WATERBORNE CALCIUM AND NITRITE INTERACTION: SURVIVAL, GROWTH, HEMATOLOGICAL AND METABOLIC PARAMETERS IN SILVER CATFISH

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ABSTRACT

The objective of this study was to determine the effect of different waterborne nitrite (NO_2^-) and calcium (Ca^{2+}) levels on growth, biochemical and hematological parameters of silver catfish juvenile (*Rhamdia quelen*). Fish were submitted to low (0.05 mg L^{-1}) or high (1.3 mg L^{-1}) NO_2 and low (7 mg L^{-1}) or high (14 mg L^{-1}) Ca^{2+} levels (four replicates) for 60 days. At the end of the experimental period, fish exposed to high NO_2 showed lower weight gain, biomass and specific growth rate than those maintained at low NO_2, irrespective of Ca^{2+} levels. Fish exposed to high NO_2/low Ca^{2+} presented higher lactate levels in the muscle than control fish, but an increase of waterborne Ca^{2+} levels avoided this increase. Fish kept at high NO_2^-/high Ca^{2+} showed higher lactate levels in the liver than those exposed to low NO_2/high Ca^{2+}. Exposure to high NO_2 or high Ca^{2+} alone reduced hepatic glycogen, protein and glucose levels. Fish kept at high NO_2/high Ca^{2+} presented a decrease in hemoglobin levels compared to those kept at low NO_2/high Ca^{2+}. Therefore, the use of 14 mg L^{-1} Ca^{2+} in water did not minimize the toxicity of nitrite for *Ramdia quelen*.

Key words: biochemical parameters; hardness; nitrogen compound.

INTERAÇÃO DO CÁLCIO E NITRITO NA ÁGUA: SOBREVIVÊNCIA, CRESCIMENTO, PARÂMETROS HEMATOLÓGICOS E METABÓLICOS EM JUNDIÁ

RESUMO

O objetivo deste estudo foi determinar o efeito de diferentes níveis de nitrito (NO_2^-) e cálcio (Ca^{2+}) no crescimento, parâmetros bioquímicos e hematológicos de juvenis de jundiá (*Rhamdia quelen*). Os peixes foram submetidos a níveis baixos (0,05 mg L^{-1}) ou elevados (1,3 mg L^{-1}) de NO_2 e baixos (7 mg L^{-1}) ou elevados (14 mg L^{-1}) de Ca^{2+} (quatro repetições) por 60 dias. No final do período experimental, peixes expostos a alto NO_2^- apresentaram ganho de peso, biomassa e taxa de crescimento específico menores do que aqueles mantidos em baixos níveis de NO_2 independentemente dos níveis de Ca^{2+}. Peixes expostos a alto NO_2/baixo Ca^{2+} apresentaram níveis de lactato mais elevados no músculo do que peixes do grupo controle, mas um aumento dos níveis de Ca^{2+} na água evitou este aumento. Peixes mantidos em alto NO_2/alto Ca^{2+} mostraram níveis de lactato mais elevados no fígado do que aqueles expostos a baixo NO_2/alto Ca^{2+}. A exposição a níveis altos de NO_2 ou Ca^{2+} reduziu os níveis de glicogênio, proteína e glicose hepáticos. Peixes mantidos em alto NO_2/alto Ca^{2+} apresentaram uma diminuição nos níveis de hemoglobina em comparação com aqueles mantidos em baixo NO_2/alto Ca^{2+}. Portanto, a utilização de 14 mg L^{-1} de Ca^{2+} na água não minimizou a toxicidade de nitrito para *R. quelen*.

Palavras-chave: parâmetros bioquímicos; dureza; composto nitrogenado.

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INTRODUCTION

Nitrite (NO_2^-) is produced by the oxidation of ammonia, the main nitrogenous compound excreted by fish, and can reach very high levels in systems with high stocking densities and/or when an imbalance occurs to disrupt the normal function of biological filters in recirculating systems (JENSEN, 2003). Nitrite acts on the oxygen transport process by oxidizing Fe^{2+} to Fe^{3+}, which is unable to bind and carry oxygen (TILAK *et al*., 2007). This causes a modification of hemoglobin configuration, resulting in methemoglobin (MADISON and WANG, 2006), which does not bind oxygen, causing tissue anoxia (JENSEN, 2003; TILAK *et al*., 2007) and lower oxygen uptake (LEFEVRE *et al*.,2011).

Nitrite uptake in fish through the gill membrane is related to branchial Cl^- uptake rates (JENSEN, 2003) because NO_2^- competes with Cl^- in the Cl^-/HCO_3 cotransporter (TOMASSO and GROSELL, 2005). Consequently, the increase in waterborne Cl^- levels reduces NO_2^- toxicity (KROUPOVA *et al*., 2005; YANBO *et al*., 2006; BOUDREAUX *et al*., 2007). Ca^{2+} plays a key role in ion regulation by reducing the permeability of biological membranes and thus the diffusive flow of ions to water (WOOD and MCDONALD, 1988; GONZALEZ, 1996). The increase of waterborne Ca^{2+} can then reduce Cl^- loss in freshwater fish and the activity of the Cl^-/HCO_3 cotransporter, reducing NO_2^- uptake and toxicity. Studies have demonstrated that an increase of waterborne $CaCl_2$ has a stronger effect on reducing acute NO_2^- toxicity than an increase of waterborne NaCl in some species (TOMASSO *et al*., 1980; WEIRICH *et al*., 1993; KROUPOVA *et al*., 2005), but not in others (ATWOOD *et al*., 2001; KROUPOVA *et al*., 2005). However, these studies were conducted with $CaCl_2$ and therefore the presence of Cl^- may have affected the results.

Moreover, no studies have investigated whether waterborne Ca^{2+} may reduce the deleterious effect of NO_2^- on fish growth. Therefore, the aim of the present study was to verify if waterborne Ca^{2+} can protect against the effect of long-term NO_2^- exposure, evaluating growth, biochemical and hematological parameters in the silver catfish (*Rhamdia quelen*), the main native species raised in South Brazil (BALDISSEROTTO, 2009).

METHODS

Fish and experimental design

Silver catfish (8.9±0.2 g and 15.0±0.8 cm, voucher nº. UFRGS 20413) 160 animals were randomly distributed in a recirculating aquaculture system (10 fish per tank) containing 16 continuously aerated polypropylene tanks (40 L). A 12/12 light/dark photoperiod was used. After 15 days of acclimation, fish were submitted to four treatments with two NO_2^- x two Ca^{2+} levels: low NO_2^-/low Ca^{2+}(control) − 0.05 mg L^{-1} NO_2^- + 7mg L^{-1} Ca^{2+}; low NO_2^-/high Ca^{2+}− 0.05 mg L^{-1} NO_2^- + 14mg L^{-1} Ca^{2+}; high NO_2^-/low Ca^{2+}−1.3 mg L^{-1} NO_2^- + 7mg L^{-1} Ca^{2+}; high NO_2^-/high Ca^{2+}−1.3 mg L^{-1} NO_2^- + 14mg L^{-1} Ca^{2+} (four replicates each) for 60 days. The high NO_2^- and Ca^{2+} levels were obtained by addition of sodium nitrite ($NaNO_2$) and calcium carbonate ($CaCO_3$) to the water. The high NO_2^- level chosen is close to levels that provoked silver catfish mortality within 20−40 days (LIMA *et al*., 2011). The high Ca^{2+} level chosen reduced the deleterious effect of acidic water (COPATTI *et al*., 2011a, 2011b) and high ammonia (FERREIRA *et al*., 2013) on silver catfish growth.

Throughout the acclimation and experimental periods, fish were fed twice daily to satiety with Supra juvenile (32% crude protein and maximum 2.0% Ca^{2+} according to manufacturer). Feces and residues were removed daily by siphoning, and 80% of the water of the recirculation system was replaced with water containing NO_2^- and Ca^{2+} levels previously adjusted to experimental values, mainly to maintain NO_2^- levels within the expected range. Fish were fasted for 24 h and were then sedated with eugenol 40µL L^{-1} for 3 min (CUNHA *et al*., 2010) before each biometry (0, 30 and 60 days). The methodology of this study was approved by the Ethics Committee and Animal Welfare Committee of the Universidade Federal de Santa Maria (process n. 108/2014).

Water quality parameters

Dissolved oxygen levels, temperature (Y5512 oximeter YSI Inc. Yellow Springs, USA) and pH (pHmeter DMPH-2, Digimed, São Paulo, Brazil) were determined daily. Temperature in the laboratory was kept constant by an air conditioner. Nitrite and total ammonia levels were determined daily according to BOYD (1998) and VERDOUW *et al*. (1977) respectively. Un-ionized ammonia levels were calculated according to COLT (2002), and water hardness and total alkalinity levels were calculated weekly following Eaton et al. (2005). Waterborne Na^+, K^+ and Ca^{2+} levels were measured using a Micronal B286 flame photometer (São Paulo, Brazil) and Cl- levels were measured according to ZALL *et al*. (1956).

Growth parameters

Growth parameters evaluated were: survival (%) = number of fish at the end of each analyzed period/initial fish number x 100; weight gain (WG,

g) = final weight (g) - initial weight (g); biomass = average weight (g) x number of fish at the end of each analyzed period; specific growth rate (SGR, %) = 100 x [(ln final weight (g) - ln initial weight (g)/time (days)]; food intake (FI) =quantity of food consumed (g)/number of fish at the end of each analyzed period; apparent food conversion (AFC) = food provided (g)/weight gain (g).

Hematological and biochemical parameters

Eight fish per treatment (n=8) were collected at the end of the experimental period (60 days) and sedated with eugenol 40µL L^{-1} for 3 min (CUNHA *et al.*, 2010). Blood was collected from the caudal vein with heparinized syringes and stored in Eppendorf tubes. The hematocrit (HT) was assessed by the microhematocrit method after blood centrifugation for 5 min at 3800 g. The hemoglobin (Hb) concentration was determined by the method of cyanomethemoglobin with a commercial kit (Bioclin) after centrifuging the mixture to remove free cores of erythrocytes. Erythrocyte count was performed in a Neubauer chamber after 1:200 dilutions in Natt and Herrick solution. Blood smears were stained with "Romanowsky" for white blood cell count, and leukocyte differential was performed using an indirect method by counting the total leukocyte number in 2000 cells in the smears (THRALL, 2006). The blood was centrifuged at 1500 g for 5 min at 4°C in a refrigerated centrifuge to obtain plasma. The plasma protein levels were determined using a colorimetric kit (Bio Tecnica®, Varginha, MG, Brazil), and absorbance readings were performed using a spectrophotometer (Biospectro SP-220; Curitiba, PR, Brazil).

Fish were then euthanized by section of the spinal cord. Samples of liver and muscle were frozen and stored at -20°C for further analysis. Liver and muscle glycogen were determined according to BIDINOTTO *et al*. (1997) and protein according to LOWRY *et al*. (1951). Tissue samples were homogenized with 10% trichloroacetic acid using a Potter-Elvejhem homogenizer and centrifuged at 1000 xg for 10 min. The supernatant was used to determine the levels of lactate (HARROWER and BROWN, 1972) and glucose (PARK and JOHNSON, 1949).

Statistical analysis

The homogeneity of variances and normality were verified by Levene and Kolmogorov-Smirnov's tests, respectively. Data were analyzed by a two-way ANOVA (NO_2^- x Ca^{2+} levels), followed by the Tukey test, and the minimum significance level was $p<0.05$. The analysis was performed using Statistica software (version 7.1). Data were presented as mean ± SEM.

RESULTS

Water quality parameters were within the expected values for silver catfish, while NO_2^- and Ca^{2+}levels were according to the treatments (Table 1). After 30 and 60 days, fish maintained at high NO_2^-/ high Ca^{2+} presented significantly lower feed intake than those kept at low NO_2^-/high Ca^{2+}. At 60 days, fish exposed to high NO_2^- showed significantly lower weight gain, biomass and specific growth rate than those maintained at low NO_2^-, irrespective of Ca^{2+} levels. Survival and feed conversion rate were not affected significantly by any treatment (Table 2).

Table 1. Physicochemical parameters of water in the experimental tanks.

Parameters	Treatments			
	low NO_2^-/ low Ca^{2+}(C)	low NO_2^-/ high Ca^{2+}	high NO_2^-/ low a^{2+}	high NO_2^-/ high Ca^{2+}
pH	7.48±0.0	7.19±0.1	7.49±0.03	7.10±0.02
Dissolved oxygen (mg/L)	7.8±0.02	7.7±0.04	7.5±0.02	7.5±0.02
Temperature (°C)	22.6±0.1	22.6±0.1	22.6±0.2	22.7±0.1
Un-ionized ammonia (mg L^{-1})	0.012±0.001	0.011±0.001	0.011±0.001	0.010±0.001
Total ammonia nitrogen (mg L^{-1})	0.46±0.017	0.51±0.006	0.50±0.022	0.49±0.014
Nitrite (mg L^{-1})	$0.045±0.001^{aA}$	$0.046±0.003^{aA}$	$1.308±0.002^{aB}$	$1.242±0.009^{aB}$
Hardness (mg $CaCO_3$ L^{-1})	$20.9±0.001^{aA}$	$44.9±0.001^{bA}$	$25.3±0.001^{aA}$	$40.4±0.001^{bA}$
Alkalinity (mg $CaCaCO_3$ L^{-1})	38.25±0.2	48.12±0.2	38.75±0.3	46.37±0.2
Ca^{2+} (mg L^{-1})	$7.0±0.1^{aA}$	$16.5±0.1^{bA}$	$8.7±0.1^{aA}$	$14.8±0.1^{bA}$
Na^+ (mg L^{-1})	44.9±4.3	38.7±0.8	36.3±0.4	39.6±0.4
K^+ (mg L^{-1})	16.3±1.1	14.5±0.2	13.6±0.4	14.0±0.3
Cl^- (mg L^{-1})	94.1±0.1	138.5±18.7	136.7±6.7	97.3±7.6

Control (C): 0.05 mg L^{-1} NO_2^- + 7 mg L^{-1} Ca^{2+}; low NO_2^-/high Ca^{2+}: 0.05 mg L^{-1} NO_2^- + 14 mg L^{-1} Ca^{2+}; high NO_2^-/low Ca^{2+}: 1.3 mg L^{-1} NO_2^- + 7 mg L^{-1} Ca^{2+}; high NO_2^-/high Ca^{2+}: 1.3 mg L^{-1} NO_2^- + 14 mg L^{-1} Ca^{2+}. Data as mean ± SEM (n=4).
Different lowercase letters in the same row indicate statistically significant differences ($P< 0.05$) between Ca^{2+} levels at the same nitrite level. Different capital letters in the same row indicate statistically significant differences ($P<0.05$) between NO_2^- levels at the same calcium level. Two-way ANOVA and Tukey test ($p<0.05$).

Table 2. Survival and growth parameters of *Rhamdia quelen* juveniles exposed to different waterborne nitrite and calcium levels for 30 and 60 days.

	Treatments			
Days	low NO_2^-/ low Ca^{2+}(C)	low NO_2^-/ high Ca^{2+}	high NO_2^-/ low Ca^{2+}	high NO_2^-/ high Ca^{2+}
		Survival (%)		
30	97.5±2.5	87.5±4.7	77.5±9.4	87.5±7.5
60	95±2.8	87.5±4.7	70±8.1	85± 8.6
		Weight gain (g)		
30	7.51±0.75	9.23±1.84	3.64±0.47	4.08±0.67
60	21.27±2.74^{aA}	26.90±3.89^{aA}	10.29±0.83^{aB}	9.75±1.82^{aB}
		Biomass (g)		
30	159.2±7.7	166.1±25.3	98.8±11.3	106.8±12.5
60	285.5±22.6^{aA}	322.4±51.2^{aA}	136.6±19.1^{aB}	151.5±18.3^{aB}
		Specific growth rate (%)		
30	2.04±0.16	2.23±0.33	1.11±0.14	1.31±0.19
60	2.02±0.16^{aA}	2.22±0.19^{aA}	1.25±0.08^{aB}	1.28±0.17^{aB}
		Feed intake (g)		
30	6.73±0.48^{aA}	10.06±1.34^{aA}	6.58±1.05^{aA}	5.55±0.66^{aB}
60	21.3±2.61^{aA}	26.90±3.93^{aA}	10.3±1.41^{aA}	9.75±1.62^{aB}
		Feed conversion rate		
30	0.90±0.04	1.13±0.09	1.81±0.17	1.56±0.38
60	1.12±0.18	0.99±0.01	1.33±0.05	1.30±0.23

Control (C): 0.05 mg L^{-1} NO_2^- + 7 mg L^{-1} Ca^{2+}; low NO_2^-/high Ca^{2+}: 0.05 mg L^{-1} NO_2^- + 14 mg L^{-1} Ca^{2+}; high NO_2^-/low Ca^{2+}: 1.3 mg L^{-1} NO_2^- + 7 mg L^{-1} Ca^{2+}; high NO_2^-/high Ca^{2+}: 1.3 mg L^{-1} NO_2^- + 14 mg L^{-1} Ca^{2+}. Data as mean ± SEM (n=4). Different lowercase letters in the same row indicate statistically significant differences (P<0.05) between Ca^{2+} levels at the same nitrite level. Different capital letters in the same row indicate statistically significant differences (P<0.05) between NO_2^- levels at the same calcium level. Two-way ANOVA and Tukey test (p<0.05).

Silver catfish at high NO_2^-/high Ca^{2+} presented a significant decrease in hemoglobin levels compared to those kept at low NO_2^-/high Ca^{2+}. The other analyzed hematological parameters did not differ significantly between treatments (Table 3).Silver catfish exposed to high NO_2^-/low Ca^{2+} presented significantly higher lactate levels in muscle than control fish, and increased waterborne Ca^{2+} levels avoided this effect. Fish kept at high NO_2^-/high Ca^{2+} showed significantly higher lactate levels in the liver than those exposed to low NO_2^-/high Ca^{2+}. Exposure to high NO_2^-/low Ca^{2+} or low NO_2^-/high Ca^{2+} significantly reduced hepatic glycogen, protein and glucose levels. Hepatic protein levels were more reduced in fish kept at high NO_2^-/high Ca^{2+} compared to those maintained at low NO_2^-/high Ca^{2+}. Silver catfish exposed to high NO_2^-/low Ca^{2+} significantly increased glycogen and reduced glucose in the muscle. The increase of waterborne Ca^{2+} (high NO_2^-/high Ca^{2+} group) avoided this glycogen alteration but, also in the muscle, there was reduced protein compared to the low NO_2^-/high Ca^{2+} group and reduced glucose compared to the high NO_2^-/high Ca^{2+} group (Table 4).

Table 3. Hematological parameters of *Rhamdia quelen* juveniles exposed to different waterborne nitrite and calcium levels for 60 days.

Parameters	Treatments			
	low NO_2^-/ low Ca^{2+}(C)	low NO_2^-/ high Ca^{2+}	high NO_2^-/ low Ca^{2+}	high NO_2^-/ high Ca^{2+}
Hematocrit (%) RBC (x 10^6 µL $^{-1}$)	26.7± 2.9 146.7± 23.9	28.0± 1.2 159.5± 8.2	25.7± 1.0 161.7± 9.9	22.0± 2.3 127.0± 12.9
Hemoglobin (g dL^{-1})	5.3± 0.6^{aA}	6.2± 0.5^{aA}	4.9± 0.2^{aA}	4.1± 0.4^{aB}
MCV (fL)	188.6±13.6	176.2± 7.5	160.2± 8.1	175.1± 15.4
MCHC (g dL^{-1})	20.0±1.4	22.2±1.4	19.1±0.1	19.1±1.8
Leukocytes (x 10^3 µL^{-1})	12.7±1.8	13.9±0.8	13.4±2.3	9.5±2.4
TPP (g dL^{-1})	5.1±0.3	4.9±0.09	5.4±0.2	5.0±0.1

Continuação Tabela 3.

Lymphocytes (%)	58.0±5.5	44.0±2.2	56.0±7.5	53.5±6.6
Neutrophils (%)	35.2±6.0	50.7±3.0	36.0±6.5	36.5±5.6
Monocytes (%)	6.7±1.2	5.2±0.8	8.0±1.4	10.0±3.1
Eosinophils (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

RBC: Number of red blood cells, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, TPP: Total plasma protein. Control (C): 0.05 mg L-1 NO2- + 7 mg L-1 Ca2+, low NO2-/high Ca2+: 0.05 mg L-1 NO2- + 14 mg L-1 Ca2+; high NO2-/ low Ca2+: 1.3 mg L-1 NO2- + 7 mg L-1 Ca2+; high NO2-/high Ca2+: 1.3 mg L-1 NO2- + 14 mg L-1 Ca2+. Data as mean ± SEM (n=8). Different lowercase letters in the same row indicate statistically significant differences (P< 0.05) between Ca2+ levels at the same nitrite level. Different capital letters in the same row indicate statistically significant differences (P<0.05) between NO2- levels at the same calcium level Two-way ANOVA and Tukey test (p<0.05).

Table 4. Metabolic parameters of liver and muscle of *Rhamdia quelen* juveniles exposed to different waterborne nitrite and calcium levels in water for 60 days.

Parameters	Treatments			
	low NO_2^-/ low Ca^{2+}(C)	low NO_2^-/ high Ca^{2+}	high NO_2^-/ low Ca^{2+}	high NO_2^-/ high Ca^{2+}
		Liver		
Lactate (µmol g^{-1})	2.2±0.3^{aA}	2.3±0.1^{aA}	3.4±0.5^{aA}	4.2±0.4^{aB}
Glycogen (µmol g^{-1})	40.6±2.3^{aA}	25.2±1.1^{bA}	30.7±3.0^{aB}	26.48±2.1^{bA}
Protein (mg g^{-1})	415.5±39.0^{aA}	257.8±21.4^{bA}	209.5±22.6^{aB}	184.8±9.8^{aB}
Glucose (µmol g-^{1})	161.4±10.6^{aA}	75.6 ±11.3^{bA}	46.7±6.0^{aB}	58.0±5.9^{aA}
		Muscle		
Lactate (µmol g^{-1})	23.6±1.0^{aA}	28.2±2.1^{aA}	35.1±2.1^{aB}	30.0±1.5^{aA}
Glycogen (µmol g-^{1})	1.7±0.2^{aA}	1.3±0.1^{aB}	2.7±0.3^{aA}	2.0±0.2^{bA}
Protein (mg g^{-1})	256.4±18.5^{aA}	285.3±12.5^{aA}	293.3±35.4^{aA}	148.9±6.4^{aB}
Glucose (µmol g-^{1})	17.5±2.6^{aA}	13.3±0.9^{aA}	4.9±0.5^{aB}	2.9±0.1^{bB}

Control (C): 0.05 mg L^{-1} NO_2^- + 7 mg L^{-1} Ca^{2+}; low NO_2^-/high Ca^{2+}: 0.05 mg L^{-1} NO_2^- + 14 mg L^{-1} Ca^{2+}; high NO_2^-/low Ca^{2+}: 1.3 mg L^{-1} NO_2^- + 7 mg L^{-1} Ca^{2+}; high NO_2^-/high Ca^{2+}: 1.3 mg L^{-1} NO_2^- + 14 mg L^{-1} Ca^{2+}. Data as mean ± SEM (n=8). Different lowercase letters in the same row indicate statistically significant differences (P< 0.05) between Ca^{2+} levels at the same nitrite level. Different capital letters in the same row indicate statistically significant differences (P< 0.05) between NO_2^- levels at the same calcium level. Two-way ANOVA and Tukey test (p<0.05).

DISCUSSION

The present study demonstrated that exposure to 1.3 mg L^{-1} NO_2^- reduced growth of silver catfish and the increase of Ca^{+2} levels did not minimize toxicity. A previous study showed that 100% mortality was observed in silver catfish maintained at 1.52 mg L^{-1} NO_2^-, but that exposure to levels of up to 1.19 mg L^{-1} NO_2^- did not affect survival or growth (LIMA *et al*., 2011). Therefore, silver catfish has a limited NO_2^- concentration range from reduced growth to total mortality. Similar results have been found in other fish: rainbow trout (*Oncorhynchus mykiss*) exposed to 1.0 mg L^{-1} NO_2^- showed no change in growth, but at 3.0 mg L^{-1} NO_2^-, growth was reduced, and 65% mortality was found after 28 days (KROUPOVA *et al*., 2008). Furthermore, channel catfish (*Ictalurus punctatus*) showed reduced growth at 1.6 mg L^{-1} NO_2^- and mortality started at 3.71 mg L^{-1} NO_2^- (COLT *et al*., 1981). On the other hand, silver perch (*Bidyanus bidyanus*) exposed to 1.43 mg L^{-1} NO_2^- had reduced growth, but survival was not affected at 16.2 mg L^{-1} (higher levels were not tested) (FRANCES *et al*., 1998). This demonstrates limited NO_2^- concentration range to induce reduced growth and to provoke mortality in several species. The increase of Ca^{+2} levels at low NO_2^- levels did not change silver catfish growth, in agreement with COPATTI *et al*. (2011a), that showed that exposure of silver catfish up to 180 mg $CaCO_3$ L^{-1} at pH 7.0 did not change growth compared to lower water hardness.

Nitrite penetrates red blood cells and oxidizes

iron, transforming hemoglobin to methemoglobin, which does not bind oxygen (KROUPOVA *et al*., 2008; WUERTZ *et al*., 2013). Matrinxã (*Brycon amazonicus*), *Labeo rohita*, walleye (*Sander vitreus*) and rainbow trout (*Oncorhynchus mykiss*) exposed to high NO_2^- levels presented lower hematocrit, total hemoglobin, and number of red blood cells than control fish (AVILEZ *et al*., 2004; MADISON and WANG, 2006; KROUPOVA *et al*., 2008; CIJI *et al*., 2013). Conversely, exposure to 1.3 mg L^{-1} NO_2^- did not affect hematological parameters in silver catfish. It is likely that these parameters are affected in silver catfish only at lethal NO_2^- levels, because WUERTZ *et al*. (2013) showed that pike-perch (*Sander lucioperca*) significantly increased methemoglobin levels after exposure to 3.5 mg L^{-1} NO_2^- for 32 days, while safe NO_2^- levels for growth were 0.061 mg L^{-1}. Besides inducing methemoglobin formation, high NO_2^- levels have also provoked hyperplasia of the lamellar epithelium in rainbow trout (KROUPOVA *et al*., 2008), but not in silver perch (FRANCES *et al*., 1998). This change in lamellar epithelium and methemoglobin may contribute to tissue hypoxia, thereby increasing anaerobic metabolism (AVILEZ *et al*., 2012). Turbot (*Scophthalmus maximus*) exposed to high NO_2^- levels increased plasma glucose and cortisol levels, probably as a response to hypoxia stress. The higher lactate levels in the muscle (present study) and liver (LIMA *et al*., 2011) of silver catfish exposed to high NO_2^- levels indicate tissue hypoxia. Consequently, the lower hepatic and muscular glucose levels and lower hepatic glycogen observed in silver catfish exposed to high NO_2^- levels (present study and LIMA *et al*., 2011) may be due to release of carbohydrate stores to the blood to provide energy to cope with hypoxia. Glycogen mobilization was proposed as the preferential metabolism reaction assumed in *Hoplias malabaricus* and *B. amazonicus* exposed to high NO_2^- levels (MORAES *et al*., 1998; AVILEZ *et al*., 2012). Overall, liver and muscle protein content reduces when anaerobic metabolism is used, because protein synthesis is one of the main energy consuming processes, accounting for 18–26% of cellular energy costs (HAWKINS, 1991). Nitrite exposure reduced serum protein, albumin and globulin levels in *L. rohita*, which may be related to the use of proteins to meet the increased energetic demand (CIJI *et al*., 2014). This, alongside avoiding spending energy in protein synthesis, may also be the reason for the reduction of hepatic protein seen in silver catfish exposed to high NO_2^- levels.

Despite the protective effect of the high Ca^{2+} level used in the present study against acidic water (COPATTI *et al*., 2011a, 2011b) and high ammonia (FERREIRA *et al*., 2013) on silver catfish growth, it was ineffective against the effect of high NO_2^- levels on growth and most biochemical parameters of this species. Hypoxia inhibited Ca^{2+} uptake in zebrafish, *Danio rerio* (KWONG *et al*., 2016), and consequently the exposure of silver catfish to high Ca^{2+} levels may facilitate the maintenance of plasma Ca^{2+} levels and partially reduce the effect of tissue hypoxia (higher muscle lactate levels) provoked by high NO_2^- levels. The high Ca^{2+} level alone was sufficient to reduce hepatic carbohydrate and protein levels of silver catfish in the present study. Previous work has shown that silver catfish exposed to 120 mg $CaCO_3$ L^{-1} (three-fold higher Ca^{2+} level of the present study) for five days presented higher plasma glucose, lactate and triglyceride levels than those maintained at 25 mg $CaCO_3$ L^{-1} (BALDISSEROTTO *et al*., 2014) (similar conditions to the control group of the present study), suggesting that high Ca^{2+} levels can induce some metabolic changes that apparently are not enough to alter growth.

CONCLUSIONS

In conclusion, contrary to expectation, the use of 14 mg L^{-1} Ca^{2+} in the water did not minimize nitrite effects on growth and biochemical parameters of silver catfish (*Rhamdia quelen*).

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