ウナギ用北洋魚粉飼料における適正AlおよびFe添加量について

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Quantitative Requirements of Aluminum and Iron in the Formulated Diets and Its Interrelation with other Minerals in Young Eel*[1,2]

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Feeding study was conducted to determine the quantitative requrements of Al and Fe, and to examine the interrelationship with the other minerals in the Japanese eel *Anguilla japonica*. The experimental diets used were those based on white fish meal supplemented with 0, 15, 30, 50, 75, and 100 μg/g of Al as aluminum chloride and 0, 100, 150, 200, 300, and 400 μg/g of Fe as ferrous sulfate. The fish on diet supplemented with 15 μg/g level of Al showed the best growth among all groups. In case of Fe, the low levels of Fe supplementation in diet resulted in higher growth rate and feed efficiency. On the other hand, the highest level of Al supplementary diet (100 μg/g) and Fe supplementary diet (400 μg/g) did not cause any side effect on growth. Among the groups treated with high dietary Fe, Zn and Mn contents in the bone decreased remarkably.

Introduction

Due to expansion of aquaculture and the need for a balanced diet for culturing fish, numerous experiments have been conducted and data concerning the qualitative and quantitative requirement of fish for carbohydrate, protein, fatty acids, and some vitamins have been accumulated.[1-4] There has been a lot of studies concerning the mineral requirement of fish, and a few reports are available on both quality and quantity of minerals to be added to fish diet.[5-7] On the other hand, it was pointed out that short-body dwarfism, including malformation of head and eyes cataract was also found in rainbow trout fed on white fish meal diet twithout any supplementation of trace element.[8] These effects may be attributable to the deficiency of minerals in their diets.

In preliminary experiments employing the viewpoint of quantitative requirements of Al and Fe it was found that these are accepted as essential elements[9] and its interrelationship with other trace metals in eel.

Materials and Methods

Test Diet

Basic compositions of the experimental diets are presented in Table 1. The two rearing experiments (Experiment Al and Fe) were conducted. The test diets for Experiment Al were supplemented with six different level of Al *i.e.*, 0, 15, 30, 50, 75, and 100 μg/g in the form of aluminum chloride anhydrous ($AlCl_3$) and Al at levels of 74, 80, 97, 108, 120, 130 μg/g diet, respectively. The diets used in Experiment Fe were supplemented with 0. 100, 150, 200, 300, and 400 μg/g by using ferrous sulfate ($FeSO_4$) and Fe levels are 420, 530, 590, 640, 710, 880 μg/g diet, respectively.

The composition of the mineral mixture used in this experiment is shown in Table 2. Mineral mixture consisted of basal mineral mixture and basal trace element mixture excluding Al and Fe.

Fish, Rearing and Feeding Management

Elvers of the *Anguilla japonica*, were procued from a fish dealer in Hamamatsu City Sizuoka Prefecture. During the preliminary feeding period, they were trained to eat live tubificids and a com-

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*1 Studies on Quantity of Trace Elements in the Formulated Diets for the Young Eel *Anguilla japonica*-I.

*2 Part of this study was presented at the Annual Autumn Meeting of the Japanese Society of Scientific Fisheries on October of 1986.

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Table 1. Composition of experimental diet

Ingredient	%
White fish meal	71
α-Starch	20
Carboxymethyl cellulose	3
Vitamin mixture	1
Major mineral mixture*	4.75
Basal trace element mixture*	0.25

* Composition of the mineral and trace element mixture are show in Table 2.

Table 2. Composition of mineral mixture

Major mineral mixture

Chemical	Composition (%)
$Ca(CH_3CHOHCOO)_2$	34.2
K_2SO_4	25.0
Na_2HPO_4	21.1
KH_2PO_4	8.7
$(MgCO_3)_4Mg(OH)_2$	3.0
$MgSO_4$	3.0
Basal trace element mixture	5.0
	To make 100

Basal trace element mixture

Chemical	Content (g)	
	Al-free	Fe-free
Ferric ammonium citrate ·Brown	60.0	0 *
$CuSO_4 \cdot 5\ H_2O$	0.3	0.3
$MnSO_4 \cdot 5\text{–}6\ H_2O$	1.2	1.2
KIO_3	0.6	0.6
NaF	0.6	0.6
$CoCl_2$	2.0	2.0
$ZnSO_4$	5.0	5.0
$AlCl_3$	0	0.3

* Ferric ammonium citrate. Brown was replaced by cellulose powder in the mineral mixure.

mercial eel diet for more than two months prior to use. In Experiment Al, 1,800 well grown fish weighing 0.84 g in average body weight were selected and divided randomly into 6 groups. In Experiment Fe, fish with mean body weight of 0.87 g were used.

Each group of 300 fish were kept in out-door concrete tank having a capacity of 0.8 m^3. These tanks had a continuous flow of fresh water. The fresh water replacement occurred in approximately two hours. Water temperature was around 23.0$\pm$1.0°C during the experimental period.

Fish on each dietary regimen were offered the same weights of feed. Initially feeding was done once a day in the morning at 10% of the body weight, and subsequently the amount of feed was increased according to their appetite. Wet type diets were made by mixing sufficient water (1.5～1.8 times) to form a dough-like mass.

The feeding experiments were carried out for 120 days and the fishes were weighted on 30th, 60th, 90th and 120th day. At the end of feeding experiment, growth factor and feed efficiency were calculated.

Chemical Analysis

Chemical anlysis such as metal composition in the diets, fish tissues, and white fish meal which is commonly used as a major protein source in a formulated eel diet were conducted. All analyses of metals except phosphorus, were carried out by atomic absorption spectrophotometry[10] using a Hitach Absorption Spectrophotometer (Model 170–50 A). Phosphorus was determined spectrophotometrically by the method of Chen *et al.*[11]

Results and Discussion

Experiment of Aluminum Supplementation

The feeding experiment was conducted for 120 days and the results are summerized in Table 3. Fish on diet supplemented with 15 μg/g level of Al (diet no. 2) was observed to have the best growth in these experimental groups. However, even the highest level of Al supplemented diet no. 6 (100 μg/g of diet) did not disturb the growth rate, feed efficiency and survival rate.

In Table 4, the hepatosomatic index data are presented along with the data on condition factor. The hepatosomatic index was found to be higher in fish on diet supplemented with 15～100 μg/g diet of Al (diet no. 2～6). Whereas this index was slightly lower without supplementation of Al. Although, almost no variation in the condition factor (12.1～14.4) was observed either. The fish on a diet without supplemental with Al appeared to have comparatively lower value than remaining groups. Values of hepatosomatic index and condition factor were seemed to vary in the similar pattern as that of the growth response. Therefore, it is reasonable to assume that supplementation of 20 μg/g diet of Al can be the optimal level for healthy growth of eel, when the white fish meal contained about 60 μg/g of Al Table 5).

Fig. 1 illustrates the concentrations of trace metals in the bone of eel at the end of the feeding experiment. Results obtained in this study in-

Table 3. Results of trace metal feeding experiment (Experiment: Al)

	Diet no.					
	1 (0 μg/g)	2 (15 μg/g)	3 (30 μg/g)	4 (50 μg/g)	5 (75 μg/g)	6 (100 μg/g)
No. of fish at start	300	300	300	300	300	300
Average body weight						
at start (g)	0.84	0.84	0.84	0.85	0.85	0.82
at 30 days (g)	2.12	1.92	1.97	2.11	1.97	1.94
at 60 days (g)	4.15	3.91	3.56	3.92	3.58	3.93
at 90 days (g)	6.53	6.50	5.73	6.28	5.65	5.27
at 120 days (g)	9.07	10.70	8.76	9.59	8.31	8.78
Growth factor*1	9.80	11.70	9.43	10.30	8.78	9.71
Feed efficiency (%)*2	58.3	64.0	53.0	62.2	52.3	55.5
Survival rate (%)	90.0	83.3	85.7	90.3	89.3	87.3

*1 $\text{Growth factor} = \frac{\text{Final average body weight (g)} - \text{Initial average body weight (g)}}{\text{Initial average body weight (g)}}$.

*2 $\text{Food efficiency} = \frac{\text{Gain in body weight (g)}}{\text{Total amount of diet consumed (g)}} \times 100$.

Table 4. Hepatosomatic index and condition factor of eels in various levels of dietary Al feeding experiment

Diet no.	Hepatosomatic*1 index (%)	Condition*2 factor
1 (0 μg/g)	2.06±0.28	12.1±1.0
2 (15 μg/g)	2.43±0.49	14.4±1.6
3 (30 μg/g)	2.24±0.33	13.2±1.2
4 (50 μg/g)	2.28±0.52	13.7±1.3
5 (75 μg/g)	2.30±0.22	13.2±0.6
6 (100 μg/g)	2.41±0.48	13.5±1.8

*1 Liver weight (g) × 100/body weight (g).
*2 Body weight (g) × 100/body length^3 (cm).

Table 5. Mineral composition of white fish meal and tubificids

	White fish meal		Tubificids*1
	Exp.-Al	Exp.-Fe	
Ca (mg/g)	44.70	46.50	2.00
Mg (mg/g)	2.30	2.33	0.10
Na (mg/g)	8.06	8.30	0.50
K (mg/g)	1.58	1.74	0.50
P (mg/g)	29.50	29.10	1.20
Fe (μg/g)	310.00	419.00	86.80
Cu (μg/g)	4.58	4.30	1.80
Mn (μg/g)	9.81	10.30	2.10
Zn (μg/g)	20.60	14.10	12.90
Co (μg/g)	18.30	18.80	0.08
Al (μg/g)	64.00	77.70	N.D.*2

*1 Wet weight basis.
*1 N.D.: not detected.

dicate that the amount of supplemental Al showed there no significant change in the trace metals levels of the bone. The previous work of Ondreicka *et al.*[12] who obtained a increase of Al in food by 2-folds greatly increased fecal excretion without significantly affecting Al retention in the tissues in rats. Therefore it is resonable to assume that any change of other metals in the bone could not be recongnized in this experiment.

Experiment of Iron Supplementation

The results of the feeding experiment are summerized in Table 6. Eels fed on diet without supplemental Fe showed the highest growth rate and feed efficiency among the dietary treatments. This was mainly due to the very high level of iron in white fish meal used in the experiment. Generally, the white fish meal has a Fe concentration in the range of 200～300 μg/g. However, it was found that the white fish meal used in this experiment contained a much higher level of Fe, *i.e.*, 420 μg/g (Table 5). This may be due to the to the variation on the preparation of white fish meal in the ship. Also this can result from contamination via the grinding and bioling apparatus.[9]

Hepatosomatic index and condition factor of experimental fish are shown in Table 7. Eels fed on diet without supplemental Fe and those supplemented wit 100～300 μg/g of Fe (diet no. 7～12) demonstrated normal values of hepatosomatic index of 2.14～2.72. However, fish fed on diet supplemented with 400 μg/g of Fe (diet no. 12) showed very low value without affecting the growth and survival.

Fig. 2 shows the results of analysis for minerals

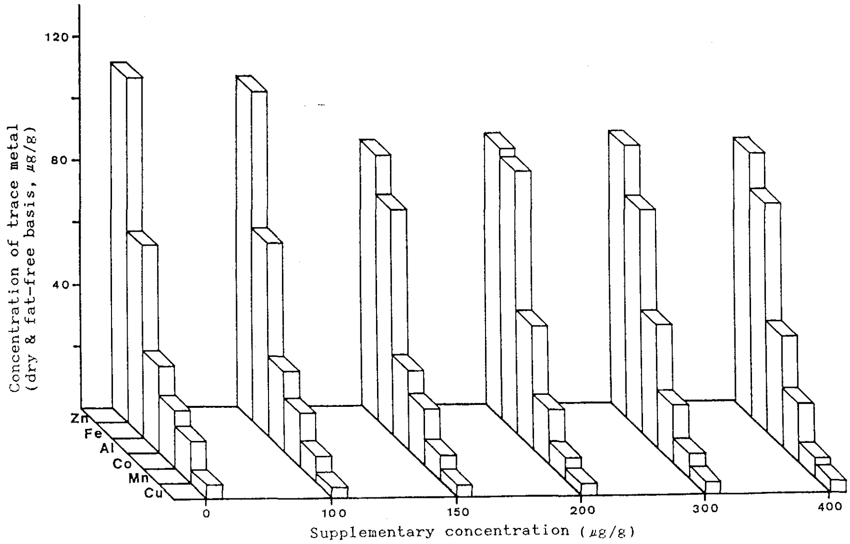

Fig. 1. Effect of supplementary Al concentration in diet on different trace metals levels in the bone of eel.

Table 6. Results of trace matal feeding experiment (Experiment: Fe)

	Diet no.					
	7 (0 μg/g)	8 (100 μg/g)	9 (150 μg/g)	10 (200 μg/g)	11 (300 μg/g)	12 (400 μg/g)
No. of fish at start	300	300	300	300	300	300
Average body weight						
at start (g)	0.87	0.88	0.86	0.87	0.87	0.89
at 30 days (g)	2.34	2.20	2.23	2.14	2.32	2.36
at 60 days (g)	4.52	4.14	4.07	4.38	4.32	4.10
at 90 days (g)	6.54	5.64	5.83	5.22	5.49	5.86
at 120 days (g)	9.46	8.88	8.27	7.09	7.33	8.21
Growth factor	9.87	9.09	8.62	7.15	7.43	8.22
Feed efficience (%)	60.4	51.2	49.8	43.5	46.8	50.0
Survival rate (%)	89.7	82.0	85.7	89.0	91.7	87.7

Table 7. Hepatosomatic index and condition factor of eels in various levels of dietary Fe feeding experiment

Diet no.	Hepatosomatic index (%)	Condition factor
7 (0 μg/g)	2.41±0.37	16.4±4.7
8 (100 μg/g)	2.72±0.65	13.6±2.0
9 (150 μg/g)	2.14±0.32	13.5±2.4
10 (200 μg/g)	2.35±0.32	12.8±1.4
11 (300 μg/g)	2.34±0.68	12.6±1.3
12 (400 μg/g)	1.68±0.29	12.4±1.3

in the liver of fish fed on the diets supplemented with various Fe levels. It illustrates that Fe administration causes significant increase of Fe level in the liver. Fe supplemented diet in the range of 0 to 400 g/g yielded a Fe level of 72.7 to 172 μg/g (wet weight) in the liver.

Fig. 3 shows the trace metal composition in the bone. A marked decrease in Zn and Mn content of the bone was indicated among those treated with high dietary Fe. Eels fed on a diet without supplemental Fe demonstrated high levels of Zn (110±5.1 μg/g dry fat-free basis) and Mn (13±2.3 μg/g) in the bone. On the contrary, a diet supplemented with 400 μg/g of Fe showed low levels of Zn (85±6.3 μg/g) and Mn (5.7±1.3 μg/g) in the bone. Ogino and Yang,[13] Watanabe *et al.*[14] reported that in rainbow trout the Zn content of the bone was inversely propotional to the dietary Fe level. The same result was also found in the present investigation. High intakes of Zn,

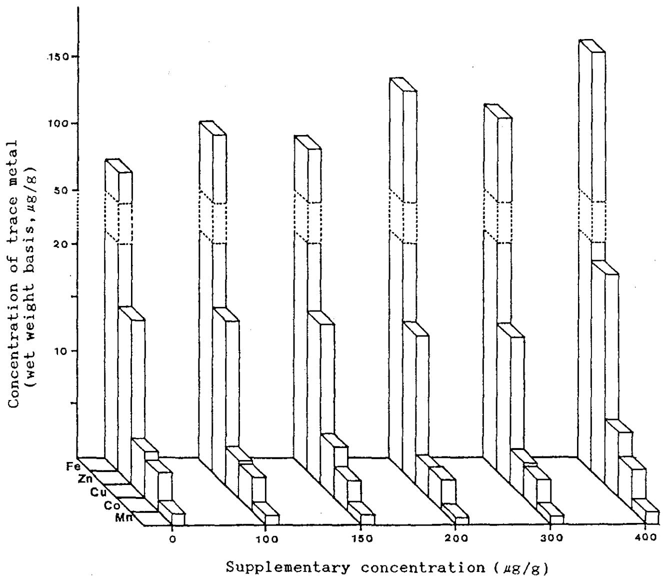

Fig. 2. Effect of supplementary Fe concentration in diet on different trace metals levels in the liver of eel.

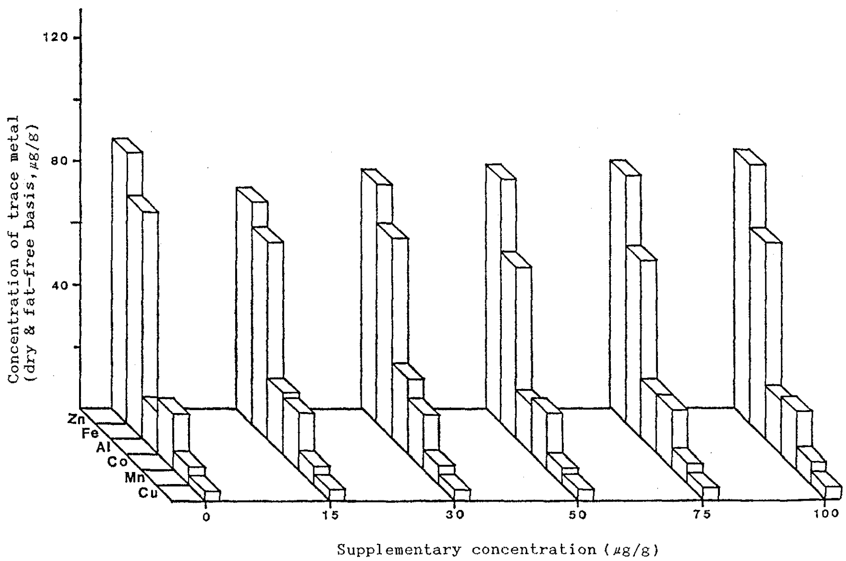

Fig. 3. Effect of supplementary Fe concentraction in diet on different trace metals levels in the bone of eel.

Cu, Mn, and Co interfere with Fe absorption through competition of absorption binding sites, and may block it almost completely in the case of human being.[15)]

Clearly, this experiment requires further examination, particularly since, at present, it is not possible to quantitate the amounts of supplemental Fe in the white fish meal diet. Nevertheless, it revealed that without supplemental Fe group relatively high growth that would otherwise indicate a availability of Fe in the white fish meal for this fish. The ingredients of meals and diets affect dietary Fe requirements profoundly through their effects on absorption. Therefore, this should be considered in detail aspect in further studies.

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