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Effects of Dietary Tricalcium Phosphate on the Growth, Feed Efficiency and Mineralization of Bone in Young Red Sea Bream*

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A rearing experiment was conducted for a 12 weeks period to investigate the effects of dietary tricalcium phosphate (TCP) on young red sea bream in aspects of growth, feed utilization and bone mineralization. The fish fed the diet with a low level (0.2%) of Ca supplement from TCP (diet 2) showed growth similar to the fish fed 0.2% Ca from Ca–lactate (diet 1). Fish fed the diet with a high level (2.5%) of Ca supplement from TCP (diet 3) showed significantly poor growth than the fish fed diet 1. Feed efficiency and condition factor were lower in the fish fed diets 2 and 3 compared to diet 1. TCP in the diets 2 and 3 decreased the Zn content of bone. These results indicate that inclusion of a high level of TCP to the purified diet decreases the growth, feed efficiency and Zn content of bone in red sea bream.

INTRODUCTION

It has been reported that dietary tricalcium phosphate negatively affects the growth and normal mineral contents, particularly Zn and Mn contents, in some fresh water fish (Watanabe *et al.*, 1988, 1997). Satoh *et al.* (1987a) observed that supplementation of 7% TCP to a semi-purified diet greatly reduced growth rate and feed efficiency and whole body Zn concentration in rainbow trout. The availability of Zn from fish meal diets was low to some salmonids due to the presence of TCP derived from hard tissues (Hardy and Shearer, 1985; Satoh *et al.*, 1987b). In contrast to the fresh water species, studies on the effects of TCP on marine fish are scarcely available. We observed recently that TCP in a diet affects the growth and mineral contents of bone in tiger puffer (Hossain and Furuichi, 1998). On the other hand, although dietary TCP affected the mineral contents of bone, there was no effect of TCP on the growth in redlip mullet (Hossain and Furuichi, 2000). In the present study the effects of dietary TCP on red sea bream have been investigated.

MATERIALS AND METHODS

Experimental Diets

Three casein based purified diets were formulated as diet 1 (0.2% supplemental Ca from Ca–lactate), diet 2 (0.2% supplemental Ca from tricalcium phosphate, TCP) and diet 3 (2.5% supplemental Ca from TCP). The compositions of the diets are shown in Table 1.

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The process of diet preparation was similar to that reported in the previous paper (Hossain and Furuichi, 1999b). The proximate and mineral compositions of the diets are presented in Table 2.

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

Diet no.	1	2	3
Casein	50	50	50
Amino acid mix*[1]	4	4	4
α-Potato starch	7	7	7
Dextrin	10	10	10
Pollack liver oil	10	10	10
Vitamin mix*[2]	3	3	3
Mineral mix*[3]	5	5	5
Ca-lactate	1.54	–	–
$Ca_3(PO_4)_2$	-	0.52	6.52
Carboxymethylcellulose	4	4	4
α-Cellulose	5.46	6.48	0.48

*[1] Amino acid mixture: arginine·HCl, alanine, glycine, and aspartate·Na, 25% each.

*[2] Halver (1957).

*[3] Mineral mix (in 100 g mixture): 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 no.	1	3	4
Proximate composition			
Moisture (%)	20.9	20.9	20.9
Crude protein (% db)*	51.3	52.6	52.8
Crude lipid (% db)	9.1	9.4	9.0
Crude ash (% db)	5.1	5.4	11.0
Mineral composition (db)			
Ca (%)	0.24	0.23	2.54
P (%)	1.00	1.12	2.37
K (%)	0.19	0.19	0.19
Mg (μg/g)	420	370	430
Fe (μg/g)	280	290	300
Zn (μg/g)	48	48	42
Mn (μg/g)	16	15	17
Cu (μg/g)	11	14	11

* db, dry basis.

Experimental Fish and Rearing Methods

Young red sea bream (*Pagrus major*) produced and reared in Fishery Research Laboratory, Kyushu University, were used for the experiment. The fish were acclimatized in the indoor flow-through aquaria for 2 weeks, during which they were fed the experimental diet 1. On the beginning of the experiment, fish were weighed individually, selected and distributed to 150 ℓ aquaria in such a manner that average body weight and size variations in three experimental groups were almost similar. The number of fish in a group was 30 (av. body wt. 11.5 g).

The fish were reared for a 12 weeks period, during which experimental diets were fed to satiation 2 times a day at 0900 and 1530 h. Rearing temperature ranged from 20.0 to 24.0 °C. Sand-filtered sea water was supplied to the aquaria continuously at a flow rate of 3–4 ℓ/min. Biweekly weighing and other rearing methods were the same as those reported previously (Hossain and Furuichi, 1999).

Sample Collection and Analytical Methods

At the end of the feeding experiment, the fish were starved for 15–20 h and anaesthetized with MS-222. Body length and body weight were measured. Then, blood samples were collected from the cuvierian duct with a 1 ml heparinized syringe. Liver was collected immediately from all the fish and weighed to calculate the hepatosomatic index and preserved at -20 °C for further analyses. After removing the internal organs, the whole body was washed with distilled water and preserved at -20 °C for bone collection.

Determinations of hematological characteristics and proximate and mineral compositions of the diet, liver and bone were the same as those described previously (Hossain and Furuichi, 1999).

The data, when applicable, were subjected to analysis of variance, and significance of difference ($P<0.05$) was determined by the Fisher's PLSD test.

RESULTS

The change in body weight over the experimental period is shown in Fig. 1. The differences in the average body weights were noticed from the 6 th week of the experiment, indicating the need of a relatively longer period for the experiment with Ca requirement. At the end of the experiment, survival rate was 93.3% in all the treatments (Table 3). The maximum growth was obtained in the fish fed diet 1 containing easily digestible Ca (0.2%) from Ca-lactate. The fish fed diet 2 supplemented with a low level of TCP (0.2% Ca) showed the statistically same growth as that of the fish fed diet 1. Significantly poor growth was observed in the fish fed diet 3 supplemented with a high level of TCP (2.5% Ca) compared to the fish fed other two diets. Daily feed consumption was higher in the fish fed diets 2 and 3 with TCP supplements than the fish fed control diet 1. However, a high level of dietary TCP decreased the feed efficiency and condition factor of fish.

The hematocrit value, and hemoglobin and plasma protein contents were similar in fish fed all the diets (Table 4). Plasma tryglyceride was slightly higher in the fish fed diets 2 and 3 compared to the fish fed diet 1. No difference was noticed in plasma P, Ca, Mg and Fe contents among the fish fed different diets.

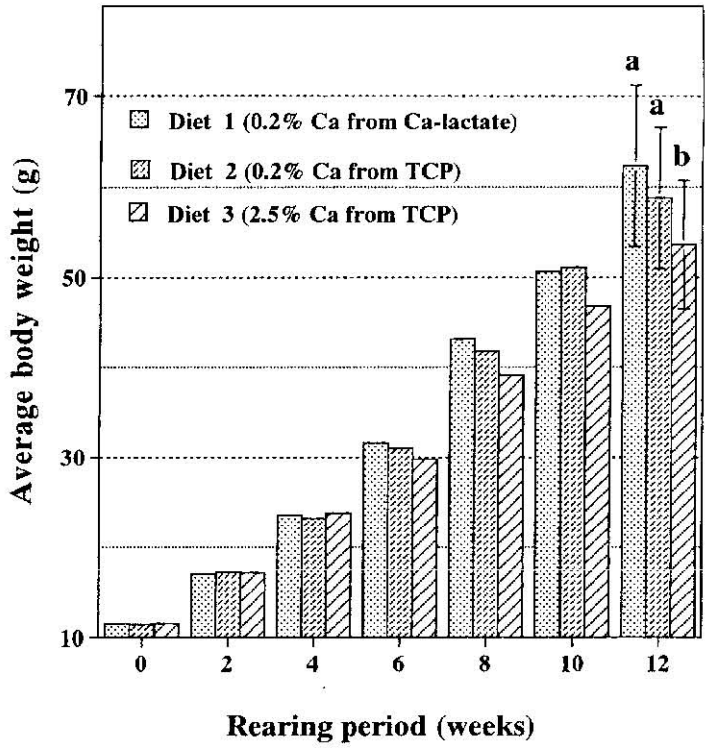

Fig. 1. Growth of red sea bream fed the experimental diets. At the end of 12 weeks rearing, different letters indicate significant differences ($P<0.05$, Fisher's PLSD test).

Table 3. Growth and feed utilization of red sea bream fed the experimental diets

Diet no.	1	2	3
Av. body wt. (g)			
Initial	11.6±0.9	11.5±0.8	11.6±0.8
Final*	62.3±8.9[a]	58.8±7.8[a]	53.6±7.1[b]
Weight gain (%)	437	411	362
Daily feed consumption (% of body wt.)	2.28	2.35	2.41
Feed efficiency (%)	91.8	86.6	80.6
Condition factor*	3.61±0.18[a]	3.55±0.13[ab]	3.46±0.22[b]
Survival rate (%)	93.3	93.3	93.3

* Values (mean±SD) in the same row bearing different letters are significantly different ($P<0.05$, Fisher's PLSD test).

Table 4. Blood characteristics of red sea bream fed the experimental diets

Diet no.	1	2	3
Hematocrit (%)*	28.9±3.5	28.8±3.9	30.5±2.7
Hemoglobin (g/100 ml)*	4.7±1.1	4.8±0.9	4.7±0.6
Plasma protein (g/100 ml)*	4.3±0.6	4.1±0.6	4.1±0.4
Plasma triglyceride (mg/100 ml)	448	490	498
Plasma P (mg/100 ml)	11.7	9.6	10.6
Plasma Ca (mg/100 ml)	13.8	12.3	13.1
Plasma Mg (mg/100 ml)	4.5	4.9	4.6
Plasma Fe (mg/100 ml)	86	100	97

* No significant difference ($P>0.05$, Fisher's PLSD test).

Table 5. Proximate composition of liver and hepatosomatic index of red sea bream fed the experimental diets

Diet no.	1	2	3
Moisture (%)	65.0	64.8	66.9
Crude protein (% db)*1	30.3	30.7	29.9
Crude lipid (% db)	39.4	41.2	35.0
Crude ash (% db)	31.0	31.0	36.0
Glycogen (% db)	10.0	23.3	26.6
HSI (%)*2	2.33	2.17	2.26

*1 db, dry basis.

*2 Hepatosomatic index: liver weight (g)×100/body weight (g).

Table 6. Lipid, ash and mineral contents in the vertebrae of red sea bream fed the experimental diets (dry basis)

Diet no.	1	2	3
Crude lipid (%)	20.6	21.1	22.4
Crude ash (%)	54.3	53.3	53.4
Ca (%)	27.5	26.2	26.9
P (%)	11.4	10.2	10.0
Mg (%)	0.23	0.22	0.21
K (μg/g)	122	130	131
Fe (μg/g)	50.2	51.1	47.7
Zn (μg/g)	66.7	55.1	56.1
Mn (μg/g)	25.4	28.3	24.1
Cu (μg/g)	1.8	2.7	3.0

Moisture and protein contents of the liver were almost similar in all the fish, but low lipid and slightly higher ash contents were detected in the fish fed diet 3 (Table 5). Liver glycogen contents were higher in the fish fed diets 2 and 3 than the fish fed diet 1. Hepatosomatic index values were similar in fish fed all the diets.

The results of bone analyses are presented in Table 6. Bone lipid and ash contents were similar in all the fish. Ca, P, Mg, Mn and K contents of bone were not affected by dietary TCP. However, dietary TCP decreased Fe and Zn contents and increased Cu content of bone.

DISCUSSION

A high level of Ca from TCP decreased the growth of red sea bream. On the other hand, a low level of Ca from TCP did not affect the growth. These indicate that only a high level of TCP in the diet affects the growth of red sea bream. In the previous studies, decreased weight gain and low bone mineral contents were observed in tiger puffer fed the diets supplemented with TCP (Hossain and Furuichi, 1998). On the other hand, dietary TCP affected bone mineral contents but not the weight gain in redlip mullet (Hossain and Furuichi, 2000). Satoh *et al.* (1987a) reported that a high level of TCP (7%) in a semi-purified diet reduced the growth and feed efficiency of rainbow trout and supplementation of 80 μg Zn/kg diet was necessary to obtain the normal growth, feed efficiency, and mineral contents of whole body similar to those in fish fed a TCP-free diet with 40 μg Zn/kg. They suggested that dietary TCP affected the availability of Zn from a diet to fish. In the present study, low levels of Zn contents was found in the bone of fish fed TCP supplemented diets. The inhibitory effect of dietary TCP to the bioavailability of Zn have been reported in some other studies (Hardy and Shearer, 1985; Satoh *et al.*, 1987b; Gatlin and Phillips, 1989). However, no effect of supplemental dietary TCP on weight gain and Zn content in vertebrae was found in case of channel catfish (Satoh *et al.*, 1989). Satoh *et al.* (1991) observed that the availability of Mn from white fish meal was low in rainbow trout and suggested that, similar to in case of Zn, TCP in white fish meal may reduce the availability of Mn. However, bone Mn content, in the present study, did not affect by dietary TCP.

From the above results, it may be concluded that an excessive TCP supplement to a diet affects the growth, feed utilization and Zn content in bone of red sea bream.

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