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Essentiality of Ca Supplement to White Fish Meal Diet for Tiger Puffer*

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The essentiality of Calcium (Ca) supplement to a white fish meal diet for tiger puffer *Takifugu rubripes* was investigated with two rearing experiments. Fish were fed on a white fish meal diet with or without Ca supplement for 12 weeks and 14 weeks in experiments 1 and 2, respectively. Poorer growth and feed efficiency were found in the Ca non-supplemented group. A deletion of Ca from the diet increased the bone lipid, Zn, and Cu contents. The present study suggests that Ca supplement to a white fish meal diet is essential for proper growth and feed efficiency of tiger puffer.

INTRODUCTION

Calcium (Ca) and phosphorus (P) are two major elements to fish. In white fish meal Ca and P mainly exist as the complex form of hydroxyapatite which derived from hard tissues such as bone and scales. Most of fish can not dissolve and completely digest the hydroxyapatite as a source of Ca and P. Therefore, the supplement of P is essential in a fish meal diet and was recomended at the level of 500–800 mg P per 100 g diet for several fish (Andrews *et al.*, 1973; Ogino and Takeda, 1976, 1978; Ogino *et al.*, 1979; Sakamoto and Yone, 1979; Yone and Toshima, 1979). However, the investigation with a supplement of Ca in a fish meal diet for fish is extremely few due to the difficulty in the experiment, because fish can easily intake Ca from surrounding water in addition to the dietary source. In the previous study (Furuichi *et al*, 1997), we found the poor growth and feed efficiency and lower Ca level of the bone in tiger puffer *Takifugu rubripes* fed on a white fish meal diet without a mineral mixture supplement. This suggests that Ca supplement is essential in a fish meal diet for tiger puffer.

MATERIALS AND METHODS

Experimental diets

The composition of a basal diet is shown in Table 1. White fish meal was used as a protein source. Mineral mixtures with or without Ca (Control and -Ca, respectively) supplemented to the basal diet are appeared in Table 2. Easily digestible Ca-lactate was used as a Ca source for the control diet. The proximate and mineral compositions of the experimental diets are shown in Table 3. The procedure for diet preparation was similar

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Table 1. Basal diet for tiger puffer

Ingredient	(%)
White fish meal	60
α-Potato starch	10
Dextrin	10
Pollack liver oil	5
Vitamin mixture[*1]	3
Mineral mixture[*2]	8
Carboxymethylcellulose	4

[*1] Halver's vitamin mixture (1957) + α-Cellulose.

[*2] See Table 2.

Table 2. Mineral mixture supplemented to the basal diet (%)

	Diet	
	Cont.	-Ca
KCl	6.54	6.54
$MgSO_4 \cdot 7H_2O$	6.81	6.81
$NaH_2PO_4 \cdot 2H_2O$	42.81	42.81
Fe-citrate	1.48	1.48
Ca-lactate	24.51	–
Trace element[*]	1.00	1.00
α-Cellulose	16.85	41.36

[*] $AlCl_3 \cdot 6H_2O$ 0.9, $ZnSO_4 \cdot 7H_2O$ 17.6, $MnSO_4 \cdot 4\text{–}6H_2O$ 3.95, CuCl 0.55, KI 0.85, $CoCl_3 \cdot 6H_2O$ 5.15, α-Cellulose 71.0 (%).

Table 3. Proximate and mineral compositions of the experimental diets for tiger puffer

	Diet	
	Cont.	-Ca
(Proximate composition)		
Moisture (%)	25.3	25.8
Crude protein (% d.m.)[*]	47.3	47.3
Crude lipid (% d.m.)	9.2	9.0
Crude ash (% d.m.)	16.6	16.2
(Mineral composition)		
P (%)	2.72	2.72
Ca (%)	3.78	3.34
Mg (mg/100 g)	145	148
K (mg/100 g)	610	618
Fe (mg/100 g)	29.9	30.3
Zn (mg/100 g)	7.22	7.77
Mn (mg/100 g)	1.09	1.10
Cu (mg/100 g)	0.30	0.23

[*] d.m. = Dry matter.

to that reported previously (Furuichi *et al.*, 1997).

Experimental fish and rearing methods

Two rearing experiments (exp. 1 and 2) were conducted in the present study. Juvenile tiger puffer *Takifugu rubripes* were used for the experiments. After acclimatizing to the indoor rearing condition, the fish (average body wt. 1.3 g in exp. 1 and 1.7 g in exp. 2) were introduced to the respective 200 ℓ polycarbonate round tank as a group of 30 individuals. Rearing temperature was within 21.5–26.0 °C in exp. 1 and 21.6–28.8 °C in exp. 2. The fish were fed with the experimental diets to satiation twice a day and reared for 12 weeks in exp. 1 and 14 weeks in exp. 2. Biweekly weighing and rearing methods were similar to those of the previous study (Furuichi *et al.*, 1997).

Sample collection and analytical methods

At the end of the experiment, the fish were anesthetized with over exposure to MS-222 (3-aminobenzoic acid ethyl ester) after 15–16 h starving. Body weight and body length were recorded. The sample collection and chemical analysis, which were conducted only for exp. 2, were done by the methods mentioned previously (Furuichi *et al.*, 1997). Student's T-test was applied to determine the significance in difference between treatment means ($p < 0.05$).

RESULTS

The biweekly growth is shown in Fig. 1. Details of the growth performance are presented in Table 4. The growth of the Ca non-supplemented group started to decrease from the 4th week in exp. 1 and 2. At the end of the experimental period, the growth was

Table 4. Growth performances of tiger puffer fed on the experimental diets

	Diet	
	Cont.	-Ca
(Experiment 1)		
Average body weight (g)		
At start	1.3±0.1	1.3±0.1
After 12 weeks	57.1±9.5	49.5±11.7*2
Survival rate (%)	86.7	86.7
Average weight gain (%)	4290	3710
Feed efficiency (%)	85.3	81.6
Condition factor*1	33.9±1.9	31.6±2.0
(Experiment 2)		
Average body weight (g)		
At start	1.7±0.1	1.7±0.1
After 14 weeks	80.0±12.8	68.6±15.6*2
Survival rate (%)	76.7	80.0
Average weight gain (%)	4610	3940
Feed efficiency (%)	89.1	77.4
Condition factor*1	35.2±4.0	38.8±4.2

*1 Condition factor = Body weight in g × 100/(body length in cm)^3.
*2 Significant ($p < 0.05$).

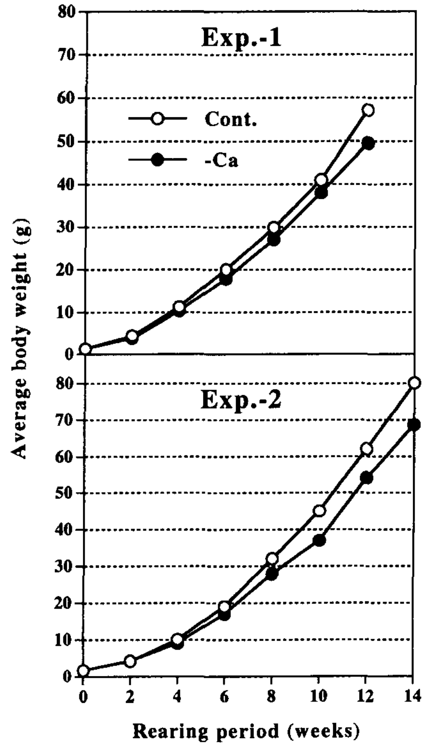

Fig. 1. Growth of tiger puffer fed on the experimental diets with or without Ca supplemwnt.

significantly lower in the Ca non-supplemented group than the supplemented one in both experiments. Feed efficiency was also low in the Ca non-supplemented group, but no significant difference was observed in the condition factor in both experiments.

Results of the blood analysis are shown in Table 5. Hematocrit value was significantly high in the Ca non-supplemented group. No significant difference in hemoglobin content was found between the groups. Plasma P and Mg contents were slightly high in the Ca non-supplemented group compared to the supplemented group. Plasma Fe content was lower in the Ca non-supplemented group. No differences between the groups were observed in plasma protein, triglyceride, cholesterol, Ca and K contents and ALP, GPT, and GOT activities.

Hepatosomatic index and proximate compositions of the liver and muscle are presented in Table 6. Hepatosomatic index was slightly higher in the Ca non-supplemented group, but the difference was not significant. Moisture, crude protein, crude lipid, crude ash and glycogen contents of the liver were similar in both groups. The proximate composition of muscle appeared independent of Ca supplement.

Results of the analysis of bone are presented in Fig. 2. Lipid content of the bone was high in the Ca non-supplemented group. No difference was found in bone ash content

Table 5. Blood characteristics of tiger puffer fed on the experimental diets with or without Ca supplement (Exp. 2)

	Diet	
	Cont.	-Ca
Hemoglobin (g/100 ml)	5.3±0.54	5.8±0.55
Hematocrit (%)	22.7±2.6	26.0±2.7*
(Plasma)		
Total protein (g/100 ml)	3.7±0.5	4.1±0.5
Triglyceride (mg/100 ml)	268	228
Total cholesterol (mg/100 ml)	185	207
P (mg/100 ml)	7.9	9.7
Ca (mg/100 ml)	12.5	14.4
Ca/P ratio	1.58	1.48
Mg (mg/100 ml)	3.1	4.2
K (mg/100 ml)	0.16	0.14
Fe (mg/100 ml)	115	81
GOT (Karmen Unit)	83	47
GPT (Karmen Unit)	22	25
ALP (K.-A. Unit)	6.6	7.4

* Significant ($p<0.05$).

Table 6. Proximate compositions of liver and dorsal muscle and hepatosomatic index of tiger puffer fed on the experimental diets with or without Ca supplement (Exp. 2)

	Diet	
	Cont.	-Ca
(Dorsal muscle)		
Moisture (%)	79.3	79.6
Crude protein (% d.m.)	93.7	96.1
Crude lipid (% d.m.)	3.9	3.9
Crude ash (% d.m.)	6.8	6.8
(Liver)		
Moisture (%)	33.2	33.1
Crude protein (% d.m.)	10.6	9.7
Crude lipid (% d.m.)	87.1	85.9
Crude ash (% d.m.)	0.9	0.7
Glycogen (% d.m)	2.4	2.1
Hepatosomatic index*1	8.5±1.3	9.7±2.1*2

*1 Liver wt (g) ×100/body wt (g).
*2 Not significant ($p<0.05$).

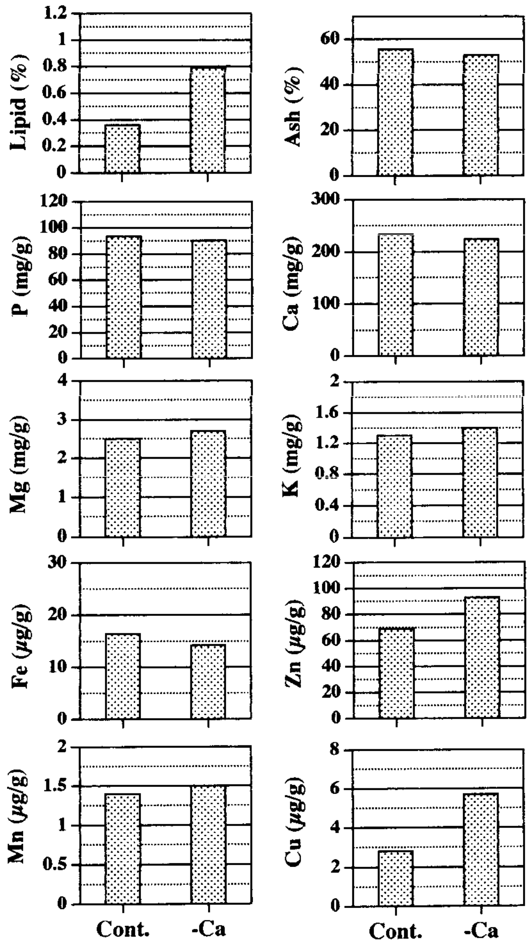

Fig. 2. Lipid, ash and mineral compositions of vertebrae of tiger puffer fed on the experimental diets with or without Ca supplement in experiment 2 (dry matter basis).

between the groups. A deletion of Ca from the mineral mixture supplement increased the Zn and Cu contents in the bone. However, P, Ca, Mg, K and Mn contents of the bone were similar in two groups.

DISCUSSION

In the present experiment, lacking of the Ca supplement to a fish meal diet caused the poor growth and feed efficiency in tiger puffer. P and Ca in fish meal exist as a complex hydroxyapatite which is difficult to digest. P from fish meal have been reported to be less available to the fish (Takamatsu *et al.*, 1975; Shitanda *et al.*, 1979; Yone and Toshima, 1979; Watanabe *et al.*, 1980 a,b). In the present study, Ca in fish meal was also found less available for tiger puffer. Tiger puffer being a stomachless fish cannot fully dissolved and digest the complex of Ca and P in fish meal. On the other hand, the environmental Ca did not satisfy the requirement of tiger puffer. Fish can actively absorb Ca from surrounding water (Lovelace and Podoliak, 1952; Ichikawa and Oguri, 1961; Templeton and Brown, 1963; Lall, 1979; Love, 1980; Cowey, 1992) and a dietary supplement was found unnecessary for some fish species (Ogino and Takeda, 1976, 1978; Sakamoto and Yone, 1976). Sakamoto (1981) reported that red sea bream increased Ca absorption from sea water when Ca in a diet was low and reversely decreased the absorption when the diet contained a high amount of Ca. In the present case, a large amount of Ca from fish meal in the Ca non-supplemented group might affect the Ca absorption from sea water. On the other hand, Ca in fish meal was not available to this species which was revealed by the poor growth and feed efficiency in the Ca non-supplemented group.

Zn and Cu contents of the bone were high in the Ca non-supplemented group. Furuichi *et al.** also found similar results in the experiment on Ca requirement with tiger puffer, where high Zn and Cu contents in the bone were detected in Ca non-supplemented or low Ca supplemented fish meal diets.

From the above results, it may be concluded that tiger puffer probably could not sufficiently use Ca from sea water and/or fish meal when fed on a fish meal diet. Hence, easily digestible Ca supplement is recommendable to the fish meal diet for tiger puffer.

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