UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

EFEITO DO ÓLEO ESSENCIAL DE *Lippia alba* NOS PARÂMETROS OXIDATIVOS DO JUNDIÁ (*Rhamdia quelen*) EXPOSTO A DIFERENTES CONCENTRAÇÕES DE OXIGÊNIO

DISSERTAÇÃO DE MESTRADO

Cati Reckelberg Azambuja

Santa Maria, RS, Brasil

2009

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por

Cati Reckelberg Azambuja

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Farmacologia, Área de Concentração em Farmacologia Aplicada à Produção Animal, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Farmacologia**.

Orientadora: Profª Drª Susana Francisca Llesuy

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A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

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elaborada por
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como requisito parcial para obtenção do grau de
Mestre em Farmacologia

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Santa Maria, 1º de setembro de 2009.

Ao meu filho ***Arthur****, que entre os desafios e conquistas, conciliou nossos momentos de diversão e estudos!*

Ao meu namorado ***Filippe Júnior****, que ajudou a manter o equilíbrio de nossas vidas pessoal e profissional!*

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"A mente que se abre a uma nova idéia jamais volta ao seu tamanho original."
(Albert Einstein)

RESUMO

Dissertação de Mestrado
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EFEITO DO ÓLEO ESSENCIAL DE *Lippia alba* NOS PARÂMETROS OXIDATIVOS DO JUNDIÁ (*Rhamdia quelen*) EXPOSTO A DIFERENTES CONCENTRAÇÕES DE OXIGÊNIO

AUTORA: CATI RECKELBERG AZAMBUJA
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Data e Local da Defesa: Santa Maria, 1º de setembro de 2009.

Juvenis de jundiá (*Rhamdia quelen*) foram expostos ao óleo essencial de *Lippia alba* (*L. alba*) e transportados em sacos plásticos por períodos diferentes (5, 6 e 7 h) produzindo diferentes concentrações finais de oxigênio. Os biomarcadores de estresse oxidativo, lipoperoxidação (LPO), catalase (CAT), superóxido dismutase (SOD), e glutationa-S-transferase (GST) foram mensurados em fígado, brânquias e cérebro de peixes. Os juvenis foram separados em 6 grupos de tratamento diferentes de acordo com a presença ou não do óleo essencial de *L. alba* na água (10 µL/L) e o tempo de transporte, o qual determinou a concentração final de oxigênio dissolvido no interior dos sacos: Cinco horas: hiperóxia (13.25 ± 0.35 mg/L O_2); hiperóxia com *L. alba* (11.27 ± 0.22 mg/L O_2); Seis horas: normóxia (7.35 ± 0.35 mg/L O_2); normóxia com *L. alba* (7.29 ± 0.40 mg/L O_2); Sete horas: hipóxia (2.29 ± 0.36 mg/L O_2); hipóxia com *L. alba* (3.82 ± 0.7 mg/L O_2). A adição do óleo essencial de *L. alba* causou um aumento da LPO nos tecidos expostos a hiperóxia e uma redução da GST nos peixes mantidos sob hiperóxia e hipóxia comparado aqueles sob normóxia. Nos tecidos houve uma redução da LPO e GST e um aumento da SOD nas espécies sob hipóxia e uma redução da GST naqueles sob hiperóxia com óleo. Estes resultados sugerem que a presença do óleo essencial de *L. alba* melhora o estado redox nos tecidos avaliados, ambos sob hiperóxia e sob hipóxia.

Palavras-Chave: Estresse oxidativo, enzimas antioxidantes, peroxidação lipídica, disponibilidade de oxigênio, *Lippia alba*, peixe.

ABSTRACT

Dissertation of Master's Degree
Post-Graduate Program in Pharmacology
Federal University of Santa Maria

EFFECT OF THE ESSENTIAL OIL OF *Lippia alba* ON OXIDATIVE PARAMETERS OF SILVER CATFISH (*Rhamdia quelen*) EXPOSED A DIFFERENTS OXYGEN LEVELS

AUTHOR: CATI RECKELBERG AZAMBUJA
ADVISER: SUSANA FRANCISCA LLESUY

Date and Place of Defense: September 1st, 2009, Santa Maria.

Juvenile silver fish (*Rhamdia quelen*) were exposed to the essential oil of *Lippia alba* (*L. alba*) and transported in plastic bags for different periods (5, 6 and 7 h) yielding final different oxygen levels. The biomarkers of oxidative stress, lipoperoxidation (LPO), catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) were measured in the liver, gills and brain of the fish. The juveniles were assigned to 6 different treatment groups according to the presence or not of the essential oil of *L. alba* in water (10 µL/L) and the length of transportation, which determined the final concentration of dissolved oxygen inside the bags: Five hours: hyperoxia (13.25 ± 0.35 mg/L O_2); hyperoxia with *L. alba* (11.27 ± 0.22 mg/L O_2); Six hours: normoxia (7.35 ± 0.35 mg/L O_2); normoxia with *L. alba* (7.29 ± 0.40 mg/L O_2); Seven hours: hypoxia (2.29 ± 0.36 mg/L O_2); hypoxia *with L. alba* (3.82 ± 0.7 mg/L O_2). The addition of essential oil of *L. alba* causes an increase of LPO in the tissues exposed to hyperoxia and a reduction of GST in the fish kept under hyperoxia and hypoxia as compared to those under normoxia. In the tissues there is a reduction of LPO and GST and an increase of SOD in the specimens under hypoxia and a reduction of GST in those under hyperoxia with the oil. These results suggest that the presence of the essential oil of *L. alba* improves the redox state in the evaluated tissues, both under hyperoxia and under hypoxia.

Keywords: Oxidative stress, antioxidant enzymes, lipid peroxidation, oxygen availability, *Lippia alba*, fish.

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LISTA DE ABREVIATURAS E SIGLAS

ATP	Adenosina trifosfato
BHA	Hidroxianisol butilado
$CaCO_3$	Carbonato de cálcio
CAT	Catalase
CDNB	1-cloro-2,4-dinitrobenzeno
CO_2	Dióxido de carbono
DNA	Ácido desoxirribonucleico
DPPH	Radical 2,2-difenil-1-picril-hidrazil
EAO	Espécies ativas de oxigênio
EDTA	Ácido etilenodiamino tetra-acético
FRAP	Capacidade antioxidante do plasma
G6PHD	Glicose-6-fosfato desidrogenase
GPx	Glutationa peroxidase
GR	Glutationa redutase
GSH	Glutationa
GSSG	Dissulfeto de glutationa
GST	Glutationa-S-transferase
H_2O_2	Peróxido de hidrogênio
H_2SO_4	Ácido sulfúrico
$HO^{\bullet}$	Radical hidroxila
IC_{50}	Concentração inibitória de 50%
KCl	Cloreto de potássio
L. alba	*Lippia Alba*
LPO	Peroxidação lipídica
NADPH	Nicotinamida adenina dinucleotídeo fosfato
NaOH	Hidróxido de sódio
O_2	Oxigênio molecular
$O_2^{\bullet -}$	Ânion radical superóxido
OD	Oxigênio dissolvido
PMSF	Fluoreto fenil metilsulfonil
SOD	Superóxido dismutase
TBA	Ácido tiobarbitúrico
TBARS	Substâncias reativas ao ácido tiobarbitúrico
TCA	Ácido tricloroacético

SUMÁRIO

1 INTRODUÇÃO

A piscicultura, como qualquer outra atividade, busca excelência no seu desenvolvimento, ou seja, busca uma alta produtividade aliada ao menor custo, sem perder qualidade. Para que sejam cumpridos estes requisitos, faz-se necessário entender toda a dinâmica que envolve a criação de determinada espécie.

O jundiá é uma espécie nativa, muito bem adaptada ao nosso ambiente, de bom valor econômico e que tem despertado o interesse dos pesquisadores a respeito das particularidades da sua criação.

Segundo IWAMA et al. (1999), os estressores de peixes encontram-se agrupados em três categorias distintas: a) ambientais – como os níveis de oxigênio dissolvido; b) físicos – como o transporte e c) biológicos – como os patogênicos.

Diante da grande variedade de fatores que podem alterar os padrões normais de equilíbrio ou homeostase nos ecossistemas aquáticos, o organismo, em resposta ao estímulo, desenvolve mecanismos adaptativos, de compensação das alterações bioquímicas e fisiológicas sofridas (BARTON, 2002).

Um ambiente aquático é caracterizado por acentuada heterogeneidade temporal e espacial no conteúdo de oxigênio, devido à temperatura da água, salinidade e fluxos (LUSHCHAK e BAGNYUKOVA, 2006). Da mesma forma, o transporte de peixes vivos, prática rotineira em piscicultura, pode causar estresse agudo (BARTON e IWAMA, 1991) e resultar em baixas taxas de sobrevivência, de crescimento, de redução na capacidade reprodutiva e da eficiência no sistema imunológico (MAULE et al., 1989).

Segundo GOLOMBIESKI (2004), o sistema de transporte de peixes mais utilizado é o sistema fechado (em sacos plásticos). Entretanto, a utilização de sacos plásticos para o transporte acarreta problemas devido ao acúmulo de metabólitos pela alteração do pH da água, pelo aumento da concentração de dióxido de carbono (CO_2) e, também, pela depleção dos níveis de oxigênio dissolvido (OD) na água (AMEND et al., 1982).

Além disso, os níveis de oxigênio dissolvido são determinantes para os sistemas de piscicultura e, portanto, manter um nível apropriado na água é fundamental para a produtividade.

A hiperóxia é caracterizada pelo aumento dos níveis de oxigênio dissolvido na água. A situação de supersaturação de oxigênio, utilizada para transporte de peixes em sacos plásticos, como anteriormente referido, é comum em sistemas de piscicultura. Sob esta condição, LUSHCHAK e BAGNYUKOVA (2006) afirmam que as enzimas antioxidantes atuam através de *up-regulation*.

A hipóxia, segundo ROSSO et al. (2006) pode ser causada pelo consumo do oxigênio, quando houver necessidade de um tempo maior de transporte. A hipóxia / anóxia, são caracterizadas pela diminuição ou ausência do oxigênio (LUSHCHAK e BAGNYUKOVA, 2006). Segundo GARCIA SAMPAIO et al. (2008) em situações de hipóxia severa, a redução gradual de elétrons na cadeia mitocondrial pode resultar em um desbalanço oxidativo, levando à formação aumentada de espécies ativas de oxigênio (EAO) e, consequentemente, mudanças no potencial redox.

2 REVISÃO BIBLIOGRÁFICA

2.1 *Rhamdia quelen*

O *Rhamdia quelen* no Brasil pode ser encontrado com os seguintes nomes: jundiá-tinga, jandiá, jandiá-tinga, mandi e sapipoca; em inglês, é chamado por *silver catfish*. Taxonomicamente, este peixe pertence ao Reino - Animalia, Filo - Chordata, Classe - Actinopterygii, Subclasse - Teleostei, Ordem - Siluriformes, Família - Heptapteridae, Gênero - *Rhamdia*, Espécie - *Rhamdia quelen* (BALDISSEROTTO, 2004) (Figura 1).

A coloração do couro do jundiá varia de tons de marrom-avermelhado claro a cinza, sendo a parte ventral do corpo mais clara. A intensidade da sua coloração varia conforme a luminosidade do ambiente onde habita. O comprimento máximo teórico calculado das fêmeas é de aproximadamente 66,5 cm e dos machos de 52,0 cm. O tempo de vida teórico estipulado também é maior em fêmeas, 21 anos, enquanto para os machos é de apenas 11 anos. A espécie habita águas calmas com fundo de areia e lama, junto às margens de lagos e rios. Possui hábitos noturnos escondendo-se durante o dia entre pedras e troncos, saindo à noite para alimentar-se. Os adultos possuem uma variada alimentação que inclui peixes, crustáceos, insetos, restos vegetais e detritos orgânicos, sendo omnívoros. As larvas alimentam-se de zooplâncton (BALDISSEROTTO, 2004).

Figura 1- Exemplar de jundiá, *Rhamdia quelen* (Heptapteridae). Fonte: http://www.fishbase.org

O jundiá *Rhamdia quelem* (Heptapteridae) é uma espécie endêmica que pode resistir invernos frios e crescer rapidamente no verão (BARCELLOS et al., 2003; BARCELLOS et al., 2004), além de ser de grande importância econômica no Rio Grande do Sul, bem adaptada e muito utilizada em viveiros de piscicultura por ser um peixe bastante consumido pela população. Pode ser encontrado desde o centro da Argentina até o sul do México (GOMES et al., 2000). No Brasil possui ampla distribuição, estando presente na maioria dos estados (SILFVERGRIP, 1996).

A sua sobrevivência, assim como o seu crescimento, depende de diversos fatores como os níveis de oxigênio na água, o transporte, a temperatura, o pH, os resíduos nitrogenados, o manuseio, entre outros. Quando os peixes são submetidos à alteração de um destes fatores ocorrem modificações no seu metabolismo, e constituem os desvios da homeostase, ou seja, situações de estresse em peixes podem causar ainda uma demanda energética maior que a sustentável pelo metabolismo aeróbio.

2.2 Balanço Oxidativo

Seres aeróbios, como plantas, fungos, bactérias e animais, necessitam consumir oxigênio (O_2) para produzir eficientemente a energia que mantém as funções celulares, assim como a atividade das enzimas que catalisam os processos metabólicos dependentes de oxigênio (CADENAS, 1989).

A molécula de oxigênio, em seu estado natural, é um bi-radical, possui dois elétrons não-pareados, é relativamente pouco solúvel em água e tem um alto potencial de redução (CHANG, 2008) (Figura 2).

A metabolização do oxigênio nos animais ocorre principalmente na mitocôndria através da cadeia de transporte de elétrons, gerando energia metabólica na forma de adenosina trifosfato (ATP) pela oxidação de nutrientes (HALLIWELL e GUTTERIDGE, 1999) (Figura 3).

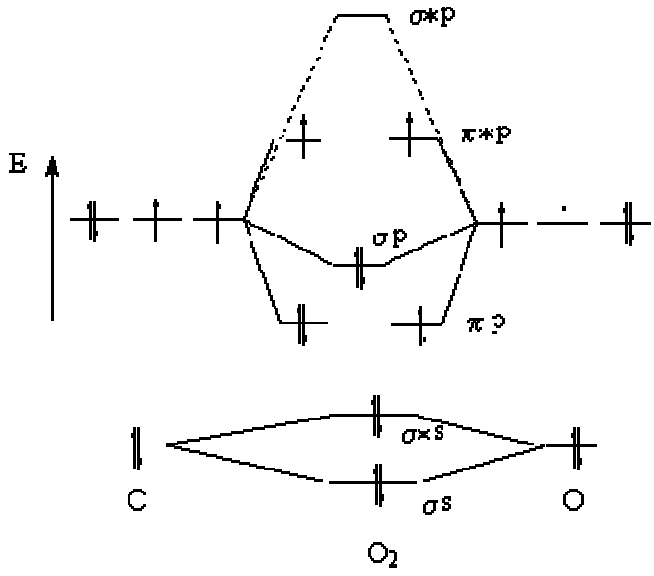

Figura 2 - Teoria dos orbitais moleculares do oxigênio (CHANG, 2008)

Porém, o mesmo elemento essencial à sobrevivência apresenta, igualmente, consequências deletérias aos organismos expostos a ele, notadamente quando em concentrações superiores às encontradas na atmosfera (21%) (HALLIWELL e GUTTERIDGE, 1999). Em certas condições, é responsável por reações químicas muito tóxicas para as células (BOVERIS e CHANCE, 1973; PAVANATO e LLESUY, 2008).

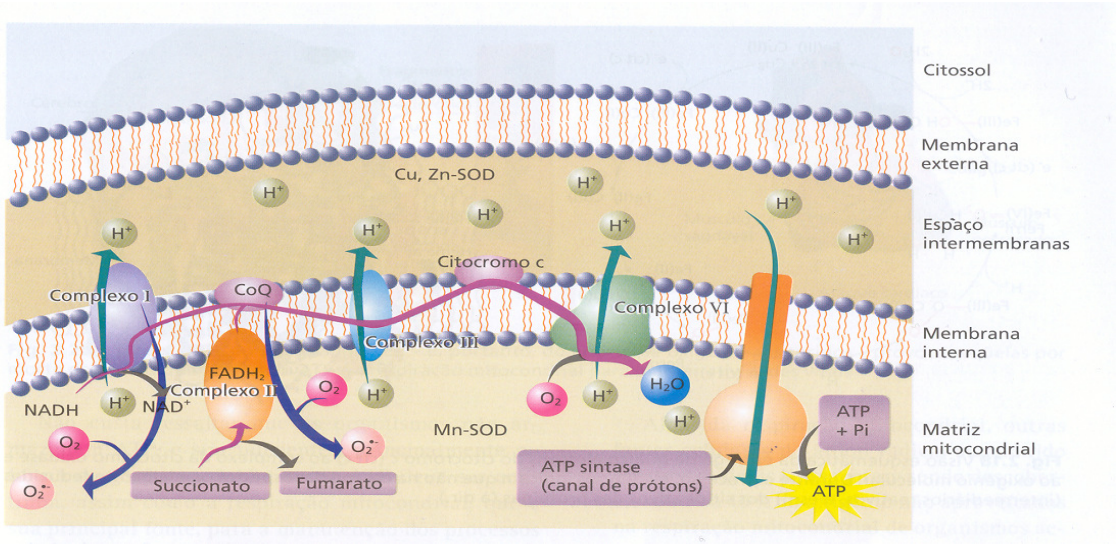

Figura 3 - Metabolização do oxigênio na mitocôndria (AUGUSTO, 2006).

Entre 2 a 7% do oxigênio consumido pelos organismos aeróbios podem sofrer redução completa pela citocromo oxidase, várias reações enzimáticas e não enzimáticas resultam na redução parcial do O_2, gerando espécies radicais como o ânion superóxido ($O_2^{\bullet}$-) e o radical hidroxila ($HO^{\bullet}$), mas também as não-radicais como o peróxido de hidrogênio (H_2O_2) (Figura 4). Todas essas são denominadas espécies ativas de oxigênio, por serem capazes de existir de forma independente (PAVANATO e LLESUY, 2008).

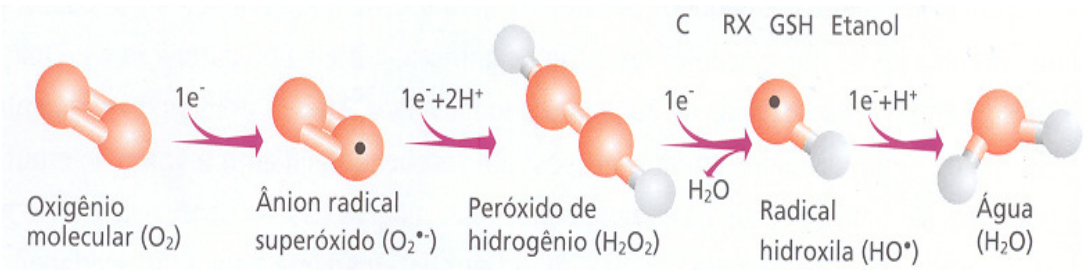

Figura 4 - Etapas de um elétron na redução do oxigênio, levando à formação das espécies reativas de oxigênio – ânion radical superóxido, peróxido de hidrogênio e radical hidroxila (AUGUSTO, 2006).

As EAO podem ser geradas de forma endógena, durante o metabolismo celular, de forma exógena através de fatores físicos e reações de produtos químicos tóxicos, ou ainda por uma combinação destes processos e condições. Dentre estas espécies ativas encontramos os radicais livres que podem ser definidos como qualquer átomo ou molécula com existência independente, contendo um ou mais elétrons não pareados (PRYOR, 1976) nos orbitais externos, o que os torna extremamente instáveis (HALLIWELL, 1992). Quimicamente, os radicais livres caracterizam-se pelo caráter paramagnético, alto grau de reatividade química e curta vida média.

O ânion radical superóxido é muito menos reativo do que o radical hidroxila, e é produto da redução do oxigênio com um elétron. O $O_2^{\bullet}$- é o principal produto das EAO e pode levar à formação de outras.

O peróxido de hidrogênio, por não possuir elétrons desemparelhados, não é considerado um radical livre, valendo-lhe uma menor reatividade do que aqueles classificados como radicais. O H_2O_2 é o produto da redução de dois elétrons de O_2 e decompõem-se rapidamente. Porém, esta EAO atravessa facilmente as membranas

celulares e, ao receber mais um elétron, normalmente de ferro ou cobre, dá origem ao radical hidroxila, através da reação de Fenton (HALLIWELL e GUTTERIDGE, 1999) (Figura 5).

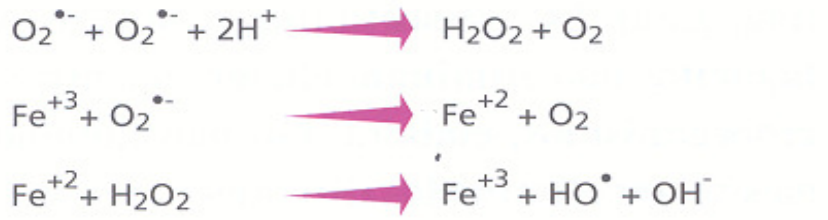

Figura 5: Formação do radical hidroxila (AUGUSTO, 2006).

O radical hidroxila, além de ser muito mais reativo, pode formar-se através de várias reações (HALLIWELL e GUTTERIDGE, 1984; STOREY, 1996; HALLIWELL e GUTTERIDGE, 1999). Em sistemas biológicos, os radicais livres reagem com os elétrons das biomoléculas que estão à sua volta, ou seja, proteínas, lipídios, carboidratos e ácidos nucléicos. Cada vez que uma biomolécula perde um elétron, esta sofre uma modificação na sua forma e função, podendo perder a sua utilidade no organismo (SIES, 1991; HALLIWELL, 1992; RAMOS et al., 2000).

Diferentes situações são geradoras de constante produção de radicais livres *in vivo*, obrigando os organismos a desenvolverem e manterem, sistemas antioxidantes de defesa, proteção, assim como de reparação, a fim de evitar o acúmulo de moléculas alteradas por oxidação (HALLIWELL, 1992; YU, 1994; RAMOS et al., 2000).

Os antioxidantes são definidos como qualquer substância que, quando está presente em baixas concentrações – comparadas com aquelas de um substrato oxidável – retarda significativamente ou impede a oxidação daquele substrato (o termo substrato oxidável inclui macromoléculas, tais como proteínas, lipídios, hidratos de carbono e DNA). Os antioxidantes podem atuar em distintos níveis como a prevenção da formação e interceptação das EAO ou de seus precursores, reparação das macromoléculas danificadas e regulação da produção de defesas

antioxidantes endógenas. A ação protetora ou o mecanismo de ação dos antioxidantes depende das particularidades de geração da espécie ativa. (HALLIWELL e GUTTERIDGE, 1999).

O sistema antioxidante pode ser de natureza endógena ou exógena. As defesas endógenas do organismo é formado pelos sistemas antioxidantes não-enzimático e enzimático (YU, 1994; PAVANATO e LLESUY, 2008) (Tabela 1).

Tabela 1 - Componentes do sistema de proteção antioxidante.

Sistema de Defesa Antioxidante		
Endógeno		**Exógeno**
Não enzimático	***Enzimático***	• β-caroteno • Vitamina C • Vitamina E • Fenóis simples e Ác. fenólicos • Flavonóides • Isoflavonóides • Quinonas • Xantonas • Lignóides • α-pironas e γ-pironas
• Bilirrubina	• SOD	
• Ácido Úrico	• CAT	
• Glutationa	• GPx	

Fonte: Adaptado de VANNUCCHI et al. (1998).

O desequilíbrio entre os antioxidantes e os pró-oxidantes é o que se denomina estresse oxidativo (SIES, 1985) que, no sistema biológico, está associado ao aumento na velocidade da geração de espécies oxidantes e/ou à diminuição na atividade dos sistemas de defesa, ou ambos, resultando em aumento sustentado das concentrações em estado estacionário de espécies ativas de oxigênio (GONZÁLEZ-FLECHA et al., 1991).

Nos peixes existem inúmeras situações que induzem o desequilíbrio das reações de óxido-redução gerando EAO, entre elas, repetidos episódios de alterações agudas ou crônicas na concentração de oxigênio dissolvido (OD) (VIDELA et al., 1995; BRAUN et al., 2006). As transições entre hipóxia/anóxia e normóxia ou entre normóxia e hiperóxia podem resultar em estresse oxidativo (SIES, 1991; STOREY, 1996; HALLIWELL e GUTTERIDGE, 1999; LUSHCHAK et al., 2001; HERMES-LIMA, 2004). Fatores estressantes como estes, e as alterações em

decorrência deles, podem afetar as taxas de crescimento, a resistência a doenças e, consequentemente, a qualidade da produção.

Nas situações de desbalanço oxidativo pode-se utilizar antioxidantes exógenos para diminuir esse dano oxidativo. Neste contexto, a muito tempo se sabe que a *Lippia alba* (Mill.) N.E. Brown (Verbenaceae) possui propriedades antioxidantes. Na medicina popular é utilizada como planta medicinal de ação calmante, sedativa, anticonvulsivante, espasmolítica suave, analgésica, ansiolítica, miorrelaxante, antiulcerogênica, antimicrobiana, antifungica, larvicida (CHATURVEDI et al., 1976; ADESINA, 1982; ASHOKY et al., 1988; BOORHEM, 1999; MORS et al., 2000; VIANA et al., 2000; PASCUAL et al., 2001; VALE et al., 2002; ZETÓLA et al., 2002; STASHENKO et al., 2004; DUARTE et al., 2005).

2.3 *Lippia alba*

A *L. alba* é uma espécie nativa promissora e que apresenta uma série de estudos pré-clínicos que reforçam muitas das atividades de seu uso popular (HEINZMANN e DE BARROS, 2007), e, segundo PASCUAL et al. (2001), dentre seus metabólitos secundários está descrita a presença de flavonóides sulfatados na posição 4, entre outros.

L. alba é uma planta arbustiva (Figura 6) com ampla distribuição nas Américas Central e do Sul, sendo encontrada em praticamente todas as regiões do Brasil (BIASI e COSTA, 2003) sendo conhecida por diversos nomes populares, como erva cidreira de arbusto, erva cidreira do campo, alecrim do campo, alecrim selvagem, cidreira brava, falsa melissa, erva cidreira brasileira, cidró, cidrão, entre outros (MARTINS et al., 1995; SILVA JUNIOR, 1998; HEINZMANN e DE BARROS, 2007).

A espécie enquadra-se nas seguintes categorias taxonômicas: Reino – Plantae, Divisão – Magnoliophyta, Classe – Magnoliopsida, Ordem – Lamiales, Família – Verbenaceae, Gênero – *Lippia*, Espécie - *Lippia alba.*

Figura 6 - Exemplar de *Lippia alba* (Mill.) N.E. Brown (Verbenaceae). Fonte: http://lucid-state.org

O aroma da planta está relacionado aos constituintes predominantes dos óleos essenciais desta espécie, os quais podem variar em teor e composição química, assim como em quantidade extraída (DE BARROS et al., 2009), em função de diversos fatores abióticos como luz, temperatura, água, solo e altitude (LIMA et al., 2003). Ainda, segundo DE BARROS et al. (2009), a variabilidade na composição do óleo essencial das folhas de *L. alba* foi demonstrada anteriormente em função das diferentes regiões do ramo vegetal, das espécies e horários de coleta, bem como a metodologia empregada na sua extração. Fisicamente, estas substâncias são líquidas, voláteis e, geralmente, com um aroma agradável e intenso, podendo ser incolores ou ligeiramente amareladas (RAVEN et al., 2001; LIMA et al., 2003).

STASHENKO et al. (2004) demonstraram que a atividade antioxidante do óleo essencial de *L. alba* exibiu efeito similar à vitamina E ao 2-(*ter*-butil)-4-methoxifenol (BHA), e RAMOS et al. (2003) comprovaram a toxicidade do extrato frente ao dano oxidativo induzido. Na maior parte dos estudos desenvolvidos, as espécies mamíferas são as escolhidas como modelo de estudo para estresse oxidativo e os mecanismos envolvidos em danos e respostas celulares (LUSHCHAK e BAGNYUKOVA, 2006). Entretanto, os peixes são de especial interesse por causa das particularidades das propriedades do ambiente aquático e sua relação com os organismos (WINSTON e DI GIULIO, 1991; NIKINMAA e REES, 2005). Além disso, RAU et al. (2004) afirmaram que tecidos de peixes podem ser mais sensíveis ao estresse oxidativo do que células de ratos.

Considerando a importância dos peixes como um produto de alto valor nutricional e a sua exposição a diferentes fatores ambientais que podem levar ao

estresse, como hipóxia e hiperóxia, além das propriedades calmantes e sedativas verificadas em vários trabalhos com o óleo essencial da *L. Alba,* propôs-se para esta pesquisa o estudo da ação do óleo essencial de *L. alba* sobre os parâmetros oxidativos de diferentes tecidos do jundiá, submetidos a distintos níveis de oxigênio durante o transporte.

3 OBJETIVOS

3.1 Geral

Verificar a ação do óleo essencial de *L. alba* nos parâmetros oxidativos do jundiá (*Rhamdia quelen*) exposto a diferentes concentrações de oxigênio durante o transporte.

3.2 Específicos

Determinar os níveis de lipoperoxidação nas brânquias, fígado e cérebro de jundiás submetidos a diferentes concentrações de oxigênio e ao óleo essencial de *L. alba* durante o transporte.

Determinar a atividade da catalase nas brânquias, fígado e cérebro de jundiás submetidos a diferentes concentrações de oxigênio e ao óleo essencial de *L. alba* durante o transporte.

Determinar a atividade da superóxido dismutase nas brânquias, fígado e cérebro de jundiás submetidos a diferentes concentrações de oxigênio e ao óleo essencial de *L. alba* durante o transporte.

Determinar a atividade da glutationa-S-transferase nas brânquias, fígado e cérebro de jundiás submetidos a diferentes concentrações de oxigênio e ao óleo essencial de *L. alba* durante o transporte.

4 MANUSCRITO

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Effect of the essential oil of *Lippia alba* on the oxidative stress balance in silver catfish (*Rhamdia quelen*) exposed to different oxygen levels

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Abstract

Juvenile silver fish (*Rhamdia quelen*) were exposed to the essential oil of *Lippia alba* (*L. alba*) and transported in plastic bags for different periods (5, 6 and 7 h) yielding final different oxygen levels. The biomarkers of oxidative stress, lipoperoxidation (LPO), catalase (CAT), superoxide dismutase (SOD), and glutathione-S-transferase (GST) were measured in the liver, gills and brain of the fish. The juveniles were assigned to 6 different treatment groups according to the presence or not of the essential oil of *L. alba* in water (10 µL/L) and the length of transportation, which determined the final concentration of dissolved oxygen inside the bags: Five hours: hyperoxia (13.25 ± 0.35 mg/L O_2); hyperoxia with *L. alba* (11.27 ± 0.22 mg/L O_2); Six hours: normoxia (7.35 ± 0.35 mg/L O_2); normoxia with *L. alba* (7.29 ± 0.40 mg/L O_2); Seven hours: hypoxia (2.29 ± 0.36 mg/L O_2); hypoxia *with L. alba* (3.82 ± 0.7 mg/L O_2). The addition of essential oil of *L. alba* causes an increase of LPO in the tissues exposed to hyperoxia and a reduction of GST in the fish kept under hyperoxia and hypoxia as compared to those under normoxia. In the tissues there is a reduction of LPO and GST and an increase of SOD in the specimens under hypoxia and a reduction of GST in those under hyperoxia with the oil. These results suggest that the presence of the essential oil of *L. alba* improves the redox state in the evaluated tissues, both under hyperoxia and under hypoxia.

Keywords: Oxidative stress, antioxidant enzymes, lipid peroxidation, oxygen availability, *Lippia alba*, fish.

1. Introduction

The most usual system of juvenile fish transportation in Brazil is the closed system using plastic bags. Fish farmers inflate the bags with pure oxygen and consequently there is a rapid change of dissolved oxygen levels through transportation (Gomes et al., 1999; Golombieski et al., 2003). There is a wide range of oxygen tolerance among fishes. Cold-adapted species usually need high oxygen levels, while cyprinid species can survive from full anoxia to hyperoxia (Love, 1980; Lushchak and Bagnyukova, 2006; Lushchak et al., 2001; Van Den Thillart and Van Waarde, 1985). Fishes tolerant to hypoxic/anoxic conditions evolve a number of physiological adaptations including metabolic rate depression, blood flow rearrangement mainly to brain and heart, and effective ways of energy production (Nilsson and Renshaw, 2004). Fish exposition to anoxia and hypoxia may result in oxidative stress although it is not evidenced yet directly. Some clues could be given by an increase in activities of antioxidant enzymes under anoxic conditions (Lushchak et al., 2001). Usually antioxidant enzymes are upregulated by an increase in intracellular ROS levels. These may result from increased ROS level due to reduction of cytochromes of mitochondrial electron transport chain and their leakage to residual oxygen molecules. Anoxia and hypoxia tolerant fishes, successfully surviving low oxygen conditions, undergo a new danger after oxygen resumption. The electron transport chain being reduced under hypoxic state can produce elevated levels of ROS during reoxygenation that may cause oxidative stress. Therefore, these species must evolve well-developed antioxidant systems. Hyperoxia itself is a state that promotes generation of elevated ROS levels.

Every aerobic organism depends on the presence of oxygen for the generation

of energy through oxidative phosphorilation. This process is associated with a tetravalent reduction of the oxygen molecule in the mitochondrial chain, using 90% of the compound (Kelly et al., 1998; Storey, 1996; Winston and Di Giulio, 1991). The remaining 10% can generate distinct cell fractions, products of the univalent reduction, the so-called reactive oxygen species (ROS), which include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^\bullet$) among others (Halliwell and Gutteridge, 1999).

Once produced, ROS may damage cellular components and tissues particularly targeting proteins, lipids, and nucleic acids, often leading to cumulative organ injury. Oxidative stress arises if ROS generation prevails over their degradation (Sies, 1991). In order to protect against oxidative stress, organisms have developed antioxidant systems consisting of low-molecular weight compounds (glutathione, ascorbic and uric acid, tocopherols, etc.) and proteins including antioxidant enzymes. The last are superoxide dismutase (SOD) and catalase (CAT) which decompose O_2^- and H_2O_2, respectively, and glutathione-dependent enzymes, glutathione peroxidase (GPx) which detoxifies both H_2O_2 and organic hydroperoxides, glutathione-S-transferase (GST) which detoxifies various compounds by conjugating them with glutathione, glutathione reductase (GR) which reduces oxidized glutathione using nicotinamide adenine dinucleotide phosphate reduced (NADPH) , and associated enzymes like glucose-6-phosphate dehydrogenase (G6PDH) which supplies reduced equivalents for GR (Halliwell and Gutteridge, 1999).

Lippia alba (Mill.) N. E. Brown (Verbenaceae), popularly known as *cidreira* herb, is a native plant widely distributed in Brazil. In the popular medicine it is used as analgesic for abdominal pains, hemorrhoids, toothache, as febrifuge, in colds and

hepatic diseases. Pharmacological studies have evidenced its analgesic, spasmolytic, and antibacterial activities, as well as the absence of toxic effects in animals. Secondary metabolites described for *L. alba* include flavonoids, tannins, iridoids triterpenic saponins, resins, mucilage and essential oil. Terpenoids, especially mono- and sesquiterpenoids, have different functions, as protection against oxidative damage and low oxygen levels (De Barros et al., 2009). Binding assays were performed on two central nervous system inhibitory targets: benzodiazepine and $GABA_A$ receptors. The most active compound was luteolin-7-diglucuronide, with half maximal inhibitory concentrations (IC_{50}) of 101 and 40 µM, respectively. Fifteen compounds isolated from *L. alba* were tested for their radical scavenging capacities against diphenylpicrylhydrazyl (DPPH). Four of the major compounds (verbascoside, calceolarioside E, luteolin-7-diglucuronide and theveside) were also tested for their antioxidant activity against superoxide radical-anion in cell-free (hypoxanthine-xanthine oxidase) and cellular (PMA-stimulated neutrophil granulocytes) systems (Hennebelle et al., 2008). The essential oil of *L. alba* presents sedative and anesthetics properties in the silver catfish, *Rhamdia quelen* (patent PI0706182-0 from Brazilian Institute of Industrial Protection). Therefore, this study was designed to investigate if this essential oil has antioxidant properties in silver catfish transported in plastic bags.

2. Materials and methods

2.1.Reagents

Phenylmethylsulfonyl fluoride (PMSF), 1-chloro-2,4-dinitrobenzene (CDNB), L-glutathione reduced (GSH), epinephrine, and glycine were purchased from Sigma Chemical Co. (USA). Hydrogen peroxide, trichloroacetic acid (TCA), thiobarbituric

acid (TBA), and albumin were purchased from Merck. All other reagents were of analytical grade.

2.2. Animals and experimental conditions

Silver catfish juveniles (64.5 ± 6.1 g and 18.85 ± 0.57 cm) were obtained from the laboratory of fish culture at the Universidade Federal de Santa Maria (southern Brazil). Fish were kept in continuously aerated tanks (250 L) with dechlorinated well water for at least two weeks before experimental use. The temperature and pH of the water were kept at 22 ± 1.0°C and 7.6 ± 0.2, respectively. The juveniles were fed always at the same time of the day (1 p.m) and the quantity of food offered corresponded to 5% of their biomass/day during the acclimation period. Thirty minutes after the feeding, the feces and food remains were siphoned out. The volume of water withdrawn during the siphoning was about 15% of the total volume of the tanks, and the same volume was immediately replenished at the same conditions of the water that was in the tanks before feeding.

In preliminary experiments, silver catfish juveniles were placed in continuously aerated 40-L tanks and exposed for 5 hours to various concentrations of essential oil of *L. alba* (10 animals per concentration). A concentration of 10 µL/L proved to be the highest one not causing sedation in silver catfish.

Juveniles were weighed and placed in plastic bags (5 L) with 2 L of water that were inflated with oxygen and tied with rubber strings. The load density was 140 - 200 g/L (10 specimens per bag), and the fish were transported on paved road for 5-7 h inside these bags. As the bags were inflated with oxygen, all the groups were exposed to hyperoxia, but on account of oxygen consumption by the fish, the dissolved oxygen levels were gradually decreasing over time. The juveniles were

assigned to 6 treatments (in triplicates) according to the presence or not of *L. alba* essential oil in water (10 µL/L) and the time they remained inside the bags.

Five hours: 1 - hyperoxia (13.25 ± 0.35 mg/L O_2); 2 - hyperoxia with *L.alba* (11.27 ± 0.22 mg/L O_2);

Six hours: 3 - normoxia (7.35 ± 0.35 mg/L O_2); 4 - normoxia with *L.alba* (7.29 ± 0.40 mg/L O_2);

Seven hours: 5 - hypoxia (2.29 ± 0.36 mg/L O_2); 6 - hypoxia with *L.alba* (3.82 ± 0.7 mg/L O_2).

After the experimental period, the fish were killed by spinal sectioning and tissues (gills, liver and brain) were removed, weighed separately and immediately frozen in liquid argon. The tissues were then stocked in freezer at -70°C for subsequent analysis of enzymatic activity.

2.3 Plant material

L. alba (Mill.) N. E. Brown was cultivated in *São Luiz Gonzaga*, state of *Rio Grande do Sul*, Brazil. The aerial parts of the plant were collected in January 2006. The plant material was identified by botanist Dr. Gilberto Dolejal Zanetti, (Department of Industrial Pharmacy, *Universidade Federal de Santa Maria* - UFSM). A voucher specimen (SMDB No. 10050) was deposited in the herbarium of the Department of Biology, UFSM.

2.4 Essential oil extraction and analysis

The essential oil was obtained from fresh leaves of the plant by hydrodistillation for 2 h using a Clevenger type apparatus (Farmacopéia Brasileira, 2000).

2.5 Water parameters

Before and after each experiment, water samples were collected from each plastic bag to determine the water quality parameters. Water alkalinity (39.6 ± 0.8 mg/L $CaCO_3$) was determined by the sulfuric acid (H_2SO_4) method (Greenberg et al., 1976), dissolved oxygen with a YSI model Y5512 oxygen meter, and water pH with a Quimix 400A pH meter. Water hardness (20.7 ± 1.04 mg/L $CaCO_3$) was determined by the EDTA titrimetric method, and total ammonia (8.2 ± 0.91 mg/L) and non-ionized ammonia (0.04 ± 0.02 mg/L) by the direct nesslerization method (Greenberg et al., 1976).

2.6. Oxidative stress parameters

Tissue were homogenized in medium consisting of 120 mM KCl and 30 mM sodium phosphate buffer (pH 7.4) containing 1 mM PMSF. The homogenates were centrifuged at 1000 x*g* for 10 min (Buege and Aust, 1978), between 0 and 4°C to eliminate nuclei and cell debris, and the supernatant fraction thus obtained was frozen at -70ºC for further measurements. The supernatants were used for analysis of lipid peroxidation (LPO), catalase (CAT), glutathione S-transferase (GST), and superoxide dismutase (SOD).

Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) using the method described by Buege and Aust (1978). Aliquots of the supernatant were added to a pyrex tube that contained TCA (10%) and TBA (0.67%) and incubated at 100°C for 15 min. The mixture was allowed to cool on ice for 5 min. The mixture was centrifuged at 1000 xg for 15 min, in order to extract the resulting chromogen (Schiff's base). The absorbance of the organic phase was determined at

535 nm in a spectrophotometer. The results were reported as nmol/mg protein. The protein content of the homogenate was measured using the method described in Lowry et al. (1951) using bovine serum albumin as the standard.

Catalase activity was evaluated by measuring the decrease in the absorption at 240 nm in a reaction medium consisting of 50 mM phosphate buffer (pH 7.4) and 2 mM H_2O_2, thereby determining the pseudo-first-order reaction constant (*k'*) of the decrease in H_2O_2 absorption, method described by Boveris and Chance (1973). The results were reported as pmol/mg protein.

Glutathione S-transferase activity towards CDNB was determined spectrophotometrically at 340 nm using the method described in Habig et al. (1974). Activity was calculated from the changes in absorbance at 340 nm using the extinction coefficient of 9.6 mmol/cm. One unit of GST activity was defined as the amount of enzyme catalyzing the conjugation of 1 µmol of CDNB with GSH per minute at 25°C.

Total superoxide dismutase activity was determined as the inhibition rate of autocatalytic adenochrome generation at 480 nm in a reaction medium containing 1 mM epinephrine and 50 mM glycine/NaOH (pH 10.2). Enzyme activity was expressed as SOD units/mg protein. One SOD unit was defined as the amount of enzyme needed for 50% inhibition of adenochrome formation, as described by Misra and Fridovich (1972).

2.7. Statistical analysis

Data are reported as mean ± SEM (N). Homogeneity of variances among groups was tested with the Levene test. Data presented homogeneous variances, and comparisons between different treatments were made by two-way ANOVA and Tukey-

Kramer Multiple Comparisons test. Analysis was performed using the software Graph Pad InStat (version 3.06), and the minimum significance level was set at $P < 0.05$.

3. Results

In the gills of the silver catfish under hyperoxia there were no significant changes in the TBARS, CAT and SOD levels. However, the GST activity was significantly decreased (44%) in the gills of these specimens, while in those under hypoxia such decrease was 35%, though not significant. The SOD activity in the gills of silver catfish was significantly smaller (44 %) for fish under hypoxia (Table 1).

The fish transported under hypoxia showed a significant decrease (63%) in liver lipoperoxidation as compared to the normoxic group. The CAT activity in liver was significantly smaller both in fish under hyperoxia (52 %) and in those under hypoxia (51 %) as compared to fish under normoxia. The GST activity in liver increased significantly (around 212 %) in silver catfish under hyperoxia, while in those under hypoxia it decreased significantly (around 69 %) as compared to those under normoxia. The SOD activity in liver was significantly smaller (68%) in the animals under hypoxia as compared to those under normoxia (Table 2).

TBARS levels in brain were significantly higher (480%) in animals under hypoxia than in those under normoxia. As for the fish exposed to hyperoxia there were significant changes in lipoperoxidation in brain. Concerning the antioxidant enzymes, values tend to decrease under hypoxia, though not significantly. Under hyperoxia, there was a 184% increase in brain CAT, the levels of the other enzymes remaining close to those of animals kept under normoxia (Table 3).

When the essential oil of *L. alba* was added, there was a significant reduction of lipoperoxidation in the gills of fish under hypoxia and hyperoxia (71% and 39%,

respectively) as compared to those under normoxia. As regards the antioxidant enzymes activity in gills, there were no changes across the groups with the essential oil of *L. alba*. The addition of the essential oil of *L. alba* to the water of animals exposed to hyperoxia caused a significant increase of 40% in gill CAT activity as compared to specimens under hyperoxia without this oil, but the other enzymatic parameters were not significantly affected. However, in the animals kept under hypoxia the addition of essential oil of *L. alba* significantly reduced the TBARS levels (60%) and led to a tendency of increase in the gill SOD activity (71%) (Table 1).

Silver catfish under normoxia with essential oil of *L. alba* presented a significant reduction (61%) of the liver TBARS levels, whereas the other enzymes did not present significant changes as compared to those under normoxia and without this oil. The addition of essential oil of *L. alba* leads to a tendency of increase of lipoperoxidation in silver catfish under hypoxia as compared to those under normoxia. Furthermore, the levels of antioxidant enzymes CAT, GST and SOD in the liver of fish under hyperoxia with *L. alba* decreased significantly by 55%, 67% and around 48%, respectively, compared to those under normoxia with the essential oil. The GST activity under hyperoxia with the essential oil of *L. alba* was also significantly reduced as compared to those under hyperoxia without the oil. In the fish under hypoxia with the oil, the levels of liver enzymes were not significantly changed as compared to fish under normoxia with the oil. The presence of *L. alba* essential oil in silver catfish under hyperoxia significantly reduced the liver TBARS, GST and SOD levels as compared to those under hyperoxia without the oil. Concerning the fish under hypoxia with the oil, the behavior of the liver oxidative parameters was the opposite, all of them rising significantly as compared to those under hypoxia without the oil (Table 2).

The addition of *L. alba* essential oil did not affect significantly the TBARS, CAT, GST and SOD levels in the brain of silver catfish under normoxia as compared with specimens under normoxia not exposed to the oil. However, animals under hyperoxia with the oil had a significant increase of lipoperoxidation in brain (525 %) as compared to those under normoxia. As for the CAT and SOD activities in brain of fish under hyperoxia, there were no significant changes when the antioxidant was added. The GST activity was significantly decreased in silver catfish under hyperoxia (66%) and hypoxia (78%) with *L. alba* essential oil as compared to those under normoxia with the oil. In the groups under hyperoxia the addition of the oil did not significantly affect lipoperoxidation and CAT and SOD activities, but it significantly reduced brain GST activity (57%) as compared to specimens not exposed to the oil. Under hypoxia the addition of the oil significantly reduced lipoperoxidation (57%) and GST activity (56%), but CAT activity was not significantly changed and SOD activity was significantly increased (152 %) in brain as compared to fish without the oil (Table 3).

4. Discussion

It is well known that an increase in environmental oxygen levels can lead to increased ROS generation (Halliwell and Gutteridge, 1989; Hermes-Lima, 2004). Therefore, this research was undertaken to monitor changes in the levels of products of free radical damage lipids oxidation as well as the activities of antioxidant and associated enzymes under hyperoxia and hypoxia. The data on effects of hyperoxic conditions on oxidative damage to tissues and antioxidant defenses are limited. Rainbow trout, *Oncorhynchus mykiss*, treated with ozone or hyperoxia for 4 h and with assays performed during a 48 h recovery period after exposure presented no

effect on TBARS content in liver, but ozone exposure increased it in the gills after only 1 h. The authors explained that the weak effect on lipid peroxidation was due to the adequate antioxidant defenses under the given conditions (Ritola et al., 2002).

Rainbow trout exposed to hyperoxia (16 mg/L dissolved oxygen levels) and fed different levels of ascorbic acid (10, 100, 1000 mg/kg food) for 18 weeks showed an increase of ferric reducing ability of plasma (FRAP) independently of dissolved oxygen when was treated with ascorbic acid (Dabrowski et al., 2004). In our experiments hyperoxia did not cause any significant changes in CAT, LPO and SOD in the gills of silver catfish, but GST decreased significantly. The decrease of this enzyme occurs because the gills are the first organ exposed to hyperoxia, and this detoxifying enzyme is likely to act in order to avoid oxidative damage. As too few studies on fish gills and oxidative stress have been conducted, we cannot discuss these results.

The liver is the most frequently studied organ in analyzing the oxidative unbalance. Exposure of goldfish, *Carassius auratus*, to transient short-term hyperoxia stress (18-20 mg/L O_2 for 3–6 h) resulted in an accumulation of TBARS in the liver. However, the activities of the main antioxidant enzymes, SOD and CAT, were not altered under hyperoxia. These results suggest that hyperoxia stimulated an enhancement of defenses against LPO or mechanisms for enhancing the catabolism of peroxidation products (Lushchak et al., 2005).

Silver catfish exposed to hyperoxia showed an insignificant increase in liver TBARS content, which could be due to the GST levels being 3-fold higher than the normoxic levels. The activity of SOD was not altered, but CAT decreased under hyperoxia conditions. These results suggest that liver GST plays an important role in detoxifying end products of LPO accumulated under hyperoxia stress.

The brain is rich in polyunsaturated acids, so hyperoxia should generate oxidative alterations. Exposure of goldfish to transient short-term hyperoxia stress (18-20 mg/L O_2 for 3–6 h) increased TBARS in the brain, but the activities of SOD, CAT and GST were not altered (Lushchak et al., 2005). In our study, the brain CAT activity increased 2.5-fold in silver catfish exposed to hyperoxia, but the other parameters did not change compared with those maintained in normoxia.

It is generally established that reduced environmental oxygen concentration (termed hypoxia) or its full absence (termed anoxia) reduces ROS level (Halliwell and Gutteridge, 1999; Hermes-Lima, 2004; Storey, 1996). Usually, hypoxia tolerant species successfully survive oxygen insult due to metabolic depression (Hochachka and Somero, 2002), although other biochemical and physiological features contributes into the tolerance. Studies on anoxia/hypoxia effects on the goldfish (Lushchak et al., 2001) and the common carp, *Cyprinus carpio* (Lushchak et al., 2005), generally fit the above mentioned concept.

Hypoxia is a widely studied model, but few fish gill analyses have been carried out. In the estuarine fish *Leiostomus xanthurus* exposed to 2.0 mg/L O_2, there was a reduction in the SOD activity (Cooper et al., 2002). In the gills of silver catfish there was a significant decrease in the SOD activity in fish under hypoxia, which may be a sign of oxidative stress, considering that there was no significant alterations in the TBARS, CAT and GST levels in the same group.

The liver has been widely studied as a metabolizing organ. In piapara, *Leporinus elongatus*, the TBARS levels were not affected, but oxidized glutathione (GSSG) levels were significantly increased during severe hypoxia (1.92 mg/L O_2) (Wilhelm Filho et al., 2005). Lushchak and Bagnyukova (2007), studying the liver of rotan *Percottus glenii* under hypoxia (0.4 mg/L O_2), observed a decrease in TBARS

and CAT and no alteration in the GST levels, though SOD was increased as compared to specimens under normoxia. Exposure to hypoxia (around 2 mg/L O_2) in pacu *Piaractus mesopotamicus*, significantly decreased CAT and GPx enzyme in liver, while SOD and lipoperoxidation (FOX measured) did not change during the experiment (Garcia Sampaio et al., 2008). Cooper et al. (2002) performed three experiments with different levels of oxygen (0.8, 2.0, 4.0, 8.0 mg/L O_2) where they found different SOD and CAT values in liver. In a experiment with hypoxia (0.9 mg/L O2), Lushchak et al. (2005) found in the common carp an increase in the TBARS and a reduction of antioxidant enzyme GPx, although SOD and CAT were not altered. Lushchak et al. (2001), whey they studied oxidative stress and antioxidant defenses in goldfish, found that antioxidant enzymes do not have a specific behavioral pattern, for example, SOD and GST were not altered, CAT was increased and GPx was decreased. In silver catfish submitted to a long-term hypoxia model (30 days), Braun et al. (2008) found increased TBARS levels in liver and muscle along with increased SOD in the same tissues, probably compensating the lipid damage. In our study we found decreased TBARS in hypoxia-exposed specimens just like Luschchak et al. (2007) and a reduction in the antioxidant enzymes CAT, GST and SOD activity, a similar pattern to the one observed by Garcia Sampaio et al. (2008).

The brain is an organ that is markedly affected by hypoxia. In our results brain lipoperoxidation was increased and brain enzymes showed little change. Working with rotan, Luschchak et al. (2007) found that SOD would be an enzymatic marker of the brain oxidative alterations, as there was a 45% reduction of SOD in the three hypoxia groups investigated. When Luschchak et al. (2005) performed an experiment with common carp under hypoxia (0.9 mg/L O_2), they found that CAT and GPx increased significantly. Luschchak et al. (2001) analyzed the brain of goldfish under

hypoxia and observed that SOD was decreased and GPx was increased in relation to specimens under normoxia. As can be seen, our results do not correspond to the results reported by other authors, suggesting that due to the particular species and the length of time the fish were submitted to hypoxia, the oxidative parameters in the three organs do not respond to the classical theory established for oxidative stress. This response fits the hypothesis of preparative adaptation to oxidative stress initially proposed and developed by Buzadzic et al. (1998).

In the gills of fish kept in hyperoxia with essential oil of *L. alba* we observed a significant increase in the CAT levels. In the liver, the presence of this oil decreased the TBARS levels as well the three antioxidant enzymes measured under hyperoxia. In the brain, we observed increased lipoperoxidation and decreased GST. As can be seen, a little quantity of essential oil added improves the antioxidant levels in the tissues or compensates the generated oxidative stress.

In the hypoxia process, the addition of essential oil of *L. alba* also produced alterations in the oxidative balance. In the gills, the presence of this oil significantly reduced the oxidative stress, as there was a decrease of LPO and a tendency of increase of SOD. In the liver, an oxidative balance was observed, with an increase of enzymatic antioxidants GST and SOD. In the brain, we found decreased LPO and GST and a significant increase of SOD.

These results suggest that the presence of essential oil of *L. alba* improves the redox state of the evaluated tissues, under both hyperoxia and hypoxia. It seems that the mechanism evolved to improve the redox state is the adaptation to oxidative stress through the increase of SOD activity, an enzyme that degrades the first ROS that is formed in the univalent reduction of oxygen. As a conclusion of this work, we suggest the use of essential oil of *L. alba* at a concentration of 10 µL/L as an

antioxidant for the brief periods of hypoxia or hyperoxia which occur during the transportation of fish from fish culture stations to their destination, thus improving the well-being of the animals as well as their quality for consumption.

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Additional components

Table 1. TBARS determination, CAT, GST and SOD activity in gills of silver catfish exposed to different oxygen levels and essential oil of *L. alba*

Group	TBARS (nmol/mg protein)	CAT (nmol/mg protein)	GST (µmol/min/mg protein)	SOD (USOD/mg protein)
Without essential oil				
Normoxia	4.95 ± 0.54	0.26 ± 0.02	11.14 ± 0.84	1.00 ± 0.14
Hyperoxia	4.78 ± 1.00	0.27 ± 0.02	6.24 ± 0.87^{+}	1.15 ± 0.11
Hypoxia	4.95 ± 0.72	0.31 ± 0.03	7.19 ± 0.81	0.56 ± 0.07^{+}
With essential oil				
Normoxia	6.90 ± 0.69	0.32 ± 0.01	6.28 ± 0.50	0.98 ± 0.10
Hyperoxia	4.25 ± 0.65^{+}	0.38 ± 0.02*	7.61 ± 0.55	1.22 ± 0.17
Hypoxia	1.98 ± 0.20^{+*}	0.31 ± 0.04	7.23 ± 1.39	0.96 ± 0.11

Values are reported as mean ± S.E.M, n = 10. $^{+}$ significantly different from the respective normoxia group * significantly different from the same group without essential oil of *L. alba* ($P < 0.05$), both determined by two-way ANOVA and Tukey comparison of mean values.

Table 2. TBARS determination, CAT, GST and SOD activity in liver of silver catfish exposed to different oxygen levels and essential oil of *L. alba*

Group	TBARS (nmol/mg protein)	CAT (nmol/mg protein)	GST (mmol/min/mg protein)	SOD (USOD/mg protein)
Without essential oil				
Normoxia	1.29 ± 0.12	14.86 ± 0.68	0.16 ± 0.04	13.71 ± 0.92
Hyperoxia	1.44 ± 0.16	7.11 ± 1.34^{+}	0.50 ± 0.03^{+}	16.02 ± 1.77
Hypoxia	0.48 ± 0.14^{+}	7.27 ± 0.56^{+}	0.05 ± 0.01^{+}	4.44 ± 0.60^{+}
With essential oil				
Normoxia	0.47 ± 0.09*	13.09 ± 1.85	0.18 ± 0.02	15.62 ± 1.31
Hyperoxia	0.43 ± 0.08*	5.96 ± 0.64^{+}	0.06 ± 0.01^{+*}	8.15 ± 1.13^{+}
Hypoxia	0.86 ± 0.15	10.13 ± 1.8	0.13 ± 0.01	13.64 ± 1.58*

Values are reported as mean ± S.E.M, n = 10. $^{+}$ significantly different from the respective normoxia group * significantly different from the same group without essential oil of *L. alba* ($P < 0.05$), both determined by two-way ANOVA and Tukey comparison of mean values.

Table 3. TBARS determination, CAT, GST and SOD activity in brain of silver catfish exposed to different oxygen levels and essential oil of *L. alba*

Group	TBARS (nmol/mg protein)	CAT (nmol/mg protein)	GST (µmol/min/mg protein)	SOD (USOD/ mg protein)
Without essential oil				
Normoxia	2.21 ± 0.81	0.31 ± 0.09	36.80 ± 5.46	1.32 ± 0.25
Hyperoxia	3.59 ± 0.92	0.88 ± 0.17^{+}	33.43 ± 5.50	1.31 ± 0.31
Hypoxia	12.80 ± 2.90^{+}	0.17 ± 0.06	20.55 ± 3.95	0.88 ± 0.36
With essential oil				
Normoxia	1.16 ± 0.14	0.26 ± 0.09	41.85 ± 4.56	2.15 ± 0.27
Hyperoxia	7.25 ± 1.32^{+}	0.56 ± 0.17	14.32 ± 4.80^{+*}	2.19 ± 0.46
Hypoxia	5.50 ± 1.44*	0.14 ± 0.02	9.00 ± 1.32^{+*}	3.14 ± 0.59*

Values are reported as mean ± S.E.M, n = 10. $^{+}$ significantly different from the respective normoxia group * significantly different from the same group without essential oil of *L. alba* ($P < 0.05$), both determined by two-way ANOVA and Tukey comparison of mean values.

5 CONCLUSÕES

Os parâmetros oxidativos dos tecidos expostos a hipóxia não correspondem à teoria clássica para dano oxidativo, provavelmente pelo tempo de exposição à hipóxia, assim como pela espécie.

A melhora do estado redox dos tecidos, tanto em hiperóxia quanto em hipóxia, parece ocorrer através da adaptação ao estresse oxidativo.

Sugerimos o uso do óleo essencial de *L. alba* na concentração de 10 µL/L como antioxidante em períodos curtos de hipóxia ou hiperóxia que ocorrem durante o transporte de peixes entre as estações de piscicultura.

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