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Short communication

Fluctuations in water temperature affected waterborne cadmium toxicity: Hematology, anaerobic glucose pathway, and oxidative stress status of Nile tilapia, *Oreochromis niloticus* (L.)

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ABSTRACT

The increase of water temperature has already been observed because of global climatic changes. Beside fluctuations in temperature, aquatic ecosystems may be stressed by the discharge of heated wastewater and toxic chemicals such as heavy metals. Therefore, this study was conducted to investigate effects of water temperature and/or waterborne cadmium (Cd) on hematology (red blood cells, RBCs; hematocrit, Ht; and hemoglobin, Hb), anaerobic glucolytic metabolism (lactate dehydrogenase, LDH; phosphofructokinase, PFK; and pyruvate kinase, PK) as well as of the pentose pathway (glycose-6-phosphate dehydrogenase, G6PDH), and oxidative defense (sodium dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx and Glutathion transferase, GST) of Nile tilapia. Fish (26.0 ± 0.38 g) were acclimated to 20, 24, 28, or 32 °C and co-exposed to 0.0 or 0.5 mg Cd/L for 8 weeks in triplicates. Two-way ANOVA revealed that all variables were significantly affected by water temperature, Cd exposure, and their interaction. Hematological variables increased significantly by increasing water temperature up to 28 °C, after which they decreased significantly at 32 °C. Additionally, these variables were lower in Cd-exposed fish group than those of Cd-free ones. Irrespective to Cd exposure, there are no significant differences in hematological variables in fish reared at 24 and 28 °C. Hepatic glycolytic and oxidative system enzymes activities increased with increasing water temperature up to 32 °C. In Cd-free fish groups, there are no significant differences in activities of these enzymes in fish reared at 24 and 28 °C. The Cd exposure caused significant increases in the activities of LDH and PK and significant decreases in PFK and G6PDH activities with increasing water temperature compared to Cd-free fish groups. Meanwhile, Cd-exposed fish showed highest activities of oxidative stress enzymes as compared to Cd-free fish groups. The changes in hepatic enzymes suggest that their activities were optimized when fish were reared at 24–28 °C. Furthermore, at 32 °C, the overall increment in the hepatic enzyme activities of Cd-exposed fish seems to be contributed to enhance Cd toxic effects.

1. Introduction

The aquaculture of Nile tilapia (*Oreochromis niloticus*) goes back to Ancient Egypt and nowadays it is one of the most widely cultured freshwater fish worldwide. It was introduced into many tropical, subtropical, and temperate regions of the world because of its delicious meat and high-market value (El-Sayed, 2006). This fish species may inhabit polluted water bodies for different seasons and be exposed to elevated water temperatures, which might constitute a kind of stress. In addition to daily and seasonal temperature changes, water bodies may suffer from the discharge of heated wastewater and/or heavy metals (Cairns et al., 1975; Fazio et al., 2014; Savorelli et al., 2017). Furthermore, high water temperature intends to increase the diffusion rate, accelerating chemical reactions, and metals accumulation that may elevate their toxic action (Heugens et al., 2001; Sokolova and Lannig, 2008). Water temperature could also affect metabolic activity, kinetics, and oxidative stress in fish (Malek et al., 2004; Heise et al., 2006; Kammer et al., 2011; Simčič et al., 2015).

In aquatic ecosystems, cadmium (Cd) has been discharged from mining, metal processing, and sewage effluent (Torre et al., 2013; Fazio et al., 2014). Even though Cd in the environment is considered toxic, its emissions have not yet ceased and this heavy metal continues to be bioaccumulated in fish threating their health (Bervoets et al., 2009; Abdel-Tawwab and Wafeek, 2010). To supply the energy demand for

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detoxification and repair processes in Cd-exposed fish, the glycogen/glucose is mobilized in the liver. Each metabolic pathway is continuously regulated in order to maintain homeostasis and control the metabolic flux (Brooks and Storey, 1995). Carvalho and Fernandes (2008) reported that in the anaerobic metabolism of glucose such key enzymes as hexokinase (HK) and phosphofructokinase (PFK) are at the beginning of the glycolytic sequence and the pyruvate kinase (PK) and lactate dehydrogenase (LDH) are at the terminal sequence of the glycolytic pathway. The glucose-6-phosphate dehydrogenase (G6PDH) is also a key enzyme of the pentose-phosphate shunt.

The oxidative stress in aquatic organisms is more profound during nutritional deficiency, elevated temperature, hypoxia, and exposure to xenobiotics (Avanzo et al., 2002; Hwang and Lin, 2002; Kolkovski et al., 2000; Radhakrishnan et al., 2014; Romeo et al., 2000). The Cd toxicity may be associated with oxidative damage for the production of reactive oxygen species (ROS) (Bagchi et al., 2000; Almeida et al., 2002; Shi et al., 2005). Fish have developed antioxidant defense mechanisms to scavenge ROS and consequently control the oxidative damage they induce. Fish are susceptible to ROS and have developed antioxidant defense including antioxidant enzymes such as sodium dismutase (SOD), catalase (CAT), glutathion peroxidase (GPx), and glutathion transferase (GST) (Martínez-Álvarez et al., 2005). Changes in the activities of these enzymes have been proposed as biomarkers of contaminant-mediated prooxidant challenge (Bagnyukova et al., 2006). These enzymes may scavenge unwanted O_2^- and H_2O_2, and ROOH produced by free radicals. For example, SOD catalyzes superoxide radical dismutation: $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. The resulting H_2O_2 in turn is decomposed by the enzymes GPx and CAT (Rzeuski et al., 1998). Abdel-Tawwab et al. (2017) found that the response of SOD, CAT, and GPx was related to environmental exposure to heavy metals.

In previous study, Abdel-Tawwab and Wafeek (2014) evaluated effects of water temperature and/or waterborne Cd toxicity on growth performance and metallothionein-Cd distribution in different organs of Nile tilapia. They found that Cd toxicity increased with increasing water temperature and that could be associated with increased Cd accumulation in the different fish organs. It was hypothesize that both water temperature and Cd exposure could cause metabolic alterations inducing free radical production and oxidative defenses. Therefore, the present study intended to evaluate interactive effects of water temperature and waterborne Cd toxicity on hematology and hepatic enzymes of anaerobic glucolytic pathway and oxidative stress status of Nile tilapia.

2. Materials and methods

2.1. Fish maintenance and experimental setup

This study was based on a 4 × 2 factorial design with four water temperatures (20, 24, 28, or 32 °C) and two Cd concentrations (0.0 or 0.5 mg/L) in triplicates. Nile tilapia (26.0 ± 0.38 g) were stocked at a density of 20 fish per 140-L aquarium and divided into four groups in separate aquaria of water temperature 20, 24, 28 or 32 °C meanwhile they were exposed to 0.0 or 0.5 mg Cd/L for 8 weeks. Each aquarium was supplied with compressed air via air-stones from air pumps at a 10 h/14 h light/dark cycle. Fish were fed a 25% crude protein diet up to satiation twice daily at 9:00 and 14:00 h. A half of aquarium's water was siphoned daily along with fish feaces and replaced with an equal volume of water maintaining the same water temperature and Cd concentration.

2.2. Hematological parameters

Five fish from each aquarium were not fed for 24 h before sampling and were anaesthetized with tricane methanesulfonate (30 mg/L) and blood was collected with a hypodermic syringe from the caudal vein. The blood collection lasted < 3 min. in order to avoid cortisol rise induced by the manipulation during sampling. The extracted blood was set in Eppendorf tubes contained heparin, used as anticoagulant, for hematology including: red blood cells (RBCs) counting, hematocrit (Ht), and hemoglobin (Hb). Red blood cells (RBCs) were counted under the light microscope using a Neubauer haemocytometer after blood dilution with phosphate-buffered saline. In order to determine the hematocrit (Ht) value, the blood was transferred to draw-in micro-hematocrit tubes and centrifuged for 5 min at 10,000*g*. Hemoglobin levels were determined colorimetrically by measuring the formation of cyanomethemoglobin according to Van Kampen and Zijlstra (1961).

2.3. Enzyme activities assays

Five fish from each aquarium were euthanized and rapidly killed by decapitation and the liver immediately removed and processed, and then snap-frozen in liquid nitrogen and stored at − 80 °C for later assay. About 0.5 g of liver tissue was homogenized after addition of 5.0 mL of 10.0 mM Tris buffer (pH 7.5) for detection of enzyme activities. The extracts were centrifuged at 15,000*g* for 10 min at 4 °C. Aliquots of supernatant were taken for analysis of enzymes activities. Enzyme activities were assayed according to the manufacturer's instructions (MyBioSource Inc., San Diego, California, USA). Lactate dehydrogenase (LDH) activity was measured by the method of Bergmeyer (1974). Pyruvate kinase (PK) activity was measured by the method of Bucher and Pfleiderer, 1955). Phosphofructokinase (PFK) activity was measured by the method of Layzer et al. (1969). Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured by the method of Bergmeyer (1974). Superoxide dismutase (SOD) activity was measured at 550 nm (McCord and Fridovich, 1969). Catalase (CAT) activity was estimated at 240 nm following the method of Aebi (1984). Activity of glutathione peroxidase (GPx) was measured at 340 nm according to Paglia and Valentine (1967). Glutathion transferase (GST) activity was determined with 1-chloro-2-dinitrobenzene (CDNB) as a substrate (Habig et al., 1974).

2.4. Statistical analysis

The obtained data were performed for homogeneity of variance by using the Bartlett's test. The two-way ANOVA was used to determine the significant effect of water temperature and Cd concentrations as factors. Duncan's test with 95% confidence limit was applied to compare the means values whenever data were significant. All the statistical analyses were done using the SPSS program version 20 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

3. Results

Changes in RBCs, Ht, and Hb values were significantly affected by water temperature, Cd exposure, and their interaction ($P < 0.05$; Table 1). These variables increased significantly by increasing water temperature up to 28 °C, after which they decreased significantly at 32 °C. Additionally, these variables were lower in Cd-exposed fish group than those of Cd-free ones. Lowest values were observed at 20 °C in Cd-exposed fish group. On the other hand, their highest values were obtained at Cd-free fish groups reared at 28 °C. Irrespective to Cd exposure, there are no significant differences in hematological variables in fish reared at 24 and 28 °C.

Two-way ANOVA showed that hepatic LDH, PK, PFK, and G6PDH activities were significantly affected by water temperature, Cd exposure, and their interaction ($P < 0.05$; Table 2). Their activities increased with increasing water temperature up to 32 °C. In Cd-free fish groups, there are no significant differences in activities of these enzymes in fish reared at 24 and 28 °C. The Cd exposure caused significant increases in LDH and PK activities and significant decreases in PFK and G6PDH activities with increasing water temperature compared to the Cd-free fish groups. At 20 °C, Cd-free fish group