Front. Agric. China 2009, 3(1): 78–83
DOI 10.1007/s11703-009-0012-3

RESEARCH ARTICLE

Huitao LI, Kangsen MAI, Qinghui AI, Chunxiao ZHANG, Lu ZHANG

Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of large yellow croaker, *Pseudosciaena crocea* R.

Abstract Cadmium (Cd) is a toxic environmental pollutant with a long biological half-life and can produce both hepatic and renal injuries in mammals and fish. Squid viscera meal (SVM), an effective attractant for aquatic animals, is widely used as an ingredient in aquafeeds. However, SVM is rich in Cd and its complexes. A study was conducted to evaluate the effects of dietary SVM on the growth and Cd deposition in the tissues of large yellow croaker, *Pseudosciaena crocea* R. Three practical diets were formulated to contain a 0, 50 and 100 g·kg^{-1} SVM diet, correspondingly containing a 0.21, 7.26 and 12.08 mg Cd·kg^{-1} diet. Each diet was randomly assigned to triplicate groups of 100 juveniles of large yellow croaker (mean initial weight, 9.75 ± 0.35 g) in floating sea cages (1.0 m × 1.0 m × 1.5 m). Fish were fed twice daily (05:00 and 17:00) to satiation for 8 weeks. The results showed that there were no significant differences in fish survival among the three dietary treatments, but significant higher specific growth rates (SGR) were observed in the fish fed diets with 50 or 100 g·kg^{-1} SVM diet compared to the control group ($P < 0.05$). The cadmium concentrations in fish tissues (muscle, liver, kidney and gill) were significantly influenced by the dietary SVM. The cadmium concentrations in all tissues significantly increased with increasing dietary Cd levels ($P < 0.05$). In all the dietary treatments, the highest Cd level was always observed in the kidney, followed by the liver and the gill. Fish fed diets with 50 and 100 g·kg^{-1} SVM had significantly higher Cd accumulations in the kidney (2.65, 4.44 mg·kg^{-1}), liver (0.58, 0.93 mg·kg^{-1}) and gill (0.35, 0.53 mg·kg^{-1}) compared with the control group (0.42, 0.26 and 0.12 mg·kg^{-1}, respectively). The Cd level in fish muscle, however, was undetectable in all treatments. Therefore, based on these results, accumulation of Cd in edible tissue (muscle) of farmed large yellow croaker is not a food safety issue. However, long-term feeding of diets with SVM may result in accumulation of Cd in the kidney, liver and gills of fish.

Keywords large yellow croaker (*Pseudosciaena crocea* R.), squid viscera meal, growth, cadmium accumulation

1 Introduction

Squid (or cuttlefish) viscera meal (SVM), which is a by-product of seafood processes, makes feeds more attractive to most aquaculture animals, and therefore it is widely used as an attractant in aquafeeds (Liang et al., 2000) in many regions. However, studies report that SVM contains substantial amounts of cadmium (Bustamante et al., 2002). FVO (1998) reported that significant levels of Cd can be found in the viscera of the squids *Loligo* (up to 13 mg·kg^{-1}) and *Illex* (up to 86 mg·kg^{-1}). The Cd levels in squid hepatopancreas often exceed the maximum residue limits (MRLs) (0.5 mg·kg^{-1}) for cephalopods recommended by the European Union (EU), although the Cd in their mantle and tentacles (the parts normally eaten) is within acceptable levels (FVO, 2002). Thus, care should be taken when supplementing SVM to aquafeeds. So far, however, only Mai et al. (2006a) has studied the effects of SVM supplementation to aquafeeds on Cd accumulation in the tissues of Japanese seabass (*Lateolabrax japonicus*). More studies on other species need to be conducted to evaluate their safety for human consumption.

Since 1993, when the International Agency for Research

Received May 6, 2008; accepted June 8, 2008

Huitao LI, Kangsen MAI (✉), Qinghui AI, Chunxiao ZHANG, Lu ZHANG
The Key Laboratory of Mariculture (Ministry of Education of the People's Republic of China), Ocean University of China, Qingdao 266003, China
E-mail: kmai@mail.ouc.edu.cn

on Cancer (IARC) classified Cd and its compounds as being carcinogenic to human, many studies have reported the characteristics of cadmium (Cd) (Goering et al., 1995; Waalkes, 2000). Some studies have found that Cd exposure can influence the repair and expression of RNA and DNA (Degraeve, 1981; Beyersmann and Hechtenberg, 1997; Calevro et al., 1998; Gerhard et al., 1998), and these effects have been associated with cancer (Waalkes, 1995; Waalkes and Misra, 1996; Waalkes, 2000).

Unlike mammals, which take up Cd mainly via the gut, fish have two sites for Cd intake, the gill and the intestines (Dang et al., 2001). Many studies have demonstrated that fish can accumulate Cd when exposed to waterborne Cd and show a decreasing order of Cd concentration in gill, kidney, liver, and muscle tissues (Mallatt, 1985; McDonald and Wood, 1993; Heath, 1995; Wendelaar Bonga, 1997; Caurant et al., 1999; de Conto Cinier et al., 1999; Hollis et al., 1999; Romeo et al., 1999; Al-Yousuf et al., 2000). Some studies have shown that fish also can accumulate Cd from diets and this contributes to a major portion of Cd load in fish (Dallinger et al., 1987; Hardy, 1996; Berntssen and Lundebye, 2001; Mai et al., 2006a). However, other studies have also shown a different manner of Cd distribution in different tissues (organs).

A series of contamination incidents in aquaculture products in recent years indicate that the safety for human consumption of products from aquaculture is of growing significance in public health. Many international organizations, such as the Joint FAO/WHO, EU and WHO, have discussed this issue. The contamination due to heavy metals is one of the issues in food safety. Cd is an inorganic toxicant and environmental pollutant which can be accumulated in animal tissues, including those of humans, through the food web. When humans consume fish with high levels of accumulated Cd, 3%–7% of the ingested Cd is absorbed (Krajnc et al., 1987), transported to other parts of the body and bound to metallothioneins. If the accumulated Cd exceeds the metallothionein-producing capacity, damage occurs (Friberg et al., 1986). Whereas 50%–85% of the body Cd burden is stored in the kidney and liver, an adult excretes only about 0.007% of the body burden per day (Krajnc et al., 1987; WHO, 1989, 1992). Therefore, the Cd level from aquaculture products must be given attention, and Cd accumulation in fish and its kinetics in fish tissues (or organs) should be investigated.

Large yellow croaker (*Pseudosciaena crocea* R.) is one of the commercially important marine fishes in China, and has been widely cultured in recent years. A few preliminary studies have been conducted on the nutrient requirements and replacement of fishmeal for this fish (Duan et al., 2001; Mai et al., 2006b; Ai et al., 2007; Li et al., 2007). The present study was designed to investigate the effects of dietary SVM on the growth and Cd accumulation in the gill, kidney, liver and muscle of this fish after an 8-week feeding trial with feeds having graded levels of Cd derived from SVM supplementation.

2 Materials and methods

2.1 Experimental diets

Using fish meal, soybean meal, menhaden fish oil and wheat meal as major ingredients, together with other essential trace components, we formulated three practical diets (Diets 1, 2 and 3) to supplement a 0, 50 or 100 $g \cdot kg^{-1}$ SVM diet (Cd level 116.4 $mg \cdot kg^{-1}$ dry weight; Cishan Fisheries, Shandong, China), resulting in final Cd concentrations of 0.21, 7.26 and 12.08 $mg \cdot kg^{-1}$ in the diets, respectively (Table 1) (Mai et al., 2006a). The contents of protein and lipid of these diets were about 44% and 11%, respectively, which are considered to be sufficient to support optimal growth of large yellow croaker (Duan et al., 2001).

Ingredients were ground into fine powder through a 320 μm mesh. All the ingredients were thoroughly mixed with menhaden fish oil, and water was added to produce a stiff dough, which was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China). The pellets were dried for about 12 h in a ventilated oven at 60°C, broken up and sieved into proper pellet sizes (1.5 mm × 2.0 mm and 2.5 mm × 3.0 mm). All diets were sealed in bags and stored at −15°C until use.

2.2 Experimental procedure

The feeding trial was conducted at Xihu bay of Ningbo, Zhejiang Province, China. Large yellow croaker juveniles were obtained from a commercial hatchery. Before the initiation of the experiment, the fish were stocked in a floating sea cage (3.0 m × 3.0 m × 3.0 m) for two weeks. During this period, all fish were fed with diet 1 (Table 1) to acclimate to the experimental diet and conditions.

At the start of the experiment, the fish were fasted for 24 h and weighed after being anesthetized with eugenol (1∶10000; Shanghai Reagent Corp, China). Fish of similar sizes (average weight of 9.75 ± 0.35 g) were distributed into 9 sea cages (1.0 m × 1.0 m × 1.5 m) at a density of 100 fish per cage. Each diet was fed to satiation to triplicate groups of fish twice (05:00 and 17:00) daily for 8 weeks. During the experimental period, the water temperature was maintained at 26.5°C–32.5°C, and salinity at 25.0–28.0 $g \cdot L^{-1}$. Dissolved oxygen was more than 7 $mg \cdot L^{-1}$. The Cd concentration of the seawater was 0.5 ± 0.1 $\mu g \cdot L^{-1}$ as determined by ICP (ICP-OES; Vista-mpx, Varian, USA). The fish were reared under a natural light cycle throughout the experiment.

2.3 Sampling and analysis

At the end of the experiment, the fish were fasted for 24 h. Total number and mean body weight of fish in each cage were determined. Twenty fish from each cage were randomly collected, half for the analysis of whole body

Table 1 Formulation and proximate composition of experimental diets for large yellow croaker

ingredient /g·kg^{-1}	diet (g SVM·kg^{-1} diet)		
	diet 1 (0)	diet 2 (50)	diet 3 (100)
fish meal	500.0	470.0	420.0
soybean meal	90.0	90.0	90.0
beer yeast	30.0	30.0	30.0
wheat flour	258.5	238.5	238.5
fish oil	30.0	30.0	30.0
soybean oil	25.0	25.0	25.0
mould inhibitors	1.0	1.0	1.0
ethoxyquin	0.5	0.5	0.5
lecithin	25.0	25.0	25.0
vitamin premix	20.0	20.0	20.0
mineral premix[e]	20.0	20.0	20.0
squid viscera meal (SVM)	0.0	50.0	100.0
analyzed chemical composition			
crude protein /%	44.4	44.6	44.4
crude lipid /%	11.5	11.5	11.0
cadmium /mg·kg^{-1}	0.21	7.26	12.08

Note: SVM obtained from Cishan Fisheries (Shandong, China), with 34.5% crude protein (dry matter), 17.0% crude lipid (dry matter), and cadmium concentration 116.4 mg·kg^{-1} dry matter; fish meal, obtained from Cishan Fisheries (Shandong, China), with 68.9% crude protein (dry matter), and 10.1% crude lipid (dry matter); and soybean meal, obtained from Liulu Oli Lit (Heilongjiang Province, China), with 46.4% crude protein (dry matter), and 1.9% crude lipid (dry matter); mould inhibitors: 50% calcium propionic acid and 50% fumaric acid; vitamin premix (mg or g·kg^{-1} diet): thiamin 25 mg; riboflavin 45 mg, pyridoxine HCl 20 mg, vitamin B_{12} 0.1 mg, vitamin K_3 10 mg, inositol 800 mg, pantothenic acid 60 mg, niacin acid 200 mg, folic acid 20 mg, biotin 1.20 mg, retinol acetate 32 mg, cholecalciferol 5 mg, DL-α-tocopherol 120 mg, ascorbic acid 2000 mg, choline chloride 2500 mg, ethoxyquin 150 mg and wheat middling 14.0117 g; mineral premix (mg or g·kg^{-1} diet): NaF 2 mg, KI 0.8 mg, $CoCl_2·6H_2O$ (1%) 50 mg, $CuSO_4·5H_2O$ 10 mg, $FeSO_4·H_2O$ 80 mg, $ZnSO_4·H_2O$ 50 mg, $MnSO_4·H_2O$ 60 mg, $MgSO_4·7H_2O$ 1200 mg, $Ca(H_2PO_4)_2·H_2O$ 3000 mg, NaCl 100 mg and Zoelite 15.4472 g.

composition and the rest for the determination of tissue concentration of Cd. The samples of whole fish and diets were dried at 105°C to a constant weight to determine the water content. The dried fish from each replicate were pooled, smashed and mixed completely. Crude protein was determined by the Kjeldahl method, crude lipid by the Soxhlet's method, and ash by combustion at 550°C in a muffle furnace (AOAC, 1995). The fish tissues (muscle, gill, liver and kidney) were separated carefully. To prevent contamination between samples, the tray and tools were washed in 0.1 mol·L^{-1} nitric acid and subsequently rinsed with distilled water before dissecting each part and every fish. The pooled samples from each replicate were freeze-dried in a freeze dryer (ALPHA 1-2, Martin Christ, Germany) for 24 h. The Cd concentrations in the samples of fish tissues and diets were measured by an inductively coupled plasma-atomic emission spectrophotometer (ICP-OES; Vista-mpx, Varian, USA) at 228.8 nm after digestion with perchloric acid.

2.4 Calculation and statistical analysis

The specific growth rate (SGR) of the fish was determined with the following equation:

$$SGR = [(\ln W_t - \ln W_o)/t] \times 100,$$

where W_t and W_o are the final and initial mean body weights, respectively, and t is the duration of the experiment in days.

Data from each treatment were subjected to one-way ANOVA with SPSS 10.0 for Windows. When overall differences were significant ($P < 0.05$), Tukey's test was used to compare the means between individual treatments (Zar, 1984).

3 Results

3.1 Survival and growth

The survival rates, ranging from 89.3% to 91.7%, were generally high and independent of the dietary treatments (Table 2). The specific growth rate (SGR) of the fish was significantly affected by SVM supplementation. Fish fed diets with 50 and 100 g·kg^{-1} SVM diet produced significantly higher SGR (1.7% and 1.8%·day^{-1}, respectively) compared with the fish fed the control diet (1.5%·day^{-1}) ($P < 0.05$), but no significant difference was found between the two SVM levels (Table 2).

3.2 Body composition

The dietary SVM supplementation did not significantly influence the approximate composition of the fish body, water content (70.3%–71.3%), protein (16.6%–17.3%), lipid (8.6%–8.0%) and ash (3.5%–4.1%) (Table 3).

3.3 Cd accumulation in fish tissues

The SVM supplementation significantly affected Cd distribution in the kidney, liver and gill of the fish ($P < 0.05$), but Cd was undetectable in fish muscle regardless of dietary treatment (Table 4). Cd concentrations in all these three tissues significantly increased with increasing Cd levels in the diets. When dietary Cd increased from 0.26 to 7.26 and 12.08 $mg \cdot kg^{-1}$, the corresponding Cd levels in different tissues were as follows: kidney, 0.42, 2.65 and 4.44 $mg \cdot kg^{-1}$; liver, 0.26, 0.58 and 0.94 $mg \cdot kg^{-1}$; and gills, 0.12, 0.35 and 0.53 $mg \cdot kg^{-1}$. Fish kidney always had the highest Cd level, followed by liver and gill no matter what diet the fish was fed with.

4 Discussion

As in the case of Japanese seabass (Mai et al., 2006a), we also found a positive relationship between the growth performance of large yellow croaker and supplementation with squid viscera meal (SVM), suggesting that SVM is an effective attractant for fish feeding, and consequently enhances fish growth. This is the reason why SVM is widely used in aquaculture feeds.

In the present study, the Cd levels in the selected tissues (kidney, liver, gill and muscle) of large yellow croaker

Table 2 Survival and growth of large yellow croaker, fed diets with different Cd levels from squid viscera meal for 8 weeks

diet ($g \cdot kg^{-1}$ SVM)	dietary Cd level /$mg \cdot kg^{-1}$	survival /%	final body weight /g	SGR /$\% \cdot day^{-1}$
diet 1 (0)	0.21	89.3 ± 0.7a	22.75	1.5 ± 0.1b
diet 2 (50)	7.26	91.7 ± 0.3a	25.43	1.7 ± 0.0a
diet 3 (100)	12.08	91.0 ± 0.6a	26.29	1.8 ± 0.0a
one-way ANOVA				
F-value		4.97		15.93
P-value		0.05		0.00

Note: The values in this table are means ± SD of three replicates sea cages. The initial mean body weight of the experimental fish is (9.75 ± 0.35) g. Means in the same column sharing a same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$).

Table 3 Body composition of *Pseudosciaena crocea* R., fed diets with different Cd levels from squid viscera meal for 8 weeks

diet ($g \cdot kg^{-1}$ SVM)	dietary Cd level/$mg \cdot kg^{-1}$	water content /%	protein /% w.w.	lipid /% w.w.	ash /% w.w.
diet 1/ (0)	0.21	70.3 ± 0.9	16.6 ± 0.5	8.6 ± 0.3	4.1 ± 0.2
diet 2/ (50)	7.26	71.3 ± 0.5	16.9 ± 0.3	8.3 ± 0.4	3.5 ± 0.2
diet 3/ (100)	12.08	71.0 ± 0.2	17.3 ± 0.2	8.0 ± 0.3	3.6 ± 0.2
one-way ANOVA					
F- value		0.74	1.18	0.85	2.89
P- value		0.52	0.37	0.47	0.13

Note: The values in this table are means ± SD of three replicates. Means in the same column sharing a same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$); w.w. represents wet weight.

Table 4 Cadmium accumulations in the tissues of *Pseudosciaena crocea* R., fed diets with different Cd levels from squid viscera meal for 8 weeks (dry matter basis)

diet ($g \cdot kg^{-1}$ SVM)	dietary Cd level /$mg \cdot kg^{-1}$	cadmium concentrations /$mg \cdot kg^{-1}$			
		gill	liver	kidney	muscle
diet 1 (0)	0.21	0.12 ± 0.00c	0.26 ± 0.01c	0.42 ± 0.01c	ND
diet 2 (50)	7.26	0.35 ± 0.01b	0.58 ± 0.01b	2.65 ± 0.01b	ND
diet 3 (100)	12.08	0.53 ± 0.01a	0.94 ± 0.01a	4.44 ± 0.01a	ND
one-way ANOVA					
F- value		1572.00	4484.00	85072.00	
P- value		0	0	0	

Note: The values in this table are means ± SD of three replicates. Means in the same column sharing a same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$); ND represents not detectable.

were slightly lower than those in the counterpart tissues in Japanese seabass (Mai et al., 2006a). In both fish species, however, the highest Cd levels were always recorded in the kidney, followed by the liver and the gill, and the Cd in fish muscle was always undetectable. Furthermore, the Cd contents in the kidney, liver and gill were significantly correlated with the dietary Cd levels. The fact that Cd levels varied with fish tissues can probably be explained by three factors as suggested by Mai et al. (2006a). Firstly, the elimination of Cd in different organs occurs at different rates, and the loss of accumulated Cd is rapid and immediate in muscle while it is slow in the kidney and liver (de Conto Cinier et al., 1999). Secondly, Cd may be transferred from muscle to the liver and kidney for excretion. Thirdly, the metal may be bound weakly to ligands in muscle, but strongly to ligands in the kidney and liver (Kuroshima, 1987). From this point of view, the different parts of the fish should be used separately to avoid subsequent Cd toxicity. Generally, only muscular tissues of fish are consumed as food for human, whereas kidney, liver and gills are discarded. Therefore, the risk is limited for humans eating fish muscle fed with SVM supplementation diets. However, the feeding trial of 56 d is much shorter than the time needed for growth to a marketable size of this fish, which usually takes 1 to 2 years. Therefore, a long-term feeding trial is necessary to further investigate Cd accumulation and its toxicity to large yellow croaker in aquaculture for fish health and human food safety.

Compared with Japanese seabass (Mai et al., 2006a), large yellow croaker had a slower growth rate when both fish species with almost the same initial weight (10.89 ± 0.21 g and 9.75 ± 0.35 g, respectively) were cultured under the same conditions. As mentioned above, the Cd levels in the tissues of large yellow croaker were slightly lower than those in the corresponding tissues of Japanese seabass. It may be inferred that the growth rate would affect the Cd accumulation of fish. However, the feeding trials only lasted for 56 d. Whether the above conclusion is suitable for all fish species or not, more precise long-term feeding trials and comparative studies should be further conducted.

The present study showed that 50–100 $g \cdot kg^{-1}$ SVM supplemented in fish feeds elevated the dietary Cd levels up to 7.26–12.08 $mg \cdot kg^{-1}$, which are much higher than the maximum concentration of Cd (0.5 $mg \cdot kg^{-1}$ dry weight) allowed in fish feeds by the EU. It was found that these feeds resulted in high levels of Cd (0.35–4.44 $mg \cdot kg^{-1}$ dry weight) in the kidney, liver and gills of large yellow croaker. It is worth noticing that the control feed (Cd 0.21 $mg \cdot kg^{-1}$) without SVM supplementation produced Cd levels as high as 0.12–0.42 $mg \cdot kg^{-1}$ dry weight in these fish organs (Table 4), suggesting that fish organs are able to accumulate Cd from diets and probably water. A serious issue can be raised that the by-product (viscera) of the fish, which have high concentrations of Cd, may become contaminants to the environment. In addition, in some countries more and more fishmeal is made from the by-product of fish processing. Therefore, the contamination could then be directly introduced to other aquatic species from the raw materials. In China, the feeds for prawn also use SVM as an attractant. This can also be a food safety risk. Therefore, action must be taken to evaluate the risk of SVM and a new attractant must be exploited to replace the SVM which has potential hazards.

Acknowledgements This study was supported by the National Key Technologies R & D Program for the 10th and 11th Five-year Plan of China (Nos. 2001BA505B-06; 2006BAD03B03), and PCSIRT. We thank Wang X. and Liu X. for their help in diet preparation. Thanks are also due to Wan M., Wang Z., Chen J., Shentu J., Tan F., Li J. and Zhang H. for their assistances in the study.

References

Ai Q, Mai K, Zhang L, Tan B, Zhang W, Xu W, Li H (2007). Effects of dietary β-1, 3 glucan on innate immune response of large yellow croaker, *Pseudosciaena crocea*. Fish & Shellfish Immun, 22: 394–402

Al-Yousuf M H, El-Shahawi M S, Al-Ghais S M (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. Sci Total Environ, 256: 87–94

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis of the Association of Official Analytical Chemists. 16th ed. Arlington, VA, USA: Association of Official Analytical Chemists

Berntssen M H, Lundebye A K (2001). Energetics in Atlantic salmon (*Salmon salar* L.) parr fed elevated dietary cadmium. Comp Biochem Physiol C Toxicol Pharmacol, 128(3): 311–323

Beyersmann D, Hechtenberg S (1997). Cadmium, gene regulation, and cellular signalling in mammalian cells. Toxicol Appl Pharm, 144: 247–261

Bustamante P, Cosson R P, Gallien I, Caurant F, Miramand P (2002). Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. Mar Environ Res, 53: 227–241

Calevro F, Beyersmann D, Hartwig A (1998). Effect of cadmium (II) on the extent of oxidative DNA damage in primary brain cell cultures from *Pleurodeles* larvae. Toxicol Lett, 94: 217–225

Caurant F, Bustamante P, Bordes M, Miramand P (1999). Bioaccumulation of cadmium, copper and zinc in some tissues of three species of marine turtles stranded along the French Atlantic coasts. Mar Pollut Bull, 36: 1085–1091

Dallinger R, Prosi F, Segner H, Back H (1987). Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. Oecologia, 73: 91–98

Dang Z C, Berntssen M H G, Lundebye A K, Flik G, Wendelaar Bonga S E, Lock R A C (2001). Metallothionein and cortisol receptor expression in gills of Atlantic salmon, *Salmo salar*, exposed to dietary cadmium. Aquat Toxicol, 53: 91–101

de Conto Cinier C, Petit-Ramel M, Faure R, Garin D, Bouvet Y (1999). Kinetics of cadmium accumulation and elimination in carp *Cyprinus*

carpio tissues. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol, 122(3): 345–352

Degraeve N (1981). Carcinogenic, teratogenic and mutagenic effects of cadmium. Mutat Res, 86: 115–135

Duan Q, Mai K, Zhong H, Si L, Wang X (2001). Studies on the nutrition of the large yellow croaker, *Pseudosciaena crocea* R. I: growth response to graded levels of dietary protein and lipid. Aquac Res, 32 (Suppl 1): 46–52

Food and Veterinary Office (FVO) (1998). Report on a Routine Mission to the Falkland Islands. Dublin, Ireland: Food and Veterinary Office, 6

Food and Veterinary Office (FVO) (2002). Inspection mission to the Falklands from 20 to 26 January 2003 in order to review the animal health situation and the office controls in place over the production of red meat intended for export to the European Union. Food and Veterinary Office, Dublin, Ireland. DG (SANCO)/8525/2002

Friberg L, Nordberg G F, Vouk V B (1986). Handbook of the Toxicology of Metals, Vol. II. Amsterdam: Elsevier, 130–184

Gerhard I, Monga B, Waldbrenner A, Runnenbaum B (1998). Heavy metals and fertility. J Toxicol Environ Health, 54: 593–611

Goering P L, Waalkes M P, Klaassen C D (1995) Toxicology of cadmium. In: Goyer R.A, Cherian M G, eds. Handbook of Experimental Pharmacology, Vol. 115. Toxicology of Metals: Biochemical Aspects. Berlin: Springer-Verlag, 189–214

Hardy R D (1996). Dietary exposure to toxic metals in fish. In: Taylor E W, ed. Toxicology of Aquatic Pollution: Physiological, Cellular and Molecular Approaches. Cambridge: Cambridge University Press, 29–59

Heath A G (1995). Water pollution and fish physiology. In: Heath A C, ed. Uptake, Accumulation, Biotransformation, and Excretion of Xenobiotics. New York: Lewis CRC, 96–97

Hollis L, McGeer J C, McDonald D G, Wood C M (1999). Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long term sublethal Cd exposure in rainbow trout. Aquat Toxicol, 46: 101–119

Krajnc E I, Van Gestel C A M, Mulder H C M (1987). Integrated criteria document. Cadmium–Effects. Appendix. National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands (Report no. 758476004)

Kuroshima R (1987). Cadmium accumulation and its effect on calcium metabolism in the girella *Girella punctata* suring a long-term exposure. Bull Jap Soc Sci Fish, 53: 445–450

Li H, Mai K, Ai Q, Zhang L, Zhang C, Zhang W, Liufu Z (2007). Apparent digestibility of selected protein ingredients for larger yellow croaker *Pseudosciaena crocea*. Acta Hydrobiologica Sinica, 31(3): 80–86 (in Chinese)

Liang M, Yu H, Chang Q, Chen C, Sun S (2000). Feeding attraction activities of food attractants for 3 species of fishes. J Fish Sci China, 7: 60–63 (in Chinese)

Mai K, Li H, Ai Q, Duan Q, Xu W, Zhang C, Zhang L, Tan B, Liufu Z (2006a). Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of Japanese seabass, *Lateolabrax japonicus* (Cuvier 1828). Aquac Res, 37: 1063–1069

Mai K, Zhang C, Ai Q, Duan Q, Xu W, Zhang L, Liufu Z, Tan B (2006b). Dietary phosphorus requirement of large yellow croaker, *Pseudosciaena crocea* R. Aquaculture, 251: 346–353

Mallatt J (1985). Fish gill structure changes induced by toxicants and other irritants: a statistical review. Can J Fish Aquat Sci, 52: 2016–2030

McDonald D G, Wood C M (1993). Branchial mechanisms of acclimation to metals in freshwater fish. In: Rankin J C, Jensen F B, eds. Fish Ecophysiology. London: Chapman and Hall, 297–332

Romeo M, Siau Y, Sidoumou Z, Gnassia-Barelli M (1999). Heavy metal distribution in different fish species from the Mauritania Coast. Sci Total Environ, 232: 169–175

Waalkes M P, Misra R R (1996). Cadmium carcinogenicity and genotoxicity. In: Chang L W, ed. Toxicology of Metals. Boca Raton, FL, USA: CRC Press, 231–244

Waalkes M P (1995). In: Berthan G, ed. Handbook on Metal-Ligand Interactions of Biological Fluids 2. New York: Marcel Dekker, 471–482

Waalkes M P (2000). Cadmium carcinogenesis in review. J Inorg Biochem, 79: 241–244

Wendelaar Bonga S E (1997). The stress response in fish. Physiol Rev, 66: 591–625

World Health Organization (WHO) (1989). Toxicological Evaluation of Certain Food Additives and Contaminants. Cambridge: Cambridge University Press, 163–219

World Health Organization (WHO) (1992). Cadmium Environmental Health Criteria No. 134. Geneva: World Health Organization

Zar J H (1984). Production. In: Zar J H, ed, Biostatistical Analysis, Prentice-Hall. NJ: Englewood Cliffs, 293–305