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Dietary bioavailability of cadmium, inorganic mercury, and zinc to a marine fish: Effects of food composition and type

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ABSTRACT

Elevated metal burdens in farmed fish can pose potential risks to public health as a result of fish consumption. It has been recognized that dietary exposure is a dominant route for metal accumulation in fish. In this study, we first assessed the metal contamination in commercial fish diet and its raw materials. Squid viscera meal, kelp meal and rapeseed meal had metal-specific contamination and contributed to high metal levels in commercial fish diet. We then quantified the metal assimilation efficiency (AE) in a juvenile marine fish blackhead seabream *Acanthopagrus schlegelii* using a radiotracer technique to assess the dietary bioavailability of cadmium (Cd), inorganic mercury (Hg(II)), and zinc (Zn). Different sources of protein in artificial diets did not significantly affect the metal AEs, except for Hg(II) from corn meal and Zn from corn meal and rapeseed meal. Generally, metal AEs tended to decrease with increasing dietary metal concentrations. Compared to meso-2,3-dimercaptosuccinic acid (DMSA) and L-cysteine in commercial fish diet, metal additives (i.e., 3000 μg Fe/g or 200 μg Cu/g) were more influential on metal AEs, probably due to metal competition. The relative bioavailability of metals in natural prey vs. artificial diet was inconsistent and complicated by metal chemical species and gut passage time. Our results indicated that metal contamination in commercial fish diet was probably underestimated in fish aquaculture.

1. Introduction

Marine fish is an important source of high-quality animal protein and long-chain polyunsaturated fatty acids, but is also a potential source of toxic metals to higher trophic levels due to fish consumption. One classical example is the Minamata disease in Japan in the 1950s caused by methylmercury (MeHg) poisoning as a result of marine fish consumption. Methylmercury is a neurotoxin and can cause myocardial infarction and coronary heart diseases to human. Recently, the safety of farmed fish has received wide attention, considering that aquaculture production nowadays accounts for 80% of consumed fish in China, which is the world's largest fish producer (FAO, 2010). In particular, dietary exposure is recognized as a predominant route for metal bioaccumulation in fish (Wang, 2002; Wang and Rainbow, 2008). Therefore, farmed fish suffer a potential risk of dietary metal exposure and consequently poses a hazard to the consumers.

In normal fish farming practices, the caged fish are fed with different types of food. One type is the natural prey including forage fish or trash fish, fish viscera, and squid viscera as the by-products of seafood processing. Another type is the dried artificial pellets produced by different animal feed factories. Traditional components of dried pellet feeds include the fish meal, squid viscera meal, fish oil, bean meal, corn gluten, wheat, rapeseed, and other supplementary ingredients such as metal additives, vitamins and amino acids (Kader et al., 2010; Olsen, 2011). The feed composition varies greatly among producers, depending on their price, availability and target animals. In the future, plant raw materials may become more important for artificial fish diet preparation than the fish meal (Olsen, 2011). Among the different natural preys, the forage fish and fish meal are rich in organic mercury residues, and squid viscera meal (SVM, used as additives to attract fish and stimulate growth) is a potentially high cadmium source (Dang and Wang, 2009; Mai et al., 2006). For example, as high as 51.2 μg Cd/g (Dang and Wang, 2009) and 35–253 ng Hg/g (Choi and Cech, 1998) in commercial fish diet have been documented, as opposed to 0.5 μg Cd/g and 100 ng Hg/g in the European Union fish feed regulation. Metal contamination in commercial fish feeds is thus mainly attributed to the high metal levels in the raw materials. On the other hand, metal contamination in natural prey has been well reported. A better understanding of factors influencing dietary metal bioavailability in farmed fish is thus requisite to ensure the safety of farmed fish.

Dietary assimilation efficiency (AE) is an effective measure of dietary metal bioavailability. The AE quantifies the efficiency at which an ingested dietary metal is assimilated by the fish following digestion and absorption within the digestive tract. Over the past decade, this parameter has been quantified in marine fish fed a variety of natural preys, including macroalgae (Chan et al., 2003), zooplankton (Ni et al., 2000), and bivalves (Dang and Wang, 2010). However, there

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is very little information on the bioavailability of metals from artificial diets. Given the potentials of metal contamination in fish diets, it is important to quantify the bioavailability of metals from fish diets to farmed fish. Furthermore, since artificial diets are generally mixed from different raw materials at different proportions, it is also important to examine the variability of metal assimilation as influenced by different diet compositions. In addition, assessment of the relative metal bioavailability from natural prey vs. commercial fish diet may provide information for management practices to alleviate the metal contamination in fish farming.

In the present study, we therefore quantified the assimilation efficiency of Cd, Hg(II), and Zn by juvenile blackhead seabream *Acanthopagrus schlegelii*, using a radiotracer methodology. We focused on two nonessential metals Cd, Hg(II), and one essential metal Zn in this study, primarily because these metals are of particular concerns in fish diets, and the availability of their respective radiotracers. For Hg, MeHg is the most common form of mercury found in fish, and its bioavailability is much higher than the inorganic Hg(II) form. Our recent measurements in the artificial feeds (pellets) produced in China found that inorganic Hg was probably the dominant form of Hg, e.g., the percentage of MeHg in the artificial feed was only 18–22% of the total Hg contents (Onsanit et al., 2012). In the present study, we therefore focused on Hg(II) instead of MeHg. For artificial fish diets, we quantified the influences of raw material component, diet proportion (animal protein versus plant protein) and metal concentration on metal assimilation. The influences of metal additives (Fe, Cu and Cd) and chelating agents (meso-2, 3-dimercaptosuccinic acid and L-cysteine) on metal assimilation were also examined. We further compared the metal assimilation from natural prey (fish flesh, squid viscera, and mussel tissues) and artificial diet.

2. Materials and methods

2.1. Fish and metals

Juvenile blackhead seabream *A. schlegelii* (3–4 cm length, approximately 0.8–2.0 g wet weight) was obtained from a fish farm in Sai Kung, Hong Kong. Black seabream is a domesticated marine carnivorous fish, and wildly distributed in southern China, especially in Hong Kong, Guangdong, and Fujian provinces. Fish were maintained in sand-filtered seawater (~30 psu) with aeration and fed with commercial fish diet (referred as control feed hereinafter, purchased from a company in Xiamen, China) twice a day at a daily ration of 5% body weight for 5 days before any experiments. After acclimation, fish were randomly separated and maintained in glass tanks containing 30 L of sand-filtered seawater with aeration.

Stable metals used in this study were the standard solutions from Perkin Elmer (as $CuCl_2$, $ZnCl_2$ and $CdCl_2$, 1000 μg/L, in 2% HNO_3). The radioisotopes ^{203}Hg(II) (as $HgCl_2$), ^{109}Cd (as $CdCl_2$) and ^{65}Zn (as $ZnCl_2$) were used as radiotracers to quantify the metal assimilation efficiency (see below). These isotopes were purchased from Eckert and Ziegler, California, USA. Compared to the background metal levels in fish diet, spiked radioactive metal concentrations were negligible in this study.

2.2. Artificial diets

Based on the information provided by the fish feed company, feed formula was prepared with raw material powders including fish meal (30%), soybean meal (20%), squid viscera meal (4.8%), corn protein meal (4.8%), rapeseed meal (4.8%), kelp powder (4.8%), starch (24%), yeast bear powder (4.0%), bean oil (0.8%), and $CaHPO_4$ powder (2.0%). This fish formula served as the control for all the assimilation experiments. In the first experiment (Expt. I), artificial diets with various protein sources were prepared, including 69% of any protein source (i.e., fish meal, SVM, soybean meal, corn meal, kelp meal, or rapeseed meal), with binder (24% starch + 4% yeast beer powder), 1% of bean oil and 2% of $CaHPO_4$. The ingredients were mixed with deionized water at a ratio of 3:2 (w/v), loaded in a pellet machine and cut into 2 mm small grains. Finally, the grains were dried at room temperature for 2 days. These pellets were stored at −20 °C until further usage. The prepared pellets were fed to the fish during the acclimation period. Before the experiment, the radioisotopes ^{203}Hg(II), ^{109}Cd and ^{65}Zn were mixed with solutions of $HgCl_2$, $CdCl_2$ and $ZnCl_2$ to result in a concentration of 6.37 μg/mL for Hg(II), 4.98 μg/mL for Cd and 29.5 μg/mL for Zn. The metal and radioisotope mixture was then spiked to the diets and carefully mixed at feed ingredient: solution ratio of 3:2 (w/v). They were then placed in a 5 mL plastic syringe and compressed into a line of diet. Finally they were dried at room temperature for 1 day and then cut into 2 mm small pellets prior to use. Metal radioactivity in spiked diet was individually monitored, and the calculated total metal concentrations in the spiked diet were 4.78 μg/g for Hg(II), 0.56 μg/g for Cd and 1.53 μg/g for Zn, respectively.

The second experiment examined the influences of animal versus plant protein source on metal bioavailability from artificial diet (Expt. II). Fish meal and bean meal are the two major components widely used as main ingredients of commercial diets. Three artificial diet formula, with 80% fish meal + 10% bean meal + 10% starch (Formula I), 45% fish meal + 45% bean meal + 10% starch (Formula II), or 10% fish meal + 80% bean meal + 10% starch (Formula III), were prepared. The formulated diet was then spiked with negligible amount of radioactive Hg(II), Cd and Zn simultaneously, as described previously, and stored at −40 °C until AE quantification.

In the third experiment (Expt. III), the control fish diets purchased directly from the company were incubated with different concentrations of stable metals and radioisotopes. The final metal concentrations in these diets were 0.5, 1, 5, 15 and 50 μg/g for Hg(II), 0.6, 3, 15, 45 and 100 μg/g for Cd, 57, 100, 200, 400 and 800 μg/g for Zn. These diets with different metal concentrations were then fed to the fish and the metal AEs were quantified.

The fourth experiment (Expt. IV) examined the influences of L-cysteine ($C_3H_7NO_2S$) or DMSA (meso-2,3-dimercaptosuccinic acid, $C_4H_6O_4S_2$) as a chelating mixture in diet on metal assimilation. The 1 mg/mL stock solution of L-cysteine or DMSA was freshly prepared to avoid oxidation. These chemicals were then added together with radioisotopes and mixed with the control commercial diet. The nominal concentration was 20 μg/g for L-cysteine and 20 μg/g for DMSA in the 5 g diets powder. The spiked chelating agents were sufficient to complex metals in the diet. The metal assimilation was then quantified.

The fifth experiment (Expt. V) examined the influence of amendment of Fe, Cu and Cd on the assimilation of Cd, Hg(II) and Zn. The control artificial diets were supplemented with 3000 μg/g Fe, 200 μg/g Cu, or 100 μg/g Cd, respectively, and mixed well with radioisotopes to quantify the metal AE.

2.3. Live prey

The natural prey considered in this study included the mussel *Perna viridis*, squid *Loligo* sp. and planktivorous fish *Mugil cephalus*, which are widely distributed in the Indo-Pacific region, especially in southern China. Live mussels, squid and juvenile mullet were all collected from Sai Kung, Hong Kong, and maintained in flow-through filtered seawater. Diatoms *Thalassiosira pseudonana*, which was maintained in the f/2 medium (f/10 levels for trace metals but without EDTA), was exposed to 5.28 μg ^{203}Hg(II)/L, 3.45 μg ^{109}Cd/L and 10.4 μg ^{65}Zn/L for 2 days. After 2 days of radiolabeling, 63% of Hg, 65% of Cd and 61% of Zn were radiolabeled onto the diatom cells. Afterwards, the algal cells were filtered onto 0.22 μm membranes, rinsed with seawater, and fed to the green mussels for 2 days.