ニジマスによる魚粉中のZnおよび各種Zn化合物の利用性

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Availability to Rainbow Trout of Zinc in White Fish Meal and of Various Zinc Compounds*1

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A feeding experiment was conducted to examine availability to rainbow trout of Zn contained in white fish meal and that of various Zn compounds ($ZnSO_4$, $ZnNO_3$, $ZnCl_2$, $5ZnO \cdot 2CO_3$) and to determine a minumum supplementary Zn level to white fish meal based diet for normal growth of rainbow trout. Fingerlings weighing 0.3 g in average were fed with the test diets for 40 weeks at water temperature of 5–20°C. The lowest growth and the highest rate of occurrence of dwarfism together with the lens cataract were observed in fish receiving a diet without supplementary Zn(No-Zn) and these symptoms were effectively improved by addition of Zn at a level more than 40 μg/g with $ZnSO_4$ or $ZnNO_3$. Zn content of vertebrae was lowest in fish fed No-Zn diet and was proportional to dietary Zn levels, reaching a plateau by adding more than 40 μg/g of Zn.

The results of this experiment have demonstrated that supplementation of Zn at more than 40 μg/g to white fish meal diet is necessary to obtain normal growth of rainbow trout without the appearance of dwarfism and cataract and that $ZnSO_4$ or $ZnNO_3$ are found to be suitable as a Zn source for this purpose. The results have also indicated that the availability of Zn in white fish meal to rainbow trout was very low, judging from the appearance of deficiency symptoms observed in the fish fed with the diet without supplementary Zn which contained enough amount of Zn to satisfy apparent Zn requirement of rainbow trout.

In the previous work,[1-6] long term experiments were conducted with rainbow trout and carp to determine effect of trace element deletion from the mineral mixture in white fish meal based diets on growth, spawning and mineral composition of vertebrae. In rainbow trout, feeding a zinc (Zn) deleted diet resulted in reduced growth and appearance of dwarfism and eye lens cataract in high levels together with a low Zn content in every tissue. In carp, feeding a manganese (Mn) deleted diet also resulted in reduced growth and appearance of dwarfism and eye lens cataract together with a low Mn content in vertebrae.

Ogino and Yang[7] reported that the requirement of rainbow trout for Zn was 15–30 μg/g diet when determined with purified diets. The Zn deleted diet contained an enough amount of Zn derived from white fish meal which satisfies the Zn requirement, but deficiency symptoms such as dwarfism and lens cataract were induced by the Zn deleted fish meal diet. This suggests low availability of Zn in white fish meal to rainbow trout. Gatlin III and Willson[8,9] also reported that a minimum dietary Zn requirement of channel catfish was quite higher in practical diets containing fish meal than purified diets containing spray-dried egg white as a protein source.

Thus, this study was conducted to examine availability to rainbow trout of Zn contained in white fish meal and that of various Zn compounds and to determine a minimum supplementary Zn level to white fish meal based diet for normal growth of rainbow trout.

Materials and Methods

Experimental Diet

Composition of the basal diet, supplementary Zn levels and forms of Zn compounds used are shown in Tables 1 and 2, respectively. White fish meal was used as protein source. The composition of the mineral mixture used was the Ogino salt[10] mixture which satisfies mineral requirements of carp and rainbow trout at a level of 5%. Zn was deleted from the mineral mixture in order to examine availability of Zn in white fish meal and of various inorganic Zn compounds. In diet 1, Zn was deleted from the mineral mixture (No-Zn). Diets 2–5 were supplemented with respectively 10, 20, 40 and 80 μg Zn/g diet using

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Table 1. Composition of the experimental diets for rainbow trout

Ingredient	%
White fish meal	55
α-Starch	20
Dextrin	5
Lipid*1	10
Mineral mixture*2	5
Vitamin mixture	1.5
Choline chloride	0.5
Cellulose	3

*1 Soybean oil: pollock liver oil=3:2, containing 1% DL-α-tocopheryl acetate.
*2 Zn-free Ogino salt mixture.

Table 2. Supplementary forms and levels of Zn to the experimental diets for rainbow trout

Diet no.	Supplementary Zn form	Supplementary Zn level (μg/g)
1	Zn-free	0
2	$ZnSO_4$	10
3	$ZnSO_4$	20
4	$ZnSO_4$	40
5	$ZnSO_4$	80
6	$ZnNO_3$	10
7	$ZnNO_3$	20
8	$ZnNO_3$	40
9	$ZnCl_2$	20
10	$5ZnO \cdot 2CO_3$	40

$ZnSO_4$ as a Zn source. Diets 6–8 were supplemented with 10, 20 and 40 μg Zn/g diet using $ZnNO_3$. Diets 9 and 10 were supplemented with 20 and 40 μg Zn/g diet using $ZnCl_2$ and $5ZnO \cdot 2CO_3$, respectively. Proximate and mineral compositions of the experimental diets and the white fish meal used are shown in Table 3. There was no significant difference in proximate composition of all the experimental diets, being about 39% in crude protein, 17% in crude lipid and 12% in crude ash. Mineral contents except Zn showed no difference in each diet, and were enough to satisfy mineral requirement of rainbow trout.[10] Zn content of No-Zn diet was 40 μg/g diet, all derived from white fish meal. Diets supplemented with 10, 20, 40 and 80 μg of Zn contained 50, 60 80 and 120 μg Zn/g diet without regard to supplementary Zn forms, respectively. No-Zn diet also contained an enough amount of Zn to satisfy the Zn requirement (15–30 μg/g) of rainbow trout reported by Ogino and Yang.[7] These experimental diets were provided to fish in a dry pellet form as described in the previous paper.[4]

Feeding of Rainbow Trout

Fingerling rainbow trouts were used as the experimental animals. Eyed eggs obtained from the Okutama Branch of the Tokyo Fisheries Experimental Station were hatched and the fry were

Table 3. Proximate and mineral compositions of the experimental diets and white fish meal used for rainbow trout

Experimental diet no.	1	2	3	4	5	6	7	8	9	10	white fish
Supplementary Zn form		$ZnSO_4$	$ZnSO_4$	$ZnSO_4$	$ZnSO_4$	$ZnNO_3$	$ZnNO_3$	$ZnNO_3$	$ZnCl_2$	$5ZnO \cdot 2CO_3$	
Zn supplement in diet (μg/g)	0	10	20	40	80	10	20	40	20	40	
Moisture %	1.8	2.7	2.3	2.4	2.4	2.6	2.7	2.4	2.3	2.6	9.2
Crude protein %	39.8	39.1	39.3	39.1	39.5	39.2	39.9	39.4	39.4	39.3	68.1
Crude lipid %	16.8	17.5	16.8	17.5	17.4	17.0	17.3	16.9	17.4	16.7	10.1
Crude ash %	12.6	11.5	12.0	12.1	12.5	12.5	12.7	12.7	12.6	12.6	12.6
K mg/g	8.2	6.5	7.8	7.6	8.3	7.3	7.2	7.4	7.1	6.2	3.7
Na mg/g	6.7	6.3	6.4	6.4	6.3	6.3	6.4	6.3	6.4	6.5	6.1
Ca mg/g	23.6	25.0	24.7	25.8	25.5	26.3	25.3	26.6	26.4	26.7	21.5
Mg mg/g	2.0	2.0	2.0	2.1	2.0	2.0	2.0	2.1	2.0	2.0	2.1
P mg/g	21.0	20.2	21.0	21.6	20.8	21.3	20.6	21.9	20.8	20.2	23.8
Zn μg/g	39.5	49.1	60.4	80.2	117.0	50.0	58.6	81.1	58.8	79.3	63.5
Mn μg/g	23.8	22.3	22.9	22.9	22.5	21.8	22.3	22.6	22.8	23.0	4.5
Fe μg/g	306.1	260.0	275.8	245.4	269.3	238.4	276.4	268.2	247.2	270.5	104.5
Cu μg/g	6.9	5.8	6.9	5.9	6.6	5.9	6.7	6.8	6.7	6.6	2.7

Table 4. Effect of supplement of various forms of Zn to the fish meal diets on the growth and appearance of lens cataract and dwarfism of rainbow trout (40 weeks)

Diet no.	Supplemental Zn form	Zn added to diet ($\mu g/g$)	Dietary Zn level ($\mu g/g$)	Av. body wt. (g) Initial	Av. body wt. (g) Final	Growth rate	Feed efficiency*	Dwarfism (%)	Cataract (%)
1		0	39.5	0.26	114.2	439	0.96	67	28
2	$ZnSO_4$	10	49.1	0.25	123.3	492	1.04	50	36
3	$ZnSO_4$	20	60.4	0.25	144.0	575	1.07	56	33
4	$ZnSO_4$	40	80.2	0.26	143.3	550	1.06	0	0
5	$ZnSO_4$	80	117.0	0.27	137.0	506	1.12	0	0
6	$ZnNO_3$	10	50.0	0.25	123.7	494	1.02	40	30
7	$ZnNO_3$	20	58.6	0.28	135.3	482	1.06	30	20
8	$ZnNO_3$	40	81.1	0.27	134.2	496	1.12	0	0
9	$ZnCl_2$	20	58.8	0.26	124.9	479	1.10	30	30
10	$5ZnO \cdot 2CO_3$	40	79.3	0.26	132.0	507	1.06	16	0

* g gain/g feed.

kept on a commercial rainbow trout diet for 3 months. Fingerlings weighing 0.3 g in average were then devided into 10 lots of 40 fish each. Feeding was conducted for 40 weeks at water temperature of 5–20°C.

Chemical Analyses

At the end of the feeding trial, 10 fish were taken from each lot for the determination of mineral compositions in vertebrae. Vertebrae taken out from fish body were defatted with ethyl alcohol and then dried at 110°C for 3 h. Concentration of each mineral except phosphorus (P) in both diet and vertebrae of fish fed the experimental diets was determined directly after nitric acid-percloric acid digestion by using atomic absorption spectrophotometer (Hitachi ZA 180–80). The flameless method with graphite atomizer was used for determination of cupper (Cu) content which is, in general, quite low to be analysed by the flame method. Preparation and analytical conditions for P in the experimental diets and vertebrae were the same as those described previously.[3)]

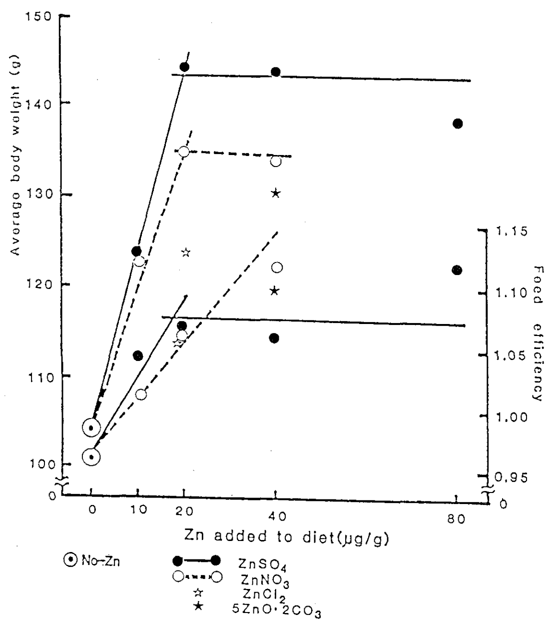

Fig. 1. Effect of dietary levels and forms of Zn on growth and feed efficiency in rainbow trout.

Results and Discussion

Growth

Results of the feeding experiment for 40 weeks are shown in Table 4 and Fig. 1. Effects of dietary levels and forms of Zn on growth of fish became significant from around the 12th week of feeding. The lowest growth was obtained in the fish receiving No-Zn diet and was effectively improved by supplementation of dietary Zn without regards to forms of Zn compounds, in agreement with our previous data and the result obtained by Ketola.[11)] The growth was proportional to supplementary Zn levels, and reached a plateau by adding Zn at a level more than 20 $\mu g/g$ diet. When growth was compared among fish receiving diets supplemented with different Zn compounds at a level of 20 μg Zn/g diet, it was highest in rainbow trout fed the diet with supplement of $ZnSO_4$, medium in fish fed the diet with $ZnNO_3$, and lowest in those fed $ZnCl_2$. In a supplementary Zn level of 40 μg Zn/g, the growth was lowest in fish fed the diet with $5ZnO \cdot 2CO_3$.

Short body dwarfism was recognized in fish fed diets containing supplementary Zn at a level less than 20 $\mu g/g$ diet without regard to forms of Zn

Table 5. Effect of supplement of various forms of Zn to the fish meal diets on the mineral compositions of the vertebrae from rainbow trout (on lipid-free basis)

		Experimental diet									
		1	2	3	4	5	6	7	8	9	10
Supplementary Zn form			$ZnSO_4$	$ZnSO_4$	$ZnSO_4$	$ZnSO_4$	$ZnNO_3$	$ZnNO_3$	$ZnNO_3$	$ZnCl_2$	$5ZnO \cdot 2CO_3$
Zn added to diet (μg/g)		0	10	20	40	80	10	20	40	20	40
Ash	%	39.9	43.4	44.2	43.0	43.9	46.7	47.7	49.0	49.8	49.3
K	mg/g	3.6	5.2	5.3	5.2	5.3	5.2	5.0	6.3	5.7	6.0
Na	mg/g	3.1	3.9	3.7	3.6	3.4	3.2	3.8	3.6	3.8	3.5
Ca	mg/g	119.3	126.3	136.1	137.0	141.4	141.5	147.4	149.1	146.2	149.5
Mg	mg/g	2.7	3.2	2.9	3.0	2.8	3.1	3.4	3.5	3.4	3.3
P	mg/g	80.1	81.4	84.3	88.4	84.1	85.0	88.5	91.6	91.0	92.5
Zn	μg/g	33.9	46.9	54.4	102.7	108.0	47.8	69.9	98.8	50.9	106.6
Mn	μg/g	9.4	8.8	13.4	12.5	13.4	13.9	15.9	15.8	11.5	14.7
Fe	μg/g	7.9	10.9	12.3	12.2	12.6	13.2	14.2	14.6	18.3	18.8
Cu	μg/g	3.3	3.7	3.4	3.6	3.8	3.6	3.5	4.1	4.9	4.9

compounds. The highest rate of occurrence of dwarfism was observed in the fish receiving No-Zn diet. The length of each vertebra of the deformed fish was shortened, as already observed in the previous experiments.[3,4,6] This symptom was found to be effectively prevented by addition of Zn at a level more than 40 μg/g with $ZnSO_4$ or $ZnNO_3$. But supplement of Zn with $5ZnO \cdot 2CO_3$ at the same level could not avoid completely the appearance of dwarfism. Eye lens cataract was also induced by feeding diets containing supplementary Zn at a level of less than 20 μg/g diet with any kinds of Zn forms. This deficiency symptom was completely prevented by addition of Zn at a level more than 40 μg/g diet with any Zn compounds.

Mineral Composition of Vertebrae

The mineral composition of vertebrae in fish fed the experimental diets is shown in Table 5. The relationship between dietary Zn levels and the Zn content of vertebrae is also shown in Fig. 2. The ash content was also lowest in this experiment in the vertebrae of fish fed No-Zn diet. As elevation of supplementary Zn levels, the ash content increased and reached a plateau by adding more than 20 μg of Zn/g with any kinds of Zn compounds. The same tendency was observed in calcium (Ca), P and Mn contents in vertebrae. On the other hand, Zn content was lowest in fish fed No-Zn diet, as observed in the previous experiments with rainbow trout and carp. The Zn content reached a plateau by adding more than 40 μg of Zn/g diet. In a supplementary level of 20 μg/g with three kinds of Zn compounds, the Zn level was lowest in vertebrae of fish fed the diet containing $ZnCl_2$ as a Zn source. It is suggested that there is some relationship between appearance of deficiency symptoms and Zn content in vertebrae, judging from the results that the appearance of dwarfism and lens cataract was avoided by supplementation of more than 40 μg Zn per g

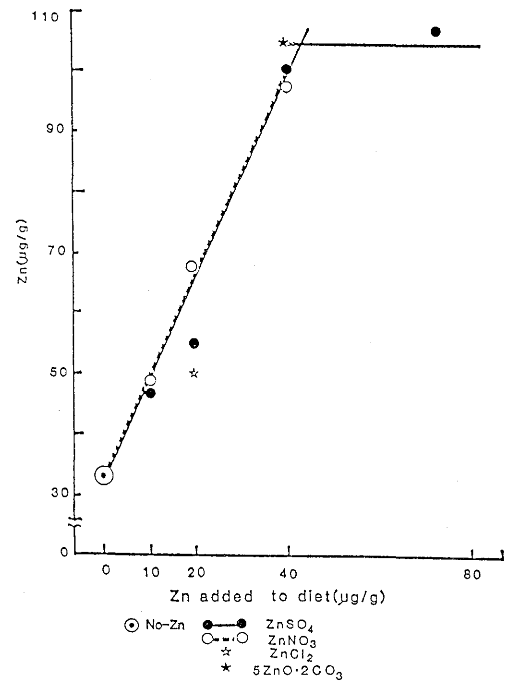

Fig. 2. Effect of dietary levels and forms of Zn on Zn content of vertebrae in rainbow trout.

diet and that Zn content in vertebrae reached a plateau by addition of the same level of Zn.

The results of this experiment have demonstrated that supplementation of Zn at more than 40 μg/g to white fish meal diets is necessary to obtain normal growth of rainbow trout without the appearance of dwarfism and cataract and $ZnSO_4$ or $ZnNO_3$ are most suitable as a Zn source for this purpose. However, this supplementary level is quite higher than the requirement of rainbow trout for Zn (15–30 μg/g) determined with semipurified diets by Ogino and Yang.[7] One of the marked differences between semipurified diets and this white fish meal based diets used in this experiment is the ash content. The ash content is much higher in white fish meal based diets that semipurified diets. The higher ash content of white fish meal is due to hydroxyapatite (mainly in the form of tricalcium phosphate) derived from fish bones of raw materials. Likuski and Forbes,[12] and Forbes *et al.*[13] reported that a high level of Ca or phytate in diet reduced bioavailability of Zn to rat. These results suggest that tricalcium phosphate in white fish meal may decrease availability of Zn in white fish meal or diet, leading to increase of an optimum Zn supplementary level in fish meal diets in comparison with semipurified diets low in tricalcium phosphate. Gatlin III and Wilson[8,9] also reported a similar result in channel catfish. The optimum supplemental Zn level for the practical diet containing 42% soybean meal and 11% menhaden fish meal is 150 mg Zn/kg diet, being much higher than the value of 20 mg/kg diet for the semipurified diet.

The results of this experiment have also demonstrated that the availability of Zn in white fish meal to rainbow trout was very low, judging from the appearance of deficiency symptoms observed in the fish fed the diet without supplementary Zn which contained an enough amount of Zn to satisfy apparent Zn requirement of rainbow trout. The availability of inorganic Zn compounds to rainbow trout was found to be highest in $ZnSO_4$ and lowest in $ZnCl_2$.

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