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Alleviation effect of dietary cerium and its complex with chitosan oligosaccharide on cadmium accumulation in juvenile turbot, *Scophthalmus maximus* L., under cadmium stress

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Abstract

An 8 weeks feeding trial was conducted to investigate the effect of dietary cerium (Ce) and its complex with chitosan oligosaccharide (COS-Ce) on growth performance and cadmium (Cd) accumulation of turbot, *Scophthalmus maximus* L. under Cd stress. The basal diet (Diet 0) was formulated without Cd and cerium as the control. Seven other experimental diets (Diets 1–7) were formulated with supplementation of 50 mg Cd^{2+}/kg feed, 50 mg Cd^{2+}/kg and 50 mg Ce^{3+}/kg feed, 50 mg Cd^{2+}/kg and 100 mg Ce^{3+}/kg feed, 50 mg Cd^{2+}/kg and 200 mg Ce^{3+}/kg feed, 50 mg Cd^{2+}/kg and 50 mg COS-Ce/kg feed, 50 mg Cd^{2+}/kg and 100 mg COS-Ce/kg feed, and 50 mg Cd^{2+}/kg +200 mg COS-Ce/kg feed. Results of the present study showed that, compared with the control group, the condition factor in fish fed the diet with 50 mg Cd^{2+}/kg feed (Diet 1) was significantly lower, whereas the Cd concentration in liver and kidney of fish fed the diet with 50 mg/kg Cd^{2+} (Diet 1) was significantly higher ($P < 0.05$). The high Cd accumulation of fish fed diets with sole 50 mg/kg Cd (Diet 1) could be significantly decreased by 51.72% after supplementation of 200 mg COS-Ce/kg in the diet (Diet 7). These results suggested that elevated COS-Ce could effectively protect against dietary Cd accumulation in turbot.

Keywords: cerium, cadmium, turbot, *Scophthalmus maximus* L., growth, residue

Introduction

Cadmium (Cd) is toxic to aquatic organism and represented a serious health hazard to human through the food web (Kruzynski 2004). Cadmium in aquatic product comes from polluted rearing water and byproducts of fish (squid viscera meal, an effective attractant in aquafeeds) (Mai, Li, Ai, Duan, Xu, Zhang, Zhang, Tan & Liufu 2006). The basis of Cd toxicity came from substitution of other metal ions (mainly Zn^{2+}, Cu^{2+} and Ca^{2+}) in metabolism (Chang 1992), which may be influenced by salinity and calcium concentration.

Several methods have been used to decrease Cd accumulation in organisms. One approach has been to transplant fish from contaminated lakes to pristine lakes for depuration. The biological half lives of Cd in the gills and gut decreased to 18 and 37 days respectively (Kraemer, Campbell & Hare 2005). The second method was that Cd concentration was decreased by chelants, such as chitosan oligosaccharide complex with calcium, magnesium and rare earth elements (Sun 2009; Li 2011). Moreover, Cd uptake could be reduced by competition between elevated dietary calcium and Cd (Baldisserotto, Chowdhury & Wood 2005). It has long been known that when animals were exposed to Cd, there were low intake levels of essential metals in tissues of the organism on the one hand, and on the other hand the Cd toxicity decreased at high contents of essential metals.

Many studies showed that dietary iron loading can protect animals from Cd-induced damage.

Spivey Fox (1974) proved that high intake levels of essential nutrients can decrease Cd damage to some extent because of low Cd concentration in Japanese quail kidney. Some reports deeply discussed the interaction between Cd and zinc (Brzóska & Moniuszko-Jakoniuk 2001) or between Cd and calcium (Brzóska & Moniuszko-Jakoniuk 1998).

Rare earths elements (REE) have been extensively used as micro-fertilizers in China for about 40 years. Based on the similar ion radius, some exporters proved that REE can be used as regulator in pollution ecology. Huang and Zhou (2006) showed that lanthanum can decrease Cd accumulation possibly influenced by the root microscopic structures change of kidney bean and corn. The relationship between rare earth elements loading and Cd exposure was also studied in loach (Sun 2008), duckweed (Ma 2004), waterweed (Wang 2005) and crucian (Jiao, Liu & Qu 2009).

Consequently, the objective of this study was to examine the effects of elevated dietary cerium (Ce) or chitosan oligosaccharide complex with Ce and Cd^{2+} (alone and in combination) on survival, Cd and cerium accumulation in several internal compartments and activity of hepatic enzymes in juvenile turbot. This study was conducted to offer important information for the safety of aquaculture polluted by Cd.

Materials and methods

Chitosan oligosaccharide complex with cerium

Chitosan oligosaccharide (viscosity-average molecular weight was 2298.9 Da) was provided by Qingdao Honghai Bio-Tech. Co. Ltd, China.

Preparation of chitosan oligosaccharide complex with cerium (COS-Ce) was determined by a modified method of Wang (2005). The process of COS-Ce preparation was as follows. Firstly, 500 mL 0.05 mol L^{-1} Ce $(NH_4)_2(NO_3)_6$ was supplemented with 1000 mL 10 mg mL^{-1} chitosan oligosaccharide. The solution was stirred for 3 h at the room temperature, adjusted to pH 7 with dilute ammonia solution and incubated at 25°C for 12 h. Then the acetone-ethanol media in volume ratio 1:1 was added to the reaction products in drops. Finally, the sedimentation was vacuum dried for about 12 h under the condition of 50°C.

Experimental diets

Using fish meal (provided by Qingdao Great Seven Bio-Tech. Co., Ltd, China) as protein sources, fish oil and soybean lecithin as lipid sources, the basal diet was formulated. The basal diet was used as the control diet (Table 1). To prepare the experimental diets, the control diet was supplemented with $CdCl_2 \cdot 2H_2O$(provided by Laiyang Economic and Technological Development Zone Fine Chemical Co., Ltd., China. Analytical Reagent 99% purity), COS-Ce and/or $CeCl_3 \cdot 7H_2O$ (provided by Tianjin Jinbolan Fine Chemical Co., Ltd., China. Analytical Reagent 99% purity). Fish were randomly fed with each of seven experimental diets: 50 mg Cd^{2+}/kg feed, 50 mgCd^{2+}/kg+50 mgCe^{3+}/kg feed, 50 mgCd^{2+}/kg+100 mgCe^{3+}/kg feed, 50 mgCd^{2+}/kg+200 mgCe^{3+}/kg feed, 50 mg Cd^{2+}/kg+50 mg COS-Ce/kg feed, 50 mg Cd^{2+}/kg+100 mg COS-Ce/kg feed, 50 mg Cd^{2+}/kg +200 mg COS-Ce/kg feed (Table 2). All the ingredients were thoroughly mixed with fish oil. $CdCl_2.2H_2O$, $CeCl_3.7H_2O$ and/or COS-Ce were dissolved in the water and added to produce stiff dough. The resulting 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 50°C. The dietary pellets (1.5 × 3.0 mm) were sealed in a sample bag and stored at −20°C until used.

Table 1 Formulation and chemical composition of the basic diets (% dry weight)

Ingredients	
White fish meal*	66.00
Wheat meal*	23.65
Fish oil*	7.00
Soybean lecithin*	1.00
Vitamin & Mineral premix †	2.00
Choline chlorine (95%)	0.20
Ethoxyquin	0.05
Calcium propionate	0.10
Proximate composition	
Crude protein (% dry matter)	52.78
Crude lipid (% dry matter)	12.76
Ash (% dry matter)	13.23

*Kindly provided by Qingdao Great Seven Bio-Tech. Co., Ltd, China. White fish meal contained 74.33% crude protein (dry weight basis), 6.69% crude lipid and 19.74% ash. Wheat meal contained 15.73% crude protein, 1.44% crude lipid and 0.85% ash.

†Kindly provided by Qingdao Jinhaili Aquatic Technology Company, China.

Table 2 The contents of Cd, Ce and COS-Ce (mg kg^{-1}) in experiment diets*

Diet No.	Cd	Ce	COS-Ce
Diet 0	–	–	–
Diet 1	50	–	–
Diet 2	50	50	–
Diet 3	50	100	–
Diet 4	50	200	–
Diet 5	50	–	50
Diet 6	50	–	100
Diet 7	50	–	200

*– Means 0.

Experimental animals

Juvenile turbot were purchased from a commercial farm in Jiaonan, Qingdao, China. Fish were maintained for 1 week in a 200 L cylindrical fibreglass tank supplied with natural seawater. Fish were hand-fed to apparent satiation twice a day with a commercial feed (NO.2 feed provided by Qingdao Great Seven Bio-Tech. Co., Ltd, China) to acclimate to the experimental conditions.

An 8-week growth trial was conducted in an indoor flowing water system. Following a 7 d acclimation period, fish of similar size (initial average weight of 12.77 ± 0.06 g) were randomly distributed into 24 200 L cylindrical fibreglass tanks, and each tank was stocked with 30 fish. Each diet was randomly assigned to triplicate tanks twice a day (08:00 and 19:00 hours). Uneaten feed and faeces at the bottom of tanks were siphoned daily. Dead fish were removed daily and mortality was recorded. Air stones in each tank was used to maintain the dissolved oxygen concentration at 7 mg L^{-1} or more. Turbot were reared under a natural light cycle from May 8 to July 3 2011. During the experimental period, the water temperature fluctuated from 19 to 20°C, pH from 7.5 to 7.8 and salinity from 24‰ to 27‰. The rare earth elements and Cd composition of the seawater was cerium 6.70 μg L^{-1}, europium 5.72 μg L^{-1}, lanthanum 4.91 μg L^{-1}, samarium 2.47 μg L^{-1}, terbium 2.26 μg L^{-1}, neodymium 1.86 μg L^{-1}, scandium 0.10 μg L^{-1}, dysprosium 0.02 μg L^{-1} and Cd 10.8 μg L^{-1} respectively.

Analysis and measurement

Sample collection

Fish were fasted overnight prior to sampling. At the end of the experiment, nine fish per tank were randomly collected and anaesthetized with eugenol (1,10000; Shanghai Reagent, China) and blood was collected from the caudal vein with heparinized 1 mL syringes. Blood was allowed to be centrifuged at 3000 × *g* for 10 min to separate plasma. Fish were then sacrificed by a blow to the head, and gill, kidney, liver and muscle were removed. The remaining segments were cooked for 10 min in a microwave and all muscle was removed to separate the bone. After a 30-day feeding period, faecal collection was conducted 5–6 h after feeding. Gill, kidney, liver, muscle bone and faecal samples were frozen and stored at −80°C until analysis of total ionic contents and enzyme activity.

Measurement of the whole-body composition

At the termination of feeding experiment, three fish from each replicate were randomly collected and stored frozen (−20°C) for determination of proximate carcass composition. Proximate composition analysis on fish body was performed by standard methods of Association of Official Analytical Chemists (1995). Samples of fish were dried to a constant weight at 105°C to determine moisture. The dried fish were pooled, smashed and mixed completely. Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method; lipid by ether extraction using Soxhlet; ash by combustion at 550°C in a muffle furnace.

Cd and Ce contents analysis

Gill, kidney, liver, muscle, bone and faeces of turbot were digested in 10 mL mixed acid at volume ratio of HNO_3 to $HClO_4$ 4,1 overnight at the room temperature. Afterwards, the solution was digested by heating with DigiBlock digestion apparatus (ED 36, LabTech Ltd., Beijing, China). Then the solution was dissolved with distilled water into a constant volume of 25 mL volumetric flask. Cd concentrations in these tissues were analysed with the atomic absorption spectrophotometer with air-acetylene oxide flame atomization (AA-6800, Shimadzu Scientific Instruments Ins. Japan).

The concentrations of Ce in samples were determined by inductively coupled plasma-atomic emission spectrophotometer (ICP-OES, VISTA-MPX, VARIAN, USA) after HNO_3-$HClO_4$ mixture digestion.

Metallothionein content

The MT content was measured using Ellman's reaction based on spectrophotometry at 412 nm.

Briefly, liver samples were homogenized at 9:1 ratio in a buffer of Tris-HCl 20 mM, pH 8.0, supplemented with 250 mM saccharose and centrifuged (105 000 g, 4°C) for 60 min. Then, 50 μL supernatants was incubated with 10 μL 1.2N HCl and 200 μL 0.1 mM EDTA for 10 min. Then 200 μL of Ellman's solution containing 1 mM EDTA, 5 mM DTNB and 6N guanidine hydrochloride was added to each tube. After 3 min, the sample was added with a solution of 0.1M PBS (pH 7.3) until the volume was 5 mL. The absorbance of each tube was evaluated at 412 nm. The MT values were expressed as mg g^{-1} in liver.

Ca^{2+}-ATPase

Ca^{2+}-ATPase was assayed according to the method of Marín, Proverbio and Proverbio (1986). The concentration of Ca^{2+}-ATPase was measured by quantifying inorganic phosphate decomposed from the hydrolysis of ATP. The ATPase activity was expressed as U mg $prot^{-1}$ in liver.

Calculations and statistical analysis

The following variables were calculated,

$$\text{Condition factors} = W/L^3 \times 100$$

$$\text{Survival}(\%)100 \times (N_t)/(N_0)$$

where N_t and N_O were final and initial number of fish, respectively, t was duration of experimental days. W was final body weight and L was body length.

All data from three independent replicates were subjected to one-way analysis of variance in SPSS 18.0 for Windows. Levene's test for homogeneity of variances was used, while square-root arcsine data transformation was used for all percentage data. Differences between the means were tested by Tukey's multiple range tests. The level of significance was chosen at $P < 0.05$ and the results were presented as means ± S.E.M. (standard error of the mean) ($n = 3$).

Results

Survival and growth

There was no significant difference in survival among dietary treatments ($P > 0.05$), which ranged from 83.33% to 96.67% (Table 3), and the highest survival was found in fish fed with the 50 mg Cd^{2+}/kg feed +200 mg COS-Ce/kg feed diet (Diet 7). There were no significant differences in specific growth rate among dietary treatments. The specific growth rate in fish fed diets with Cd + Ce feed (from Diet 2 to Diet 4) decreased with the increase in Ce (ranged from 1.90 to 1.83% d^{-1}), while the specific growth rate in fish fed diets with Cd + COS-Ce feed (from Diet 5 to Diet 7) increased with increase in COS-Ce (ranged from 1.73 to 1.83% d^{-1}). The condition factors of turbot fed diets with supplementation of Cd^{2+} ($P < 0.05$) were significantly lower compared with the control group. Condition factors tended to increase with increasing dietary (from Diet 5 to Diet 7) COS-Ce in the Cd-treated fish (ranged from 3.37 to 3.78 g cm^{-3}). When fish was fed the diet with 50 mg Cd^{2+}/kg feed +100 mg COS-Ce/kg feed (Diet 6), condition factor was significantly higher than that in fish fed the Cd diet (Diet 2) ($P < 0.05$).

Table 3 Effects of Ce and COS-Ce on survival and condition factors of *Scophthalmus maximus* L.under Cd stress*

Diet No.	Initial body weight (g)	Final body weight (g)	Specific growth rate (% d^{-1})	Condition factors r(g cm^{-3})	Survival (%)
Diet 0	12.81 ± 0.03	41.43 ± 0.84	1.96 ± 0.03	3.84 ± 0.15^{c}	85.56 ± 5.89
Diet 1	12.78 ± 0.01	40.37 ± 0.80	1.89 ± 0.03	3.39 ± 0.03^{ab}	93.33 ± 3.33
Diet 2	12.82 ± 0.01	40.03 ± 0.95	1.90 ± 0.04	3.50 ± 0.06^{abc}	95.52 ± 1.09
Diet 3	12.73 ± 0.10	36.85 ± 1.05	1.82 ± 0.02	3.25 ± 0.05^{a}	93.33 ± 0.00
Diet 4	12.80 ± 0.02	38.48 ± 0.49	1.83 ± 0.02	3.34 ± 0.05^{ab}	83.33 ± 8.38
Diet 5	12.85 ± 0.04	36.37 ± 1.28	1.73 ± 0.06	3.37 ± 0.02^{ab}	86.67 ± 5.09
Diet 6	12.66 ± 0.17	37.09 ± 2.47	1.78 ± 0.09	3.65 ± 0.05^{bc}	90.00 ± 5.09
Diet 7	12.71 ± 0.16	38.14 ± 0.37	1.83 ± 0.02	3.78 ± 0.09^{v}	96.67 ± 3.33

*Data presented as means ± S.E.M ($n = 3$); Data within the same column with the same superscripts were not significantly different determined by Tukey's multiple test ($P > 0.05$).

Body composition

There were no significant differences in body composition (protein, lipid, ash and moisture) among dietary treatments ($P > 0.05$). The whole-body moisture ranged from 75.67% to 77.92%, the whole-body protein content ranged from 49.63% to 52.81%, the whole-body lipid content ranged from 10.48% to 13.30% and whole-body ash content ranged from 15.98% to 17.58% (Table 4).

Cd^{2+} accumulation

The Cd concentrations in tissues of turbot were in the following order: liver > kidney > gill> bone > muscle (Table 5). The Cd concentration in liver and kidney of fish fed the diet with 50 mg Cd^{2+}/kg feed (Diet 1) was significantly higher than those of fish fed with the control diet (Diet 0) ($P < 0.05$).

After the 8-week feeding trial, fish fed the diet with 50 mg Cd^{2+}/kg diet and 50 mgCe^{3+}/kg diet (Diet 2) showed higher Cd^{2+} uptake in tissues than that fed the control diet. However, the Cd concentrations in liver, kidney, gill and muscle were lower relative to fish fed the diet with Cd^{2+} (Diet 1). Compared with fish fed the control diet, fish fed the diet with 50 mg Cd^{2+}/kg feed +100 mg COS-Ce/kg feed (Diet 6) showed higher Cd^{2+} uptake in tissues than those fed the control diet except the bone segment, but actually the Cd^{2+} concentration in liver, kidney, gill, bone and muscle was significantly lower relative to fish fed the Cd^{2+} treatment diet (Diet 1). Compared with fish fed the diet with Cd^{2+} (Diet 1), fish fed the diet with 50 mg Cd^{2+}/kg feed +200 mg COS-Ce/kg feed (Diet 7) showed a significant decrease of accumulated Cd in muscle ($P < 0.05$), and the overall reduction was approximately 51.72%. Fish fed diets with Cd+Ce and Cd+COS-Ce supplementation showed a significant increase in the Cd accumulation in faeces ($P < 0.05$) compared with the control diet. The presence of elevated Ce^{3+} or COS-Ce together with Cd^{2+} in diets (i.e. Diet 2 and Diet 6) was clearly protective against chronic Cd accumulation in liver, kidney, gill and muscle.

Table 4 The whole-body composition of turbot fed experimental diets for 8 weeks*

Diet No.	Moisture (%)	Crude protein (% d.w.)	Crude lipid (% d.w.)	Ash (% d.w.)
Diet 0	76.08 ± 0.16	52.12 ± .1.61	12.67 ± 1.55	15.98 ± 0.44
Diet 1	75.67 ± 0.19	52.31 ± 0.73	13.30 ± 0.49	16.48 ± 0.34
Diet 2	75.82 ± 0.19	50.64 ± 4.10	12.44 ± 0.21	16.39 ± 0.31
Diet 3	75.93 ± 0.21	49.63 ± 0.12	10.55 ± 0.81	17.14 ± 0.21
Diet 4	77.92 ± 1.61	51.82 ± 1.01	10.48 ± 0.74	17.58 ± 0.72
Diet 5	75.67 ± 0.11	51.63 ± 2.13	10.76 ± 0.86	16.93 ± 0.17
Diet 6	75.83 ± 0.33	49.91 ± 0.32	12.03 ± 0.86	16.98 ± 0.37
Diet 7	75.85 ± 0.20	52.81 ± 1.13	10.97 ± 0.26	15.90 ± 0.84

*Data presented as means ± S.E.M ($n = 3$); Values of crude protein, crude lipid and ash were expressed on a dry-weight basis.

Table 5 Effects of Ce or COS-Ce on Cd accumulation (mg kg^{-1}) in tissues of *Scophthalmus maximus* L.under Cd stress (dry matter basis)*

Diet No.	Liver	Kidney	Gill	Bone	Muscle	Faeces
Diet 0	4.06 ± 0.61^{a}	1.56 ± 0.20^{a}	3.13 ± 0.13	1.10 ± 0.20	0.32 ± 0.04^{ab}	12.07 ± 0.27^{a}
Diet 1	21.25 ± 6.14^{b}	6.11 ± 0.46^{b}	5.12 ± 1.57	1.05 ± 0.11	0.58 ± 0.10^{bc}	60.39 ± 1.33^{b}
Diet 2	16.49 ± 2.45^{ab}	4.96 ± 0.65^{ab}	4.64 ± 0.35	1.25 ± 0.12	0.45 ± 0.04^{abc}	64.03 ± 4.32^{b}
Diet 3	19.59 ± 0.75^{b}	4.56 ± 1.37^{ab}	4.43 ± 0.19	1.07 ± 0.14	0.57 ± 0.04^{abc}	67.16 ± 2.70^{b}
Diet 4	21.56 ± 3.82^{b}	5.52 ± 1.43^{b}	4.80 ± 0.32	0.89 ± 0.22	0.50 ± 0.04^{abc}	65.31 ± 4.55^{b}
Diet 5	27.41 ± 1.00^{b}	4.81 ± 0.70^{ab}	3.92 ± 0.33	0.67 ± 0.20	0.63 ± 0.06^{c}	57.33 ± 2.22^{b}
Diet 6	15.57 ± 0.68^{ab}	4.38 ± 0.51^{ab}	4.00 ± 0.41	0.93 ± 0.24	0.43 ± 0.08^{abc}	55.90 ± 2.59^{b}
Diet 7	17.24 ± 2.54^{ab}	6.26 ± 0.48^{b}	4.13 ± 0.33	0.93 ± 0.17	0.28 ± 0.02^{a}	60.45 ± 0.20^{b}

*Data presented as means ± S.E.M ($n = 3$); Data within the same column with the same superscripts were not significantly different determined by Tukey's multiple test ($P > 0.05$).

Table 6 Effects of Ce or COS-Ce on cerium accumulation (mg kg^{-1}) in tissues of *Scophthalmus maximus* L.under Cd stress (dry matter basis)*

Diet No.	Liver	Kidney	Gill	Bone	Muscle	Feces
Diet 0	1.32 ± 0.06^{ab}	1.82 ± 0.41	1.75 ± 0.03	0.41 ± 0.34	0.42 ± 0.00	7.51 ± 0.08^{a}
Diet 1	1.14 ± 0.32^{a}	2.22 ± 1.22	2.15 ± 0.03	0.82 ± 0.01	0.53 ± 0.00	8.21 ± 2.72^{a}
Diet 2	1.15 ± 0.01^{a}	5.59 ± 1.93	5.01 ± 1.51	1.20 ± 0.41	0.59 ± 0.16	40.85 ± 2.53^{a}
Diet 3	1.11 ± 0.10^{a}	3.95 ± 1.56	5.51 ± 1.03	1.22 ± 0.40	0.50 ± 0.20	75.41 ± 6.45^{a}
Diet 4	1.33 ± 0.04^{ab}	4.72 ± 0.83	5.08 ± 1.45	1.22 ± 0.39	0.55 ± 0.13	148.85 ± 12.20^{a}
Diet 5	1.76 ± 0.02^{bc}	5.30 ± 0.85	4.95 ± 1.58	1.03 ± 0.01	0.61 ± 0.17	116.74 ± 3.25^{a}
Diet 6	1.34 ± 0.01^{ab}	6.25 ± 0.87	4.89 ± 1.64	0.65 ± 0.02	0.42 ± 0.04	396.93 ± 38.48^{bc}
Diet 7	1.95 ± 0.02^{c}	6.34 ± 1.76	5.12 ± 1.41	0.67 ± 0.01	0.40 ± 0.06	725.38 ± 25.48^{c}

*Data presented as means ± S.E.M (n = 3); Data within the same column with the same superscripts were not significantly different determined by Tukey's multiple test ($P > 0.05$).

Ce^{3+} accumulation

Total Ce^{3+} concentrations were highest in gill, followed by kidney and liver, and the lowest values were found in bone and muscle (Table 6). Because whole gill including cartilaginous tissue was analysed, undoubtedly Ce^{3+} concentrations in gill were higher than those in bone. Compared with fish fed the control diet and the Cd treatment diet (Diet 1), fish fed the diet with 50 mg Cd^{2+}/kg feed +200 mg COS-Ce/kg feed diet (Diet 7) showed a significant increase in the Ce^{3+} accumulation in liver ($P < 0.05$). However, no significant differences in total Ce^{3+} levels were observed among kidney, gill and bone ($P > 0.05$). Compared with fish fed the Cd+Ce treatment diet (Diet 1), fish fed the 50 mg Cd^{2+}/kg feed +200 mg COS-Ce/kg feed diet (Diet 7) showed a significant increase in the Ce^{3+} accumulation in the faeces ($P < 0.05$).

Compared with the control group, fish fed the diet with the only Cd supplementation (Diet1) had an approximate threefold elevation of metallothione concentrations in liver. Also, fish fed diets with 50 mg Cd^{2+}/kg and 50 mg Ce^{3+}/kg feed (Diet 2) and 50 mg Cd^{2+}/kg and 100 mg COS-Ce/kg feed (Diet 6) had more MT concentrations than the control diet. There were no significant differences in hepatic Ca^{2+}-ATPase activity among dietary treatments ($P > 0.05$), although the highest Ca^{2+}-ATPase activity was detected in fish fed the 50 mg Cd^{2+}/kg feed +100 mgCe^{3+}/kg feed diet (Diet 3) (Table 7).

Table 7 Effects of Ce or COS-Ce on metallothionein content and Ca^{2+}-ATPase activity in liver from *Scophthalmus maximus* L.under Cd stress*

Diet No.	Metallothionein (mg g^{-1})	Ca^{2+}-ATPase (U mg $prot^{-1}$)
Diet 0	34.82 ± 9.27	0.13 ± 0.00
Diet 1	108.02 ± 8.51	0.15 ± 0.06
Diet 2	71.24 ± 5.36	0.15 ± 0.00
Diet 3	18.46 ± 1.67	0.34 ± 0.24
Diet 4	24.62 ± 5.31	0.12 ± 0.06
Diet 5	19.32 ± 3.03	0.12 ± 0.05
Diet 6	90.14 ± 5.73	0.21 ± 0.09
Diet 7	79.45 ± 4.60	0.24 ± 0.00

*Data presented as means ± S.E.M (n = 3).

Discussion

In this study, survival and growth performance were not significantly affected by dietary Cd stress or the supplementation of cerium and COS-Ce. This was inconsistent with the findings of Mahaffey, Capar, Gladen and Fowler (1981) who found that growth performance of rats was significantly inhibited after exposure to the diet with 50 mg Cd^{2+}/kg. Abdel-Tawwab and Wafeek (2014) found that growth performance of fish was significantly decreased when reared in water with Cd at a concentration of 0.5 mg/L for 8 weeks. In this study, condition factor was significantly affected by the supplementation of Cd. This could be due to bone deformity caused by high dose of Cd. Fish fed diets with 50 mg Cd^{2+}/kg (Diet 1) showed the highest percentage of bone deformity. Deformity caused by Cd has been found in *Chironomus riparius* (Di Veroli, Santoro, Pallottini, Selvaggi, Scardazza, Cappelletti & Goretti 2014) and *Mytilus edulis* (Sunila & Lindström 1985). In this study, condition factor increased significantly as dietary COS-Ce increased from 50 to 200 mg/kg. On the contrary, no significance was observed in condition factor as

dietary Ce increased. Thus, COS-Ce rather than Ce could exert much more important role in alleviating the bone deformity of turbot.

Recently, more and more attention has been paid to food safety of aquaculture products. Cd (Cd) is believed to be one of the most abundant and ubiquitously distributed toxins in the aquatic system. Previous study showed that distribution of Cd levels was in the order of kidney > liver > gill in Atlantic salmon (Berntssen & Lundebye 2001). In the present study, Cd accumulation was highest in liver, followed by kidney, gill, bone and muscle, which was consistent with the findings of Abdel-Tawwab and Wafeek (2014). This indicated that Cd are prone to accumulate in liver and kidney, rather than muscle which is the edible part of most fish species including turbot.

In this study, the Cd accumulation was increased by at least two times after the sole supplementation of 50 mg/kg Cd in the diet (Diet 1). Fish fed the diet with 100 mg COS-Ce/kg (Diet 6) decreased the deposition of Cd by 28.31% in kidney, 26.73% in liver and 25.86% in muscle, which demonstrated clearly the protective effects of COS-Ce against chronic Cd accumulation in tissues of turbot. Many studies have found that Cd toxicity took effect through the calcium channel, and thus calcium channels blockers (i.e. Dihydropyridines, Verapamil and Diltiazem) could protect animals from Cd toxicity (Hinkle, Kinsella & Osterhoudt 1987). Liu (2006) conjectured that COS could induce activation of Ca^{2+}/calmodium-dependent protein kinase II(CaMK II), which could mediate calcium concentration. Ce has been found to be a calcium channels blocker by interfering the Ca^{2+}-ATPase, which could regulate calcium concentration of internal and external cells (Qiu, Li, Chen, Zhou, Li & Zhang 2003). Dietary Ce could alleviate the Cd stress on carps according to the findings of Qu (2008). Therefore, in this study, the double beneficial effects of COS and Ce on Cd alleviation could account for the higher ability of COS-Ce in decreasing Cd accumulation. Indeed, Cd concentration in faeces increased significantly as dietary COS-Ce increased from 50 to 200 mg/kg. This could be attributed to be accomplished by metallothionein (MT), which is an intracellular protein to protect against Cd toxicity (Klaassen, Liu & Choudhuri 1999). However, in this study, no significant differences were detected in MT among dietary treatments. Our previous study has found that COS-Ce could increase immunity turbot (Cui, Xu, Ai, Wang & Mai 2012), which could be responsible for the elevated survival (86.67% to 96.67%) with the increase in dietary COS-Ce although no statistical differences were observed.

In the present study, the highest Ce accumulation was found in kidney, followed by gill, liver, bone and muscle (Table 6). However, the highest concentration of cerium was found in liver of rats, and in a decreasing order for bone and kidney (Nakamura, Tsumura & Tonogai 1997). The differences could be due to different species. In the present study, the remaining Ce concentration in muscle of fish fed diet with 50–200 mg/kg Ce diet was below 0.63 mg/kg and showed no significant differences among dietary treatments. Up to now, no safety utmost level of Ce was given for humans. Previous studies on rats have found that oral median lethal dose-values for rare earth elements-nitrates ranged from 900 to 1750 mg/kg body weight (De Boer, Verweij, van der Velde-Koerts & Mennes 1996) and median dose-values of cerium chloride by intraperitoneal injection was 353 mg/kg body weight (Qin, Chen & Li 2002). Thus, the Ce concentration in tissues was far below the median lethal dose observed in rats and it was possible the accumulation of Ce was among the safety range for humans when Ce was added at a concentration of 200 mg/kg in the diets.

In conclusion, feeding a dose (200 mg/kg) of COS-Ce with Cd significantly increased condition factors compared with the only Cd-treated group ($P < 0.05$). Dietary COS-Ce could protect turbot against Cd accumulation, with maximum reduction observed in muscle of fish fed the diet with 200 mg/kg COS-Ce. Only trace amount of Ce was found to deposit in muscle of experimental fish when the supplementation of Ce or COS-Ce was as high as 200 mg/kg. Following studies are needed to investigate the alleviation effect of Ce and COS-Ce on Cd accumulation in turbot in a longer experiment duration, and clarify the mechanisms involved in this process.

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