Aquaculture 420–421 (2014) 187–192

Contents lists available at ScienceDirect

Aquaculture

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

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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ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form 2 November 2013
Accepted 5 November 2013
Available online 14 November 2013

Keywords:
Turbot
$ZnSO_4$
Chelated Zn
Requirement
Nutrition

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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0044-8486/$ – see front matter
http://dx.doi.org/10.1016/j.aquaculture.2013.11.003

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.