Ecotoxicology and Environmental Safety 137 (2017) 272–280

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Dietary taurine supplementation ameliorates the lethal effect of phenanthrene but not the bioaccumulation in a marine teleost, red sea bream, *Pagrus major*

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ARTICLE INFO

Keywords:
Taurine
Amelioration
Phenanthrene
Hepatic metabolomics
Lethal toxicity
Bioaccumulation

ABSTRACT

The present study was performed to evaluate the effect of dietary taurine on the hepatic metabolic profiles of red sea bream (*Pagrus major*) and on phenanthrene (a polyaromatic hydrocarbon) toxicity and bioaccumulation. The fish were fed a diet supplemented with 0% (TAU0%), 0.5% (TAU0.5%), or 5% (TAU5%) taurine for 40–55 d and subjected to phenanthrene acute toxicity and bioaccumulation tests. Taurine deficiency in feed severely affected the hepatic metabolic profiles of fish, which indicated a complementary physiological response to taurine deficiency. For the acute toxicity test, fish were fed the test diets for 55 d and were then exposed to 0–893 μg/L phenanthrene for 96 h. Tolerance to phenanthrene was significantly improved by 0.5% of taurine inclusion in feed relative to TAU0%, but not by 5.0% inclusion. Reduced glutathione in the liver, which acts as an oxygen-free radical scavenger, was associated with a reduction in the toxicity of phenanthrene. For the bioaccumulation test, fish were fed the test diets for 40 d and were thereafter chronically exposed to 20 μg/L phenanthrene for 13 d followed by depuration for 3 d. The activity of hepatic biomarker, ethoxyresorufin-O-deethylase, was increased by phenanthrene exposure in the taurine inclusion groups. However, phenanthrene concentrations in the liver and muscle of fish fed TAU5.0% tended to be higher than those of fish fed TAU0% and TAU0.5% during the exposure period. These results indicate that 0.5% of taurine inclusion in feed plays an important role in the alleviation of phenanthrene toxicity but not bioaccumulation. Furthermore, larger amount of taurine inclusion (TAU5%) did not show marked beneficial effects against phenanthrene exposure. This study provides insight about a major concern of environmental contaminants into aquatic environment and can be effectively used for improvement of aquaculture.

1. Introduction

Release of ubiquitous environmental contaminants into aquatic environment has been a cause of general concern for decades and has led a number of researchers to examine the effects of such contaminants on aquatic organisms. Polycyclic aromatic hydrocarbons (PAHs), mainly produced due to incomplete combustion of fossil fuels, have attracted much attention in this regard. The three benzene-ring containing compound, phenanthrene (phe), is one of the most abundant PAH. Several studies have demonstrated toxic biological effects of phe on aquatic organisms such as diatoms, gastropods, crustaceans, and fish (U.S. Environmental Protection Agency, 2006). Furthermore, higher bioaccumulation of phe compared to other PAHs, such as pyrene, chrysene, and benzo[a]pyrene was observed in the red sea bream, *Pagrus major*, a commercial fish species (Cheikyula et al., 2008). Generally, phe occurs in low concentrations (dozens of nanogram per liter) in the marine environment (Latimer and Zheng, 2003; Zhang et al., 2014). However, exceptionally high levels of phe ranging from 14.6 μg/L (Vrana et al., 2001) to 1460 μg/L (Anyakora et al., 2005) have been reported. These values are high enough to impair the growth and physiological functions of fish (Payne et al., 2003). Considering the profound impact of such concentrations on the environment, phe is included in the Environmental Protection Agency's priority pollutant list (Smith et al., 1989).

Taurine (2-amino ethanesulfonic acid), a derivative of a sulfur-containing amino acid, is a conditionally essential nutrient, that

Abbreviations: GC-MS, gas chromatography - mass spectrometry; Glm, Generalized linear model; GSH, Glutathione

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http://dx.doi.org/10.1016/j.ecoenv.2016.12.005
Received 22 August 2016; Received in revised form 17 November 2016; Accepted 3 December 2016
Available online 19 December 2016
0147-6513/

performs multifaceted roles in fish physiology. These roles include growth promotion (Lim et al., 2013; Matsunari et al., 2008a), anti-oxidative action (Han et al., 2014), and neuroprotection (Lima et al., 2001). This nutrient is mainly synthesized in liver (Yokoyama et al., 2001). However, marine fish species do not biosynthesize sufficient amounts of taurine because the enzymes involved in its production are either present at low levels or are missing. Therefore, in aquaculture, marine fish require taurine supplementation in their diet (Matsunari et al., 2008a) In the wild, these fishes are believed to acquire the nutrient by feeding on prey animals, such as mysids (*Archaeomysis vulgaris*), which contain large amounts of taurine (Park et al., 2000).

Taurine is a promising candidate for improving liver function (Takeuchi, 2014). We have observed that it plays an important role in the alleviation of cadmium toxicity and bioaccumulation (Hano et al., 2016). However, little is known about the effects of taurine administration on the toxicity and bioaccumulation of organic xenobiotics, such as phe, in marine fish. In order to understand these effects, it is best to monitor the liver, which detoxifies and accumulates PAHs (Cheikyula et al., 2008), and is the organ most impacted by taurine deficiency (Salze and Davis, 2015).

Red sea bream, *Pagrus major*, are among the important commercial fish species in Japan, and are extensively studied marine fish species (Matsunari et al., 2008a, 2008b; Cheikyula et al., 2008). Recently, metabolomics has been recognized as an important "omics" science in systems biology because it can explain the snapshots of health condition of the organism by comprehensively evaluating the changes of low-molecular weight endogenous metabolites (Patti et al., 2012). It has also great potential to assess toxicity of xenobiotics, including PAHs (Williams et al., 2009) and heavy oil (Kokushi et al., 2012) and to identify even the slight effects, which is expected to be undetectable responses in traditional toxicity tests based on mortality and inferior growth. Therefore, we employed gas chromatography mass spectrometry (GC-MS) based metabolomics techniques to globally characterize the alterations of metabolites in the liver caused by taurine deficiency and/or phe exposure.

The present study was performed to unravel the potential beneficial effect of dietary taurine against phe, as the environmental pollutant. To achieve this goal, marine teleost fish, *P major*, were provided with the graded concentrations of taurine supplementation in feed, the fish were then subjected to phe exposure tests: acute toxicity or bioaccumulation tests. In addition to the hepatic metabolomics, we also measured the activity of ethoxyresorufin-*O*-deethylase (EROD), which is a measurement of the inducibility of CYP1A enzymes responsible for catalyzing phe biotransformation (Costa et al., 2011) and investigated its possible connections between taurine supplementation and phe exposure.

2. Materials and methods

2.1. Diet and fish

Artificial diets containing 0%, 0.5%, and 5.0% taurine were prepared in the laboratory, according to the procedure described by Hano et al. (2016). The composition of the diets and analytical contents including taurine, protein and lipids are shown in Table S1. These concentrations were selected because a dietary taurine concentration of 0.5% is reported to be optimal for maximum growth in red sea bream (Matsunari et al., 2008b) and approximately 5.0% taurine is reported in mysids, an important food for marine fish in the wild (Park et al., 2000).

Red sea bream juveniles with an approximate total length (TL) of 30 mm and body weight (BW) of 0.5 g were purchased from A-marine Kindai Co. (Wakayama, Japan) and kept at 20 °C under a 14-h light and 10-h dark cycle during the acclimation and experimental periods. Fish were maintained in a flow-through system containing seawater that had been filtered through sand and activated carbon. The fish were acclimated in 60-L glass tanks (600 × 300 × 360 mm; 150 individuals per tank) and fed a commercial diet (Otohime EP-0, Marubeni Nisshin Feed, Tokyo, Japan) daily. After acclimation for 60 d, all fish were fed the TAU0% diet for 10 d. Thereafter, red sea bream, which had a BW of 2.3 ± 0.4 g (mean ± standard deviation) and TL of 54 ± 3.5 mm, were divided into six tanks, each tank consisting of 100 individuals, and used for feeding trial (Fig. 1). Each group was housed in one of the tanks used for acclimation; two tanks were assigned to each of the three experimental-diet groups; TAU0%, TAU0.5%, and TAU5.0%. Fish were reared not to exceed a density of 10 kg/m^3, which is recommended by the Japanese Ministry of Agriculture, Forestry and Fisheries (1999) for fish aquaculture.

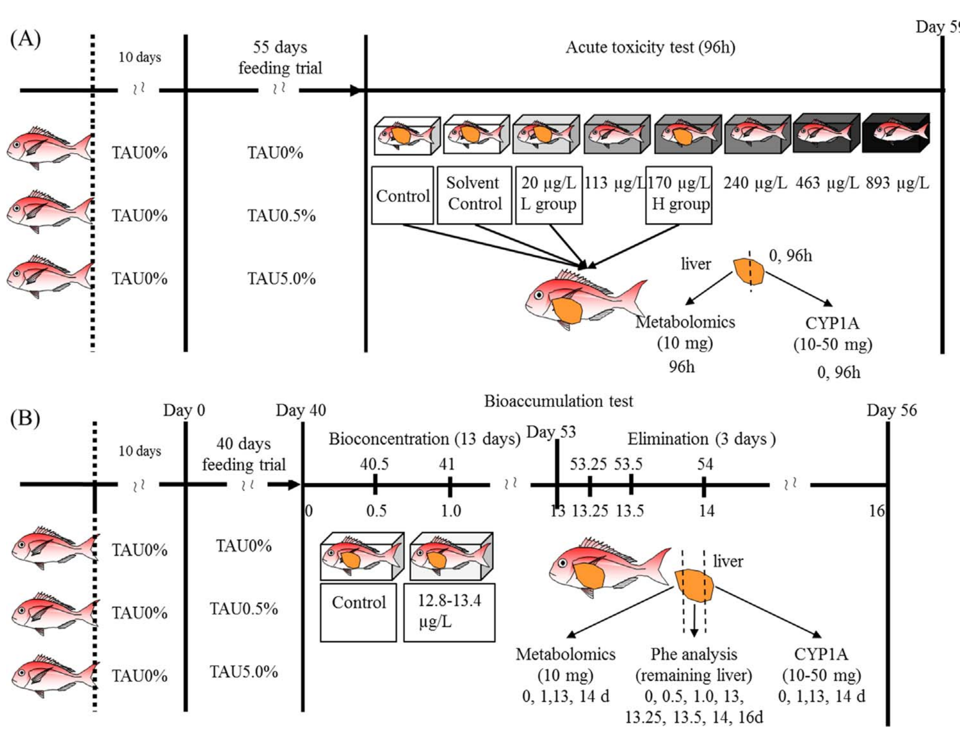

Fig. 1. Experimental scheme for (A) Acute toxicity and (B) Bioaccumulation test.