コイの亜鉛およびマンガン利用に及ぼす第三リン酸カルシウムの影響

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Effect of Supplemental Tricalcium Phosphate on Zinc and Manganese Availability to Common Carp*1

Shuichi Satoh,*2 Kazuhiro Izume,*2 Toshio Takeuchi,*2 and Takeshi Watanabe*2

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Two feeding experiments were conducted to determine the effect of supplemental tricalcium phosphate on zinc (Zn) and manganese (Mn) availability to common carp, using semipurified diet. The growth was lowest in fish fed the diet without supplemental Zn or Mn, and was improved by supplementation of Zn and Mn irrespective of dietary tricalcium phosphate level. However, supplement of tricalcium phosphate to the diet having insufficient amount of Mn induced higher incidence of short body dwarfism. Dietary tricalcium phosphate decreased Zn and Mn absorption, leading to reduced levels of Zn and Mn in vertebrae. Thus, tricalcium phosphate in diet was found to be one of the inhibitors against Zn and Mn bioavailability to common carp. Zn and Mn requirements of common carp were reconfirmed to be 15–30 and 13–15 μg/g, respectively.

It is reported that calcium phosphate or phytic acid contained in the diet affected the zinc (Zn) availability to salmonid fish[1-3] and channel catfish.[4,5] These reports have suggested that tricalcium phosphate contained in fish meal may inhibit availability of Zn in the meal and diet to rainbow trout, leading to poor growth, eye lens cataract and short body dwarfism. The supplementation of Zn above 40 μg/g diet to white fish meal diets was found to be essential to obtain normal growth of rainbow trout.[6] On the other hand, it was reported that availability of manganese (Mn) contained in white fish meal to common carp *Cyprinus carpio* was very high, and may not be affected by tricalcium phosphate in diet, quite different from the case of Zn for rainbow trout.[7,8] This may be due to the absence of the stomach in carp which can not dissolve tricalcium phosphate.[9]

Thus, following the previous experiments two feeding studies were conducted to examine the effect dietary tricalcium phosphate on availability to common carp of Zn and Mn in diet. In the first experiment (Experiment I) common carp were fed diets containing different levels of supplemental Zn and tricalcium phosphate in order to determine the effect of tricalcium phosphate on availability of Zn. The second experiment (Experiment II) was conducted to determine the effect of tricalcium phosphate on the availability of Mn by feeding diets containing different levels of tricalcium phosphate and Mn.

Materials and Methods

Experimental Diet

Composition of the experimental diets and the combination of dietary tricalcium phosphate and supplemental Zn and Mn levels for Experiments I and II are shown in Tables 1 and 2. Egg albumin used in both Experiments I and II was denatured by boiling with hot ethyl alcohol for 5 h.[9] The mineral mixture used was the Ogino salt mixture[9] which satisfies mineral requirements of common carp at a supplemental level of 5% in the diet. Zn or Mn was deleted from the mineral mixture to examine the effect of tricalcium phosphate on avaialbility of Zn or Mn. Tricalcium phosphate was supplemented to diets 5–8 and 14–18 at a level of 7%, which showed the same level of tricalcium phosphate as white fish meal based diets used in previous experiments.[7,8] Zn as $ZnSO_4$ which is one of the most suitable Zn sources for rainbow trout[6] was supplemented to diets 1–4 and 5–8 at levels of 0, 10, 20, and 40 μg/g diet, respectively (Table 2). Mn as $MnSO_4$ which is one of the most suitable Mn sources for common carp[7] was supplemented to diets 9–13 and 14–18 at levels of 0, 5, 10, 15, and 20 μg/g diet, respectively (Table 2). Diets

*1 Mineral Nutrition in Fish-XXV.

*2 Fish Nutrition Laboratory, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan (佐藤秀一, 井瓜一宏, 竹内俊郎, 渡邉 武: 東京水産大学水族栄養学研究室).

Table 1. Composition of the experimental diets used for common carp in Experiments I and II (%)

Ingredient	Diet no.			
	1–4	5–8	9–13	14–18
Egg albumin*1	45	45	45	45
α-Starch	20	20	20	20
Dextrin	5	5	5	5
Lipid*2	12	12	12	12
Tricalcium phosphate	0	7	0	7
Vitamin mixture*3	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5
Mineral mixture	5*4	5*4	5*5	5*5
Cellulose	11	4	11	4

*1 Egg albumin was denatured by boiling with ethyl alcohol for 5 h.
*2 Soybean oil: pollock liver oil=3: 2, containing 1% DL-α-tocopheryl acetate.
*3 Composition of the vitamin mixture was the same as that reported previously.[10]
*4 Zn was deleted from Ogino salt mixture.[9]
*5 Mn was deleted from Ogino salt mixture.[9]

Table 2. Combination of supplemental tricalcium phosphate, Zn, and Mn levels in the experimental diets

Zn in diet (μg/g)	Mn in diet (μg/g)	$Ca_3(PO_4)_2$ in diet	
		0%	7%
Experiment I			
0	20	Diet 1	Diet 5
10	20	Diet 2	Diet 6
20	20	Diet 3	Diet 7
40	20	Diet 4	Diet 8
Experiment II			
40	0	Diet 9	Diet 14
40	5	Diet 10	Diet 15
40	10	Diet 11	Diet 16
40	15	Diet 12	Diet 17
40	20	Diet 13	Diet 18

4 and 13 were control diets. All the test diets used in Experiment II were also supplemented with 0.5% of chromic oxide for determination of apparent digestibility (absorption) of minerals.

Mineral compositions of the experimental diets used in Experiments I and II are shown in Tables 3 and 4, respectively. The diets supplemented with 0 and 7% tricalcium phosphate contained about 2 and 28 mg Ca/g diet and 10 and 23 mg phosphorus (P)/g diet, respectively. Diets supplemented with 0, 10, 20, and 40 μg Zn/g diet and 0, 5, 10, 15, and 20 μg Mn/g diet contained almost equivalent amounts of Zn and Mn provided to the diets. All diets contained sufficient other minerals to satisfy the mineral requirements of common carp. These experimental diets were given to fish in dry pellet form as described in the previous paper.[7,8]

Feeding and Chemical Analyses

Fingerlings of common carp were used as the experimental animals. Carp were obtained from Sankyo Suisan Co., Ltd. They were fed a commercial carp diet for three months and then divided into 8 and 10 lots of 30 fish each, respectively for Experiments I (3.0 g on average) and II (2.0 g on average), and fed the diets shown in Tables 3 and 4 for 14 and 15 weeks in Experiments I and II, resperctively, at water temperature of 15–25°C. Feces were collected in order to determine the absorption of minerals in Experiment II. Collection of feces was carried out by the same method described previously.[3] Preparation of analytical samples and analytical conditions for chromic oxide and minerals in the test diets, feces and vertebrae were the same as those described previously.[3]

Table 3. Mineral compositions of the experimental diets used in Experiment I

	Diet no.							
	1	2	3	4	5	6	7	8
Ca (mg/g)	1.6	1.6	1.5	1.6	29.4	29.7	30.1	31.1
P (mg/g)	9.4	9.7	9.9	9.5	21.2	23.2	21.2	21.5
Mg (mg/g)	1.2	1.2	1.1	1.2	1.3	1.3	1.3	1.3
K (mg/g)	8.7	9.2	8.4	8.9	9.0	9.5	9.3	9.6
Na (mg/g)	8.2	8.4	8.1	8.0	8.1	8.3	8.0	8.1
Zn (μg/g)	2.6	12.4	22.6	41.2	2.4	10.8	22.9	42.8
Mn (μg/g)	20.7	19.9	20.0	21.6	19.5	19.9	20.4	20.1
Fe (μg/g)	258	273	261	250	283	291	278	277
Cu (μg/g)	3.1	3.2	3.2	3.1	3.1	3.0	3.1	3.2

Table 4. Mineral compositions of the experimental diets used in Experiment II

	Diet no.									
	9	10	11	12	13	14	15	16	17	18
Ca (mg/g)	2.0	2.0	2.0	1.9	1.9	28.0	28.3	28.1	28.1	28.0
P (mg/g)	10.0	10.2	10.1	10.2	10.2	23.2	23.2	23.2	23.3	23.3
Mg (mg/g)	1.2	1.2	1.2	1.2	1.2	1.3	1.3	1.3	1.3	1.3
K (mg/g)	9.2	9.4	9.2	9.2	9.1	9.0	9.1	9.1	9.0	9.0
Na (mg/g)	8.1	8.1	8.1	8.0	8.1	8.1	8.1	8.1	8.0	8.2
Zn (μg/g)	41.4	41.9	39.9	40.5	40.1	40.9	40.8	41.8	41.3	41.6
Mn (μg/g)	1.0	5.3	9.9	14.7	20.1	1.1	5.3	10.3	14.6	20.0
Fe (μg/g)	241	244	244	244	246	243	245	245	245	245
Cu (μg/g)	3.1	2.9	3.0	3.2	2.9	2.9	3.1	2.9	2.9	3.0

Table 5. Effect of supplemental tricalcium phosphate and Zn on growth and feed efficiency of common carp

Diet no.	Supplemental		Av. body wt.(g)		Growth rate (%)	Feed efficiency*
	$Ca_3(PO_4)_2$ (%)	Zn (μg/g)	Initial	Final		
1	0	0	3.09	35.78	1058	1.09
2	0	10	2.89	42.43	1368	1.12
3	0	20	2.94	43.21	1370	1.10
4	0	40	2.97	42.82	1342	1.10
5	7	0	2.96	32.19	988	0.98
6	7	10	2.92	40.97	1279	1.05
7	7	20	2.93	42.27	1348	1.10
8	7	40	2.94	41.08	1297	1.06

* g gain/g feed.

Results and Discussion

Experiment I

Results of the feeding experiment of 14 weeks and the effect of dietary tricalcium phosphate and Zn levels on growth are shown in Table 5 and Fig. 1, respectively. The lowest growth was obtained in fish receiving a diet without supplement of Zn irrespective of dietary tricalcium phosphate levels, and was effectively improved by supplement of dietary Zn, as observed in our previous experiments with carp[11] and rainbow trout.[3,6] The growth reached a plateau by feeding the diet supplemented with more than 10 μg Zn/g diet, this level was slightly lower than Zn requirement of common carp reported by Ogino and Yang.[12] When the growth was compared between the fish receiving diets containing 0 and 7% tricalcium phosphate, it was slightly higher in fish fed diets without supplemental tricalcium phosphate than that of fish on 7% tricalcium phosphate in any Zn levels. Neither shortbody dwarfism nor

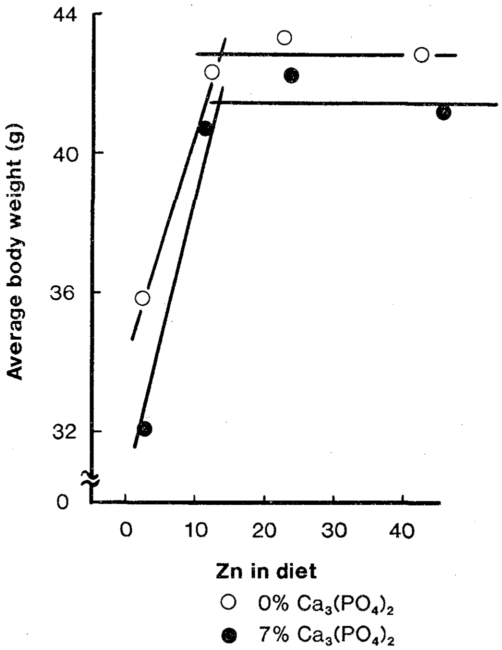

Fig. 1. Combined effect of tricalcium phosphate and Zn in diet on growth of common carp.

eye lens cataract were recognized, being different from the previous experiment with rainbow trout.[3)]

Effect of dietary tricalcium phosphate and Zn levels on the mineral composition of vertebrae of fish are shown in Table 6. Zn content was lowest in fish receiving a diet without supplement of Zn, and was increased as elevation of supplemental Zn levels in any tricalcium phosphate levels. When Zn content in vertebrae was compared among fish fed the diet supplemented with different levels of tricalcium phosphate at the same supplemental Zn level, it was higher in carp receiving the diets without tricalcium phosphate than that of fish fed the 7% tricalcium phosphate diets. The Mn content was strongly affected by the addition of tricalcium phosphate. Namely, the Mn content of fish fed 7% tricalcium phosphate was markedly lower than that of fish fed the tricalcium phosphate free diet regardless of dietary Zn levels. The Mn content was 1/3 to 1/5 of that of fish fed the tricalcium free diet. However, there was no marked difference in other mineral contents, *e. g.* Ca, P.

Experiment II

Results of the feeding experiment for 15 weeks are summarized in Table 7. The growth was lowest in fish receiving the diet without supplemental Mn, and was effectively improved by addition of Mn, and reached a near plateau by feeding the diets supplemented with more than 10 μg Mn/g diet in each tricalcium phosphate level. This Mn level was slightly lower than Mn requirement of common carp reported by Ogino and Yang.[13)] When growth was compared with fish receiving the diets with or without tricalcium phosphate at the same supplemental Mn level, it was slightly higher in carp fed diets without tricalcium phosphate than that of the fish receiving the 7% tricalcium phosphate diets. Feed efficiency was very high in all treatments, being 1.3–1.4 without difference among the experimental diets groups. The highest occurrence of short body dwarfish was recognized in fish fed the diet without supplemental Mn regardless of dietary tricalcium phosphate levels. The short body dwafism decreased with elevation of supplemental Mn level and was completely prevented

Table 6. Effect of supplemental tricalcium phosphate and Zn levels on the mineral composition of vertebrae of common carp (lipid free dry basis)

		Diet no.							
		1	2	3	4	5	6	7	8
Supplemental	$Ca_3(PO_4)_2$ (%)	0	0	0	0	7	7	7	7
	Zn (μg/g)	0	10	20	40	0	10	20	40
Crude ash (%)		51.8	51.8	52.8	50.8	51.3	51.3	52.2	53.6
Ca	(mg/g)	172	181	186	176	181	184	180	185
P	(mg/g)	87.1	92.9	93.3	93.3	93.4	91.5	87.3	88.5
Mg	(mg/g)	2.8	3.0	2.6	2.9	3.0	3.1	3.3	2.9
K	(mg/g)	3.1	3.0	3.2	3.1	3.5	3.6	3.6	3.6
Na	(mg/g)	2.6	2.8	2.9	3.1	2.9	3.0	2.7	2.9
Zn	(μg/g)	41.2	76.6	118	141	54.1	55.2	85.2	128
Mn	(μg/g)	13.5	16.4	14.9	12.3	4.7	4.3	3.6	3.1

Table 7. Effect of supplemental tricalcium phosphate and Mn on the growth of common carp

Diet no.	Supplemental		Av. body wt. (g)		Growth rate (%)	Feed efficiency*	Short body dwarfism (%)
	$Ca_3(PO_4)_2$ (%)	Mn (μg/g)	Initial	Final			
9	0	0	2.00	55.00	2650	1.30	26.7
10	0	5	2.01	63.38	3053	1.36	14.8
11	0	10	2.00	75.14	3657	1.34	6.7
12	0	15	2.05	73.84	3592	1.37	0
13	0	20	2.00	74.88	3644	1.38	0
14	7	0	2.00	50.47	2424	1.34	43.3
15	7	5	2.00	57.60	2780	1.36	36.7
16	7	10	2.00	66.00	3200	1.38	16.7
17	7	15	2.00	64.84	3142	1.34	3.3
18	7	20	2.00	65.49	3175	1.37	0

* g gain/g feed.

Table 8. Effect of supplemental tricalcium phosphate and Mn on the mineral composition of vertebrae of common carp (lipid free dry basis)

	Diet no.									
	9	10	11	12	13	14	15	16	17	18
Supplemental $Ca_3(PO_4)_2$(%)	0	0	0	0	0	7	7	7	7	7
Supplemental Mn (μg/g)	0	5	10	15	20	0	5	10	15	20
Crude ash (%)	53.0	53.4	54.1	53.7	54.3	53.3	53.7	54.5	54.4	53.7
Ca (mg/g)	189	191	191	192	192	187	190	192	191	191
P (mg/g)	93.6	94.2	95.2	96.0	95.3	92.5	92.7	93.8	94.0	93.8
Mg (mg/g)	2.9	3.1	3.0	3.2	2.9	2.9	3.0	3.0	3.0	3.0
K (mg/g)	3.3	3.1	3.1	3.3	3.1	2.7	2.9	2.7	2.8	3.0
Na (mg/g)	5.4	5.6	5.7	5.3	5.4	5.4	5.3	5.5	5.2	5.3
Zn (μg/g)	121.7	130.9	139.3	133.2	136.5	79.8	85.5	97.2	91.6	94.6
Mn (μg/g)	0.5	4.5	7.9	9.8	11.6	0.5	1.0	1.5	1.7	1.9

by addition of 15 μg Mn/g diet in the 0% tricalcium phosphate diet, and by 20 μg Mn/g diet supplement to the 7% tricalcium phosphate diet. In previous experiments,[7,8] fish meal based diets containing 13–15 μg Mn/g diet which was derived from fish meal and supplemental Mn avoided shortbody dwarfism. The relationship between the occurrence rate of short body dewarfism and dietary Mn levels supported the information of Mn requirement of carp which was reported by Ogino and Yang.[13]

Effect of dietary Mn and tricalcium phosphate on mineral composition of vertebrae is shown in Table 8. There was no difference in the contents of crude ash and macro elements. Mn content was strongly affected by the addition of tricalcium phosphate in a manner similar to Experiment I. Namely, Mn content was lowest in the vertebrae of fish fed the diet without supplemental Mn, and was effectively increased in fish fed the 0% tricalcium phosphate diets as elevation of supplemental Mn. However, the Mn content of fish fed the 7% tricalcium phosphate diets was slightly increased by supplementation of Mn, but could not reach the same level as fish receiving the 0% tricalcium phosphate diets. Zn content was higher in carp receiving diets without tricalcium phosphate than that of fish fed the 7% tricalcium phosphate diet, when Zn content in vertebrae was compared among fish fed the diets with or without tricalcium phosphate at the same supplemental Mn level. Zn content was lowest in fish fed the diet without supplemental Mn among each dietary tricalcium level, and was increased by supplementation of Mn. However, Zn content of fish fed the 7% tricalcium phosphate diets was much lower than that of fish receiving 0% tricalcium phosphate diets at the same supplemental Mn level.

Effect of dietary tricalcium phosphate on mineral absorption is shown in Table 9. Absorption of P in tricalcium phosphate-free diets showed

Table 9. Effect of supplemental tricalcium phosphate on mineral absorption in common carp (%)

Diet no.	Supplemental		Mineral absorption			
	Mn (μg/g)	$Ca_3(PO_4)_2$ (%)	P	Mg	Mn	Zn
9	0	0	94.6	91.1	0.0	82.9
10	5	0	95.6	91.4	64.4	84.6
11	10	0	97.0	91.7	69.7	90.6
12	15	0	97.3	92.3	73.9	89.2
13	20	0	97.8	92.3	80.6	89.2
14	0	7	33.5	83.4	0.0	45.1
15	5	7	35.9	84.1	10.7	49.9
16	10	7	36.2	86.6	18.4	55.8
17	15	7	38.4	87.1	18.5	56.3
18	20	7	39.4	87.2	18.7	56.2

more than 95%, these diets contained only available phosphate. However, P absorption was decreased by addition of tricalcium phosphate which was very low in availability to carp,[9] only P derived from the mineral mixture was absorbed. Absorption of Mg was slightly reduced from 92% to 83–87% by addition of tricalcium phosphate. Absorption of Mn was markedly reduced by supplementing tricalcium phosphate. These values indicate that only the tricalcium phosphate free diet supplemented with 15 and 20 μg Mn/g contained enough of available Mn to satisfy the Mn requirement of the fish. Zn absorption was also reduced from 83–89% to 45–56% by supplement of tricalcium phosphate, however these values were still higher than those obtained with rainbow trout previously.[3]

These results obtained in this study clearly indicate that supplementation of tricalcium phosphate to semipurified diet at the same level as white fish meal based diet reduces Zn and Mn content in vertebrae. However, the diets containing 15–30 μg Zn/g, which satisfy Zn requirement of carp, performed good growth regardless of supplemental tricalcium phosphate. These results suggested that tricalcium phosphate was not dissolved in the intestine due to a smaller amount of gastric juice in carp than in rainbow trout. Consequently, the effect of tricalcium phosphate on Zn absorption was much less than that found in rainbow trout, judging from the result of Experiment II. Thus, ill effect of tricalcium phosphate on Zn utilization appeared to be relatively lower in carp than in rainbow trout. It was reported that common carp contains a higher amount of Zn in several tissues than other fishes,[14] and that the absorption rate of Zn in carp might be higher than other fishes.[15] This fact may explain one of the reasons why Zn deficiency is scarcely observed in carp.

The diets supplemented with insufficient amount of Mn (0, 5, 10 μg/g) together with 7% tricalcium phosphate induced lower Mn content in vertebrae and higher incidence of short body dwarfism than those without tricalcium phosphate. However, effect of tricalcium phosphate on growth of carp was very weak, being quite different from the case of rainbow trout which was strongly affected by tricalcium phosphate.[3] However, tricalcium phosphate supplementation to carp diet reduced absorption and retention of Mn, promoted excretion of Mn, Mn content of vertebrae decreased accordingly. It is reported that the Mn content of vertebrae in carp fed fish meal based diets is ten times lower than that of rainbow trout. When the Mn content of vertebrae was compared between carp and rainbow trout fed egg albumin based diets in this and previous experiments,[3] Mn content of carp fed the tricalcium phosphate supplemented diet was one tenth of that of rainbow trout, as observed in previous experiments. The Mn content of carp effectively increased by depletion of tricalcium phosphate from the diets, although the level was still a half of rainbow trout. Thus, it is suggested that tricalcium phosphate in fish meal based diets induces a much lower Mn content in carp vertebrae than in rainbow trout since the availability of Mn was affected by tricalcium phosphate in the meal.

The results of current study suggested that tricalcium phosphate in diet reduced Zn and Mn retention in vertebrae of carp, however, the effect of tricalcium phosphate supplementation on the growth of carp was much weaker than the case of rainbow trout. The current study also reconfirmed that Zn and Mn requirements of carp are 15–30 and 13–15 μg/g diet, respectively.

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