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Comparative study on the bioavailability of chelated or inorganic zinc in diets containing tricalcium phosphate and phytate to turbot (*Scophthalmus maximus*)

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ABSTRACT

A 2 × 6 factorial experiment was conducted to evaluate the effects of chelated (Mintrex™ Zn, Zn–M) or inorganic ($ZnSO_4 \cdot 7H_2O$, Zn–S) zinc as dietary zinc sources on growth, feed utilization, tissue zinc deposition and anti-oxidative responses of turbot (*Scophthalmus maximus*). Semi-purified diets were made to contain tricalcium phosphate and sodium phytate at levels of 2% and 0.5%, respectively, to resemble levels in practical diets. Ten experimental diets were made by adding either Zn–S or Zn–M to the basal diet to achieve five levels of dietary zinc (15, 45, 75, 105 and 135 mg/kg diet) for each zinc source, respectively. The basal control diet and ten experimental diets were fed to groups (n = 5) of juvenile turbot (initial mean weight: 4.78 g) for 8 weeks. Results showed that the specific growth rate (SGR), feed intake (FI), feed efficiency (FE), whole body and bone zinc concentration, whole body crude lipid content, serum superoxide dismutase (SOD) activity and glutathione peroxidase (GSH-PX) activity in serum or liver of turbot were significantly improved by zinc supplementation ($P < 0.05$). There was no significant difference in the growth of turbot between the two zinc sources ($P > 0.05$). On the basis of SGR, the dietary zinc requirement of juvenile turbot was estimated to be 60.2 mg/kg, using broken-line regression analysis.

1. Introduction

Zinc is required for normal growth, development, and function in animals. It functions as a cofactor in several enzyme systems and is a component of a large number of metallo-enzymes (NRC, 2011). Zinc deficiency leads to mortality, anorexia, poor growth, cataract, skin erosion and oxidative damage in fish such as rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*) and tilapia (*Oreochromis niloticus*) (Eid and Ghonim, 1994; Gatlin and Wilson, 1983; Kucukbay et al., 2006; Ogino and Yang, 1978). Previous studies showed that requirements of dietary zinc for fish ranged from 15 to 40 mg/kg diet (Watanabe et al., 1997). The zinc requirement varies with the feed ingredients. Gatlin and Wilson (1983) indicated that zinc requirement of channel catfish was 20 mg/kg in purified diets, but 150 mg/kg in practical diets (Gatlin and Wilson, 1984). Fish meal and plant protein sources used in practical diets contain anti-nutritional factors (e.g., tricalcium phosphate and phytate) that inhibit zinc availability (Apines et al., 2003; Richardson et al., 1985; Satoh et al., 1987, 1989). Thus, increased levels of zinc are required to overcome the inhibitory effects of tricalcium phosphate or phytate (Davis et al., 1993; Gatlin and Phillips, 1989).

Organic minerals are important trace mineral sources, because they protect trace elements from forming insoluble complexes (such as with phytate) in the digestive tract and facilitates transport across the intestinal mucosa (Ashmead, 1993). It was confirmed that organic zinc had higher bioavailability than inorganic zinc in terrestrial vertebrates and aquatic animals, such as the chick (Wedekind et al., 1992), abalone (*Haliotis discus hannai*) (Tan and Mai, 2001), channel catfish (Paripatananont and Lovell, 1995) and rainbow trout (Apines et al., 2001). However, it was also suggested in some other studies that substitution of organic zinc for inorganic zinc did not lead to improvement in growth of pig (Swinkels et al., 1996; Wedekind et al., 1994), chick (Pimentel et al., 1991) or tilapia (Do Carmo E Sá et al., 2005; Zhao et al., 2011).

Mintrex™ Zn is a relatively new type of organically bound Zn that has become available on the market. It is a Zn chelated with 2-hydroxy-4 (methylthio) butanoic acid (HMTBa), which is the hydroxy analog of methionine. In previous studies, it was found that HMTBa from this Zn source was fully available as a methionine source in broiler chicks (Yi et al., 2007) and hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) (Savolainen and Gatlin, 2010). Meanwhile, Zn from Mintrex Zn was more bioavailable than Zn from $ZnSO_4$ in chicks and poults (Dibner, 2005; Yuan et al., 2011).

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Turbot (*Scophthalmus maximus*) is a commercially important marine fish species. It is farmed on the Atlantic coast of Europe as well as on the Pacific coast of Asia including China, Korea and Japan. However, there is no published data on the mineral nutrition for this species. The aim of this study was to comparatively analyze the effects of chelated or inorganic dietary zinc sources on growth performance, feed utilization and physiological responses in *S. maximus* fed semi-purified diets containing tricalcium phosphate and sodium phytate at levels typically found in practical diets.

2. Materials and methods

2.1. Experimental diets

Semi-purified diets supplemented with 2% of tricalcium phosphate and 0.5% of sodium phytate were used in the present study. The basal diet was formulated with casein, gelatin (casein:gelatin = 4:1) and white fish meal as the intact protein sources to contain 48.5% crude protein, 11% crude lipid (Table 1). The basal diet containing 36.2 ± 0.7 mg/kg of zinc was used as the control. Ten experimental diets were formulated based on the basal diet and were supplemented with 15, 45, 75, 105 and 135 mg/kg diet zinc using either $ZnSO_4 \cdot 7H_2O$ (Zn–S, 22.63% zinc; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) or Mintrex™ Zn (Zn–M, 14% zinc, 80% HMTBa; Novus International Inc., St. Charles, MO, USA) on an equivalent basis, respectively. Methionine levels in diets were balanced by adding Mera™ Met (84% HMTBa; Novus International Inc., St. Charles, MO, USA). Final zinc concentrations in the five Zn–S supplemented diets (diets 2–6; n = 3) were 54.4 ± 0.4, 76.2 ± 4.0, 106.5 ± 4.7, 124.8 ± 4.3 and 165.6 ± 6.6 mg/kg, respectively, as analyzed by an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; VISTA-MPX, Varian, Palo Alto, USA). Those for the five Zn–M supplemented diets (diets 7–11; n = 3) were 48.8 ± 1.6, 75.4 ± 4.3, 110.9 ± 5.2, 128.7 ± 5.0 and 167.2 ± 5.7 mg/kg, respectively.

Diet ingredients were ground into fine powder through a 246-μm mesh. Then all the ingredients were thoroughly mixed with the fish oil, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China) and dried for about 12 h in a ventilated oven at 45 °C, and stored at −20 °C until used.

2.2. Feeding trial

Juvenile turbot were obtained from a commercial farm in Laizhou, Shandong, China. Prior to the start of the feeding trial, fish were acclimatized to the zinc-deficient basal diet for two weeks. Groups of fish (initial weight: 4.78 ± 0.01 g) were then randomly assigned to the basal control diet or one of the 10 experimental diets. There were 11 groups 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

Table 1
Formulation and proximate composition of the experimental diets (% dry matter).

Ingredients	Diet number (added each zinc source level mg/kg diet)										
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11
	(0)	$ZnSO_4 \cdot 7H_2O$ (15)	$ZnSO_4 \cdot 7H_2O$ (45)	$ZnSO_4 \cdot 7H_2O$ (75)	$ZnSO_4 \cdot 7H_2O$ (105)	$ZnSO_4 \cdot 7H_2O$ (135)	Chelated Zn (15)	Chelated Zn (45)	Chelated Zn (75)	Chelated Zn (105)	Chelated Zn (135)
Casein[a]	34	34	34	34	34	34	34	34	34	34	34
Gelatin[a]	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
White fish meal[a]	10	10	10	10	10	10	10	10	10	10	10
Alpha starch	10	10	10	10	10	10	10	10	10	10	10
Dextrine	15	15	15	15	15	15	15	15	15	15	15
Microcrystalline cellulose	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16	3.16
Fish oil	11	11	11	11	11	11	11	11	11	11	11
Zinc-free mineral premix[b]	2	2	2	2	2	2	2	2	2	2	2
Vitamin premix[c]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ca $(H_2PO_4)_2 \cdot H_2O$	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Attractant[d]	1	1	1	1	1	1	1	1	1	1	1
Taurine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Calcium propionate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium phytate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Tricalcium phosphate	2	2	2	2	2	2	2	2	2	2	2
Mera™ Met (mg/kg diet)[e]	918.4	918.4	918.4	918.4	918.4	918.4	816.3	612.2	408.2	204.1	0
$ZnSO_4 \cdot 7H_2O$ (mg/kg diet)[f]	0	66.3	198.9	331.4	464	596.6	0	0	0	0	0
Mintrex™ Zn (mg/kg diet)[g]	0	0	0	0	0	0	107.1	321.4	535.7	750.0	964.3
Proximate composition (N = 3)											
Zinc (mg/kg diet)	36.2	54.4	76.2	106.5	124.8	165.6	48.8	75.4	110.9	128.7	167.2
Crude protein	48.5	49.7	48.4	49.8	49.2	49.0	49.6	48.8	49.0	49.9	49.5
Crude lipid	11.0	11.2	11.5	11.8	10.5	10.3	11.7	11.5	11.5	10.7	11.2
Moisture	8.6	7.7	8.2	9.4	10.1	8.6	9.7	9.1	9.9	9.7	9.7
Ash	5.4	5.3	5.4	5.4	5.3	5.3	5.3	5.4	5.4	5.4	5.3

[a] Casein (Hua Ling Casein Company Limited, Gansu Province, China), crude protein 96.9%, crude lipid 0.53%; Gelatin (Yi Xin Bio-tech Co. Ltd., Shandong, China), crude protein 99.3%, crude lipid 0.21%; white fish meal (Great Seven Bio-tech Co. Ltd., Shandong, China), crude protein 71.3%, crude lipid 6.89%.

[b] Mineral premix (g/kg diet): $MgSO_4 \cdot H_2O$, 1.200; $CuSO_4 \cdot 5H_2O$, 0.010; $FeSO_4 \cdot H_2O$, 0.080; $MnSO_4 \cdot H_2O$, 0.045; $CoCl_2 \cdot 6H_2O$ (1%), 0.050; $Ca(IO_3)_2$ (1%), 0.060; Na_2SeO_3 (1%), 0.020; microcrystalline cellulose, 18.485.

[c] Vitamin premix (g/kg 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.

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

[e] Mera™ Met, contained 84% 2-hydroxy-4-(methylthio) butanoic acid (HMTBa), Novus International, Inc., St. Charles, Missouri 63304, USA.

[f] $ZnSO_4 \cdot 7H_2O$, contained 22.63% zinc, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

[g] Mintrex™ Zn, contained 14% zinc, 80% HMTBa, Novus International, Inc., St. Charles, Missouri 63304, USA.

re-circulating seawater system for 8 weeks. Water in system was renewed for 2 times per day. About 150 L water in each tank was exchanged by new seawater each time. 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 18 °C, salinity from 29 to 30‰, and dissolved oxygen was >5 mg/L. Concentration of zinc in seawater was 16.49 ± 0.26 μg/L.

2.3. Sample collection and chemical analysis

At the termination of the feeding trial, fish were not fed for 24 h, and were then counted and weighed. Four fish per tank were randomly selected for the determination of whole body composition. Nine other fish per tank were anesthetized with eugenol (1:10,000) (Shanghai Reagent Corp, China) for blood collection. Samples of blood were stored at 4 °C for 5 h and then centrifuged at 4000 ×*g* for 10 min to obtain serum. The fish were then dissected to obtain samples of liver, muscle and bone. Bones were dried for 2 h at 105 °C, then ether extracted in a Soxhlet apparatus for 3 h to remove lipid. Sampling of the experimental fish followed the Guidelines for the Care and Use of Laboratory Animals of Ocean University of China.

Feed ingredients, experimental diets and fish whole bodies were analyzed for dry matter, crude protein, crude lipid and ash using standard methods of 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 was analyzed by ether extraction using the Soxhlet method (36680-analyer, BUCHI, Flawil, Switzerland). Combustion at 550 °C in a muffle furnace was used to determine the ash content.

Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) in serum were determined by colorimetric method using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The liver samples were homogenized in 9 volumes (v/w) of ice-cold (0 °C) normal saline. Activities of SOD, CAT and GSH-PX in liver homogenates were determined using the same kits as for serum. Thiobarbituric acid reactive substance (TBARs) in liver was analyzed using a QuantiChrom™ TBARs assay kit (Bas-biotech, Inc., Chengdu, China). Zinc concentrations in the serum, liver, muscle, bone and whole body were measured by ICP-OES.

2.4. Calculations and statistical analysis

The growth and feed utilization were expressed as follows:

Specific growth rate (SGR, %/day) = 100 × ln (final body weight / initial body weight) / days.
Feed efficiency (FE, %) = 100 × (body weight gain) / (feed fed).
Feed intake (FI, %/day) = 100 × feed fed / [(initial body weight + final body weight) / 2] / days.

The interaction between the dietary zinc sources and levels was evaluated to a two-factorial design (2 × 6). Two-way ANOVA was conducted for all the data using SPSS 16.0. When significant differences ($P < 0.05$) were observed, Duncan's multiple range test was used to compare differences among treatments. Dietary zinc requirements of juvenile turbot were estimated by broken-line regression analysis (Robbins, 1986; Robbins et al., 1979).

3. Results

3.1. Growth and survival

No mortalities occurred during the feeding trial. Fish fed the basal diet showed the significantly lowest SGR, FE and FI ($P < 0.05$, Table 2). SGR and FI increased significantly with the increasing supplemented zinc levels from 15 to 45 mg/kg diet, regardless of the dietary zinc sources ($P < 0.05$). However with further increase of dietary zinc supplementation, both SGR and FI remained relatively constant.

There was no significant effect of dietary zinc sources on SGR, FE and FI ($P > 0.05$). Data on SGR in the two zinc source treatments were pooled for the following analysis. Broken-line regression analysis of SGR indicated that the dietary zinc requirement of juvenile turbot was estimated to be 60.2 mg/kg (Fig. 1).

Table 2
Effects of $ZnSO_4$ (Zn–S) and chelated Zn (Zn–M) on growth and feed utilization of turbot (n = 5).

Sources	Supplemented levels (mg/kg diet)	Initial weight (g)	Final weight (g)	SGR[1] (%/day)	FI[2] (%/day)	FE[3] (%)
Control	0	4.78 ± 0.00	22.66 ± 0.54	2.78 ± 0.04	1.63 ± 0.01	104.08 ± 2.16
Zn–S	15	4.78 ± 0.01	24.82 ± 0.39	2.94 ± 0.03	1.68 ± 0.03	109.82 ± 1.37
	45	4.78 ± 0.00	26.07 ± 0.73	3.03 ± 0.05	1.74 ± 0.01	109.48 ± 2.21
	75	4.78 ± 0.00	25.41 ± 0.15	2.98 ± 0.01	1.70 ± 0.02	110.45 ± 1.40
	105	4.78 ± 0.01	25.78 ± 0.47	3.01 ± 0.03	1.71 ± 0.02	110.47 ± 1.35
	135	4.78 ± 0.00	25.85 ± 0.62	3.01 ± 0.04	1.68 ± 0.02	112.93 ± 1.97
Zn–M	15	4.78 ± 0.00	24.59 ± 0.47	2.92 ± 0.03	1.64 ± 0.01	111.91 ± 2.11
	45	4.78 ± 0.00	26.18 ± 0.90	3.03 ± 0.04	1.69 ± 0.02	113.32 ± 3.13
	75	4.78 ± 0.00	26.05 ± 0.42	3.03 ± 0.03	1.67 ± 0.02	113.09 ± 1.99
	105	4.78 ± 0.00	25.50 ± 0.49	2.99 ± 0.03	1.67 ± 0.02	112.49 ± 1.52
	135	4.78 ± 0.01	25.30 ± 1.03	2.97 ± 0.08	1.70 ± 0.03	109.34 ± 4.38
Zinc source	Zn–S		25.10 ± 0.29	2.96 ± 0.02	1.69 ± 0.01	109.54 ± 0.83
	Zn–M		25.05 ± 0.34	2.95 ± 0.02	1.67 ± 0.01	110.71 ± 1.18
Zinc level	0		22.66 ± 0.54^{c}	2.78 ± 0.04^{c}	1.63 ± 0.01^{c}	104.08 ± 2.16^{b}
	15		24.70 ± 0.29^{b}	2.93 ± 0.02^{b}	1.66 ± 0.02^{bc}	110.86 ± 1.24^{a}
	45		26.13 ± 0.55^{a}	3.03 ± 0.04^{a}	1.72 ± 0.01^{a}	111.40 ± 1.92^{a}
	75		25.73 ± 0.24^{ab}	3.01 ± 0.02^{ab}	1.70 ± 0.02^{ab}	111.77 ± 1.23^{a}
	105		25.64 ± 0.32^{ab}	3.00 ± 0.02^{ab}	1.69 ± 0.02^{ab}	111.48 ± 1.02^{a}
	135		25.58 ± 0.57^{ab}	2.99 ± 0.04^{ab}	1.69 ± 0.02^{ab}	111.13 ± 2.34^{a}
Source		0.305	0.889	0.821	0.123	0.384
Level		0.948	0.000	0.000	0.015	0.011
Source × level		0.853	0.948	0.935	0.573	0.656

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

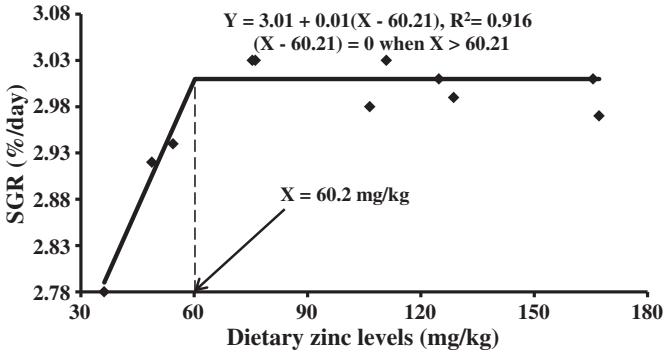

Fig. 1. Relationship between dietary zinc levels and the specific growth rate (SGR) of turbot fed diets containing graded levels of zinc for 8 weeks. X represents the zinc requirement of turbot.

3.2. Tissue zinc concentrations

Tissue zinc concentrations of turbot fed experimental diets are presented in Table 3. The zinc concentration in bone or in the whole body significantly increased with the dietary zinc levels from 0 to 105 mg/kg diet, regardless of the dietary zinc source ($P < 0.05$). No significant increase of zinc concentration in the bone was observed with further increase in dietary zinc supplementation. In addition, zinc concentrations in the muscle, liver and serum were not significantly influenced by the different dietary treatments ($P > 0.05$).

3.3. Whole body composition

Dietary treatment did not significantly influence crude protein or moisture content in the whole body (Table 4). Fish fed the control diet showed significantly lower crude lipid content but higher ash content than those fed zinc supplemented diets ($P < 0.05$). Two-way ANOVA showed that dietary zinc sources significantly influenced the crude lipid contents ($P < 0.05$). Fish fed Zn–S diets exhibited significantly higher whole body crude lipid contents than those fed Zn–M diets.

Table 4
Effects of $ZnSO_4$ (Zn–S) and chelated Zn (Zn–M) on whole body composition of turbot (n = 5).

Sources	Supplemented levels (mg/kg diet)	Whole body composition (% on a wet weight)			
		Crude protein	Crude lipid	Moisture	Ash
Control	0	15.1 ± 0.1	2.8 ± 0.1	78.6 ± 0.4	3.6 ± 0.1
Zn–S	15	15.1 ± 0.1	3.2 ± 0.0	78.4 ± 0.2	3.4 ± 0.1
	45	15.2 ± 0.1	3.4 ± 0.1	78.1 ± 0.3	3.3 ± 0.1
	75	15.1 ± 0.1	3.3 ± 0.1	78.5 ± 0.1	3.3 ± 0.0
	105	15.4 ± 0.1	3.1 ± 0.2	78.2 ± 0.2	3.3 ± 0.1
	135	15.2 ± 0.1	3.1 ± 0.1	78.3 ± 0.2	3.4 ± 0.0
Zn–M	15	15.1 ± 0.1	3.0 ± 0.1	78.6 ± 0.2	3.4 ± 0.0
	45	15.0 ± 0.1	3.2 ± 0.1	78.6 ± 0.2	3.4 ± 0.0
	75	15.1 ± 0.1	3.1 ± 0.1	78.6 ± 0.2	3.3 ± 0.1
	105	15.2 ± 0.1	2.8 ± 0.1	78.8 ± 0.2	3.3 ± 0.1
	135	15.0 ± 0.1	3.1 ± 0.1	78.7 ± 0.2	3.4 ± 0.1
Zinc source	Zn–S	15.2 ± 0.0	3.2 ± 0.1	78.4 ± 0.1	3.4 ± 0.0
	Zn–M	15.1 ± 0.0	3.0 ± 0.1	78.7 ± 0.1	3.4 ± 0.0
Zinc level	0	15.1 ± 0.1	2.8 ± 0.1^{c}	78.6 ± 0.4	3.6 ± 0.1^{a}
	15	15.1 ± 0.1	3.1 ± 0.1^{ab}	78.5 ± 0.1	3.4 ± 0.0^{b}
	45	15.1 ± 0.1	3.3 ± 0.1^{a}	78.4 ± 0.2	3.4 ± 0.0^{b}
	75	15.1 ± 0.1	3.2 ± 0.1^{a}	78.5 ± 0.1	3.3 ± 0.0^{b}
	105	15.3 ± 0.1	3.0 ± 0.1^{bc}	78.5 ± 0.2	3.3 ± 0.0^{b}
	135	15.1 ± 0.1	3.1 ± 0.1^{ab}	78.5 ± 0.2	3.4 ± 0.0^{b}
Source		0.082	0.021	0.032	0.707
Level		0.156	0.002	0.953	0.000
Source × level		0.233	0.657	0.754	0.998

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

3.4. Antioxidant responses in serum or liver

Data on the antioxidant responses in serum or liver are shown in Table 5. Activity of SOD in serum significantly increased as zinc supplementation increased from 0 to 75 mg/kg ($P < 0.05$). GSH-PX activity in serum also significantly increased as zinc supplementation increased from 0 to 105 mg/kg ($P < 0.05$). Diet supplemented with 45 mg/kg zinc resulted in significantly higher GSH-PX activity in liver ($P < 0.05$). No differences were found in SOD activity in the liver, CAT activity in the serum and liver, and TBARs levels in the liver ($P > 0.05$). Dietary zinc sources significantly influenced the SOD activity in serum and the GSH-PX activity in the liver ($P < 0.05$). Fish fed Zn–S diets showed

Table 3
Effects of $ZnSO_4$ (Zn–S) and chelated Zn (Zn–M) on zinc concentration in the whole body, bone, muscle, liver and serum of turbot (n = 5).

Sources	Supplemented levels (mg/kg diet)	Whole body (mg/kg)	Bone (mg/kg)	Muscle (mg/kg)	Liver (mg/kg)	Serum (μg/mL)
Control	0	75.6 ± 9.8	72.9 ± 2.1	66.1 ± 8.3	106.8 ± 22.2	25.9 ± 3.3
Zn–S	15	84.3 ± 9.7	72.6 ± 4.3	68.0 ± 9.1	102.9 ± 19.6	28.0 ± 2.1
	45	115.5 ± 5.4	94.5 ± 10.2	59.7 ± 6.0	110.8 ± 22.4	32.6 ± 2.5
	75	124.6 ± 20.4	105.5 ± 9.8	68.1 ± 6.6	104.5 ± 21.6	31.4 ± 3.1
	105	211.0 ± 26.8	134.9 ± 14.8	73.7 ± 7.8	117.6 ± 27.6	30.9 ± 1.3
	135	160.7 ± 27.2	136.9 ± 13.9	82.5 ± 8.1	107.7 ± 13.7	36.8 ± 2.4
Zn–M	15	80.1 ± 3.9	81.4 ± 5.5	65.0 ± 12.8	88.5 ± 8.1	33.6 ± 2.1
	45	101.0 ± 9.5	97.2 ± 12.4	95.4 ± 9.0	106.5 ± 24.4	29.3 ± 2.1
	75	120.9 ± 10.0	109.9 ± 13.2	66.4 ± 8.7	125.9 ± 34.3	35.6 ± 2.7
	105	166.8 ± 15.2	146.9 ± 18.0	73.1 ± 9.8	93.4 ± 19.9	33.4 ± 3.7
	135	153.8 ± 24.4	138.2 ± 14.4	65.7 ± 5.9	80.3 ± 9.3	27.8 ± 3.7
Zinc source	Zn–S	128.6 ± 11.0	102.6 ± 6.2	69.7 ± 3.2	108.4 ± 8.1	30.9 ± 1.1
	Zn–M	116.4 ± 8.2	107.7 ± 6.8	72.0 ± 4.0	100.2 ± 8.5	30.9 ± 1.3
Zinc level	0	75.6 ± 9.8^{c}	72.9 ± 2.1^{c}	66.1 ± 8.3	106.8 ± 22.2	25.9 ± 3.3
	15	82.2 ± 5.0^{c}	77.0 ± 3.6^{c}	66.5 ± 7.4	95.7 ± 10.3	30.8 ± 1.7
	45	108.2 ± 5.7^{bc}	95.8 ± 7.6^{bc}	77.6 ± 7.8	108.7 ± 15.6	30.9 ± 1.6
	75	122.8 ± 10.7^{b}	107.7 ± 7.8^{b}	67.2 ± 5.2	115.2 ± 19.5	33.5 ± 2.1
	105	188.9 ± 16.3^{a}	140.9 ± 11.2^{a}	73.4 ± 5.9	105.5 ± 16.6	32.1 ± 1.9
	135	157.2 ± 17.3^{a}	137.6 ± 9.4^{a}	74.1 ± 5.5	94.0 ± 9.0	32.3 ± 2.6
Source		0.201	0.457	0.647	0.423	0.988
Level		0.000	0.000	0.671	0.279	0.122
Source × level		0.774	0.994	0.082	0.346	0.112

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

Table 5
Effects of $ZnSO_4$ (Zn–S) and chelated Zn (Zn–M) on antioxidant responses in the serum or liver of turbot (n = 5).

Sources	Supplemented levels (mg/kg diet)	Serum SOD (U/mL)	Liver SOD (U/mg prot)	Serum CAT (U/mL)	Liver CAT (U/mg prot)	Serum GSH-PX (U/mL)	Liver GSH-PX (U/mg prot)	TBARs (μm MDA equivalents)
Control	0	66.2 ± 10.2	64.1 ± 9.7	3.8 ± 0.5	14.7 ± 2.1	28.4 ± 5.0	29.2 ± 4.9	4.2 ± 0.3
Zn–S	15	69.6 ± 7.3	52.9 ± 6.3	3.1 ± 0.2	12.7 ± 1.0	33.3 ± 2.3	31.1 ± 0.5	2.6 ± 0.4
	45	69.0 ± 7.4	97.0 ± 11.8	5.2 ± 0.8	22.7 ± 6.6	49.7 ± 11.5	36.9 ± 6.8	5.1 ± 1.0
	75	107.5 ± 10.9	74.0 ± 10.6	4.1 ± 0.8	16.9 ± 3.6	35.5 ± 6.7	37.5 ± 3.2	4.3 ± 1.1
	105	138.5 ± 20.0	73.8 ± 9.1	5.5 ± 0.7	15.8 ± 1.8	65.7 ± 3.1	30.5 ± 1.4	4.9 ± 1.1
	135	86.8 ± 12.5	57.9 ± 5.8	3.9 ± 0.4	16.9 ± 1.8	34.6 ± 6.7	35.4 ± 1.8	6.0 ± 1.1
Zn–M	15	48.2 ± 10.5	67.1 ± 5.8	3.8 ± 0.5	15.9 ± 2.1	38.2 ± 6.7	35.9 ± 1.7	4.7 ± 0.5
	45	52.0 ± 15.5	68.2 ± 6.3	4.0 ± 0.5	14.3 ± 1.4	42.3 ± 3.4	63.2 ± 7.00	4.5 ± 1.3
	75	107.0 ± 17.4	69.7 ± 9.2	3.9 ± 0.7	16.1 ± 4.8	43.3 ± 7.5	36.9 ± 5.1	4.3 ± 1.3
	105	76.1 ± 21.2	71.1 ± 1.8	3.9 ± 0.3	12.0 ± 2.7	62.6 ± 9.0	42.1 ± 5.9	3.0 ± 1.1
	135	100.6 ± 11.5	65.3 ± 16.9	3.4 ± 0.2	9.4 ± 2.4	57.6 ± 8.2	42.9 ± 1.5	6.2 ± 1.5
Zinc source	Zn–S	88.4 ± 6.7	69.2 ± 4.4	4.3 ± 0.3	16.3 ± 1.2	42.4 ± 3.8	33.4 ± 1.5	4.5 ± 0.4
	Zn–M	77.8 ± 5.9	67.5 ± 3.4	3.8 ± 0.2	13.6 ± 1.2	45.7 ± 3.5	41.7 ± 3.0	4.6 ± 0.4
Zinc level	0	66.2 ± 10.2^{b}	64.1 ± 9.7	3.8 ± 0.5	14.7 ± 2.1	28.4 ± 5.0^{c}	29.2 ± 4.9^{b}	4.2 ± 0.3
	15	60.4 ± 6.3^{b}	58.2 ± 5.0	3.4 ± 0.3	14.1 ± 1.2	35.4 ± 3.0^{bc}	33.5 ± 1.4^{b}	3.7 ± 0.5
	45	61.7 ± 6.2^{b}	84.7 ± 8.7	4.6 ± 0.5	18.5 ± 3.6	46.0 ± 5.7^{b}	50.0 ± 7.3^{a}	4.8 ± 0.8
	75	107.2 ± 6.5^{a}	71.8 ± 6.5	4.0 ± 0.5	16.4 ± 2.9	40.0 ± 5.0^{bc}	37.2 ± 2.7^{b}	4.3 ± 0.8
	105	102.8 ± 15.8^{a}	72.6 ± 4.9	4.8 ± 0.5	14.1 ± 1.6	64.3 ± 3.8^{a}	37.1 ± 4.0^{b}	4.0 ± 0.8
	135	93.7 ± 6.9^{a}	61.0 ± 7.3	3.6 ± 0.2	13.2 ± 2.0	47.7 ± 6.9^{ab}	39.2 ± 2.0^{b}	6.1 ± 0.9
Source		0.020	0.667	0.489	0.096	0.304	0.004	0.955
Level		0.000	0.180	0.158	0.527	0.001	0.003	0.208
Source × level		0.015	0.335	0.137	0.352	0.319	0.064	0.567

SOD, superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; TBARs, thiobarbituric acid reactive substance.
Values (means ± S.E.M.) in the same column sharing a common superscript letter were not significantly different ($P > 0.05$).

significantly higher serum SOD activity than those fed Zn–M diets. However, fish fed Zn–M diets displayed significantly higher GSH-PX activity in the liver than those fed Zn–S diets.

4. Discussion

Previous studies have shown that the zinc requirements of fish fed practical diets were higher than those fed semi-purified diets (Gatlin and Wilson, 1983, 1984; Satoh et al., 1987). One of the reasons is that the anti-nutritional substances in ingredients of practical diets, such as tricalcium phosphate and phytate, inhibit zinc availability in fish (Apines et al., 2003; Richardson et al., 1985; Satoh et al., 1989). Typically, 60–70% fish meal and 10–15% plant products are used in the commercial feeds for turbot in China. These feeds contain approximately 2–5% calcium and 0.3–0.5% phytate. Hence, in the present study, 2% of tricalcium phosphate and 0.5% of sodium phytate were added to the experimental semi-purified diets to achieve levels similar to those seen in commercial diets.

In the present study, growth response, bone zinc concentration and antioxidant response were improved by dietary Zn–S or Zn–M supplementation. It is suggested that turbot can utilize the chelated Zn as a dietary zinc source. Positive effects of chelated zinc (Mintrex™ Zn) on growth and bone zinc deposition were also observed in broiler chickens (Yuan et al., 2011). In the present study, low growth performance was observed in fish fed zinc-deficient diet (basal diet). Unlike in rainbow trout (Ogino and Yang, 1978), however, no eye cataracts or skin erosion was observed. The basal diet in the Ogino and Yang (1978) trial had far lower levels of Zn than the present study (1 versus 36 mg/kg). This is more likely to have been the main factor why negative health related effects of Zn deficiency were noted in rainbow trout versus the turbot in the present study.

In the present study, broken-line regression analysis of SGR data indicated that the dietary zinc requirement of juvenile turbot was 60.2 mg/kg (Fig. 1). These results are higher than those reported in some other aquatic animals, such as rainbow trout (15–30 mg/kg diet) (Ogino and Yang, 1978), Nile tilapia (30–44.5 mg/kg diet) (Do Carmo E Sá et al., 2004; Eid and Ghonim, 1994), grass shrimp (*Penaeus monodon*) (32 mg/kg diet) (Shiau and Jiang, 2006). In addition to the species difference, one of the reasons could be the presence of tricalcium phosphate and phytate supplemented into diets in the present study. These anti-nutritional substances may have inhibited zinc availability leading to a higher requirement for zinc (Apines et al., 2003; Gatlin and Phillips, 1989).

The role of tissue zinc deposition in the animal is important. Whenever necessary, these tissues quickly provide zinc to the organism. In the present study, whole body zinc concentrations increased with the increasing of dietary zinc levels. These results are in agreement with previous studies on Atlantic salmon (*Salmo salar*) (Maage and Julshamn, 1993), rainbow trout (Kucukbay et al., 2006), grass shrimp (Shiau and Jiang, 2006) and abalone (Tan and Mai, 2001). As demonstrated by Jeng and Sun (1981) in common carp, zinc firstly accumulated in the digestive tract, followed by skeletal tissues, and then skin and muscle. Meanwhile, previous studies showed that zinc was mainly stored in the bone and liver. Therefore, zinc levels in these organs were the useful indices for evaluating zinc status (Apines et al., 2003). This was confirmed with tissue zinc from the present study (Table 3). In the present study, the bone zinc concentration increased and then plateaued with increasing zinc supplemented level. This was agreed with previous studies on channel catfish, Nile tilapia and hybrid striped bass (Buentello et al., 2009; Do Carmo E Sá et al., 2004; Gatlin and Wilson, 1984). However, zinc concentrations in the muscle, liver and serum did not reflect zinc intake like those in whole body and bone in the present study. Besides bone, zinc increased in whole body may be related to the zinc increased in the skin, scale, blood cell, gill and viscera except the liver, which were effective organs for zinc deposition in fish (Jeng and Sun, 1981; Sun and Jeng, 1998). Further study is needed.

The present study found no significant difference in zinc bioavailability between the two zinc sources. Similar results have been reported in pig (Swinkels et al., 1996) and tilapia (Zhao et al., 2011). However, some studies reported that the bioavailability of zinc from organic zinc was better than inorganic zinc for birds (Wedekind et al., 1992; Yuan et al., 2011), channel catfish (Paripatananont and Lovell, 1995) or abalone (Tan and Mai, 2001). The difference could be related to different species, diet types or chelated zinc sources (Do Carmo E Sá et al., 2005).

Zinc plays an important role in enhancing antioxidant status and decreasing lipid peroxidation (Anderson et al., 2001). In general, antioxidant enzymes, such as SOD, CAT and GSH-PX, are the main constituents of an organism's antioxidant system (Mallick and Mohn, 2000). Superoxide dismutase is the first enzyme to respond against oxygen radicals (Winston and Di Giulio, 1991). In the present study, serum

SOD activities were significantly increased by dietary zinc, regardless of the zinc sources. This could be due to the fact that zinc is a component of Cu–Zn–SOD (Shiau and Jiang, 2006). A similar trend for GSH-PX activity in the serum and liver was also found in the present study and is similar to that observed in Jian carp (Feng et al., 2011). In addition, in the present study, crude lipid contents in the whole body were significantly increased as dietary zinc supplementation increasing from 0 mg/kg to 75 mg/kg. This could be due to the increase in anti-oxidative activity in turbot (e.g., SOD and GSH-PX activities), which decreased lipid peroxidation (Mallick and Mohn, 2000). Kucukbay et al. (2006) reported that the serum, liver and whole body malondialdehyde (MDA) concentrations in rainbow trout linearly decreased with dietary zinc supplementations. However, in the present study, liver TBARs concentrations were not significantly influenced by dietary zinc supplementations, regardless of the zinc sources. At this time point, it is difficult to explain why there were different influences of dietary zinc on MDA concentrations between rainbow trout and turbot. Further studies are needed to explain the relationship between zinc and lipid oxidation in fish.

5. Conclusion

Supplementation of dietary chelated or inorganic zinc improved the growth, feed utilization, bone zinc deposition and anti-oxidant responses of turbot. There was no significant difference in the zinc bioavailability between the two zinc sources. Based on SGR, the dietary zinc requirement of juvenile turbot was estimated to be 60.2 mg/kg.

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