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Effect of Deletion of Calcium Supplement from Purified Diet on Growth and Bone Mineralization in Red Sea Bream

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Young red sea bream (av. body wt. 11.5 g) were fed the purified casein based diets with or without calcium (Ca) supplement for 12 weeks at a water temperature of 20.0–24.0 °C. After the feeding trials, growth performances, blood characteristics and compositions of liver and bone were examined. Average final body weight, condition factor and hepatosomatic index were not affected by the unsupplementation of dietary Ca. A lacking of Ca supplement to the diet did not affect the haematological characteristics including plasma Ca and phosphorus (P) contents. Bone Ca content was independent of dietary Ca supplement. It appeared that young red sea bream adequately absorbed Ca from sea water and that Ca supplement to a purified diet might not be necessary.

INTRODUCTION

An appreciable amount of dissolved calcium (Ca) exists in aquatic environment, especially in sea water, which may be absorbed by fish to meet specific metabolic requirements. Therefore, limited attention has been paid to the studies on Ca nutrition of marine fish. However, our previous studies demonstrated a need for supplementation of dietary Ca in tiger puffer (Furuichi *et al.*, 1997a, b; Hossain and Furuichi, 1998) and redlip mullet.*[2] On the other hand, black sea bream appeared to satisfy their Ca requirements through absorption from surrounding sea water (Hossain and Furuichi, unpubl. data). Therefore, the present study was designed to evaluate the effect of deletion of Ca on growth and bone mineralization of red sea bream *Pagrus major*, one of the commercially important fish in Japan and adjacent areas.

MATERIALS AND METHODS

Experimental Diets and Feeding Regime

Two purified diets with or without Ca supplement were formulated (Table 1). Vitamin-free milk casein, dextrin and α-starch (gelatinized starch), and pollack liver oil were used as protein, digestible carbohydrate and lipid sources, respectively. A mixture of vitamins (Halver, 1957) was added to the diets. Ca (0.2%) was supplied only to the control diet through Ca-lactate. The ingredients were mixed thoroughly and an aliquot of water (20%) was added. Pellets were prepared with a laboratory pellet mill and cut into an appropriate size. The diets were then dried in an air flow drier at 60 °C for 60 min and

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stocked under −20 °C. The proximate and mineral compositions of the diets are presented in Table 2.

Young red sea bream were accustomed to a casein diet in indoor 150–*l* rectangular tanks for two weeks before the experiment was started. At the start of the experiment, the fish were weighed individually, selected and distributed to the tanks in such a manner that average body weight and size variation in two tanks were almost similar. The number of fish for a tank was 30 (average body weight 11.5 g). The fish were reared for 12 weeks, during which the experimental diets were fed 2 times a day at 0900 and 1530 h. Diets

Table 1. Composition of the experimental diets for red sea bream

Diet	cont.	no Ca
Ingredient (%)		
Casein	50	50
Amino acid mix.*1	4	4
α–Potato starch	7	7
Dextrin	10	10
Pollack liver oil	10	10
Vitamin mix.*2	3	3
Mineral mix.*3	5	5
Ca–lactate	1.54	–
Carboxymethylcellulose	4	4
α–Cellulose	5.46	7

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

*2 Halver's vitamin mixture (1957).

*3 Mineral mixture (in 100 g): Major element in g: KCl 7.68, $MgSO_4 \cdot 5H_2O$ 8.16, $NaH_2PO_4 \cdot 2H_2O$ 68.52, Fe–citrate 2.40 and cellulose 12.69; Minor element in mg: $AlCl_3 \cdot 6H_2O$ 90.0, $ZnSO_4 \cdot 7H_2O$ 264.0, $MnSO_4 \cdot 5H_2O$ 175.3, CuCl 15.7; KI 3.7 and $CoCl_2 \cdot 6H_2O$ 1.3.

Table 2. Proximate and mineral compositions of the experimental diets for red sea bream

Diet	cont.	no Ca
*Proximate composition (% dm)**		
Moisture	20.9	21.5
Crude protein	51.3	52.0
Crude lipid	9.1	9.3
Crude ash	5.1	5.0
*Mineral composition (dm)**		
Ca (%)	0.24	0.03
P (%)	1.00	1.05
K (%)	0.19	0.18
Mg (μg/g)	420	370
Fe (μg/g)	280	270
Zn (μg/g)	48	52
Mn (μg/g)	16	16
Cu (μg/g)	11	12

* dm, dry matter.

were offered as long as the fish continued to feed. Each tank was provided with adequate air and continuous sea water (3–4 *l*/min) supplies. Water temperature ranged from 20.0 to 24.0 °C during the experiment. Ca in the rearing water was around 400 mg/*l*. Tanks were cleaned daily by siphoning before the mourning feeding. Biweekly body weight of the fish was recorded after anesthetizing with MS–222. After every weighing, each tank was treated with sodium nifurstyrenate (sodium salt of 5–nitro–2–(p–carboxy styryl)–furan) to prevent any bacterial attack that may be caused due to handling (Sugimoto *et al.*, 1976).

Sampling and Analysis

At the end of the experiment, the body weight and body length of the fish were measured individually to calculate the condition factor. Blood samples were collected from the cuvierian duct with a 1 ml heparinized syringe. Each liver was weighed to calculate the hepatosomatic index. Pooled samples of all the liver and fish of each treatment were stored at −20 °C for chemical analysis and bone collection, respectively.

Hematocrit, plasma protein and hemoglobin were measured with a Kubota Hematocrit Reader (Hesser, 1960), an ATAGO Serum–Protein Refractometer and a spectrophotometer (Hitachi, U–2000) by the cyanmethemoglobin method (Wintrobe, 1956), respectively. Plasma Ca, P, Mg, Fe and triglyceride were measured using a Rapid Blood Analyzer (RaBA Super, Chugai Pharmaceutical Co.).

Bone was separated from the whole body after steaming on a boiling water bath for a few minutes, then cleaned, washed with distilled water and dried in an oven. The dried bone samples were ground in small particles and subjected to digestion with nitric acid and perchloric acid. Mineral contents, except P, in the digested samples were determined by an atomic absorption spectrometer (Perkin–Elmer 3300) using flame absorption techniques. P in the digested bone samples was determined spectrophotometrically by the molybdate method (Taussky and Shorr, 1953).

Student's T–test was applied to determine the significance in difference between the treatment means ($p < 0.05$).

RESULTS

Growth performances and feed utilization of red sea bream fed the experimental diets are shown in Fig. 1 and Table 3. The survival rates at the end of the experiment were 93.3% and 100% in the fish fed the control diet and Ca unsupplemented diet, respectively. No significant difference was observed in the average final body weight between the two treatment groups. Daily feed consumption was similar in both groups. A deletion of Ca supplement from the diet showed slightly lower feed efficiency in the fish compared to the fish fed the control diet. However, condition factor was not affected by dietary Ca.

Hematocrit values, and hemoglobin and plasma protein contents were similar in both groups fed the control and Ca unsupplemented diets (Table 4). A deletion of Ca supplement from the diet slightly increased the plasma triglyceride. Plasma P, Ca and Fe were not affected by the lacking of Ca supplement to the diet. However, slightly lower plasma Mg content was detected in the fish fed the control diet with Ca supplement.

No prominent differences were detected in liver moisture, protein, lipid, glycogen and ash contents among the fish fed the control or Ca unsupplemented diets (Table 5). Hepatosomatic index appeared to be independent of dietary Ca supplement.

The results of bone analyses are presented in Table 6. Bone lipid and ash contents were similar in both groups. Ca and P contents of the bone appeared to be independent of dietary Ca supplement. A deletion of supplemental Ca from the diet did not affect the bone Mg, K, Fe, Zn and Mn contents. Cu content of the bone was markedly increased by the deletion of Ca supplement from the diet.

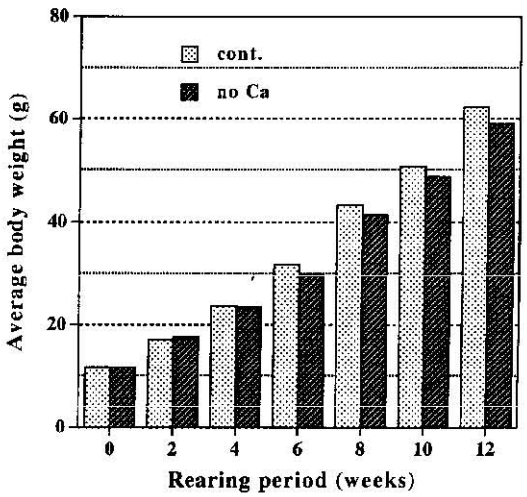

Fig. 1. Fortnightly growth of red sea bream fed the experimental diets with or without Ca supplement.

Table 3. Growth and feed utilization of red sea bream fed the experimental diets with or without Ca supplement

Diet	cont.	no Ca
No. of fish at initial	30	30
Survival rate (%)	93.3	100
Av. body wt. (g)		
Initial	11.6±0.9	11.5±0.8
Final*[1]	62.3±8.9	59.0±7.7*[2]
Weight gain(%)	437	413
Daily feed consumption (% of body wt.)	2.28	2.27
Feed efficiency (%)	91.8	84.8
Condition factor*[3]	3.61±0.18	3.61±0.17*[2]

*[1] Calculated from all the fish of each tanks.

*[2] No significant difference ($P>0.05$).

*[3] Condition factor: Body weight (g)×100/(body length in cm)^3, (n=15).

Table 4. Blood characteristics of red sea bream fed the experimental diets with or without Ca supplement

Diet		cont.	no Ca
Hematocrit	(%)	28.9±3.5	28.6±3.0*
Hemoglobin	(g/100 ml)	4.7±1.1	4.9±1.1*
Plasma protein	(g/100 ml)	4.3±0.6	4.4±0.4*
Plasma triglyceride	(mg/100 ml)	448	512
Plasma P	(mg/100 ml)	11.7	10.6
Plasma Ca	(mg/100 ml)	13.8	13.6
Plasma Mg	(mg/100 ml)	4.5	6.1
Plasma Fe	(mg/100 ml)	86	79

* No significant difference ($p>0.05$; n=10).

Table 5. Proximate composition of liver and hepatosomatic index of red sea bream fed the diets with or without Ca supplement

Diet		cont.	no Ca
Moisture	(%)	65.0	65.4
Crude protein	(% dm)*1	30.3	30.1
Crude lipid	(% dm)	39.4	40.5
Glycogen	(% dm)	18.9	23.1
Crude ash	(% dm)	3.1	3.2
HSI*2		2.33±0.40	2.32±0.37

*1 dm, dry matter.

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

Table 6. Lipid, ash, and mineral composition of bone of red sea bream fed the experimental diets with or without Ca supplement*

Diet		cont.	no Ca
Crude lipid	(%)	20.6	19.1
Crude ash	(%)	54.3	55.5
Ca	(%)	27.5	26.9
P	(%)	11.4	10.5
Mg	(%)	0.23	0.24
K	(μg/g)	122	125
Fe	(μg/g)	50.2	54.6
Zn	(μg/g)	66.7	64.4
Mn	(μg/g)	25.4	27.7
Cu	(μg/g)	1.8	2.5

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

DISCUSSION

The similar growth in the fish fed the control and Ca unsupplemented diet revealed that red sea bream might absorb adequate Ca from sea water for its normal growth and feed utilization. It is well known that fish have a capability to absorb Ca from surrounding water (Lovelace and Podoliak, 1952; Ichikawa and Oguri, 1961; Templeton and Brown, 1963; Andrews *et al.*, 1973; Lall, 1979; Love, 1980; Cowey, 1992). However, it is important to find out whether Ca absorption from sea water is adequate for fish or not. Sakamoto and Yone (1976) reported that Ca supplement was dispensable in a purified diet for red sea bream. Black sea bream, a member of same family sparidae as red sea bream, appeared to absorb adequate Ca from sea water for growth and bone mineralization (Hossain and Furuichi, unpubl. data). Environmental Ca was found not to be sufficiently available in tiger puffer (Furuichi *et al.*, 1997a, b; Hossain and Furuichi, 1998) and redlip mullet.*[2] On the other hand, from the growth, hematological, and liver and bone mineralization data in the present study, it appeared that Ca absorption from sea water may be adequate for red sea bream.

In the study with radio isotope, we observed that red sea bream of average body weight 1.0 g had increased the absorption of ^{45}Ca from sea water when Ca was deleted from a purified diet (Hossain and Furuichi, unpubl. data). Sakamoto (1981) also reported that absorption of radioactive Ca in red sea bream increased with the decrease of dietary Ca levels. Therefore, the present investigation suggests that a dietary Ca supplement may not be necessary for young red sea bream.

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