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Requirement of Yellow Croaker *Nibea albiflora* for Dietary Phosphorus*

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Fingerling yellow croaker *Nibeaalbiflora*, with initial mean weight of 20g were fed semi-purified test diets containing different levels of phosphorus (0.3-0.9%) for 13 weeks. Deficiency of phosphorus reduced growth and feed efficiency. In vertebrae an increase of ash and a decrease of lipid was observed with increasing dietary phosphorus. Hematocrit, hemoglobin and serum total protein remain to be unaffected. However, serum triglyceride and total cholesterol were maximum at the level of 0.65% dietary phosphorus. Results of growth and mineral contents of serum, bone and liver suggest that the minimum requirement for dietary phosphorus is approximately 0.65%.

INTRODUCTION

Detailed studies on the nutrition of yellow croaker *Nibea albiflora* are scarcely available despite its importance in fishery and aquaculture resources in Japan and other adjacent countries. Few studies have dealt with the development and maturation of yellow croaker (Kakuda *et al.*, 1980, 1981; Takita, 1974). There is only one study that reported the dietary protein requirement (Han *et al.*, 1994). The culture of yellow croaker is receiving a special attention in Japan. Therefore, further research is needed for better understanding of the nutritional requirements for efficient production of this species. Investigations concerning the mineral nutrition of the yellow croaker have received almost no attention. In our laboratory a series of studies were conducted to determine various mineral requirements of fingerling yellow croaker.

Phosphorus is an essential element for the formation of bone, maintenance of entire cellular membrane of the soft tissues and for lipid and carbohydrate metabolism. The required levels of phosphorus are the highest in comparison to all inorganic ions for most fishes. The dietary phosphorus was found to range from 0.5-0.9% available phosphorus for many freshwater and marine fishes (Andrews et al., 1973; Sakamoto and Yone, 1978; Ogino *et al.*, 1979; Ogino and Takeda, 1976, 1978; Yone and Toshima, 1979).

This study was designed to examine the efficiency of dietary phosphorus on growth, food conversion, hematology and chemical composition of the soft tissues and bone in yellow croaker.

*Contribution from Fish, Res. Lab., Kyushu University, No. 208

MATERIALS AND METHODS

A feeding study was conducted with zero year-old yellow croaker fingerlings. The fish were produced from broodstock held in captivity at the Fishery Research Laboratory of Kyushu University, Fukuoka-Japan.

Experimental diet

The composition of the experimental mineral mixture is shown in Table 1. Sodium phosphate monobasic ($NaH_2PO_4.2H_2O$) was substituted in place of α -cellulose in order to

Table 1. Mineral mixture with various P levels added to test diets.

Diet No.	I	II	III	IV	V
Supplemental P (mg/100 diet)	0	100	300	400	550
Dietary P (mg/100g diet)*	322	416	652	761	888
KCl (g)	26.15	26.15	26.15	26.15	26.15
$MgSO_4.7H_2O$	27.25	27.25	27.25	27.25	27.25
$NaH_2PO_4.2H_2O$	0	25.16	75.53	100.75	138.40
Fe-citrate	5.9	5.9	5.9	5.9	5.9
Ca-lactate	98.05	98.05	98.05	98.05	98.05
Trace elements(mg)					
$AlCl_3.6H_2O$	35.6	35.6	35.6	35.6	35.6
$ZnSO_4.7H_2O$	710	710	710	710	710
$MnSO_4.4\text{-}6H_2O$	159.2	159.2	159.2	159.2	159.2
CuCl	22	22	22	22	22
KI	34	34	34	34	34
$CoCl_2.6H_2O$	208.8	208.8	208.8	208.8	208.8
α -Cellulose (g)	241.50	216.32	165.97	140.65	103.04
Total (g)	400	400	400	400	400

*One gram casein and one gram squid meal contain 5.9 and 7.8 mg P in dry basis, respectively.

obtain 5 levels (O-0.55%) of supplemental phosphorus. Basal diet (Table 2) were formulated from a commercially obtained ingredients: vitamin free casein and squid meal were used as the protein source, dextrin and α -starch (gelatinized starch) as a source for digestible carbohydrate and pollack liver oil as lipid source. The vitamin mixture (Halver, 1957)+α -cellulose was also used. To each test diet the mineral mixture was supplemented at 8% level. Since casein and squid meal contain considerable amount of phosphorus (5.9 and 7.8 mg P in dry basis), the level of dietary phosphorus in each test diet was determined colorimetrically by the molybdenum blue method (See Table 1).

Table 2. Composition of the 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	1
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 1.
*4 Carboxymethylcellulose.
*5 DL-Alanine, 0.3g; L-Asp Na, 0.3g; 5'-ribonucleaotide. Na, 0.032g. Glu. Na, 0.368g
*6 Digestible energy (assumed from the values for carp (Ogino et al., 1976): 4kal/g protein, 8kcal/g lipid and 3.5kcal/g digestible carbohydrate).

Experimental design

Prior to initiation of the experiment, the fish underwent a 20 days conditioning period during which they readily adjusted to a semi-purified diet and acclimated to indoor-laboratory conditions. Diet No.4 which containing adequate amounts of phosphorus (0.7%) was fed as the conditioning diet. Experiments were conducted in a 150-L rectangular flow-through aquaria with flow rates of approximately 1.2-1.8 *l*/min. To each tank sufficient aeration (400-600 ml/min.) was also supplemented. Rearing sea water was maintained at 21-22.5°C.

Fish and Feeding

Croaker fingerlings with average body weight 20.7±3.25 grams were sorted into five groups of 30 fish each, and placed in an individual tank. Fish groups were fed 2-3 times/day until satiation with test diets adjusted previously to be appropriate to fish size. Feed was introduced gradually to avoid any leftovers. Feeding period was extended to 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, weight gain, feed efficiency, daily growth rate, daily feed intake, condition factor, gonadosomatic (GSI), and hepatosomatic (HSI) indices were measured individually.

Blood samples were taken by cardiac puncture from 10 fish in each group. Hemo-

globin content (Hb), hematocrit value (Ht) and serum total protein were determined by cyanmethemoglobin method, microhematocrit method and ATAGO hand refractometer, respectively. Inorganic P, Ca, triglyceride and total cholesterol in pooled samples of blood serum were quantified by RaBA-Super (Rapid Blood Analyzer) and Unikit (Chugai Pharmaceutical Co.). A composite samples of vertebrae and/or liver were combined from all fish of each experimental group for proximate analysis. Moisture was measured in a triplicate of subsamples dried in electrical oven at 105°C for 24 hours. Ash was determined in duplicate samples ignited over night at 550°C in electric muffle furnace. Crude protein and crude lipid were estimated using microKjeldal and Soxhlet methods, respectively. Inorganic P, Ca, Cu, Zn and Mn contents of samples of vertebrae and liver were determined by Perkin-Elmer (3300) Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Dietary phosphorus at level 0.32% had substantial effect on growth rate (Fig. 1). The response of yellow croaker fingerlings to increasing levels of phosphorus is presented in Table 3. Dietary phosphorus supplementation up to 0.65% increased growth, average weight gain and feed efficiency, addition beyond this level had no further effect. Daily growth rate, daily feed intake and condition factor exhibited almost similar values among the five experimental groups. HSI was significantly lower ($P<0.05$) at dietary phosphorus 0.32% compared to other groups. No significant difference in GSI, however, was detected among the five groups.

Effects of dietary graded levels of phosphorus on hematological characteristics and

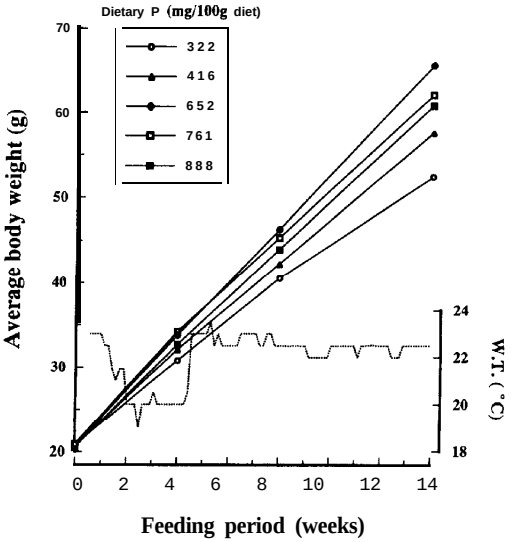

Fig. 1. Growth of yellow croaker fed on semi-purified test diets with graded levels of dietary phosphorus for 14 weeks. Right panel shows the change in water temperature (W.T.).

Table 3. Effect of dietary phosphorus on growth and efficiency of feed utilization of yellow croaker.

Dietary P (mg/100g diet)	322	416	652	761	888
Average body weight (g)					
at start	20.8±2.9	20.6±3.2	20.7±3.1	20.9±3.5	20.5±3.4
after 14 weeks	52.4±8.8[b]	57.6±11.0[b]	65.5rt14.7'	62.0±11.3[a]	60.8±16.3[ab]
Average weight gain (%)	152	178	216	197	196
Feed efficiency (%)	73.0	70.4	86.0	78.8	87.1
Daily growth' rate (%)	0.90	1.00	1.09	1.04	1.05
Daily feed intake (%)	1.30	1.43	1.26	1.32	1.29
Gonadosomatic index*[1] (Male)	1.17±0.59[a]	1.18±0.38[a]	1.47±0.55[a]	1.23±0.42[a]	1.15±0.62[a]
(Female)	10.26±5.05[a]	6.31±4.35[a]	8.40±4.07[a]	10.11±3.87[a]	7.51±4.10[a]
Hepatosomatic index*'	1.76±0.53[b]	2.46±0.78[a]	2.23±0.68[a]	2.42±0.69[a]	2.37±0.77[a]
Condition factor*'	2.00±0.20[a]	2.00±0.17[a]	2.02±0.16[a]	1.96±0.23[a]	2.08±0.16[a]

Values within the same row which bears different letters are significantly different, $P<0.05$ (Fisher's LSD test and Student's t-test).
*1 Gonad weight (g) ×100/body weight (g).
*2 Liver weight (g) ×100/body weight (g).
*3 Body weight (g) ×10^3[body length (cm)]'.

Table 4. Effect of dietary phosphorus on the hematological characteristics and chemical components of the blood serum of yellow croaker.

Dietary P (mg/100g diet)	322	416	652	761	888
Hematocrit (%)	28.9±4.3[b]	30.2±2.6[a]	30.4±4.0[ab]	32.4±1.6[a]	29.8±2.6[ab]
Hb (g/100ml)	6.5±1.4[b]	8.5±0.6[a]	7.8±1.1[a]	8.6±1.5[a]	7.5±0.8[b]
Total protein (g/100ml)	2.4±0.5[a]	2.9±0.2[a]	2.8±0.5[a]	2.4±0.4[a]	2.3±0.2[a]
Triglyceride (mg/100ml)	166	387	370	306	148
Total cholesterol (mg/100ml)	68	103	106	88	63
Ca (mg/100ml)	7.9	7.4	6.8	7.2	7.2
P (mg/100ml)	3.2	4.9	4.5	4.7	5.3
Ca/P	2.5	1.5	1.5	1.6	1.2
K (mEq/100ml)	2.6	3.0	3.0	3.1	2.2
Mg (mg/1 00ml)	1.9	1.2	0.9	1.2	0.9

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

serum mineral contents are summarized in Table 4. Ht and Hb values were significantly lower ($P<0.05$) in fish group fed the dietary phosphorus 0.32% than the other groups. It was not verified yet if the reduction in Ht value found in channel catfish *Ictalurus punctatus* fed on diets deficient with Ca could be as a result of blood dilution due to osmoregularity changes or to the absolute reduction of blood cell level or size (Andrews *et al., 1973).* In this study the serum calcium showed almost similar values for all groups and serum phosphorus level was remarkably lower specially in the group fed the lowest P

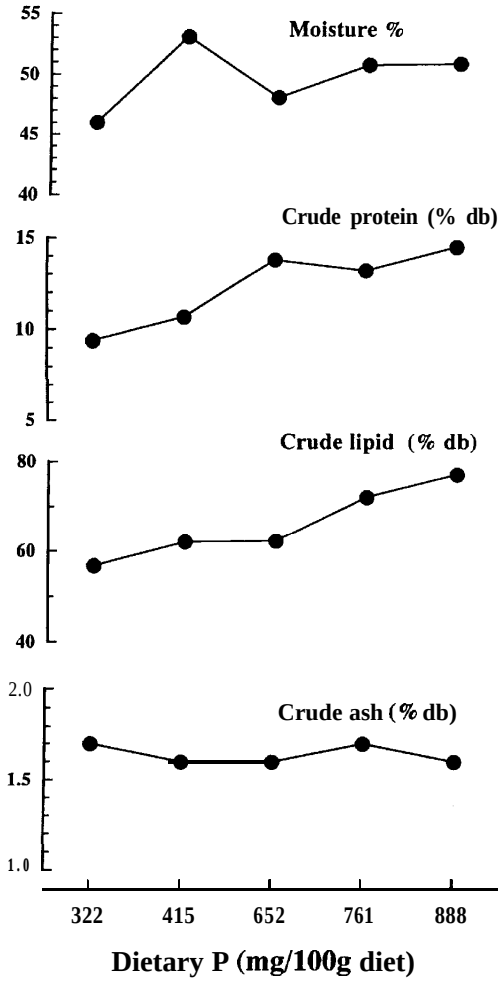

Fig. 2. Proximate composition of liver of yellow croaker fed on diets containing graded levels of dietary phosphorus for 14 weeks (Values in dry weight basis).

level (0.32%). This resulted in an increase of the Ca/P ratio (2.5) which is the highest among the experimental groups the reason that could be attributed to the change in Ht and Hb values. On the other hand, no significant difference (k-0.05) was detected in the value of serum total protein among the fish groups fed different dietary P. However, serum triglyceride and total cholesterol were minimum at 0.32% dietary phosphorus level which could be related to the reduced growth of this group.

A slight increase was observed in both protein and lipid with almost similar values of ash contents in liver with increasing dietary phosphorus (Fig. 2). Liver mineral contents were comparable in all the groups and showed no obvious trend with the change in

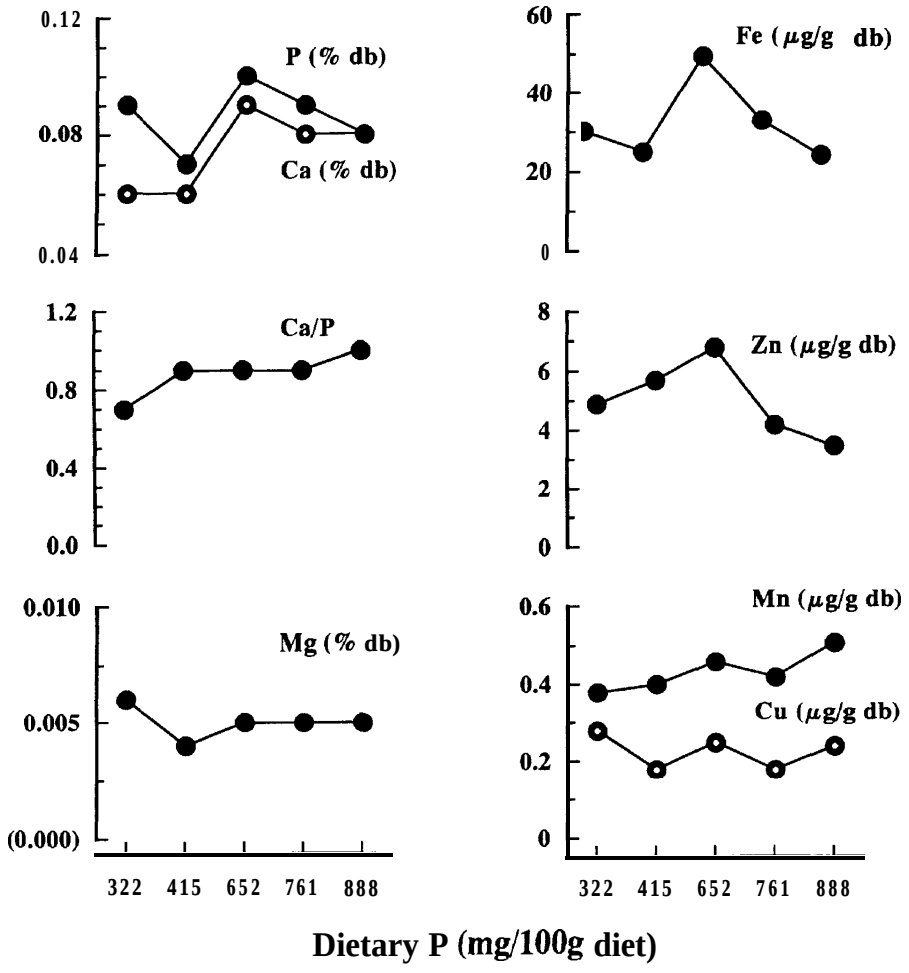

Fig. 3. Effects of dietary phosphorus on mineral contents of the liver of yellow croaker (Values in dry weight basis).

dietary phosphorus (Fig. 3).

Lipid, ash and mineral contents of vertebrae are shown in Fig. 4. An increase in lipid content and a decrease in ash content of vertebrae was observed at lower dietary phosphorus. Sakamoto and Yone (1978) reported similar influence on the lipid content of red sea bream *Pagrus major* and suggested that the decrease in dietary phosphorus levels may promote the absorption of dietary lipid or inhibit the glycogenesis in the fish resulting in the acceleration of protein and carbohydrate transformation into lipid. Bone ash, Ca and P contents were maximum at 0.65 % level of dietary phosphorus, suggesting that the requirement of phosphorus for optimal growth is similar to that for maximum mineralization of vertebrae. However, several reports have shown that the dietary

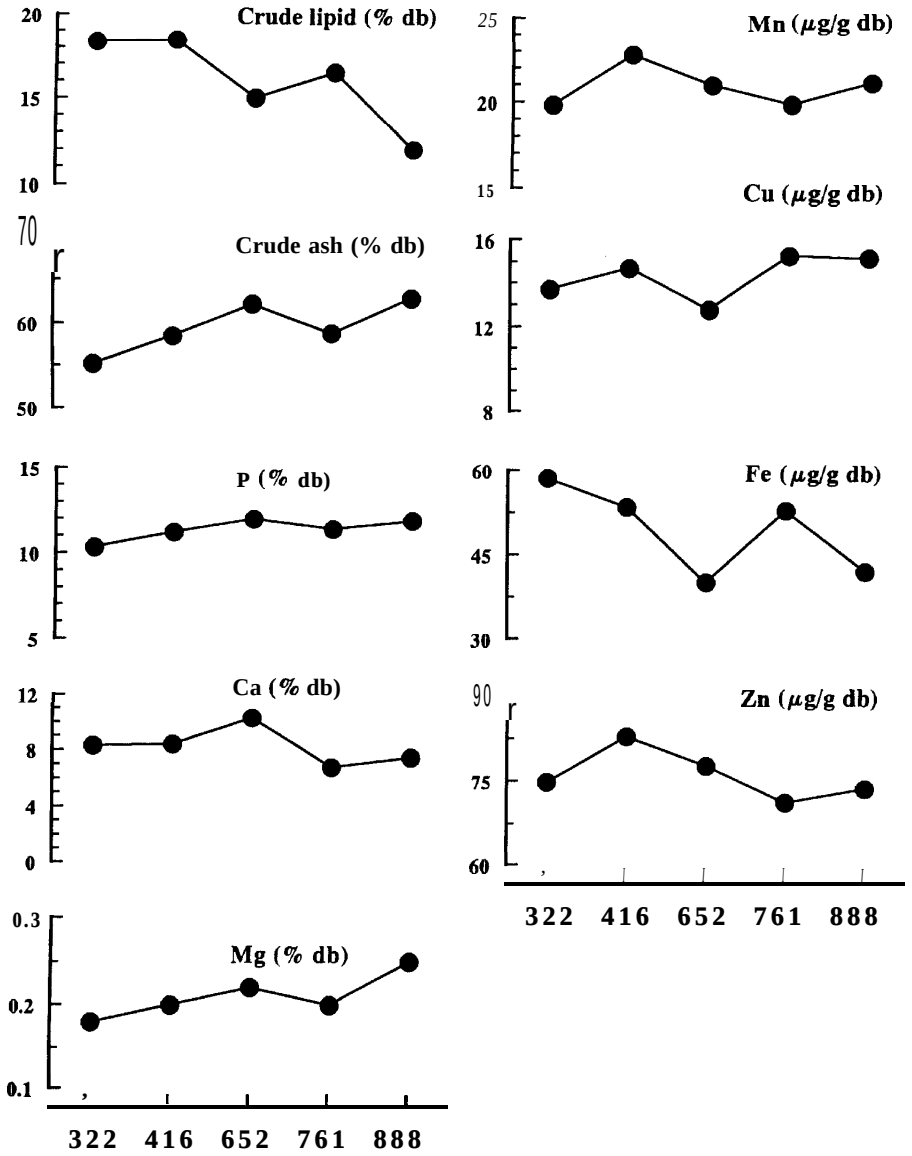

Fig. 4. Effects of dietary phosphorus on lipid, ash and mineral contents of the vertebrae of yellow croaker (Values in dry weight basis).

phosphorus requirements for optimum mineralization of bone in rainbow trout *Oncorhynchus mykiss*, channel catfish *Ictalurus punctatus* and carp *Cyprinus carpio* are above that for optimum growth (Ketola *et al.*, 1994; Andrews *et al.*, 1973; Ogino *et al.*,

1973). The available phosphorus level required to maintain normal growth of fingerling yellow croaker was estimated to be 0.65-0.75% of diet which is similar to the requirement values 0.6-0.8% available phosphorus for carp and rainbow trout (Ogino and Takeda, 1976, 1978) and red sea bream (Sakamoto and Yone, 1978), while higher than the requirement value of 0.4% reported for channel catfish (Wilson et al., 1982).

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