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ScienceDirect
Aquaculture 264 (2007) 236–246
Aquaculture
www.elsevier.com/locate/aqua-online

The use of calcium pre-exposure as a protective agent against environmental copper toxicity for juvenile Nile tilapia, *Oreochromis niloticus* (L.)

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Received 29 June 2006; received in revised form 2 December 2006; accepted 11 December 2006

Abstract

This study was carried out to evaluate the response of Nile tilapia, *Oreochromis niloticus* (L.), pre-exposed to calcium oxide for 4 days, and exposed to copper for 6 weeks. Fish (8.3 ± 0.1 g) were stocked in 1.5 m^3 fiberglass tank and exposed to calcium oxide at a rate of 100 mg Ca^{2+} L^{-1} for 4 days. After that, fish were transferred and randomly distributed at a rate of 15 fish per 100-L aquarium. Then, fish were post-exposed to concentrations of 0, 0.503, or 1.25 mg Cu^{2+} L^{-1} (T1, T2 and T3, respectively). Three other groups were not pre-exposed to Ca and exposed to the same Cu concentrations (T4, T5 and T6, respectively). A fish diet containing 30% crude protein was offered to fish at a daily rate of 3% of live body weight twice daily; 5 days a week for 6 weeks. The final weight, weight gain and SGR were affected significantly by both Cu concentrations and Ca pre-exposure ($P<0.05$). Fish pre-exposed to Ca (T2 and T3) exhibited better growth compared to non-exposed groups (T5 and T6). Fish mortality increased significantly with increasing the Cu concentration ($P<0.05$). Feed intake reduced, while FCR increased significantly with increasing the Cu concentration ($P<0.05$). The feed intake and FCR in T2 and T3 were better than those of T5 and T6 groups. Moisture content was not significantly differed at different treatments ($P>0.05$). Crude protein decreased significantly at T6 ($P<0.05$). Total lipids in T2 and T3 were higher than those of T5 and T6 ($P<0.05$). Ash content and Cu residues in T2 and T3 were significantly lower than those of T5 and T6 ($P<0.05$). Fulton condition factor, liver somatic index, and viscera somatic index were affected significantly by Cu toxicity (T5 and T6; $P>0.05$), while they exhibited non-significant differences in T1–T4 groups. RBCs counts, Hct and Hb values were significantly affected by Cu toxicity ($P<0.05$), while they were similar to control group in Ca pre-exposed groups ($P>0.05$). Uric acid, creatinine, and AST were significantly affected by both Ca pre-exposure and Cu toxicity ($P<0.05$). There was no significant changes in uric acid among T2, T3 and T5 ($P>0.05$). The highest value of uric acid was obtained at T6 ($P<0.05$). Creatinine, AST and ALT in T2 and T3 were lower than those of T5 and T6 ($P<0.05$). Lipids in plasma and liver were high, while protein in plasma and liver were low in T5 and T6 ($P<0.05$). Histological sections were done in fish's gills, liver and kidney in all treatments (T1–T6). The pathologic lesions were observed due to Cu toxicity. These damages became

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doi:10.1016/j.aquaculture.2006.12.020

severe with increasing Cu concentration. Tissue alterations in T2 and T3 were less than that in T5 and T6. The present study displayed that Ca pre-exposure may play a factor then it reduced Cu toxicity resulting in a better fish growth.

Keywords: Calcium; Liming agent; Nile tilapia; Copper toxicity; Copper residue; Growth; Somatic indices; Physiological functions; Gills; Liver; Kidney; Histology

1. Introduction

Copper sulfate is often used as an algaecide in commercial and recreational fish ponds to control growth of phytoplankton and filamentous algae and to control certain fish disease (Boyd, 1990; Tucker and Robinson, 1990). Boyd (1990) stated that the concentrations of copper sulfate used for phytoplankton control are seldom directly toxic to fish, but do kill large numbers of invertebrate food organisms such as rotifers, cladocerans and copepods. However, above a specific concentration, copper is toxic to fish including such cultured species as salmonids, cyprinids and catfish (Wurts and Perschbacher, 1994).

Nile tilapia is a native fish species of Egypt that has become popular species worldwide mainly as a valuable fish, easy to breed and grow in a variety of aquaculture systems (El-Sayed, 2006). However, Nile tilapia is an omnivorous fish consuming detritus, phytoplankton and zooplankton (Abdelghany, 1993; Abdel-Tawwab, 2000; Abdel-Tawwab and El-Marakby, 2004). Thus, treating plankton with copper compounds may lead to copper bioaccumulation reaching a toxic level in fish.

The impact of copper on the aquatic environment is complex and depends on the physicochemical characteristics of water (Laurén and Mcdonald, 1986; Erickson et al., 1996; Mazon and Fernandes, 1999; Tao et al., 1999; Takasusuki et al., 2004). Therefore, recommendations for the safe use of copper sulfate have been based on hardness (Sawyer et al., 1989; Perschbacher and Wurts, 1999), total alkalinity (Boyd, 1990; Reardon and Harrell, 1990; Perschbacher and Wurts, 1999), and pH (Masuda and Boyd, 1993) of the water. High concentration of calcium, which is a major component of hardness, is also thought to limit copper toxicity by protecting the ion-regulating mechanisms at the gills from the disruptive effects of copper (Pagenkopf, 1983). One means to increase the uptake of calcium by aquatic organisms is to increase the level of environmental calcium through the application of liming agents. Chakraborti and Mukherjee (1995) found that the total plasma calcium level of common carp (40–50 g) raised in tap water (0.15 mM Ca^{2+}) remains within 3 mM, while fish kept in freshwater rich in calcium exhibited hypercalcemic responses. Calcium supplied through liming can reduce the uptake of heavy metals (Raddum et al., 1986; Andersson and Borg, 1988). The objective of the present study was to evaluate the use of calcium pre-exposure as a protective agent against environmental copper toxicity for Nile Tilapia, *Oreochromis niloticus* (L.) expressed in growth, feed utilization, and physiological functions.

2. Materials and methods

2.1. Experimental procedures

Healthy Nile tilapia, *O. niloticus* (L.) were collected from the nursery ponds of Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish (8.3±0.1 g) were acclimated to laboratory conditions in indoor tanks for 2 weeks. Afterwards, experimental fish stock have been randomly divided into two groups. While the first group was held in calcium oxide (+Ca) for 4 days, the rest of experimental fish were not exposed to calcium oxide (−Ca). For +Ca, a solution of 100 mg Ca^{2+} L^{-1} of calcium oxide was prepared in 1.5 m^3 tank. After the 4 days of Ca exposure or non-exposure, 18 100-L aquaria were randomly allocated to the three-replicate experiment whereas each aquarium was stocked at a rate of 15 fish. The design of the experiment was as follows:

Treatment	Ca pre-exposure	Cu concentration (mg L^{-1})
T1	Yes	0
T2	Yes	0.503
T3	Yes	1.25
T4	No	0
T5	No	0.503
T6	No	1.25

Each aquarium was supplied with compressed air via air-stones from air pumps. Well-aerated water was provided from a storage fiberglass tank. The ambient temperature throughout the study ranged from 26 to 28 °C. Dead fish once observed at any aquarium were removed and recorded. Fish were offered 30% crude