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Essentiality of dietary calcium supplement in fingerling scorpion fish (*Sebastiscus marmoratus*)

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Abstract

Essentiality of dietary calcium (Ca) supplement was investigated in fingerling scorpion fish. Four purified diets were formulated with supplementation of 0.2% Ca from Ca-lactate, no supplemental Ca, and 0.2 and 2.5% Ca from tricalcium phosphate (TCP), respectively. Each experimental diet was fed to three replicate groups of fish (mean weight, 0.8 g) for 12 weeks at a water temperature of 24 ± 0.2°C. Significantly lower weight gain was observed in fish fed the Ca-unsupplemented diet compared to the control diet with 0.2% Ca supplement from Ca-lactate. The growth of fish fed the diet with a low level (0.2%) of Ca from TCP was similar to that of fish fed the control diet. However, a high level (2.5%) of dietary Ca from TCP significantly decreased growth. Calcium and phosphorus contents in whole body and vertebrae were similar in fish fed all the diets. A high level of dietary Ca from TCP decreased some trace elements in whole body, liver and vertebrae. Results suggest that scorpion fish do not obtain adequate Ca from seawater for normal growth and a dietary Ca supplement is necessary. Although this species can use a low level of dietary TCP as a source of Ca for growth, excessive dietary TCP depresses growth and negatively affects the contents of some trace elements in tissues. Readily available Ca (mono- or dibasic Ca) supplement to the diet appears to be essential for optimum growth and for maintaining normal mineral levels in tissues of scorpion fish.

Keywords: Scorpion fish; Calcium; Growth; Bone mineralization

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1. Introduction

It is generally accepted that fish can absorb calcium (Ca) from the surrounding water to fulfill their metabolic requirements (Lovelace and Podoliak, 1952; Ichikawa and

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Oguri, 1961; Love, 1980). Seawater contains an appreciable amount of dissolved Ca that may be a Ca source to marine fish. Therefore, very few studies have been conducted on the dietary Ca requirements of marine fish. In previous studies we observed that the tiger puffer (*Takifugu rubripes*), giant croaker (*Nibea japonica*) and redlip mullet (*Liza haematocheila*) could not absorb adequate Ca from seawater to fulfill their requirements (Furuichi et al., 1997; Hossain and Furuichi, 1999a, 2000). In contrast, Ca absorption from seawater by red sea bream (*Pagrus major*) and black sea bream (*Acanthopagrus schlegeli*) was adequate to fulfill their requirements (Sakamoto and Yone, 1976; Hossain and Furuichi, 1999b). It appears that the utilization of Ca from seawater by marine fish varies. In fish meal, Ca combines with phosphorus (P) and forms the complex hydroxyapatite and/or tricalcium phosphate (TCP). Due to the complexity of its structure, Ca and P from TCP have been reported to be less available to some fish species (Takamatsu et al., 1975; Shitanda et al., 1979; Watanabe et al., 1980; Hossain and Furuichi, 1998). In the present study, we investigated whether or not fingerling scorpion fish need dietary Ca supplementation for optimum growth, feed utilization and for maintaining normal mineral content in tissues.

2. Materials and methods

2.1. Experimental diets

Four experimental diets were formulated from purified ingredients with casein (vitamin free, Ca 0.2 g kg^{-1}; Wako, Japan) as a protein source and pollack liver oil

Table 1
Composition of the experimental diets for scorpion fish

Diet no.	1	2	3	4
Ca supplement [%] (source)[a]	0.2 (Ca-L)	No Ca	0.2 (TCP)	2.5 (TCP)
Ingredient [%]				
Casein (vitamin-free)	50	50	50	50
Alpha-starch	7	7	7	7
Dextrin	10	10	10	10
Pollack liver oil	10	10	10	10
Vitamin mix[b]	3	3	3	3
Mineral mix[c]	5	5	5	5
Ca-lactate	1.54	–	–	–
$Ca_3(PO_4)_2$	–	–	0.52	6.52
Feeding stimulants[d]	2	2	2	2
Carboxymethylcellulose	4	4	4	4
Alpha-cellulose	7.46	9.00	8.48	2.48

[a] Ca-L, Ca-lactate; TCP, tricalcium phosphate.

[b] Vitamin mixture (mg/kg diet): thiamin-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.

[c] Mineral mixture (mg/kg diet): KCl, 3840; $MgSO_4 \cdot 5H_2O$, 4080; $NaH_2PO_4 \cdot 2H_2O$, 34,260; Fe-citrate, 1200; $AlCl_3 \cdot 6H_2O$, 45; $ZnSO_4 \cdot 7H_2O$, 132; $MnSO_4 \cdot 5H_2O$, 877; CuCl, 7.9; KI, 1.9; $CoCl_2 \cdot 6H_2O$, 0.7.

[d] Feeding stimulants (g/kg diet): inosine, 1.92; alanine, 2.40; methionine, 0.56; proline, 3.66; serine, 1.46; arginine·HCl, 10.00.

(Riken Vitamin, Japan) as a lipid source (Table 1). A vitamin mixture and a Ca-free mineral mixture were added to the diets. Diet 1 (control diet) contained 0.2% Ca from Ca-lactate. Diet 2 was without any Ca supplement. Diets 3 and 4 were supplemented with low (0.2%) and high (2.5%) levels of Ca, respectively, from TCP [$Ca_3(PO_4)_2$]. A low level of Ca in diet 3 was the same as that in control diet 1 and a high level of Ca in diet 4 was similar to that derived from 60% fish meal in a fish meal diet. A mixture of feeding stimulants was added to all the diets to increase acceptability (Takaoka et al., 1990). Ingredients for each diet were mixed thoroughly with an appropriate amount of deionized water and pelleted by passing the moistened mixture through a laboratory pellet mill. Diets were dried at 60°C for 1 h in an air drying oven and stored at −20°C until fed. Proximate and mineral compositions of the diets are shown in Table 2.

2.2. Fish and feeding trial

Fingerling scorpion fish (*Sebastiscus marmoratus*) were transported from Yamaguchi Mariculture (Japan) to the Fishery Research Laboratory, Kyushu University and reared in a 500-l tank until use. Prior to the start of the experiment, fish were transferred to indoor 150-l rectangular tanks and acclimated for 2 weeks to experimental conditions during which they were fed the control diet 1. In the beginning of the experiment, fish were weighed individually, selected and distributed into each of the 150-l rectangular tanks (initial weight: 0.8 g, 30 fish per tank, three replicates per treatment). Tanks were continuously supplied with sand-filtered seawater (2–3 l min^{-1} $tank^{-1}$, salinity 32–34 g l^{-1}, Ca approximately 400 mg l^{-1}) and aeration. A daily light:dark cycle of 12:12 h was maintained throughout the experiment. Rearing temperature was maintained at 24.0 ± 0.2°C.

Table 2

Proximate and mineral composition of the experimental diets

Diet no.	1	2	3	4
Ca supplement [%] (source)[a]	0.2 (Ca-L)	No Ca	0.2 (TCP)	2.5 (TCP)
Proximate composition				
Moisture [%]	21.3	19.9	21.1	21.8
Crude protein [% dm][b]	51.0	51.2	52.4	51.0
Crude lipid [% dm]	7.9	7.9	8.4	7.4
Ash [% dm]	5.0	4.8	5.0	10.7
Mineral content (dm)				
Ca [%]	0.22	0.02	0.23	2.50
P [%]	1.04	0.97	1.08	2.40
K [%]	0.22	0.21	0.22	0.21
Mg [$\mu g\ g^{-1}$]	445	412	393	411
Fe [$\mu g\ g^{-1}$]	254	251	259	260
Zn [$\mu g\ g^{-1}$]	58	51	56	56
Mn [$\mu g\ g^{-1}$]	19	19	17	20
Cu [$\mu g\ g^{-1}$]	10	8	11	9

[a] Ca-L, Ca-lactate; TCP, tricalcium phosphate.

[b] dm, Dry matter.

Fish were fed the experimental diets three times a day to near satiation for 12 weeks. Fish were weighed once every 2 weeks to monitor growth and health. After weighing, fish were bathed in 1 mg l^{-1} sodium nifurstyrenate (sodium salt of 5-nitro-2-(*p*-carboxy styryl)-furan) to reduce the risk of bacterial infections as a result of stress during the weighing process (Sugimoto et al., 1976).

2.3. Sample collection and analysis

At the end of the feeding trial, all fish were individually weighed and body lengths were measured. Livers were collected from 10 fish in each tank, weighed individually and stored as a pooled sample for subsequent analyses. After removing other viscera, the 10 carcasses (from which livers had been collected) were washed with distilled water and stored at −20°C for subsequent vertebral collection. The other 10 carcasses from each tank were pooled, washed with distilled water, and stored at −20°C for whole body mineral determination.

For vertebral collection, carcasses were defrosted at room temperature, and then steamed over boiling water. Vertebrae were separated from whole carcasses, washed with distilled water to remove adhering tissues and dried in an oven at 110°C for 2 h. Dried samples were ground to fine particles and stored at −20°C to be used for mineral determination.

Proximate compositions of diets, whole body and vertebrae were analyzed as follows. Lipid content was determined by ether extraction method, protein by Kjeldahl method, ash was quantified after combustion at 560°C for 12 h and moisture content was determined after heating at 105°C until constant weight.

Mineral determinations were performed on diet, whole body, liver and vertebral samples after wet digestion with a nitric acid–perchloric acid mixture. All the minerals except P in the digested samples were determined with an atomic absorption spectrophotometer (Perkin-Elmer 3300, Perkin Elmer, USA) using flame absorption techniques.

Table 3
Weight gain, feed efficiency, condition factor and survival of scorpion fish fed the experimental diets for 12 weeks[1]

Diet no.	1	2	3	4
Ca supplement [%] (source)[2]	0.2 (Ca-L)	No Ca	0.2 (TCP)	2.5 (TCP)
Initial body wt. [g]	0.82 ± 0.08	0.82 ± 0.09	0.82 ± 0.08	0.83 ± 0.09
Final body wt. [g]	5.28 ± 0.72^a	4.84 ± 0.58^b	5.24 ± 0.76^a	4.87 ± 0.65^b
Average wt. gain [%]	544 ± 23^a	490 ± 24^b	539 ± 20^a	487 ± 26^b
Feed efficiency [%][3]	72.8 ± 1.9^a	68.3 ± 0.9^b	71.1 ± 1.2^a	67.2 ± 0.6^b
Condition factor[4]	3.12 ± 0.20^a	2.98 ± 0.19^b	3.06 ± 0.14^a	3.11 ± 0.25^a
Survival [%]	92.2	93.3	97.8	94.4

[1] Average of three replicate groups of fish. Means(± S.D.) in the same row with different superscripts are significantly different ($P < 0.05$) as determined by Fisher's PLSD test.

[2] Ca-L, Ca-lactate; TCP, tricalcium phosphate.

[3] Wet weight gain (g) × 100/dry feed intake (g).

[4] Body weight (g) × 100/(body length in cm)^3.

Table 4
Whole body moisture, lipid, ash and mineral content of scorpion fish fed the experimental diets for 12 weeks[1]

Diet no.	1	2	3	4
Ca supplement [%] (source)[2]	0.2 (Ca-L)	No Ca	0.2 (TCP)	2.5 (TCP)
Moisture [%]	69.9 ± 0.5	70.1 ± 0.4	70.0 ± 0.7	70.3 ± 0.5
Crude lipid [% dm][3]	23.1 ± 0.3	23.6 ± 0.2	23.4 ± 0.4	23.0 ± 0.3
Ash [% dm]	16.7 ± 0.2	16.7 ± 0.1	16.7 ± 0.5	17.8 ± 0.2
Ca [% dm]	5.39 ± 0.37	5.44 ± 0.18	5.52 ± 0.39	5.61 ± 0.40
P [% dm]	3.35 ± 0.09	3.25 ± 0.16	3.27 ± 0.16	3.37 ± 0.26
K [% dm]	1.50 ± 0.08	1.45 ± 0.10	1.42 ± 0.07	1.44 ± 0.01
Mg [μg g^{-1} dm]	210 ± 4	210 ± 7	200 ± 6	210 ± 5
Fe [μg g^{-1} dm]	156 ± 4	151 ± 3	157 ± 2	155 ± 2
Zn [μg g^{-1} dm]	70.1 ± 3.1^a	71.7 ± 2.8^a	71.7 ± 1.8^a	47.7 ± 4.0^b
Mn [μg g^{-1} dm]	10.8 ± 0.8^a	11.4 ± 1.1^a	11.8 ± 1.4^a	8.1 ± 1.5^b
Cu [μg g^{-1} dm]	10.9 ± 1.6	11.8 ± 1.8	12.7 ± 1.3	11.7 ± 1.5

[1] Average of three replicate groups of fish. Means(± S.D.) in the same row with different superscripts are significantly different ($P < 0.05$) as determined by Fisher's PLSD test.
[2] Ca-L, Ca-lactate; TCP, tricalcium phosphate.
[3] dm, Dry matter basis.

P in the samples was determined colorimetrically according to the molybdate method described by Taussky and Shorr (1953).

Data were subjected to analysis of variance and Fisher's protected least significant difference test (Fisher's PLSD test) to determine difference in means ($P < 0.05$).

3. Results

Weight gain of scorpion fish fed the Ca-unsupplemented diet 2 was significantly lower than that of fish fed the control diet 1 with a Ca supplement from Ca-lactate

Table 5
Liver mineral composition of scorpion fish fed the experimental diets for 12 weeks[1]

Diet no.	1	2	3	4
Ca supplement [%] (source)[2]	0.2 (Ca-L)	No Ca	0.2 (TCP)	2.5 (TCP)
Ca [μg g^{-1}]	120 ± 4.6	114 ± 5.8	113 ± 5.1	122 ± 4.8
P [%]	0.47 ± 0.01	0.45 ± 0.02	0.46 ± 0.00	0.45 ± 0.01
K [%]	1.14 ± 0.01	1.07 ± 0.03	1.10 ± 0.01	1.07 ± 0.01
Mg [μg g^{-1}]	520 ± 22	540 ± 15	550 ± 12	520 ± 11
Fe [μg g^{-1}]	91 ± 3^a	92 ± 4^a	90 ± 2^a	75 ± 3^b
Zn [μg g^{-1}]	65 ± 1.1^a	64 ± 0.8^a	63 ± 0.8^a	56 ± 1.1^b
Mn [μg g^{-1}]	4.9 ± 0.3	5.0 ± 0.6	5.6 ± 0.3	5.3 ± 0.2
Cu [μg g^{-1}]	9.5 ± 0.2	9.7 ± 0.2	9.5 ± 0.1	9.3 ± 0.4

[1] Average of three replicate groups of fish. Data for mineral composition are on dry matter basis. Means(± S.D.) in the same row with different superscripts are significantly different ($P < 0.05$) as determined by Fisher's PLSD test.
[2] Ca-L, Ca-lactate; TCP, tricalcium phosphate.

Table 6
Ash, lipid and mineral content of vertebrae of scorpion fish fed the experimental diets for 12 weeks[1]

Diet no.	1	2	3	4
Ca supplement [%] (source)[2]	0.2 (Ca-L)	No Ca	0.2 (TCP)	2.5 (TCP)
Crude lipid [%]	12.3 ± 0.3	13.0 ± 0.2	12.0 ± 0.6	12.8 ± 0.5
Ash [%]	62.5 ± 0.5	63.4 ± 0.4	62.4 ± 0.6	61.8 ± 0.8
Ca [%]	22.1 ± 1.1	21.3 ± 1.3	22.3 ± 0.8	22.4 ± 1.1
P [%]	13.0 ± 0.1	12.5 ± 0.6	13.1 ± 0.2	13.3 ± 0.6
Mg [%]	0.83 ± 0.04	0.83 ± 0.04	0.85 ± 0.06	0.84 ± 0.06
K [$\mu g\ g^{-1}$]	163 ± 3	170 ± 8	165 ± 3	169 ± 9
Fe [$\mu g\ g^{-1}$]	128 ± 4^a	121 ± 6^a	124 ± 4^a	99 ± 5^b
Zn [$\mu g\ g^{-1}$]	164 ± 8^a	162 ± 8^a	158 ± 2^a	76 ± 5^b
Mn [$\mu g\ g^{-1}$]	34.6 ± 1.9^a	33.7 ± 0.9^a	35.2 ± 1.2^a	21.6 ± 1.0^b
Cu [$\mu g\ g^{-1}$]	6.5 ± 0.4^a	4.4 ± 0.7^b	4.2 ± 0.5^b	3.9 ± 0.8^b

[1] Average of three replicate groups of fish. Data are on dry matter basis. Means(±S.D.) in the same row with different superscripts are significantly different ($P < 0.05$) as determined by Fisher's PLSD test.

[2] Ca-L, Ca-lactate; TCP, tricalcium phosphate.

(Table 3). The Ca-unsupplemented diet also resulted in poorer feed efficiency and condition factor values. The Ca-unsupplementated diet had no effect on the mineral content in whole body, liver and vertebrae (Tables 4–6) except a decreased Cu content of vertebrae. A low (0.2%) Ca supplement from TCP to the diet 3 increased weight gain, feed efficiency and condition factor similar to those of diet 1. Diet 3 also did not affect the mineral content of whole body, liver and vertebrae. However, a high level (2.5%) of Ca supplement from TCP in diet 4 significantly decreased weight gain and feed efficiency of the fish compared to diet 1. A high level of dietary TCP significantly affected some mineral contents in the tissues, such as Zn in whole body, liver and vertebrae, Mn in whole body and vertebrae, Fe in liver and vertebrae and Cu in vertebrae.

4. Discussion

A lack of Ca supplementation to the diet resulted in significantly poorer growth and feed efficiency in scorpion fish. This is in contrast with the generally accepted view that fish do not require a Ca supplement, because they can easily absorb Ca from the surrounding water (Lovelace and Podoliak, 1952; Ichikawa and Oguri, 1961; Templeton and Brown,1963; Love, 1980). Dietary Ca is not required for some fish species held in freshwater such as common carp, rainbow trout, chum salmon, channel catfish and guppy (*Poecilia reticulata*) (Ogino and Takeda, 1976, 1978; Lovell and Li, 1978; Watanabe et al., 1980; Shim and Ho, 1989). On the other hand, the present study indicates that Ca uptake from seawater was not adequate for proper growth of scorpion fish. Although seawater contains a large amount of dissolved Ca, scorpion fish may need dietary Ca in addition to the Ca absorbed from seawater. Takagi et al. (1989) observed that both water and dietary Ca were necessary for normal calcification of regenerating scales in tilapia. Some marine fish such as tiger puffer, giant croaker and redlip mullet also showed a necessity for dietary Ca supplementation when fed purified or semi-purified diets (Hossain and Furuichi, 1998, 1999a, 2000). In contrast, Sakamoto and Yone (1976) reported that a dietary Ca supplement was dispensable for red sea bream fed a purified diet. A dietary Ca supplement was reported not to be essential in tilapia (*Tilapia mossambica*) when they are reared in artificial seawater (Boroughs et al., 1957). Therefore, the Ca requirements in marine fishes appears to be species-specific. Tomiyama et al. (1956) investigated the absorption of ^{45}Ca from seawater in marine fishes, where the highest absorption rate was noticed in the sardine (*Sardinia melanostica*) followed with the scomber (*Scomber japonicus*) and globe fish (*Sphaeroides alboplumbeus*), and the lowest in the scorpaenoid fish, which indicated that the more active the fish is, the more radiocalcium is absorbed. The necessity of dietary Ca supplement found for scorpion fish (a sluggish fish and a member of scorpaenoid) in the present study may be associated with their low absorption of Ca from seawater. In this study, although growth was poor in fish fed the Ca-unsupplemented diet, the Ca content of whole body and vertebrae were not affected by the lack of dietary Ca supplement. In other words, Ca absorption from seawater was sufficient for maintaining normal tissue Ca but not for normal growth. We obtained similar results in our pervious studies with tiger puffer, giant croaker and redlip mullet (Hossain and Furuichi, 1998, 1999a, 2000). Dietary Ca also affected the growth but not vertebral Ca and P contents of channel catfish and blue tilapia when reared in Ca-free water (Robinson et al., 1986, 1987). However, O'Connell and Gatlin (1994) observed in blue tilapia that a dietary Ca supplement increased the bone Ca content when they were reared in low-Ca water. Mugiya and Watabe (1977) observed the mobilization of scale Ca but not bone Ca into plasma following estrogen injection in goldfish and killifish (*Fundulus heteroclitus*). These findings may indicate that fish maintain bone Ca content, leaving inadequate Ca for other physiological processes when faced with Ca inadequacy and incurred poor growth and feed utilization.

In the present study, scorpion fish fed the Ca-lactate-supplemented and low TCP-supplemented diets had similar growth performances, which indicated that a low level of Ca from TCP could support the growth of scorpion fish. However, a high level dietary TCP supplement significantly decreased the growth and feed efficiency. As P availability from TCP is reportedly low in fish, the effects of a high level of P in the diet alone may be considered negligible. Zn and Mn contents of whole body and vertebrae and Fe and Cu contents of the vertebrae were also negatively affected by dietary TCP. It has been reported that TCP contained in fish meal decreased the availability of Zn and Mn in fish diets (Hardy and Shearer, 1985; Satoh et al., 1987a,b, 1992a,b). On the other hand, Satoh et al. (1989) found no effect of supplemental dietary TCP on weight gain and Zn content in vertebrae of channel catfish, which might be due to a relatively lower TCP level in the diet compared to that in the present study.

The present experiment suggests that Ca uptake from seawater is not sufficient for the proper growth and feed utilization of fingerling scorpion fish. Although this fish may use TCP as a dietary Ca source, a high TCP supplement to the diet reduces weight gain, feed efficiency, and decreases trace mineral content in tissues.

Therefore, a readily available Ca (mono- or dibasic Ca) supplement to the diet is essential for fingerling scorpion fish.

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