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El-Zibdeh, Mohammad

Fishery Research Laboratory, Faculty of Agriculture, Kyushu University

Yoshimatsu, Takao

Fishery Research Laboratory, Faculty of Agriculture, Kyushu University

Matsui, Seiichi

Fishery Research Laboratory, Faculty of Agriculture, Kyushu University

Furuichi, Masayuki

Fishery Research Laboratory, Faculty of Agriculture, Kyushu University

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## **Effects of the Deletion of Ca or Trace Elements from Purified Diet on Growth and Efficiency of Feed Utilization of Redlip Mullet\***

**Mohammad El-Zibdeh, Takao Yoshimatsu, Seiichi Matsui  
and Masayuki Furuichi**

Fishery Research Laboratory, Faculty of Agriculture, Kyushu University,  
Fukuoka 811-33, Japan

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Purified diets with and without supplement of Ca or trace elements were fed to fingerling redlip mullet, *Liza haematocheila*, (initial body weight: 4.98 g) for 13 weeks at 22-24.5 °C. The essentiality for these minerals was examined in terms of weight gain, feed efficiency, condition factor, hepatosomatic index and the chemical analysis of blood, liver and vertebrae. Feeding fish groups with the Ca or trace elements deficient diet resulted in reduced growth compared to the control fish. No difference, however, was noted in the hematological characteristics among all groups. Ca and inorganic P levels in blood serum and lipid content of vertebrae were decreased distinctly in fish fed Ca deficient diet. In fish group fed trace elements deficient diet, lower levels of Cu and Mn and increased levels of lipid were detected in vertebrae and liver compared to the control group. From these findings, it appears that the supplement of Ca and trace elements in diet, for redlip mullet, is required.

### INTRODUCTION

Mulletts are widely spread throughout tropical and temperate seas and cultured intensively in several countries (Wu, 1984; Nash et al., 1980). Like other mullets, redlip mullet *L. haematocheila* is considered to be an important food source in the far east and the adjacent Asian countries.

The requirement of redlip mullet for various nutrients such as protein and lipid have been determined (Arakawa et al., 1980; Yoshimatsu et al., 1992, 1993). However, investigations concerning the mineral nutrition of this fish have received almost no attention.

In our laboratory a series of studies were conducted to determine various mineral requirements for redlip mullet. The present experiment was performed in order to examine the effect of the deletion of calcium or various trace elements (T.E.) from diet on growth, food conversion, hematology and mineral composition of redlip mullet.

### MATERIALS AND METHODS

#### **Diets**

The basal diet was formulated from purified ingredients; vitamin free milk casein as the protein source, dextrin and  $\alpha$ -starch (gelatinized starch) as the digestible carbohydrate

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\* Contribution from Fish. Res. Lab., Kyushu University, No. 212.

source, pollack liver oil as lipid source and various vitamins. Three mineral mixtures with (control) and without Ca or T.E. were prepared independently (Table 1). To each diet the mineral mixture was supplemented at 8% level.

### Experimental Design

Experiments were conducted in 150-L rectangular flow-through aquaria (flow and aeration rates of 1.2-1.8 l/min. and 400-600 ml/min., respectively). Rearing water temperature was maintained at 22-24.5 °C, controlled by using a heating system.

**Table 1.** Composition of the basal diet fed to redlip mullet,.

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

\*1. Halvers vitamin mixture (1957)+α -Cellulose.

\*2. Mineral mixture supplemented to test diets.

Test group	Control	No-Ca	No-T. E.
<u>Major elements (g)</u>			
KCl	26.15	27.25	26.15
MgSO <sub>4</sub> . 7H <sub>2</sub> O	27.25	27.25	27.25
NaHPO <sub>4</sub> . 2H <sub>2</sub> O	171.25	171.25	171.25
Fe-citrate	5.91	5.91	5.91
Ca-lactate	98.04	.....	98.04
<u>Trace elements (mg)</u>			
AlCl <sub>3</sub> . 6H <sub>2</sub> O	35.60	35.60	.....
ZnSO <sub>4</sub> . 7H <sub>2</sub> O	710.00	710.00	.....
MnSO <sub>4</sub> . 4-6H <sub>2</sub> O	159.20	159.20	.....
CuCl <sub>2</sub>	22.00	22.00	.....
KI	34.00	34.00	.....
CoCl <sub>2</sub> . 6H <sub>2</sub> O	208.80	208.80	.....
α -Cellulose (g)	70.23	168.27	71.40
Total (g)	400	400	400

\*3. Carboxymethylcellulose.

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

Prior to initiation of the experiment, the fish underwent a 2-3 weeks conditioning period during which they were readily adjusted to a purified diet and acclimated to indoor-laboratory conditions. The control diet was fed to fish during the conditioning period.

### **Fish and Feeding**

At the start of the experiment, redlip mullet fingerlings (average body weight  $4.98 \pm 0.11$  g) were counted into three groups of 30 fish in each aquarium. Pellet size of the experimental diet was adjusted according to the progress in fish growth. Fish groups were fed 2-3 times/day ad libitum for 13 weeks.

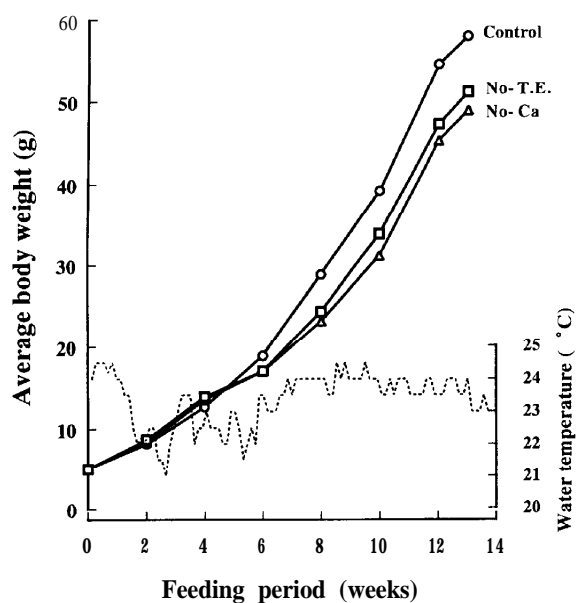
### **Measurements and Analytical Methods**

Body weight of individual fish from each experimental group was measured at a biweekly interval. Blood samples were taken by cardiac puncture from 10 fish in each group for hematocrit, hemoglobin and serum total protein measurements. Calcium and inorganic phosphorus in pooled samples of blood serum were quantified by Rapid Blood Analyzer (RaBA Super). A composite samples of vertebrae and/or liver were combined from all fish of each group and subjected for proximate analysis. Mineral contents of vertebrae were determined by a Per-kin-Elmer (3300) Atomic Absorption Spectrophotometer.

## **RESULTS AND DISCUSSION**

Growth of fish fed on the test diet without supplements of either Ca or T.E. was significantly lower ( $P < 0.05$ ) than that of fish fed the control diet (Fig. 1 and Table 2). Feed efficiency, condition factor and hepatosomatic index were not influenced by either Ca or T.E. deficient diets.

Hematocrit value and hemoglobin content together with serum total protein, Ca and P contents are shown in Fig. 2. The deletion of Ca or T.E. from diets did not alter hematocrit value, hemoglobin and serum total protein contents. Ca and P levels in blood serum of the fish given diet without Ca were significantly lower ( $P < 0.05$ ) than the control fish. Similarly, Ca content of vertebrae of the same group was slightly lower than the control fish, whereas, no change was observed in P content (Fig. 3). These results could demonstrate the incapability of redlip mullet to meet its requirement of Ca by direct absorption of adequate amount of Ca from water (Templeton and Brown, 1963; Ichikawa and Oguri, 1961). However, in similar feeding trials conducted on channel catfish, carp and rainbow trout, fishes were able to assimilate Ca directly from surrounding water (Andrews *et al.*, 1976; Ogino and Takeda, 1976, 1978). The deletion of T.E. from the diet resulted in a drastic decrease in both Cu and Mn contents in vertebrae, while Zn remains similar to the control fish (Fig. 3). In rainbow trout the deficiency of dietary Mn 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 minerals in body tissue of both species, however, such effects were not induced by the deletion of Zn (Ogino and Yang, 1980; Satoh *et al.*, 1983 a, b, c). The reduced growth in fish group fed T.E. deficient diet observed in the present study could be attributed to the



**Fig. 1.** Growth of redlip mullet fed on purified test diets with (control) and without calcium (No-Ca) or trace elements (No-T.E.) supplements for 13 weeks. Dotted line shows the change in the rearing water temperature.

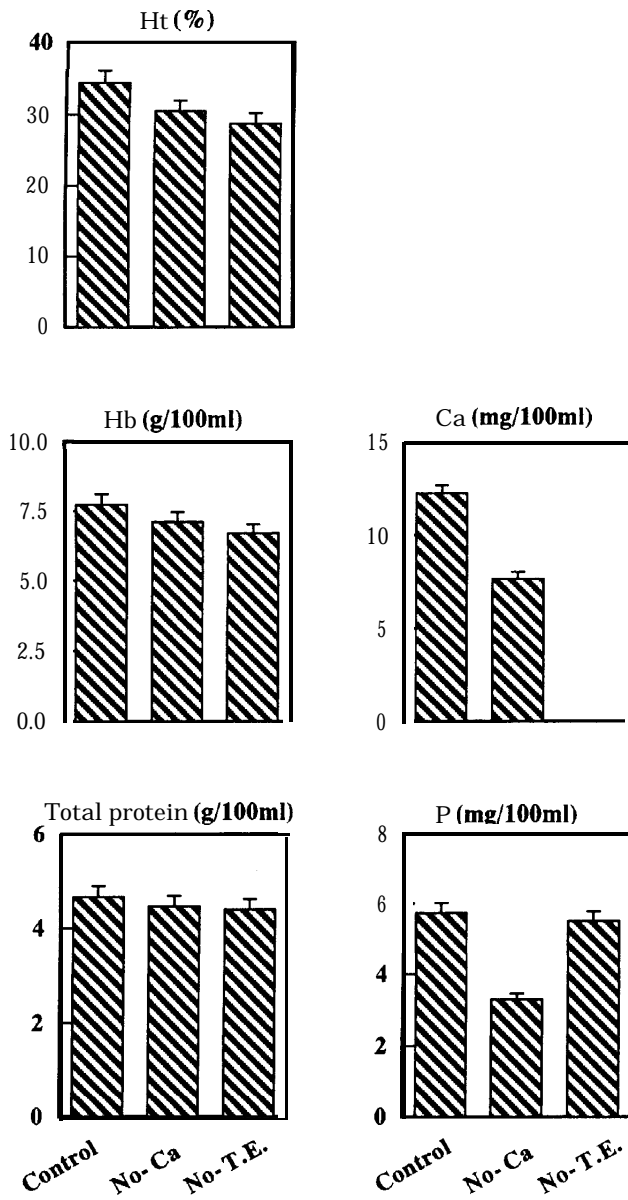
**Table 2.** Effect of feeding calcium (No-Ca) or trace elements (No-T. E.) deficient diets on growth and efficiency of feed utilization of redlip mullet.

Experimental group	Control	No-Ca	No-T. E.
Number of fish at start	30	30	30
after 13 weeks	27	27	26
Average body weight (g)			
at start	5.0±0.10	5.0±0.10	5.0±0.10
after 13 weeks	58.0±3.1 <sup>a</sup>	48.9±2.57 <sup>b</sup>	51.1±2.46 <sup>b</sup>
Average weight gain (%)	944	785	790
Feed efficiency (%)	95	91	95
Hepatosomatic index*1	10.6±0.05 <sup>a</sup>	1.18±0.07 <sup>a</sup>	1.19±0.07 <sup>a</sup>
Condition factor*2	12.53±0.36 <sup>a</sup>	12.53±0.16 <sup>a</sup>	12.73±0.23 <sup>a</sup>

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)

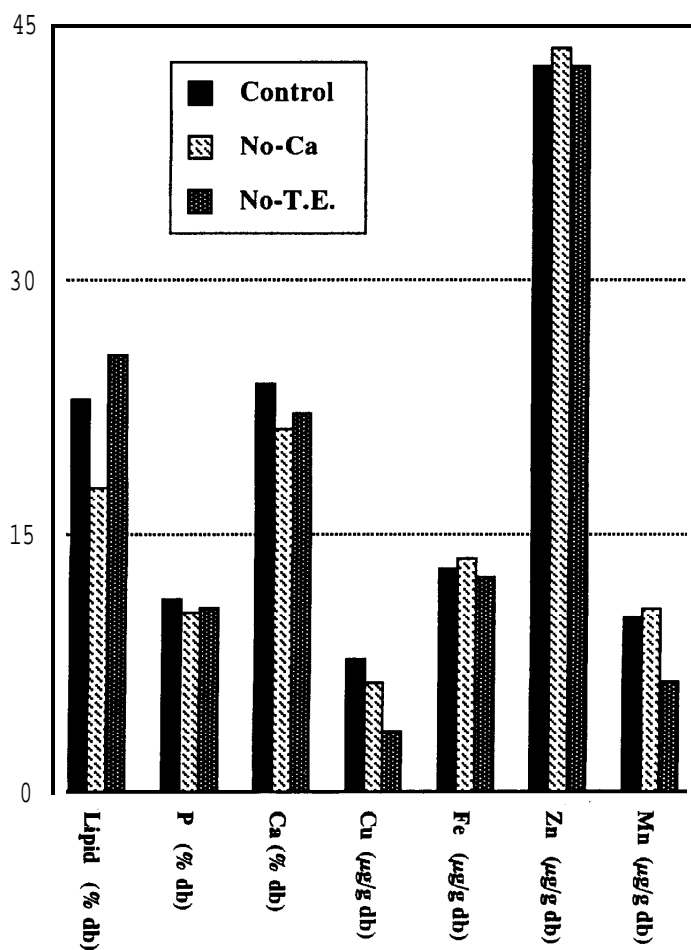
\*2 Body weight (g)  $\times 10^3$  / [body length (cm)]<sup>3</sup>.



**Fig. 2.** Influence of feeding the diets with (control) and without calcium (No-Ca) or trace elements (No-T.E.) supplements upon hematocrit, hemoglobin and serum total protein, Ca and inorganic P levels of redlip mullet.

inadequacy of trace elements in the test diets given to redlip mullet,

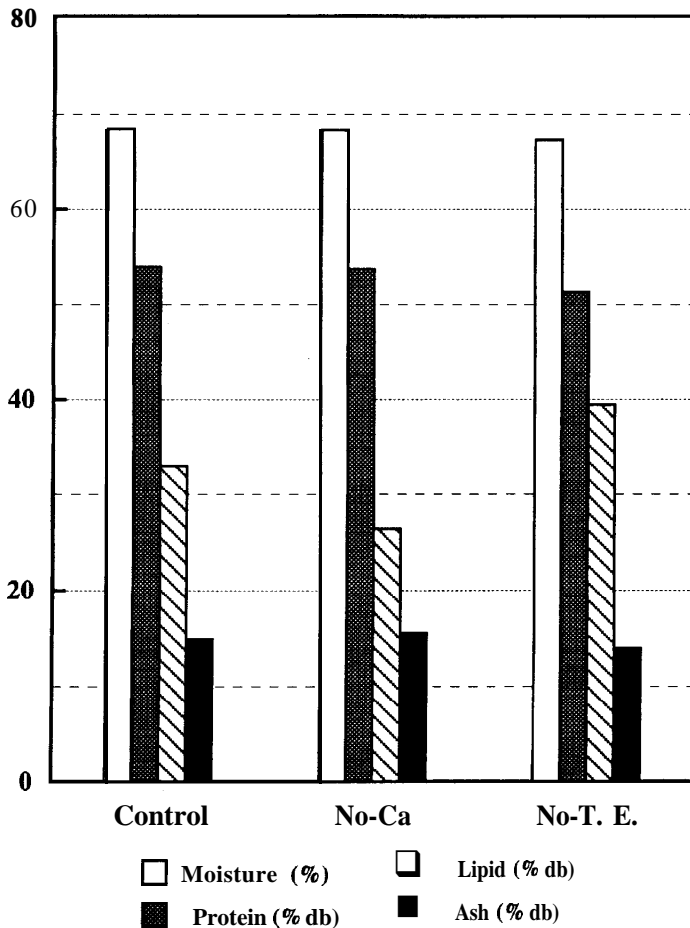
Almost no variations in the moisture, crude protein and ash contents of liver in both Ca and T.E. deficient fish were observed in comparison with the control (Fig. 4). In vertebrae of fish group received Ca deficient diet, decreased lipid content was detected, while increased level was observed in those fed T.E. deficient diet than the control (Fig. 3). The reason for this incident was unknown, however, the poor appetite and thus the feed intake in fish fed the Ca deficient diet could inversely affect the growth, resulted in a reduced lipid content of the body tissue. On the other hand, low level of Zn in fish tissues



**Fig. 3.** Effect of feeding the diets with (control) and without calcium (No-Ca) or trace elements (No-T.E.) on lipid and mineral contents of vertebrae of redlip mullet. db; dry basis.

was reported to have an inhibitory effect on the digestible enzymatic activity resulted in low levels of protein and lipid in rainbow trout (Ogino and Yang, 1978). Nevertheless, in this experiment both vertebrae and liver of fish fed the T.E. deficient diet have shown higher lipid contents than that of the control. It is possible therefore that an abrupt inadequacy of some T.E. other than Zn could be related to this phenomenon, since both Mn and Mo deficiency tends to result in abnormal metabolism in mammals (Ashley, 1972). The same thing may be intimated in the case of redlip mullet and influence the lipid levels in various body tissues.

Judging from the results of the present experiment, the addition of Ca as well as T.E.



**Fig. 4.** Proximate composition of liver of redlip mullet fed on control and calcium (No-Ca) or trace elements (No-T.E.) deficient diets for 13 weeks. db; dry basis.



to diet for redlip mullet is necessary to maintain normal growth and chemical constituents of the body.

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