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Effects of Clomazone Herbicide on hematological and some parameters of protein and carbohydrate metabolism of silver catfish *Rhamdia quelen*

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Abstract

The effects of clomazone (0.5 and 1.0 mg/L) according to nominal concentrations used in paddy rice fields (0.4–0.7 mg/L) on protein and carbohydrate metabolism and haematological parameters were evaluated in silver catfish (*Rhamdia quelen*) after 12, 24, 48, 96 and 192 h of exposure with a recovery period of 96 and 192 h. Liver glycogen increased significantly ($P<0.05$) in all periods and concentrations tested. The maximum glycogen increase reaches 250% after 12 h of exposure. Muscle glycogen reduced significantly after 24, 48, 96 and 192 h for both clomazone concentrations ($P<0.05$). Significantly elevated plasma glucose values ($P<0.05$) and variation in glucose in the liver and muscle of exposed fish were observed. Muscle lactate levels increased after 12, 24 and 48 h of clomazone exposure (22–67%), but reduced in the liver ($P<0.05$). Protein levels were enhanced in the liver and white muscle, except at 96 and 192 h of exposure, whereas it increased in the plasma in the period from 48 to 96 h ($P<0.05$). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly elevated in the plasma ($P<0.05$). In the liver, ALT increased after 24 h, while AST activity was enhanced only after 12 h of exposure. Hematocrit contents were reduced after 96 and 192 h of exposure. Most of the metabolic disorders observed did not persist after the recovery period, except for the liver AST and ALT activity. Clomazone concentrations used in this study appear safe to fish, *Rhamdia quelen*, because overall parameters can be recovered after 96 and 192 h in clean water. ALT and AST activity may be an early biomarker of clomazone toxicity.

Keywords: Herbicide; Clomazone; Silver catfish (*Rhamdia quelen*); Metabolism; Aminotransferases

1. Introduction

The aquatic environment is continuously being contaminated with chemicals from agriculture activities. Hundreds of pesticides of different chemical structures are extensively used to control a wide variety of agricultural pests and can contaminate aquatic habitats due to leaching and runoff water from treated areas. The pesticides may produce an immense disruption of the ecological balance causing damage to non-target organisms including fish of commercial importance (Oruç and Üner, 1999; Bretaud et al., 2000).

Several biochemical and physiological responses occur when a toxicant enters an organism, which may be an acclimation of the organism or may lead to toxicity (Begum, 2004). Fish blood is sensitive to pollution-induced stress and changes in the hematological and metabolic parameters can be used as toxicity

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indicators of the pesticides and herbicides (Roche and Bogé, 2000; Sancho et al., 2000). Liver damage can be identified by alterations in the activities of enzymes such as aspartate aminotransferase (AST; EC 2.6.1.1) and alanine aminotransferase (ALT; EC 2.6.1.2) (Oruç and Üner, 1999; Poleksíc and Karan, 1999).

Clomazone herbicide (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) is widely used against weeds in paddy rice fields in Southern Brazil (Jonsson et al., 1998). Clomazone residues were detected in 90% of water samples collected from rivers of the rice cultivation regions (Zanella et al., 2002). It may cause groundwater contamination due to its high water solubility ($1100\,mg\,L^{-1}$) and relatively long half-life dissipation averaging from 28 to 84 days (Colby et al., 1989).

Silver catfish, *Rhamdia quelen* (Heptapteridae), was chosen for this research, since the effect of clomazone on fish species, particularly on this one, is scarcely studied. It was also chosen because it is a commercially relevant species for fisheries in the southern region of Brazil. The silver catfish survives in cold winters and grows rapidly in summer, reaching a body weight of 600–800 g in 8 months (Barcellos et al., 2004). In addition, there is no information available, regarding the biochemical response of silver catfish after the exposure to clomazone. Thus, the aim of this study was to verify the effects of clomazone on silver catfish (*R. quelen*), at the concentrations used in rice fields, through the measurement of biochemical and hematological parameters as toxicity indicators.

2. Material and methods

2.1. Fish

Young silver catfish (*R. quelen*) of both sexes with an average weight of 50.0 ± 10.0 g and length of 15 ± 1.0 cm were acquired from a university fish farm (UFSM) and were acclimated to laboratory conditions for 15 days. They were kept in tanks (250 L) with tap water under a natural photoperiod (12 h light–12 h dark). Water was oxygen saturated through constant aeration in a static system. Water conditions were checked every day as follows: temperature $21 \pm 1.0\,^{\circ}C$, pH 7.6 ± 0.2 units, dissolved oxygen 6.4 ± 0.3 mg/L, non-ionized ammonia 0.007 ± 0.001 mg/L, nitrite 0.03 ± 0.01 mg/L, alkalinity 65 ± 5.4 mg/L $CaCO_3$ and hardness 20 ± 1.5 mg/L $CaCO_3$. All water parameters were determined according to Boyd and Tucker (1992). During the acclimation period, fish were fed ad libitum two times a day (08:30 and 17:30 h) with commercial fish pellets (42% crude protein, Supra, Brazil). During the experimental period fish were fed at 72 h only for the period of 192 h of exposure. Fish exposed for 12, 24, 48 and 96 h were not fed during the experimental period in accordance with other toxicity studies (de Aguiar et al., 2004). Fecal remains and food residues were removed by suction every other day.

2.2. Experimental design

The herbicide used in this study was obtained commercially from the FMC Corporation (Gamit—36% purity, Philadelphia, United States). Previous experiments carried out in our laboratory established 7.32 mg/L as the LC_{50} 96 h for clomazone (Miron et al., 2004). The concentration usually recommended in rice fields is from 0.4 to 0.7 mg/L (Rodrigues and Almeida, 1998) and thus we choose clomazone concentrations of 0.5 and 1.0 mg/L for the experiments. After acclimation, fish were transferred to glass aquaria (45 L) with controlled aeration and temperature. Groups of 8 fish per aquaria (duplicates) were exposed for 12, 24, 48, 96 and 192 h to clomazone: 0.5 and 1.0 mg/L (nominal concentration) and the control group were kept in water without herbicide. Stock solutions were prepared by dissolving clomazone in water and added to the experimental aquaria. The clomazone concentration was monitored in tanks every 2 days by high-performance liquid chromatography (HPLC) method according to Zanella et al. (2002). Herbicide concentration in the water after 48 h was approximately 90% of the initial concentration (data not shown). The water in the aquaria was renewed every 48 h to maintain the herbicide concentration constant during the exposure period. After each period of exposure the fish blood was quickly collected for determining plasma parameters. The fishes were killed by punching the spinal cord behind the opercula. White muscle and liver samples were removed, washed in 150 mM saline solution, packed in Teflon tubes and kept at $-4\,^{\circ}C$ for analyses of transaminases activities, glycogen, lactate, glucose and protein levels.

Two series (duplicate) of four extra glass tanks (8 fish/tank) were used for recovery periods as follows. After exposure to both clomazone concentrations, the fish were kept in herbicide-free water for 96 and 192 h to recover (after 96 and 192 h of exposure, respectively). Control fish were sampled at each experimental period. The blood and liver samples were collected as reported above to determine plasma parameters, AST (EC 2.6.1.1) and ALT (EC 2.6.1.2) activities.

2.3. Analytical procedures

Blood was collected from caudal vein into 3 ml heparinized syringes. One aliquot of the sample was used for hemoglobin and hematocrit determinations. Another blood aliquot was centrifuged at 3000*g* for 10 min and plasma was separated. Hemoglobin contents (Hb) were determined spectrophotometrically (500 nm) using the cyanomethemoglobin method. Hematocrit