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Effects of the Deletion of Ca or Trace Elements from Semi-purified Diet on Growth and Feed Utilization of Yellow Croaker, *Nibea albiflora**

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This study was conducted to determine the effect of the deletion of Ca or trace elements from semi-purified diets on growth and body composition of the yellow croaker *Nibea albiflora*. Fish with mean average weight 28.8 ± 2.5 g were fed on test diets with (control group) and without supplement of Ca or trace elements for 14 weeks at 20.0-22.5°C. No significant difference was observed in the growth of fish groups fed on diets deficient with Ca or trace elements and the control group. No apparent effect of feeding diets without Ca or trace elements was detected in hematological characteristics in all test groups. However, serum contents of Ca and inorganic P were higher in fish group received Ca deficient diet compared to the control. Bone contents of Mn, Cu and Zn decreased distinctly in fish group fed deficient trace elements diet. No obvious effect on the mineral contents of liver was seen in fish from all test groups. It appears from these findings that, For growth performance, yellow croaker can meet their requirements of Ca or trace elements by direct assimilation of these minerals from the surrounding water but for ideal bone mineralization the addition of trace elements in diet seems to be necessary.

INTRODUCTION

Knowledge in the area of yellow croaker *Nibea albiflora* nutrition are still limited. Although, protein requirements has been reported (Han *et al.*, 1994), all of the few studies available have dealt only with the development and maturation of this species in nature (Kakuda *et al.*, 1980; 1981 and Taketa, 1974). Further advancement in nutritional research is needed as commercial yellow croaker production becomes more intensive mostly in the countries of far east like Japan and Korea.

There have been almost no studies on the requirement of yellow croaker for various dietary minerals. Our laboratory has determined the phosphorus requirement for fingerling croaker (El-Zibdeh *et al.*, 1995).

Studies by several workers (Ishac and Dollar, 1967; Satoh *et al.*, 1983a; Ogino and Yang, 1978; 1979; Murai *et al.*, 1981) have revealed that the supplementation of various trace elements such as Zn, Mn, Cu, are essential for the maintenance of fish health. Moreover, the deletion of single trace element such as Zn or Mn from diets was found to affect the growth in carp and rainbow trout more severely than the deletion of total trace elements (Satoh *et al.*, 1983ab). On the other hand, No correlation was found between dietary deficient Ca and the growth rate of Carp and rainbow trout (Ogino and Takeda, 1976; 1978; Sakamoto and Yone, 1976). The present study was conducted to determine precisely the effect of the deletion of calcium or trace minerals from diet on growth and

* Contribution from Fish. Res. Lab., Kyushu University, No. 209.

mineral composition of yellow croaker.

MATERIALS AND METHODS

Diets

Basal diet (Table 1) was formulated from commercially obtained ingredients: Vitamin free casein and squid meal were added as the protein source, dextrin and α -starch (gelatinized starch) as the digestible carbohydrate source and pollack liver oil as lipid source in addition to the vitamin mixture (Halver's 1957+ α -cellulose). Three mineral mixtures were prepared, one with complete mineral mixture as a control. In the remaining two mixtures, Ca or T.E. were deleted and replaced with corresponding amount of α -cellulose (Table 2). To each diet the mineral mixture was supplemented at 8% level.

Fish and Feeding

Experiments were conducted in a 150-L rectangular flow-through aquaria with flow rates of approximately 1.2-1.8 l/min. Rearing water temperature was maintained at 20-22.5°C. Prior to initiation of the experiment, the fish underwent a 2-3 weeks conditioning period during which they fed on the control diet containing complete mineral mixture.

At the start of the experiment, yellow croaker fingerlings (average body weight

Table 1. Composition of basal diet for yellow croaker.

Ingredients	%
Casein* ¹	50
Squid meal	5
Dextrin	10
α -Starch	5
Pollack liver oil	10
Vitamin mixture* ²	3
Mineral mixture* ³	8
CMC* ⁴	3
Attactants* ⁵	
α -Cellulose	5
Total	100
DE (Kcal/100g diet)* ⁶	353

*1 Vitamin free milk casein.

*2 Halver's vitamin mixture (1957)+ α -Cellulose.

*3 See Table 2.

*4 Carboxymethylcellulose.

*5 DL-Alanine, 0.3g; L-Asp Na, 0.3g; 5'-ribonucleotide. Na, 0.032g. Glu. Na, 0.368g

*6 Digestible energy (assumed from the values for carp (Ogino *et al.*, 1976): 4kcal/g protein, 8kcal/g lipid and 3.5kcal/g digestible carbohydrate).

Table 2. Mineral mixture supplemented to test diets.

Experimental group	Control	-Ca	-T.E.*
KCl (g)	26.15	26.15	26.15
MgSO ₄ ·7H ₂ O	27.25	27.25	27.25
NaH ₂ PO ₄ ·2H ₂ O	171.25	171.25	171.25
Fe-citrate	5.91	5.91	5.91
Ca-lactate	98.04		98.04
T.E.* (mg)			
AlCl ₃ ·6H ₂ O	35.60	35.60	
ZnSO ₄ ·7H ₂ O	710.00	710.00	
MnSO ₄ ·4·6H ₂ O	159.20	159.20	
CuCl	22.00	22.00	
KI	34.00	34.00	
CoCl ₂ ·6H ₂ O	208.80	208.80	
α -Cellulose (g)	70.23	168.27	71.40
Total (g)	400	400	400

Levels of minerals in test diets

Diet	Control	-Ca	-T.E.*
Ca (%)†	0.33	0.09	0.38
Mn (μ g/g)†	5.7	5.0	0.9
Cu (μ g/g)†	4.1	4.8	0.1
Zn (μ g/g)†	67.5	60.1	24.5

†Dry weight basis.

*Trace elements.

28.9±2.5 g) were counted into groups of 20 fish in each aquarium. Fish were fed on test diets 2-3 times/day to satiation for a period of 14 weeks.

Measurements and Analytical Methods

Body weight of individual fish from each experimental group was measured at four week intervals. At the end of feeding trial blood samples were taken by cardiac puncture from 10 fish randomly selected from each group. Hematocrit value (Ht), hemoglobin content (Hb) and serum total protein were determined by the microhematocrit method, cyanmethemoglobin method and ATAGO hand refractometer, respectively. Measurements of Ca, inorganic P, and Mg in pooled samples of blood serum were quantified by RaBA Super (Rapid Blood Analyzer). Composite samples of vertebrae and/or liver were combined from all fish of each group and subjected for the estimation of moisture, crude protein, crude lipid and crude ash. Inorganic P in liver and vertebrae was measured by molybdenum blue method, levels of Ca, Mg, Mn, Cu, Zn and Fe were determined by Atomic Absorption Spectrophotometer (Perkin-Elmer 3300).

The analysis of variance (ANOVA), Fisher's LSD test and student's t-test were employed at a $P < 0.05$ level to determine whether a significant difference existed among groups.

RESULTS

Growth curves of yellow croaker fed the three experimental diets are presented in Fig. 1. No significant difference was observed in the growth of fish groups fed the calcium or trace elements deficient diets compared to the control group. As shown in Table 3 the only significant difference observed was in the female GSI, namely, the control group had lower value in comparison to the other two groups.

Effects on the hematological characteristics and chemical components of the blood serum of yellow croaker are presented in Table 4. No significant differences were seen in Ht, Hb and serum total protein values among the three groups. Triglyceride and total cholesterol together with Ca and inorganic P were considerably higher in the group fed calcium deficient diet. A slight increase in Fe content was measured in both Ca and T.E. dietary deficient groups, however, Mg contents were similar among all groups.

Lipid, ash and mineral contents of vertebrae are shown in Fig. 2. A decrease of crude lipid and a little increase in ash content were observed in fish group fed on the Ca deficient diet compared to the control.

Results of proximate composition of liver are plotted in Fig.3. Crude protein in T.E. deficient fish and crude lipid in Ca deficient fish showed notably lower values than the control group. The effect of feeding diets either deficient with Ca or trace elements on mineral content of liver was visible on the slight increase of all measured elements except Fe which showed similar values to that in the control group (Fig. 4).

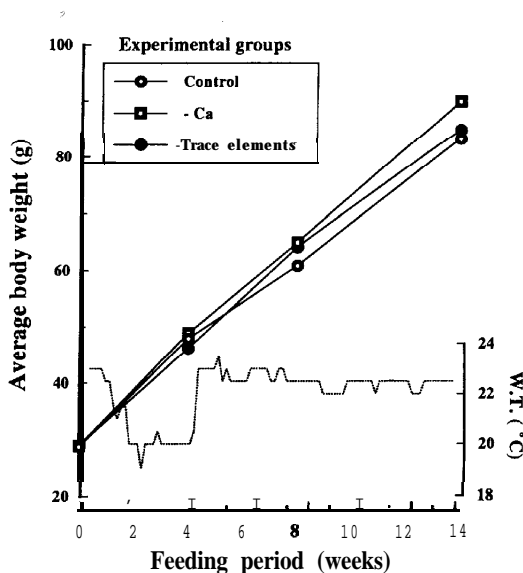


Fig. 1. Growth of yellow croaker fed on semi-purified test diets with and without Ca or trace elements supplements for 14 weeks. Right panel shows the change in rearing water temperature (W.T.).

Table 3. Effect of Ca or trace elements deficient diets on growth and efficiency of feed utilization of yellow croaker.

Experimental group	Control	-Ca	-T.E.
Average body weight (g)			
at start	28.7±2.8	28.9±2.6	28.9±2.9
after 14 weeks	83.3±10.8	89.9±14.9 ^a	84.9±15.2 ^a
Average weight gain (%)	190	211	187
Feed efficiency (%)	86.3	88.5	85.6
Daily growth rate (%)	1.07	1.13	1.08
Daily feed intake (%)	1.28	1.26	1.24
Hepatosomatic index* ¹	2.66±0.79 ^a	2.40±0.47 ^a	2.25±0.57 ^b
Gonadosomatic index* ²			
(female)	2.53±1.30 ^a	7.55±5.00 ^b	7.31±4.47 ^b
(Male)	1.26±0.79 ^a	1.80±0.74 ^a	1.28±0.46 ^a
Condition factor* ³	2.00±0.15 ^a	2.04±0.14 ^a	2.06±0.16 ^a

Values within the same row which bears different letters are significantly different, $P < 0.05$ (ANOVA, Fisher's LSD test).

*1 Liver weight (g) $\times 100$ /body weight (g).

*2 Gonad weight (g) $\times 100$ /body weight (g).

*3 Body weight (g) $\times 10^3$ /[body length (cm)]³.

Table 4. Effect of Ca or trace elements deficient diets on the hematological characteristics and chemical composition of the blood serum of yellow croaker.

Experimental group	Control	-Ca	-T.E.
Hematocrit (%)	28.3±3.1 ^a	29.1±2.8 ^a	30.8±2.7 ^a
Hemoglobin (g/100ml)	7.5±1.0 ^a	7.3±0.8 ^a	8.0±0.7 ^a
Total protein (mg/100ml)	2.6±0.3	2.71±0.3	2.8±0.3
Triglyceride (mg/100ml)	181	332	200
Total cholesterol (mg/100ml)	48	96	71
Ca (mg/100ml)	6.0	11.6	6.4
P (mg/100ml)	4.4	7.4	5.3
Ca/P	1.4	1.6	1.2
Fe (µg/100ml)	98	118	138
Mg (mg/100ml)	1.0	1.2	1.1

Values within the same row which bears different letters are significantly different, $P < 0.05$ (ANOVA, Fisher's LSD test).

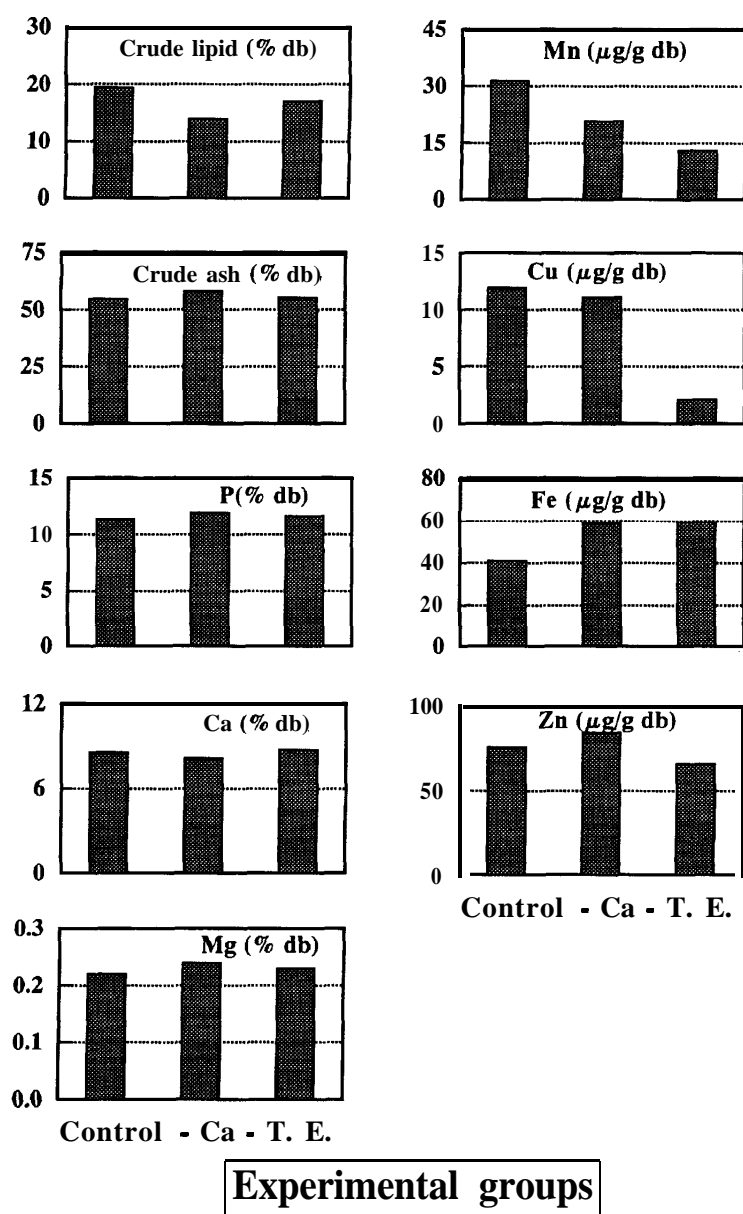


Fig. 2. Effects of feeding the control and Ca or trace elements (T.E.) deficient diets on lipid, ash and mineral contents of the vertebrae of yellow croaker (db; values in dry weight basis).

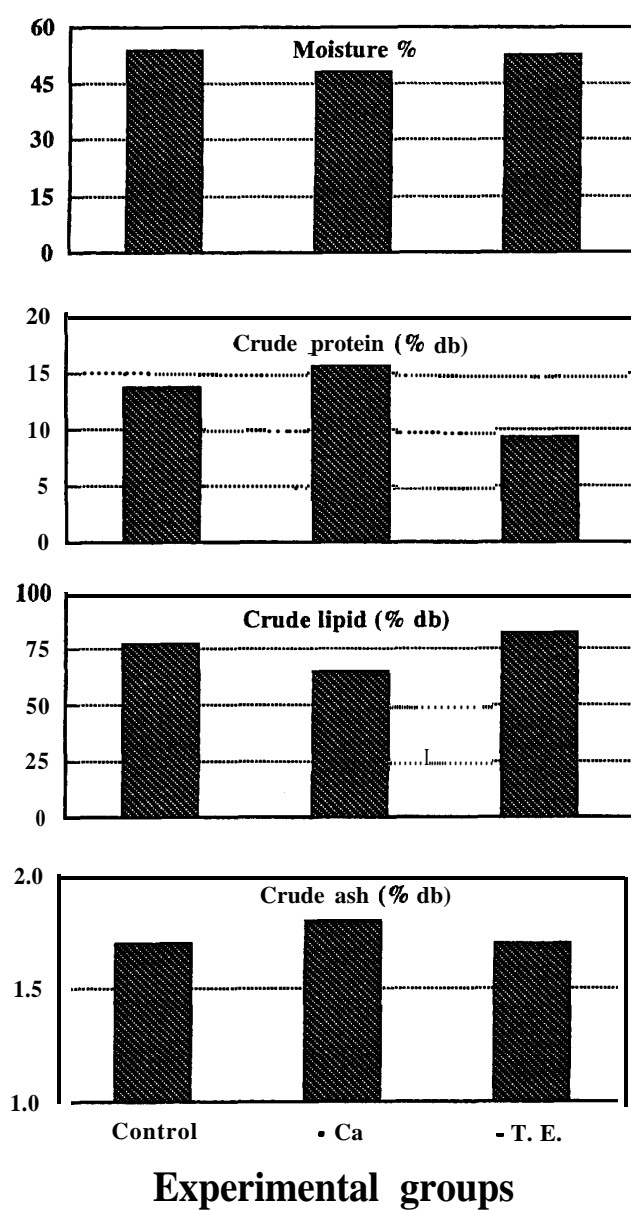


Fig. 3. Proximate composition of liver of yellow croaker fed on the control and Ca or trace elements (T.E.) deficient diets for 14 weeks (db; Values in dry weight basis).

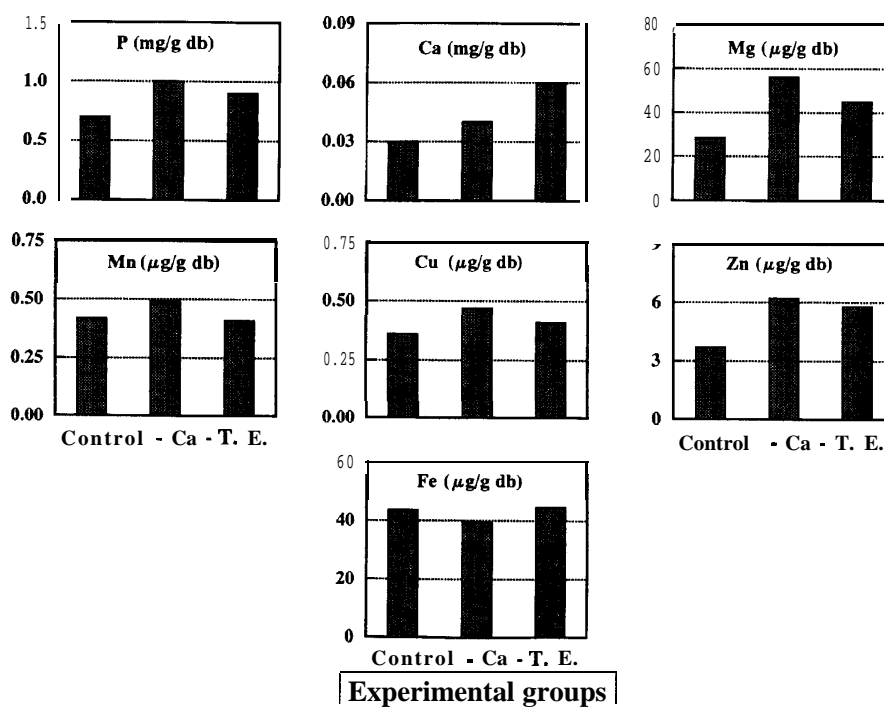


Fig. 4. Effects of feeding the control and Ca or trace elements (T.E.) deficient diets on mineral contents of the liver of yellow croaker (db; Values in dry weight basis).

DISCUSSION

The results of the present study showed that feeding yellow croaker on diets deficient with either Ca or trace elements had no severity on the growth of fish. Various studies have indicated that fish can utilize Ca directly from environmental water or diet and any sign of Ca deficiency in several species like rainbow trout, carp and catfish was not developed (Ogino and Takeda, 1976, 1978; Andrews *et al.*, 1973). The lack of any significant difference in growth performance between the group fed Ca deficient diet and the control indicates that feeding with deficient Ca diets to yellow croaker at constant level of available P (0.7%) had no apparent effect on fish. Moreover, mineral contents of vertebrae showed that P as well as Ca concentrations were similar to the control. These results may indicate that the yellow croaker have the ability to balance the ratio of Ca/P in both serum and bone by controlling the Ca requirement through the absorption from surrounding water (Templeton and Brown, 1963; Ichikawa and Oguri, 1961) and any sign of deficiency could not be developed. Results from this study agreed with those reported for carp, rainbow trout (Ogino and Takeda, 1976; 1978) and red sea bream (Sakamoto and Yone, 1976). On the other hand, the addition of Ca in diet was found to be essential to maintain optimum growth and bone mineralization in redlip mullet (El-Zibdeh *et al.*, 1995). The discrepancies between the various species could be related to

the difference in feeding habitats. Lower content of lipid in vertebrae was observed in fish received the Ca deficient diet than the control. A similar result was noted in vertebrae of the redlip mullet fed Ca deficient diet and suggested to be as a result of low feed intake and poor appetite (El-Zibdeh et al., unpublished). The reason in the case of yellow croaker is unknown, however, it is probable that the increased levels of Ca observed in some body tissues prevents lipid accumulation.

Trace elements could be present in most rearing water in amounts that would prevent the development of obvious deficiency symptoms (Wolf, 1951). It has been reported that feeding with Mn deficient diet in rainbow trout and tilapia developed malformation such as the shortening of the body length and feeding with Cu deficient diet resulted in poor growth of carp in addition to the distinct decrease of these two minerals in body tissue (Ogino and Yang, 1980; Satoh et al., 1983ab). Similarly, Zn was found also to be essential for the prevention of eye cataract and poor growth. Feeding with low dietary Zn below 15 ppm was found to affect growth rate and mortality as well as tissue contents of Zn, Cu and Fe in carp (Ogino and Yang, 1979). The growth of fish group received diet without trace elements observed in the present study was similar to that of the control group, both of which exhibited normal growth and no mortalities, suggesting that the requirement of trace elements for normal growth in yellow croaker can be met from water. However, the supplement of trace elements mixture in diet is required for optimal mineralization of body tissue.

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