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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*

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Abstract

A growth trial was conducted to evaluate the effects of chelated (Mintrex™ Mn, Mn-M) or inorganic ($MnSO_4 \cdot H_2O$, Mn-S) manganese (Mn) on growth, feed utilization, tissue Mn deposition and liver superoxide dismutase (SOD) activity in turbot *Scophthalmus maximus*. A semi-purified basal diet was formulated to be deficient in Mn (3.7 mg kg^{-1}) and contained tricalcium phosphate and sodium phytate at levels of 20 and 5 g kg^{-1}, respectively. Ten other diets were made by adding five levels (5, 10, 20, 35 and 55 mg Mn kg^{-1} diet) of either the Mn-M or Mn-S to the basal diet, respectively. The 11 experimental diets were fed to groups of turbot (mean initial weight: 4.6 g) for 8 weeks. Results showed that the specific growth rate (SGR), feed intake, whole body Mn/vertebra Mn concentration and Mn-SOD activity in liver were significantly improved by Mn supplementation ($P < 0.05$). On the basis of SGR, vertebra Mn concentration or liver Mn-SOD activity data, dietary Mn requirement was estimated to be 10.5, 46.3 or 12.9 mg kg^{-1} for turbot fed Mn-S, and the same was estimated to be 7.6, 43.0 or 22.5 mg kg^{-1} for turbot fed Mn-M, respectively. There was no significant difference in growth, feed intake, whole body Mn concentration or vertebra Mn concentration between the two dietary Mn sources ($P > 0.05$).

KEY WORDS: chelated Mn, $MnSO_4$, nutrition, requirement, turbot

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Introduction

Manganese (Mn) functions as a cofactor in several enzyme systems, including urea synthesis from ammonia, amino acid metabolism, fatty acid metabolism and glucose oxidation (NRC 2011). It is an essential trace element for growth, reproduction and prevention of skeletal abnormalities in terrestrial animals and fish (Liu *et al.* 2012).

Requirement for dietary Mn in fish varies from 2.4 to 24.9 mg kg^{-1} according to the published studies on Atlantic salmon *Salmo salar* (Lorentzen *et al.* 1996; Maage *et al.* 2000), channel catfish *Ictalurus punctatus* (Gatlin & Wilson 1984), cobia *Rachycentron canadum* L. (Liu *et al.* 2012), common carp *Cyprinus carpio* (Ogino & Yang 1980; Satoh *et al.* 1987), gibel carp *Carassius auratus gibelio* (Pan *et al.* 2008), rainbow trout *Oncorhynchus mykiss* (Ogino & Yang 1980; Satoh *et al.* 1991), tilapia *Oreochromis niloticus* × *O. aureus* (Lin *et al.* 2008) and yellow catfish *Pelteobagrus fulvidraco* (Tan *et al.* 2012). In addition to species, differences in the dietary ingredients could be one of the reasons leading to the observed variation in dietary Mn requirements from different studies. Maage *et al.* (2000) reported that the Mn requirement of Atlantic salmon was 7.5–10.5 mg kg^{-1} when fed semi-purified diets. Whereas it was 15 mg kg^{-1} diet in salmon fed practical diets (Lorentzen *et al.* 1996). This was also observed in studies on rainbow trout (Ogino & Yang 1980; Satoh *et al.* 1991). The presence of inhibitory factors (e.g., tricalcium phosphate and phytate) in practical diets decreases Mn availability in fish (Satoh *et al.* 1991, 1992; Apines *et al.* 2004).

Both inorganic Mn and organic Mn can be used in practical diets for aquaculture animals. Some studies showed that organic Mn had better bioavailability than the inorganic forms in fish (Paripatananont & Lovell 1997; Satoh *et al.* 2001; Apines *et al.* 2004). Mintrex™ Mn (Mn-M) is a

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relatively new type of organically bound Mn that has become available on the market. It is a Mn chelated with 2-hydroxy-4 (methylthio) butanoic acid (HMTBa), which is the hydroxy analog of methionine. Previous studies in poultry birds demonstrated that Mn-M had higher Mn bioavailability than Mn sulphate (Mn-S) (Yan & Waldroup 2006; Zhao *et al*. 2010; Yuan *et al*. 2011). However, there are still no published data available on Mn nutrition of turbot (*Scophthalmus maximus*), which is a commercially important mariculture fish species in China. Hence, the aim of this study was to comparatively analyse the effects of Mn-M or Mn-S on growth performance, feed utilization and physiological responses in turbot, and also to determine the dietary Mn requirement of this species.

Materials and methods

Experimental diets

A basal semi-purified diet was formulated with casein, gelatin (casein : gelatin = 4 : 1) and white fish meal (Table 1) to contain 485 g kg^{-1} crude protein, 100 g kg^{-1} crude lipid and 3.7 mg kg^{-1}Mn. The basal diet contained tricalcium phosphate and sodium phytate as trace mineral antagonists at levels of 20 and 5 g kg^{-1}, respectively, to resemble levels typically found in practical diets. The basal diet (Diet 1) was used as the control. The other ten experimental diets were formulated by supplementing with 5, 10, 20, 35 and 55 mg Mn kg^{-1} diet using either $MnSO_4{\cdot}H_2O$ (Mn-S, 318 g Mn kg^{-1}; Sinopharm Chemcial Reagent Co. Ltd, Shanghai, China) or Mintrex™ Mn (Mn-M, 130 g Mn kg^{-1}, 760 g HMTBa kg^{-1}; Novus International Inc., St. Charles, MO, USA) on an equivalent basis. HMTBa levels in diets were balanced by adding Mera™ Met (840 g HMTBa kg^{-1}; Novus International Inc.). Final Mn contents in the five Mn-S supplemented diets (Diet 2–6) were 7.2, 13.0, 21.6, 35.6 and 50.5 mg kg^{-1}, respectively, as analysed by an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, Varian, Palo Alto, USA). Those for the five Mn-M supplemented diets (Diet 7–11) were 7.5, 12.4, 23.1, 35.8 and 50.3 mg kg^{-1}, respectively.

Feeding trial

Juvenile turbot were obtained from a commercial farm in Laizhou, Shandong, China. Prior to the start of the feeding trial, fish were acclimated to the basal diet for 2 weeks. Then groups of fish (initial weight: 4.64 ± 0.01 g) were randomly assigned the basal control diet or one of the 10 experimental diets. There were 11 groups in all with 5 replicates per group. Each tank (300 L) stocked with 30 fish was used as a replicate. The feeding trial was conducted in an indoor re-circulating seawater system for 8 weeks. Fish were hand-fed to apparent satiation twice daily at 07:30 and 19:30, respectively. During the feeding trial, water temperature ranged from 16 to 19 °C, salinity from 29 to 30‰, and dissolved oxygen was more than 5 mg L^{-1}. Concentration of Mn in seawater was <0.005 mg L^{-1}.

Table 1 Formulation and proximate composition of the basal diet (g kg^{-1} diet)

Ingredients	g kg^{-1}
Casein	340
Gelatin	85
White fish meal	100
Alpha starch	100
Dextrin	183.3
Fish oil	110
Manganese-free mineral premix[1]	20
Vitamin premix[2]	15
Choline chloride	2
$Ca(H_2PO_4)_2{\cdot}H_2O$	3
Attractant[3]	10
Taurine	5
Mould inhibitor	1
Antioxidant	0.5
Sodium phytate	5
Tricalcium phosphate	20
Mera™ Met (mg kg^{-1} diet)[4]	241.3
Proximate composition	
Crude protein (g kg^{-1} diet)	485
Crude lipid (g kg^{-1} diet)	100
Moisture (g kg^{-1} diet)	80
Ash (g kg^{-1} diet)	51
Manganese (mg kg^{-1} diet)	3.7

[1] Mineral premix (g kg^{-1} diet): $MgSO_4{\cdot}H_2O$, 1.200; $CuSO_4{\cdot}5H_2O$, 0.010; $FeSO_4{\cdot}H_2O$, 0.080; $ZnSO_4{\cdot}7H_2O$, 0.050; $CoCl_2{\cdot}6H_2O$ (1%), 0.050; $Ca(IO_3)_2$ (1%), 0.060; Na_2SeO_3 (1%), 0.020; Microcrystalline cellulose, 18.485.

[2] Vitamin premix (g kg^{-1} diet): thiamin, 0.025; riboflavin, 0.045; pyridoxine HCl, 0.020; vitamin B12, 0.010; vitamin K3, 0.010; inositol, 0.800; pantothenic acid, 0.060; niacin acid, 0.200; folic acid, 0.020; biotin, 0.060; retinal acetate, 0.032; cholecalciferol, 0.005; α-tocopherol, 0.240; ascorbic acid, 2.000; ethoxyquin 0.003; Microcrystalline Cellulose, 11.470.

[3] Attractant, Betaine: DMPT: Glycine: Alanine: 5-inosinyl phosphate inosine = 4 : 2 : 2 : 1 : 1.

[4] Mera™ Met, contained 840 g 2-hydroxy-4-(methylthio) butanoic acid (HMTBa) kg^{-1}, Novus International Inc., St. Charless, MO, USA.

Sample collection and chemical analysis

At the termination of the feeding trial, fish were not fed for 24 h, then were counted and weighed. Five fish per tank

were randomly selected for the determination of whole body composition. An additional five fish per tank were anesthetized with eugenol (1 : 10 000) (Shanghai Reagent Corp., Shanghai, China). Blood was collected from the caudal vein with 1-mL syringes. The fish were then dissected to obtain liver, muscle and vertebra. Blood was stored in 4 °C for 5 h and then centrifuged at 4000 ***g*** for 10 min to obtain serum. Vertebrae were dried for 2 h at 105 °C, then ether extracted in a Soxhlet apparatus for 3 h to remove lipid.

Feed ingredients, experimental diets and fish whole bodies were analysed for dry matter, crude protein, crude lipid and ash using standard methods of Association of Official Analytical Chemists (AOAC) (1995). Samples were dried to a 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 at 550 °C in muffle furnace. Activities of total superoxide dismutase (T-SOD) and Mn superoxide dismutase (Mn-SOD) in liver were analysed using an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively. The protein contents in liver samples were determined by coomassie brilliant blue method using commercial kit (Nanjing Jiancheng Bioengineering Institute). Mn concentrations in serum, liver, muscle, vertebra and whole body were measured by ICP-OES.

Calculations and statistical analysis

Growth and feed utilization were expressed as follows:

$$\text{Specific growth rate (SGR, \% day}^{-1}) = \frac{100 \times \ln(\text{final body weight/initial body weight})}{\text{days}}.$$

$$\text{Feed efficiency (FE)} = \text{body weight gain/feed fed}.$$

$$\text{Feed intake (FI, \% day}^{-1}) = \frac{100 \times \text{feed fed}/[(\text{initial body weight} + \text{final body weight})/2]}{\text{days}}.$$

Two separate one-way analysis of variance (ANOVA) was used to analyse the data from the treatments of dietary Mn-S or Mn-M, respectively. When significant differences ($P < 0.05$) were observed, Duncan's multiple range test was used to compare differences among treatments. T-test was used to compare the difference between the two dietary Mn sources. Dietary Mn requirements of juvenile turbot were estimated by broken-line regression analysis (Robbins *et al.* 1979).

Results

Growth and feed utilization

Turbot fed the basal control diet (Diet 1) showed the significantly lowest SGR and FI ($P < 0.05$). The SGR and FI were significantly increased with dietary Mn levels, regardless of the dietary Mn sources ($P < 0.05$, Tables 2 & 3). T-test showed that there was no significant difference in SGR, FI and FE between the two Mn sources at the same supplemented Mn level ($P > 0.05$). Broken-line regression analysis of SGR data estimated the dietary Mn requirement of juvenile turbot to be 10.5 mg kg^{-1} based on Mn-S (Fig. 1a) or 7.6 mg kg^{-1} based on Mn-M (Fig. 1b).

Tissue Mn concentrations

Tissue Mn concentrations of turbot fed experimental diets are presented in Tables 4 & 5. Whole body Mn concentra-

Table 2 Effects of $MnSO_4$ (Mn-S) on the growth and feed utilization of turbot ($n = 5$)

Diet no.	Manganese levels (mg kg^{-1} diet)	Initial weight (g)	Final weight (g)	SGR[1] (% day^{-1})	FI[2] (% day^{-1})	FE[3]
1	3.7	4.63 ± 0.00	22.78 ± 0.47^{c}	2.84 ± 0.04^{c}	1.61 ± 0.02^{c}	1.47 ± 0.02
2	7.2	4.63 ± 0.01	24.03 ± 0.20^{bc}	2.95 ± 0.02^{b}	1.67 ± 0.01^{bc}	1.45 ± 0.01
3	13.0	4.64 ± 0.00	25.34 ± 0.62^{ab}	3.01 ± 0.06^{ab}	1.73 ± 0.02^{a}	1.43 ± 0.01
4	21.6	4.64 ± 0.00	25.75 ± 0.83^{a}	3.07 ± 0.06^{a}	1.74 ± 0.01^{a}	1.42 ± 0.02
5	35.6	4.64 ± 0.01	26.04 ± 0.46^{a}	3.07 ± 0.06^{a}	1.68 ± 0.05^{ab}	1.49 ± 0.03
6	50.5	4.65 ± 0.02	25.43 ± 0.16^{ab}	3.04 ± 0.01^{ab}	1.71 ± 0.01^{ab}	1.45 ± 0.01

Values (means ± SEM) in the same column sharing a common superscript letter were not significantly different ($P > 0.05$).
[1] SGR (Specific growth rate,% day^{-1}) = 100 × ln (final body weight/initial body weight)/days.
[2] FI (feed intake,% day^{-1}) = 100 × feed fed/[days × (initial body weight + final body weight)/2].
[3] FE (feed efficiency) = body weight gain/feed fed.

Table 3 Effects of chelated Mn (Mn-M) on growth and feed utilization of turbot (n = 5)

Diet no.	Manganese levels (mg kg^{-1} diet)	Initial weight (g)	Final weight (g)	SGR[1] (% day^{-1})	FI[2] (% day^{-1})	FE[3]
1	3.7	4.63 ± 0.00	22.78 ± 0.47^{b}	2.84 ± 0.04^{b}	1.61 ± 0.02^{b}	1.47 ± 0.02
7	7.5	4.64 ± 0.00	25.15 ± 0.65^{a}	3.02 ± 0.05^{a}	1.68 ± 0.02^{ab}	1.47 ± 0.02
8	12.4	4.63 ± 0.00	25.88 ± 0.19^{a}	3.07 ± 0.01^{a}	1.71 ± 0.01^{a}	1.46 ± 0.01
9	23.1	4.63 ± 0.00	24.65 ± 0.02^{a}	2.99 ± 0.00^{a}	1.67 ± 0.05^{ab}	1.47 ± 0.01
10	35.8	4.64 ± 0.00	25.04 ± 0.55^{a}	3.01 ± 0.04^{a}	1.75 ± 0.03^{a}	1.43 ± 0.03
11	50.3	4.63 ± 0.01	25.28 ± 0.75^{a}	3.03 ± 0.05^{a}	1.75 ± 0.03^{a}	1.44 ± 0.03

Values (means ± SEM) in the same column sharing a common superscript letter were not significantly different ($P > 0.05$).
[1] SGR (specific growth rate,% day^{-1}) = 100 × ln (final body weight/initial body weight)/days.
[2] FI (feed intake,% day^{-1}) = 100 × feed fed/[days × (initial body weight + final body weight)/2].
[3] FE (feed efficiency) = body weight gain/feed fed.

tion increased linearly as dietary Mn levels increasing, regardless of the dietary Mn sources ($P < 0.05$). When Mn-S used as the dietary Mn source, vertebra Mn concentration significantly increased with increasing dietary Mn levels from 3.7 to 35.6 mg kg^{-1} ($P < 0.05$), then plateaued above these levels ($P > 0.05$). The same trend was found in Mn-M data. However, both the two Mn sources had no significant effect on the Mn concentration in muscle, liver and serum ($P > 0.05$). T-test showed that there was no significant difference in whole body, vertebra and muscle Mn concentration between the two Mn sources at the same supplemented Mn level ($P > 0.05$). However, fish fed Mn-M diets had significantly higher Mn concentrations in serum than those fed Mn-S diets at the 55 mg kg^{-1} supplemented Mn level ($P < 0.05$), and the lower Mn concentrations in serum than those fed Mn-S diets at the 5, 10 or 35 supplemented Mn level ($P < 0.05$).

Broken-line regression analysis of vertebra Mn concentration data estimated the dietary Mn requirement of juvenile turbot to be 46.3 mg kg^{-1} based on Mn-S (Fig. 1c) or 43.0 mg kg^{-1} based on Mn-M (Fig. 1d).

Whole body composition

Both the two dietary, Mn sources had no significant effect on the whole body composition ($P > 0.05$; Tables 6 & 7). However, t-test showed that fish fed Mn-M diets had significantly lower crude lipid content than those fed Mn-S diets at the 20 or 55 mg kg^{-1} supplemented Mn level ($P < 0.05$).

Liver SOD activity

Liver SOD activities of turbot are presented in Tables 6 & 7. When used Mn-S as Mn source, liver Mn-SOD activity significantly increased with the increasing dietary Mn levels from 3.7 to 13.0 mg kg^{-1}, then decreased with Mn levels from 21.6 to 50.5 mg kg^{-1} ($P < 0.05$, Table 6). Mn-M data followed the same trend (Table 7). T-test showed that diet supplemented with 10 mg Mn kg^{-1} diet by Mn-S resulted in significantly higher liver Mn-SOD activity than that by Mn-M ($P < 0.05$). Diet supplemented with 35 and 55 mg Mn kg^{-1} diet by Mn-M had significantly higher liver Mn-SOD activity than by Mn-S ($P < 0.05$). Fish fed Mn-S diets had significantly higher liver Mn-SOD activity than those fed Mn-S diets at 5, 35 or 55 mg kg^{-1} supplemented Mn level ($P < 0.05$).

Broken-line regression analysis of liver Mn-SOD activity data estimated the dietary Mn requirement of juvenile turbot to be 12.9 mg kg^{-1} based on Mn-S (Fig. 1e) or 22.5 mg kg^{-1} based on Mn-M (Fig. 1f).

Discussion

Previous studies have shown that inhibitory substances (e.g., tricalcium phosphate and phytate) in ingredients of practical diets (e.g., fish meal and/or plant protein sources) could decrease Mn availability in fish (Satoh *et al*. 1991, 1992; Apines *et al*. 2004). Hence, the Mn requirements of fish fed practical diets were higher than those fed purified diets (Ogino & Yang 1980; Satoh *et al*. 1991; Lorentzen *et al*. 1996; Maage *et al*. 2000). Typically, 600–700 g kg^{-1} fish meal and 100–150 g kg^{-1} plant products are used in commercial feeds for turbot in China (Ren 2011). These feeds approximately contain 20–50 g kg^{-1} calcium and 3–5 g kg^{-1} phytate. Therefore, in the present study, 20 g kg^{-1} of tricalcium phosphate and 5 g kg^{-1} of sodium phytate were added to the experimental semi-purified diets to resemble levels found in commercial feeds.

In the present study, low SGR and FI were observed in fish fed the Mn-deficient basal diet. However, typical deficiency symptoms (e.g., lens cataracts and short body dwarfism)

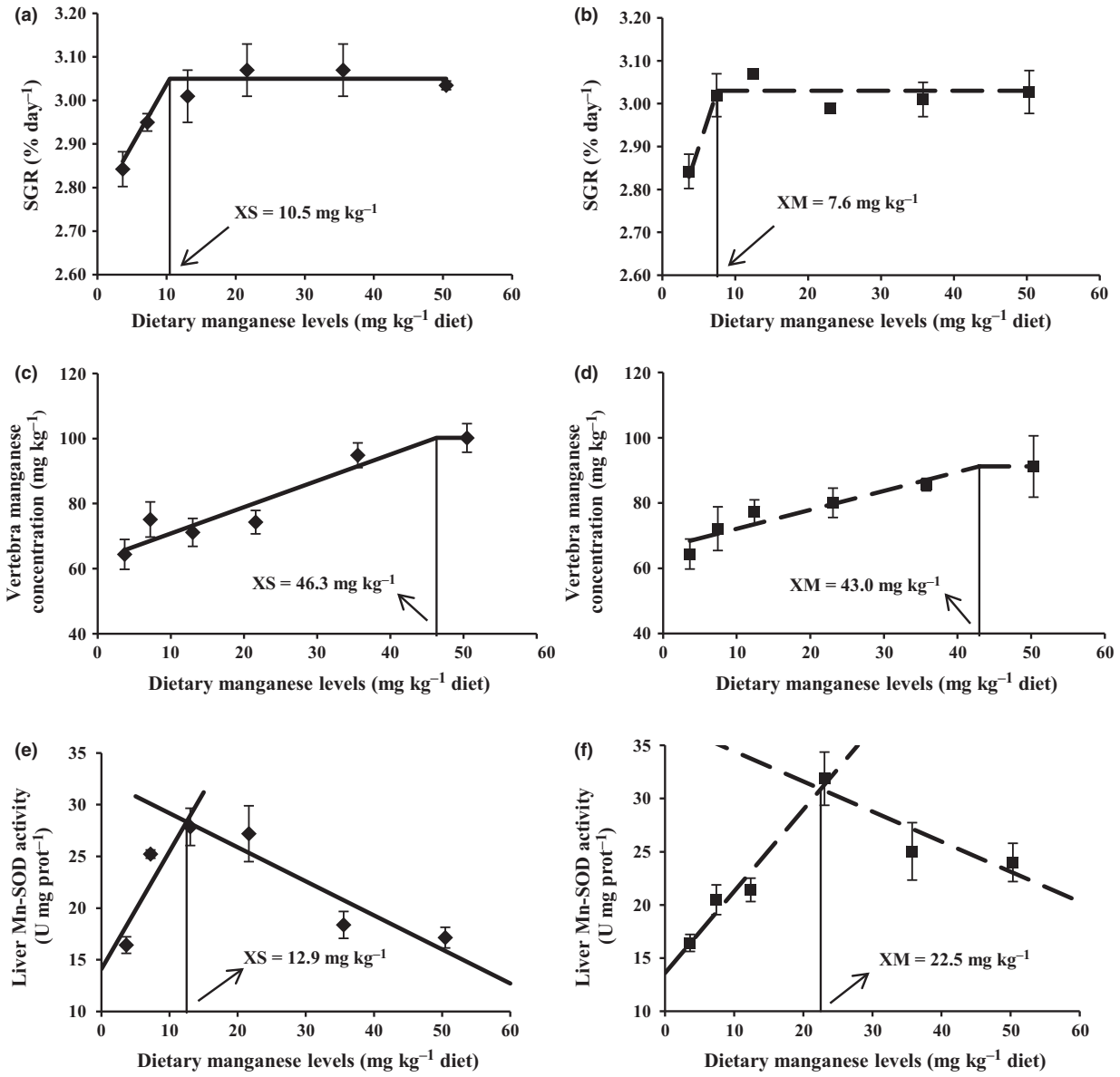

Figure 1 Relationship between dietary manganese levels and the specific growth rate (SGR) (a, b), vertebra manganese concentration (c, d) and activity of manganese superoxide dismutase (Mn-SOD) in liver (e, f) of turbot fed diets containing graded levels of two manganese sources for 8 weeks. Diamonds and solid lines represent data from fish fed $MnSO_4 \cdot H_2O$ (Mn-S) (a, c, e), squares and dotted lines represent data from fish fed Mintrex Mn (Mn-M) (b, d, f); XS represents the manganese requirement of turbot fed Mn-S, and XM represents the manganese requirement of turbot fed Mn-M. The regression equations of the six models were listed as follows:

a. $Y = 3.05 + 0.03(X - 10.47)$, $(X - 10.47) = 0$ when $X > 10.47$, $R^2 = 0.937$

b. $Y = 3.03 + 0.05(X - 7.56)$, $(X - 7.56) = 0$ when $X > 7.56$, $R^2 = 0.890$

c. $Y = 100.21 + 0.81(X - 46.33)$, $(X - 46.33) = 0$ when $X > 46.33$, $R^2 = 0.905$

d. $Y = 91.24 + 0.58(X - 42.96)$, $(X - 42.96) = 0$ when $X > 42.96$, $R^2 = 0.923$

e. $Y = 1.14X + 14.08$, $R^2 = 0.818$ (ascent line) $Y = -0.33X + 32.47$, $R^2 = 0.887$ (descent line)

f. $Y = 0.77X + 13.62$, $R^2 = 0.965$ (ascent line) $Y = -0.03X + 37.29$, $R^2 = 0.817$ (descent line)

reported in common carp and rainbow trout (Ogino & Yang 1980; Satoh *et al.* 1992) were not found in the present study. In the present study, SGR, FI, whole body and vertebra Mn concentration, liver Mn-SOD activity were improved by either dietary Mn-S or Mn-M supplementation. These results suggested that turbot can utilize both

Table 4 Effects of $MnSO_4$ (Mn-S) on manganese concentration in the whole body, vertebra, muscle, liver and serum of turbot (n = 5)

Diet no.	Manganese levels (mg kg^{-1} diet)	Whole body (mg kg^{-1} dry weight)	Vertebra (mg kg^{-1} lipid free dry matter)	Muscle (mg kg^{-1} dry weight)	Liver (mg kg^{-1} wet weight)	Serum (μg ml^{-1})
1	3.7	3.1 ± 1.0^{c}	64.4 ± 4.6^{b}	1.9 ± 0.3	1.1 ± 0.1	65.1 ± 13.1
2	7.2	5.1 ± 1.2^{bc}	75.1 ± 5.4^{b}	2.3 ± 0.4	1.6 ± 0.4	47.8 ± 15.2
3	13.0	9.8 ± 1.9^{b}	71.2 ± 4.3^{b}	2.2 ± 0.4	1.7 ± 0.1	68.4 ± 26.4
4	21.6	6.7 ± 1.3^{bc}	74.3 ± 3.6^{b}	2.0 ± 0.4	1.2 ± 0.2	52.9 ± 6.9
5	35.6	10.5 ± 1.2^{b}	94.9 ± 3.8^{a}	2.9 ± 0.8	1.3 ± 0.2	75.6 ± 18.9
6	50.5	15.8 ± 3.0^{a}	100.2 ± 4.4^{a}	2.3 ± 0.4	0.9 ± 0.1	48.3 ± 4.2

Values (means ± SEM) in the same column sharing a common superscript letter were not significantly different (P > 0.05).

Table 5 Effects of chelated Mn (Mn-M) on manganese concentration in whole body, vertebra, muscle, liver and serum of turbot (n = 5)

Diet no.	Manganese levels (mg kg^{-1} diet)	Whole body (mg kg^{-1} dry weight)	Vertebra (mg kg^{-1} lipid free dry matter)	Muscle (mg kg^{-1} dry weight)	Liver (mg kg^{-1} wet weight)	Serum (μg mL^{-1})
1	3.7	3.1 ± 1.0^{b}	64.4 ± 4.6^{c}	1.9 ± 0.3	1.1 ± 0.1	65.1 ± 13.1
7	7.5	4.3 ± 1.1^{b}	72.2 ± 6.7^{bc}	2.0 ± 0.1	1.0 ± 0.2	50.9 ± 12.7
8	12.4	5.7 ± 2.0^{ab}	77.3 ± 3.7^{abc}	2.0 ± 0.2	1.1 ± 0.1	103.5 ± 23.9
9	23.1	6.6 ± 1.4^{ab}	80.1 ± 4.5^{abc}	2.2 ± 0.2	1.0 ± 0.1	76.9 ± 23.7
10	35.8	10.4 ± 2.4^{a}	85.7 ± 1.9^{ab}	1.9 ± 0.1	1.0 ± 0.1	98.9 ± 28.3
11	50.3	11.3 ± 2.1^{a}	91.2 ± 9.4^{a}	2.3 ± 0.5	1.0 ± 0.1	124.3 ± 11.2

Values (means ± SEM) in the same column sharing a common superscript letter were not significantly different (P > 0.05).

Table 6 Effects of $MnSO_4$ (Mn-S) on total liver activity of superoxide dismutase (T-SOD), liver activity of manganese superoxide dismutase (Mn-SOD) and whole body composition of turbot (n = 5)

Diet no.	Manganese levels (mg kg^{-1} diet)	Liver T-SOD (U mg $prot^{-1}$)	Liver Mn-SOD (U mg $prot^{-1}$)	Whole body composition (g kg^{-1} fresh weight)			
				Crude protein	Crude lipid	Moisture	Ash
1	3.7	48.4 ± 3.2	16.4 ± 0.8^{b}	146.3 ± 0.5	34.2 ± 0.8	786.2 ± 2.5	32.6 ± 0.4
2	7.2	52.3 ± 5.4	25.2 ± 0.4^{a}	156.3 ± 1.5	35.0 ± 1.5	785.4 ± 2.3	32.8 ± 0.8
3	13.0	52.8 ± 2.0	27.9 ± 1.8^{a}	149.9 ± 1.9	36.0 ± 1.2	781.7 ± 2.3	32.9 ± 0.5
4	21.6	54.4 ± 4.4	27.2 ± 2.7^{a}	147.0 ± 2.0	36.7 ± 1.4	782.0 ± 2.1	32.4 ± 0.5
5	35.6	48.3 ± 2.5	18.4 ± 1.3^{b}	145.1 ± 3.0	36.1 ± 1.2	782.4 ± 1.1	33.8 ± 0.8
6	50.5	46.6 ± 1.2	17.2 ± 1.0^{b}	148.0 ± 0.3	36.9 ± 1.7	781.8 ± 1.5	32.8 ± 0.6

Values (means ± SEM) in the same column sharing a common superscript letter were not significantly different (P > 0.05).

Table 7 Effects of chelated Mn (Mn-M) on total liver activity of superoxide dismutase (T-SOD), liver activity of manganese superoxide dismutase (Mn-SOD) and whole body composition of turbot (n = 5)

Diet no.	Manganese levels (mg kg^{-1} diet)	Liver T-SOD (U mg $prot^{-1}$)	Liver Mn-SOD (U mg $prot^{-1}$)	Whole body composition (g kg^{-1} fresh weight)			
				Crude protein	Crude lipid	Moisture	Ash
1	3.7	48.4 ± 3.2	16.4 ± 0.8^{c}	146.3 ± 0.5	34.2 ± 0.8	786.2 ± 2.5	32.6 ± 0.4
7	7.5	55.4 ± 0.9	20.5 ± 1.4^{bc}	149.2 ± 1.8	34.6 ± 0.7	785.2 ± 2.7	32.6 ± 0.4
8	12.4	54.0 ± 3.8	21.4 ± 1.1^{bc}	148.1 ± 1.5	34.8 ± 0.8	783.6 ± 2.3	32.8 ± 0.3
9	23.1	62.3 ± 5.8	31.9 ± 2.5^{a}	147.5 ± 1.2	34.8 ± 2.0	785.0 ± 3.2	32.9 ± 0.3
10	35.8	56.5 ± 3.5	25.0 ± 2.7^{b}	150.4 ± 2.0	34.4 ± 1.3	783.7 ± 2.7	33.1 ± 0.6
11	50.3	52.3 ± 2.9	24.0 ± 1.8^{b}	150.1 ± 3.2	32.9 ± 0.8	785.4 ± 3.1	32.5 ± 0.4

Values (means ± SEM) in the same column sharing a common superscript letter were not significantly different (P > 0.05).

the manganese sources tested. Positive effect of chelated Mn on tibia Mn deposition was also observed in young broilers (Yan & Waldroup 2006).

Broken-line regression analysis of SGR data indicated that the dietary Mn requirement of juvenile turbot was estimated to be 10.5 mg kg^{-1} based on Mn-S (Fig. 1a) or 7.6 mg kg^{-1} based on Mn-M (Fig. 1b) in the present study. These values are higher than those reported in some other fish species, such as yellow catfish (5.5 mg kg^{-1}) (Tan *et al*. 2012) and channel catfish (2.4 mg kg^{-1}) (Gatlin & Wilson 1984), but lower than common carp and rainbow trout (12–13 mg kg^{-1}) (Ogino & Yang 1980); gibel carp (13.77 mg kg^{-1}) (Pan *et al*. 2008); cobia (21.72 mg kg^{-1}) (Liu *et al*. 2012). The difference could be related to different species, diet types, experimental conditions and methods of data analysis (Satoh *et al*. 2001; Liu *et al*. 2012). There was no significant difference between the effects of two Mn sources in the present study. However, the Mn requirement of turbot based on Mn-M was lower than that based on Mn-S. One of the reasons could be the dietary tricalcium phosphate and phytate supplementation in the present study. These inhibitory substances can inhibit Mn availability (Satoh *et al*. 1991, 1992; Apines *et al*. 2004). Organic minerals could protect trace elements from forming insoluble complexes and improve bioavailability, with antagonists such as tricalcium phosphate or phytate in the digestive tract, and this facilitates mineral transport across the intestinal mucosa (Ashmead 1993).

Manganese concentration in whole body or bone of fish is also a method to measure the Mn requirement of fish (Lorentzen *et al*. 1996; Maage *et al*. 2000; Pan *et al*. 2008; Liu *et al*. 2012). In the present study, the whole body Mn concentration in turbot increased linearly with dietary Mn levels. Hence, it was not an appropriate parameter to estimate the Mn requirement for turbot. Similar result was also found in grouper *Epinephelus coioides* (Ye *et al*. 2009). Mn is widely distributed in fish tissues, but the highest concentration is found in bone (Lall 2002). Broken-line regression analysis of vertebra Mn concentration indicated that the dietary Mn requirement of juvenile turbot was estimated to be 46.3 mg kg^{-1} based on Mn-S (Fig. 1c) or 43.0 mg kg^{-1} based on Mn-M (Fig. 1d) in the present study. The result was higher than those based on SGR data. This shows that vertebrae are the major reservoir of Mn in turbot as previously described in common carp and rainbow trout (Satoh *et al*. 1987, 2001). Thus, dietary Mn required to maintain vertebral Mn concentration is higher than those required for maximal weight gain. The Mn requirements in the present study were also higher than those reported in some other fish species, such as gibel carp (13.63 mg kg^{-1}) (Pan *et al*. 2008) and cobia (24.93 mg kg^{-1}) (Liu *et al*. 2012). In addition to the species difference, one of the reasons could be the dietary tricalcium phosphate and phytate supplementation in the present study. These inhibitory substances can decrease Mn availability (Satoh *et al*. 1991, 1992; Apines *et al*. 2004).

Manganese can be involved in biochemical systems as metalloenzyme, for example the superoxide dismutase (Lin *et al*. 2008). Liver Mn-SOD activity has been shown to be decreased in Mn-deficient rainbow trout (Knox *et al*. 1981). In the present study, liver Mn-SOD activity increased and then decreased with dietary Mn levels increasing. Similar trend was also found in the yellow catfish *Pelteobagrus fulvidraco* (Tan *et al*. 2012) and tilapia, *Oreochromis niloticus* × *O. aureus* (Lin *et al*. 2008). Broken-line regression analysis of liver Mn-SOD activity data indicated that the dietary Mn requirement of juvenile turbot was estimated to be 12.9 mg kg^{-1} based on Mn-S (Fig. 1e) or 22.5 mg kg^{-1} based on Mn-M (Fig. 1f). The Mn requirements in the present study were higher than those reported in some other fish species, such as tilapia (6.96 mg kg^{-1}) (Lin *et al*. 2008) and yellow catfish (6.4 mg kg^{-1}) (Tan *et al*. 2012). The major reason is the dietary tricalcium phosphate and phytate supplementation. In addition, an impact of species differences cannot be completed eliminated.

With respect to the relative bioavailability of organic Mn to inorganic Mn, the amino acid-chelated Mn was more available than Mn sulphate in lambs (Henry *et al*. 1992), chicks (Fly *et al*. 1989; Henry *et al*. 1989; Yan & Waldroup 2006) and fish (Satoh *et al*. 2001). In the present study, the Mn requirement of turbot fed Mn-M diet was lower than those fed Mn-S diet on the basis of SGR or vertebra Mn concentration. However, there was no significant difference between the effects of two Mn sources. The difference is probably species specific, variations in intestinal Mn absorption rate and feed efficiency. Moreover, the disparity could be related to different diet types or chelated Mn types.

Dietary supplemented Mn decreased abdominal fat content in broilers (Lu *et al*. 2006). In fish, Tan *et al*. (2012) reported that hepatic lipase activity, which was a main lipid metabolic enzyme, was observed to be the highest for yellow catfish fed a diet containing 12 mg Mn kg^{-1} diet. In the present study, fish fed Mn-M diets had lower whole body crude lipid content than those fed Mn-S. In addition, fish fed Mn-M diet had significantly higher T-SOD activity than those fed Mn-S at the 5, 35 or 55 mg kg^{-1} supplemented

Mn level ($P < 0.05$). Meanwhile, higher Mn-SOD activity was found in Mn-M groups when the dietary supplemented Mn levels ranging from 35 to 55 mg kg^{-1}. The reason could be that fish fed Mn-M needs higher SOD activity to decrease lipid peroxidation, because SOD is the first enzyme to respond against oxygen radicals from lipid peroxidation (Winston & Di Giulio 1991). However, further study is needed to clarify the mechanism of Mn-M on lipid metabolism.

Conclusion

Supplementation of dietary Mn-M or Mn-S can improve growth, feed intake, manganese deposition in vertebra and whole body, and activity of Mn-SOD in liver of turbot. On the basis of SGR, vertebra Mn concentration or liver Mn-SOD activity data, dietary Mn requirement was estimated to be 10.5, 46.3 or 12.9 mg kg^{-1} for juvenile turbot fed Mn-S and that was estimated to be 7.6, 43.0 or 22.5 mg kg^{-1} for fish fed Mn-M, respectively. There was no significant difference in the growth, feed intake, whole body Mn concentration and vertebra Mn concentration between the two dietary Mn sources.

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