COMPARISON BETWEEN BIOCHEMICAL RESPONSES OF THE TELEOST PACU AND ITS HYBRID TAMBACU (*Piaractus mesopotamicus* x *Colossoma macropomum*) TO SHORT TERM NITRITE EXPOSURE

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ABSTRACT

Aquatic environmental factors are very changeable in short periods. Among these factors are pH, temperature, dissolved oxygen, ammonia and ions. Nitrite, as one ion naturally present in aquatic systems, deserves particular consideration as it is highly toxic for many species. Among fish, nitrite may have harmful effects, such as methemoglobin (MtHb) formation, disruption to the gill and hepatic structure, which could result in hemolytic anemia and cell hypoxia by reducing the functional hemoglobin content. In this work, we compared hematological and metabolical responses of pacu and its hybrid tambacu exposed to 20 ppm of environmental nitrite. It was observed that the MtHb content was less than 18% in tambacu while pacu reached nearly 8%. These data reflect specific differences in nitrite uptake by the gill. The hematocrit of both fish was distinct; pacu did not have a typical response of poisoning by nitrite. This fact shows less skill of the hybrid to cope with environmental nitrite. Incipient hemolytic anemia was observed in pacu and both species presented a neoglycogenic profile. The glucose-provider character of the liver was more evident in tambacu. The white muscle of both species presented distinct metabolic behavior. While in pacu the white muscle was predominantly oxidative, in tambaqui the lactic fermentation was the most important metabolic profile. Metabolic and hematological observations in both species show that they present distinct metabolical strategies to cope with toxic effects of nitrite and there is no evidence that the hybrid is more resistant to nitrite.

Keywords: nitrite, metabolism, fish, adaptation, pacu, tambaqui.

RESUMO

Comparação entre as respostas bioquímicas do teleosteo pacu e de seu híbrido tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*) à exposição aguda ao nitrito

Os fatores ambientais nos meios aquáticos são muito flutuantes em curtos intervalos de tempo. Valores de pH, temperatura, oxigênio dissolvido, amônia e íons podem estar freqüentemente variando. Entre esses íons, o nitrito merece especial atenção por ser altamente tóxico para muitas espécies. Entre os peixes, o nitrito pode apresentar efeitos danosos como a formação de metahemoglobina (MtHB), lesão às estruturas branquiais e hepática, podendo levar a quadros de anemia hemolítica e hipóxia celular pela redução do teor de hemoglobina funcional. Neste trabalho, foram comparadas as respostas hematológicas e metabólicas do pacu e de seu híbrido tambacu expostos a 20 ppm de nitrito ambiental, e verificou-se que o teor de MtHb no tambacu foi menor que 18%, enquanto no pacu atingiu valores de 8%. Esses valores refletem diferenças específicas na captação de nitrito pelas brânquias. O hematócrito de ambas as espécies foi diferente; o pacu não apresentou uma resposta típica à intoxicação pelo nitrito. Este fato revelou uma diminuição na capacidade do híbrido em resistir ao nitrito ambiental. Observou-se no pacu um princípio de anemia hemolítica. As duas espécies mostraram um perfil bioquímico neoglicogênico. O papel glicemiante do fígado foi mais evidente no tambacu. O músculo branco de ambas as espécies mostrou um comportamento metabólico distinto. Enquanto o músculo do pacu foi predominantemente oxidativo, o músculo branco do tambaqui exposto ao nitrito realizou fermentação láctica. As observações metabólicas e hematológicas em ambas as espécies indicam que estas apresentam estratégias metabólicas diferentes para enfrentar os efeitos tóxicos do nitrito ambiental, não sendo evidenciada qualquer vantagem do híbrido neste particular.

Palavras-chave: nitrito, metabolismo, peixes, adaptação, pacu, tambaqui.

INTRODUCTION

Aquatic organisms are exposed to many environmental factors, which fluctuate in short periods of time. Natural aquatic environments are very dynamic and, therefore a number of parameters are variable but usually in a predictable way. Among them, pH, temperature, dissolved oxygen, ammonia and ions can be pointed out. These factors, throughout the evolution process, have enabled animals to adapt to their habitats (Hochachka & Somero, 2002). However, human interference in natural processes has changed many characteristics of various environments. An example of this is sewage dumping in rivers and lakes. Furthermore, in artificial systems, such as fish farms, these parameters vary greatly, especially in high-density systems. Nitrite ion is one factor that can be of major importance in both natural and artificial aquatic environments because of its toxicity. Nitrite is a natural anion which is found in aquatic environments due to the nitrification process. Although its concentrations are usually low (Eddy & Williams, 1987), various processes can interfere in the balance of the nitrogen cycle. Among them, pollution of the aquatic environment is known to cause a considerable increase in nitrite concentration (Nikinmaa, 1992). As a matter of fact, the level of nitrite is used as an indicator of impacted areas. Nitrite toxicity to fish, as a result of high environmental levels, is due to various processes including the formation of methemoglobin, which is a non-functional hemoglobin form and the cytolysis of hepatocytes and gill chloride-cells (Arillo *et al.*, 1984). Fish can be especially sensitive to nitrite because of its competition with chloride to bind sites in the chloride-cells (Bath & Eddy, 1980). Nitrite can actually be found in high concentrations in plasma of fish exposed to high nitrite levels. (Margiocco *et al.*, 1983)

Methemoglobin formation can induce cyanosis and a physiological state similar to that found in cases of environmental hypoxia (Smith & Russo, 1975). On the other hand, the damage caused to gills and liver by nitrite alters two of the most important regulatory organs in fish and can, therefore, cause serious problems in maintaining homeostasis. Moreover, nitrite is also known to induce hemolytic anemia (Scarano *et al.*, 1984), which can aggravate the metabolic hypoxia stress. Pacu (*Piaractus mesopotamicus*) and its hybrid tambacu (*Piaractus mesopotamicus* x *Colossoma macropomum*) are bred on a large scale in Brazil. This hybrid is considered to be more resistant to many environmental impacts. The exposure of these fish to nitrite in some types of cultivation systems is usual and different degrees of sensibility of both species can sustain advantages of cultivation of one over the other. The aim of this work is to evaluate and compare the effects of environmental nitrite on the hematology and intermediary metabolism of both fish.

MATERIALS AND METHODS

Juveniles of *P. mesopotamicus* and the hybrid tambacu were kept for four weeks in 2,000 L tanks supplied with aerated, thermostatic and filtered water, under natural photoperiod for acclimatization. The fish, weighing 51.21 ± 16.50 g, were transferred to two experimental 200 L tanks (six tambacu and ten *P. mesopotamicus*) where they were maintained for five days. The water conditions in these tanks were equal to the original. Twenty-four hours before the experiment feeding was discontinued. Pre-dissolved $NaNO_2$ was added to one of the tanks to a final concentration of 20 ppm. The other tank was kept as a control. After eight hours of nitrite exposure, the fish were killed and blood, white muscle as well as liver samples were collected and frozen in liquid nitrogen for posterior biochemical analysis.

The aliquots of the blood samples were withdrawn in heparinized syringes and used for hematocrit (Lima *et al.*, 1969), hemoglobin (Drabkin, 1948) and methemoglobin (Benesch *et al.*, 1973) determinations. The remains were centrifuged and plasma samples were obtained, in which the contents of glucose (Dubois *et al.*, 1956), lactate (Harrower & Brown, 1972) and pyruvate (Lu, 1939) were determined. In the tissues, the same metabolites were quantified in 1:10 w/v extracts of 10% trichloroacetic acid (TCA). In addition, the glycogen content was determined (Bidinotto *et al.*, 1997). The data obtained were analyzed for normality using the KOLMOGOROV-SMIRNOV test and afterwards the means were compared using the parametric T-Student test. All tests were done at 5% level of confidence using the GRAPHPAD PRISM™ software.

RESULTS

The hematological parameters (Table 1) changed in both species. In tambacu, both the hematocrit and RBC reduced significantly. In pacu, the hematocrit increased while the RBC was reduced. Furthermore, the hemoglobin from the pacu exposed to nitrite was lower when compared to the control.

The metabolic profile was also altered in both fish (Table 2). In pacu, the glucose decreased in the liver and white muscle, while the glycemia remained constant. There was an increase in liver glycogen in the liver, while muscular glycogen levels decreased. Pyruvate increased in the white muscle and plasma, while lactate levels were lower in the muscle of exposed fish. Lactate/Pyruvate (L/P) ratios were lower in white muscle and plasma of fish submitted to nitrite.

In tambacu, glucose levels were higher in the liver and plasma. The glycogen content of the liver and white muscle also rose in exposed fish. There was an increase in plasmatic pyruvate, while in the liver this metabolite decreased. Fish exposed to nitrite presented increased lactate concentrations in the liver and plasma, but the L/P ratio only changed in plasma.

DISCUSSION

Significant amounts of MtHb reduce the contents of functional hemoglobin (Hb) and a consequent hypoxia is predictable. However, when compared to environmental hypoxia caused by a reduction of partial pressure of oxygen (PO_2), the functional hypoxia is essentially different. Functional hypoxia is a consequence not from the lack of oxygen, but rather from the impossibility of carrying the available oxygen due to a decrease in the functional Hb concentrations. The contents of MtHb observed in tambacu exposed to nitrite (Table 1) were low (the highest was approximately 18%) as compared with values reported (Souza *et al.*, 1995; Bath & Eddy, 1980; Schoore *et al.*, 1995; Tucker *et al.*, 1989; Hilmy *et al.*, 1987). In fact, similar MtHb concentrations were found in *Hoplias malabaricus* exposed to 30 ppm of environmental nitrite (Moraes *et al.*, 1998). Lower MtHb concentration in both species (Pacu and Tambacu) can reflect a particular ability to recover basal values of Hb or specific differences in the nitrite uptake by the gills.

The hematological responses observed in fish exposed to nitrite differ to those reported for environmental hypoxia; nitrite should cause

TABLE 1
Hematological parameters of tambacu and *Piaractus mesopotamicus* exposed to nitrite.

Blood parameter	*P. mesopotamicus*		Tambacu	
	Control	20 ppm	Control	20 ppm
Hematocrit	29.25 ± 1.06	36.70 ± 1.46**	28.33 ± 0.99	23.42 ± 0.49**
RBC	3.84 ± 0.15	2.95 ± 0.32*	3.24 ± 0.35	2.33 ± 0.16*
MCV	0.76×10^{-10}	1.24×10^{-10}	0.93×10^{-10}	1.00×10^{-10}
Hemoglobin	9.42 ± 0.18	8.69 ± 0.21*	12.03 ± 0.39	11.89 ± 0.32
Methemoglobin	0	8.84 ± 1.37	2.71 ± 0.66	18.67 ± 1.46

Fish were exposed to 20 ppm of nitrite $N\text{-}NO_2$ for eight hours. Hematocrit and methemoglobin are expressed in values (%) ± S.E; hemoglobin is expressed in g/dL, RBC in millions of cells per mm^3 and MCV in mL. Significant differences are indicated by (*) when $p < 0.05$ and (**) when $p < 0.001$).

TABLE 2
Metabolic intermediates in tambacu and *Piaractus mesopotamicus* (pacu) exposed nitrite.

Piaractus mesopotamicus						
Parameter			**Tissue**			
	Liver		**WM**		**Plasma**	
	Control	**Exposed**	**Control**	**Exposed**	**Control**	**Exposed**
glycogen	480,7 ± 20	544,8 ± 15*	17,01 ± 0,5	14,49 ± 0,8*	-	-
glucose	647,0 ± 49	496,1 ± 13**	26,9 ± 1	23,8 ± 1*	6,1 ± 0,1	6,6 ± 0,2
pyruvate	1,36 ± 0,1	1,40 ± 0,1	0,45 ± 0,1	0,90 ± 0,1**	1,02 ± 0,1	1,9 ± 0,1**
lactate	7,75 ± 0,5	7,32 ± 0,2	43,02 ± 1	37,09 ± 1*	2,40 ± 0,3	2,7 ± 0,2
L/P	5,93 ± 0,3	5,42 ± 0,4	112,6 ± 0,1	46,3 ± 5**	2,4 ± 0,3	1,40 ± 0,1**
Tambacu						
Parameter			**Tissue**			
	Liver		**WM**		**Plasma**	
	Control	**Exposed**	**Control**	**Exposed**	**Control**	**Exposed**
glycogen	329,4 ± 28	438,6 ± 34*	6,6 ± 0,5	10,4 ± 0,7**	-	-
glucose	396,7 ± 25	484,4 ± 25*	12,25 ± 1	11,75 ± 0,2	3,4 ± 0,5	5,1 ± 0,1**
pyruvate	1,55 ± 0,1	1,01 ± 0,1**	0,92 ± 0,1	1,09 ± 0,1	0,77 ± 0,1	1,19 ± 0,1**
lactate	9,96 ± 0,6	6,85 ± 0,3**	30,52 ± 2,0	30,14 ± 1,9	3,66 ± 0,1	4,71 ± 0,3**
L/P	6,3 ± 0,3	7,0 ± 0,2	29,4 ± 1,6	27,7 ± 2,4	4,0 ± 0,4	2,7 ± 0,3*

The fish were exposed to 20 ppm of nitrite $N\text{-}NO_2$ for eight hours. The values of metabolite concentration are expressed in µmols/mg of wet tissue ± S.E (except the L/P- ratio [lactate]/[pyruvate]). Significant differences are indicated by (*) when $p < 0.05$) and (**) when $p < 0,01$.

hemolytic anemia (Scarano & Soroglia, 1984). The hematocrit responses observed in study species after exposure to nitrite were different. Pacu did not present a typical hematological response to nitrite poisoning. We actually found an increase in hematocrit of pacu, which is a common response to many stressors or even the environmental hypoxia. Hemoglobin concentration in pacu was significantly reduced and this might be a consequence of an initial process of cleaning the red cells affected by MtHb formation. This can be a strategy which is not observed in the hybrid and should support the hemolytic anemia referred to above, as well as a more efficient or prompt response to nitrite in pacu. On the other hand, the number of red cells (RBC) decreased in both study species. This is usually observed in fish exposed to low Po_2 (Fievet *et al.*, 1987; Peterson, 1990; Moraes *et al.*, 1995, 1996). However, considering the opposite responses observed in the hematocrit of studied species, the larger red cell volume (MCV) of pacu suggests the presence of regulatory effectors in the Hb within its red blood cells. This set of hematological responses suggests that these animals were exposed to a lack of oxygen supply, at least to some extent. In addition, the responses were slightly different and the hybrid appears to be less able to cope with the environmental nitrite.

One of the major strategies to survive hypoxia is the regulation of the glycolytic pathway (Hochachka & Somero, 2002). The liver of both fish showed a glucogenic profile, however the tambacu´s liver appears to have a neoglucogenic outline since in this tissue there was a significant reduction in the lactate concentration while the L/P (lactate/pyruvate) ratio was constant due to a reduction of pyruvate. The alterations in glucose and glycogen content can be understood in two non exclusive ways: glucose can be exported to other tissues, especially the brain, or it can be converted into glycogen. The tambacu´s liver provided glucose in a more evident way than pacu under a nitrite effect. If plasma concentrations of lactate and pyruvate between the study species are compared, it is clear that there is a fermentative response in tambacu. The level of lactate was significantly increased, as well as the L/P ratio in this fish.

The white muscle is adapted to sustain its activity in the anaerobic consumption of glucose (Hochachka & Somero, 2002). However, to accomplish this status the white muscle must adjust the set of glycolytic enzymes. Moreover, white muscle can sustain higher levels of lactate than other tissues (Hochachka, 1980). We observed two quite different metabolic responses of white muscle in both fish to cope with nitrite. The liver might export the excess of glucose, produced by neoglucogenesis, contributing to the white muscle glycogen synthesis and supporting the white muscle energetic demand. Lactate fermentation was the metabolic preference of the white muscle in tambacu. This statement is supported by having observed a significant increase of glucose and the L/P ratio in the plasma. The lactate surplus should be exported increasing the lactemia. On the other hand, the tendency to increase the pyruvate plus reduce the L/P ratio and the lactate concentration in the white muscle of pacu suggest that the white muscle was prevalently oxidative in this species. The tambacu´s white muscle presented the same strategy reported to *C. macropomum* exposed to nitrite (Souza *et al.*, 1995). The metabolic profile found in the liver and white muscle of *P. mesopotamicus* is very similar to the one presented by the fish exposed to severe environmental hypoxia (Moraes *et al.*, 1997).

The metabolical observations plus the hematological response in pacu and tambacu indicate that even low levels of methemoglobin were able to trigger a set of metabolic responses to cope with a reduction in oxygen availability. In addition, the present findings point out different metabolic and hematological strategies of two close related fish. The first strategy to cope with functional hypoxia resulting from environmental nitrite is connected to hematological adjustments. The usual reduction of the number of red cells to likely increase the blood fluidity was observed. Very low levels of MetHb suggest a blood enzymatic mechanism to recover Hb (Avilez *et al.*, 2004), however the hybrid seems to be less effective. Hepatic neoglucogenesis was observed in the hybrid followed by a white muscle fermentative profile but an oxidative preference was observed in pacu. In conclusion, the metabolic strategies were common in some aspects of the environmental hypoxia only reported for many fish species, and the comparison between *P. mesopotamicus* and its hybrid with *C. macropomum* suggests no advantages of hybrid-descendents to cope with environmental nitrite.

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