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Necessity of dietary calcium supplement in file fish (*Monacanthus cirrhifer*)

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Abstract
File fish, *Monacanthus cirrhifer*, juveniles with initial mean body weight of 0.27 g were fed purified diets with or without calcium (Ca) supplement for 10 weeks at a water temperature of 27.0±1.4°C. Growth was significantly low in fish fed diet without Ca supplement than fish fed diet with Ca supplement. Feed efficiency and condition of fish were also significantly decreased in absence of a dietary Ca supplementation. Minerals contents of bone were similar in both the treatment groups and did not appear as a suitable indicator of Ca requirement. It appeared that Ca supplement to the purified diet is necessary for file fish for their proper growth and feed utilization.

Key words: File fish, Ca requirement, Bone mineralization

Introduction

The importance of Ca as an important nutrient for fish is well established. Until recent time, it was generally accepted that a dietary Ca supplementation may not be necessary for fish. Because, it has been reported that different species of fish can easily absorb calcium (Ca) from surrounding water through the gills, intestine and other organs (Love 1980, Ichii and Mugiya 1983, Ishihara and Mugiya, 1987, Takagi and Yamada 1992). However, our recent studies revealed that some marine species require a Ca supplement to the diet (Hossain and Furuichi 1998, 1999, 2000a,b). Therefore, further study is necessary to investigate whether Ca absorption from seawater is sufficient for marine fishes, i.e. whether they need a dietary Ca supplement. Accordingly, important aquaculture species need be tested for dietary Ca requirements. In the present study, the necessary of dietary Ca supplement for file fish *(Monacanthus cirrhifer)* has been investigated.

Materials and methods

Experimental diets

The composition of the experimental diets is shown in Table 1. Best quality ingredients were used for diet preparation. Protein sources were 50% vitamin-free casein. Lipid and sugar sources were pollack liver oil and starch and dextrin, respectively. Vitamin mixture, amino acid mixture and mineral mixture were prepared separately and added to the diets. Ca-lactate was supplied only to the control diet (with Ca) to obtain 0.3% Ca. Pollack liver oil was weighed upon the portion of casein weighed previously and was mixed with other ingredients. Ingredients were mixed 500 g at a time to facilitate a complete mixing. The mixed powder was then mixed with 20% distilled water and was pelleted with a laboratory type pelleter with sieve of appropriate mesh size. Pelleted diets were then cut into small spices appropriate to the mouth size of the fish to be fed. Then the feed was half dried with an air flow dryer at 60^{0}C and stocked at -20^{0}C until use. The proximate and mineral compositions of the experimental diets are shown in Table 2.

Table 1. Composition of the experimental diets for file fish

Diet	Control	No Ca supplement
Ingredient (%)		
Casein[1]	50	50
Amino acid premix[2]	4	4
Starch, pregelatinised	7	10
Dextrin	10	15
Pollack liver oil	10	10
Vitamin premix[3]	3	3
Mineral premix[4]	6	6
Carboxymethylene cellulose	4	5
Ca-lactate	2.308	-
Alpha-cellulose	3.692	6

[1] Vitamin free, from milk, 200 mg Ca/kg casein.

[2] Amino acid premix (g/kg diet): Arginine-HCl, 10; alanine, 10; glycine, 10; aspartate-Na, 10.

[3] Vitamin premix (mg/kg diet): Thiamine-HCl, 60; riboflavin, 200; pyridoxine-HCl, 40; vitamin B_{12}, 0.09; nicotinic acid, 800; Ca-pantothenate, 280; inositol, 4000; biotin, 6; folic acid, 15; PABA, 400; choline chloride, 8000; ascorbic acid, 2000; alpha-tocopherol, 400; menadione, 40; beta-carotene, 12; vitamin D_3, 0.05.

[4] Mineral mixture (mg/kg diet): KCl 3840; $MgSO_4 \cdot 5H_2O$ 4080; $NaH_2PO_4 \cdot 2H_2O$, 34,260; Fe-citrate, 1200; $AlCl_3.6H_20$, 45; CuCl, 7.9; KI, 1.9; $CoCl_2.6H_2O$, 0.7.

Table 2. Proximate and mineral compositions of the experimental diets for file fish

Ingredient (%)	Diet	
	Control	No Ca supplement
Proximate composition (% dm)[*1]		
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)		
Calcium (%)	0.34	0.03
Phosphorus (%)	1.00	1.05
Potassium (%)	0.19	0.18
Magnesium (μg/g)	385	390
Iron (μg/g)	260	255
Zinc (μg/g)	40.2	40.5
Manganese (μg/g)	22.4	23.0
Copper (μg/g)	10.2	12.4

[*1]dm = dry matter.

Fish and rearing procedure

Juvenile file fish attaching with the floating sea weeds/debris were collected using a scoop net from the open bay. The fish of similar size were shorted and adapted in rearing conditions for 2 weeks before starting of the experiment. Fish were fed the control diet during adaptation. The rearing experiment was carried out in 100-L round polycarbonate tanks with a continuous water flow of 1.5-2.0 L/min. Water temperature was 27.0±1.4^{0}C. The rearing water contained approximately 400 mg Ca/L. A daily light:dark cycle of 12 h:12 h was maintained. At the beginning of the feeding trial, fish (average initial weight of 0.27 g) were weighed and distributed to 6 rearing tanks (three tanks for each treatment) as a group of 100 fish. The fish were fed the experimental diets to satiation twice a day for 10 weeks.

Sample collection

Final sampling was done after 16 h starving. Any abnormalities in external features were monitored and recorded. Fish of each tank were counted to record survivability. Then body length and weight were measured. After removing the internal organs, the whole body was washed with distilled water and preserved at -20^{0}C for bone collection. For bone collection, preserved whole body carcasses were defrosted at room temperature, and then steamed on a boiling water bath for a few minutes. Vertebral column was separated from the body and cleaned in distilled water using a brush. Then each of the vertebrae was separated from the vertebral column and all together were rinsed vigorously to be cleaned properly. Finally, the vertebral bone samples were washed with distilled water for several times, and soaked on a cleaned filter paper. After drying in an

oven for 24 h at 105^{0}C, the samples were ground in fine grains using a mortar and pestle. The samples were then preserved in clean glass vials for further analysis.

Analytical methods

The proximate composition of experimental diets was analyzed according to the methods given in Association of Official Analytical Chemists (AOAC, 1980). For mineral determination, dried bone samples were digested with wet digestion method with a nitric acid-perchloric acid mixture. Minerals, except phosphorus, in the digested samples were determined with an Atomic Absorption Spectrophotometer (Perkin-Elmer 3300, Perkin Elmer, USA). Phosphorus in the digested samples was determined colorimetrically according to the molybdate method described by Taussky and Shorr (1953).

Statistical analysis

Data were analyzed for significant differences with student T-test using a statistical package (SPSS package programme).

Results and discussion

After the 10 weeks of rearing period, average final body weight and weight gain of file fish fed the diet without Ca supplement were significantly lower than that fed the diet with a Ca supplement (control diet) as shown in Table 3. Survival rate was 78.3 and 82.0 in fish fed control diet and Ca unsupplemented diet, respectively and was not statistically different from each other. Significantly lower condition factor was observed in fish fed Ca unsupplemented diet compared to the control diet. A deletion of Ca from diet decreased the feed efficiency of file fish. There were no differences in ash and mineral contents of vertebrae between two treatment groups except that an unsupplementation of Ca to the diet decreased the Fe content of vertebrae (Table 4).

Table 3. Growth and feed utilization of file fish after 10 months rearing period fed the experimental diets with or without Ca supplement

	Diet	
	Control	No Ca supplement
No. of fish at initial	100	100
Survival rate (%)	78.5	82.0
Av. body weight at initial (g)	0.27±0.02	0.27±0.02
Av. final body weight (g)[1]	2.47±0.47a	2.09±0.55b
Weight gain (%)[1]	815±13a	674±15b
Condition factor[1,2]	3.71±0.06a	3.57±0.05b
Feed efficiency (%)	91.8±1.8a	87.9±2.1b

[1]Significant difference ($p<0.05$), [2]Condition factor: Body weight (g) x 100/(total length in cm)^3.

Table 4. Ash and mineral composition of bone of file fish[1]

	Diet	
	Control	No Ca supplement
Crude ash (%)	55.8±0.7	55.5±0.7
Calcium (%)	22.4±0.3	21.9±0.5
Phosphorus (%)	10.2±0.7	10.5±0.3
Magnesium (%)	0.69±0.08	0.69±0.26
Potassium (μg/g)	92.3±13.9	99.8±18.2
Iron (μg/g)[2]	189±20a	165±12b
Zinc (μg/g)	120.0±1.8	116.9±6.8
Manganese (μg/g)	97.9±1.2	102.4±5.9
Copper (μg/g)	7.8±0.2	7.6±0.8

[1]Dry matter basis. Average values (mean±SD) of composite sample of bones from all the fish of each tank. [2]Significant difference.

It has been reported that fish can actively absorb Ca from surrounding water (Ogino and Takeda 1978, Love 1980, Takagi and Yamada 1992). Therefore, it is generally accepted that a dietary Ca supplement may not be necessary for marine fishes. However in the present study, the poor growth of fish feed Ca unsupplemented diet indicated that Ca absorption from seawater by file fish was not sufficient for their growth. Similar poor growth was observed in tiger puffer, Japanese flounder and scorpion fish in some previous studies (Hossain and Furuichi 1998, 2000a,b), which supports the findings of the present experiment. When experiencing Ca inadequacy, file fish maintained bone Ca content (Table 4), probably providing inadequate Ca for other physiological process leading to poor growth and food utilization. From the above discussion, it is clear that Ca uptake from seawater is not sufficient for proper growth and feed utilization of file fish and they need a dietary Ca supplement.

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