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Effects of Dietary Tricalcium Phosphate on the Growth and Mineral Availability in Japanese Flounder*

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A 10-week feeding trial was conducted to investigate the effects of dietary tricalcium phosphate (TCP) on growth and mineral availability in juvenile Japanese flounder (*Paralichthys olivaceus*). A 0.25% Ca supplement from Ca-lactate to the diet (diet 2) significantly increased the growth of fish compared to the basal diet 1 without a Ca supplement. However, a dietary supplement of 2.5% Ca from TCP (diet 3) did not improve the growth and decreased Zn and Mn contents of bone when compared to the basal diet 1. A 0.25% Ca supplement from Ca-lactate to the diet (diet 4), in addition to the 2.5% Ca supplement from TCP, also could not improve the growth and Zn and Mn contents of bone. A Zn supplement to the diet (diet 5), in addition to the 2.5% Ca supplement from TCP, increased the Zn content of bone but did not improve the growth. The present study suggests that dietary TCP introduces decreased growth and low availability of Zn and Mn in Japanese flounder and that easily digestible Ca supplementation to the TCP-riched diet alone can not improve the growth and mineral availability. In a TCP-riched diet (fish meal diet, for example), both easily digestible Ca and higher amounts of Zn and Mn supplements may be necessary for obtaining improved growth and mineral availability in juvenile Japanese flounder.

INTRODUCTION

It has been reported that tricalcium phosphate (TCP) interacts with minerals in a diet. Low availability of Zn in the presence of TCP has been reported for some fishes (Richardson *et al.*, 1985; Satoh *et al.*, 1987a,b). In the previous study, we observed that a high level of dietary TCP decreased the weight gain and reduced some trace elements in bone of Japanese flounder (Hossain and Furuichi, 2000). However it was not clear from the previous study that how dietary TCP decreases the growth in this species. The poor growth might be attributed to a deficiency of Ca, because Ca from TCP was less available in some fish. Another possible reason for the poor growth might be due to the less availability of Zn in the presence of TCP. In the present study we have investigated the possible causes of poor growth introduced by TCP in Japanese flounder.

MATERIALS AND METHODS

Experimental Diets

A basal diet (diet 1) was formulated with 45% vitamin-free casein and 10% squid meal as dietary protein sources (Table 1). A Ca-free mineral mixture was supplied to the

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Table 1. Composition of the basal diet for Japanese flounder

Ingredient	(%)
Casein	45
Squid meal	10
α -Starch	5
Dextrin	10
Pollack liver oil	10
Vitamin mix* ¹	3
Mineral mix* ²	5
Feeding stimulants* ³	2
Guar gum	3
α -Cellulose	7

*¹ Vitamin mixture: Halver (1957).

*² Ca-free mineral mixture (in 100 g mixture): major elements in g: KCl 7.68; MgSO₄·5H₂O 8.16; NaH₂PO₄·2H₂O 68.52; Fe-citrate 2.40; cellulose 12.69; minor elements in mg: AlCl₃·6H₂O 90.0; ZnSO₄·7H₂O 264.0; MnSO₄·5H₂O 175.3; CuCl 15.7; KI 3.7; CoCl₂·6H₂O 1.3.

*³ Feeding stimulants (Takaoka *et al.*, 1995): alanine 6.50; aspartate·Na 1.05; betaine 33.95; glycine 16.45; serine 1.20; and cellulose 40.85 (%).

Table 2. Proximate and mineral composition of the experimental diets for Japanese flounder

Diet no.	1	2	3	4	5
Ca from Ca-lactate (%)	0	0.25	0	0.25	0
Ca from TCP (%)	0	0	2.5	2.5	2.5
Zn (mg/kg)	30	30	30	30	80
<i>Proximate composition (% dm)*</i>					
Moisture	16.9	18.0	17.9	18.0	17.5
Crude protein	49.5	49.2	49.7	49.0	50.0
Crude lipid	10.5	10.6	10.2	10.3	10.0
Crude ash	4.5	4.6	9.6	10.5	10.1
<i>Mineral composition (dm)</i>					
Ca (%)	0.02	0.28	2.55	2.78	2.52
P (%)	0.98	1.00	0.96	1.00	0.91
K (%)	0.20	0.20	0.20	0.21	0.23
Mg (μ g/g)	380	370	370	360	370
Fe (μ g/g)	270	270	280	280	270
Zn (μ g/g)	49	47	49	50	95
Mn (μ g/g)	25	24	23	25	26
Cu (μ g/g)	12	14	12	13	11

* dm, dry matter basis.

basal diet to provide 30 mg Zn/kg diet in addition to other minerals. Diet 2 was obtained by supplying 0.25% Ca from Ca-lactate to the basal diet 1. Diets 3–5 were supplied with 2.5% Ca from tricalcium phosphate (TCP). In addition to TCP supplementation, 0.25% Ca from Ca-lactate and 80 mg/kg Zn from Zn-sulfate were supplied to diets 4 and 5, respectively. The procedure for diet preparation was the same as that reported previously (Hossain and Furuichi, 1999). The proximate and mineral compositions of the experimental diets are shown in Table 2.

Fish and Feeding Trial

Juvenile Japanese flounder were obtained from Fukuoka Prefectural High School of Fisheries and acclimated to the laboratory conditions at Fukuoka Mariculture Corporation for 2 weeks. The fish were fed the experimental diet 2 during acclimatization. The feeding trial was carried out in 100 l round polycarbonate tanks with a running water system with filtered sea water at a flow rate of 2–3 l/min. Water temperature during the feeding trial was 27.0 ± 1.4 °C. At the beginning of the feeding trial, the fish (average body weight 0.38 g) were weighed individually and distributed to 5 rearing tanks (35 fish/tank). Differences in the mean body weight of fish among the tanks and standard deviation within a tank were kept minimum. The fish were fed the experimental diets to satiation twice a day for 10 weeks. Biweekly weighing and other rearing methods were the same as those described previously (Hossain and Furuichi, 1999).

Sample Collection and Analysis

The fish were anaesthetized with over exposure to MS-222 after 18–24 h starving at the termination of rearing experiment. Body weight and body length were recorded. The sample collection and chemical analyses were done by the methods mentioned previously (Hossain and Furuichi, 1999). The data were analyzed for significance using Fisher's Protected Least Significant Difference (Fisher's PLSD, $P < 0.05$).

RESULTS AND DISCUSSION

A 0.25% Ca supplement through Ca-lactate to the diet (diet 2) significantly increased the growth and feed efficiency of fish compared to the basal diet without a Ca supplement (Table 3). These results are further confirmation of our previous study where poor growth was observed in Japanese flounder when Ca was not supplemented to the diet, which indicated that Japanese flounder could not absorb adequate Ca from sea water to support the growth (Hossain and Furuichi, 2000a). A high level of TCP supplement (2.5% Ca) to the diet (diet 3) could not improve the growth of fish compared to the basal diet, indicating that Ca from TCP was not possibly available to Japanese flounder. Furthermore, remarkably low bone Zn content was observed in fish fed the diet 3 (Table 4). Dietary TCP in diet 3 also tended to decrease the Mn content of bone. These indicate that dietary TCP decreased the availability of Zn and Mn in Japanese flounder. A growth depression due to the dietary TCP also has been reported for some other species such as tiger puffer and red sea bream (Hossain and Furuichi, 1998, 2000b). Low bioavailability of Zn due to the dietary TCP has been reported for some fresh water fishes (Hardy and Shearer, 1985; Satoh *et al.*, 1987a, b; Gatlin and Phillips, 1989). A minimum dietary Zn

requirement of channel catfish and rainbow trout was quite higher in practical diets containing fish meal than purified or semi-purified diet due to the presence of TCP in fish meal (Gatlin and Wilson, 1984; Satoh *et al.*, 1987a). A 0.25% Ca supplement to the diet through Ca-lactate, in addition to TCP (diet 4), could not improve the growth and bone Zn and Mn contents. A higher amount of Zn supplementation to the diet (diet 5), in addition to TCP, improved the Zn content in bone, however, could not increase the growth. These results indicate that in a TCP-riched diet, either easily digestible Ca or Zn supplement can not improve the growth of Japanese flounder. The combined addition of

Table 3. Growth performances of Japanese flounder fed the experimental diets

Diet no.	1	2	3	4	5
Ca from Ca-lactate (%)	0	0.25	0	0.25	0
Ca from TCP (%)	0	0	2.5	2.5	2.5
Zn (mg/kg)	30	30	30	30	80
Av. body wt. (g)					
Initial	0.38±0.06	0.38±0.06	0.38±0.07	0.38±0.06	0.38±0.06
Final ^{*1,2}	11.1±2.6 ^b	13.0±3.8 ^a	11.1±2.8 ^b	10.3±2.8 ^c	10.1±2.5 ^b
Weight gain (%)	2820	3320	2820	2610	2560
Feed efficiency (%) ^{*3}	128	142	134	126	106
Condition factor ^{*2,4}	1.63±0.10 ^b	1.73±0.10 ^a	1.65±0.07 ^b	1.53±0.10 ^c	1.66±0.12 ^{ab}
Survival rate (%)	85.7	82.9	94.3	97.1	82.9

*¹ Calculated from all the fish of each tanks.

*² Values (mean±SD) in the same row with different letters are significantly different ($P<0.05$).

*³ $100 \times$ wet weight gain/dry feed intake.

*⁴ Body weight (g) $\times 100 / (\text{total length in cm})^3$, (n=15).

Table 4. Ash, lipid and mineral contents in the bone of Japanese flounder fed the experimental diets*

Diet no.	1	2	3	4	5
Ca from Ca-lactate (%)	0	0.25	0	0.25	0
Ca from TCP (%)	0	0	2.5	2.5	2.5
Zn (mg/kg)	30	30	30	30	80
Crude ash (%)	65.2	67.2	68.3	67.6	68.0
Crude lipid (%)	2.40	2.29	2.20	2.16	2.34
Ca (%)	26.5	26.5	26.7	25.7	25.4
P (%)	11.8	11.4	12.0	11.5	11.5
Mg (%)	0.51	0.46	0.49	0.48	0.52
K ($\mu\text{g/g}$)	264	258	248	260	254
Fe ($\mu\text{g/g}$)	280	292	270	275	282
Zn ($\mu\text{g/g}$)	242	253	170	150	239
Mn ($\mu\text{g/g}$)	139	138	128	118	121
Cu ($\mu\text{g/g}$)	7.6	7.5	7.2	6.8	7.1

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

easily digestible Ca and Zn to the diet might be suitable to improve the growth and mineral contents of bone. As TCP decreased the availability of Mn, a supplementation of Mn to TCP-riched diet also should be considered.

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