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Changes in Copper Content of Allogynogenetic Silver Crucian Carp after Application of Copper Sulfate to Fishponds

Jinlan Liu and Guang Yang*

Key Laboratory of Aquatic Ecology and Aquaculture of Tianjin, Department of Fishery Science, Tianjin Agricultural University, No. 22 Jinjing Rd., Xiqing District, Tianjin 300384, P.R. China

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Abstract

Copper sulfate was applied to freshwater fishponds containing allogynogenetic silver crucian carp and changes in the copper content in fish tissues were measured. The copper content in blood, gill, amd liver went up quickly, but remained high for a relatively short time. Twenty-four hours after application, the copper content in the gill and liver reached their highest levels, 4.846 mg/kg and 6.624 mg/kg, respectively. The copper content in the blood reached its maximum of 22.125 mg/l three days after application. The copper content in the kidney also reached its maximum, 17.472 mg/kg, on day 3 but the high copper content prevailed until the end of the 20-day observation. The copper content in the muscle reached its maximum, 1.873 mg/kg, on day 7 and its minimum, 0.6 mg/kg, after two weeks.

Introduction

Copper sulfate is often used to prevent and treat diseases of cultured aquatic animals because of its high efficiency, wide range of anti-bacterial effects, and affordability. However, if aquatic animals are exposed to a concentrated copper solution, copper may accumulate in their bodies (Lan, 1998; Liu and Tao, 1999) and affect growth, metabolism, and disease resistance (Chen and Zhang, 1988; Xiao and Shi, 1999; Chen and Lin, 2001). Further, copper can be traced in humans via the food chain.

The accumulation of copper in the body of fish is often used as an indication of pollution deriving from mining activities, pond treatments, or other sources (Liu and Tao, 1999; Farkas et al., 2003; Kraemer et al., 2005). Consumers have often expressed their concern about the food safety of fish raised in fishponds treated with copper sulfate. The aim of this research was to

* Corresponding author. Tel.: +86-22-89587238, fax: +86-22-23792176,
e-mail: yg9558@163.com

study the changes in copper content in various tissues of allogynogenetic silver crucian carp (ASCC) after copper sulfate was applied to a freshwater fishpond using a dose normally employed for treating fish disease. The results of this experiment will provide information on the assimilation of copper sulfate in ASSC tissues and its withdrawal time.

Materials and Methods

Experimental fish. ASCC (133-350 g; mean 240 g) were reared outdoor in two fishponds, one of 350 m^2 with a water depth of 0.65 m and the second of 450 m^2 with a water depth of 0.71 m. Feeding was carried out twice a day (8:00-9:00 and 16:00-17:00) with the equivalent of 3-4% of the total fish body weight.

*Application of copper sulfate***.** Copper sulfate (purity 99.0%), 115 g for the first pond and 160 g for the second, was dissolved in two small containers and evenly distributed into the ponds. The final concentration of copper sulfate in both ponds was about 0.5 mg/l.

Collection of samples. Every day at 8:00-9:00, four fish were captured from each pond and weighed. Meanwhile, water samples from the middle layer (30±5 cm) were collected and water temperature and pH were measured. Blood was collected from the tail artery using a syringe with a small amount of heparin. The fish were then dissected and 3-4 g of branchial filaments and back muscles as well as the whole liver and kidney were removed. Tissues of any two fish from a single pond were pooled together as a sample and each sample was weighed.

Treatment of samples and analysis of copper content. The water samples were filtrated using a net with 40 mesh/cm^2. Blood was treated using a method called wet digestion (Che and Cai, 2004) as follows: 4 ml blood, 4 ml concentrated nitric acid (HNO_3), 1 ml concentrated perchloric acid ($HCLO_4$), and 4 ml distilled H_2O were added in sequence to a 50-ml flask. The mixture was digested by boiling in a water bath until the solution became black. The solution was cooled to room temperature and transferred to a 25-ml flask. Distilled H_2O was added to the calibration line of the flask and the solution was filtrated through neutral filter paper.

Samples of gill, muscle, liver, and kidney tissues were treated using the dry ash method (Che and Cai, 2004) as follows: the samples were first dried at 90-100°C for 1 h and then dried at 100-110°C for 2 h in a drying oven. After treatment in a stove at 400-500°C for 5-6 h, the sample became ash. The ash was cooled and transferred to a crucible, followed by addition of 3 mol/l HCl until the ash was just immersed. The combination of ash and HCl was heated in a water bath of 90-100°C for 1 h. Finally the combination was diluted to 50 ml with distilled H_2O and filtrated through neutral filter paper.

The copper content of the treated samples was measured using flame atomic absorption spectrophotometry. For water and blood, the copper content was expressed in mg/l; for tissues, it was expressed in mg/kg.

Results

Before copper sulfate was applied to the fishponds, no copper was detected in the water and the baseline levels in the fish blood, gill, liver, kidney, and muscle were 5.667 mg/l, 1.362 mg/kg, 1.438 mg/kg, 7.026 mg/kg, and 0.563 mg/kg, respectively. On the first three days after application of the copper sulfate, the copper content in the water remained around 0.45 mg/l, nearly equal to the concentration immediately after treatment (Fig. 1). On day 4, the copper content dropped, reaching 0.175 mg/l on day 5. It continued to drop and, by day 13, no copper was detected in the water. In the blood, the copper content reached its highest value (22.125 mg/l) on day 3, after which it eventually stabilized at 10 mg/l. The content in the gills rose rapidly and reached 4.846 mg/kg, about three times as before treatment. The content dropped to 1.949 mg/kg on day 4, reached a secondary peak of 4.020 mg/kg on day 6. and gradually decreased until it stabilized at 1.540 mg/kg. In the liver, the copper content reached its highest level (6.624 mg/kg) 24 h after treatment, then gradually decreased until the end of the experiment. Similar to

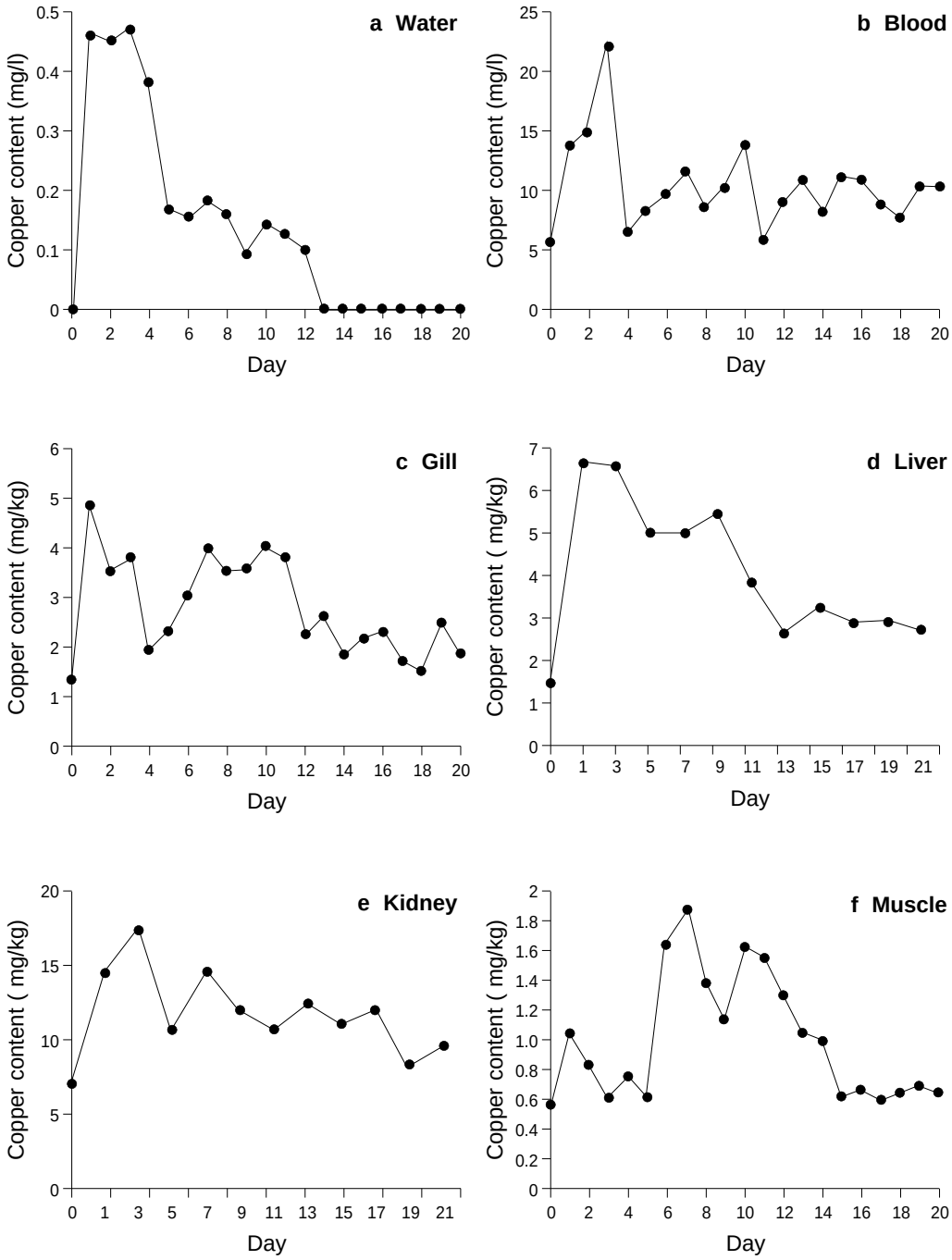

Fig. 1. Changes in copper content in (a) fishpond water, and in (b) blood, (c) gill, (d) liver, (e) kidney, and (f) muscle tissues of allogynogenetic silver crucian carp after application of copper sulfate to fishponds

the trend in blood, the copper content in the kidney steadily rose until it peaked at 17.472 mg/kg on day 3, then gradually decreased to around 10 mg/kg. In the muscle, the copper content rose to 1.046 mg/kg one day after treatment, gradually decreased during the following five days, reached a second peak of 1.873 mg/kg on days 5-7, and slowly declined to around 0.6 mg/kg.

Discussion

Copper content in the water. The copper content in the water increased shortly after application and remained elevated for three days, much longer than in channel catfish ponds where it lasted a few minutes only (Liu et al., 2006). By day 5, it dropped approximately 65% to 0.175 mg/l. This may be due to stirring of the water by the fish and a relatively high water temperature. ASCC are probably more active than channel catfish. In our experiment, the copper content in the water remained high for three days mainly because of the high water temperature (25-27°C) and the fish movement. On days 4 and 5, the copper content dropped rapidly because of a decrease in water temperature (16-18°C) and less movement of the fish.

Copper content in fish tissues. Copper ion is a coenzyme for certain enzymes and an essential component of pigments. It is essential for synthesizing hemoglobin (Hb), formation of skeletons, and maintaining the lecithin level in the nervous system (Harper, 1985). Therefore, there is always a small amount of essential copper ions in the body of animals. In this experiment, the amount of copper in the animals on day 0 represents the usual amount of essential copper ions in the body of ASCC.

Most copper immediately combines with albumin in blood plasma and is subsequently distributed to the liver and other organs (Harper, 1985). The copper combines with liver albumin is then released back into the blood, resulting in a fluctuating level of copper in the blood. During long-term exposure to waterborne copper, the concentration in plasma achieves a steady-state situation after a few hours or days by toxicokinetic analysis (Carbonell, 1994). In this experiment, the copper content in the blood of ASCC increased during the first week, then returned to its baseline and remained relatively stable. Results indicate that blood, the main carrier of material through the body, has a very strong affinity with copper but also has the physiological capability to maintain its own stability.

The copper content in all tested tissues increased after copper sulfate was applied to the fishponds. However, changes in the copper content differed in different tissues. The gill absorbed the most copper from the water, probably due to its negatively charged surface cells that can directly absorb free copper ions and enhance Cu tolerance by gill repair when exposed to a high Cu dose (Tate-Boldt and Kolok, 2008). In this experiment, the change in copper content in the gills was consistent with the physiological function of the gill, which rapidly absorbed copper ions followed by a decrease caused by gill damage and a gradual increase owing to gill repair 13 days after treatment.

The copper content in the liver rose after application, then gradually decreased until the end of the experiment. This result is consistent with those in fishes from polluted water (Liu and Tao, 1999; Kraemer et al., 2005). Cu concentration in the liver of channel catfish far exceeded that in meat and bone (Liu et al., 2006). Thus, it can be inferred not only that copper ions can highly accumulate in the liver of fishes, but also that they cannot be easily eliminated.

The copper content in the kidney increased during the first three days and then decreased gradually to around 10 mg/kg, higher than the base line. This suggests that fish kidneys may be the main storage organ that tolerates copper ions.

The copper content in the muscle, the main edible part of the fish, remained low throughout the experiment as reported for other fishes (Farkas et al., 2003; Kraemer et al., 2005; Liu et al., 2006). The level was lower than China's standard of food health which is 10.0 mg/kg (Che and Cai, 2004) and the Food and Agriculture Organization (FAO) limit of 20.0 mg/kg (Abou-Arab et al., 1996).

No appreciable Cu leaked into the neighboring groundwater after copper sulfate was applied to channel catfish ponds at a rate of once a week for 16 summer weeks (Liu et al., 2006). Therefore, taking into account the high efficiency of copper sulfate in killing parasites and harmful algae boom, the low leaking of copper into the surrounding environment, the need for fish as a food source, and the above results, it can be concluded that the use of copper sulfate as a drug in aquaculture is safe and adoptable. Because the copper level in the fish meat was relatively low, it may be further concluded that copper-treated fish are fit for human consumption according to Chinese standards and FAO limits. However, in view of the accumulation in the various organs, the authors recommend a withdrawal time of more than 15 days.

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