Ecotoxicology and Environmental Safety 76 (2012) 126–134

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

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

Protective effects of calcium on copper toxicity in *Pelteobagrus fulvidraco*: Copper accumulation, enzymatic activities, histology

Q.-L. Chen [a], Z. Luo [a,*], J.-L. Zheng [a], X.-D. Li [b], C.-X. Liu [a], Y.-H. Zhao [b], Y. Gong [a]

[a] Fishery College, Huazhong Agricultural University, Wuhan 430070, China
[b] Postgraduate Research Base, Panjin Guanghe Fishery Co. Ltd., Panjin 124200, China

ARTICLE INFO

Article history:
Received 30 July 2011
Received in revised form
3 October 2011
Accepted 6 October 2011
Available online 21 October 2011

Keywords:
Pelteobagrus fulvidraco
Copper toxicity
Calcium
Hepatic metabolism
Histology
Copper accumulation

ABSTRACT

The present study was conducted to determine interactive effects of waterborne co-exposure of copper (Cu) and calcium (Ca) on Cu accumulation, enzymatic activities and histology in yellow catfish *Pelteobagrus fulvidraco* and test the prediction that Ca could protect against Cu—induced toxicity in the fish species. Yellow catfish were exposed to 0, 1.0, 2.0 mg Cu/l, in combination with 0 and 50 mg Ca/l. Waterborne Cu and Ca co-exposure influenced the majority of tested enzymatic activities (succinate dehydrogenase, malic dehydrogenase, lactate dehydrogenase, lipoprotein lipase and hepatic lipase), and changed Cu contents in several organs (gill, liver, kidney, gastrointestine and muscle). For histological observations, at the same Ca level, waterborne Cu exposure induced injuries in gills and liver. However, Ca addition seemed to mitigate the severity of Cu—induced injuries. Thus, our study demonstrated that Ca had the capacity to reduce Cu toxicity in *P. fulvidraco*.

1. Introduction

Despite the essential role of copper (Cu) for a number of key metabolic enzymes (Watanabe et al., 1997), the toxicity of Cu in fish has aroused extensive concern because of the increasing Cu concentration in aquatic system from the increasing industrial activities and the use of $CuSO_4$ as a fungicide in agricultural practices as well as in the control of algae and pathogens in fish culture ponds (Boyd and Massaut, 1999; Carvalho and Fernandes, 2008). In fish, Cu toxicity includes the alteration of the severe histological changes in the gills and liver (Sola et al., 1995; Karan et al., 1998; Dautremepuits et al., 2004; Monteiro et al., 2009), tissue Cu accumulation (Monteiro et al., 2009), oxidative stress (Wendelaar Bonga, 1997), the damage of hepatic intermediary metabolism (Taylor et al., 2000; Liu et al., 2010) and reduced growth (De Boeck et al., 1997; Liu et al., 2010). At present, these studies mentioned above were conducted to determine the toxicity of a single Cu exposure. In fact, the impact of waterborne Cu exposure on the aquatic environment is complex and depends on the physicochemical characteristics of water, especially hardness. Calcium, as a main cation of hardness, has been reported to have a direct ameliorative effect against Cu toxicity (Miller and Mackay, 1980; Spry and Wiener, 1991; Wurts and Perschbacher, 1994). Recently, Abdel-Tawwab et al. (2007) reported that the pre-exposure to Ca could reduce the harmful effect of Cu against fish. However, to our knowledge, the effect of the co-exposure of the Cu and Ca on the Cu toxicity in fish is not yet well studied.

Yellow catfish, *Pelteobagrus fulvidraco*, is regarded as a good candidate for freshwater culture in China for its delicious meat and high market value (Tan et al., 2010). At present, several studies have been conducted to determine the characteristics of nutrient physiology for the fish species in our laboratory (Tan et al., 2009; Luo et al., 2010, 2011). The fish is currently employed to monitor environmental pollution because of their advantageous characteristics: they constitute the major component in most freshwater ecosystems in China, and they are easily cultured in the laboratory. As the representative of the benthic fish in most aquatic ecosystems, the species can be used to study the bioaccumulation rates of waterborne heavy metals. The purpose of the present study was to assess the protective effect of Ca on Cu toxicity in juvenile yellow catfish. We examined several hepatic enzymatic activities involved in intermediary metabolism, such as succinate dehydrogenase (SDH, EC 1.3.99.1), lactate dehydrogenase (LDH, EC 1.1.1.27), malic dehydrogenase (MDH, EC 1.1.1.40), lipoprotein lipase (LPL, EC 3.1.1.34) and hepatic lipase (HL, EC 3.1.1.3), and Cu accumulation and histology of yellow catfish exposed to waterborne Cu in combination with Ca addition.

2. Materials and methods

The experiment was conducted in Panjin Guanghe Fisheries Co., Ltd, Panjin, China. For the experiment, Cu was added as $CuSO_4 \cdot 5H_2O$ and Ca as $CaCl_2$

* Corresponding author. Fax: +86 27 8728 2114.
E-mail address: luozhi99@yahoo.com.cn (Z. Luo).

0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.10.007

(AR, Shanghai Sinopharm Group Corporation, Shanghai, China). They were dissolved in distilled water for stock concentrations, respectively. Individual test solutions during the experiment were obtained by adding the appropriate volume of the primary stock to the dilution water. The Cu and Ca concentrations in the test tanks were measured using the flame atomic absorption spectrometry. We assure that the experiments performed on animals, animal care and all protocols followed the ethical guidelines of Huazhong Agricultural University for the care and use of laboratory animals.

2.1. Experimental procedures

A 3×2 factorial design was used in this study. Six exposure treatments were used to contain three nominal waterborne Cu concentrations (0, 1 and 2 mg Cu/l, respectively), each with two measured levels of Ca (10 and 50 mg Ca/l, respectively). Cu and Ca concentrations of freshwater (background) used in the experiment were approximately 0.001 mg/l and 11 mg/l, respectively. Accordingly, actual metal concentrations in water for each treatment after flame atomic absorption spectrophotometry measurement were (T1) 0 mg/l Cu+10 mg/l Ca, (T2) 1 mg/l Cu+10 mg/l Ca, (T3) 2 mg/l Cu+10 mg/l Ca, (T4) 0 mg/l Cu+50 mg/l Ca, (T5) 1 mg/l Cu+50 mg/l Ca and (T6) 2 mg/l Cu+50 mg/l Ca (Table 1).

Seven hundred juvenile yellow catfish were bought from a local fish pond (Panjin, China) and kept in twenty 300-l circular fiberglass tanks for two-week acclimatization. During the acclimatization period, the fish were fed minced trash fish twice a day. Afterwards, 450 uniform-sized fish (initial body weight: 24.06 ± 1.20 g, means $\pm$ SEM) were stocked in 18 fiberglass tanks, with 25 fish for each tank. They were exposed to six treatments mentioned above, with three replicates for each treatment. The experiment was carried out in static aquarium system, and continuously aerated to maintain dissolved oxygen near saturation. During the experimental, fish were fed six percent body weight per day (two meals per day) with trash fish, and residual food was removed 20 min after feeding in order to avoid the contamination and adsorbing metal ion. Meantime, to ensure water quality and maintain Ca and Cu levels, water was renewed 100 percent every day. The experiment continued for fifteen days.

The experiment was conducted at ambient temperature and subjected to natural photoperiod (approximately 14 h light/10 h darkness). Water quality parameters were monitored twice a week in the morning. The ranges of the parameters were as followed: temperature, 24.1–25.7 °C; pH 8.1–8.3; dissolved oxygen $\geq$ 6.3 mg/l; NH_4-N 0.051–0.078 mg/l. To obtain actual Cu and Ca concentrations in the aquaria, water samples were collected from the test aquaria after 1 and 24 h of renewing the test solutions. Water samples were analyzed during the experimental period by the method of flame atomic absorption spectrometry measurement and shown in Table 1.

2.2. Sampling and samples analysis

Sampling occurred on the zero, seventh and fifteenth day of the exposure. On each sampling day, fish were starved for one day prior to sampling. Then all fish were counted to determine survival. Eight fish per tank were randomly selected, euthanized (MS-222 at 10 mg l^{-1}) and dissected in ice to obtain gill, liver, kidney, gastrointestine tract, white muscle and vertebrae samples. For histological observation, the left lobe of liver (sliced into 3 mm thick slabs) and the second gill arch of the right side of each fish were collected, fixed in ten percent neutral buffered formalin for 24 h and prepared for histological analysis. For enzymatic activities, liver samples were removed immediately using sterile forceps, frozen in liquid nitrogen, and stored at −80 °C (not longer than two weeks) for further processing. For Cu content measurement, gills, liver, white muscle, kidney, gastrointestine and vertebrae samples were obtained and stored at −80 °C for further analysis.

2.2.1. Cu contents in tissues

For Cu content determinations, gills, liver, white muscle, kidney, gastrointestine and vertebrae samples were dried at 80 °C to constant weights and then digested using concentrated nitric acid and microwave (Midea, Midea Group, Guangdong, China) digestion. The digested samples were diluted to appropriate concentrations for flame atomic absorption spectrophotometry measurements (Shanghai Precision and Scientific Instrument Co., Ltd., Shanghai, China). These analyses were conducted in duplicate. The Cu concentrations in the tissues were expressed as μg g^{-1} wet weight.

Table 1
Nominal and measured concentrations of waterborne Cu and Ca in each treatment during the experiment.

Treatment	Nominal concentration (mg/l)		Measured concentration (mg/l)	
	Cu	Ca	Cu	Ca
T1 (control)	0	0	0.001 ± 0.00	11.02 ± 0.53
T2	1	0	0.98 ± 0.01	10.86 ± 0.48
T3	2	0	1.95 ± 0.03	10.77 ± 0.66
T4	0	40	0.001 ± 0.00	51.21 ± 1.51
T5	1	40	0.96 ± 0.01	49.78 ± 1.74
T6	2	40	1.93 ± 0.02	52.06 ± 1.23

2.2.2. Assays for enzymatic activities

For enzymatic analysis, liver was homogenized in 0.1 M Tris–HCl buffer at 4 °C, pH 7.4, to make a ten percent (W/V) homogenate. The homogenates were centrifuged at 16,000*g* for 5 min at 4 °C and then the supernatants were collected for enzymatic analysis. The assays were run in triplicates. The following enzyme activities were measured: LPL activity was measured using labeled triolein-^{3}H as a substrate, according to the modified methods by Ballart et al. (2003), SDH activity as described by Slater and Bonner (1952) in an incubation medium containing 0.1 M phosphate buffer (pH 7.6), 0.9 mM sodium azide, 0.9 mM 1-methoxyphenazine methylsulphate, 1.5 mM nitroblue tetrazolium, 5.6 mM EDTA-disodium salt and 48 mM succinate disodium salt, LDH activity measured as a change of absorbance at 340 nm min^{-1} and 30 °C according to Jones and Sidell (1982), MDH according to Ochoa (1955) on the basis of property of SOD to inhibit autooxidation of epinephrine to adrenaline at pH 10.5, HL following Ehnholm et al. (1975) and modified by Burgaya et al. (1989) on the basis of a unit (U) of enzyme activity corresponding to the amount of enzyme that releases 1 μmol of oleate per minute. Soluble protein content of liver homogenate was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as standard. The assays were run in triplicates. All enzyme activities were expressed as U (units) per mg of soluble protein.

2.2.3. Histology of gills and liver

In the present study, seven fish from each treatment were dissected to obtain liver and gills samples for histological observation. For each fish, three subsamples were prepared. They were fixed for 24 h in ten percent neutral buffered formalin. After dehydration in graded concentrations of ethanol, the samples were embedded in paraffin wax. Sagittal sections of 6 to 8 μm thickness were stained with hematoxylin–eosin (Woods and Ellis, 1994). Histological preparations were randomly examined with 60 microscope fields for each sub-samples, and the results from each observation were then combined for the overall results. The mean prevalence of each histological parameter was categorized according to Velmurugan et al. (2007).

2.3. Statistical analysis

The results were presented as mean $\pm$ standard error of mean (SEM). Data from each treatment were subjected to one-way analysis of variance (ANOVA) and two-way ANOVA, where appropriate. When overall differences were significant ($P < 0.05$), Turkey's test was used to compare the mean values among the treatments. Statistical analysis was performed using the SPSS 10.0 for Windows (SPSS, Michigan Avenue, Chicago, IL, USA).

3. Results

3.1. Cu content in tissues

Survival rate was high (98.5 percent) throughout the experiment and showed no significant difference among the treatments ($P > 0.05$) (data not shown). Cu accumulation in the gills, liver, kidney, gastrointestinal tract, muscle and vertebrae of juvenile yellow catfish co-exposed to different Cu and Ca concentration on the seventh and fifteenth day was shown in Tables 2 and 3, respectively. Generally speaking, during the experiment, Cu concentrations in several tissues followed the order: liver > gills > kidney > vertebrae > gastrointestinal tract > muscle. On the seventh day, with the same Ca level, Cu contents in gills, liver, kidney, digestive and muscle increased with increasing waterborne Cu concentration ($P < 0.05$), but vertebrae Cu content remained relatively stable ($P > 0.05$). With the same Cu level, increasing Ca levels significantly reduced muscle Cu content ($P < 0.05$), but did not influence Cu contents in other tested tissues ($P > 0.05$). The interaction of Cu and Ca significantly influenced Cu contents in gills and muscle ($P < 0.05$), but not in liver, kidney, gastrointestinal tract and vertebrae ($P > 0.05$).