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Dietary Calcium Requirement of Giant Croaker *Nibea japonica*

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The requirement for dietary calcium (Ca) of giant croaker *Nibea japonica* was investigated by feeding them with purified diets containing different levels of Ca for 10 weeks at temperatures of 23.0–25.5°C. The rearing water used contained 400 mg Ca/l. Dietary Ca levels were found to affect greatly the growth and feed utilization. Ca contents of vertebrae were independent of dietary Ca supplements. The results indicated that a minimum Ca level of 0.10% of the dry diet was required to maintain the normal growth and feed utilization of juvenile giant croaker.

INTRODUCTION

Calcium (Ca) is available to marine fish not only from the dietary sources but also from surrounding sea water that contains an appreciable amount of dissolved Ca which can be utilized by fish to meet a part or all of their metabolic Ca requirements (Lovelace and Podoliak, 1952; Ichikawa and Oguri, 1961; Love, 1980). However, in the previous studies, we observed that Ca absorption from the surrounding water alone could not fulfill the requirements of some marine fish and that dietary Ca supplements were necessary in tiger puffer (Furuichi *et al.*, 1997a, b; Hossain and Furuichi, 1998), redlip mullet*² and Japanese flounder (Hossain and Furuichi, unpubl. data). Therefore, it is necessary to investigate the dietary Ca requirements of different important marine fish. Giant croaker *Nibea japonica* is one of the largest sciaenids and is distributed along the southern coasts of Japan and the East China Sea (Masuda *et al.*, 1984). In Japan, the culture of this species started in the late 1960's (Tabaru *et al.*, 1988). However, informations on the nutritional requirements of this species are scarcely available in spite of its obvious importance in fish culture. The objective of the present study was to determine the minimum dietary Ca requirement of juvenile giant croaker.

MATERIALS AND METHODS

Experimental Diets

The composition of the basal diet is shown in Table 1. Purified ingredients were used. Vitamin-free casein and pollack liver oil were used as dietary protein and lipid sources, respectively. Dextrin and starch were used as a dietary carbohydrate source. A vitamin mixture and a Ca-free mineral mixture were added. Ca-lactate was supplied to the basal diet in expense of cellulose to obtain dietary supplemental Ca levels of 0, 0.05, 0.1, 0.2,

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*² M. A. Hossain and M. Furuichi: Abst. Metg. Japan. Soc. Fisheries Sci., September, 1997, p. 45.

and 0.4% of dry diet in diets 1, 2, 3, 4, and 5, respectively. Ingredients were mixed thoroughly, moistened with an aliquot of water and pelleted with a laboratory pelleting machine. Pelleted diets were manually cut to an appropriate size and stored under -20°C after drying in an air flow drier at 60°C for 60 min. The diet for a week was thawed and applied to the fish. The proximate and mineral compositions of the diets are presented in Table 2.

Table 1. Composition of the basal diet for giant croaker

Ingredient	%
Casein	50
Amino acid mix.* ¹	4
α -Starch	7
Dextrin	10
Pollack liver oil	10
Vitamin mix.* ²	3
Mineral mix.* ³	5
Carboxymethylcellulose	4
α -Cellulose	7

*¹ Amino acid: arginine·HCl, alanine, glycine, and aspartate·Na (25% each).

*² Halver's vitamin mixture (1957) + α -cellulose.

*³ Ca-free mineral mixture (in 100 g): Major element in g: KCl 7.68, $\text{MgSO}_4 \cdot 5\text{H}_2\text{O}$ 8.16, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 68.52, Fe-citrate 2.40 and α -cellulose 12.69; Minor element in mg: $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 90.0, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 264.0, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ 175.3, CuCl 15.7, KI 3.7 and $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 1.3.

Table 2. Proximate and mineral compositions of the experimental diets for giant croaker

Diet no.	1	2	3	4	5
Supplemental Ca level (% of diet)	0	0.05	0.10	0.20	0.40
<i>Proximate composition (% of dry matter)</i>					
Moisture	12.8	14.3	12.7	13.5	14.6
Crude protein	50.1	51.5	50.7	51.9	52.2
Crude lipid	9.2	9.8	10.3	9.5	10.0
Crude ash	4.7	4.8	5.0	5.0	5.2
<i>Mineral Content (dry matter basis)</i>					
Ca (%)	0.02	0.08	0.12	0.23	0.42
P (%)	0.87	0.89	0.79	0.85	0.83
K (%)	0.22	0.21	0.23	0.23	0.21
Mg ($\mu\text{g/g}$)	400	390	430	400	410
Fe ($\mu\text{g/g}$)	270	260	280	270	270
Zn ($\mu\text{g/g}$)	41	40	39	43	40
Mn ($\mu\text{g/g}$)	22	23	20	19	22
Cu ($\mu\text{g/g}$)	11	13	12	12	11

Fish and Rearing

Juvenile giant croaker used in the present experiment were produced in our laboratory from broodstock held in captivity. Prior to the initiation of the experiment, fish were transferred to indoor culture tanks and adapted to a casein diet for two weeks. Then, the fish were starved for 24 h, weighed individually and divided into 5 groups. Each group containing 30 fish (av. body wt. 0.55 g) was placed in a 200-l round polycarbonate tank. The fish were reared for 10 weeks and fed the experimental diets to satiation twice a day. Tanks were provided with sufficient air supply and continuous sand-filtered sea water supply (3–4 l/min). Rearing water temperature was 23.0–25.5°C. Ca content in rearing water was around 400 mg/l. A diurnal cycle of 12 h dark: 12 h light was maintained. Tank bottom was cleaned everyday before the morning feeding. The fish were weighed for every 2 weeks, after which they were subjected to an antibiotic bath to prevent possible diseases caused by handling.

Analytical Methods

At the end of the experiment, the fish were starved for 20–24 h to empty the digestive tract, then anesthetized with MS-222 and body length and body weight were measured. Blood was collected from 10 fish of each tank. A small portion of the collected blood was subjected to hematocrit and plasma protein determination. After the remaining blood was centrifuged at 3000 rpm for 15 min, plasma was collected and analyzed for Ca content according to the OCPC method (Gitelman, 1967). Liver from all fish of each tank was collected and weighed individually and stored as a composite sample for further chemical analyses. Carcasses were washed with distilled water and stored under -20°C for subsequent vertebral collection. Carcasses were defrosted at a room temperature, steamed over a boiling water bath for a few min., then vertebrae were separated carefully from the carcasses and cleaned with brush and distilled water. After drying in an oven, vertebral samples were digested with a conc. nitric acid and 70% perchloric acid mixture. P in the digested samples were determined by the molybdate method (Taussky and Shorr, 1953). Ca, Mg, K, Fe, Zn, Mn and Cu contents were determined with a Perkin-Elmer (Model 3300) atomic absorption spectrophotometer using flame absorption technique. Data, when applicable, were analyzed by Fisher's Protected Least Significance Difference test with a StatView 4.5 program for Macintosh computer ($p < 0.05$).

RESULTS AND DISCUSSION

Average final body weight of the fish increased with the increasing levels of dietary Ca supplement (Fig. 1, Table 3). A 0.05% Ca supplement (diet 2) to the basal diet (diet 1) increased the average final body weight, but the difference was not statistically significant. However, Ca supplements of more than 0.1% (diets 3, 4 and 5) to the basal diet significantly increased the average final body weight of the fish. A Ca supplement to a purified or fish meal diet improved the growth in tiger puffer (Furuichi *et al.*, 1997a, b; Hossain and Furuichi, 1998). Redlip mullet also required a dietary Ca supplement.*² Survival data was not related to the dietary Ca supplement in this study. Feed efficiency was low in the fish fed Ca unsupplemented diet 1, but increased in the fish fed other diets with different levels of Ca supplement.

A dietary Ca supplement did not affect the plasma protein and Ca contents of the fish (Table 4). Lower hematocrit values were detected in blood of the fish fed diets 4 and 5. Ca supplements of 0.1 to 0.4% in the diets 3 to 5 increased the Ca content of the liver

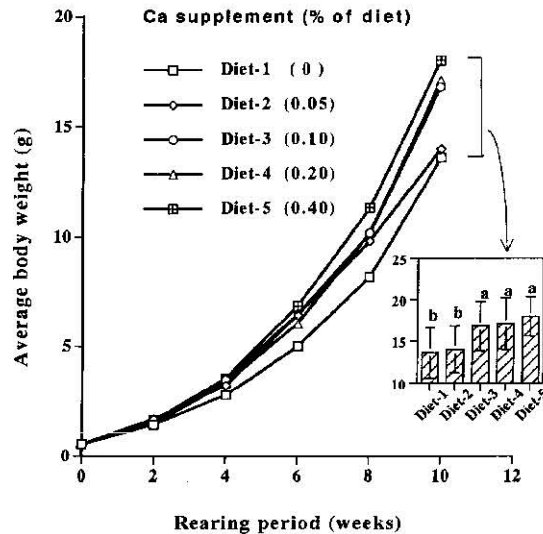


Fig. 1. Growth of giant croaker fed the experimental diets with different Ca levels. Different letters (inset) indicate significant differences ($p < 0.05$).

Table 3. Performances of giant croaker fed the experimental diets with different Ca levels

Diet no.	1	2	3	4	5
Supplemental Ca level (% of diet)	0	0.05	0.10	0.20	0.40
No. of fish at start	30	30	30	30	30
Survival rate (%)	81.5	88.9	85.2	96.3	88.9
Av. body wt. (g)					
Start	0.56 ± 0.11	0.56 ± 0.10	0.55 ± 0.10	0.55 ± 0.11	0.55 ± 0.11
End ^{*1}	13.6 ± 3.4 ^b	14.0 ± 2.8 ^b	16.8 ± 3.0 ^a	17.1 ± 3.1 ^a	18.0 ± 2.4 ^a
Av. wt. gain (%)	2330	2400	2960	3010	3170
Daily food consumption (%)	2.96	2.38	2.45	2.57	2.30
Feed efficiency (%)	84.4	108.1	107.8	106.7	110.1
Condition factor ^{*2}	1.61 ± 0.09	1.65 ± 0.11	1.66 ± 0.12	1.63 ± 0.07	1.64 ± 0.07

^{*1} Values (mean ± SD of all fish of each tank) bearing different letters are significantly different ($p < 0.05$).

^{*2} Condition factor = Body weight (g) × 100 / (body length in cm)³. No significant difference ($p > 0.05$; $n = 15$).

(Table 5). P, Mg, K, Fe, Zn and Mn contents of the liver were not affected by a dietary Ca supplement. However, liver Cu content increased with increasing dietary Ca levels. Hepatosomatic index showed no relation with dietary Ca supplements.

Ca content of vertebrae might be a suitable indicator of Ca status in fish. However, in this study, Ca content of vertebrae was not affected by dietary Ca (Table 6). Similar results were also found in our previous studies with tiger puffer and redlip mullet, where a lacking of dietary Ca suppressed the growth, but did not affect the vertebral Ca concentration. Robinson *et al.* (1984) reared tilapia in a Ca-free water and found no suppression of vertebral Ca content but a decrease in growth due to the lack of dietary Ca. They concluded that a dietary insufficiency of Ca decreased the growth but did not affect the vertebral mineralization. In this study, dietary Ca supplements up to 0.4% did not affect the P, Mg, K, Zn, Cu and Fe contents of vertebrae.

From the above study, it is apparent that a minimum of 0.1% Ca supplement to the diet is necessary for giant croaker for normal growth and feed utilization.

Table 4. Hematocrit, plasma protein and plasma Ca contents of giant croaker fed the experimental diets with different Ca levels*

Diet no.	1	2	3	4	5
Supplemental Ca level (% of diet)	0	0.05	0.10	0.20	0.40
Hematocrit (%)	28.4±2.3 ^a	30.0±2.5 ^a	28.7±2.9 ^a	24.9±2.2 ^a	24.1±1.7 ^b
Plasma protein (g/100 ml)	2.78±0.49 ^a	2.64±0.50 ^a	2.74±0.29 ^a	2.66±0.36 ^a	2.45±0.30 ^a
Plasma Ca (mg/100 ml)	9.9	9.8	10.0	10.1	10.0

* Values (mean±SD) in the same row bearing different letters are significantly different ($p < 0.05$; $n=10$).

Table 5. Mineral contents in the liver and hepatosomatic indices of giant croaker fed the experimental diets with different Ca levels (dry matter basis)

Diet no.	1	2	3	4	5
Supplemental Ca level (% of diet)	0	0.05	0.10	0.20	0.40
Mg (%)	0.077	0.079	0.085	0.089	0.094
K (%)	1.88	1.72	1.80	1.86	2.07
P (%)	1.14	1.03	1.16	1.11	1.11
Ca (μg/g)	141	120	189	172	177
Zn (μg/g)	60	61	66	69	69
Mn (μg/g)	6.7	6.5	6.6	6.4	6.7
Cu (μg/g)	8.8	8.1	9.9	9.4	10.4
Fe (μg/g)	148	174	148	161	178
HSI*	1.50±0.29	1.47±0.37	1.52±0.39	1.50±0.41	1.47±0.34

* Hepatosomatic index: liver wt. (g) × 100/body wt. (g). No significant difference ($p > 0.05$; $n=10$).

Table 6. Ash, lipid and mineral contents in the bone of giant croaker fed the experimental diets with different Ca levels*

Diet no.	1	2	3	4	5
Supplemental Ca level (% of diet)	0	0.05	0.10	0.20	0.40
Ash (%)	55.0	53.3	52.9	55.4	55.7
Lipid (%)	8.7	12.4	13.2	11.9	11.6
Ca (%)	20.0	19.9	19.2	19.7	20.9
P (%)	12.1	11.6	11.6	12.4	12.3
Mg (%)	0.60	0.59	0.56	0.67	0.65
K ($\mu\text{g/g}$)	115	104	117	107	116
Zn ($\mu\text{g/g}$)	91	87	89	87	83
Mn ($\mu\text{g/g}$)	68	62	65	67	64
Cu ($\mu\text{g/g}$)	15	13	16	16	14
Fe ($\mu\text{g/g}$)	75	79	68	72	67

* Dry matter basis. Analytical values of composite sample of vertebrae from all the fish of each tank.

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