

## MANGANESE REQUIREMENTS OF COMMON CARP *CYPRINUS CARPIO* FINGERLIGS

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### Abstract

A total number of 360 common carp fry with an average weight  $1.14 \pm 0.01$  g were divided into six experimental treatments. Manganese was added at different levels 3, 5, 8, 11, 14 and 17 mg Mn kg<sup>-1</sup> diet to a formulated basal diet contained casein and gelatin as sources of protein. Fish were fed frequently a diet of 25% crude protein at different feeding rates 7, 5 and 3% of live body weight daily. The optimum fish growth, weight gain and specific growth rate (SGR) were obtained at Mn levels from 8–14 mg/kg diet. Feed consumed was significantly higher with fish fed 14 mg/kg diet ( $p \leq 0.05$ ). Subsequently, FER and APU were significantly increased when fish were fed Mn levels from 8-14 mg/kg diet, while FCR and PER were not significantly affected. Dry matter and fat of fish body decreased significantly with increasing dietary Mn levels, while protein and ash appeared no significant different. There were significant variations in fish body Mg, Zn, P, K and Ca by dietary manganese levels, manganese concentration in whole fish body was positively correlated with Mn levels in the diet.

**Key words:** Common carp, dietary manganese, growth performance and body minerals content

### INTRODUCTION

Manganese (Mn) is essential for normal growth, reproduction and prevention of skeletal abnormalities in terrestrial animals (Hurley *et al.*, 1987) and fish (Lall, 1989). Manganese acts through its ability to sustain proper enzyme activity either

as a metal cofactor for a number of enzyme which form metal enzyme complexes. The most important biological function of Mn is as a cofactor of enzymes, particularly in energy metabolism (Wapnir, 1990). It is a vital factor in lipid and carbohydrate metabolism too.

Manganese (Mn) content of the water is not sufficient to cover the requirement of the fish, diet is considered a more significant source of the element (Srivastava and Agrawal, 1983; NRC, 1993). Although the uptake of manganese from water has been demonstrated (Miller *et al.*, 1980; Srivastava and Agarwal, 1983), it is more efficiently absorbed from feed. Effects of dietary Mn has been examined on fish species such as rainbow trout (Ogino and Yang, 1980; Knox *et al.*, 1981; Satoh *et al.*, 1983a), common carp (Ogino and Yang, 1980; Satoh *et al.*, 1983b), channel catfish (Gatlin and Wilson, 1984) and Atlantic salmon (Maage *et al.*, 2000) and Nile tilapia (El-Marakby. 2006).

Manganese deficiency caused reduced growth and skeletal abnormalities in rainbow trout, carp, and tilapia (Ishak and Dollar, 1968; Ogino and Yang, 1980; Yamamoto *et al.*, 1983). In rainbow trout, low manganese intake decreased the activities of copper-zinc superoxide dismutase and manganese-superoxide dismutase in cardiac muscle, liver, suppressed manganese and calcium concentrations of the vertebrae (Knox *et al.*, 1981).

The purpose of this study is to investigate the effect of dietary supplemental manganese levels on growth and feed utilization of common carp *Cyprinus carpio* fingerlings.

## **MATERIALS AND METHODS**

### **Diets Preparation**

A manganese free mineral premix was firstly prepared (Table 1). Traces of Mn in casein and gelatin were calculated from NRC (1993), and Mn-sulfate was added to adjust the tested Mn levels. L-cellulose was used as an inert bulkier to adjust the ingredients in the all tested diets. Purified casein/gelatin basal diets

were prepared by thoroughly mixing the dry ingredients with oil and then warm water was added until stiff dough resulted then passed through a mincer (2 mm diameter) and dried in a forced convection air drier at 65°C. Each pelleted diet was placed in plastic bags and stored at -10°C.

### **Fish and Husbandry**

Fifteen weeks experiment was conducted at Department of Animal Production and Fish Resources, Suez Canal University. Common carp *Cyprinus carpio* weighing  $1.14 \pm 0.01$  g/fish were acclimated indoor tank, for 2 weeks to laboratory conditions. The fish were distributed randomly at a rate of 20 fish per 160-L glass aquarium capacity. Each aquarium was supplied with compressed air via air-stones from air compressor. The fish were assigned to six groups with three replicates each. The fish groups fed diets containing different levels of manganese 3, 5, 8, 11, 14 and 17 mg Mn kg<sup>-1</sup> diet. Fish were fed frequently a diet containing 25% crude protein (Table 1). During the course of the experiment, all fish from each aquarium were collected every two weeks and weighed. Feeding was carried out 6 days/week, 3 times /day and the ration was adjusted each time the fish were weighed. The daily feeding rate was 7% of fish body weight for the first four weeks, then reduced to 5% for the next four weeks and further reduced to 3% for the remaining period of the study because fish were getting bigger in size.

Faeces and feed residues were removed by siphoning from each aquarium. De-chlorinated tap water was used throughout the study. In order to avoid accumulation of the metabolites, the half water of the aquarium was changed daily. Water quality parameter including pH, ammonia and alkalinity were periodically measured, water quality was found to be within the acceptable range for growth (Stickney, 1979).

### **Proximate Analysis of Diet and Fish**

The basal diet and samples of 10 fish from each treatment were analyzed using standard methods of the Association of Official Analytical Chemists (AOAC, 1990) for determination of moisture, crude protein, total lipids and ash.

### Minerals Contents

Ash was dissolved in mixture of conc. HCl: HNO<sub>3</sub> (1:1 v/v) and appropriately diluted with deionized water to constant volume. The studied elements were determined using atomic absorption spectrophotometer (Perkin Elmer model 2280). Sodium and potassium were determined by flame photometry (AOAC, 1990).

### Statistical Analysis

One- way analysis of variance was used to test the effects of the treatments on growth and feed utilization parameter. Duncan's Multiple Range Test (Duncan, 1955) was applied to compare the significance of the various parameters among the tested treatments.

## RESULTS AND DISCUSSION

Fish fed diets, in which Mn level up to 5 mg/kg till 14 mg/kg showed good growth performance in terms of final weight (g/fish), weight gain (g/fish), percent increase in weight, growth rate (%/day) in comparable to fish fed 3 mg Mn kg<sup>-1</sup> (Table 2).

The maximum growth performance was obtained at 5-14 mg Mn kg<sup>-1</sup> diet and fish response to the Mn levels in the diet. This result indicates that a level of 5-14 mg Mn kg<sup>-1</sup> diet may be sufficient to cover Mn requirement. In comparison with Mn requirements reported for other species using purified diet, it seems that common carp has higher Mn requirement 8-14 mg Mn kg<sup>-1</sup> diet than Nile tilapia 5-8 Mn kg<sup>-1</sup> diet, Atlantic salmon 7.5-10.5 mg Mn kg<sup>-1</sup> diet, rainbow trout 12-13 mg Mn kg<sup>-1</sup> diet and channel catfish 2-4 mg Mn kg<sup>-1</sup> diet (El-Marakby, 2006; Ogino and Yang, 1980; Satoh *et al.*, 1987; Satoh *et al.*, 2001; Gatlin and Welson, 1984 and Maage *et al.*, 2000). In this study, the lowest growth was obtained when common carp fed basal diet 3 mg Mn kg<sup>-1</sup> and 17 mg Mn kg<sup>-1</sup> that might have been related to problems with appetite (Rumsey and Ketola, 1975 and Helland *et al.*, 1991).

In general survival rate in fish fed all the experimental diets were high and ranged from 86.0 to 96.6% (Table 2). The survival rate was lower in group of fish fed diet containing 3 mg Mn kg<sup>-1</sup> than that fed Mn-enriched diets.

Fish fed diets in which different Mn levels had similar or even higher feed utilization efficiencies in terms of feed consumed (g/fish), FER and APU to fish fed Mn level diet from 8-17 mg Mn kg<sup>-1</sup> diet than that fed diet contain 3 and 5 mg Mn kg<sup>-1</sup> diet (Table 3). It was noticed that the addition of Mn to fish diets enhanced FCR and PER. These results indicating that, the necessity of Mn for fish growth and feed utilization. Meanwhile, Mn deficiency in the diet impaired cellular immune response in rainbow trout (Inoue *et al.*, 1998), and was associated with poor growth, depressed feed intake, skeletal abnormalities (dwarfism), eye lens cataracts, and increased mortality (Knox *et al.*, 1981).

The results of the proximate analysis of whole body common carp fed different levels of Mn for dry matter, protein; fat and ash at the end of the study are shown in Table 4. Dry matter and fat percentages were decreased with the increase of Mn levels in the diets ( $P < 0.05$ ). Fat and dry matter percentages were significantly lower in fish group fed 17 mg Mn kg<sup>-1</sup>. Protein and ash content in whole fish body was slightly increased insignificantly with increasing the dietary Mn levels ( $P < 0.05$ ).

Minerals concentration in fish body is shown in Table (5). Contents of magnesium, manganese, zinc, phosphorus, potassium and calcium in fish body were significantly changed, however Mn content in fish body was increased by increasing dietary Mn levels. In this regard, Gatlin and Wilson (1984), Lorentzen and Maage (1999) and Satoh *et al.* (2001) reported that the incorporation of high amount of Mn resulted an increase in whole body and bone Mn content. El-Marakby (2006) found a good positively correlation between Mn concentration in diet and Mn concentration in the body of Nile tilapia.

In conclusion, the study clearly indicated that the common carp fingerlings require 5-14 mg Mn kg<sup>-1</sup> dry diet to maintain the optimum growth performance and feed utilization efficiency.

Table 1. Composition and proximate chemical analyses (% on dry matter basis) of the experimental diet containing 25% crude protein.

Ingredients	%
Casein	23.4
Gelatin	6.0
Dextrin	20.0
Corn starch	16.4
L-cellulose	19.2
Corn oil	3.00
Fish oil	3.00
Vitamin mixture <sup>(1)</sup>	2.00
Mn-free mineral mixture <sup>(2)</sup>	4.00
Carboxymethyl cellulose	2.00
Calcium carbonate	1.00
Chemical analysis (%)	
Dry matter	90.3
Crude protein	25.1
Ether extract	6.3
Crude fibers	19.6
Nitrogen free extract <sup>(3)</sup>	42.15
Ash	6.85
Gross energy (Kcal/kg) <sup>(4)</sup>	4607

<sup>(1)</sup> Each one kg of vitamin mixture contains: vitamin A 72000 IU; E 60 mg; B<sub>1</sub> 6 mg; B<sub>3</sub> 12000 IU; B<sub>6</sub> 9 mg; B<sub>12</sub> 0.06 mg; C 12 mg; Pantothenic acid 60 mg; Nicotinic acid 120 mg; Folic acid 6 mg; Biotin 0.3 mg; Choline chloride 3 mg.

<sup>(2)</sup> Each one kg of mineral mixture contains: zinc sulfate heptahydrate 3.0 g; magnesium sulphate 0.335 g; cuprous chloride 0.10 g; calcium phosphate monobasic 135.8 g; calcium lactate 327.0 g; ferric citrate 29.7 g; potassium phosphate dibasic anhydrous 239.8 g; sodium phosphate monobasic 87.2; sodium chloride 43.6 g; aluminium chloride anhydrous 0.15 g; potassium iodide 0.15 g; cobalt chloride 1.0 g; sodium selenite 11 mg and L-cellulose 132.25 g.

<sup>(3)</sup> Nitrogen free extract (NFE) = 100 - (protein + lipid + ash + fiber)

<sup>(4)</sup> Gross energy (GE): Calculated after Jobling (1983) as 5.65, 9.45 and 4.2 Kcal/g for protein, lipid and carbohydrates, respectively.

Table 2. Growth performance of Common carp; *Cyprinus carpio* fed diets containing different levels of dietary Mn.

Items	Mn levels (mg kg <sup>-1</sup> diet)					
	3 mg	5 mg	8 mg	11 mg	14 mg	17 mg
Initial weight (g/fish)	1.15 ±0.05	1.14 ±0.01	1.13 ±0.01	1.14 ±0.01	1.13 ±0.01	1.12 ±0.01
Final weight <sup>1</sup> (g/fish)	27.43 <sup>b</sup> ± 0.386	30.30 <sup>ab</sup> ± 1.266	31.74 <sup>a</sup> ±1.622	31.84 <sup>a</sup> ±1.159	32.84 <sup>a</sup> ±1.241	30.80 <sup>ab</sup> ±1.459
Weight gain (g/fish)	26.28 <sup>b</sup> ± 0.265	29.16 <sup>ab</sup> ± 1.414	30.61 <sup>ab</sup> ±1.664	30.7 <sup>ab</sup> ± 0.317	31.71 <sup>a</sup> ±0.649	29.68 <sup>ab</sup> ±1.379
Increase in weight (%)	2285.22 <sup>b</sup> ±75.002	2557.89 <sup>ab</sup> ± 85.281	2708.85 <sup>a</sup> ±110.136	2692.98 <sup>a</sup> ±60.371	2806.19 <sup>a</sup> ±76.530	2650.0 <sup>a</sup> ±85.654
Growth rate <sup>2</sup> (g/day)	0.250 <sup>b</sup> ±0.002	0.278 <sup>ab</sup> ±0.013	0.292 <sup>ab</sup> ± 0.016	0.292 <sup>ab</sup> ± 0.003	0.302 <sup>a</sup> ± 0.006	0.283 <sup>ab</sup> ± 0.013
SGR <sup>3</sup> (%/day)	3.18 <sup>b</sup> ± 0.031	3.28 <sup>ab</sup> ± 0.035	3.34 <sup>a</sup> ± 0.04	3.35 <sup>a</sup> ± 0.028	3.36 <sup>a</sup> ± 0.029	3.32 <sup>a</sup> ± 0.032
Survival rate (%)	86.6	88.3	96.6	96.6	95.0	91.66

a,b Means with different superscripts in the same row are significantly different at P<0.05.

1- Weight gain = Final weight – initial weight.

2 – Growth rate (g/day) = Weight gain/ time (days).

3- Specific growth rate (%/day) = (Ln final weight – Ln initial weight/time (days) ×100.

Table 3. Feed utilization efficiency parameters of Common carp; *Cyprinus carpio* fed formulated diets containing different levels of dietary Mn.

Items	Mn levels (mg kg <sup>-1</sup> diet)					
	3 mg	5 mg	8 mg	11 mg	14 mg	17 mg
Feed consumed (g /fish)	41.14 <sup>b</sup> ± 8.240	43.94 <sup>ab</sup> ± 9.762	43.33 <sup>ab</sup> ± 7.401	43.8 <sup>ab</sup> ± 8.196	45.77 <sup>a</sup> ± 1.6234	43.19 <sup>ab</sup> ± 0.2228
FCR <sup>1</sup>	1.56 ± 0.0404	1.51 ± 0.0404	1.42 ± 0.0436	1.43 ± 0.0306	1.44 ± 0.0404	1.46 ± 0.0379
FER <sup>2</sup>	0.66 <sup>b</sup> ± 0.0118	0.66 <sup>b</sup> ± 0.0119	0.70 <sup>a</sup> ± 0.0837	0.70 <sup>a</sup> ± 0.0837	0.69 <sup>ab</sup> ± 0.0691	0.68 <sup>ab</sup> ± 0.0103
PER <sup>3</sup>	2.65 ± 0.0569	2.65 ± 0.0896	2.83 ± 0.0643	2.80 ± 0.0493	2.77 ± 0.0702	2.75 ± 0.0656
APU <sup>4</sup>	136.9 <sup>b</sup> ± 0.6658	138.76 <sup>b</sup> ± 0.9054	151.52 <sup>a</sup> ± 1.0046	151.78 <sup>a</sup> ± 1.5543	150.96 <sup>a</sup> ± 1.1548	149.95 <sup>a</sup> ± 0.8202

a,b Means with different superscripts in the same row are significantly different at P<0.05.

1- Feed conversion ratio (FCR) = feed intake (g) / weight gain.

2- Feed efficiency ratio (FER) = body weight gain (g)/ feed intake(g)

3- Protein efficiency ratio (PER) = gain in weight (g)/ protein intake in feed (g).

4- Apparent protein utilization (APU) = Protein gain in fish (g) / protein intake in feed (g) × 100.

Table 4. Proximate composition (% on dry matter basis) of whole body of common carp fed experimental diets containing different levels of Mn.

Items	Mn levels (mg kg <sup>-1</sup> diet)					
	3 mg	5 mg	8 mg	11 mg	14 mg	17 mg
Dry matter	34.85 <sup>a</sup> ± 1.2905	33.79 <sup>ab</sup> ± 0.7895	32.58 <sup>ab</sup> ± 1.3009	31.90 <sup>ab</sup> ± 0.9268	31.50 <sup>ab</sup> ± 1.1758	30.64 <sup>b</sup> ± 1.2385
Protein	51.68 ± 0.7780	52.30 ± 1.0095	53.60 ± 1.3987	54.15 ± 1.0404	54.45 ± 1.6958	54.50 ± 1.1859
Fat	31.50 <sup>a</sup> ± 1.3029	30.80 <sup>ab</sup> ± 1.1820	29.20 <sup>ab</sup> ± 1.2553	28.20 <sup>ab</sup> ± 0.9412	28.05 <sup>ab</sup> ± 0.5168	27.80 <sup>b</sup> ± 0.7112
Ash	16.80 ± 0.7217	16.95 ± 0.5809	17.26 ± 0.9280	17.65 ± 0.5965	17.50 ± 0.6062	17.70 ± 0.8573

a,b Means with different superscripts in the same row are significantly different at P<0.05.



Table 5. Changes in some elements in whole fish body (ppm) of common carp fed diets containing different levels of Mn.

Items	Mn levels (mg kg <sup>-1</sup> diet)					
	3 mg	5 mg	8 mg	11 mg	14 mg	17 mg
Mg	10.7883 <sup>b</sup> ± 0.0267	11.1542 <sup>ab</sup> ± 0.2374	10.9775 <sup>b</sup> ± 0.0635	11.4975 <sup>a</sup> ± 0.0686	10.3537 <sup>c</sup> ± 0.0329	10.9375 <sup>b</sup> ± 0.1405
Mn	0.2407 <sup>c</sup> ± 0.071	0.2242 <sup>c</sup> ± 0.0111	0.3359 <sup>c</sup> ± 0.0143	0.3353 <sup>b</sup> ± 0.004	0.3400 <sup>b</sup> ± 0.004	0.4922 <sup>a</sup> ± 0.035
Zn	15.4487 <sup>d</sup> ± 0.1527	19.2740 <sup>a</sup> ± 0.1764	17.7992 <sup>c</sup> ± 0.2372	18.1505 <sup>bc</sup> ± 0.0416	18.5597 <sup>b</sup> ± 0.0974	19.3390 <sup>a</sup> ± 0.2121
P	310.00 <sup>d</sup> ± 15.00	598.33 <sup>c</sup> ± 9.279	762.00 <sup>a</sup> ± 4.163	771.67 <sup>a</sup> ± 8.819	703.33 <sup>b</sup> ± 28.038	666.00 <sup>b</sup> ± 8.327
K	1520.00 <sup>d</sup> ± 35012	2790.00 <sup>c</sup> ± 415.25	3340.00 <sup>b</sup> ± 106.93	4116.67 <sup>a</sup> ± 44.10	3593.33 <sup>ab</sup> ± 173.33	3593.33 <sup>ab</sup> ± 128.63
Ca	2155.00 <sup>d</sup> ± 78.16	4086.67 <sup>a</sup> ± 63.33	3530.00 <sup>b</sup> ± 25.12	4291.67 <sup>a</sup> ± 40.86	2960.00 <sup>c</sup> ± 317.70	2810.00 <sup>c</sup> ± 173.49

a, b, c, d Means with different superscripts in the same row are significantly different at P<0.05.

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## الإحتياجات الغذائية من المنجنيز لإصبعيات سمك المبروك

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تمت الدراسة على ٣٦٠ لإصبعيات مبروك عادي (متوسط وزن  $1.14 \pm 0.01$  جم) قسمت إلى ستة معاملات لكل منها ثلاث مكررات. تم إضافة المنجنيز بتركيزات ٣، ٥، ٨، ١١، ١٤، ١٧ مجم/كجم عليقة إلى ٢٥% بروتين مصدره الكازين و الجيلاتين بمعدل تغذية يومية متدرجة حسب الوزن و هي ٧، ٥، ٣% من وزن الأسماك لمدة ١٠٥ يوم. أظهرت النتائج أن أفضل نمو وأعلى معدل نمو نسبي وأفضل زيادة في الوزن وأعلى كفاءة غذائية كانت في المجاميع التي تغذت على عليقة محتوية على من ٨ الى ١٤ مجم منجنيز/كجم علف.

وقد تأثرت كل من المادة الجافة ونسبة الدهن في الأسماك بينما لم تتأثر كل من نسبة البروتين وأيضاً نسبة الرماد بإضافة المنجنيز في العليقة.

كان هناك تداخل معنوي لتركيز كل من الماغنسيوم والزنك والفوسفور والبوتاسيوم والكالسيوم في جسم الأسماك ومستويات المنجنيز في العليقة. وأيضاً كان هناك ارتباط إيجابي لتركيز المنجنيز في جسم الأسماك ومستوياته في العليقة.

ويستنتج من هذه الدراسة أن الإحتياجات الغذائية لإصبعيات سمك المبروك العادي تتراوح بين ٥-١٤ مجم/كجم عليقة.