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Acute toxicity of nitrite to matrinxã, *Brycon cephalus* (GÜNTHER, 1869), (Teleostei-Characidae)

Toxicidade aguda ao nitrito em matrinxã, *Brycon cephalus* (GÜNTHER, 1869), (Teleostei-Characidae)

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ABSTRACT

Nitrite leads to many physiological and hematological disturbances followed by lethality. This work reports the lethal concentration of nitrite to juvenile matrinxã. Experiments were done at 24ºC, pH 6.7-7.0 under natural photoperiod. Fish were exposed to 0.2- 0.5- 1.0 and 2.0mg L^{-1} of nitrite N-NO_2 for 96h. The 96-h LC50 of nitrite-N was calculated by the trimmed Spearman-Karber method (0.86± 0.05mg L^{-1}; 0.57-1.30 to 95%CI) and it was concluded that matrinxã is very sensitive to environmental nitrite. Therefore, care must be taken to prevent accumulation of nitrite in intensive culture systems of matrinxã.

Key words: *LC50, nitrite, matrinxã, **Brycon cephalus**, toxicity.*

RESUMO

O nitrito ocasiona vários distúrbios fisiológicos e hematológicos levando à mortalidade. Este trabalho relata as concentrações letais de nitrito em juvenis de matrinxã. Os experimentos foram feitos a 24ºC, pH 6,7-7,0 sob foto-período natural. Os peixes foram expostos a 0,2- 0,5- 1,0 e 2,0mg L^{-1} de nitrito N-NO_2 por 96h. O CL50 96-h de nitrito-N foi calculado pelo método de Spearman-Karber (0,86± 0,05mg L^{-1}; 0,57-1,30 para 95%IC) e concluiu-se que o matrinxã é muito sensível ao nitrito ambiental. Portanto, deve-se ter cuidado em sistemas de cultura intensivo de matrinxã e prevenir o acúmulo de nitrito.

Palavras-chave: *LC50, nitrito, matrinxã, **Brycon cephalus**, toxicidade.*

INTRODUCTION

Several fish from the wild have been domesticated in Brazil and many of them are promising as new species in aquaculture. Among those, the teleost matrinxã (***Brycon cephalus***, GÜNTHER, 1869), originally from Amazon Basin, has several desirable traits for fish culture, including high growth rate, an appetite for commercial pellets and tolerance of a wide range of temperature and pH. However, information concerning environmental requirements for successful rearing is limited. In addition, basic data on sensitive to environmental toxicants, such as nitrite, are lacking.

Ammonia is the principal nitrogenous excretory product of fish. Its oxidation results in nitrite, an intermediary chemical product of the nitrogen cycle. Nitrite is usually present at low environmental concentrations, but pollution can unbalance the nitrogen cycle leading to elevated values. Chemical residues from industrial activities when dumped in water environments can raise the nitrite levels (NIKINMAA, 1992; HECKMAN et al., 1997). Also, high stocking densities in fish commercial culture systems can lead to nitrite accumulation (DIAB et al., 1993). The increase of water nitrite concentrations can result in many physiological disturbances (HAGOPIAN & RILEY, 1998; HARGREAVES, 1998).

In the blood, nitrite oxidizes hemoglobin to methemoglobin, a non-functional Fe^{3+} molecule (CAMERON, 1971; BATH & EDDY, 1980; JENSEN, 2003), and can be raised in the plasma leading to biochemical changes (BATH & EDDY 1980; SCHOORE et al., 1995; GROSELL & JENSEN, 2000) and hematological disorders, as hemolytic

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anemia (SCARANO & SAROGLIA, 1984; SCARANO et al., 1984). Nitrite also possesses pharmacological properties (JENSEN, 2003) and can be covalently combined to organic compounds resulting in nitro amines, which are very oncogenic derivatives (WOLFF & WASSERMAN, 1972; HECKMAN et al., 1997). However, the biological activities of nitrite are dependent on whole animal uptake, which are species specific. The goal of this study was to determine the median-lethal concentration (96-h LC50) of nitrite to juvenile matrinxã.

MATERIAL AND METHODS

Juveniles matrinxã were obtained from the fish farm Águas Claras, Mococa, SP, transported by pick-up to the Adaptive Biochemistry Laboratory, Genetics Department at Sao Carlos University, and stocked in 2,000-L re-circulated, bio-filtered tanks. The water (24 ± 1.0°C; pH 6.7-7.0) was continuously aerated and temperature, pH, hardness, oxygen and nitrite were daily monitored. The fish were kept for acclimatization under a natural photoperiod (February-April), fed on commercial pellets with 35% protein, two times daily *ad libitum*, and no nitrite was recorded in the tanks. Feeding was discontinued 24h before the initiation of toxicity trials.

Median-lethal concentrations were determined in fiberglass tanks, each containing ten fish and 200L of continuously aerated water. At the time of the experimentation the juveniles weighed 45.0 ± 11.85g. The experiments consisted of a control tank and four concentrations of nitrite. The experiments were conducted in triplicate and results are expressed as percent of mortality. Nominal nitrite (nitrite-N) concentrations (as sodium nitrite) in the tanks were 0.2, 0.5,1.0 and 2.0mg L^{-1} $N\text{-}NO_2$. Water was renewed every 24h and nitrite was added to maintain the initial concentration in a semi-static system for 96h. Fish were not fed during the experiment and dead fish were daily removed. Fish mortality was recorded and the 96-h LC50 $N\text{-}NO_2$ was calculated by the trimmed Spearman-Karber method (HAMILTON et al., 1978). Water quality was monitored every day in the course of the experiment. Ammonia was determined by nesslerization modified from GENTZKOW & MASEN (1942). Hardness was determined by titration with eriochrome black and expressed as mg of $CaCO_3$ L^{-1} (APHA, 1980). Temperature and oxygen levels were determined to the nearest 1°C and 0.1mg L^{-1} respectively, with an oxygen-temperature-conductivity-pH meter Check Mate II Corning. The median temperature of water (23 ± 0.9°C) and the oxygen level (5.9 ± 0.21mg L^{-1}) were constant. A slight increase of hardness and conductivity was observed but these changes were not significant. There was a rise in pH likely attributed to ammonia released by the fish (Table 1). Alkalinity was determined by sulfuric titration (GOLDSTEIN & CLYMO, 1969) and no changes were observed. Chloride concentration was determined as APHA (1980). Water nitrite concentrations were determined with sulfanilamide and N-1 (naphtyl) ethylene-diamine bichloridrate at 540nm (TAVARES, 1994). Nitrite concentrations were constant in the experimental tanks and practically absent in the control. Because fish nitrite responses are different among the species, a wider range of nitrite concentrations was previously tested to determine the present range for the LC50 assay.

RESULTS AND DISCUSSION

To date, the maximum nitrite concentration allowed for environmental waters in Brazil is 1.0 ppm pursuant to CONAMA resolution, nº 20 (BRASIL, 1986). Matrinxã was revealed to be very sensitive to nitrite. The 96-h LC50 of nitrite-N to juvenile matrinxã was 0.86 ± 0.05mg L^{-1} (0.57 – 1.30; 95% CI). All fish survived in the control tank. One, two, nine and ten fish died in the 0.2, 0.5,1.0 and 2.0mg L^{-1} $N\text{-}NO_2$ treatments, respectively (Table 1). The range of sensitivity to environmental nitrite is very wide among fish. These differences may be related to environmental adaptations and/or organismal traits (LEWIS & MORRIS, 1986; ARANA, 1997). The 96-h LC50 of nitrite-N of channel catfish ***Ictalurus punctatus*** is 7 mg/L (PALACHEK & TOMASSO, 1984). The rainbow trout ***Onchorinchus mykiss*** tolerates 0.24mg L^{-1} at 10°C whereas ***Onchorinchus tschawytscha*** survives at 4.7mg L^{-1}, and the tolerance of ***Micropterus salmoides*** reaches 140mg L^{-1}. However, the rainbow trout in brackish water tolerates 22mg L^{-1} (RUSSO & THURSTON, 1977). Tambaqui, ***Colossoma macropomum***, a Neotropical fish from Amazonia is very sensitive to nitrite. Its 96-h LC50 is 0.54mg L^{-1} nitrite $N\text{-}NO_2$ (COSTA et al., 2004). There are very few data on the nitrite toxicity in Amazonian fish. The wide biodiversity observed in the Amazon and the potential of the species for aquaculture drive the studies concerning their environmental characteristics very relevant. The level of environmental chloride is important in determining the toxicity of nitrite to fish. Chloride competes with nitrite at the level of chloride cell of the gills (GAINO et al., 1984).

High concentrations of chloride ions reduce the toxicity of nitrite. Seawater fish are usually less

sensitive to nitrite than freshwater fish. The 96-h LC50 of nitrite-N for European eel ***Anguilla anguilla*** can range from 84.0mg L^{-1} in freshwater to 812.0mg L^{-1} in full strength seawater (salinity = 36.0mg L^{-1}) (SAROGLIA et al., 1981). This has been attributed to the ability of some species to exclude the nitrite entry at the gill membrane. Some species lack this ability and actually concentrate nitrite in the plasma when it is present in the environment. This uptake is proposed to be an active mechanism (PERRONE & MEADE, 1977; PALECHEK & TOMASSO, 1984). Another hypothesis assumes that the inter-specific differences in nitrite resistance lie in chloride influx rates (WILLIAMS & EDDY, 1986). Species with higher influx rates have concomitantly higher nitrite uptake rates. These species should be more susceptible to nitrite toxicosis in lower nitrite environmental concentrations. The chloride ion should compete with nitrite uptake at the gill surface. Therefore, the response of freshwater fish to nitrite is very dependent on the type of water (EDDY et al., 1983; GAINO et al., 1984). Our study was conducted in low levels of chloride (0.010 ± 0.0016mM), and they were kept constant at values near the natural freshwater environment. Brazilian continental waters are ion-poor and have low Cl^- concentrations (ESTEVES, 1988). Amazonian waters vary from 0.0006mM in the Negro River to 0.089 mM Cl^- in Solimões River (FURCH, 1984). Most Amazonian rivers possesses low levels of the major ions, and chloride concentration is also low (VAL & HONCZARYK, 1995). Matrinxã originated from very soft water and the high nitrite sensitivity observed in matrinxã was expected.

Other ions also influence the rate of nitrite uptake in fish. The hybrid sunshine bass (female ***Marone chrysops*** x male ***M. saxatilis***) is protected against nitrite toxicity by environmental chloride; however, the addition of 250mg L^{-1} of Ca^{2+} (as chloride) was about seven times more effective to restrain nitrite uptake (WEIRICH et al., 1993). Therefore, concentrations of nitrite and water quality are quite related, and are particularly relevant in fish culture conditions. No relevant data are supplied concerning the effects of environmental nitrite-N on the growth rate of fish, when the concentrations are lower than the 96h LC-50. Nevertheless, the use of chloride in fish culture systems may alleviate toxic effects of nitrite. The 96h LC-50 for nitrite-N established in matrinxã shows it is very sensitive. For that reason special attention must be paid to the critical levels of nitrite in order to prevent acute toxicity for the species in intensive culture systems. Further studies are necessary to investigate the protective effect of Cl^- and other ions such as Ca^{2+} on nitrite toxicity in matrinxã.

Table 1 - Water quality during exposure of juvenile matrinxã to environmental nitrite nitrogen (nitrite-N). Values for hardness, conductivity and ammonia are mean ± SD, and values for pH are ranges.

$N\text{-}NO_2$ (mg/L)	Time (h)	Percent mortality	Hardness (mg/L)	Conductivity ($\mu S/cm^3$)	$NH_3\text{-}NH_4^+$ (mg/L)	pH
Control	0	0	18 ± 0.5	63.7 ± 0.03	0.2 ± 0.01	6.0 – 6.4
	96	0	27 ± 1.1	72.3 ± 0.04	1.7 ± 0.02	6.2 – 6.6
0.2	0	0	25 ± 1.3	66.0 ± 0.04	0.4 ± 0.01	7.8 – 8.0
	96	10	29 ± 1.6	74.3 ± 0.03	1.6 ± 0.02	7.3 – 7.7
0.5	0	0	28 ± 1.2	70.0 ± 0.05	0.3 ± 0.02	7.6 – 7.7
	96	20	29 ± 1.6	76.8 ± 0.02	1.6 ± 0.02	7.6 – 7.8
1.0	0	0	23 ± 0.1	75.0 ± 0.04	0.4 ± 0.02	6.1 – 6.3
	96	90	35 ± 0.9	79.1 ± 0.03	1.2 ± 0.02	6.4 – 6.6
2.0	0	0	23 ± 0.5	86.0 ± 0.04	0.3 ± 0.01	6.5 – 6.7
	96	100	25 ± 1.0	88.3 ± 0.02	0.7 ± 0.02	6.6 – 6.8

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