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

Gabriel Cardoso NEVES¹, Carine de Freitas SOUZA², Alessandro Casale dos SANTOS², Bernardo BALDASSEROTTO², Jane Mello LOPES³

ABSTRACT
The objective of this study was to determine the effect of different waterborne nitrite (NO₂⁻) and calcium (Ca²⁺) 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) NO₂⁻ and low (7 mg L⁻¹) or high (14 mg L⁻¹) Ca²⁺ levels (four replicates) for 60 days. At the end of the experimental period, fish exposed to high NO₂⁻ showed lower weight gain, biomass and specific growth rate than those maintained at low NO₂⁻ irrespective of Ca²⁺ levels. Fish exposed to high NO₂⁻/low Ca²⁺ presented higher lactate levels in the muscle than control fish, but an increase of waterborne Ca²⁺ levels avoided this increase. Fish kept at high NO₂⁻/high Ca²⁺ showed higher lactate levels in the liver than those exposed to low NO₂⁻/high Ca²⁺. Exposure to high NO₂⁻ or high Ca²⁺ alone reduced hepatic glycogen, protein and glucose levels. Fish kept at high NO₂⁻/high Ca²⁺ presented a decrease in hemoglobin levels compared to those kept at low NO₂⁻/high Ca²⁺. Therefore, the use of 14 mg L⁻¹ Ca²⁺ in water did not minimize the toxicity of nitrite for R. 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 (NO₂⁻) e cálcio (Ca²⁺) 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 NO₂⁻ e baixos (7 mg L⁻¹) ou elevados (14 mg L⁻¹) de Ca²⁺ (quatro repetições) por 60 dias. No final do período experimental, peixes expostos a NO₂⁻ apresentaram ganho de peso, biomassa e taxa de crescimento específico menores do que aqueles mantidos em baixos níveis de NO₂⁻, independentemente dos níveis de Ca²⁺. Peixes expostos a NO₂⁻/baixo Ca²⁺ apresentaram níveis de lactato mais elevados no músculo do que os pesquisadores em baixos níveis de NO₂⁻, independentemente dos níveis de Ca²⁺. Peixes mantidos em NO₂⁻/baixo Ca²⁺ mostraram níveis de lactato mais elevados no fígado do que aqueles expostos a NO₂⁻/alto Ca²⁺. A exposição a níveis altos de NO₂⁻ ou Ca²⁺ reduziu os níveis de glicogênio, proteína e glicose hepáticas. Peixes mantidos em NO₂⁻/alto Ca²⁺ apresentaram uma diminuição nos níveis de hemoglobina em comparação com aqueles expostos em baixo NO₂⁻/alto Ca²⁺. Portanto, a utilização de 14 mg L⁻¹ de Ca²⁺ na água não minimizou a toxicidade de nitrito para R. quelen.

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

Nitrite (NO\textsubscript{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\textsuperscript{2+} to Fe\textsuperscript{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\textsuperscript{-} uptake rates (JENSEN, 2003) because NO\textsubscript{2} competes with Cl\textsuperscript{-} in the Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} cotransporter (TOMASSO and GROSELL, 2005). Consequently, the increase in waterborne Cl\textsuperscript{-} levels reduces NO\textsubscript{2} toxicity (KROUPOVA et al., 2005; YANBO et al., 2006; BOUDREAXUS et al., 2007). Ca\textsuperscript{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\textsuperscript{2+} can then reduce Cl\textsuperscript{-} loss in freshwater fish and the activity of the Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} cotransporter, reducing NO\textsubscript{2} uptake and toxicity. Studies have demonstrated that an increase of waterborne CaCl\textsubscript{2} has a stronger effect on reducing acute NO\textsubscript{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\textsubscript{2} and therefore the presence of Cl\textsuperscript{-} may have affected the results.

Moreover, no studies have investigated whether waterborne Ca\textsuperscript{2+} may reduce the deleterious effect of NO\textsubscript{2} on fish growth. Therefore, the aim of the present study was to verify if waterborne Ca\textsuperscript{2+} can protect against the effect of long-term NO\textsubscript{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\textsubscript{2} x two Ca\textsuperscript{2+} levels: low NO\textsubscript{2}/low Ca\textsuperscript{2+}(control) − 0.05 mg L\textsuperscript{-1} NO\textsubscript{2} + 7 mg L\textsuperscript{-1} Ca\textsuperscript{2+}; low NO\textsubscript{2}/high Ca\textsuperscript{2+}− 0.05 mg L\textsuperscript{-1} NO\textsubscript{2} + 14 mg L\textsuperscript{-1} Ca\textsuperscript{2+}; high NO\textsubscript{2}/low Ca\textsuperscript{2+}−1.3 mg L\textsuperscript{-1} NO\textsubscript{2} + 7 mg L\textsuperscript{-1} Ca\textsuperscript{2+}; high NO\textsubscript{2}/high Ca\textsuperscript{2+}−1.3 mg L\textsuperscript{-1} NO\textsubscript{2} + 14 mg L\textsuperscript{-1} Ca\textsuperscript{2+} (four replicates each) for 60 days. The high NO\textsubscript{2} and Ca\textsuperscript{2+} levels were obtained by addition of sodium nitrite (NaNO\textsubscript{2}) and calcium carbonate (CaCO\textsubscript{3}) to the water. The high NO\textsubscript{2} level chosen is close to levels that provoked silver catfish mortality within 20–40 days (LIMA et al., 2011). The high Ca\textsuperscript{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\textsuperscript{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\textsubscript{2} and Ca\textsuperscript{2+} levels previously adjusted to experimental values, mainly to maintain NO\textsubscript{2} levels within the expected range. Fish were fasted for 24 h and were then sedated with eugenol 40µL L\textsuperscript{-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\textsuperscript{+}, K\textsuperscript{+} and Ca\textsuperscript{2+} levels were measured using a Micronal B286 flame photometer (São Paulo, Brazil) and Cl\textsuperscript{-} 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,
RESULTS

Water quality parameters were within the expected values for silver catfish, while NO$_3^-$ and Ca$^{2+}$ levels were according to the treatments (Table 1). After 30 and 60 days, fish maintained at high NO$_3^-$/high Ca$^{2+}$ presented significantly lower feed intake than those kept at low NO$_3^-$/high Ca$^{2+}$. At 60 days, fish exposed to high NO$_3^-$ showed significantly lower weight gain, biomass and specific growth rate than those maintained at low NO$_3^-$, irrespective of Ca$^{2+}$ levels. Survival and feed conversion rate were not affected significantly by any treatment (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>low NO$_3^-$ / low Ca$^{2+}$ (C)</td>
</tr>
<tr>
<td>pH</td>
<td>7.48±0.0</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>7.8±0.02</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22.6±0.1</td>
</tr>
<tr>
<td>Un-ionized ammonia (mg L$^{-1}$)</td>
<td>0.012±0.001</td>
</tr>
<tr>
<td>Total ammonia nitrogen (mg L$^{-1}$)</td>
<td>0.46±0.017</td>
</tr>
<tr>
<td>Nitrite (mg L$^{-1}$)</td>
<td>0.045±0.001</td>
</tr>
<tr>
<td>Hardness (mg CaCO$_3$ L$^{-1}$)</td>
<td>20.9±0.011</td>
</tr>
<tr>
<td>Alkalinity (mg CaCaCO$_3$ L$^{-1}$)</td>
<td>38.25±0.2</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mg L$^{-1}$)</td>
<td>7.0±0.1</td>
</tr>
<tr>
<td>Na$^+$ (mg L$^{-1}$)</td>
<td>44.9±0.3</td>
</tr>
<tr>
<td>K$^+$ (mg L$^{-1}$)</td>
<td>16.3±0.1</td>
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<tr>
<td>Cl$^-$ (mg L$^{-1}$)</td>
<td>94.1±0.1</td>
</tr>
</tbody>
</table>

Control (C): 0.05 mg L$^{-1}$ NO$_3^-$ + 7 mg L$^{-1}$ Ca$^{2+}$; low NO$_3^-$/high Ca$^{2+}$: 0.05 mg L$^{-1}$ NO$_3^-$ + 14 mg L$^{-1}$ Ca$^{2+}$; high NO$_3^-$/low Ca$^{2+}$: 1.3 mg L$^{-1}$ NO$_3^-$ + 7 mg L$^{-1}$ Ca$^{2+}$; high NO$_3^-$/high Ca$^{2+}$: 1.3 mg L$^{-1}$ NO$_3^-$ + 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$_3^-$ levels at the same calcium level. Two-way ANOVA and Tukey test (p<0.05).

Waterborne calcium and nitrite interaction...  

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Days</th>
<th>low NO$_2$/ low Ca$^{2+}$ (C)</th>
<th>low NO$_2$/ high Ca$^{2+}$</th>
<th>high NO$_2$/ low Ca$^{2+}$</th>
<th>high NO$_2$/ high Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>97.5±2.5</td>
<td>97.5±4.7</td>
<td>77.5±9.4</td>
<td>87.5±7.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>95±2.8</td>
<td>87.5±4.7</td>
<td>70±8.1</td>
<td>85±8.6</td>
<td></td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.51±0.75</td>
<td>9.23±1.84</td>
<td>3.64±0.47</td>
<td>4.08±0.67</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>21.27±2.74$^{aA}$</td>
<td>26.90±3.89$^{aA}$</td>
<td>10.29±0.83$^{aA}$</td>
<td>9.75±1.82$^{aA}$</td>
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</tr>
<tr>
<td>Biomass (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>159.2±7.7</td>
<td>166.1±25.3</td>
<td>98.8±11.3</td>
<td>106.8±12.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>285.5±22.6$^{aA}$</td>
<td>322.4±51.2$^{aA}$</td>
<td>136.6±19.1$^{aA}$</td>
<td>151.5±18.3$^{aA}$</td>
<td></td>
</tr>
<tr>
<td>Specific growth rate (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.04±0.16</td>
<td>2.23±0.33</td>
<td>1.11±0.14</td>
<td>1.31±0.19</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2.02±0.16$^{aA}$</td>
<td>2.22±0.19$^{aA}$</td>
<td>1.25±0.08$^{aA}$</td>
<td>1.28±0.17$^{aA}$</td>
<td></td>
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<tr>
<td>Feed intake (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>30</td>
<td>6.73±0.48$^{aA}$</td>
<td>10.06±1.34$^{aA}$</td>
<td>6.58±1.05$^{aA}$</td>
<td>5.55±0.66$^{aA}$</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>21.3±2.61$^{aA}$</td>
<td>26.90±3.93$^{aA}$</td>
<td>10.3±1.41$^{aA}$</td>
<td>9.75±1.62$^{aA}$</td>
<td></td>
</tr>
<tr>
<td>Feed conversion rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.90±0.04</td>
<td>1.13±0.09</td>
<td>1.81±0.17</td>
<td>1.56±0.38</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.12±0.18</td>
<td>0.99±0.01</td>
<td>1.33±0.05</td>
<td>1.3±0.23</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>low NO$_2$/ low Ca$^{2+}$ (C)</th>
<th>low NO$_2$/ high Ca$^{2+}$</th>
<th>high NO$_2}$/ low Ca$^{2+}$</th>
<th>high NO$_2$/ high Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%) RBC (x 10$^3$ μL$^{-1}$)</td>
<td></td>
<td>26.7±2.9</td>
<td>146.7±23.9</td>
<td>28.0±1.2</td>
<td>159.5±8.2</td>
</tr>
<tr>
<td>Hemoglobin (g dL$^{-1}$)</td>
<td></td>
<td>5.3±0.6$^{aA}$</td>
<td>6.2±0.5$^{aA}$</td>
<td>4.9±0.2$^{aA}$</td>
<td>4.1±0.4$^{aA}$</td>
</tr>
<tr>
<td>MCHC (g dL$^{-1}$)</td>
<td></td>
<td>188.6±13.6</td>
<td>176.2±7.5</td>
<td>160.2±8.1</td>
<td>175.1±15.4</td>
</tr>
<tr>
<td>MCHC (g dL$^{-1}$)</td>
<td></td>
<td>20.0±1.4</td>
<td>22.2±1.4</td>
<td>19.1±1.0</td>
<td>19.1±1.8</td>
</tr>
<tr>
<td>Leukocytes (x 10$^6$ μL$^{-1}$)</td>
<td></td>
<td>12.7±1.8</td>
<td>13.9±0.8</td>
<td>13.4±2.3</td>
<td>9.5±2.4</td>
</tr>
<tr>
<td>TPP (g dL$^{-1}$)</td>
<td></td>
<td>5.1±0.3</td>
<td>4.9±0.09</td>
<td>5.4±0.2</td>
<td>5.0±0.1</td>
</tr>
</tbody>
</table>

had reduced ±0.5 ±9.8 ±0.1 ±6.4 at pH 7.0 did not change growth ±12.5 ±6.0 -1 58.0 / high Ca Ca NO NO2 2.0 -1 161.4 Metabolic parameters of liver and muscle of /low Ca: 0.05 mg L NO 2 2 (higher levels were not tested) (FRANCES levels at the same calcium level. 36.0±6.5 ±0.4 ±18.5 2 0.0±0.0 8.0±1.4 NO NO2 0.0±0.0 COLT 2 2+ 14 mg L did not affect survival or growth (LIMA; high NO 2.3 ±2.1 35.1 285.3 2 1.3 mg L NO L 2 44.0 2 0.0±0.0 148.9 2 3.4 NO 2: 1.3 mg L 2 2+ levels did not change silver catfish 293.3 35.2±6.0 ±5.5 (C): 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 NO2- 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 | | --- | --- | --- | --- | --- | --- | Liver | | low NO2/ low Ca2+(C) | low NO2/ high Ca2+ | high NO2/ low Ca2+ | high NO2/ high Ca2+ | | Lactate (μmol g-1) | 2.2±0.3a | 2.3±0.1a | 3.4±0.5a | 4.2±0.4a | | Glycogen (μmol g-1) | 40.6±2.3A | 25.2±1.1B | 30.7±3.0a | 26.4±2.1a | | Protein (mg g-1) | 415.5±39.0A | 257.8±21.4A | 209.5±22.6a | 184.8±9.8a | | Glucose (μmol g-1) | 161.4±10.6A | 75.6±11.3Ab | 46.7±6.0a | 58.0±5.9a | | | Muscle | | | | Lactate (μmol g-1) | 23.6±1.0A | 28.2±2.1Ab | 35.1±2.1a | 30.0±1.5a | | Glycogen (μmol g-1) | 1.7±0.2a | 1.3±0.1ab | 2.7±0.3a | 2.0±0.2a | | Protein (mg g-1) | 256.4±18.5A | 285.3±12.5A | 293.3±35.4A | 148.9±6.4a | | Glucose (μmol g-1) | 17.5±2.6A | 13.3±0.9A | 4.9±0.5a | 2.9±1.1a | Control (C): 0.05 mg L NO 2 7 mg L Ca; low NO2-/high Ca2+: 0.05 mg L NO2- + 14 mg L Ca2+; high NO2-/low Ca2+: 1.3 mg L NO2- + 7 mg L Ca2+; high NO2-/high Ca2+: 1.3 mg L NO2- + 14 mg L 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). DISCUSSION

The present study demonstrated that exposure to 1.3 mg L NO 2 3 reduced growth of silver catfish and the increase of Ca2+ levels did not minimize toxicity. A previous study showed that 100% mortality was observed in silver catfish maintained at 1.52 mg L NO 2, but that exposure to levels of up to 1.19 mg L NO 2 did not affect survival or growth (LIMA et al., 2011). Therefore, silver catfish has a limited NO2- 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 NO 2 showed no change in growth, but at 3.0 mg L 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 NO2- and mortality started at 3.71 mg L NO 2 (COLT et al., 1981). On the other hand, silver perch (Bidyanus bidyanus) exposed to 1.43 mg L NO 2 had reduced growth, but survival was not affected at 16.2 mg L (higher levels were not tested) (FRANCES et al., 1998). This demonstrates limited NO2- concentration range to induce reduced growth and to provoke mortality in several species. The increase of Ca2+ levels at low NO2- 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 CaCO3 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). Matrixnă (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).

ACKNOWLEDGEMENTS

The authors are grateful to the Conselho Nacional de Desenvolvimento Tecnológico (CNPq), Comissão de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Universidade Federal do Maranhão/UFMA, Universidade Federal de Santa Maria/UFSM and the Fundação de Amparo à Pesquisa do Maranhão/FAPEMA.

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