 0.2% trace metals including Al, Zn, Mn, Cu, Co, etc. but not Ca, Mg, and Fe for growth. KITABAYASHI *et al.*¹¹ have revealed the necessity of supplemental Ca in the diets of prawn juveniles. Our finding on the dietary value of supplemental K and Fe almost agreed with that on the same prawn obtained by DESHIMARU and YONE¹⁰, but not with that on the red sea bream *Chrysophrys major*, a marine fish¹²⁻¹⁵. As mentioned above, however, the information on the effects of supplemental Ca and

Mg for the prawn is conflicting.

Literatures have shown that the supplement of suitable levels of Ca to diets was effective in improving growth of the catfish *Ictalurus punctatus*¹⁶⁾, the rainbow trout *Salmo gairdneri*¹⁷⁾, and the carp *Cyprinus carpio*¹⁸⁾. The supplemental Mg has also been revealed to improve growth of the carp¹⁹⁾ and the rainbow trout^{20,21)}. Whereas, OGINO and TAKEDA²²⁾ have pointed out that growth of the carp was correlated with dietary P levels but not with Ca levels, suggesting that the carp is likely to balance the ratio of Ca/P of bodies by controlling the absorption or excretion of Ca. The red sea bream has also been shown not to require supplemental Ca²³⁾ and Mg²⁴⁾ when the diets contained small amounts of Ca (more than 0.136%) and Mg (more than 0.012%) together with sufficient amounts of Ca (more than 0.68%), presuming the possible uptake of both Ca and Mg from sea water by this fish. DESHIMARU and YONE¹⁰⁾ have thought that the prawn *P. japonicus* possibly meets the Ca requirement by uptaking Ca from environmental water, because the ⁴⁵Ca dissolved in sea water was absorbed more effectively by the prawns fed the diets without supplemental Ca as compared with the groups fed the diets with supplemental Ca²⁵⁾. In the present study, however, the significant ($P < 0.10$) interaction was found between the effects of supplemental Ca and P on the weight gain, feed conversion, and survival rate of the prawn (see Table 9). Therefore, we assume that a supplemental Ca may play some role in the effective utilization of dietary P by the prawn. This point will warrant further detailed studies in future.

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