Aquaculture 450 (2016) 74–79

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Dietary manganese requirement of juvenile large yellow croaker *Larimichthys crocea* (Richardson, 1846)

Hanle Zhang [a], Ruijian Sun [a,b], Wei Xu [a], Huihui Zhou [a], Wenbing Zhang [a,*], Kangsen Mai [a]

[a] The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China
[b] Technology Center of Tongwei Co., LTD Chengdu 610041, China

ARTICLE INFO

Article history:
Received 23 May 2015
Received in revised form 14 July 2015
Accepted 15 July 2015
Available online 21 July 2015

Keywords:
Large yellow croaker
Manganese
Requirement
Growth
Oxidative stress

ABSTRACT

A 9-week feeding trial was conducted to determine the dietary manganese (Mn) requirement of juvenile large yellow croaker *Larimichthys crocea* (Richardson, 1846) (initial mean weight 7.71 ± 0.02 g). Manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$) was added to the semi-purified basal diet to prepare 6 experimental diets with graded levels of Mn (2.33, 7.63, 13.16, 18.27, 24.05 and 41.79 mg/kg, respectively). Results showed that the specific growth rate (SGR), feed efficiency (FE), Mn concentrations in whole-body, liver and vertebra, and activities of hepatic Mn superoxide dismutase (Mn-SOD) and total SOD (T-SOD) were significantly improved by dietary Mn supplementation ($P < 0.05$). However, fish fed the basal diet with 2.33 mg/kg of Mn had the significantly highest Mn retention, proportion of hepatic Mn to whole-body Mn and activity of copper–zinc superoxide dismutase (Cu–Zn SOD) ($P < 0.05$). As biomarkers of oxidative stress, malondialdehyde (MDA) and protein carbonyl (PC) in serum were significantly higher in fish fed the basal diet ($P < 0.05$). Using the broken-line models based on SGR and hepatic Mn-SOD activity, dietary Mn requirements of the juvenile large yellow croaker were estimated to be 16.44 and 16.16 mg/kg, respectively.

Statement of relevance

This study is not a test of commercial aquaculture.

1. Introduction

Manganese (Mn) is an essential micronutrient for growth, reproduction and prevention of skeletal abnormalities in terrestrial animals. Furthermore, it plays an essential role in many cellular processes including lipid, protein and carbohydrate metabolism and serves as a cofactor or activator for many enzyme systems, such as Mn superoxide dismutase (Mn-SOD) (De Rosa et al., 1980; Andreini et al., 2008). Symptoms of manganese deficiency include increased mortality, retarded growth and skeletal abnormalities (NRC, 2011). Different from terrestrial animals, fish can absorb Mn from aquatic environment as well as feed. Dietary supplementation of Mn is often required because Mn absorbed from water is not sufficient.

Requirements of dietary Mn ranging from 2.4 to 24.93 mg/kg have been reported in various fish species including fresh water fish and marine fish, such as rainbow trout and common carp (Ogino and Yang, 1980), channel catfish (Gatlin and Wilson, 1984), grass carp (Wang and Zhao, 1994), Atlantic salmon (Maage et al., 2000), gibel carp (Pan et al., 2008), tilapia (Lin et al., 2008), grouper (Ye et al., 2009), yellow catfish (Tan et al., 2012), cobia (Liu et al., 2013) and turbot (Ma et al., 2014).

Large yellow croaker is one of the most successful marine fish culture operations in terms of the number of juveniles produced and commercial size fish production annually in China (Liu et al., 2008). However, chopped or minced trash fish is still the major diet for large yellow croaker. Given that trash fish is difficult to store and may cause diseases and environmental pollution, formulated feed with balanced nutrients is urgently needed. Dietary requirements of most nutrients have been established for this species in the last decades. In regard to the minerals, only the requirements of dietary phosphorus, iron, zinc and copper have been established (Mai et al., 2006; Zhang, 2007; Zhang et al., 2008; Cao et al., 2014). The purpose of this study was to determine the dietary Mn requirement of juvenile large yellow croaker.

2. Materials and methods

2.1. Diet preparation

The formulation and proximate composition of the experimental diets are shown in Table 1. Casein, gelatin and fish protein concentrate

* Corresponding author.
E-mail address: wzhang@ouc.edu.cn (W. Zhang).

http://dx.doi.org/10.1016/j.aquaculture.2015.07.010
0044-8486/

Table 1
Composition and proximate analysis of the basal diet (% dry matter).

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Vitamin free casein[1]	30.0	30.0	30.0	30.0	30.0	30.0
Gelatin[2]	7.6	7.6	7.6	7.6	7.6	7.6
Fish protein concentrate[3]	8.0	8.0	8.0	8.0	8.0	8.0
Dextrin[4]	25.0	25.0	25.0	25.0	25.0	25.0
Fish oil[5]	9.0	9.0	9.0	9.0	9.0	9.0
Lecithin	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin mix[6]	2.0	2.0	2.0	2.0	2.0	2.0
Mn-free mineral mix[7]	2.0	2.0	2.0	2.0	2.0	2.0
Compound attractant[8]	1.0	1.0	1.0	1.0	1.0	1.0
Taurine	0.5	0.5	0.5	0.5	0.5	0.5
Mold inhibitor[9]	0.1	0.1	0.1	0.1	0.1	0.1
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Microcrystalline cellulose	12.75	12.74	12.74	12.74	12.74	12.74
$MnSO_4 \cdot H_2O$ (mg/kg diet)	0	15.52	31.04	46.56	62.08	124.15
Proximate analysis (n = 3)						
Moisture (%)	7.8	7.5	7.4	7.8	7.6	7.5
Crude protein (%)	42.3	43.1	42.5	43.0	42.4	42.7
Crude lipid (%)	11.2	11.1	11.4	11.2	11.5	11.3
Ash (%)	7.2	6.9	7.3	7.1	7.3	7.2
Mn (mg/kg diet)	2.3	7.6	13.2	18.3	24.1	41.8
Cu (mg/kg diet)	3.9	3.2	3.5	3.4	3.7	3.2
Zn (mg/kg diet)	39.1	40.7	40.5	40.6	39.7	40.7

[1] Casein, vitamin-free: Sigma Chemical, St. Louis, MO, USA.
[2] Gelatin: Shandong Yixin Biological Technology Co., Ltd., Shandong Province, China.
[3] Fish muscle protein: Shanghai Haiqing Aquatic Bio-tech. Co., Ltd., Shanghai, China.
[4] Dextrin: Shandong Xiwang Sugar Co., Ltd., Shandong Province, China.
[5] Fish oil: Qingdao Great-seven Bio-tech. Co., Ltd., Qingdao, China.
[6] Vitamin mix (mg or g/kg diet): thiamin, 25 mg; riboflavin, 45 mg; pyridoxine·HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.2 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; microcrystalline cellulose, 14.1617 g.
[7] Mn-free mineral mix (mg or g/kg diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H2O$, 50 mg; $CoCl_2 \cdot 6H_2O$, 50 mg; Na_2SeO_3, 20 mg; $Ca(IO_3)_2$, 60 mg; $Ca(H_2PO_4)_2 \cdot H2O$, 3000 mg; microcrystalline cellulose, 15.485 g.
[8] Compound attractant (g/kg diet): betain · HCl, 4 g; dimethyl-β-propiothetin, 2 g; glycine, 2 g; alanine, 2 g; taurine, 5 g.
[9] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

were used as the dietary protein sources. Fish oil and lecithin were used as the lipid sources. Manganese sulfate monohydrate ($MnSO_4 \cdot H_2O$, Sinopharm Chemical Reagent Co., Ltd, SCR, Shanghai, China) was added into the basal diet to formulate experimental diets with 6 graded supplemented levels of Mn (0, 5, 10, 15, 20 and 40 mg/kg diet, respectively). Final dietary Mn concentrations were 2.33, 7.63, 13.16, 18.27, 24.05 and 41.79 mg/kg, respectively, as analyzed by the inductively coupled plasma–atomic emission spectrophotometer (ICP–OES; VISTA-MPX, VARIAN, USA) (Tan and Mai, 2001).

2.2. Feeding trial

Large yellow croaker juveniles were obtained from Fufa hatchery (Ningde, Fujian Province, China) and reared in sea. Prior to the start of the feeding trial, juveniles were stocked in a large sea cage (4 × 4 × 4 m) to acclimate to the experimental condition and the basal diet for 2 weeks. At the beginning of the feeding trial, the fish were not fed for 24 h, and then weighed after being anesthetized with eugenol (1:10,000) (Sinopharm Chemical Reagent Co., Ltd, SCR, Shanghai, China). Fish with similar size (initial mean weight: 7.71 ± 0.02 g) were distributed to 18 sea cages (1.5 × 1.5 × 2.0 m) at density of 70 fish per cage. Each diet was hand-fed to triplicate groups of fish twice daily (05:00 and 17:00) to apparent satiation for 9 weeks. During the feeding trial, the water temperature ranged from 27.4 to 33.2 °C, salinity varied between 30 and 33‰ and dissolved oxygen was higher than 7 mg/l. The Mn concentration in seawater was 9.2–9.6 μg/l. Fish were reared under the natural light conditions throughout the feeding trail.

2.3. Sample collection and analysis

At the start of the feeding trial, twenty fish were sampled and stored frozen (−20 °C) for proximate analysis of the initial whole-body mineral composition. At the end of the feeding trial, all treatment groups were not fed for 24 h, and then the total numbers and body weight of fish in each cage were counted and measured to calculate survival, special growth rate (SGR) and feed efficiency (FE). Five fish were randomly selected from each cage and stored frozen (−20 °C) to determine the whole-body mineral composition. Another five fish were randomly selected from each cage and dissected to obtain liver and vertebra for mineral analysis. Liver were weighed and stored frozen (−20 °C) and vertebras were prepared as described previously (Mai et al., 2006). Nine fish per cage were randomly chosen to collect serum and liver to analyze enzyme activity.

Samples of diet were dried to constant weight at 105 °C to determine moisture. Crude protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method (2300-Auto-analyzer, FOSS, Hillerød, Denmark), crude lipid by ether extraction using Soxhlet method (36680-analyzer, BUCHI, Flawil, Switzerland) and ash by combustion using a muffle furnace at 550 °C (AOAC, 1995). Mn, Cu and Zn concentrations in the diet, whole-body, liver and vertebrae were analyzed using ICP–OES (VISTA-MPX, VARIAN, USA) (Tan and Mai, 2001). Duplicate analyses were conducted for each sample.

The liver samples were homogenized in 9 volumes (v/w) of ice-cold (0 °C) normal saline, and then centrifuged at 3000 g for 20 min at 4 °C. The supernatants were collected and stored at −80 °C before the enzyme activity analysis. Activities of the hepatic T-SOD and Cu–Zn SOD were analyzed based on the enzymes' ability to inhibit the oxidation of hydroxylamine catalyzed by the xanthine–xanthine oxidase system using commercial kits. Activity of Mn-SOD was calculated by T-SOD activity minus Cu–Zn SOD activity. Protein contents in the supernatants were determined by Coomassie brilliant blue method using commercial kit. The content of malondialdehyde (MDA) in serum was determined through measuring the pink color produced by the reaction of thiobarbituric acid (TBA) with MDA at 90–100 °C using commercial kits. The content of protein carbonyl (PC) in serum was evaluated by 2, 4-dinitrophenylhydrazine (DNPH) method using commercial kits. All the commercial kits mentioned above were provided by Nanjing Jiancheng Bioengineering Institute, Nanjing, China. The parameters mentioned above were assayed by colorimetric method and absorbance was measured with the UV spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan). Duplicate analyses were conducted for each sample.

2.4. Calculations and statistical analysis

The survival, growth, feed utilization and Mn distribution were calculated by the following formulae:

$$\text{Survival rate (\%)} = 100 \times \text{final number of fish/initial number of fish.}$$

$$\text{Specific growth rate (SGR, \% d}^{-1}) = 100 \times (\text{Ln } W_t - \text{Ln } W_0) / t.$$

$$\text{Feed efficiency (FE)} = (W_t - W_0) / I.$$

$$\text{Mn retention (\%)} = 100 \times (W_t \times M_t - W_0 \times M_0) / (I \times M).$$

$$\text{Proportion of hepatic Mn to whole-body Mn (\%)} = 100 \times (W_l \times M_l) / (Wt \times Mt).$$

Where W_t, W_0 and W_l are final body weight, initial body weight and liver weight, respectively; I is feed fed as dry matter. M, M_t, M_0 and M_l represent Mn concentration in diet, final fish body, initial fish body and liver, respectively.

The results were presented as means ± SE of three replicates. Data were subjected to one-way analysis of variance (ANOVA). When overall differences were significant ($P < 0.05$), Tukey's test was used to compare the mean values among the treatments by SPSS 16.0 (SPSS Inc., Chicago, USA). The dietary Mn requirement

Table 2
Effect of dietary Mn levels on final weight, specific growth rate (SGR), feed efficiency (FE), Mn retention and survival rate of large yellow croaker.

Dietary Mn (mg/kg)	Final weight (g)	SGR (%·d^{-1})	FE	Mn retention (%)	Survival rate (%)
2.33	28.27 ± 0.03^{a}	2.06 ± 0.00^{a}	0.80 ± 0.03^{a}	29.4 ± 4.7^{b}	84.3 ± 2.9
7.63	29.37 ± 0.50^{ab}	2.12 ± 0.03^{ab}	0.84 ± 0.01^{a}	12.9 ± 1.0^{a}	82.4 ± 3.6
13.16	31.11 ± 0.40^{abc}	2.21 ± 0.02^{bc}	0.92 ± 0.02^{b}	12.4 ± 2.5^{a}	85.2 ± 4.6
18.27	32.56 ± 1.89^{c}	2.28 ± 0.09^{c}	1.03 ± 0.01^{b}	12.7 ± 1.7^{a}	85.2 ± 2.2
24.05	32.46 ± 1.24^{bc}	2.28 ± 0.06^{c}	0.99 ± 0.05^{b}	9.2 ± 0.3^{a}	84.8 ± 5.0
41.79	32.03 ± 0.88^{bc}	2.26 ± 0.05^{bc}	1.00 ± 0.03^{b}	6.9 ± 0.4^{a}	83.3 ± 8.1
ANOVA					
F value	6.869	7.859	23.140	35.654	0.171
P value	0.004	0.002	0.000	0.000	0.969

Values (means ± S.E.) in the same column sharing a common superscript letter were not significantly different.

based on SGR and Mn-SOD were estimated using broken-line model (Y = L − U (R − X)) (Robbins et al., 1979).

3. Results

3.1. Growth and feed utilization

The results on growth and feed utilization are presented in Table 2. Survival rate of juvenile large yellow croaker ranging from 82.38 to 85.24% was not significantly affected by dietary Mn. However, final weight, SGR and FE significantly increased ($P < 0.05$) when dietary Mn levels increased from 2.33 mg/kg to 18.27 mg/kg, and then leveled off with the further increasing of dietary Mn. Fish fed the basal diet had significantly higher ($P < 0.05$) Mn retention than that in the Mn-supplemented treatments. Meanwhile, no significant difference was observed among the five Mn-supplemented treatments.

Broken-line analysis of SGR showed that the requirement of dietary Mn for large yellow croaker was 16.44 mg/kg (Fig. 1).

3.2. Tissue mineral concentration and distribution

Data on mineral concentration and distribution are shown in Table 3. The significantly lowest whole-body, hepatic and vertebral Mn concentrations were found in fish fed the basal diet ($P < 0.05$). Moreover, the supplementation of dietary Mn significantly increased tissues' Mn concentrations, as dietary Mn contents ranged from 7.63 to 42.79 mg/kg ($P < 0.05$). The concentrations of Cu and Zn in the above tissues showed no significant differences among all the treatments.

The proportion of hepatic Mn to whole-body Mn was significantly higher ($P < 0.05$) in fish fed the basal diet when compared with that in fish fed diets with 13.16 and 18.27 mg Mn/kg (Table 4). However, no significant difference was observed when compared with fish fed with higher dietary Mn (≥24.05 mg/kg). The proportions of hepatic Cu and Zn to those in whole-body were not significantly affected by dietary Mn level.

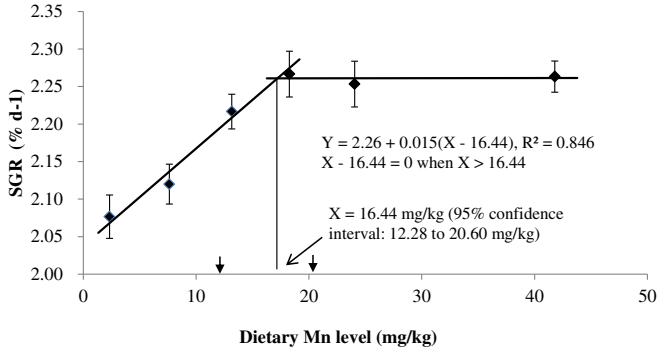

Fig. 1. Relationship between specific growth rate (SGR) and dietary Mn levels based on a broken-line regression analysis, where X represents dietary Mn requirement of large yellow croaker.

3.3. Oxidative stress and anti-oxidative response

Results related to oxidative stress and anti-oxidative response are presented in Table 5. The significantly highest serum MDA and PC contents were found in fish fed the basal diet ($P < 0.05$). Increasing dietary Mn contents from 7.63 to 18.27 mg/kg resulted in significantly decreased MDA content. Meanwhile, PC contents also decreased as dietary Mn contents increased from 7.63 to 13.16 mg/kg. Higher dietary Mn content did not result in further significant decrease of MDA and PC.

The hepatic T-SOD activity significantly increased with increasing dietary Mn levels from 2.33 to 18.27 mg/kg, and reached a plateau thereafter ($P < 0.05$). A similar trend was also found in the hepatic Mn-SOD activity. In contrast, the highest Cu–Zn SOD activity was found in fish fed the basal diet ($P < 0.05$).

Broken-line analysis of the hepatic Mn-SOD activity showed that the requirement of dietary Mn for large yellow croaker was 16.16 mg/kg (Fig. 2).

4. Discussion

In the present study, SGR increased significantly with increasing dietary Mn contents up to the optimal level. Similarly, positive relationship between growth and dietary Mn level has been reported in other fish species, such as rainbow trout (Ogino and Yang, 1980), common carp (Ogino and Yang, 1980), grass carp (Wang and Zhao, 1994), gibel carp (Pan et al., 2008), grouper (Ye et al., 2009), yellow catfish (Tan et al., 2012) and cobia (Liu et al., 2013). Thus it could be concluded that dietary Mn is essential for normal growth of large yellow croaker, and retarded growth observed herein can be ascribed to Mn deficiency.

It is noteworthy that Mn concentrations in the whole body, liver and vertebra increased constantly with increasing dietary Mn levels without plateau reached. Similarly, no plateau of increased tissue Mn concentrations was found in grouper (Ye et al., 2009), yellow catfish (Tan et al., 2012) and turbot (Ma et al., 2014). The result indicated that tissue Mn concentration was a poor indicator for the determination of Mn requirement for large yellow croaker.

In the present study, supplementation of Mn significantly increased the activity of Mn-SOD. Previous studies in fish found that Mn-SOD activity was regulated by dietary Mn contents. For example, suppressed hepatic Mn-SOD activity has been observed in Mn-deficient rainbow trout (Knox et al., 1981), tilapia (Lin et al., 2008) and cobia (Liu et al., 2013).

According to the broken-line models based on SGR and hepatic Mn-SOD activity, dietary Mn requirements of the juvenile large yellow croaker were estimated to be 16.44 and 16.16 mg/kg, respectively. This is higher than those of some fish species in previous studies, such as common carp and rainbow trout (13–15 mg Mn/kg) (Ogino and Yang, 1980), channel catfish (2.4 mg Mn/kg) (Gatlin and Wilson, 1984),

Table 3
Effect of dietary Mn levels on whole-body, hepatic and vertebral mineral concentrations of large yellow croaker.

Dietary Mn (mg/kg)	Whole-body			Liver			Vertebra		
	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
2.33	1.9 ± 0.1^{a}	1.0 ± 0.2	22.3 ± 0.9	1.9 ± 0.4^{a}	11.4 ± 1.6	62.7 ± 4.4	50.0 ± 37.2^{a}	78.2 ± 6.4	214.8 ± 20.8
7.63	2.2 ± 0.0^{ab}	0.8 ± 0.1	23.5 ± 3.4	2.5 ± 0.2^{ab}	12.5 ± 0.6	62.1 ± 3.7	66.6 ± 10.9^{ab}	89.4 ± 13.5	232.2 ± 21.5
13.16	2.7 ± 0.3^{bc}	0.8 ± 0.1	25.0 ± 4.5	2.7 ± 0.1^{b}	13.8 ± 1.7	64.5 ± 7.9	83.4 ± 10.3^{bc}	88.8 ± 2.1	227.9 ± 14.0
18.27	3.2 ± 0.2^{cd}	0.8 ± 0.1	23.3 ± 1.6	3.3 ± 0.2^{c}	12.9 ± 2.3	63.8 ± 4.8	114.9 ± 15.0^{cd}	105.3 ± 10.1	254.1 ± 40.9
24.05	3.2 ± 0.1^{d}	0.8 ± 0.1	24.3 ± 1.1	3.0 ± 0.1^{cd}	10.6 ± 1.3	67.5 ± 6.6	123.1 ± 12.2^{d}	96.9 ± 32.6	255.0 ± 37.5
41.79	3.8 ± 0.3^{e}	0.9 ± 0.1	27.2 ± 1.5	4.1 ± 0.3^{d}	10.7 ± 0.9	68.8 ± 14.5	140.1 ± 14.3^{d}	105.4 ± 14.5	211.3 ± 22.5
ANOVA									
F value	39.621	1.619	1.327	42.466	2.209	0.345	26.021	0.980	1.356
P value	0.000	0.229	0.317	0.000	0.121	0.876	0.000	0.469	0.307

Values (means ± S.E.) in the same column sharing a common superscript letter were not significantly different.
Minerals in whole-body and liver were calculated based on wet weight, while minerals in vertebra were calculated based on lipid free dry matter.

grass carp (15 mg Mn/kg) (Wang and Zhao, 1994), Atlantic salmon (7.5–10.5 mg Mn/kg) (Maage et al., 2000), gibel carp (13.77 mg Mn/kg) (Pan et al., 2008), tilapia (7 mg Mn/kg) (Lin et al., 2008) and yellow catfish (5.5–6.4 mg Mn/kg) (Tan et al., 2012). Meanwhile, higher requirements of dietary Mn were also observed in some warm water marine fish species, such as grouper (19 mg Mn/kg diet) (Ye et al., 2009) and cobia (21.72–24.93 mg Mn/kg diet) (Liu et al., 2013). The difference in dietary Mn requirement among these studies could be attributed to fish species, diet formulation and evaluating criteria (Liu et al., 2013).

Higher Mn retention, larger proportion of hepatic Mn to whole-body Mn and higher Cu–Zn SOD activity were found in fish fed the basal diet, which might be compensatory responses to the Mn-deficiency. Investigators have noted the compensatory responses to mineral deficiency in animals, which includes increased absorption and decreased excretion of mineral (Flanagan et al., 1983; Carriquiriborde et al., 2004; Bai et al., 2008; Lin et al., 2008) and changed distribution of minerals among different tissues (Davis et al., 1992; Malecki et al., 1994; Kamunde et al., 2002). Furthermore, higher activity of the Cu–Zn SOD in Mn-deficient chickens and rats than in controls were found, which were considered as compensation for Mn-deficiency (De Rosa et al., 1980; Zidenberg-Cherr et al., 1983; Malecki and Greger, 1996).

Animals can maintain stable tissue level of Mn via tight homeostatic control of both intestine absorption and endogenous excretion (Malecki et al., 1996). One may speculate that fish can increase Mn absorption and reduce Mn excretion to compensate dietary Mn deficiency. Unfortunately, there is little information on the adaptive changes to alleviate Mn-deficiency in fish. In the present study, although absorption and biliary excretion were not detected, significant higher Mn retention was observed in fish fed the basal diet which was regarded as Mn-deficient. Similar results have been obtained previously in Mn-deficient rat with 70 fold reduction in endogenous losses of Mn (Malecki et al., 1994).

Table 4
Effect of dietary Mn levels on the proportions of hepatic mineral to whole-body mineral.

Dietary Mn (mg/kg)	Mn (%)	Cu (%)	Zn (%)
2.33	1.4 ± 0.1^{b}	13.3 ± 1.0	3.5 ± 0.3
7.63	1.2 ± 0.0^{ab}	14.2 ± 1.4	2.9 ± 0.6
13.16	1.0 ± 0.2^{a}	15.4 ± 1.3	2.6 ± 0.5
18.27	1.1 ± 0.1^{a}	14.9 ± 0.4	2.8 ± 0.7
24.05	1.2 ± 0.1^{ab}	14.0 ± 2.6	2.9 ± 0.6
41.79	1.1 ± 0.1^{ab}	12.8 ± 0.4	2.6 ± 0.6
ANOVA			
F value	3.808	1.394	1.279
P value	0.027	0.294	0.335

Values (means ± S.E.) in the same column sharing a common superscript letter were not significantly different.
Values were calculated from the absolute ratio of mineral content in liver and whole-body.

Moreover, increased absorption was found in broiler intestine treated with low level of Mn (Bai et al., 2008). The result herein indicated that higher retention of Mn might be the compensatory response for Mn-deficiency. The elevated Mn retention in tilapia fed Mn-deficient diet further supports the hypothesis (Lin et al., 2008). Further study on the effect of dietary Mn on Mn absorption and endogenous loss is needed.

In the present study, a greater proportion of hepatic Mn to whole-body Mn was found in fish fed the basal diet. Similarly, Davis et al. (1992) reported that Mn-deficient rat had a greater proportion of newly absorbed ^{54}Mn distributed in liver compared with Mn-adequate one. According to Malecki et al. (1994), manganese may shift between tissue compartments to maintain essential function, such as Mn-SOD activity. Similarly, larger proportion of hepatic Cu to whole-body Cu (20% versus 10%) was found in rainbow trout fed low dietary Cu (12.6 nmol/g) compared with fish fed normal dietary Cu (50.4 nmol/g) (Kamunde et al., 2002). However, there are no published data on the effect of dietary Mn level on proportion of whole-body Mn retained in liver of fish. Accordingly, increased proportion of hepatic Mn to whole-body Mn might be compensatory response for Mn-deficiency. Moreover, investigating proportions of whole-body Mn retained in other tissues including muscle, bone, intestine, gill and serum in fish fed various levels of Mn could be an interesting topic.

Suppressed Mn-SOD activity and enhanced Cu–Zn SOD activity were found in fish fed the basal diet in the present study. Activity of Mn-SOD in the fish fed the basal diet was about 48% of that in Mn-adequate treatments (18.27 mg/kg), while Cu–Zn SOD activity was approximately 10% greater in fish fed the basal diet. Superoxide radicals ($O_2^{\bullet-}$) produced as by-products of metabolic oxidation can induce oxidative stress. Mn-SOD localized in the mitochondrial matrix and Cu–Zn SOD localized predominantly in cytoplasmic both can catalyze the dismutation of the $O_2^{\bullet-}$ (Lebovitz et al., 1996). Han et al. (2003) reported that $O2^{\bullet-}$ generated in mitochondria can be released to cytosol. Moreover, $O_2^{\bullet-}$ can upregulate the gene expression of Cu–Zn SOD (Miao and St Clair, 2009). Considering that more $O_2^{\bullet-}$ would be released from mitochondrial to cytosol in fish with lower Mn-SOD activity, the increase of Cu–Zn SOD activity might be a compensatory response for alleviating raised $O_2^{\bullet-}$ in cytosol (De Rosa et al., 1980). Similar compensatory increases of Cu–Zn SOD activity were found in rat (Zidenberg-Cherr et al., 1983; Malecki et al., 1994; Lebovitz et al., 1996; Malecki and Greger, 1996) and chicken (De Rosa et al., 1980) when Mn-SOD activity was deficient. Ma et al. (in press) observed T-SOD and Mn-SOD activity in turbot fed graded levels of Mn, while Cu–Zn SOD activity was not calculated. We calculated the data and also found that Cu–Zn SOD activity was higher (32 U/mg prot. versus 25 U/mg prot.) in fish fed Mn-deficient diet (3.7 mg/kg) than fish fed Mn-sufficient diet (13.0 mg/kg). Similarly, increased Mn-SOD activity was observed in mice (Elchuri et al., 2005; Sentman et al., 2006) and rainbow trout (Hidalgo et al., 2002) when Cu–Zn SOD activity was decreased.

Table 5
Effect of dietary Mn levels on oxidative stress and anti-oxidative response in the liver and serum of large yellow croaker.

Dietary Mn (mg/kg)	T-SOD (U/mg prot.)	Cu–Zn SOD (U/mg prot.)	Mn-SOD (U/mg prot.)	MDA (nmol/ml serum)	PC (nmol/ml serum)
2.33	213.2 ± 4.9^{a}	175.4 ± 3.5^{b}	37.8 ± 1.4^{a}	3.2 ± 0.3^{c}	13.3 ± 1.3^{b}
7.63	224.3 ± 8.1^{ab}	170.4 ± 5.4^{ab}	54.0 ± 3.8^{b}	1.9 ± 0.2^{b}	9.1 ± 1.1^{ab}
13.16	228.5 ± 8.6^{ab}	161.1 ± 4.7^{a}	67.4 ± 3.9^{bc}	1.6 ± 0.3^{ab}	7.6 ± 1.3^{a}
18.27	236.2 ± 6.7^{b}	158.8 ± 3.4^{a}	77.4 ± 5.0^{c}	1.1 ± 0.2^{a}	9.5 ± 1.7^{ab}
24.05	237.3 ± 11.7^{b}	164.8 ± 6.5^{ab}	72.6 ± 8.9^{c}	1.3 ± 0.4^{ab}	9.5 ± 2.4^{ab}
41.79	241.1 ± 7.6^{b}	162.5 ± 2.1^{a}	78.5 ± 6.1^{c}	1.4 ± 0.3^{ab}	8.0 ± 2.3^{a}
ANOVA					
F value	4.764	5.725	26.216	24.023	3.961
P value	0.012	0.006	0.000	0.000	0.024

T-SOD, total superoxide dismutase; MDA, malondialdehyde; PC, protein carbonyl.
Values (means ± S.E.) in the same column sharing a common superscript letter were not significantly different.

The reactive oxygen species (ROS) is the oxygen radical formed in the metabolism of molecular oxygen and it can initiate a wide range of oxidative damage within the cell (Elchuri et al., 2005). Malondialdehyde (MDA) and protein carbonyl (PC) are products generated during oxidative damage induced lipid peroxidation and protein damage. They were higher in fish fed the basal diet, in the present study, while supplementation of Mn decreased them. Previous studies also found that Mn-deficient rat had significantly higher lipid peroxidation (Zidenberg-Cherr et al., 1983; Malecki and Greger, 1996). The results indicated that fish fed the basal diet confronted with severer oxidative stress, which consisted with suppressed antioxidant responses in fish fed the basal diet.

5. Conclusion

It can be concluded from the present study that 1) supplementation of dietary Mn can improve growth, feed utilization, Mn deposition in whole-body, liver and vertebrae, and activity of hepatic Mn-SOD. 2) Based on SGR and Mn-SOD, dietary Mn requirements of juvenile large yellow croaker were 16.44 and 16.16 mg/kg diet, respectively. 3) Higher Mn retention, larger proportion of hepatic Mn to whole-body Mn and higher Cu–Zn SOD activity in fish fed the basal diet might be compensatory responses to dietary Mn-deficiency.

Acknowledgments

The work was supported by grants from the National Natural Science Foundation of China (No. 31372542), the National Public Sector Funding for Special Research Project of China (201003020) and the State Spark-Program China (2014GA701001). We thank X. Miao, J.J. Cao, S.Q. Lin, L. Cheng, X.X. Geng and J. Ma for their help in feeding and sampling.

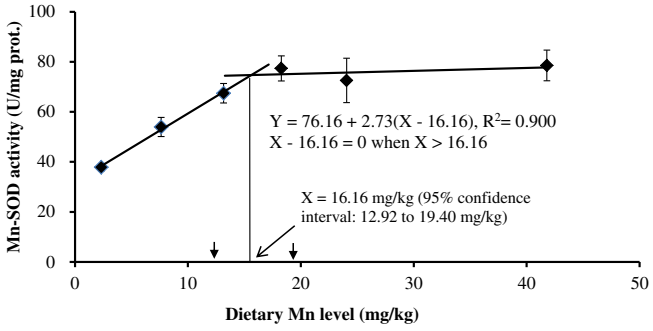

Fig. 2. Relationship between Mn-SOD activity and dietary Mn levels based on a broken-line regression analysis, where X represents the dietary Mn requirement of large yellow croaker.

References

Andreini, C., Bertini, I., Cavallaro, G., Holliday, G.L., Thornton, J.M., 2008. Metal ions in biological catalysis: from enzyme databases to general principles. J. Biol. Inorg. Chem. 13, 1205–1218.
AOAC, 1995. Official Methods of Analysis of AOAC International. AOAC, Arlington, VA.
Bai, S., Lu, L., Luo, X., Liu, B., 2008. Kinetics of manganese absorption in ligated small intestinal segments of broilers. Poult. Sci. 87, 2596–2604.
Cao, J., Miao, X., Xu, W., Li, J., Zhang, W., Mai, K., 2014. Dietary copper requirements of juvenile large yellow croaker *Larimichthys croceus*. Aquaculture 432, 346–350.
Carriquiriborde, P., Handy, R., Davies, S., 2004. Physiological modulation of iron metabolism in rainbow trout (*Oncorhynchus mykiss*) fed low and high iron diets. J. Exp. Biol. 207, 75–86.
Davis, C.D., Wolf, T.L., Greger, J., 1992. Varying levels of manganese and iron affect absorption and gut endogenous losses of manganese by rats. J. Nutr. 122, 1300–1308.
De Rosa, G., Keen, C.L., Leach, R.M., Hurley, L.S., 1980. Regulation of superoxide dismutase activity by dietary manganese. J. Nutr. 110, 795–804.
Elchuri, S., Oberley, T.D., Qi, W., Eisenstein, R.S., Jackson Roberts, L., Van Remmen, H., Epstein, C.J., Huang, T.T., 2005. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. Oncogene 24, 367–380.
Flanagan, P.R., Haist, J., Valberg, L.S., 1983. Zinc absorption, intraluminal zinc and intestinal metallothionein levels in zinc-deficient and zinc-replete rodents. J. Nutr. 113, 962–972.
Gatlin, D.M., Wilson, R.P., 1984. Studies on the manganese requirement of fingerling channel catfish. Aquaculture 41, 85–92.
Han, D., Antunes, F., Canali, R., Rettori, D., Cadenas, E., 2003. Voltage-dependent anion channels control the release of the superoxide anion from mitochondria to cytosol. J. Biol. Chem. 278, 5557–5563.
Hidalgo, M.C., Expósito, A., Palma, J.M., Higuera, M.d.l., 2002. Oxidative stress generated by dietary Zn-deficiency: studies in rainbow trout (*Oncorhynchus mykiss*). Int. J. Biochem. Cell Biol. 34, 183–193.
Kamunde, C., Grosell, M., Higgs, D., Wood, C.M., 2002. Copper metabolism in actively growing rainbow trout (*Oncorhynchus mykiss*): interactions between dietary and waterborne copper uptake. J. Exp. Biol. 205, 279–290.
Knox, D., Cowey, C.B., Adron, J.W., 1981. The effect of low dietary manganese intake on rainbow trout (*Salmo gairdneri*). Br. J. Nutr. 46, 495–501.
Lebovitz, R.M., Zhang, H., Vogel, H., Cartwright, J., Dionne, L., Lu, N., Huang, S., Matzuk, M.M., 1996. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc. Natl. Acad. Sci. 93, 9782–9787.
Lin, Y.-H., Lin, S.-M., Shiau, S.-Y., 2008. Dietary manganese requirements of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 284, 207–210.
Liu, M., Mitcheson, D., Sadovy, Y., 2008. Profile of a fishery collapse: why mariculture failed to save the large yellow croaker. Fish Fish. 9, 219–242.
Liu, K., Ai, Q.H., Mai, K.S., Zhang, W.B., Zhang, L., Zheng, S.X., 2013. Dietary manganese requirement for juvenile cobia, *Rachycentron canadum* L. Aquac. Nutr. 19, 461–467.
Ma, R., Hou, H., Mai, K., Bharadwaj, A., Ji, F., Zhang, W., 2014. Comparative study on the effects of chelated or inorganic manganese in diets containing tricalcium phosphate and phytate on the growth performance and physiological responses of turbot *Scophthalmus maximus*. Aquac. Nutr. http://dx.doi.org/10.1111/anu.12206 (in press).
Maage, A., Lygren, B., El-Mowafic, A.F.A., 2000. Manganese requirement of Atlantic salmon (*Salmo salar*) fry. Fish. Sci. 66, 1–8.
Mai, K., Zhang, C., Ai, Q., Duan, Q., Xu, W., Zhang, L., Liufu, Z., Tan, B., 2006. Dietary phosphorus requirement of large yellow croaker, *Pseudosciaena crocea* R. Aquaculture 251, 346–353.
Malecki, E.A., Greger, J., 1996. Manganese protects against heart mitochondrial lipid peroxidation in rats fed high levels of polyunsaturated fatty acids. J. Nutr. 126, 27–33.
Malecki, E.A., Huttner, D.L., Greger, J.L., 1994. Manganese status, gut endogenous losses of manganese, and antioxidant enzyme activity in rats fed varying levels of manganese and fat. Biol. Trace Elem. Res. 42, 17–29.
Malecki, E.A., Radzanowski, G.M., Radzanowski, T.J., Gallaher, D.D., Greger, J., 1996. Biliary manganese excretion in conscious rats is affected by acute and chronic manganese intake but not by dietary fat. J. Nutr. 126, 489–498.
Miao, L., St Clair, D.K., 2009. Regulation of superoxide dismutase genes: implications in disease. Free Radic. Biol. Med. 47, 344–356.

NRC, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press Washington, DC.
Ogino, C., Yang, G., 1980. Requirements of carp and rainbow trout for dietary manganese and copper. Bull. Jpn. Soc. Sci. Fish. 46, 455–458.
Pan, L., Zhu, X., Xie, S., Lei, W., Han, D., Yang, Y., 2008. Effects of dietary manganese on growth and tissue manganese concentrations of juvenile gibel carp, *Carassius auratus* gibelio. Aquac. Nutr. 14, 459–463.
Robbins, K.R., Norton, H.W., Baker, D.H., 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109, 1710–1714.
Sentman, M.L., Granstrom, M., Jakobson, H., Reaume, A., Basu, S., Marklund, S.L., 2006. Phenotypes of mice lacking extracellular superoxide dismutase and copper- and zinc-containing superoxide dismutase. J. Biol. Chem. 281, 6904–6909.
Tan, B., Mai, K., 2001. Zinc methionine and zinc sulfate as sources of dietary zinc for juvenile abalone, *Haliotis discus hannai* Ino. Aquaculture 192, 67–84.
Tan, X.Y., Xie, P., Luo, Z., Lin, H.-Z., Zhao, Y.-H., Xi, W.-Q., 2012. Dietary manganese requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*, and effects on whole body mineral composition and hepatic intermediary metabolism. Aquaculture 326–329, 68–73.
Wang, D., Zhao, L., 1994. Requirement of fingerling grasscarp (*Ctenopharyngodon idellus*) for manganese. J. Shanghai Fish. Univ. 3, 1–2 (in Chinese with English abstract).
Ye, C.X., Tian, L.X., Yang, H.J., Liang, J.J., Niu, J., Liu, Y.J., 2009. Growth performance and tissue mineral content of juvenile grouper (*Epinephelus coioides*) fed diets supplemented with various levels of manganese. Aquac. Nutr. 15, 608–614.
Zhang, J., 2007. Studies on Nutritional Physiology of Zinc and Iron for Japanese Seabass, *Lateolabrax japonicus*, and Large Yellow Croaker, *Pseudosciaena crocea* R. (MA. Sc. dissertation), Ocean University of China, China (in Chinese with English abstract).
Zhang, J., Ai, Q., Mai, K., Zhang, L., Zhang, C., Liufu, Z., 2008. Dietary zinc requirement of juvenile large yellow croaker, *Pseudosciaena crocea*. J. Fish. China 32, 417–424. (in Chinese with English abstract).
Zidenberg-Cherr, S., Keen, C.L., Lönnerdal, B., Hurley, L.S., 1983. Superoxide dismutase activity and lipid peroxidation in the rat: developmental correlations affected by manganese deficiency. J. Nutr. 113, 2498–2504.