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Quantitative Requirements of Copper and Manganese in Formulated Diets and Its Interrelation with Other Minerals in Young Eel*[1,2]

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뱀장어용 配合飼料의 適正 Cu와 Mn 添加量에 관한 研究*[1,2]

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ABSTRACT

Following the previous study on the nutritional quantity of Al, Fe, and their interrelationships with other trace metals, this study was conducted to determine the effect of supplementation of Cu and Mn to fish meal-diets on the growth of Japanese eel. The feeding experiment to determine Cu requirement was conducted using white fish meal-diets supplemented with 0, 5, 10, or 20 μg/g cupric sulfate, and that to determine Mn requirement was conducted by suppling the diet with 0, 10, 20, or 40 μg/g manganese sulfate. The results revealed that the fish on diet annexed with 5 μg/g of Cu were observed to have the best growth among these groups. In case of Mn, 20 μg/g of supplementary level promoted growth rate and 40 μg/g of diet resulted in the highest feed efficiency. On the other hand, the highest level of Cu (20 μg/g), and Mn (40 μg/g) supplementary diets did not expressed adverse effect on growth. These results indicated that the suitable Cu and Mn supplementary concentrations are 20 μg/g and 30 μg/g of formulated the white fish meal-diet respectively. The white fish meal had 1.6 μg/g of Cu and 6.1 μg/g of Mn.
KEY WORDS : eel. copper and manganese requirement, formulated diet, trace metal.

要 約

주요 蛋白質源이 北洋魚粉(White fish meal)인 뱀장어용 配合飼料에 각종 無機物質중 Cop-

*[1] Studies on Quantity of Trace Elements in the Formulated Diets for the Young Eel, *Anguilla japonica*－II.

*[2] Part of this study was presented at the 1st meeting of the Korean Aquaculture Society on November of 1988.

per와 Manganese에 대한 添加 適量을 검토하기 위한 뱀장어 飼育實驗을 실시하였다.

Cu 添加 飼育實驗 結果, 5 μg/g 添加區가 成長 및 飼料效率이 가장 좋았고, 다소 肥滿度가 높은 결과를 보였다. 한편 가장 높은 濃度인 20 μg/g 실험구에서 Cu에 의한 藥害는 나타나지 않았으며, 無添加區는 添加 실험구보다 肝 重量이 다소 낮은 경향을 보였다. 그리고 筋肉 및 肝에서의 Cu 含量, 骨格에서의 Al 含量은 飼育중 Cu 含量에 比例하여 增加하며, 肝에서의 Zn 含量은 反比例하였다.

Mn 添加 飼育實驗 結果, 20 μg/g 添加區에서 가장 좋은 成長을 나타내었으며, 40 μg/g 添加區에서 飼料效率이 가장 좋았다. 筋肉, 肝 그리고 骨格의 Mn 含量 및 肝의 Cu 含量은 Mn 添加量에 比例하여 增加하였다.

이상의 結果를 볼 때 北洋魚粉을 主 蛋白質源으로 하는 뱀장어용 配合飼料에 Cu 및 Mn의 適正 添加量은 각각 5 μg/g과 30 μg/g 정도가 적합하다고 사료된다.

INTRODUCTION

The study of nutritional requirement of trace metals in fish has, with a few exceptions, been performed in the past with formulated diet containing white fish meal as protein source. In a previous study (Park and Shimizu, 1989), it was noticed that the optimum supplementary quantity of Al and Fe for eels were 20 and 150 μg/g respectively.

In recent years, concerns have been increased on the requirement of trace elements in formulated diets for eels (Arai et al., 1974). Short-body dwarfism, including malformation of head and eye cataract was also found in rainbow trout fed on white fish meal diet without any supplementation of trace elements (Watanabe et al., 1980). These effects may be attributable to the deficiency of minerals in their diets. It has also been reported that Cu or Mn-deficient diet produces low growth rate in carp (Ogino and Yang, 1980). In case of Mn, attention has been focussed primarily on the necessity of Mn in order to obtain better growth of fish (Satoh et al., 1983 b ; Yamamoto et al., 1983). However, little attention has been paid to the quantitative requirement of the fish for this element.

The eel, *Anguilla japonica* is one of the important culture fishes not only in Korea but also in the nearby Far East Asian countries. Thus, the study of nutritional requirements of eels is of vital importance in eel culture. The present study has been carried out on quantitative requirements of Cu and Mn in formulated white fish meal diets and its interrelationships with other trace metals in eels.

MATERIALS AND METHODS

Test Diet

Basic compositions of the experimental diets are presented in Table 1. The two rearing experiments (Experiments Cu and Mn) were conducted. The test diets for Experiment Cu were supplemented with four different Cu levels i.e., 0, 5, 10, and 20 μg/g in the form of cupric sulfate ($CuSO_4 \cdot 5H_2O$). The diets used in Experiment Mn were supplemented with 0, 10, 20, and 40 μg/g by using manganese sulfate ($MnSO_4 \cdot$ 5-6 H_2O).

The composition of the mineral mixture used in these experiments is shown in Table 2. Mineral mixture consisted of a basal mineral mixture and a basal trace element mixture. In these cases, the experimental trace metal i.e., Cu or Mn was not containad in the basal mineral mixture.

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 shown in Table 2.

Table 2. Composition of mineral mixture

Major mineral mixture

Name of mineral	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

Basal trace element mixture

Name of element	Content(g)	
	Cu-free	Mn-free
Ferric ammonium citrate, Brown	60.0	60.0
$CuSO_4.5H_2O$	0	0.3
$MnSO_4.5\text{-}6H_2O$	1.2	0
KIO_3	0.6	0.6
NaF	0.6	0.6
$CoCl_2$	2.0	2.0
$ZnSO_4$	5.0	5.0
$AlCl_3$	0.3	0.3

Rearing and Feeding Management

Elvers of the *Anguilla japonica,* were obtained from a fish dealer in Hamamatsu City, Sizuoka Prefecture, Japan. The experimental conditions such as rearing and feeding method were identical to those described earlier (Park and Shimizu, 1989).

One thousand eight hundred helthy elvers, 0.38 g in average body weight, were selected and randomly divided into 4 groups, each group counting 450.

Each group was kept in 0.8 m^3 out-door concrete tank. Water was introduced continuously in each tank and turn over rate was once every two hours. Water temperature was kept at around

24.5± 1.5℃ during the experimental period.

The feeding experiments were carried out for 120 days and the fish were weighed every 30days. At the end of feeding experiment, growth rate and feed efficiency were determined.

Chemical Analyses

Metal compositions of the diets, fish tissues, and white fish meal were analized. All analyses of metals except phosphorus, were carried out by atomic absorption spectrophotometry (Analytical Methods Committee, 1960) using a Hitachi Atomatic Absorption Spectrophotometer (Model 170-50A). Phosphorus was determined spectrophotometrically by the method of Chen et al. (1956).

RESULTS AND DISCUSSION

Experiment of Copper Supplementation

The feeding experiment for copper supplementation was conducted for 120 days and the results are summerized in Table 3. The growth rate and feed efficiency of eels fed on the diet which was supplemented with 5 μg/g Cu, were higher than those of the other experimental groups.

Table 3. Results of Cu supplentation experiment.

	Cu supplemented (μg/g diet)			
	0	5	10	20
No. of fish at start	450	450	450	450
Average body weight				
ar start (g)	0.38	0.38	0.38	0.38
at 30 days (g)	0.77	0.90	0.73	0.71
at 60 days (g)	1.54	1.99	1.19	1.36
at 90 days (g)	3.00	4.40	2.06	2.78
at 120 days (g)	5.57	8.41	4.58	6.97
Growth factor	13.7	21.1	11.1	17.3
Feed efficiency (%)	48.7	69.8	33.1	46.8
Mortality (%)	14.4	8.6	9.7	18.0

The results of measuring the hepatosomatic index and condition factor of eels at the end of feeding experiments are shown in Table 4. Eels fed with a diet not supplemented with copper demonstrated a slightly lower value of hepatosomatic index (2.22). However, there were no marked differences in the values of hepatosomatic index among Cu supplemented experimental diet groups (2.53~2.77). The deletion of supplemental Cu remarkably depressed the weight gain in eels. On the other hand, the other groups showed normal values of condition factor (12.9~15.2), but there were no significant differences in the values of condition factor among different groups. It was revealed that supplementation of 5 μg/g Cu to the basal trace element mixture in formulated white fish meal diet was optimum and the white fish meal contained 1.63 μg/g (Table 5.).

Fig. 1 illustrates the concentrations of trace metals in the liver of eels at the end of the feeding experiments. It is clear that high dietary Cu ingestion leads to the increment of Cu concentration in the liver. Supplementary Cu concentration of diet in the range of 0 to 20 μg/g showed Cu level of the liver to range from 1.72± 0.13 to 3.49± 0.42 μg/g wet weight basis. In the rainbow trout the

Table 4. Hepatosomatic indices and condition factors of eels fed on various concentrations of dietary Cu

Cu concentration (μg/g diet)	Hepatosomatic [1] index (%)	Condition[2] factor
0	2.22± 0.53	12.9± 1.8
5	2.77± 0.37	15.2± 2.1
10	2.53± 0.62	13.7± 1.7
20	2.66± 0.51	14.9± 1.5

[1] Liver weight (g) x 100 / body weight (g)
[2] Body weight (g) x 100 / body length^3 (cm)

Table 5. Mineral composition of white fish meal and tubificids

	White fish meal	Tubificids*
Ca (mg/g)	51.70	2.00
Mg (mg/g)	2.23	0.10
Na (mg/g)	7.40	0.50
K (mg/g)	1.62	0.50
P (mg/g)	33.70	1.20
Fe (μg/g)	217.00	86.80
Cu (μg/g)	1.63	1.80
Mn (μg/g)	6.06	2.10
Zn (μg/g)	24.50	12.90
Co (μg/g)	19.00	0.08
Al (μg/g)	25.20	N.D.**

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

continued ingestion of Cu in excess of requirements leads to accumulation to a certain extent in the tissues, such as mitochondria, microsomes, and nuclei of the liver. But liver, acts as a key organ in the metabolism of this element (Julshamn et al., 1988). Cu retention in the liver and other tissues is also influenced by the levels of Zn, Cd, and Fe in the diet (Underwood, 1977).

A marked decrease in hepatic Fe content was observed (Fig. 1). Eel fed on Cu deficient diet demonstrated a high value of hepatic Fe concentration of 188± 22.3 μg/g. On the contrary, 125± 12.6 μg/g on the wet weight basis of hepatic Fe concentration appeared in the eels fed on a Cu-excessive diet containing 20 μg/g. According to Sourkes et al. (1968) a significant inverse correlation between hepatic Fe and Cu-levels exists in rat.

There was no significant change in the mineral concentration in the muscle and bones of the eels fed on diets with various concentrtations of supplementary Cu.

Experiment of Manganese Supplementation

The results of the feeding experiment for manganese supplementation are summerized in Table 6. Supplementary Mn of 20 μg/g in the formulated white fish meal diet promoted growth rate, and the diet supplemented with 40 μg/g gave the highest feed conversion efficiency (67.1%). The poorest

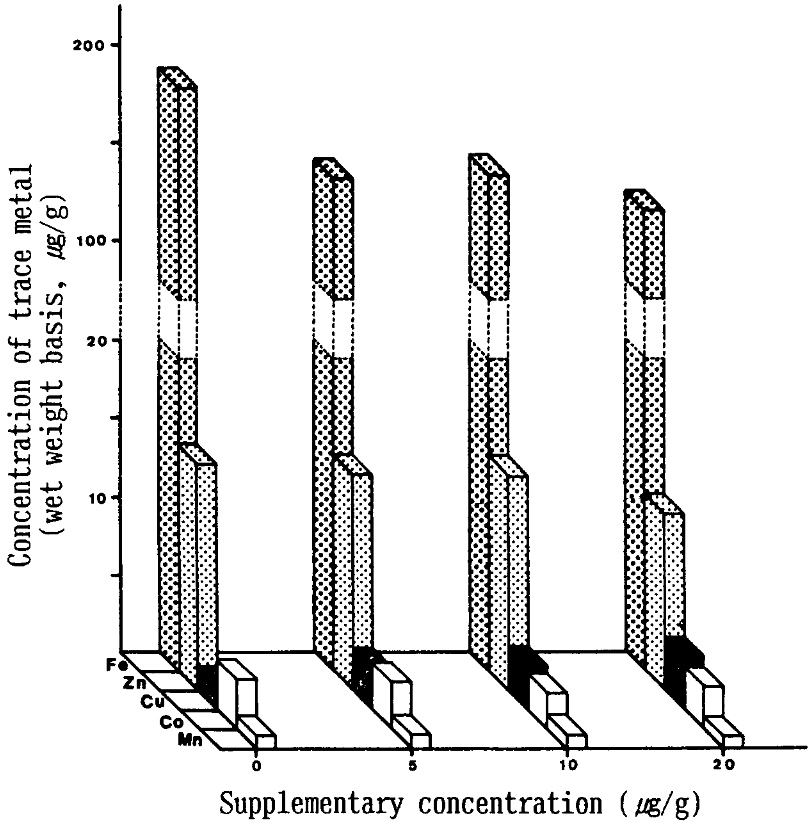

Fig. 1. Effect of supplementary Cu concentration in diet on different trace metals levels in the liver of eels.

Table 6. Results of Mn supplementation experiment

	Mn supplemented (μg/g diet)			
	0	10	20	40
No. of fish at start	450	450	450	450
Average body weight				
ar start (g)	0.38	0.38	0.38	0.38
ar 30 days (g)	0.76	0.79	0.77	0.91
at 60 days (g)	1.61	1.35	1.73	1.96
at 90 days (g)	3.20	2.63	3.32	3.45
at 120 days (g)	6.54	5.36	7.04	6.70
Growth factor	16.2	13.1	17.5	16.6
Feed efficiency (%)	55.8	37.9	58.7	67.1
Mortality (%)	11.5	5.7	10.0	8.7

growth was obtained in the diet which was supplemented with 10 μg/g diet level of Mn. However, the reasons of this poor growth are not clear. According to Yamamoto et al. (1983) and Satoh et al. (1983a) rainbow trout which were fed on white fish meal diets without a supplementation of Mn revealed that the Mn-deficient diet brought about lens cataracts and short-body dwarfism, but the growth was not much affected. Whereas, Satoh et al. (1983 b) found that the deletion of Mn from the fish meal diet reduced growth in carp. The present data revealed that the suitable Mn supplementary level to white fish meal diet is 30 μg/g for the healthy growth of eels.

The results of the condition factor and hepatosomatic index of eels at the end of the feeding experiment are shown in Table 7. Eels fed on different experimental diets demonstrated no significant differences in the hepatosomatic index and condition factor.

Table 7. Hepatosomatic indices and condition factors of eels fed on various concentrations of dietary Mn

Mn concentration (μg/g diet)	Hepatosomatic index (%)	Condition factor
0	2.82± 0.79	14.2± 1.5
10	2.62± 0.79	13.8± 1.8
20	2.99± 0.62	13.8± 1.4
40	2.72± 0.62	14.2± 1.1

The results of analytical data of trace mineral contents in the liver are shown in Fig. 2. The result indicated that there was considerable changes of the Mn, Cu, and Fe levels in the liver. A significant increase in the Mn level of the liver agrees with a earlier investigation (Maynard and Cotzias, 1955). According to this, Mn tended to be higher in mitochondria-rich tissues, such as liver, pancreas, kidney, and was more concentrated in the mitochondria than in the cytoplasm or other organelles of cells. The Cu level was also increased significantly along with Mn and Fe.

Fig. 3 shows the trace mineral composition of the bone of eels at the end of the feeding experiment. It is clear that high dietary Mn ingestion inereased Mn concentration of the bone. In the case of

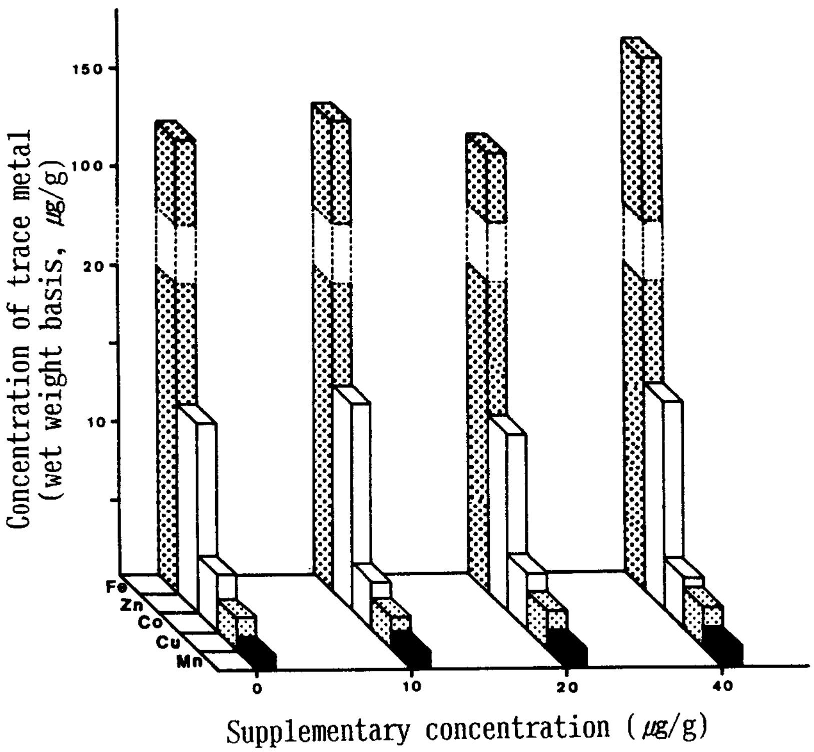

Fig. 2. Effect of supplementary Mn concentration in diet on different trace metals levels in the liver of eels.

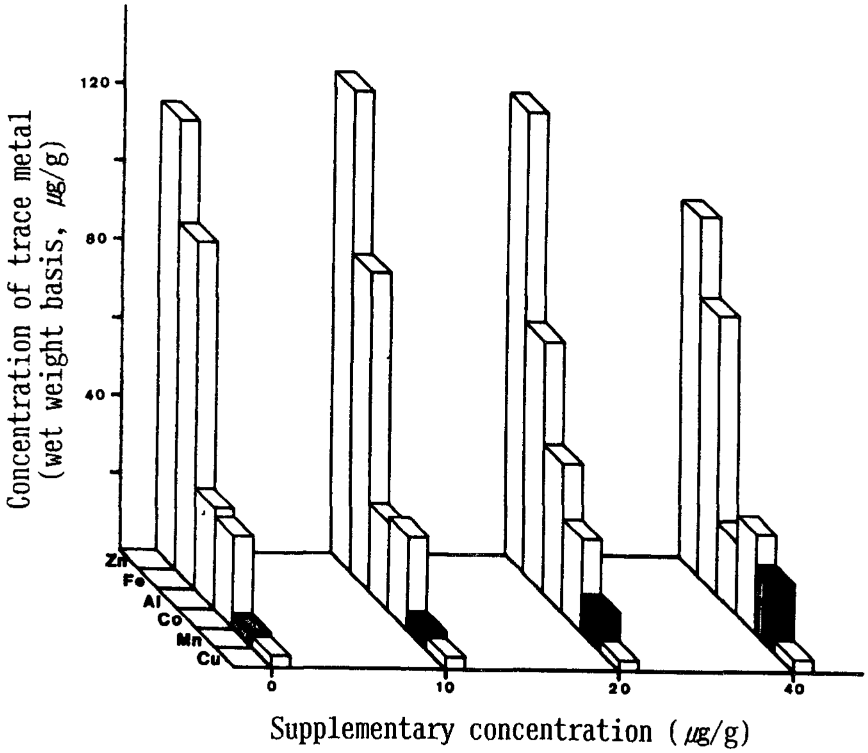

Fig. 3. Effect of supplementary Mn concentration in diet on different trace metals levels in the bone of eels.

carp, Ogino and Yang (1980) showed that Mn deleted diet induced the reduction of growth, and lower Mn concentration in the vertebrae.

CONCLUSION

To know the effects of various supplementation levels of Cu (0, 5, 10, and 20 μg/g of diet as $CuSO_4 \cdot 5H_2O$) or Mn (0, 10, 20, and 40 μg/g of diet as $MnSO_4 \cdot 5-6\ H_2O$) in the white fish meal diet on the growth, feed efficiency, hepatosomatic index, and tissue levels of Japanese eel, *Anguilla japonica* were studied.

The growth rate and feed efficiency of 5 μg/g of Cu supplemented group was better than other Cu supplemented groups. Hematosomatic index of control (0 μg/g of Cu) group was 2.22 and this is slightly lower than the other groups. Therefore, we observed that the optimum supplementary level of Cu in the white fish meal diet for young eel was 5 μg/g of diet as $CuSO_4 \cdot 5H_2O$.

The growth rate of 20 μg/g of Mn supplementary group was the highest among Mn treated group. For the feed efficiency, 40 μg/g Mn supplementary group showed the highest.

Deposit level of Cu in the liver increased as the dietary Cu level increased and that of Mn in the liver and bone also increased as the dietary Mn level increased. The deposition of Mn in the liver adversly related with Fe.

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