

SCIENTIFIC NOTE

LIPID PEROXIDATION AND SUPEROXIDE DISMUTASE ACTIVITY IN SILVER CATFISH (*Rhamdia quelen*) JUVENILES EXPOSED TO DIFFERENT DISSOLVED OXYGEN LEVELS

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ABSTRACT

Fish are usually exposed to episodes of environmental and physiological hypoxia, and are likely to produce elevated levels of reactive oxygen species. Therefore, silver catfish (*Rhamdia quelen*) juveniles were exposed to different dissolved oxygen levels (severe hypoxia: 1.96 ± 0.08 , moderate hypoxia: 3.10 ± 0.10 and normoxia: 6.15 ± 0.03 mg.L⁻¹) for 30 days to verify if these conditions may induce any oxidative stress in the liver and

muscle of this species. The levels of lipid peroxidation and superoxide dismutase activity in liver and muscle were higher in fish exposed to severe and moderate hypoxia than in those exposed to normoxia. This result suggests that low dissolved oxygen levels in the water lead to lipid peroxidation, but at the same time there is an increase of superoxide dismutase activity, maintaining the oxidative equilibrium.

KEY WORDS: Anti-oxidant enzymes, free radicals, hypoxia, normoxia.

RESUMO

NOTA CIENTÍFICA: PEROXIDAÇÃO LIPÍDICA E ATIVIDADE DA SUPEROXÍDICO DISMUTASE EM JUVENIS DE JUNDIÁ (*Rhamdia quelen*) EXPOSTOS A DIFERENTES NÍVEIS DE OXIGÊNIO DISSOLVIDO

Peixes são frequentemente expostos a episódios de hipóxia ambiental e fisiológica, e usualmente produzem níveis elevados de espécies reativas de oxigênio. Juvenis de jundiá (*Rhamdia quelen*) foram expostos a diferentes níveis de oxigênio dissolvido (hipóxia severa: $1,96 \pm 0,08$, hipóxia moderada: $3,10 \pm 0,10$ e normóxia: $6,15 \pm 0,03$ mg.L⁻¹) por trinta dias, para verificar se tais condições poderiam induzir algum estresse oxidativo no fígado e

músculo dessa espécie. Os níveis de peroxidação lipídica e a atividade da superóxido dismutase no fígado e músculo foram mais altos em peixes expostos à hipóxia severa e moderada que nos expostos à normóxia. Trata-se de resultados que sugerem que baixos níveis de oxigênio dissolvido na água levam à peroxidação lipídica, mas ao mesmo tempo há um aumento da atividade da superóxido dismutase, mantendo o equilíbrio oxidativo.

PALAVRAS-CHAVES: Enzimas anti-oxidantes, hypoxia, normóxia, radicais livres.

INTRODUCTION

Oxygen availability is a limiting growth factor and chronic hypoxia or hyperoxia may be an important environmental stressor influencing fish growth (WILHELM FILHO et al., 2005). Fish are frequently exposed to frequent episodes of environmental and physiological hypoxia, and are likely to produce elevated levels of reactive oxygen species (ROS) during or on recovery of any physiological stress (MILLER & BRZEZINSKA-SLEBODZINSKA, 1993; ABELE & PUNTARULO, 2004). If these noxious oxygen derivatives are not controlled by antioxidant defense systems, oxidative stress occurs. The direct effects include peroxidative damage to important macromolecules. Indirectly, changes induced by reactive oxygen metabolites in cellular membranes and components can modify metabolic pathways, resulting in altered physiology and possible pathology (MILLER & BRZEZINSKA-SLEBODZINSKA, 1993). However, biological systems protect themselves against free radicals (and others derived from oxygen) aggression converting these free radicals in oxygen by oxidation phenomena or reduction through antioxidant systems. Univalently reduced O_2^- are reduced to uncharged H_2O_2 either spontaneously or by superoxide dismutase (SOD) (HALLIWELL & GUTTERIDGE, 1985).

The oxidative/antioxidant status and the consequences of hypoxic/hyperoxic conditions are crucial for aquaculture (WILHELM FILHO et al., 2005). Therefore, the aim of the present study was to investigate lipid peroxidation and superoxide dismutase activity in silver catfish (*Rhamdia quelen*) juveniles exposed to different levels of dissolved oxygen to verify if hypoxia may induce any oxidative stress in the liver and muscle of this species.

MATERIAL AND METHODS

Silver catfish juveniles (6.90 ± 0.09 cm and 4.99 ± 0.18 g) were placed in polypropylene boxes (10 juveniles/box) for 30 days and fed once a day (5% of the biomass) with a 42% protein

commercial feed Supra Juvenil (Alisul Alimentos S.A., Carazinho, Brazil). Juveniles were exposed to three dissolved oxygen concentrations (in $mg.L^{-1}$): 1.96 ± 0.08 ; 3.10 ± 0.10 and 6.15 ± 0.03 , which were considered severe and moderate hypoxia and normoxia, respectively (three replicates per treatment). Dissolved oxygen and temperature ($22.4 \pm 0.3^\circ C$) were monitored with an oxygen meter (model Y 5512, YSI Inc., Yellow Springs, USA). Room temperature was maintained with an air conditioner. The pH levels (7.5 ± 0.04) were verified twice a day with pH meter DMPH-2 (Digimed, São Paulo, Brazil), total ammonia ($3.00 \pm 0.04 mg.L^{-1}$) was determined twice daily according to BOYD & TUCKER (1992), and non ionized ammonia ($0.09 \pm 0.04 mg.L^{-1}$) calculated according to PIPER et al. (1982). Water hardness ($39.25 \pm 0.34 mg.L^{-1} CaCO_3$) was analyzed by the EDTA titrimetric method and alkalinity ($26.00 \pm 1.90 mg.L^{-1} CaCO_3$) and nitrite ($0.08 \pm 0.02 mg.L^{-1}$) according to BOYD & TUCKER (1992). Removal of the uneaten feed and feces and renewal of 70% of the water (with water previously adjusted to dissolved oxygen levels according to the respective treatments) was performed daily.

After 30 days of experiment fish were sacrificed by section of spinal cord to collect muscle and liver. Tissue samples (1g) were homogenized in 1.15% p/v KCl (0-4°C). Homogenization was performed in Ultra-Turrax for 30s, and homogenized suspension centrifuged for 10 min at 1000 g at (0-4°C), according to BUGÉ & AUST (1978). The supernatant was then collected and separated into aliquots for thiobarbituric acid-reactive substances (TBARS) and superoxide dismutase (SOD) activity determinations. Determination of TBARS was used to assay endogenous lipid oxidation according to BUGÉ and AUST (1978). Total SOD activity was determined by measuring the inhibition of the rate of autocatalytic formation of adrenochrome in a reaction medium containing 1 mmol/L epinephrine and 50 mmol/L glycine-NaOH (pH = 10.2) (McCORD & FRIDOVICH, 1969).

Homogeneity of the variances among the groups was verified with the Levene test.

Data exhibited homogeneous variances, so comparisons among different treatments were made by one-way analysis of variance, followed by Tukey test. All the tests were made with the software Statistica. Minimum significance level was 95% ($P < 0.05$).

RESULTS AND DISCUSSION

The values of TBARS and SOD in liver and muscle of silver catfish exposed to severe and moderate hypoxia were significantly higher than those exposed to normoxia (Figure 1). Similar result was found in *Colossoma macropomum* and *Carassius auratus* exposed to hypoxia (MARCON & WILHELM FILHO, 1999; LUSHCHAK et al., 2001). However, apparently lipid peroxidation response changes according to species, because in *Leporinus elongatus* TBARS levels were higher in fish exposed to moderate hypoxia and normoxia than in those maintained in severe hypoxia (WILHELM et al., 2005). The SOD activity was also elevated in *Cyprinus carpio*, *C. macropomum*, *Leiostomus xanthurus* and *Leporinus elongatus* exposed to severe hypoxia (VÍG & NEMESÓK, 1989; MARCON & WILHELM FILHO, 1999; COOPER et al., 2002; WILHELM FILHO et al., 2005).

The obtained data suggests that there is an increase in lipid peroxidation and ROS generation in liver and muscle associated with a decrease on oxygen availability. The increase in lipid peroxidation in silver catfish exposed to hypoxia might induce damage in these tissues. Therefore, the higher SOD (present study) and catalase (BRAUN et al. [in press]) activities in silver catfish exposed to hypoxia indicate that a significant part of the available energy may be directed to the synthesis of antioxidants to protect the tissues against peroxidation. This could explain the lower growth of silver catfish and *L. elongatus* juveniles maintained in hypoxia reported by BRAUN et al. (in press) and WILHELM FILHO et al. (2005). It can be concluded that severe and moderate hypoxia induced lipid peroxidation and SOD activity increase in silver catfish, which may indicate a

compensatory response of this species to survive in hypoxic conditions.

This study was performed according to the Commission of Ethics and Animal Welfare (register number 23081.003801/2006-73) from the Universidade Federal de Santa Maria.

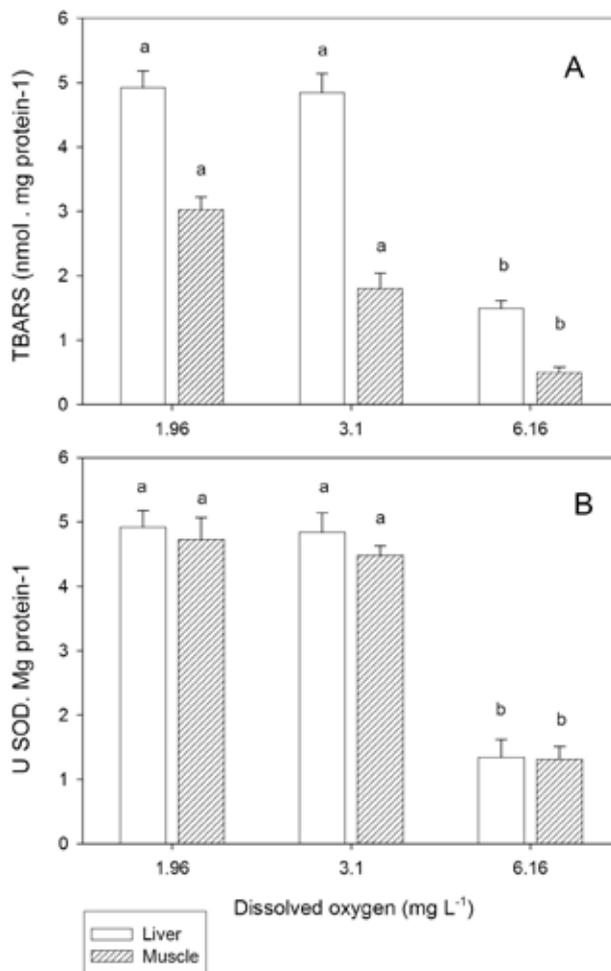


FIGURE 1. TBARS (A) and SOD (B) activities in muscle and liver of silver catfish juveniles exposed to different dissolved oxygen levels for 30 days. Different letters on the bars indicate significant difference among treatments in the same tissue by ANOVA and Tukey test ($P < 0.05$).

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REFERENCES

- ABELE, D.; PUNTARULO, S. Formation of reactive species and induction of antioxidant defense systems in polar and temperate marine invertebrates and fish. **Comparative Biochemistry and Physiology**, v. 138, p. 405-415, 2004.
- BOYD, C. E.; TUCKER, C. S. Water quality and pond soil analyses for aquaculture. **Auburn**, Auburn University, 1992.
- BRAUN, N.; LIMA, R. L.; MORAES, B.; LORO, V. L.; BALDISSEROTTO, B. Survival, growth and biochemical parameters of silver catfish, *Rhamdia quelen* (Quoy & Gaimard, 1824), juveniles exposed to different dissolved oxygen levels. **Aquaculture Research** [in press].
- BUGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. **Methods in Enzymology**, v. 52, p. 302-310, 1978.
- COOPER, R. U.; CLOUGH, L. M.; FARWELL, M. A.; WEST, T. L. Hypoxia-induced metabolic and antioxidant enzymatic activities in the in the estuarine fish *Leiostomus xanthurus*. **Journal of Experimental Marine Biology and Ecology**, v. 279, p.1-20, 2002.
- HALLIWELL, B.; GUTTERIDGE, J. M. C. Hypoxia-induced metabolic and antioxidant enzymatic activities in the estuarine fish *Leiostomus xanthurus*. **Journal of Experimental Marine Biology and Ecology**, v. 279, p.1-20, 1985.
- LUSHCHAK, V. I.; BAGNYUKOVA, T. V.; LUSHCHAK, O. V.; STOREY, J. M.; STOREY, K. B. Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. **International Journal of Biochemistry and Cell Biology**, v. 37, p.1319-1330, 2005.
- MARCON, J. L.; WILHELM FILHO, D. Antioxidant processes of the wild tambaqui, *Colossoma macropomum* (Osteichthyes, Serrasalminidae) from the Amazon. **Comparative Biochemistry and Physiology C**, v.123, p.257-263, 1999.
- McCORD, J. M.; FRIDOVICH, I. Superoxide dismutase. Enzymatic function for erythrocyte (hemocuprein). **Journal of Biological Chemistry**, v. 244, p. 6049-6055, 1969.
- MILLER, J.K.; BRZEZINSKA-SLEBODZINSKA, E. Oxidative stress, antioxidants, and animal function. **Journal of Dairy Science**, v. 76, p. 2812-2823, 1993.
- PIPER, R. G.; McELWAIN, I. B.; ORME, L. E.; McCrAREN, J. P.; FOWLER, L. G., LEONARD, J. R. **Fish hatchery management**. Washington: United States Department of the Interior Fish and Wildlife Service, 1982.
- VÍG, E.; NEMESÓK, J. The effects of hypoxia and paraquat on the superoxide dismutase activity in different organs of carp, *Cyprinus carpio*. L. **Journal of Fish Biology**, v. 35, p. 23-25, 1989.
- WILHELM FILHO, D.; TORRES, M. A.; ZANIBONI-FILHO, E.; PEDROSA, R. C. Effect of different oxygen tensions on weight gain, feed conversion, and antioxidant status in piapara, *Leporinus elongatus* (Valenciennes, 1847). **Aquaculture**, v. 244, p. 349-357, 2005.

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