Absorption of Labelled Calcium- ^{45}Ca by Prawn from Sea Water*¹

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In connection with the previous study of the mineral requirements of prawn, *Penaeus japonicus*, the absorption of radioactive calcium- ^{45}Ca from surrounding sea water by the prawn fed on a diet without supplemental calcium was compared with that of the prawn fed on a calcium-supplemented diet. The surrounding sea water was prepared to contain labelled calcium- $^{45}\text{Ca}-\text{CaCl}_2$.

The prawn were first fed on each test diet in ordinary sea water, and one hour after the final mealtime were transferred to sea water in which $^{45}\text{Ca}-\text{CaCl}_2$ was dissolved. One, four, and twenty-three hours later, the prawn were analyzed for the radioactivity incorporated into their bodies.

The radioactivity of ^{45}Ca accumulated in the prawn gradually increased with the lapse of time, and the group fed on the diet without supplemental calcium exhibited faster absorption and higher activity of ^{45}Ca than the group fed on the diet with calcium.

In the previous study¹⁾, it was noticed that prawn, *Penaeus japonicus*, fed on a diet without supplemental calcium usually grew as well as the group fed on a calcium-supplemented diet. This fact suggests that calcium existing in surrounding sea water compensates for the lack of calcium in a diet.

The present study deals with the comparison of the uptake of calcium from sea water by the prawn fed on purified diets with or without supplemental calcium.

Materials and Methods

Test Prawn

The prawn, weighing approximately one gram apiece, were divided into two groups of thirty animals each. These were maintained in seventy-liter plastic containers furnished continuously with sea water (25°C).

Test Diet

Two purified diets with or without supplemental calcium, as derived from the previous study¹⁾, were used as the test diets (Table 1). The diets were prepared into a dry pellet. The two groups were fed on the respective test diet for one week.

Experiment on Determination of ^{45}Ca Uptake

One hour after the final mealtime, the prawn which appeared to have much ingesta in the alimentary canal were selected and transferred to five-liter glass containers. Each container was filled with sea water (23.5°C) containing $^{45}\text{Ca}-\text{CaCl}_2$ (specific activity 26.7 mCi/mg Ca, New England Nuclear) at a radioactivity of 45 μCi per liter of sea water.

Analysis

At one, four, and twenty-three hours later, four to five prawn were sampled from each group. The whole body of each prawn sampled was resolved individually with nitric acid after cleaning with distilled water. Subsequently it was dried by heating, resolved with 1 ml of 2 N hydrochloric acid, and diluted with distilled water to estimate radioactivity of ^{45}Ca in their bodies. Measurement of radioactivity was made with a Beckman scintillation counter. The dioxane-naphthalene solution (10% naphthalene) with 0.7% diphenyl-oxazole (PPO) and 0.03% phenyloxazole (POPOP) was used as a scintillator. Calcium content of sea water used was determined spectrophotometrically by orthocresol-phthaleine-complexon (OCPC) method²⁾ using a calcium Test-Kit (Nippon Shoji Ltd., Osaka, Japan).

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Table 1. Composition of test diets

Ingredient	With calcium	Without calcium	Ingredient	With calcium	Without calcium
Casein·albumin (9:1) mixture* ¹	60%	60%	Minerals		
Soy bean oil	2.5	2.5	NaH ₂ PO ₄ ·2H ₂ O	10%	10%
Pollack liver oil	2.5	2.5	CaCO ₃	5	0
Glycogen	10	10	KCl	2	2
Vitamin mixture* ²	4	4	Trace metals	0.2	0.2
Cholesterol	1.8	1.8	Total	17.2	12.2
Glycine	2	2			
Minerals	17.2	12.2			
Cellulose	0	5			

*¹ 60 grams of the mixture contained 7.2 mg of calcium originating from the ingredients.

*² The composition is the same as reported previously³).

Table 2. Amount of calcium accumulated in prawn from sea water after feeding on diets with or without calcium supplementation

Diet	With calcium			Without calcium		
Exposure time (h)	1	4	23	1	4	23
No. of prawn tested	4	5	4	4	5	5
Mean body weight (g)	0.95	0.95	0.94	1.00	1.29	0.99
cpm (10 ³)/g prawn	9.6	34.7	84.5	16.2	44.6	99.4
Calcium { mg/g prawn	0.08	0.29	0.71	0.14	0.37	0.83
accumulated { mg/g prawn/h*	0.080	0.070	0.022	0.140	0.077	0.024

* Amount of calcium accumulated in the prawn per hour in the durations of 1, 1 to 4, and 4 to 23 hours of exposure.

Results and Discussion

The experimental results are summarized in Table 2.

Analysis of one ml of the radioactive sea water revealed 0.44 mg of calcium and 52700 cpm of radioactive calcium-⁴⁵Ca. The radioactivity incorporated into the prawn gradually increased with the length of time held in the sea water containing ⁴⁵Ca-CaCl₂. The levels of radioactivity, however, differed between the two groups previously fed the diets with or without the calcium supplement. The radioactivities of the prawn fed on the diet without supplemental calcium were 16200, 44600, and 9940 cpm per gram of body weight after one, four, and twenty-three hours' exposure, respectively, whereas those of the prawn fed on the diet with supplemental calcium were 9600, 34700, and 84500 cpm, correspondingly. Based on these radioactivities, the amount of calcium accumulated in the prawn from sea water was calculated to be approximately 0.83 mg in a day per gram of body weight for the former group, and approximately 0.71 mg for the latter group. Thus, the prawn fed on the diet without the calcium supplement absorbed calcium from sea water to a higher level than the calcium-supplemented diet

group. Furthermore, the amount of calcium accumulated in the prawn per hour in the durations of one hour, one to four, and four to twenty-three hours of exposure in the radioactive sea water were 0.140, 0.077, and 0.024 mg for the group fed on the diet without calcium supplementation, respectively, while those of the group fed on the calcium-supplemented diet were 0.080, 0.070, and 0.022 mg, correspondingly. Moreover, the prawn which ingested the test diets were noticed to still have some ingesta in a part of the post-intestine after one hour, and entirely excreted the ingesta in one to four hours. These results suggest that although the prawn always absorb calcium from sea water, the absorption increases with ingestion of a diet and decreases with excretion of ingesta.

In the present study, it was also found that the level of calcium absorbed by the prawn shortly after feeding was higher in the group fed on the diet without the calcium supplement than the group fed on the calcium-supplemented diet. Therefore, it is presumed that the difference in the amounts of calcium accumulated from sea water in a day between both groups is due mainly to the difference in the amounts of calcium absorbed by the prawn during the first hour after exposure to the radioactive sea water.

From these findings, it appears that the requirement of the prawn for calcium could be satisfied by calcium in the surrounding sea water when lacking in the diet.

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