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Sharine Nunes Descovi

ÓLEO ESSENCIAL DE *Nectandra grandiflora* NA DIETA DE JUNDIÁ *(Rhamdia quelen)* ANTES E DEPOIS DA HIPÓXIA: ESTRESSE OXIDATIVO E DESEMPENHO ZOOTÉCNICO

Santa Maria, RS
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Dissertação apresentada ao Curso de Pós-Graduação em Zootecnia da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Zootecnia: Produção Animal**

Orientador: Prof. Dr. Bernardo Baldisserotto

Co orientadora: Dr.ª Carine de Freitas Souza

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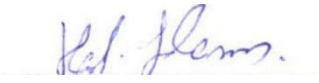

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Aos meus pais

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“Não importa o que aconteça, continue a nadar. ”

(WALTER, GRAHAM: ***Procurando nemo****, 2003)*

RESUMO

ÓLEO ESSENCIAL DE *Nectandra grandiflora* NA DIETA DE JUNDIÁ *(Rhamdia quelen)* ANTES E DEPOIS DA HIPÓXIA: ESTRESSE OXIDATIVO E DESEMPENHO ZOOTÉCNICO

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No cenário atual, o uso de profiláticos à base de produtos naturais vem crescendo devido ao seu potencial biodegradável e menor risco de toxicidade aos animais e ao meio ambiente. Estudos comprovam que os óleos essenciais têm efeitos positivos no desenvolvimento, por meio de melhorias nos níveis fisiológicos, metabólicos, antioxidantes, imunológicos e até mesmo no desempenho zootécnico de algumas espécies de peixes quando adicionados à dieta. O objetivo deste estudo foi avaliar o efeito da adição do óleo essencial de *Nectandra grandiflora* (OENG) na dieta de juvenis de jundiás e sua relação no crescimento e parâmetros pró e antioxidantes desta espécie durante a hipóxia e reoxigenação. 225 juvenis de jundiá (0,92 ± 20 g; 4,42 ± 50 cm) receberam uma dieta suplementada com OENG nas proporções: 0; 0.5; 1.0; 1.5 e 2.0 mL/kg de ração por 60 dias e posteriormente foram submetidos à hipóxia por 8 horas e reoxigenação por 2 horas. Os parâmetros de qualidade da água foram monitorados e mantidos em níveis aceitáveis para a espécie até o momento da hipóxia (oxigênio dissolvido: 2 mg/L). Amostras de fígado, músculo e brânquias foram coletadas, bem como medidas de peso e comprimento dos animais para posterior análise metabólica e de desempenho, respectivamente. Em relação ao desempenho zootécnico, os níveis de OENG testados não alteraram significativamente o crescimento dos peixes. Em relação ao estresse por hipóxia e reoxigenação, os níveis de OENG testados não apresentaram efeito antioxidante frente aos parâmetros avaliados.

Palavras-chave: Aditivo alimentar; peixe; crescimento; estresse oxidativo; reoxigenação; superóxido dismutase

ABSTRACT

ESSENTIAL OIL OF *Nectandra grandiflora* IN THE DIET OF SILVER CATFISH *(Rhamdia quelen)* BEFORE AND AFTER HYPOXIA: OXIDATIVE STRESS AND ZOOTECHNICAL PERFORMANCE

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In the current scenario, the use of prophylactics based on natural products has been growing due to their biodegradable potential and lower risk of toxicity to animals and the environment. Studies prove that essential oils have positive effects on development, through improvements in physiological, metabolic, antioxidant, immunological levels and even in the zootechnical performance of some species of fish when added to the diet. The aim of this study was to evaluate the effect of adding the essential oil of Nectandra grandiflora (OENG) to the diet of young jundiás and its relationship to the growth and pro and antioxidant parameters of this species during hypoxia and reoxygenation. 225 young jundiás (0.92 ± 20 g; 4.42 ± 50 cm) received a diet supplemented with OENG in the proportions: 0; 0.5; 1.0; 1.5 and 2.0 mL/kg of feed for 60 days and subsequently submitted to hypoxia for 8 hours and reoxygenation for 2 hours. Water quality parameters were monitored and maintained at levels acceptable for the species until the time of hypoxia (dissolved oxygen: 2 mg/L). Samples of liver, muscle and gills were collected, as well as measures of weight and length of the animals for further metabolic and performance analysis, respectively. Regarding the zootechnical performance, the tested OENG levels did not significantly alter the growth of the fish. Regarding stress due to hypoxia and reoxygenation, the tested OENG levels did not show an antioxidant effect compared to the parameters evaluated.

Keywords: food additive; fish; growth; oxidative stress; reoxygenation; superoxide dismutase

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LISTA DE ABREVIATURAS E SIGLAS

ANOVA – Análise de variância

ATP – Trifosfato de adenosina

CAT – Catalase

DNA – Ácido desoxirribonucléico

EROS – Espécies reativas ao oxigênio

GPx – Glutationa peroxidase

GSH – Glutationa reduzida

GSSH – Glutationa oxidada

GST – Glutationa S-transferase

H_2O_2 – Peróxido de hidrogênio

LPO – Lipoperoxidação lipídica

MDA - Malondialdeido

MS222 – Tricaína metanosulfato

NADPH – Nicotinamida adenina dinucleótido fosfato reduzida

$O_2^{\bullet-}$ - Ânion radical superóxido

OD – Oxigenio dissolvido

OE – Óleo essencial

OENG – Óleo Essencial de *Nectandra Grandiflora*

OH – Radical hidroxila

pH – Potencial hidrogeniônico

SOD – Superóxido dismutase

TBARS – Substâncias reativas ao ácido tiobarbitúrico

SUMÁRIO

1 INTRODUÇÃO

1.1 AQUICULTURA: ASPECTOS GERAIS

Com o aumento da demanda por proteína animal, a aquicultura é considerada o setor de alimentos que mais cresce, pois é responsável pela criação de vários animais aquáticos (GARLOCK et al., 2020), dentre seus segmentos, a piscicultura se destaca pela crescente produção a cada ano (FAO, 2020).

Em 2019, a piscicultura brasileira produziu 758.006 toneladas de peixes de cultivo, obtendo um crescimento de 4,9% sobre as 722.560 toneladas do ano anterior (PeixeBR, 2020). Tal crescimento se deve ao clima favorável, aos recursos hídricos do país, as dimensões continentais e a busca por tecnologias pelos produtores. O surgimento das novas tecnologias proporciona a expansão do cultivo intensivo da aquicultura a nível mundial, a fim de se obter maior produtividade e suprir a crescente demanda por peixe (TAKAHASHI, 2011). No entanto, o uso de altas densidades nos cultivos vem acarretando em desequilíbrio da produção, pois causa uma supressão do sistema imunológico e diminuição do status antioxidante (CHENG et al., 2015; QI et al., 2017). Contudo, novos desafios surgem devido aos recorrentes problemas de manejo, dentre eles, as baixas concentrações de oxigênio dissolvido (OD), pois limitam o desenvolvimento dos peixes.

Baixos níveis de oxigênio na agua por longos períodos acarretam em alto nível de estresse para os animais, podendo levar a morte ou então ao desenvolvimento de doenças e, consequentemente a perdas econômicas.

1.2 MARCADORES DE ESTRESSE OXIDATIVO

O estresse oxidativo é entendido como o acúmulo desbalanceado de radicais livres que sobrepõem a proteção antioxidante do organismo, causando danos oxidativos às células (HALLIWELL, 2007). O oxigênio apesar de ser essencial para a vida é também tóxico, devido a sua biotransformação em espécies reativas ao oxigênio (EROS) (HERMES-LIMA; ZENTENO-SAVIN, 2002). Alguns desses EROs são radicais livres, tais como hidroxila (OH) e os radicais superóxido (O_2•-), já o peroxido de hidrogênio (H_2O_2) são agentes oxidantes não radicais (BARBOSA et al.,

2010). Quando o sistema de defesa antioxidante está comprometido, pode-se estabelecer um desequilíbrio pró-oxidante nas células, podendo acarretar danos em proteínas celulares (HALLIWELL; CHIRICO, 1993), no ácido desoxirribonucéico (DNA) (MARNETT, 1999), bem como nos lipídeos (ESTERBAUER; CHEESEMAN, 1990).

O organismo conta com um sistema antioxidante para modificar e eliminar essas espécies reativas em excesso e assim, impedir que elas causem efeitos nocivos. Esse sistema é formado por antioxidantes endógenos representados principalmente pelas enzimas antioxidantes, das quais se destacam a superóxido dismutase (SOD), a catalase (CAT), a glutationa-S-transferase (GST) e a glutationa peroxidase (GPx), bem como por antioxidantes não-enzimaticos como a glutationa reduzida (GSH) (WU e tal, 2013). O mecanismo de ação desse sistema se resume em ação preventiva, a primeira linha de defesa, onde os antioxidantes atuam prevenindo as reações dos radicais livres com substâncias biológicas e mecanismos de reparação, ou seja, atuam interrompendo as reações de oxidação e posteriormente na reparação dos danos estruturais causados pelas espécies reativas (WU; KOSTEN; ZHANG, 2013).

De forma geral, a superóxido dismutase (SOD) catalisa a reação de transformação do radical superóxido (O_2 •-) em peróxido de hidrogênio (H_2O_2), que será degradado pela catalase ou glutationa peroxidase (RIBEIRO et al., 2005). A catalase possui a capacidade de transformar o peróxido de hidrogênio em oxigênio e água, atua melhor quando há grandes concentrações intracelulares de peróxido de hidrogênio, pois quando essa concentração é menor a glutationa peroxidase se mostra mais eficiente (HERMES-LIMA, 2004). A glutationa peroxidase (GPx) converte glutationa reduzida (GSH) em glutationa oxidada (GSSH), removendo o peróxido de hidrogênio e formando água. Sendo assim, a catalase e a glutationa peroxidase evitam acúmulo de radical superóxido e peróxido de hidrogênio, para que não seja formado o radical hidroxil, pois contra ele não existe sistema enzimático de defesa (YU, 1994).

A glutationa-S-transferase é a principal enzima de desintoxicação da segunda fase de defesa antioxidante, envolve reações de conjugação na presença de glutationa reduzida, contudo desempenha um papel importante na detoxificação e eliminação de compostos eletrofílicos, pois a partir dessa detoxificação os produtos

tornam-se mais solúveis em água (HERMES-LIMA, 2004; LIMÓN-PACHECO; GONSEBATT, 2009). A glutationa reduzida (GSH) está envolvida na degradação de peróxidos endógenos, na formação de moléculas bioativas, na detoxificação de toxinas e também participa no transporte de aminoácidos (STAMLER; SLIVKA, 1996). Além disso, auxilia na decomposição do peróxido de hidrogênio (H_2O_2), que é convertido em água em uma reação catalisada pela glutationa peroxidase (GPx), oxidando a glutationa reduzida (GSH). A glutationa oxidada é então reciclada à forma reduzida (GSSH) pela glutationa reduzida (GSH) e nicotinamida adenina dinucleótido fosfato reduzida (NADPH) (BOURAOUI, 2008).

Quando o sistema de defesa antioxidante é insuficiente ou inativado ocorrem danos lipídicos, o qual é mensurado a partir da formação de malondialdeido (MDA) (ESTERBAUER; SCHAUR; ZOLLNER, 1991). Além dos danos lipídicos, o estresse oxidativo pode gerar danos as proteínas e também ao ácido desoxirribonucléico (DNA) (MARNETT, 1999), sendo este o dano mais significativo no metabolismo celular.

1.3 OXIGÊNIO DISSOLVIDO E O ESTRESSE OXIDATIVO

Como qualquer ser aeróbico, os peixes dependem da presença de oxigênio para sobreviver. Sua primeira função no organismo é gerar energia, a qual mantem as funções celulares e as atividades das enzimas, as quais catalisam os processos metabólicos dependentes de oxigênio (CADENA, 1989). A metabolização do oxigênio ocorre principalmente na mitocôndria através da cadeia transportadora de elétrons, a qual através da oxidação de nutrientes gera energia metabólica na forma de adenosina trifosfato (ATP) (HALLEWEL e GUTTERIDGE, 2007).

A concentração de oxigênio na água varia de acordo com alguns fatores como temperatura, presença de matéria orgânica, salinidade e difusão da luz (DAVIS, 1975; MATSUO e VAL, 2003; CHIPPARI-GOMES et al., 2003). Segundo Dejours (1975), a quantia de oxigênio dissolvido na água corresponde a 1/30 da quantidade encontrada no mesmo volume de ar, ou seja, até mesmo um consumo moderado de oxigênio pelos processos biológicos ou não biológicos podem acabar diminuindo rapidamente a tensão de oxigênio no ambiente aquático. Essas mudanças nas concentrações de oxigênio podem gerar benefícios para o desempenho das funções fisiológicas ou

contribuir para o desenvolvimento de patologias (BING; OPSTEIN; BROOKS, 1975; HESS e MANSON, 1984; KOCH, 2002; MC DONOUGH e SPITZER, 1983).

Quando a concentração de oxigênio é superior à da encontrada na atmosfera, pode causar consequências deletérias ao organismo, sendo responsável por gerar reações químicas muito tóxicas para as células (BOVERIS e CHANCES, 1973; PAVANATO e LLESUY, 2008). Os níveis de oxigênio dissolvido requeridos para a maioria das espécies encontram-se entre 5 - 6 mg/L (BOYD e TUCKER, 1992; BALDISSEROTTO, 2002). Sabendo disso, situações de hipóxia crônica ou hiperóxia podem ser consideradas estressores ambientais capazes de limitar o desenvolvimento e crescimento dos animais (WILHELM FILHO et al., 2002).

O ambiente aquático está em constante mudança e consequentemente a quantidade de oxigênio dissolvido também, a partir disso, os organismos aquáticos precisam se ajustar as mudanças para garantir a sobrevivência. Para tolerar os déficits de oxigênio os peixes reduzem as taxas metabólicas, reorganizam o fluxo sanguíneo, especialmente cérebro e coração, e vias de produção de energia efetivas (NILSSON e RENSHAW, 2004). Desse modo, torna-se relevante o estudo de produtos capazes de reduzir o estresse causado pela hipóxia, visto que esse fator causa perdas econômicas, seja através de enfermidades ou pela morte dos animais.

1.4 ÓLEOS ESSENCIAIS

Os óleos essenciais (OEs) são definidos como líquidos oleosos aromáticos, obtidos de material vegetal tais como cascas, madeira, raízes, frutas, flores, sementes, folhas, galhos, brotos e ervas. São misturas complexas de substâncias de baixo peso molecular (MORAIS, 2009), com ampla variação em suas propriedades químicas (HUSSAIN et al., 2008). Os óleos essenciais extraídos de plantas contêm compostos produzidos durante o metabolismo secundário da planta e constituem um dos mais importantes grupos de matérias-primas para as indústrias de alimentos, higiene e limpeza, farmacêutica, perfumaria, entre outras. Sua obtenção pode ser feita através de fermentação, enfloração, extração ou expressão, no entanto, o método mais usual para a produção comercial é a destilação por arraste de vapor (VAN DE BRAAK, LEIJTEN, 1999).

As plantas que possuem compostos de interesse terapêutico são muito utilizadas na medicina humana e despertam interesse na aquicultura, pois são produtos considerados mais seguros quando comparados com os produtos sintéticos, convencionalmente usados. Há milênios os óleos essenciais de espécies botânicas são utilizados como fontes de conservantes alimentares e medicamentos (BURT, 2014). Alguns destes óleos essenciais vêm se destacando na piscicultura em áreas como anestesia e sedação, ação antioxidante, bem como na promoção de crescimento (ZHENG *et al*., 2009; CUNHA *et al.,* 2010; BECKER *et al.,* 2012; SACCOL *et al*., 2013; PARODI *et al.,* 2014).

A partir dos benefícios gerados pelos óleos essenciais, tem-se aumentado o interesse por produtos naturais na aquicultura (SHAKYA et al., 2015; AWAD; AWAAD, 2017), os quais geram um menor impacto ambiental relacionado a contaminação de corpos d'agua, diminuem os resíduos químicos na carne dos animais, bem como o menor risco de seleção de patógenos resistentes (COIMBRA et al, 2006).

A crescente utilização de princípios ativos vegetais como imunomoduladores na aquicultura está relacionado ao seu uso de maneira profilática durante os períodos críticos do ciclo produtivo, tais como manejos de classificação, larvicultura, transporte e vacinação, ou seja, onde se tornam mais susceptíveis aos agentes infecciosos (BRICKNELL; DALMO, 2002). A forma de administração pode ser feita através de injeção, imersão ou na dieta, sendo este último o mais prático (CHAKRABORTY; HANCZ, 2011; CHU et al., 2010). Devido aos resultados positivos que os óleos essenciais vêm demonstrando na prevenção e tratamento de diversas enfermidades, muitos estudos vêm sendo realizados a fim de comprovar sua eficácia na promoção de crescimento e melhoria nas respostas metabólicas de peixes.

1.5 CARACTERÍSTICAS GERAIS DA *Nectandra grandiflora*

Conhecida popularmente como canela-amarela (LORENZI, 1982; JURINITZ; JARENKOW, 2003), a *Nectandra grandiflora* (Figura 1) faz parte da família Lauraceae, a qual apresenta grande importância econômica devido a suas espécies apresentarem ampla aplicação (MARQUES, 2001; MELO et al., 2006). O gênero

Nectandra encontra-se entre os mais importantes dentre as Lauraceas (ALVES; SARTORI, 2009), destaca-se pelo potencial madeireiro e também pelos seus óleos essenciais, os quais possuem alto valor econômico e em sua maioria são encontrados no lenho, nas folhas e na casca (MARQUES, 2001; VIEIRA; BIZZO; DESCHAMPS, 2009).

Estudos químicos e farmacológicos realizados com essa espécie evidenciaram atividade antitumoral do extrato etanólico de sua casca, o qual produziu efeito inibitório do sarcoma 180 e do carcinoma de Ehrlich implantados em ratos (MORENO et al., 1993). Enquanto o extrato etanólico obtido das folhas mostrou atividade antioxidante frente ao β-caroteno (RIBEIRO et al., 2005). Na medicina tradicional, a *Nectandra grandiflora* é utilizada como anti-reumática, diurética e digestiva (PIO-CORRÊA,1984; RAGGI, 2008). Para peixes, o óleo essencial de *Nectandra grandiflora* parece apresentar efeito poupador sobre os níveis de oxigênio dissolvido na água de *Colossoma macropomum* quando adicionado 30 µL/L na água e demonstra ainda uma condição fisiológica melhorada após transporte para a espécie (BARBAS et al., 2020). Em estudos de Tondolo et al., (2013) com o óleo essencial de *Nectandra megapotamica*, verificou-se que este óleo apresenta atividade anestésica no robalo peba *(Centropomus parallelus).*

Figura 1: Exemplar de *Nectandra grandiflora*.

Fonte: Flora digital, UFSC.

1.6 JUNDIÁ *(Rhamdia quelen)*

Pertencente a ordem dos Siluriformes e da família Heptapteridae, o jundiá *(Rhamdia quelen)* (Figura 2) é um bagre bentônico de grande importância ecológica e econômica no sul do Brasil (BALDISSEROTTO, 2009). Esta espécie caracteriza-se por apresentar o corpo sem escamas, boca ausente de dentes e barbilhões de forma cilíndrica, com um comprimento que varia proporcionalmente ao tamanho do espécime (GUEDES, 1980), possui coloração acinzentada-escura e ventre branco. Sua alimentação é a base de peixes como lambaris e guarús, crustáceos, insetos aquáticos e terrestres e restos vegetais, (CASATTI et al., 2001; BALDISSEROTTO, 2004; CASTRO, 2006; OYAKAWA et al., 2006), o que lhe confere o habito alimentar onnívoro.

O jundiá apresenta bom desenvolvimento nos primeiros anos de vida, seu comprimento aproxima-se de 66,5 cm nas fêmeas e 52 cm nos machos. A maturidade sexual ocorre logo no primeiro ano e o período reprodutivo compreende os meses de agosto a março (GOMES et al., 2000). Sua carne saborosa, com ausência de espinhos intramusculares (BARCELLOS, 2004), baixo teor de gordura e sabor agradável, tem boa aceitação pelos consumidores. Sua boa adaptação a diferentes ambientes, bem como fácil indução a reprodução (DIEMER et al., 2011) chamam a atenção dos produtores, além disso, na última década essa espécie vem sendo utilizada como modelo experimental para vários extrativos vegetais (CUNHA et al., 2010; SILVA et al., 2012, 2013; GRESSLER et al., 2014; PARODI et al., 2014; TONI et al., 2014).

Figura 2 – Exemplar de Jundiá (*Rhamdia quelen)*

Fonte: minhapescaria.blogspot.com

REFERÊNCIAS

ALVES, F.M.; SARTORI, A.L.B. *Nectandra* Rol. Ex Rottb. (Lauraceae) no Mato Grosso do Sul, Brasil. **Acta Botanica Brasilica**, v. 23, n. 1, p. 118-129, 2009.

ANUÁRIO PEIXE BR da Piscicultura 2020. **Peixe BR: Associação Brasileira de Piscicultura.** Disponível em: https://www.peixebr.com.br/anuario-peixebr-2020/. Acesso em: 19 dez. 2020.

AWAD, E.; AWAAD, A. Role of medicinal plants on grwth performance and imune status in fish. **Fish and Shellfish Immunology**, v. 67, p. 40-54, 2017.

BALDISSEROTTO, B. Biologia do Jundiá. In: BALDISSEROTTO, B.; RADÜNZ NETO, J. **Criação de Jundiá**. Santa Maria: Editora UFSM, 2004, p. 67-72.

BALDISSEROTTO, B. Fisiologia de peixes aplicada a piscicultura. **Santa Maria**: Ed. UFSM, 2002.

BALDISSEROTTO, B. Piscicultura continental no Rio Grande do Sul: situação atual, problemas e perspectivas para o futuro. **Ciência Rural**, v. 39, p. 291-299, 2009.

BARBAS, L.A.L. et al. Stress relieving potential of two plant-based sedatives in the transport of juvenile tambaqui *Colossoma macropomum.* **Aquaculture**, v. 520. https://doi.org/10.1016/j.aquaculture.2019.734681, 2020.

BARBOSA, K. B. F. et al. Estresse oxidativo: conceito, implicações e fatores modulatórios. **Revista de Nutrição**, v. 23, n. 4, p. 629-643, 2010.

BARCELLOS, L. J. G. et al. Nursery rearing of jundiá *Rhamdia quelen* (Quoy & Gaimard) in cages: cage type, stocking density and stress response to confinement. **Aquaculture**, v. 232, p. 383-394, 2004.

BECKER, A. G. et al. Transpotation of silver catfish, *Rhamdia quelen*, in water with eugenol and the essential oil of *Lippia alba*. **Fish Physiology Biochemistry**, v. 38, p. 789-796, 2012.

BING, O.H.; APSTEIN, C.S.; BROOKS, W.W. Factors influencing tolerance of cardiac muscle to hypoxia. **Recent Advances in Studies in Cardiac Structure and Metabolism.** V. 10, p. 343-354, 1975.

BOURAOUI, Z. Acute effects of cádmium on liver phase I and phase II enzymes and metallothionein accumulation on sea bream *Sparus aurata*. **Fish Physiology and Biochemistry,** v. 72, p. 248-254, 2008.

BOVERIS, A.; CHANCE, B. The mitochondrial generation of hydrogen peroxide. **Biochemical Journal**. v. 134, p. 707-716, 1973.

BOYD, C.E. & TUCKER, C.S. Water quality and soil analyses for aquaculture. **Auburn: Auburn University**.1992.

BRICKNELL, I.; DALMO, R.A. The use of in immunostimulants in fish larval aquaculture. **Fish and Shellfish Immunology**, v. 19, p. 457-472, 2005.

CADENAS, E. Biochemistry of oxygen tocity. **Annual Review of Biochemistry**, v. 58, p. 79-110, 1989.

CASATTI, L; LANGEANI, F.; CASTRO, R. M. C. Peixes de riacho do Parque Estadual Morro do Diabo, bacia do alto rio Paraná, SP. **Biota Neotropica**, V.1, P. 1-15, 2001.

CASTRO, R. M. C.; CASATTI, L. Testing the ecomorphological hypothesis in a headwater rifles fish assemblage of the river São Francisco, southeastern Brazil. **Neotroplical Ichthyology**. V. 4, n. 2, p. 203-214, 2006.

CHAKRABORTY, S.B.; HANCZ, c. Application of phytochemicals as immunostimulant, antipathogenic and antistress agentes in finfish culture. **Reviews in Aquaculture**, v.3, p. 103-119, 2011.

CHIPPARI-GOMES et al. Hypoxia tolerance and adaptations in fishes: the case of Amazon Cichlids. In: VAL, A. L. & KAPOOR, B. G. (Eds). Fish adaptations. New Hampshire, **Science Publisheres**, p.37-49, 2003.

CHU, C.; ZHANG, Q.Z.; LUO, F. 2010. Effect of twenty Chinese herbal medicines on killing trophonts, cysts and theronts of *Ichthyophthirius multifiliis* in vitro. **Freshwater Fisheries**, v. 40, p. 55-60, 2010.

COIMBRA, J.L.; SOARES, A.C.F.; GARRIDO, M.S.; SOUZA, C.S.; RIBEIRO, F.L.B. Toxicidade de extratos vegetais a *Scutellonema bradys*. **Pesquisa Agropecuária Brasileira**, v.41, n. 7, p. 1209-1211, 2006.

CUNHA, M. A. et al. Essential oil of *Lippia alba*: a new anesthetic for silver catfish, *Rhamdia quelen*. **Aquaculture**, v. 306, p. 403-406, 2010.

DAVIS, J.C. Minimal dissolved oxygen requiriments of aquatic life with emphasis on Canadian species: a review. **Journal fish research board Canada**. V. 32, p. 2295-2332, 1975.

DEJOURS, P. **Principles of Comparative Respiratory Physiology**. Amsterdam. North-Holland, 1975.

DIEMER, O. et al. Níveis de fósforo total na alimentação de juvenis de jundiá criados em tanques-rede. **Pesquisa agropecuária tropical**, v. 41, n. 4, p. 559-563, 2011. Disponível em: < https://www.scielo.br/scielo.php?script=sci_arttext&pid=S1983-40632011000400017> Acesso em: 09 dez. 2020.

ESTERBAUER, H.; CHEESEMAN, K. H. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. **Methods in Enzymology**, New York, v. 186, p. 407-421, 1990.

ESTERBAUER, H.; SCHAUR, R.J.; ZOLLNER, H. Chemistry and Biochemistry of 4-hydroxynoneal malondialdehyde and related aldehydes. **Free Radical Biology and Meducine**, New York, v. 11, p. 81-128, 1991.

FAO – FOOD AND AGRICULTURE ORGANIZATION. **The State of World Fisheries and Aquaculture.** Rome: FAO, 2020. Disponível em: http://www.fao.org/3/ca9229en/ca9229en.pdf. Acesso em: 19 dez. 2020.

GARLOCK, T., ASCHE, F., ANDERSON, J., BJORNDAL, T., KUMAR, G., LORENZEN, K., ROPICKI, A., SMITH, M.D., TVETERAS, R. A global blue revolution: aquaculture growth across regions, species, and countries. **Reviews in Fisheries Science & Aquaculture**. 28, 107-116, 2020.

GOMES, L. C.; GOLOMBIESKI, J.I.; GOMES, A.R.C.; BALDISSEROTTO, B. Biologia do jundiá *Rhamdia quelen* (Teleostei, pimelodidae). **Ciência Rural**, v. 30, n. 1, p. 179-185, 2000.

GRESLER, L. T. et al. Silver catfish *Rhamdia quelen* immersion anestesia with essential oil of *Aloysia triphylla* (L'Hérit) Britton or tricaine methanesulfonate: effect on stress response and antioxidante status. **Aquaculture Research**, v. 45, p. 1061-1071, 2014.

GUEDES, D.S. **Contribuição ao estudo da sistemática e alimentação de jundiás *(Rhamdia spp)* na região central do Rio Grande do Sul (Pisces, Pimelodidae).** 1980. 99 f. Dissertação (Mestrado em Zootecnia) – Universidade Federal de Santa Maria, Santa Maria, 1980.

HALLIWELL, B.; CHIRICO, S. Lipid peroxidation: its mechanism, measurement, and significance. **The American Journal of Clinical Nutrition**, v. 57, p. 715-725, 1993.

HALLIWELL, B.; GUTTERIDGE, L. Free Radicals in Biology and Medicine. Nova York: Oxford University Press, v.1, 851p, 2007.

HERMES-LIMA, M. **Oxygen in biology and biochemistry: role of free radicals.** In: STOREY, K.B. (ed) Functional metabolismo: regulation and adaptation. New York, John Wiley & Sons, Inc., p. 319-368, 2004.

HERMES-LIMA, M.; ZENTENO-SAVÍN, T. Animal response to drastic changes in oxygen availability and physiological oxidative stress. **Comparative Biochemistry and Physiology Part** C: Toxicology & Pharmacology, v. 133, p. 537-556, 2002.

HESS, M.L.; MANSON, N.H. Molecular oxygen: friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. **Journal of Mollecular and Cellular Cardiology**. V. 16, p. 969-985, 1984.

HUSSAIN, AI, ANWAR, F., SHERAZI, STH E PRZYBYLSKI, R. The chemical composition and antioxidant and antimicrobial activities of essential oils of basil *(Ocimum basilicum)* depend on seasonal variations. ***Food Chem.*** 108, 986–995. doi: 10.1016 / j.foodchem.2007.12.010, 2008.

JURINITZ, C. F.; JARENKOW, J. A. Estrutura de uma floresta estacional no Rio Grande do Sul. **Revista Brasileira de Botânica**, v. 26, p. 475-487, 2003.

KOCH, C.L. Measurement of absolute oxygen levels in cells and tissues usig oxygen sensors and 2-nitroimidazole EF5. **Methods in Enzymology.** V. 352, p. 3-31, 2002.

LIMÓN-PACHECO, J.; GONSEBATT, M.E. The role of antioxidants and antioxidant related enzymes in protective responses to environmentally induced oxidative stress. **Mutation Research**, v. 674, p. 137-147, 2009.

LORENZI, H. **Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil.** Nova Odessa: Institut Plantarum, 1982, 368 p.

MARNETT, L.J. Lipid peroxidation-DNA damage by malondialdehyde. **Mutation Research**, v. 424, p. 83-95, 1999.

MARQUES, C.A. Importancia da familia Lauraceae Lindl. **Floresta e Ambiente**, v.8, p. 195-2006, 2001.

MATSUO, A. Y. O.; VAL, A.L. Fish adaptations to Amazonian blackwaters. Em: VAL, A.L.; KAPOOR, B. G. (Eds). Fish adaptations. New Hampshire, **Science Publisheres**, p.37-49, 2003.

MC DONOUGH, K.H.; SPITZER, J.J. Effects of hypoxia and reoxigenation on adult rat heart cell metabolismo. **Proceedings of the Society for Experimental Biology and Medicine.** V. 173, p. 519-526, 1983.

MELO, J. O. et al. Anti-inflammatory activity of crude extract and fractions of *Nectandra falcifolia* leaves. **Biological & Pharmaceutical Bulletin**, v.29, n. 11, p. 2241-2245, 2006.

MORAIS, LAS. Influência de fatores abióticos na composição química de óleos essenciais. ***Hortic. Bras****.* 27, 4050–4063, 2009.

MORENO, P.R.H, et al. Chemical constituents and antitumor activity of *Nectandra grandiflora*. **International Jouranl of Pharmacognosy**, v.31, p. 189-192, 1993.

NILSSON, G.E.; RENSHAW, G.M.C. Hypoxic survival strategies in two ishes: extreme anoxia tolerance in the North European crucian carp and natural hypoxic preconditioning in a coral-reef shark. **The Journal of Experimental Biology**. V. 207, p.3131-3139, 2004.

OYAKAWA, O. T.; AKAMA, A.; MAUTARI, K. C.; NOLASCO, J. C. **Peixes de riachos da Mata Atlântica**. São Paulo: Neotrópica, 2006.

PARODI, T. V et al. Anesthetic activity of *Aloysia triphylla* and effectveness in reducing stress durin transporto f albine and grey strains of silver catfish, *Rhamdia quelen*. **Fish Phisiology and Biochemistry**, v. 40, n. 2, p. 323-334, 2014.

PAVANATO, M.A.; LLESUY, S.F. Active species of oxygen and nitrogen. In: Oxidative stress and inflammation: from experimental to clinical models. MARRONI, N.P. (Org). **Canoas**: Ed. ULBRA, p. 12-24, 2008.

PIO-CORRÊA, M**. Dicionário de plantas úteis do Brasil e das exóticas cultivadas.** Rio de Janeiro: Imprensa Nacional, 1984.

RAGGI, L. Estudo da composição quimica e das atividades biologicas dos oleos volateis de espécies de Lauraceae, em diferentes épocas do ano. 2008. 67 f. Dissertação (Mestrado em Biodiversidade Vegetal e Meio Ambiente) – Instituto de Botânica da Secretaria de Estado do Meio Ambiente, **São Paulo**, 2008.

RIBEIRO, S.M.R. et al. The formation and effects of reactive oxygen species in the biological environment.. **Bioscience Journal**, v. 21, p. 133-149, 2005.

SACCOL, E. M. H et al. Addition of *Lippia alba* (Mill) N. E. Brown essential oil to the diet of the silver catfish: Na analisys of growth, metabolic and BLOOD PARAMETERS AND THE ANTIOXIDANT RESPONDE. **Aquaculture**, v. 416-417, p. 244-254, 2013.

SHAKYA, S.R. Medicinal uses of ginger (Zingiber officinale Roscoe) improves growth and enhances immunity in aquaculture. **International Journal of Chemical Studies**, v.3, n. 2, p. 83-87, 2015.

SILVA, L. L. et al. Essential oil of *Ocimum gratissimum* L.: Anesthetic sffects, mechanism of action and tolerance in silver catfish, *Rhamdia quelen*, **Aquaculture**, v. 350-353, p. 91-97, 2012.

STAMLER, J.S.; SLIKVA, A. Biological chemistry of thiols in the vasculature and in vascular-related desease. **Nutrition Reviews**, v. 54, p. 1-30, 1996.

TAKAHASHI, N. S. **Nutrição de peixes.** São Paulo: Associação Brasileira de Piscicultores e Pesqueiros, 2011. Disponível em: http://www. abrappesq.com.br/materiais.htm. Acesso em: dezembro de 2020.

TONI, C. et al. Fish anestesia: effects of the essential oil of *Hesperozygis ringens* and *Lippia alba* on the biochemistry and physiology os silver catfish (*Rhamdia quelen).* **Fish Physiology and Biochemistry,** v. 40, n. 3, p. 701-714, 2014.

VAN DE BRAAK, S. A. A. J.; LEIJTEN, G. C. J. J. Essential Oils and Oleoresins: A Survey in the Netherlands and other Major Markets in the Europen Union. CBI, Center for the Promotion of Imports from Developing Countries, **Rotterdam**, p. 116, 1999.

VIEIRA, R.F.; BIZZO, H.R.; DESCHAMPS, C. Genetic resources of aromatic plants from Brazil. **Israel Journal of Plant Sciences**, v. 58, p. 263-271, 2009.

WILHELM FILHO et al. Effect of diferente oxygen tensions on wheight gain, feed conversion, and antioxidante status in piapara, *Leporinus elongatus* (Valenciennes, 1947). **Aquaculture.** V. 244, p.349-357, 2002.

WU, Q.; KOSTEN, T. R.; ZHANG, X. Y. Freeradicals, antioxidant defense systems, and schizophrenia. **Progress Neuropsychopharmacol Biological Psychiatry**, v. 46, p. 200-206, 2013.

YU. B. P. Cellular defenses against damage from reactive oxygen species. **Physiological Reviews**, v.74, p. 139-162, 1994.

ZHENG, Z. L. et al. Evaluation of orégano essential oil *(Origanum heracleoticum L.)* on growth, antioxidante effect and resistance against *aeromonas hydrophila* in channel catfish *(Ictalurus punctatus)*. **Aquaculture**, v. 292, n. 3-4, p. 214-218, 2009.

2 OBJETIVOS

2.1 OBJETIVO GERAL

- Avaliar o efeito da adição do óleo essencial de *Nectandra grandiflora* na ração de juvenis de jundiá, seu efeito sob o desempenho zootécnico e parâmetros metabólicos dos peixes quando submetidos a hipóxia.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar se a adição do OE de *Nectandra grandiflora* na dieta de juvenis de jundiá é eficiente na promoção do crescimento para a espécie.

- Avaliar se a adição do OE de *Nectandra grandiflora* na dieta de juvenis de jundiá altera os parâmetros pró e antioxidantes desta espécie, quando submetidos ao estresse causado pela hipóxia e reoxigenação.

3 DESENVOLVIMENTO

3.1 MANUSCRITO

ESSENTIAL OIL OF NECTANDRA GRANDIFLORA IN THE DIET OF JUNDIÁ (RHAMDIA QUELEN) BEFORE AND AFTER HYPOXY: OXIDATIVE STRESS AND ZOOTECHNICAL PERFORMANCE

Essential oil of *Nectandra grandiflora* in the diet of jundiá (Rhamdia quelen) before and after hypoxy: oxidative stress and zootechnical performance

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Abstract: In the current scenario, the use of prophylactics based on natural products has been growing due to their biodegradable potential and lower risk of toxicity to animals and the environment. Studies prove that essential oils have positive effects on development, through improvements in physiological, metabolic, antioxidant, immunological levels and even in the zootechnical performance of some species of fish when added to the diet. The aim of this study was to evaluate the effect of adding the essential oil of Nectandra grandiflora (OENG) to the diet of young jundiás and its relationship to the growth and pro and antioxidant parameters of this species during hypoxia and reoxygenation. 225 young jundiás (0.92 ± 20 g; 4.42 ± 50 cm) received a diet supplemented with OENG in the proportions: 0; 0.5; 1.0; 1.5 and 2.0 mL / Kg-1 of feed for 60 days and subsequently submitted to hypoxia for 8 hours and reoxygenation for 2 hours. Water quality parameters were monitored and maintained at levels acceptable for the species until the time of hypoxia (dissolved oxygen: 2 mg / L). Samples of liver, muscle and gills were collected, as well as measures of weight and length of the animals for further metabolic and performance analysis, respectively. Regarding the zootechnical performance, the tested OENG levels did not significantly alter the growth of the fish. Regarding stress due to hypoxia and reoxygenation, the tested OENG levels did not show an antioxidant effect compared to the parameters evaluated.

Keywords: food additive; fish; growth; oxidative stress; reoxygenation; superoxide dismutase

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Introduction

With the increase in demand for animal protein, aquaculture is considered the fastest growing food sector, as it is responsible for the creation of several aquatic animals (GARLOCK et al., 2020), among its segments, fish farming stands out for the growing production every year (FAO, 2020). However, new challenges arise due to the recurrent management problems, among them, the low concentrations of dissolved oxygen (DO), as they limit the development of fish.

Hypoxia can be defined as a decrease in dissolved oxygen (DO) levels, which makes it difficult for aquatic organisms to extract. Therefore, they need to reduce the rate of energy expenditure to match the availability of oxygen (YOANN et al. 2019; ABDELTAWWAB et al. 2019). This problem results in physiological disorders and negatively affects behavior, growth, immunology and reproductive success, both individually and in the population level of fish species (WU 2002; ABDELTAWWAB et al. 2019). The hypoxia lethal threshold value is approximately 2 mg/L of dissolved oxygen for most teleost species (RABALAIS et al. 2010).

However, tolerance to hypoxia varies considerably between species and respective stages of development (EKAU et al. 2010; VAQUER-SUNYER and DUARTE, 2008). In general, fish respond by increasing oxygen transfer to tissues or reducing oxygen consumption through metabolic depression to survive, if hypoxia is inevitable (MORAES et al. 2002; DOUXFILS et al. 2012). Hypoxia-tolerant fish are in a new danger after the resumption of oxygen, the electron transport chain reduced in a hypoxic state can produce high levels of reactive oxygen species (EROS) during reoxygenation (WILHELM FILHO et al., 2001; WILHELM FILHO et al., 2002), which can cause oxidative stress.

Due to this stress, the organism has developed means to protect itself, among them, the production of antioxidant enzymes (HALLIWEL and GUTTERIDGE, 1999). Enzymes such as superoxide dismutase (SOD) catalyze the reaction of converting the radical superoxide anion ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), so that later the catalase enzyme (CAT) dismutes the hydrogen peroxide (H_2O_2) in Water. Glutathione peroxidase (GPx) has the ability to reduce hydrogen peroxide (H_2O_2), but it also acts on hydroperoxides. Glutathione-S-transferase (GST) is a detoxification enzyme and

catalyzes the conjugation of reduced glutathione (GSH) and oxidized molecules, making toxic compounds easily excreted. (LUSHCHAK; BAGNYUKOVA, 2006; LUSHCHAK, 2014).

Essential oils have been showing good results in areas such as anesthesia and sedation, antioxidant action, as well as in promoting growth (ZHENG et al., 2009; CUNHA et al., 2010; BECKER et al., 2012; SACCOL et al., 2013; PARODI et al., 2014). For fish, the essential oil of *Nectandra grandiflora* seems to have a sparing effect on the levels of oxygen dissolved in the water of *Colossoma macropomum* when added 30 µL/L in the water, it also demonstrates an improved physiological condition after transport for the species (BARBAS et al., 2020).

Popularly known as yellow cinnamon (LORENZI, 1982; JURINITZ; JARENKOW, 2003), *Nectandra grandiflora* is part of the Lauraceae family and has great economic importance due to its species having wide application (MARQUES, 2001). However, there is a lack of studies focused on the antioxidant action of this essential oil in the face of hypoxia, as well as on the growth of young silver catfish. From this, the objective of this study was to evaluate the action of *Nectandra grandiflora* essential oil on the antioxidant parameters of silver catfish *(Rhamdia quelen)* when in a hypoxia situation, as well as its action on the growth of this species when added in the diet.

Material and methods

Fish and culture conditions. The experiment was conducted in a water recirculation system at the Pisciculture Laboratory of the Federal University of Santa Maria (UFSM), Campus Santa Maria, Rio Grande do Sul (RS), Brazil. Jundiás juveniles (0.92 ± 20 g; 4.42 ± 50 cm) were captured from a local producer. The fish were randomly distributed in fifteen polypropylene tanks (30 L), 15 fish per tank, and acclimated to laboratory conditions for two weeks. The water parameters were checked daily: a temperature of 27.5 ºC ± 0.5 was obtained; dissolved oxygen 7.12 ± 0.11 mg/L (oxygen meter Y5512; YSI Inc., Yellow Springs, OH, USA); pH 7.15 ± 0.03 (pH meter DMPH -2, Digimed, São Paulo, SP, Brazil); levels of total ammoniacal nitrogen 0.84 ± 0.09 mg/L (EATON et al. 2005); and levels of non-ionized ammonia (NH3) 0.005 ± 0.001 mg/L (COLT 2002).

Alkalinity 28.5 ± 1.0 CaCO3 mg/L (BOYD & TUCKER 1992) and water hardness 25.0 ± 1.3 CaCO3 mg/L (EDTA titration method) were determined weekly. At the end of the experiment, the fish were submitted to stress due to hypoxia, with interruption of oxygenation for 48 hours, where the oxygen level reached 2mg/L, for eight hours. Experimental protocol was approved by the Animal Experimentation Committee of UFSM under registration nº 6361090518-4° C until use.

Extraction and analysis of essential oil. The leaves of Nectandra grandiflora were collected in the city of Jaguari (RS) and a specimen, botanically identified by Dr. Solon Jonas Longhi, was deposited in the Herbarium of the Department of Biology at the Federal University of Santa Maria, Santa Maria, RS, Brazil (SMDB 13,162). The essential oil was extracted from the leaves for 3 hours by hydrodistillation with Clevenger apparatus in triplicate (European Pharmacopoeia, 2010) and stored in a light-proof container at -4 °C until use. The essential oil of Nectandra grandiflora and the compounds were identified and quantified by gas chromatography coupled to the mass spectrometry method (GC-MS) (Table 1).

Diets and experimental design. Five diets were formulated (36.16% crude protein and 8.24% lipids) based on the study by Lazzari et al. (2008). The ingredients were finely ground, weighed and mixed by kneading until smooth. Different concentrations of the essential oil of N. grandiflora (0-control, 0.5, 1.0, 1.5 or 2.0 mL/kg of diet) were added to the mixture along with the canola oil (Table 2). Then, water was added to the diets and the drying was carried out in an oven with forced air circulation for 24 hours (35 °C). Finally, the pellets were broken, sifted and stored in a freezer until use. The fish received the experimental diets until apparent satiety three times a day (9 am, 1 pm and 5 pm) for 60 days. The experimental design resulted in five groups (performed in triplicate).

Table 1. Chemical composition of the EO of *Nectandra grandiflora* added to the experimental diet.

RT	Compound	KI_C	KI_L	%
10.32	α-Pinene	931	937^N	2.14
12.01	β-Pinene	973	978^N	2.27
14.61	(*Z*)-Ocimene	1039	1038^N	0.27
15.00	(*E*)-Ocimene	1049	1048^N	0.63
17.02	Linalool	1101	1101^N	4.42
20.27	(2*E*)- Hexenyl butyrate	1188	$1191^{N/A}$	0.53
27.22	β-Elemene	1393	1394^N	0.47
28.09	Aromadendrene	1421	1418^N	1.05
28.77	NI	1443	_	2.75
29.66	α-Muurolene	1471	1472^N	1.04
29.89	Allo-Aromadendrene	1479	1478^N	1.30
30.02	Amorpha-4,7(11)-diene	1483	1479^A	3.79
30.21	Valencene	1489	1490^N	1.40
30.38	Eremophilene	1495	1489^N	0.90
30.49	Biciclogermacrene	1498	1500^A	5.93
30.75	NI	1507	_	7.52
30.81	NI	1509	_	2.59
31.76	NI	1542	_	0.75
32.87	Spathulenol	1579	$1578^{N/A}$	3.48
33.05	NI	1586	_	0.75
33.19	NI	1590	_	0.57
33.36	NI	1596	_	0.58
34.96	**Dehydrofukinone epoxide**	**1653**	$\mathbf{1656^S}$	**10.07**
35.06	**Selin-11-en-4-α-ol**	**1657**	$\mathbf{1659^A}$	**4.09**
37.70	NI	1755	_	3.66
37.86	NI	1761	_	1.32
39.08	**(+)-Dehydrofukinone**	**1808**	$\mathbf{1813^S}$	**22.21**
39.40	**Eremophil-11-en-10-ol**	**1821**	$\mathbf{1824^S}$	**6.19**
42.18	Rumueno	1933	1930^N	1.62
44.72	(-)-Caureno	2040	2041^N	5.50

RT = compound retention time in the column (min.); NI= non-identified compound; KIc=calculated Kovats index; KIL=literature Kovats index; N=Nist (2008); A=Adams (2009); S=Silva (2017).

Table 2. Formulation (%) of the experimental diet.

Ingredients	**(%)**
Soybean meal	30
Meat and bone meal	35
Rice bran	12
Corn	15
Canola oil	3
Salt	1
Vitamins and minerals (premix) [a]	3
Phosphate dicalcium	1

[a] Vitamin and mineral mixture (security levels per kilogram of product) — folic acid: 250 mg, pantothenic acid: 5,000 mg, antioxidant: 0.60 g, biotin: 125 mg, cobalt: 25 mg, copper: 2000 mg, iron: 820 mg, iodo: 100 mg, manganese: 3750 mg, niacin: 5000 mg, selenium: 75 mg, vitamin A: 1,000,000 UI, vitamin B1: 1250 mg, vitamin B12: 3750 mcg, vitamin B2: 2500 mg, vitamin B6: 2485 mg, vitamin C: 28,000 mg, vitamin D3: 500,000 UI, vitamin E: 20,000 UI, vitamin K: 500 mg, zinc: 17,500 mg.

Growth performance detection. The fish in each tank were weighed and measured individually on days 0 and 60 of the experiment. From the weight and total length evaluations, the initial weight (g), final weight (g), weight gain (g) [WG = (Fw-Iw)] and Specific growth rate (% day) [SGR] were measured = 100 * (In Fw * In Iw/t)].

Tissue collection and analysis. After 60 days, five fish from each box were used for tissue collection and biochemical analysis. The remaining animals were submitted to hypoxia (2 mg/L of dissolved oxygen) for 8 hours, by turning off the water recirculation system. Of these animals, five fish from each box were used to collect tissues (gills, liver and muscle). After the hypoxia period, the remaining animals went through

reoxygenation for two hours (7 mg/L of dissolved oxygen). Of these animals, five fish from each box were used to collect tissues (gills, liver and muscle).

For the evaluation of oxidative parameters, liver, gills and muscle were homogenized in a 1:10 ratio of 30 mM sodium phosphate buffer, pH 7.4, containing 120 mM KCl according to Bueg and Aust (1978). The homogenates were centrifuged at 850 × g for 10 minutes at 4 °C and the supernatants were used to determine reactive oxygen substances (EROS), superoxide dismutase (SOD) and reduced glutathione (GSH). The levels of reactive oxygen species (EROS) were determined by the dichlorofluorescin (DCFH) oxidation method described by LeBel et al. (1992), using excitation and emission of wavelengths of 485 and 538 nm, respectively. The results were expressed as U DCF/mg protein.

Lipid lipoperoxidation was established through substances reactive to thiobarbituric acid (TBARS), carried out by the reaction of malondialdehyde (MDA) with thiobarbituric acid according to Buege and Aust (1978) at 532 nm. TBARS levels were expressed as nmol MDA/g tissue. Protein content in tissues was determined by spectrophotometer (595 nm) using Coomassie blue G dye (Read and Northcote, 1981), using bovine albumin as a standard. Superoxide dismutase (SOD) activity was evaluated spectrophotometrically as described by Marklund and Marklund (1974), recently published in detail by Souza et al. (2018). The enzymatic activity was expressed in SOD units / mg of protein. The reduced glutathione (GSH) activity was determined by the method previously described by Vardi et al (2008) and expressed in µmol GSH/g of tissue.

Statistical analysis. Data are reported as mean ± SE. The homogeneity of variances among groups was determined with the Levene test. All treatment groups were compared by two-way analysis of variance and Tukey's test: or when homogeneity of variances was not obtained by Scheirer-Ray-Hare extension of the Kruskal-Wallis test and Nemenyl test. Analyses were performad using the STATISTICA software package, version 5.1 (StatSoft, Tulsa, OK. USA), and the minimum significance level was set at $p<0,05$. Zootechnical performance data was used Levene test was applied to evaluate the homogeneitty of the variances. Data were comparad using one-way (ANOVA) followey by Tukey's test, the minimum significance level was set at $p<0,05$.

Results

Zootechnical performance. No mortality occurred during the experiment. In addition, no significant differences were observed in growth rates and other zootechnical parameters of jundiá fed diets containing essential oil of Nectandra grandiflora when compared to the control group (Table 3).

Table 3 - Performance of juvenile silver catfish *(Rhamdia quelen)* fed with diferente concentrations of OENG in the diet

	Dieta (mL EO por kg de dieta)				
	0	**0.5**	**1.0**	**1.5**	**2.0**
IW	1,0 ± 0, 11	1,02 ± 0,14	0,86 ± 0,20	0,84 ± 0,21	0,85 ± 0,13
FW	5,12 ± 0,30	5,09 ± 0,21	4,80 ± 0,34	5,11 ± 0,16	5,03 ± 0,17
WG	4,85 ± 0,09	4,06 ± 0,43	3,58 ± 0,25	4,33 ± 0,09	4,18 ± 0,28
SGR	77,49 ± 0,24	70,37 ± 3,89	65,14 ± 3,06	71,51 ± 1,11	70,14 ± 2,89

IW= Initial weigth; FW= Final weight; WG= Weight gain; SGR= Specific growth rate; p<0,05 by Tukey's test.

Reactive oxygen species (EROS). The levels of EROS in the gills (Fig. 1A) decreased during hypoxia ($p < 0.05$) and remained low in reoxygenation in all treatments. However, in reoxygenation, fish fed 1.5 and 2.0 mL/kg of OENG had lower EROS levels ($p < 0.05$) than the other groups. EROS levels in the liver did not change in any of the treatments (Fig. 1B) during normoxia and hypoxia. However, in reoxygenation there was an increase ($p < 0.05$) of these levels in the control groups, 0.5, 1.0 and 1.5 mL / kg of OENG. In the muscle, during hypoxia, fish from treatments 1.5 and 2.0 mg / kg of OENG showed an increase ($p < 0.05$) in EROS levels in relation

to their values in normoxia and the other groups. On reoxygenation, there was an increase in the levels of EROS in the fish of the control groups, 0.5 and 1.0 mL / kg of OENG in relation to normoxia and hypoxia (Fig. 1C).

Thiobarbituric acid reactive substances (TBARS). All groups that received supplementation of OENG in the diet had lower levels of TBARS in the gills. The fish in the control group showed a reduction ($p < 0.05$) in TBARS levels when exposed to hypoxia and reoxygenation, compared to normoxia. Animals fed with EONG supplementation showed an increase ($p < 0.05$) in TBARS levels during hypoxia and reoxygenation, when compared to normoxia. Fish fed 2.0 mL / kg of EONG demonstrated the lowest levels ($p < 0.05$) of TBARS on reoxygenation (Fig. 2A). In normoxia, animals fed 1.5 mL / kg of OENG had the highest levels ($p < 0.05$) of TBARS in the liver. In hypoxia, there was a reduction in the levels of hepatic TBARS in the groups fed with 0.5, 1.0 and 1.5 mL / kg of OENG in comparison with normoxia. During hypoxia, fish fed 0.5 mL / kg of OENG had lower levels ($p < 0.05$) of TBARS in the liver when compared to control. and during reoxygenation, the animals that received 1.0 mL / kg of OENG showed a reduction ($p < 0.05$) in the levels of hepatic TBARS when compared to the other treatments. During reoxygenation, the levels of hepatic TBARS in the control group were higher ($p < 0.05$) than during normoxia and hypoxia (Fig. 2B). The levels of muscle TBARS were increased ($p < 0.05$) in the fish in the control group during hypoxia and reoxygenation, when compared to the normoxia group. Fish fed with 0.5 mL / kg of OENG showed an increase ($p < 0.05$) in the levels of TBARS in the muscle, when compared to all other treatments during normoxia and during hypoxia the levels of TBARS were reduced in relation to those other groups. On exposure to reoxygenation, animals fed 0.5, 1.5 and 2.0 mL / kg of OENG showed a reduction ($p < 0.05$) when compared to animals in the control groups and 1.0 mL / kg (Fig. 2C).

Superoxide dismutase (SOD). In the gills, the animals in the control group showed an increase ($p < 0.05$) in SOD activity during exposure to reoxygenation, compared to normoxia and hypoxia. During normoxia, the groups fed 1.0 and 2.0 mL / kg of OENG, showed an increase ($p < 0.05$) in the SOD activity, in relation to the other treatments. In hypoxia, the animals in the groups fed 1.5 and 2.0 mL / kg of OENG, showed an increase ($p < 0.05$) in SOD activity when compared to the other groups. SOD activity increased ($p < 0.05$) in fish fed 0.5 and 1.0 mL / kg of OENG during reoxygenation (Fig. 3A). Hepatic SOD activity did not show significant difference ($p < 0.05$) between

treatments during normoxia. In hypoxia, animals fed 1.0 mL / kg of OENG demonstrated a reduction ($p < 0.05$) of SOD activity in the liver, compared to other treatments, while the 1.5 mL / kg treatment remained constant in all conditions. (Fig. 3B). In muscle, during normoxia there was no significant difference between treatments and the control group in relation to SOD. In hypoxia, the diet containing 2.0 mL / kg of OENG caused an increase ($p < 0.05$) in the SOD activity in the muscle, compared to the other groups. In reoxygenation, there was no difference ($p < 0.05$) in muscle SOD activity between treatments, however, compared to normoxia and hypoxia, there was an increase in activity in all treatments (Fig. 3C).

Reduced glutathione (GSH). In gills, the GSH content of the control group was reduced ($p < 0.05$) during hypoxia and reoxygenation, in relation to normoxia. Fish fed with 1.0 and 2.0 mL / kg of OENG showed an increase ($p < 0.05$) in the GSH content compared to other treatments in normoxia. During hypoxia, the addition of 1.5 mL / kg of OENG caused a reduction ($p < 0.05$) in the branchial GSH content, compared to the other groups. Fish showed a reduction in GSH levels in all treatments, both in hypoxia and in reoxygenation (Fig. 4A). The hepatic GSH content increased ($p < 0.05$) for the control group in hypoxia compared to exposure in normoxia and reoxygenation. During exposure to hypoxia, animals fed 0.5 mL / kg of OENG showed an increase ($p < 0.05$) of the GSH content in the liver, in relation to the other treatments. The groups that received diets containing 1.5 and 2.0 mL / kg of OENG showed an increase ($p < 0.05$) in the hepatic GSH content when compared to the other groups in reoxygenation (Fig. 4B). In the muscle, the GSH content in the control treatment did not change under any of the conditions. During normoxia and hypoxia, there was no significant difference between treatments. On reoxygenation, a reduction ($p < 0.05$) in the muscular GSH content was observed for fish fed with 0.5 mL / kg of OENG (Fig. 4C).

Discussion

In these studies, it was demonstrated that some plant extracts or essential oils favored the performance on the growth of some fish species, mainly due to the positive effects on the digestion, absorption and assimilation of the various nutrients (WANG et al., 2017). In our study, the results indicated that fish growth performance was not

affected by OENG concentrations in the diets, as it was not possible to observe significant differences between experimental groups.

The results found may be related to a lower dietary supplementation of OENG, especially when compared to the study carried out by Talpur and Ikhwanuddin (2012) with Asian sea bass *(Calcarífero lates)* and *Huso huso* fry (KANANI et al. 2014) fed with garlic and dried ginger powder in concentrations (5–20 g/kg of food), showing a significant improvement in growth and weight gain. In studies with Nile tilapia fed with essential oil of *Cymbopogon citratus* or *Pelargonium graveolens* in the diet, the 400 mg/kg ratio also promoted an improved growth performance (AL-SAGHEER et al., 2018). According to Saccol (2013), the addition of any concentration (0.25, 0.5, 1.0 and 2.0 mL/kg) of *L. alba* EO to the diet did not significantly interfere with the growth of jundiá, as seen in our work. However, the results of this study did not point out negative effects on growth, as was reported by Figueiredo (2017) when adding 15 mL/kg of ginger OE to the diet of Nile tilapia *(Oreochromis niloticus),* as it caused toxicity against the parameters metabolic and with that, it can be said that the inclusion of OENG does not harm the growth of silver catfish.

The interruption of the balance between oxidation and antioxidant systems due to excess formation of EROS or depletion of antioxidants, characterizes oxidative stress. In this study, a reduction ($p < 0.05$) in the levels of EROS in the gills was observed during hypoxia and reoxygenation in all treatments, which according to Halliwel and Gutteridge, (1999); Hermes-Lima, (2004); Storey (1996), is expected, since the decrease in the oxygen concentration in the environment (hypoxia) or its total absence (anoxia) decreases the level of EROS. However, in the liver and muscle tissue, an increase in EROS levels has been demonstrated after reoxygenation. According to several studies, hypoxia and reoxygenation can induce a rapid overproduction of reactive oxygen species (EROS), which can result in oxidative lesions in the liver, gills and blood of fish (LUSHCHAK E BAGNYUKOVA, 2007; XIONG et al., 2016). In addition, EROS can oxidize cellular components. In the present study, reoxygenation increased the levels of TBARS (substances reactive to thiobarbituric acid) in the gills (except the 2.0 mL/kg group), in the liver and muscle (except 0.5, 1.5 and 2.0 mL/kg) of the animals, which corroborates the results obtained by Xiong et al., (2016) where it was observed that hypoxia and reoxygenation can increase the level of TBARS in the tissues of Mugil's headach. On the other hand, it was possible to

observe that the muscle tissue showed a reduction (p> 0.05) in the levels of TBARS in fish fed with 0.5, 1.5 and 2.0 mL/kg of OENG during reoxygenation. According to Cooper et al (2002), the activity of superoxide dismutase (SOD) should increase as the oxygen concentration decreases to detoxify EROS, as observed by the authors in *Leiostomus xanthurus*. It was observed in our study that the animals fed with 2.0 mL/kg of OENG showed an increase (p <0.05) in the activity of the muscular SOD during hypoxia and the animals fed with 1.5 mL/kg of OENG maintained high (p <0.05) liver SOD activity during reoxygenation, when compared to other treatments.

Studies by Garcia et al., (2008) demonstrated that pacu *(Piaractus mesopotamicus)* exposed to hypoxia (about 2 mg/L O_2) had no change in SOD and lipoperoxidation at the liver level, however, in our study it was observed that during hypoxia, fish fed 0.5 mL/kg of OENG had a reduction (p <0.05) in TBARS levels in the liver. Similar results were found in studies by Zeppenfeld et al., (2017) with silver catfish fed 2.0 mL/kg of essential oil of *Aloysia triphylla*, where TBARS levels were lower and superoxide dismutase levels were higher in the liver and muscle of fish compared to those fed a control diet. Other studies with silver catfish observed that animals fed diets supplemented with essential oil of *Lippia alba* (lipohol chemotype of 0.5-2.0 mL/kg) obtained a stimulus in superoxide dismutase activities, as well as a reduction in lipoperoxidation in several organs compared to those fed a control diet (SACCOL et al., 2013).

Tissues and organs have different rates of metabolic activity, oxygen consumption and their levels of antioxidants are also different. Maranesi et al., (2004) confirmed that protection against LPO differs from tissue to tissue, and in some the defense mechanism appears to be less efficient. In gills, the main site for the absorption of xenobiotics due to its large surface area and permeability, the addition of *N. grandiflora* to the silver catfish diet resulted in lower levels of LPO during normoxia. In addition, higher SOD and GSH (only 1.0 and 2.0 mL EO per kg of diet, compared to the control), as well as low levels of EROS during hypoxia and reoxygenation were observed. Studies with low oxygen availability also induced an increase in antioxidant enzymes in *C. auratus* (LUSHCHAK et al., 2001) and *C. Carpio* (LUSHCHAK et al., 2005). Increased glycogen stores and antioxidant defenses during physiological states where oxygen free radical production must be reduced is considered an evolutionary adaptation and a preparatory mechanism that minimizes

potential damage due to oxidative stress during reoxygenation when oxygen consumption increases. The activity and expression of antioxidant enzymes can be specific organic, but they can also be modulated by the metabolic requirements of the tissue, therefore, each organ contains its own antioxidant capacity (LIMÓN-PACHECO and GONSEBATT, 2009). Thus, the differences observed in the tissues may be due to different rates of generation of free radicals, differences in susceptibility to oxidative damage and even the different antioxidant resources of the tissues.

Conclusion

The addition of *N. grandiflora* OE to the diet of jundiá juveniles did not influence growth. Regarding the antioxidant status, during normoxia, the addition of 1.0 and 2.0 mL/kg of OENG had a positive effect on the gills, however, during hypoxia and reoxygenation, the tested OENG levels did not have an antioxidant effect on the tissues. However, more research is needed to verify the effects that the essential oil of *Nectandra grandiflora* has on the body of the jundiá.

References

ABDEL-TAWWAB, M.N. MONIER, S.H. HOSEINIFAR, C. FAGGIO. Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. **Fish Physiol. Biochem**., 45, pp. 997-1013, 2019.

AL-SAGHEER, H.K. MAHMOUD, F.M. REDA, S.A. MAHGOUB, M.S. AYYAT. Supplementation of diets for Oreochromis niloticus with essential oil extracts from lemongrass *(Cymbopogon citratus)* and geranium *(Pelargonium graveolens)* and effects on growth, intestinal microbiota, antioxidant and imune activities. **Aquac. Nutr**., 24 (2018), pp. 1006-1014.

BECKER, A. G. et al. Transpotation of silver catfish, *Rhamdia quelen*, in water with eugenol and the essential oil of *Lippia alba*. **Fish Physiology Biochemistry**, v. 38, p. 789-796, 2012.

BOYD, C.E. & TUCKER, C.S. (1992) Water quality and soil analyses for aquaculture. **Auburn: Auburn University**.

BUEGE, J.A.; AUST. S.D.; Microsomal lipid peroxidation. Methods Enzymol. **52** (1978), pp. 302 – 310.

COLT, J. (2002) List of spreadsheets prepared as a complement. [online] Available from: http://www.fisheries.org/afs/hatchery htmLS. [accessed: 10th October 2020]. In: Wedemeyer G.A. (ed), **Fish Hatchery Management**, 2 eds., American Fisheries Society.

COOPER, R.U., CLOUGH, L.M., FARWELL, M.A., WEST, T.L. Metabolic activities and enzymatic antioxidants induced by hypoxia in estuarine fish *Leiostomus xanthurus*. **J. Exp. Mar. Biol. Ecol**., 279 (2002), pp. 1 – 20.

CUNHA, M. A. et al. Essential oil of *Lippia alba*: a new anesthetic for silver catfish, *Rhamdia quelen*. **Aquaculture**, v. 306, p. 403-406, 2010.

DOUXFILS, M.J.; DEPREZ, S.N. MANDIKI, S. MILLA, E. HENROTTE, C. MATHIE U. Physiological and proteomic responses to single and repeated hypoxia in juvenile *Eurasian perch* under domestication-clues to physiological acclimation and humoral immune modulations. **Fish. Shellfish. Immunol**., 33 (2012), pp. 1112-1122.

EATON, A.D., CLESCERI, L.S., RICE, E.W., GREENBERG, A.B. (2005) Standard methods for the examination of water and wastewater. 21 ed., **Washington: American Public Health Association**.

EKAU, W.; AUEL, H.-O. PÖRTNER, D. GILBERT. Impacts of hypoxia on the structure and processes in pelagic communities (zooplankton, macro-invertebrates and fish). **Biogeosciences,** 7 (2010), pp. 1669-1699

European Pharmacopoeia. European Directorate for the Quality of < medicines. **Pharmacopoeia, Strassbourg**, 2010.

FAO – FOOD AND AGRICULTURE ORGANIZATION. **The State of World Fisheries and Aquaculture.** Rome: FAO, 2020. Disponível em: http://www.fao.org/3/ca9229en/ca9229en.pdf. Acesso em: 19 dez. 2020.

FRANCIS, G., MAKKAR, H. P. S., & BECKER, K. (2002). Dietary supplementation with a *Quillaja saponin* mixture improves growth performance and metabolic efficiency in common carp (*Cyprinus carpio* L.). **Aquaculture,** 203, 311–320.

MORAES, I.M., et al., 2002. Biochemical and hematological responses of the banded knife fish *Gymnotus carapo* (Linnaeus, 1758) exposed to environmental hypoxia. **Braz. J. Biol**., 62, pp. 633-640.

GARLOCK, T., et al., 2020. A global blue revolution: aquaculture growth across regions, species, and countries. **Reviews in Fisheries Science & Aquaculture**. 28, 107-116.

HALLIWEL, J.M.C.B.; GUTTERIDGE. Free radicals in biology and medicine. **Int. J. Biochem. Cell Biol.**, 31 (1999), pp. 1454 – 1468.

HERMES-LIMA, M. Oxygen in biology and biochemistry: role of free radicals. In: STOREY, K.B. (ed) **Functional metabolismo: regulation and adaptation**. New York, John Wiley & Sons, Inc., p. 319-368, 2004.

JURINITZ, C. F.; JARENKOW, J. A. Estrutura de uma floresta estacional no Rio Grande do Sul. **Revista Brasileira de Botânica**, v. 26, p. 475-487, 2003.

LAZZARI, R., et al., 2008. Alimentação do jundiá *(Rhamdia quelen,* heptapteridae) com ingredientes proteicos. ***Arch. Zootec**.,* v.56, p.116, 2008.

LEBEL, C.P., ISCHIROPOULOS, H., BONDY, S.C. Evaluation of the probe 2',7' - dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress, **Chem. Res. Toxicol**. 5, 227 –230, 1992.

LIANG, Q., Y. SHENG, P. JIANG, L. JI, Y. XIA, Y. MIN, *et al.* The gender-dependent difference of liver GSH antioxidant system in mice and its influence on isoline-induced liver injury. **Toxicology**, 280 (2011), pp. 61-69.

LORENZI, H. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil. **Nova Odessa: Institut Plantarum**, 1982, 368 p.

LOWRY, O.H.,N.J ROSEBROUGH, L. FARRA, RJ RANDALL.Protein measurement with Folin Phenol reagent .**J. Biol. Chem**. v.193, p. 265–275, 1951.

LUSHCHAK V.I., et al., 2001. Oxidative stress and antioxidant defenses in goldfish Carassius auratus during anoxia and reoxygenation. **American Journal of**

Physiology-Regulatory, Integrative and Comparative Physiology. v. 280, p.100-107, 2001.

LUSHCHAK, V. I. Free radicals, reactive oxygen species, oxidative stress and its classification. **Chemico-Biological Interactions**, v. 224, pg. 164–175. 2014.

LUSHCHAK, V. I., & BAGNYUKOVA, T. V. Effects of different environmental oxygen levels on free radical processes in fish. **Comparative Biochemistry and Physiology, Part B**, v. 144, p. 283–289, 2006.

LUSHCHAK,V.I., et al., 2005. Hypoxia and recovery disrupt the free radical process and antioxidant potential in common carp tissues (*Cyprinus carpio*). **Int. J. Biochem. Cell Biol.,** 37, pp. 1319 – 1330.

MARKLUND, S., MARKLUND, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, **Eur. J. Biochem**. 47, 469 –474.

MARQUES, C. A. Importancia da família Lauraceae Lindl. **Floresta e Ambiente**, v. 8, p. 195-206, 2001.

PARODI, T. V et al. Anesthetic activity of *Aloysia triphylla* and effectveness in reducing stress durin transporto f albine and grey strains of silver catfish, *Rhamdia quelen*. **Fish Phisiology and Biochemistry**, v. 40, n. 2, p. 323-334, 2014.

RABALAIS, N.N., et al., 2010. **Dynamics and distribution of natural and human-caused hypoxia.** Biogeosciences, 7, pp. 585-619.

READ, S.M., NORTHCOTE, D. H., 1981. Minimization of variation in the response to different proteins of the Coomassie blue G dye -binding assay for protein. **Anal. Biochem.** 116:53 - 64.

SACCOL, E. M. H et al. Addition of *Lippia alba* (Mill) N. E. Brown essential oil to the diet of the silver catfish: Na analisys of growth, metabolic and blood parameters and the antioxidant response. **Aquaculture**, v. 416-417, p. 244-254, 2013.

SOUZA, C.F. et al., 2018. Serum and hepatic oxidative damage induced by a diet contaminated with fungal mycotoxin om freshwater silver catfish *Rhamdia quelen*: involvement on disease pathogenesis. **Microb. Pathog**. 124, 82–86.

STOREY K. Oxidative stress: animal adaptations in nature. Brazilian **Journal of Medical and Biological Research.** 29, 1715– 1733, 1996.

TALPUR, A. D., & IKHWANUDDIN, M. Dietary effects of garlic *(Allium sativum)* on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Lates calcarifer* (Bloch). **Aquaculture,** 364–365, 6–12, 2012

VAQUER-SUNYER, C.M. R.; DUARTE. **Thresholds of hypoxia for marine biodiversity.** Proc. Natl. Acad. Sci., 105 (2008), pp. 15452-15457.

VARDI, H.N., et al. 2008. Potent protective effect of apricot and β-carotene on methotrexate-induced intestinal oxidative damage in rats. **Food Chem. Toxicol.**, 46, pp. 3015 – 3022.

WILHELMFILHO, T.D. et al., 2002. Comparison between the antioxidant status of land mammals and divers **Comp. Biochem. Physiol.** A Mol. Integr. Physiol., 133, pp. 885 – 892.

WILHELMFILHO, T.D., et al., 2001. Influence of seasons and pollution on the oxidant defenses of the cichlid fish acara *(Geaphagus brasiliensis)*. **Braz J Med Biol Res**. v. 34, p. 719-726.

WU, R.S.S., 2002. Hypoxia: from molecular responses to ecosystem response. Mar. **Pollut. Bull**. 45, 35-45.

YOANN, T. et al., 2019. Effects of hypoxia on metabolic functions in marine organisms: observed patterns and modelling assumptions within the contexto of dynamic energy budget (DEB) theory. **J. Sea Res**. 143, 231-242.

ZEPPENFELD, C. C. et al., 2016. Essential oil of *Aloysia triphylla* as feed additive promotes growth of silver catfish *(Rhamdia quelen)*. **Aquaculture Nutrition**, v. 22 p. 933-940.

ZHENG, Z. L., et al., 2009. Evaluation of oregano essential oil *(Origanum heracleoticum L.)* on growth, antioxidant, effect and resistance against *Aeromonas hydrophila* in channel catfish *(Ictalurus punctatus)*. **Aquaculture,** 292, 214–218.

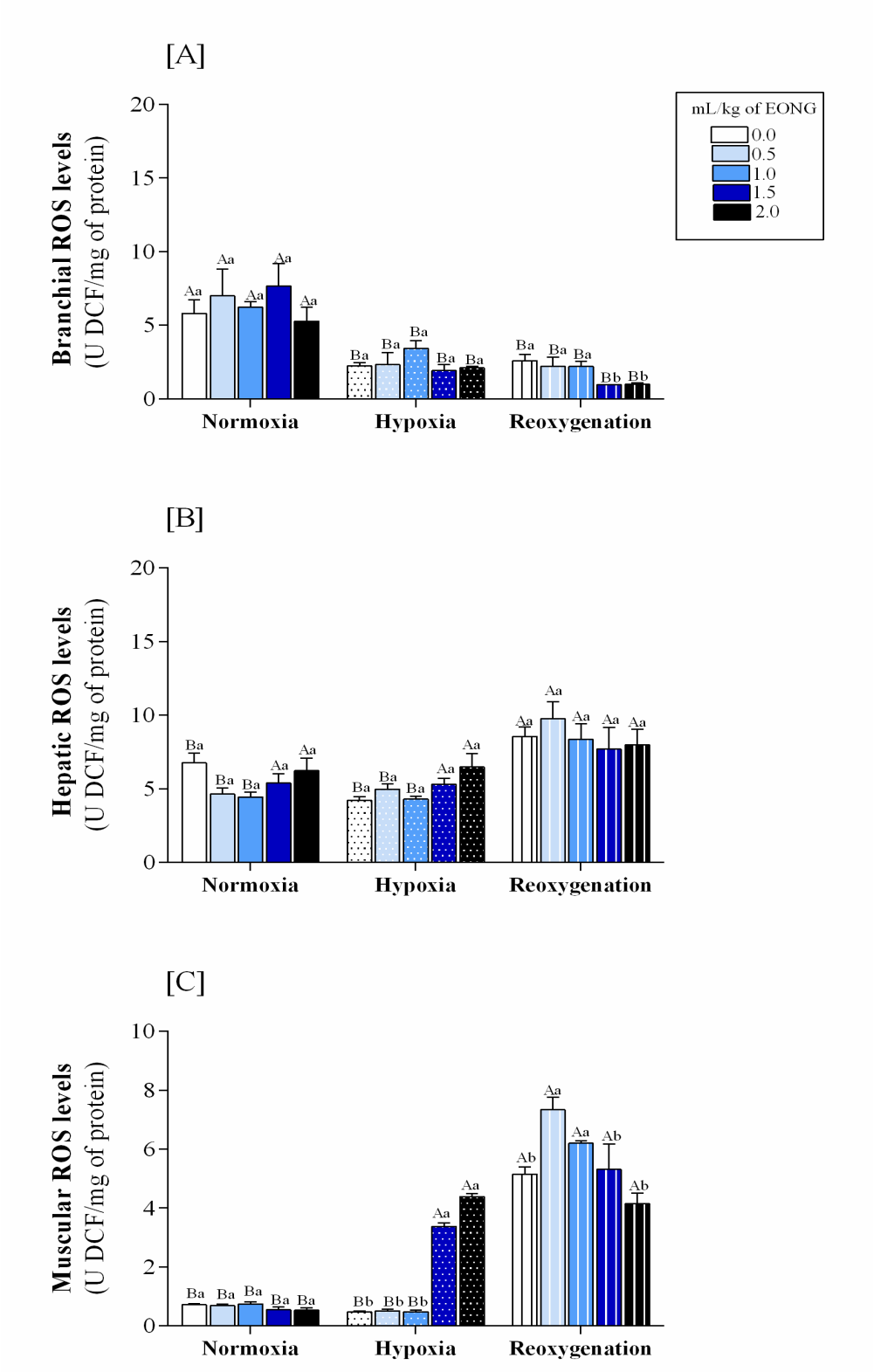

Figure 1. Reactive oxygen species (EROS) levels in gills (A), liver (B) and muscle (C) in juvenile silver catfish supplemented with different concentrations of essential oil of *N. grandiflora* (EONG), under the states of normoxia, hypoxia and reoxygenation.

*Capital letters mean difference from the same treatment in different exposures and lowercase letters represent differences in different treatments within the same exposure ($p<0,05$).

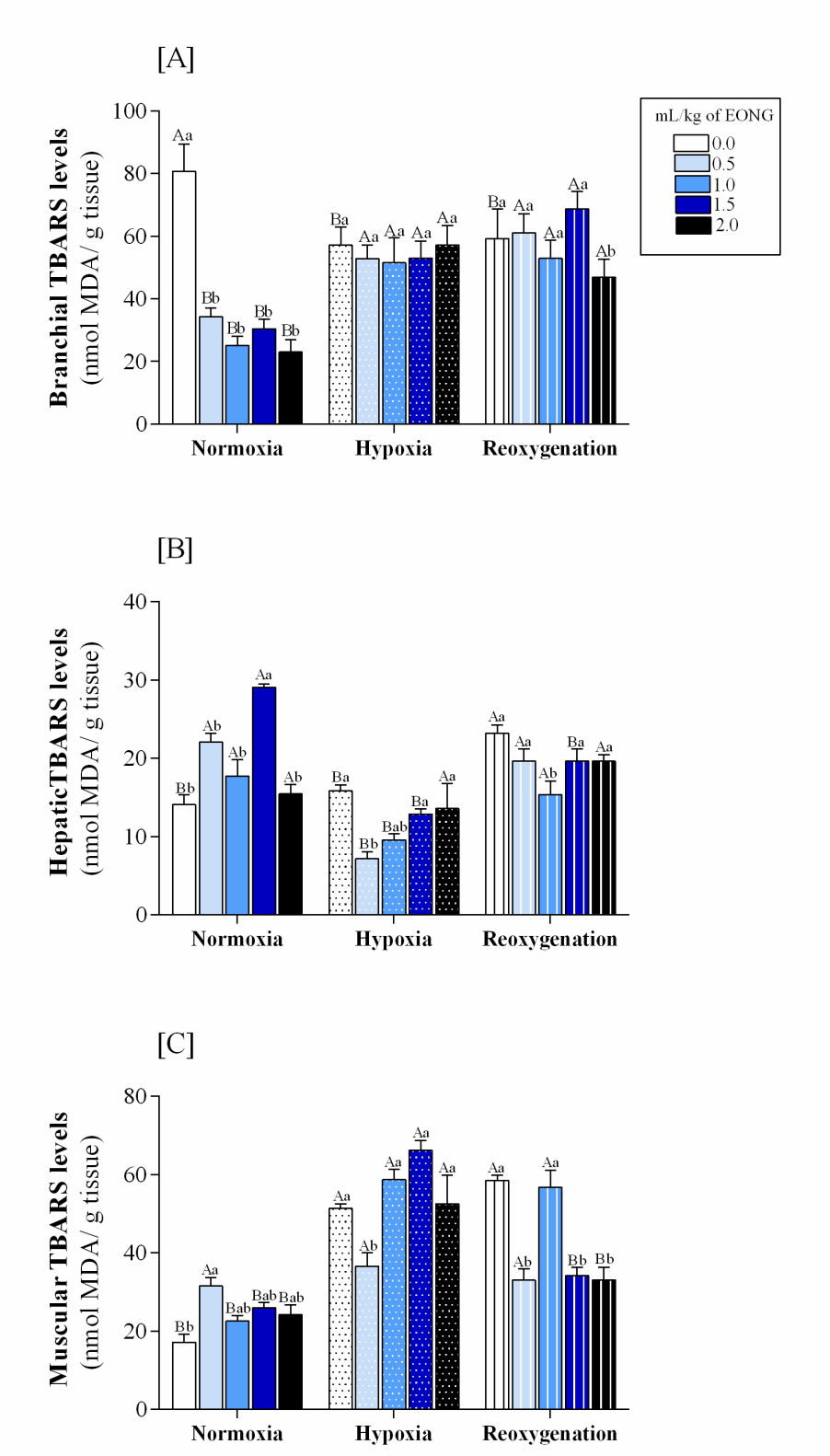

Figure 2. Reactive substances to thiobarbituric acid (TBARS) levels in gills (A), liver (B) and muscle (C) in juvenile silver catfish supplemented with different concentrations of essential oil of *N. grandiflora* (OENG), under the states of normoxia, hypoxia and reoxygenation.

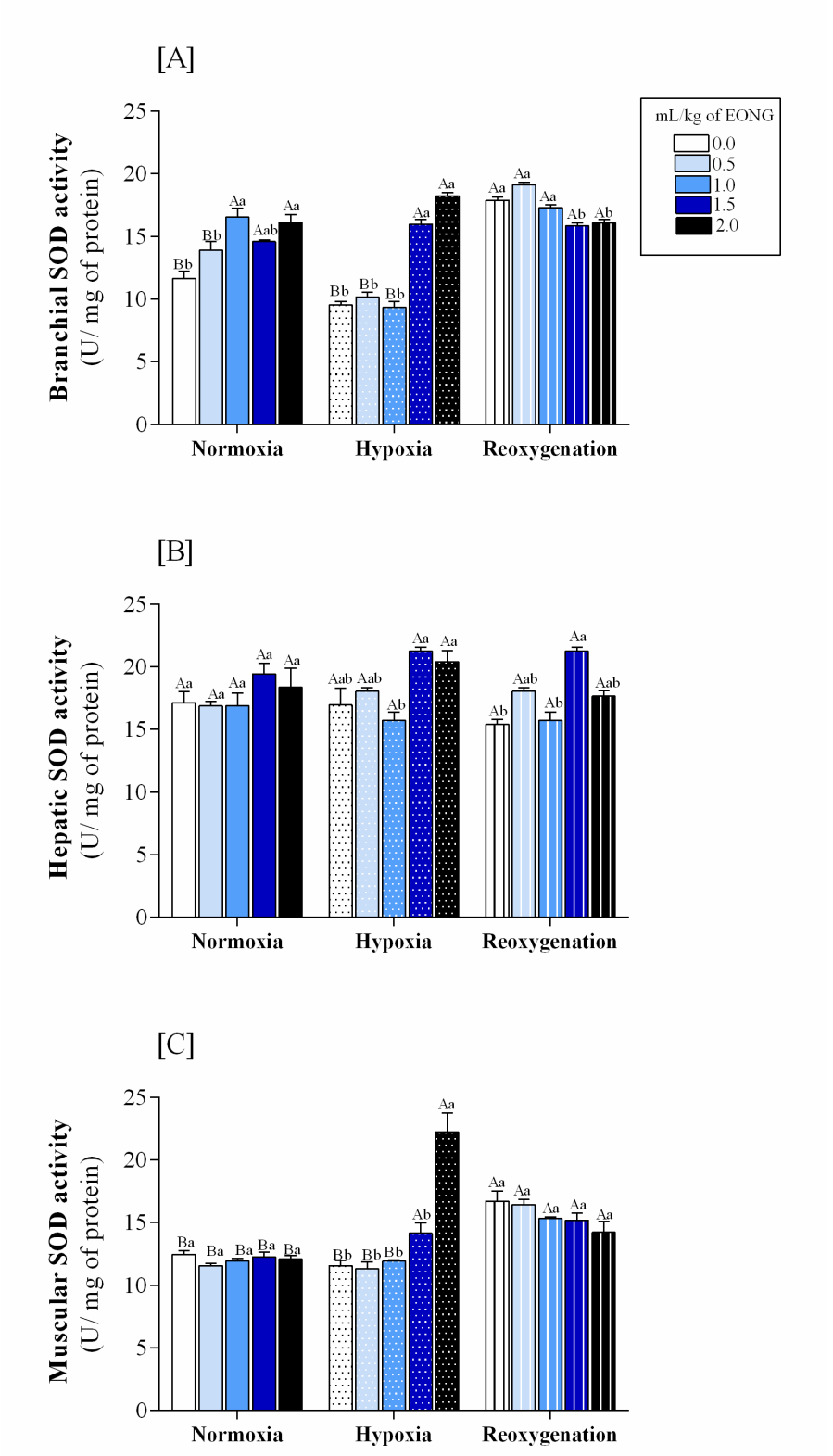

Figure 3. Superoxide dismutase (SOD) levels in gills (A), liver (B) and muscle (C) in juvenile silver catfish supplemented with different concentrations of essential oil of *Nectandra grandiflora* (OENG), under the states of normoxia, hypoxia and reoxygenation.

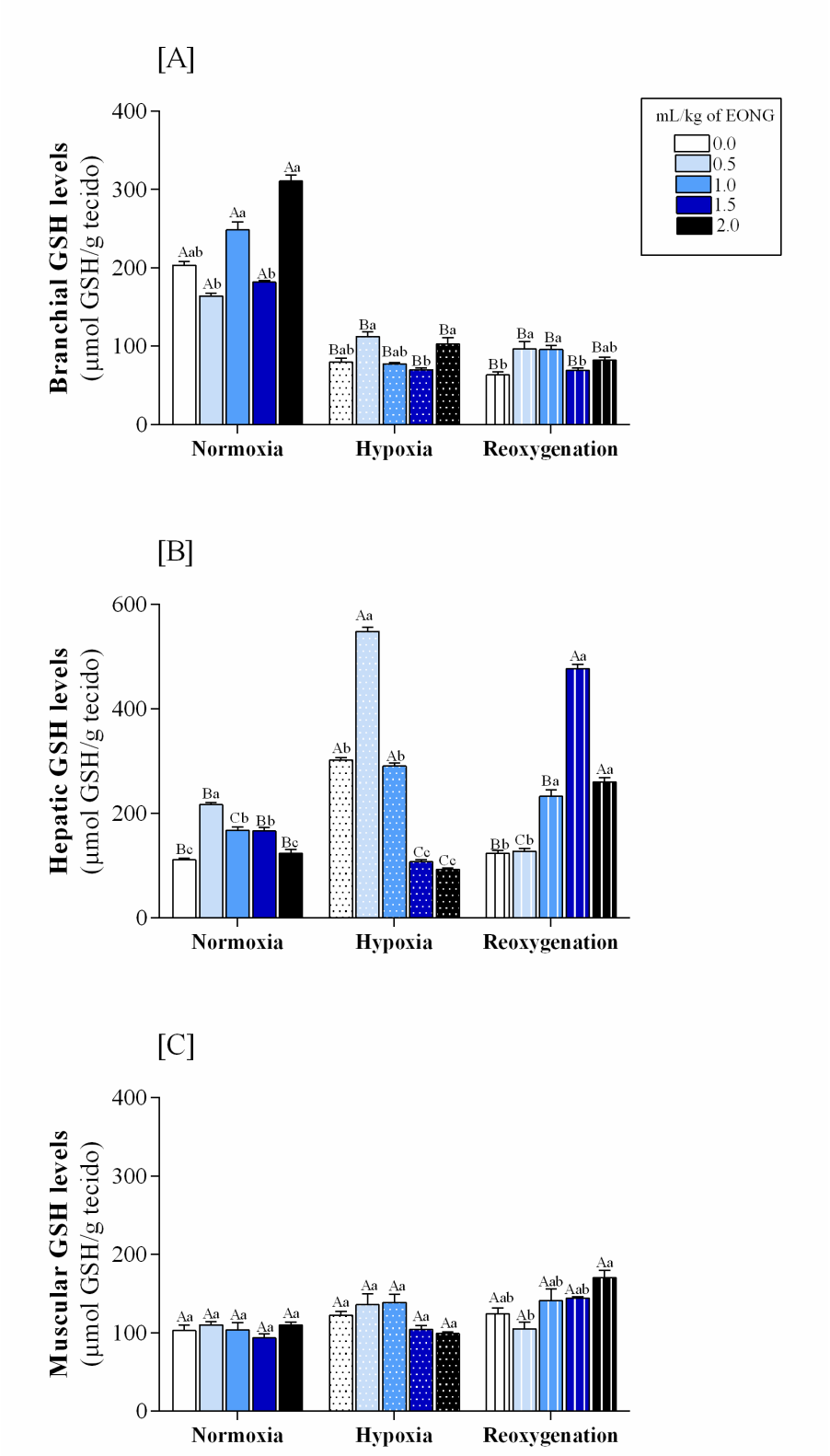

Figure 4. Reduced glutathione (GSH) levels in gills (A), liver (B) and muscle (C) in juvenile silver catfish *(Rhamdia quelen)* supplemented with different concentrations of essential oil of *Nectandra grandiflora* (OENG), under the states of normoxia, hypoxia and reoxygenation.

4 Conclusões Finais

Em relação ao desempenho de crescimento, a adição de óleo essencial de *Nectandra grandiflora* não apresentou resultados significativos em relação ao grupo controle, mas sua adição na dieta não causou prejuízos aos juvenis de jundiás, podendo ser usado como aditivo na dieta do jundiá. Em relação ao estresse gerado pela hipóxia e reoxigenação, os niveis de OENG avaliados neste estudo não demosntraram efeito antioxidante nos tecidos. Devido a esses resultados, recomenda-se maiores estudos com este óleo essencial para jundiás.