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JUNDIÁ: EFEITOS FISIOLÓGICOS DO ÓLEO ESSENCIAL DE *LIPPIA ALBA* ADICIONADO À RAÇÃO

DISSERTAÇÃO DE MESTRADO

Carine de Freitas Souza

Santa Maria, RS, Brasil

2014

JUNDIÁ: EFEITOS FISIOLÓGICOS DO ÓLEO ESSENCIAL DE *Lippia alba* ADICIONADO À RAÇÃO

Carine de Freitas Souza

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como requisito parcial para obtenção do grau de
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Santa Maria, 17 de Julho de 2014.

Aos meus pais

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A meu tio Jorge *(in memorian)*

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“Valeu a pena? Tudo vale a pena
Se a alma não é pequena.
Quem quer passar além do Bojador
Tem que passar além da dor.”
(Fernando Pessoa).

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Biodiversidade Animal
Universidade Federal de Santa Maria

JUNDIÁ: EFEITOS FISIOLÓGICOS DO ÓLEO ESSENCIAL DE *Lippia alba* ADICIONADO À RAÇÃO

AUTOR: Carine de Freitas Souza
ORIENTADOR: Bernardo Baldisserotto
Data e Local da Defesa: Santa Maria/RS, 17 de Julho de 2014

O objetivo deste estudo foi analisar o efeito do óleo essencial de *Lippia alba* (EOLA) adicionado à ração de *Rhamdia quelen* (jundiá) em parâmetros metabólicos, osmorregulatórios e endócrinos. Os peixes foram alimentados por 20 dias com diferentes concentrações de OELA (0,00; 0,25 e 0,50 mL kg^{-1} ração). Os peixes foram alimentados durante 20 dias com diferentes concentrações de OELA (0,0 - controle, 0,25 e 0,50 mL kg^{-1} de ração). Parâmetros metabólicos, Na^+, Cl^-, K^+ e cortisol no plasmas não foram afetados pela dieta, com a exceção de ALT (alanina aminotransferase), que foi maior no fígado dos peixes alimentados com 0,50 mL OELA kg^{-1} de ração. Os peixes alimentados com 0,25 mL EOLA kg^{-1} de ração, apresentaram maior atividade de Na^+/K^+-ATPase e da expressão da somatolactina, mas a atividade de H^+-ATPase e da expressão do hormônio do crescimento e da prolactina não alterou-se. O OELA pode ser utilizado como um suplemento dietético para o jundiá nas concentrações testadas, mas 0,25 mL EOLA kg^{-1} de ração, parece ser melhor do que a concentração de 0,50 mL kg^{-1}, uma vez que esta última pode estar relacionada com danos no fígado.

Palavras-chave: Plantas medicinais. Somatolactina. Na^+/K^+-ATPase. ALT. Peixe. Jundiá.

ABSTRACT

Master Dissertation
Post-Graduation in Animal Biodiversity
Federal University of Santa Maria

SILVER CATFISH: PHYSIOLOGICAL EFFECTS OF DIETARY ADDITION OF THE ESSENTIAL OIL OF *Lippia alba*

AUTHOR : CARINE DE FREITAS SOUZA
ADVISOR: BERNARDO BALDISSEROTTO
Date and place of the defense: Santa Maria, 17th 2014

The aim of this study was to evaluate the effect of the essential oil of *Lippia alba* (EOLA) as a feed additive on ionoregulatory and metabolic parameters, and pituitary hormones expression in silver catfish, *Rhamdia quelen*. Fish were fed for 20 days with different concentrations of EOLA (0.0 – control, 0.25 and 0.50 mL kg^{-1} food). Plasma Na^+, Cl^-, K^+ and cortisol, and metabolic parameters were not affected by the diet, with the exception of ALT (alanine aminotransferase), which was higher in the liver of fish fed 0.50 mL EOLA kg^{-1} food. Fish fed 0.25 mL EOLA kg^{-1} food presented higher Na^+/K^+-ATPase activity and somatolactin expression, but H^+-ATPase activity and growth hormone and prolactin expression did not change. The EOLA can be used as a dietary supplement for silver catfish at the evaluated concentrations, but using 0.25 mL EOLA kg^{-1} food seems to be more suitable than 0.50 mL EOLA kg^{-1} food since the latter may be related to liver damage.

Keywords: Medicinal plants. Somatolactin. Na^+/K^+-ATPase. ALT. Fish. Silver catfish.

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1 INTRODUÇÃO

1.1 Espécie em estudo

O jundiá (*Rhamdia quelen*) (Figura 1) pertence à ordem Siluriformes, família Heptapteridae, é um dos habitantes mais comuns dos rios do sul do Brasil e foi escolhido para este estudo devido a sua importância econômica e ecológica (BALDISSEROTTO, 2009). Morfologicamente o jundiá caracteriza-se por possuir boca sem dentes e corpo sem escamas, possuindo barbilhões de forma cilíndrica com comprimento variando proporcionalmente ao tamanho do espécime (GUEDES, 1980). É um bagre bentônico especulador do substrato. Sua alimentação tem como base insetos terrestres e aquáticos, crustáceos, restos vegetais e também peixes como os lambaris e os guarús (CASATTI *et al*., 2001; BALDISSEROTTO, 2004; CASATTI, CASTRO, 2006; OYAKAWA *et al*., 2006).

Figura 1 - Exemplar de *Rhamdia quelen* adulto.

Fonte: Carine Souza

1.2 Óleo Essencial

No cenário atual, compostos bioativos presentes em extratos naturais têm se destacado mundialmente, e a rica biodiversidade brasileira, com diversos princípios ativos desconhecidos torna-se relevante e de interesse (FREIRE, 2006). Uma parte que pode ser obtida das plantas são os óleos essenciais. Óleos essenciais são líquidos oleosos aromáticos obtidos a partir de material vegetal como flores, brotos, sementes, folhas, galhos, cascas, ervas, madeira, frutas e raízes. Eles podem ser obtidos por expressão, fermentação, enfloração ou extração, mas o método mais utilizado para produção comercial é o de destilação por

arraste de vapor (VAN DE BRAAK, LEIJTEN, 1999). Há diversos estudos recentes que relatam o uso de óleos essenciais na piscicultura para anestesia e sedação, promotor de crescimento, ação antioxidante entre outros (ZHENG *et al*., 2009; CUNHA *et al*., 2010; BECKER *et al*., 2012; SACCOL *et al*., 2013; PARODI *et al*., 2014).

A planta *Lippia alba* (Mill.) NE Brown (Figura 2), cujo óleo essencial (Figura 3) foi utilizado no presente trabalho, é um arbusto aromático popularmente conhecido como erva cidreira, pertencente à família Verbenaceae, amplamente utilizada na América Latina. Algumas espécies de *Lippia* exibem ações sedativas, e os compostos fenólicos (flavonóides) são geralmente assumidos como sendo as substâncias ativas (PASCUAL *et al*., 2001). Recentemente foram realizados alguns estudos demonstrando a eficácia do óleo essencial de *L. alba* para anestesia e sedação do jundiá (CUNHA *et al*., 2010), cavalo marinho (*Hippocampus reidi*) (CUNHA *et al*., 2011) e camarão (*Litopenaeus vannamei)* (PARODI *et al*. 2012).O composto não altera o crescimento, mas possui ação antioxidante quando adicionado à ração de jundiás (SACCOL *et al*., 2013).

Figura 2 - *Lippia alba*.

Fonte: Liana John

Figura 3 - Óleo essencial de *Lippia alba*.

Fonte: Carine Souza

1.3 O potencial de óleos essenciais em dietas para peixes

A utilização de óleos essenciais e extratos herbais como aditivos para alimentação dos peixes tem demonstrado grande potencial, necessitando ainda de ensaios a campo para se determinar a real utilização destes compostos (CAMPAGNOLO *et al*., 2013). A procura pela máxima eficiência alimentar tem promovido o uso de aditivos na ração utilizados para controlar agentes prejudiciais ao processo digestivo e assim proporcionar a melhora dos índices zootécnicos (NUNES *et al*., 2012). Portanto, o uso de diferentes aditivos naturais,vegetais e herbais vêm sendo largamente testado na dieta de animais terrestres e aquáticos (SANTOS et al., 2009). Estudos mostraram um aumento no desempenho zootécnico de tilápias nilóticas (*Oreochromis niloticus*) alimentadas com extrato de alho (*Allium sativum*) (SHALABY *et al*., 2006), juvenis de bagre do canal (*Ictalurus punctatus*) alimentados com óleo essencial de orégano *(Origanum heracleoticum L.*) (ZHENG et al., 2009) e pós-larvas de bagre do canal alimentadas com extrato de *Yucca shidigera* (KELLY, KOHLER, 2003). Outros estudos também mostraram que aditivos vegetais das plantas *Astragalus radix* e *Scutellaria radix* promoveram melhoria na imunidade em juvenis de tilápia nilótica (YIN *et al*., 2006).

1.4 Parâmetros metabólicos

Quando submetidos a uma nova condição alimentar, os peixes devem ser avaliados quanto ao seu metabolismo através de ensaios bioquímicos, uma vez que podem sofrer adaptações fisiológicas, utilizando vias metabólicas diferentes (WALKER *et al*.,1996; ROMÉO *et al*., 2000). Glicogênio e glicose podem refletir o estado metabólico dos tecidos em situações de estresse (CATTANI *et al*., 1996). Peixes estressados costumam fazer o uso de sua reserva hepática de glicogênio para disponibilizar glicose como fonte de energia para o organismo fugir ou se adaptar a mudanças ambientais (IWAMA *et al*., 2004). O lactato pode indicar o acúmulo de ácido lático decorrente de aumento de atividade física nessas situações estressoras (SILVEIRA *et al*., 2009). As proteínas estão envolvidas na adaptação fisiológica do organismo diante de situações de estresse (DE SMET, BLUST, 2001; CRESTANI *et al*., 2006), como por exemplo, a sua redução na alimentação ou exposição dos animais a agentes tóxicos (IRVING *et al*, 2003). Aspartato aminotransferase (AST) e alanina aminotransferase (ALT) são enzimas que podem fornecer informações específicas sobre a disfunção de órgãos (SRIVASTAVA *et al.,* 2004; WAGNER, CONGLETON, 2004) sendo envolvidas nas transaminações, podendo ser alteradas sob várias condições fisiológicas e patológicas.

1.5 Osmorregulação em peixes de água doce

Os peixes dulciaquícolas perdem constantemente íons para o meio externo por difusão através das brânquias e superfície do corpo, bem como pela excreção nas fezes e urina. O balanço iônico é mantido pelo influxo de íons nas brânquias (EVANS, 2011) e pela dieta (FERREIRA, BALDISSEROTTO, 2007; GARCIA *et al*., 2007). Distúrbios osmorregulatórios induzidos pelo estresse podem desordenar o balanço aquoso e mineral, resultando até mesmo na morte do animal. Sendo assim, os peixes, hiperosmóticos em relação ao meio, devem evitar ao máximo essa perda de íons e eliminar todo o excesso de água.

Na água doce, as células de cloreto são responsáveis por boa parte da absorção de Na^+ e Cl^-. As células de cloreto, localizadas no epitélio branquial, estão envolvidas na absorção de íons em peixes de água doce (BECKER *et al*., 2012). Estudos relacionam a redução de Na^+ e Cl^- plasmáticos nos peixes à redução da atividade da enzima Na^+/K^+-ATPase e a modificações bioquímicas relacionadas com potenciais transepiteliais (BURY *et al*., 1998). A Na^+/K^+-ATPase é sem dúvida a enzima mais importante na membrana plasmática da célula animal. A ação desta bomba iônica é essencial para as funções celulares, tal como a manutenção do equilíbrio osmótico e potencial de membrana e permitir o transporte ativo secundário de moléculas tais como glicose e aminoácidos (THERIEN, BLOSTEIN, 2000).

1.6 Expressão Gênica: Hormônio do crescimento, Prolactina e Somatolactina

Os principais fatores fisiológicos que regulam os processos anabólicos e, consequentemente, o crescimento muscular, são hormônios secretados pela hipófise, gônadas e seus receptores (MOMMSEN *et al*., 2001). O hormônio do crescimento (GH), a prolactina (PRL) e somatolactina (SL) são hormônios hipofisários originados a partir de uma molécula ancestral comum e desempenham um papel fundamental na regulação da homeostase, bem como de um grande número de processos fisiológicos em resposta aos desafios ambientais (RAND-WEAVER, KAWAUCHI, 1993; KANEKO, 1996). O GH possui um importante papel na aclimatação osmótica (MANCERA, MCCORMICK 1998; SANGIAO-ALVARELLOS, 2006), bem como no crescimento e no metabolismo energético dos peixes (BJÖRNSSON, 1997; CARRIÓN *et al*., 2009). A PRL controla a osmorregulação na água doce, crescimento e desenvolvimento, além de possuir efeitos sobre o metabolismo, comportamento, reprodução e imunorregulação (HIRANO, 1986; MANZON, 2002). A função definitiva da SL ainda não está clara, sendo sugerido que esteja envolvida em vários

eventos biológicos, como por exemplo, resposta ao estresse (RAND-WEAVER *et al*., 1993), regulação de Ca^{2+} (KANEKO, HIRANO, 1993), regulação ácido-base (KAKIZAWA *et al*., 1996), adaptação ao fundo (CÁNEPA *et al*., 2006), mobilização de energia (KANEKO *et al*., 1993) e também na maturação gonadal (RAND-WEAVER, SWANSON, 1993).

1.7 Cortisol

O cortisol nos peixes tem ação de glicocorticoide e mineralocorticoide, sendo liberado a partir de células interrenais do rim cefálico e desempenha um papel central na resposta ao estresse, incluindo osmorregulação (WENDELAAR BONGA, 1997; ARJONA *et al*., 2008) e regulação metabólica (MOMMSEN *et al*., 1999; BABITHA, PETER, 2010). Fatores externos, como salinidade do ambiente, estresse, maturidade e estado nutricional podem influenciar a produção de cortisol e a resposta do animal ao hormônio, a qual pode ser afetada por qualquer um dos fatores que regulam sua disponibilidade (proteínas de ligação, receptores nos tecidos alvo, captação pelos tecidos e catabolismo do hormônio) (MOMMSEN *et al*., 1999).

1.8 Referências

ARJONA, F. J. et al. The involvement of thyroid hormones and cortisol in the osmotic acclimation of *Solea senegalensis*. **Geneneral and Comparative Endocrinology**, v. 155, p. 796–803, 2008.

BABITHA, G. S.; PETER, M. C. S. Cortisol promotes and integrates the osmotic competence of the organs in North African catfish (*Clarias gariepinus* Burchell): evidence from in vivo and in situ approaches. **General and Comparative Endocrinology**, v. 168, p. 14–21, 2010.

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

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

BARTON, B. A. Stress in fish: a diversity of responses with particular reference to changes in circulating corticosteroids. **Integrative and Comparative Biology**, v. 42, p. 517–525, 2002.

BECKER, A. G. et al. Transportation 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.

BJÖRNSSON, B. T. The biology of salmon growth hormone: from daylight to dominance. **Fish Physiology Biochemistry**, v. 17, p. 9–24, 1997.

BURY, N. R. et al. Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells in vitro. **Aquatic Toxicology**, v. 40, p.193-202, 1998.

CAMPAGNOLO, R. et al. Óleos essenciais na alimentação de alevinos de tilápia do Nilo. **Revista Brasileira de Saúde e Produção Animal** , v. 14, n.3, p.565-573. 2013.

CÁNEPA, M. M. et al . Involvement of somatolactin in background adaptation of the cichlid fish *Ciclasoma dimerus* . **Journal of Experimental Zoology**, v. 305, p. 410–419, 2006.

CARRIÓN, L. R. et al. Expression of pituitary prolactin, growth hormone and somatolactin is modified in response to different stressors (salinity, crowding and food-deprivation) in gilthead sea ream *Sparus auratus*. **General and Comparative Endocrinology**, v. 162, p. 293-300, 2009.

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

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.

CATTANI O. et al. Correlation between metallothionein and energy metabolism in sea bass, Dicentrachus labrax, exposed to cadmium. **Comparative Biochemistry and Physiology – Part:C**, v. 113, p. 193–199, 1996.

CRAVEIRO, A. A. et al. Sucedâneos nacionais de bisabolol, citral, timol e carvacrol. **Ciência e Cultura**, v.40, n.7, p.604. Suppl., Deutsches Arzneibuch, 1986. Ausgabe Wissenschaftliche, 1988.

CRESTANI, M. et al. Effects of clomazone herbicide on hematological and some parameters of protein and carbohydrate metabolism of silver catfish *Rhamdia quelen*. **Ecotoxicology and Environmental Safety**, v. 65, p. 48–55, 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.

CUNHA, M. A. et al. Anesthetic induction and recovery of *Hippocampus reidi* exposed to the essential oil of *Lippia alba*. **Neotropical Ichthyology**, v. 9, p. 683-688, 2011.

DE SMET H.; BLUST, R. Stress responses and changes in protein metabolism in carp *Cyprinus carpio* during cadmium exposure. **Ecotoxicology and Environmental Safety**, v. 48, n.3, p. 255-262, 2001.

EVANS, D.H., Freshwater Fish Gill Ion Transport: August Krogh to morpholinos and microprobes. **Acta Physiologica**, v. 202, p. 349–359, 2011.

FERREIRA, F. W.; BALDISSEROTTO, B. Diet and osmorregulation. In: Baldisserotto, B.; Mancera, J. M.; Kapoor, B. G. (Ed.). **Fish osmoregulation**. New Hampshire: **Science Publishers**, 2007. p. 67-83.

FREIRE, C. M. M; MARQUES, M. O. M.; COSTA, M. Effects of seasonal variotional on the central nervous system activity of *ocimum gratissimum* L. essencial oil. Journal of Ethnopharmacology, v. 105, n. 1-2, p. 161-166, 2006.

GARCIA, L. O. et al. Salt in the Food and Water as a Supportive Therapy for *Ichthyophthirius multifiliis* Infestation on Silver Catfish, *Rhamdia quelen*, Fingerlings. **Journal of the World Aquaculture Society**,v. 38, n. 1, p. 1–11, 2007.

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.

HIRANO, T. **The spectrum of prolactin action in teleosts**. In: Ralph, C.L. Ed., Comparative Endocrinology, Developments and Directions. Alan R. Liss, New York, pp. 53–74,1986.

IRVING, E. C.; BAIRD, D.J.; CULP, J.M. Ecotoxicological responses of the mayfly Baetistri cauadatus to dietary and waterborne cadmium: implications for toxicity testing. **Environmental Toxicology and Chemistry**, v. 22, p. 1058–1064, 2003.

IWAMA, G. et al. Are hsps suitable for indicating stressed states in fish? **The Journal of Experimental Biology**, v. 204, p. 15-19, 2004.

KAKIZAWA, S.; KANEKO, T., HIRANO, T. Elevation of plasma somatolactin concentrations during acidosis in rainbow trout. **Journal of Experimental Biology**, v. 199, p. 1043–1051, 1996.

KANEKO, T. Cell biology of somatolactin. **International Review of Cytology**, v. 169, p. 1–24, 1996.

KANEKO, T.; HIRANO, T. Role of prolactin and somatolactin in calcium regulation in fish. **Journal of Experimental Biology**, v. 184, p. 31–45, 1993.

KANEKO, T.; KAKIZAWA, S.; YADA, T. Pituitary of ‘‘cobalt” variant of the rainbow trout removed from hypothalamus lacks most of pars intermedia and neurohypophysial tissue. **General and Comparative Endocrinology**, 92, 31–40, 1993.

KELLY, A.; KOHLE, C. Effects of *Yucca shidigera* Extract on Growth, Nitrogen Retention, Ammonia Excretion, and Toxicity in Channel Catfish *Ictalurus punctatus* and Hybrid Tilapia *Oreochromis mossambicus* × *O. niloticus*. **Aquacullure Socicly**, v.34, n.2, p.156-161, 2003.

MANCERA, J. M.; MCCORMICK,S.D. Osmoregulatory actions of the GH/IGF axis in non-salmonidteleosts. **Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology**, v. 121, n. 1, p. 43-48, 1998.

MANZON, L. A. The role of prolactin in fish osmoregulation: a review. **General and Comparative Endocrinology,** v. 125, p. 291–300, 2002.

MOMMSEN, T. P.; VIJAYAN, M. M. ; MOON, T. W. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. **Reviews in Fish Biology and Fisheries**, v. 9, p. 211–268, 1999.

MOMMSEN, T. P. Paradigms of growth in fish. **Comparative Biochemistry and Physiology Part B**, v. 129, p. 207-219, 2001.

NUNES, J.O. et al. Evaluation of the probitic (*Bacillus subtilis C-3102*) as additive to improve performance in broiler chicken diets. **Revista Brasileira de Zootecnia**, v. 41, n. 11, p. 2374-2378, 2012.

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

PARODI, T. V.; CUNHA, M. A.; HELDWEIN, C. G.; DE SOUZA, D. M.; MARTINS, Á. C.; GARCIA, L. DE O.; WASIELESKY, W. J. R.; MONSERRAT, J. M.; SCHMIDT D.; CARON, B. O.; HEINZMANN, B.; BALDISSEROTTO, B. The anesthetic efficacy of eugenol and the essential oils of *Lippia alba* and *Aloysia triphylla* in post-larvae and sub-adults of *Litopenaeus vannamei* (Crustacea, Penaeidae). **Comparative Biochemistry Physiology C Toxicology Pharmacology**, v. 155, p. 462-468, 2012.

PARODI, T. V et al. Anesthetic activity of *Aloysia triphylla* and effectiveness in reducing stress during transport of albine and grey strains of silver catfish, *Rhamdia quelen.* **Fish Physiology and Biochemistry**, v. 40, n. 2, p. 323-334, 2014.

PASCUAL, M. E. et al. Lippia: traditional uses, chemistry and pharmacology: a review. **Journal of Ethnopharmacology**, v. 76, n. 3, p. 201-214, 2001.

RAND-WEAVER, M.; KAWAUCHI, H. Growth hormone, prolactin and somatolactin: a structural overview. In: Hochachka, P.W., Mommsen, T.P Eds., **Biochemistry and Molecular Biology of Fishes**, vol. 2. Elsevier, Amsterdam, p. 39–56,1993.

RAND-WEAVER, M.; POTTINGER, T. G.; SUMPTER, J. S. Plasma somatolactin concentrations in salmonid fish are elevated by stress. **Journal of Endocrinology**, v. 138, p. 509–515, 1993.

RAND-WEAVER, M.; SWANSON, P. Plasma somatolactin levels in coho salmon (Oncorhynchuskisutch) during smoltification and sexual maturation. **Fish Physiology Biochemistry**, v. 11, p. 175–182, 1993.

ROMÉO, M. et al. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass (*Dicentrarchus labrax)*. **Aquatic Toxicology**, v. 48, p. 185-194, 2000.

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

SANGIAO-ALVARELLOS S., ARJONA, F.J., MIGUEZ, J.M., DEL RIO, M.P.M., SOENGAS, J.L., MANCERA, J.M. Growth hormone and prolactin actions on osmoregulation and energy metabolism of gilthead sea bream (*Sparus auratus*). Comparative biochemistry and physiology, part: A, v. 144, p. 491-500, 2006.

SANTOS, E. L.; LUDKE, M. C. M. M.; LIMA, M. R. Extratos vegetais como aditivos em rações para peixes. **Revista Eletrônica Nutritime**, v.6, n.1, p.789-800, 2009. Disponível em: <http://www.nutritime.com.br/arquivos_internos/artigos/077V6N1P789_800_JAN2009_.pdf> Acesso em: 12 Março 2014.

SHALABY, A. M. ; KHATTAB, Y. A. ; ABDEL RAHMAN, A. M. Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of nile tilapia (*Oreochromis niloticus*). **Journal of Venomous Animals and Toxins including Tropical Diseases**, v.12, n.2, p.172-201, 2006.

SILVEIRA, S. U.; LOGATO, R. P.; PONTES, C. E. Fatores estressantes em peixes. **Revista Eletrônica Nutritime**, v. 6, n. 4, p. 1001-1017, 2009. Disponível em: <http://www. nutritime.com.br/home/?pg=revista_nutritime>. Acesso em: 20 fev. 2014.

SRIVASTAVA, S.; SINHA, R.; ROY, D. T.; ANVER, C. E. Blood chemistry (electrolytes, lipoprotein and enzymes) values of black scorpion fish (*Scorpaena porcus*, 1758) in the Dardnelles. **Turkish Journal of Biology**, v. 4, p. 716–719, 2004.

THERIEN, A. G.; BLOSTEIN, R. Mechanisms of sodium pump regulation. **American Journal of Physiology - Cell Physiology,** v. 279, p. 541–566, 2000.

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 European Union.** CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, p. 116, 1999.

WAGNER T.; CONGLETON, J. L. Blood chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile Chinook salmon (*Onchorhynchustshawytscha*). **Canadian Journal of Fisheries and Aquatic Sciences**, v. 61, p. 1066–1074, 2004.

WALKER, C. H. et al. **Principle of ecotoxicology**. London: Taylor & Francis, 1996. 321p.

WENDELAAR BONGA, S. E. The stress response in fish. Physiological Reviews, v.77 , p. 591–625, 1997.

YIN, G. et al. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromisniloticus*. **Aquaculture**, v. 253, n. 1-4, p. 39-47, 2006.

ZHENG, Z. L. et al. Evaluation of oregano essential oil (*Origanum heracleoticum L.)* on growth, antioxidant 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

- Verificar a expressão dos hormônios do crescimento, prolactina e somatolactina na hipófise, bem como parâmetros metabólicos, osmorregulatórios e de estresse em jundiás (*Rhamdia quelen*) quando alimentados com ração contendo o óleo essencial de *Lippia alba*.

2.2 Objetivos específicos

- Verificar se a adição do óleo essencial de *L. alba* em rações para jundiás altera a expressão dos hormônios do crescimento, prolactina e somatolactina.

- Determinar se a adição do óleo essencial de *L. alba* em rações para jundiás altera parâmetros osmorregulatórios (atividade da Na^+/K^+-ATPase e H^+-ATPase, níveis de Na^+, K^+ e Cl^- plasmáticos) nesta espécie.

- Verificar se a adição do óleo essencial de *L. alba* em rações para jundiás altera o metabolismo de proteína, lipídios, spartato aminotransferase, alanina aminotransferase, glicose, glicogênio e lactato nesta espécie.

- Analisar se a adição do óleo essencial de *L. alba* em rações para jundiás causa alterações no cortisol plasmático.

A presente dissertação está estruturada de acordo com as normas da Universidade Federal de Santa Maria (MDT), sendo composta por um manuscrito.

3.DESENVOLVIMENTO - Manuscrito: Descreve um estudo realizado em laboratório para mensurar os efeitos do óleo essencial de *Lippia alba*, adicionado à ração de jundiás (*R. quelen*), quanto a metabolismo, osmorregulação e expressão de hormônios hipofisários.

Manuscrito encaminhado para Aquaculture Nutrition

Manuscrito - Silver catfish, *Rhamdia quelen***: effect of dietary addition of the essential oil of** *Lippia alba* **(Mill.) N. E. Brown on metabolism, osmoregulation and endocrinology**

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Abstract

The aim of this study was to evaluate the effect of the essential oil of *Lippia alba* (EOLA) as a feed additive on ionoregulatory and metabolic parameters, and pituitary hormones expression in silver catfish, *Rhamdia quelen*. Fish were fed for 20 days with different concentrations of EOLA (0.0 – control, 0.25 and 0.50 mL kg^{-1} food). Plasma Na^{+}, Cl^{-}, K^{+} and cortisol, and metabolic parameters were not affected by the diet, with the exception of ALT, which was higher in the liver of fish fed 0.50 mL EOLA kg $food^{-1}$. Fish fed 0.25 mL EOLA kg^{-1} food presented higher Na^{+}/K^{+}-ATPase activity and somatolactin expression, but H^{+}-ATPase activity and growth hormone and prolactin expression did not change. The EOLA can be used as a dietary supplement for silver catfish at the evaluated concentrations, but 0.25 mL EOLA kg^{-1} food seems to be more suitable than 0.50 mL EOLA kg^{-1} food since the latter may be related to liver damage.

Keywords: medicinal plant, growth hormone, enzymatic activity, somatolactin, prolactin, cortisol.

Introduction

The addition of herbal extracts in fish feed is increasingly seen as a safe and practical alternative to synthetic pharmaceuticals. Some studies showed that dietary addition of plants has several advantages. The addition of 0.5% of *Massa medicata, Crataegi fructus, Artemisia capillaries* or *Cnidium officinale* to the diet led to better use of lipids in stress and recovery of *Pagrus major* (Ji *et al*. 2009). The use of the oregano essential oil (*Origanum heracleoticum*) as a supplement in the food improved growth, antioxidant status and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*) (Zheng *et al*. 2009), and the extract of *Allium sativum* in the diet also promoted growth and fish health and reduced total bacteria in Nile tilapia (*Oreochromis niloticus*) (Shalaby *et al*. 2006).

The plant *Lippia alba* (Verbenaceae) is found in South and Central America and tropical areas of Africa (Terblanche & Kornelius 1996). In silver catfish *Rhamdia quelen* (Quoy & Gaimard, 1824), the essential oil of (EOLA) is an effective anesthetic (Cunha *et al*. 2010) and sedative for transport (Azambuja *et al*. 2011; Becker *et al*. 2012), can delay lipid peroxidation (LPO) during frozen storage of fillets (Veeck *et al*. 2013), and decreases LPO and increases tissue antioxidant response when added to the diet (Saccol *et al.* 2013). The EOLA is also an effective anesthetic for the sea horse (*Hipocampus heidi*) (Cunha *et al*. 2011).

The pituitary hormones control several physiological processes. Growth hormone (GH) is related to growth, metabolism (Laiz-Carrión *et al*. 2009; Sinha *et al.* 2012), and osmoregulation (Sakamoto *et al*. 1997; Sakamoto & McCormick 2006; Sangiao-Alvarellos *et al.* 2006). Prolactin (PRL) also participates in the control of growth and osmoregulation (Sakamoto *et al*. 1997; Sakamoto & McCormick 2006), while somatolactin (SL) is apparently related to energy balance, acid-base equilibrium (Kakizawa *et al*. 1996; Furukawa *et al.*

2010), and metabolism (Company *et al*. 2001). Cortisol, produced by the interrenal cells, is the main glucocorticoid and mineralocorticoid steroid in fish, and is a good indicator for assessing primary stress (Mommsen *et al*. 1999).

Silver catfish can be found from Argentina to southern Mexico (Perdices *et al*. 2002), and is the most raised native species in South Brazil (Baldisserotto 2009). A recent study demonstrated that feeding silver catfish with different levels of dietary EOLA for 60 days does not affect growth, but alters some metabolic parameters and improves the antioxidant status (Saccol *et al.* 2013). However, it is not clear whether the lack of influence of EOLA on growth is due to some transient effect upon the metabolism, osmoregulation, or endocrinology of this fish species. Consequently, the aim of this study was to evaluate metabolic, osmoregulatory and endocrinological parameters of silver catfish fed with a diet containing different levels of EOLA.

Materials and methods

Fish

The experiment was conducted in a continuously aerated recirculation system at the Laboratory of Fish Physiology, Universidade Federal de Santa Maria (UFSM). Silver catfish (n=30; 132.74 ± 10.24 g, 24.05 ± 0.55 cm) were obtained from the Fish Culture Laboratory (UFSM) and placed in 60-L tanks (4 fish/tank). The experimental protocol was approved by the Committee on Animal Experimentation - UFSM, under the registration number 46/2010.

Water sampling and analyses

The water parameters measured daily during the experimental period were: dissolved oxygen 6.85 ± 0.12 mg L^{-1}, and temperature 24.01 °C ± 0.2 (oxygen meter Y5512; YSI Inc., Yellow Springs, OH, USA); pH 7.15 ± 0.06 (DMPH-2 pH meter, Digimed, São Paulo, SP, Brazil); total ammonia nitrogen levels 0.85 ± 0.11 mg L^{-1} (Eaton *et al*. 2005); and un-ionized ammonia (NH_3) levels 0.007 ± 0.001 mg L^{-1} (Colt 2002). Alkalinity 29.5 ± 1.0 mg L^{-1} $CaCO_3$ (Boyd & Tucker 1992) and water hardness 26.0 ± 1.4 mg L^{-1} $CaCO_3$ (EDTA titrimetric method) were determined weekly.

Essential oil

The plant *L. alba* was cultivated in the Centro de Educação Superior do Norte (CESNORS-UFSM) - Frederico Westphalen Campus. A voucher specimen (SMDB 10050) was deposited in the Herbarium of the Department of Biology (UFSM). Botanical identification was made by Gilberto Dolejal Zanetti (Department of Industrial Pharmacy, UFSM). The EOLA was obtained from fresh leaves of *L. alba* by hydrodistillation in a Clevenger apparatus for 2 h (European Pharmacopoeia 2007) and stored at -20^{o}C until use. The composition of the EOLA was the same as that described by Saccol *et al.* (2013): the major components of the EO were linalool (55.26%), 1,8-cineole (7.85%),γ-muurolene (4.63%), β-caryophyllene (3.15%) and E-carveol (2.79%).

The use of EOLA in the experiment

The animals were fed to satiation once a day (15:00) with a diet (34% crude protein) formulated according to Lazzari *et al*. (2007) (Table 1). Three concentrations of the EOLA in the diet (0-control, 0.25 and 0.50 mL kg^{-1} food) were added to the ingredients together with canola oil. The EOLA concentrations were the lowest concentrations which improved antioxidant status in silver catfish (Saccol *et al.* 2013). The amount of feed offered and the unconsumed remains were weighed to determine feed intake. The fish (n=10/concentration of EOLA in the diet) were fed with the control diet for one week prior to the experiment, and then for additional 20 days with the treatment diets. Uneaten food and feces were siphoned 30 minutes after feeding, and the water removed in this process was replaced with water under the same conditions and proportions found in the system.

Sample collection and chemical analyses

After being fasted for 24 h, the fish were anesthetized with 50 mg L^{-1} eugenol and blood was collected by caudal puncture. The samples were centrifuged at 1000 xg for 5 min and the plasma was stored at -20^{o}C until analyses. The fish were then euthanized and the pituitary gland, gills, liver and muscle were excised.

Biochemical measurements

The protein content in liver and muscle was measured according to Lowry *et al*. (1951) using bovine serum albumin as standard. Plasma glucose and lactate were measured with Labtest kits (Lagoa Santa, MG, Brazil). Glycogen and glucose in the liver and muscle were determined according to Duboie (1956). Lactate in the muscle was determined as in Harrower & Brown (1972), and total lipids were determined in the liver and muscle by the

method of Bligh & Dyer (1959). Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma and liver were determined colorimetrically according to Reitman & Frankel (1957).

Ionoregulatory measurements

Plasma Cl^- levels were determined according to Zall *et al*. (1956), and Na^+ and K^+ with a flame spectrophotometer B262 (Micronal, São Paulo, Brazil). Standard solutions were made with analytical reagent grade (Vetec Merck) dissolved in deionized water, and standard curves for each ion were tested at five different concentrations. The activities of Na^+/K^+-ATPase and H^+-ATPase were assessed in the gills as described by Gibbs & Somero (1989).

RNA Extraction and cDNA synthesis

Total RNA was extracted from pituitary using Trizol reagent (Invitrogen) according to manufacture instructions. Total RNA quantity and purity were assessed by NanoDrop (Thermo Scientific, Delaware, USA; Abs 260/280 nm ratio) spectrophotometer. Ratios above 1.7 were considered pure, and samples below this threshold were discarded. Total RNA (1µg) was treated with DNase (Invitrogen) at 37 °C for 5 min to digest any contaminating DNA. The reverse transcriptase reaction was performed with iScript cDNA synthesis kit (Bio-Rad) in a final volume of 20 µL.

Pituitary expression of GH, PRL and SL mRNA

The mRNA expression was analyzed through qRT-PCR, using the StepOnePlus™ RT-

PCR system (Applied Biosystems) with Power SYBR Green PCR Master Mix (Applied Biosystems). The sequences used to design all the primers were according to Baldisserotto *et al*. (in press) using the Primer Express program v 3.3 (Applied Biosystems). The results were normalized to the expression of the constitutive gene β-actin according to Baldisserotto *et al*. (in press). The calculation of relative expression was performed as recommended by Pfaffl *et al*. (2001).

Cortisol

Plasma cortisol was determined in duplicates using an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochem Canada Inc., Canada). Absorbance was determined in spectrophotometer at 450 nm, and the inter- and intra-assay variation coefficients were 5.15±0.53% and 4.13±0.67% respectively.

Statistical Analyses

A Levene test was conducted to evaluate the homogeneity of variances. The data were compared using one-way analysis of variance (ANOVA) followed by the Tukey test. The data regarding GH were not homocedastic and were compared using the Kruskal-Wallis test followed by the multiple comparison of mean ranks for all groups. All analyzes were performed with the software Statistica 7.0 (Stat Soft, Tulsa, OK). The minimum level of significance was $P < 0.05$. The results were expressed as the mean ± standard error of the mean (SEM).

Results

Feed consumed and metabolic parameters

The amount of feed consumed per day was similar in the different treatments (g feed/kg fish): 1.70 ± 0.1 g kg^{-1} for control, 1.32 ± 0.1 g kg^{-1} for those fed 0.25 mL EOLA kg^{-1} food, and 1.36 ± 0.2 g kg^{-1} for those fed 0.50 mL EOLA kg^{-1} food. Glycogen (liver and muscle), glucose (plasma, liver and muscle) and lactate (plasma and muscle) (Fig. 1), and protein, total lipids and AST (plasma and liver) (Fig. 2) were not affected by the dietary EOLA. Hepatic ALT of fish fed 0.50 mL EOLA kg^{-1} food was significantly higher compared to the other treatments (Fig. 2).

Ions and enzyme activities

Fish fed 0.25 mL EOLA kg^{-1} food presented significantly higher Na^+/K^+-ATPase activity compared to the other treatments, but plasma Na^+, K^+ and Cl^- levels, as well as H^+-ATPase activity, were not significantly affected by the treatments (Fig. 3).

Hormones expression and plasma cortisol

Pituitary expressions, GH and PRL did not change significantly between groups, but SL expression was higher in the group treated with 0.25 mL EOLA kg^{-1} food compared to the others (Fig. 4). Dietary EOLA did not significantly affect plasma cortisol levels (Fig. 5).

Discussion

Lactate, glycogen, glucose, protein, lipids are biochemical parameters commonly used to assess the metabolic state of fish tissues (Gimeno *et al*. 1994; Pretto *et al*. 2014). Apparently, dietary addition of both EOLA concentrations for 20 days did not change silver catfish metabolism because these parameters were not affected. Silver catfish fed with dietary EOLA for 60 days also did not show significant alteration in these biochemical parameters in the plasma (Saccol *et al*. 2013). Therefore, dietary EOLA does not promote any transient or long-term change in silver catfish metabolism.

The enzymes AST and ALT are mainly used as biomarkers to assess liver damage, although they are also found in organs such as skeletal muscle, heart, pancreas and kidneys (Evans *et al*. 1996). The increased ALT activity in the liver of silver catfish fed with the highest dietary EOLA concentration suggests that there was hepatocyte damage. However, the lack of alteration in plasma ALT and in plasma and liver AST indicates that the observed increase in hepatic ALT did not cause any serious damage. In agreement with this hypothesis, silver catfish fed with up to 2.0 mL EOLA kg^{-1} food for 60 days only decreased the glucose levels in the liver, resulting in an increase in the glycogen and lactate reserves in the liver (Saccol *et al*. 2013).

The addition of EOLA to the water of transport reduced the net Na^+, K^+ and Cl^- losses in silver catfish (Becker *et al*. 2012), and immersion anesthesia with this oil increased gill Na^+/K^+-ATPase and H^+/ATPase activities in this species (Toni *et al*. 2014), indicating an osmoregulatory effect. However, the only osmoregulatory effect of dietary EOLA observed in the present experiment was the increase in gill Na^+/K^+-ATPase activity in those fish fed with 0.25 mL EOLA kg^{-1} food. The expression of SL also increased in silver catfish fed with that concentration of EOLA, indicating that both effects may be related. The Na^+/K^+-ATPase

plays a major role in fish osmoregulation (McCormick 1994; Handeland *et al*. 2003; Ban *et al*. 2007), while SL seems to be involved with acid-base regulation in rainbow trout (*Oncorhynchus mykiss*) (Kakizawa *et al*. 1996) and correction of plasma osmotic balance in Mozambique tilapia (*Oreochromis mossambicus*) exposed to acidic freshwater (Furukawa *et al*. 2010). The absence of significant difference in gill Na^+/K^+-ATPase activity and SL expression in the fish fed with 0.50 mL EOLA kg^{-1} food may be because at such concentration the effect was faster and the osmotic and/or acid-base equilibrium was fully reestablished.

Maintenance of GH expression is in accordance with the findings by Saccol *et al*. (2013), which demonstrated that dietary EOLA did not change silver catfish growth. Prolactin is considered one of the most important hormones related to freshwater adaptation, and it is essential for ion uptake as well as reduction in ion and water permeability of osmoregulatory surfaces (Sakamoto & McCormick 2006). Its unaltered expression in silver catfish fed with dietary EOLA is probably related to the unchanged plasma ion levels. Immersion anesthesia with EOLA prevented the increase in cortisol in silver catfish subjected to handling (Cunha *et al*. 2010), but dietary EOLA did not change plasma cortisol levels, indicating that it did not have an effect upon stress parameters when administered through this route.

Besides being an effective antioxidant (Saccol *et al*. 2013), 0.25 mL EOLA kg^{-1} food may be recommended to silver catfish since it prevents major changes in metabolism, osmoregulation and endocrinology. The use of the dietary supplementation with 0.50 mL EOLA kg^{-1} food requires additional investigation because it increased ALT, indicating a possible liver damage.

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References

Agulleiro, B., García-Hernandez, M.P. & García-Ayala, A. (2006) Teleost adenohypophysis: morphofuncional and developmental aspects. In: Reinecke, M; Zaccone, G.; Kapoor, B.G. (Ed.). *Fish Endocrinol*., vol.**1**, pp 289-323. Enfield, New Hampshire, USA.

Ashmore, L.J., Hrizo, S.L., Paul, S.M., Van Voorhies, W.A., Beitel, G.J., Palladino, M.J. (2009) Novel mutations affecting the Na, K ATPase alpha model complex neurological diseases and implicate the sodium pump in increased longevity. *Hum. Genet*. **126**, 431–447.

Azambuja, C.R.M., J., Riffel, A.P.K., Finamor, I.A., Garcia, L.O., Heldwein, C.G., Heinzmann, B.M., Baldisserotto, B., Pavanato, M.A. & Llesuy. S.F. (2011) Effect of the essential oil of *Lippia alba* on oxidative stress parameters in silver catfish (*Rhamdia quelen*) subjected to transport. *Aquacult. Int.*, **319**, 156 – 161.

Baldisserotto, B. (2009) Freshwater fish culture in Rio Grande do Sul State: actual situation, problems and future perspectives. *Ciênc. Rur*., **39**, 291-299.

Baldisserotto, B., Martos – Sitcha, S.A., Menezes, C., Toni, C., R.L., Garcia, L.O., Salbego, J., Mancera, J.M. & Martínez – Rodrigues, G. (in press) The effects of ammonia and hardness on the hormonal of the freshwater silver catfish, *Rhamdia quelen*. *Aquatic Toxicol.(DOI:* 10.1016/j.aquatox.2014.04.023)

Ban, M., Ando, H. & Urano, A. (2007) Effects of long-day on gill Na+,K+-ATPase gene expression and the development of seawater tolerance in sockeye salmon. *Aquaculture*, **273**,

218–226.

Barton, B.A. (1997) Stress in finfish: past, present and future a historical perspective. In: Iwama, G.K., Pickering, A.D., Sumpter, J.P., Schreck, C.B. (Eds.). *Fish stress and health in aquaculture*. Society for Experimental Biology Seminar Series 62. Cambridge University Press, New York, NY. p.1-33.

Barton, B.A., Morgan, J.D., Vijayan, M.M. (2002) Physiological and condition-related indicators of environmental stress in fish. *In*: Adams (ed.). Biological indicator of aquatic ecosystem stress, Bestherda, Maryland, American Fisheries Society, p.289-320.

Becker, A.G., Cunha, M.A., Garcia, L.O., Zeppenfeld, C.C., Parodi, T.V., Maldane, G.Morel, A.F. & Baldisserotto, B. (2012) Transportation of silver catfish, *Rhamdia quelen*, in water with eugenol and the essential oil of *Lippia alba*. *Fish Physiol. Biochem.*, **38**, 789-796.

Bernardi, G., Sordino, P. & Powers, D.A. (1993) Concordant mitochondrial and nuclear-DNA phylogenies for populations of the teleost fish *Fundulus heteroclitus*. **P** *Natl. Acad. Sci. USA.*, **90**, 9271-9274.

Björnsson, B.T. (1997) The biology of salmon growth hormone: from daylight to dominance. *Fish Physiol. Biochem.*, **17**, 9-24.

Bligh, E.G., & Dyer, W.J. (1959) A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.*, **37**, 911–917.

Boyd, C.E. & Tucker, C.S. (1992) Water quality and pond soil analyses for aquaculture. Auburn: Auburn University.

Carrión, L.R., Fuentes, R., Redruello,B., Guzmán, J.M., Del Río, M.P.M, Power, D. & Mancera, J.M. (2009) Expression of pituitary prolactin, growth hormone and somatolactin is modified in response to different stressors (salinity, crowding and food-deprivation) in gilthead sea ream *Sparus auratus*. *Gen. Comp. Endocr*., **162**, 293-300.

Colt, J. (2002) List of spreadsheets prepared as a complement. [online] Available from: http://www.fisheries.org/afs/hatchery htmLS. [accessed: 10th April 2013] . In: Wedemeyer G.A. (ed.), Fish Hatchery Management, 2 ed., American Fisheries Society.

Company, R., Astola, A., Pendon, C., Valdivia, M.M. & Perez-Sanchez, J. (2001) Somatotropic regulation of fish growth and adiposity: growth hormone (GH) and somatolactin (SL) relationship. *Comp. Biochem. Physiol*., **130**, 435-445.

Cunha, M.A., Barros, F.M.C., Garcia, L.O. , Veeck, A.P.L., Heinzmann, B.M. Loro, Emanuelli, V.L.T. & Baldisserotto, B. (2010) Essential oil of *Lippia alba*: a new anesthetic for silver catfish, *Rhamdia quelen*. *Aquaculture*, **306**, 403-406.

Cunha, M.A., Silva, B.F. Delunardo, F.A.C., Benovit, S.C., Gomes, L.C., Heinzmann, B.M. & Baldisserotto, B. (2011) Anesthetic induction and recovery of *Hippocampus reidi* exposed to the essential oil of *Lippia alba*. *Neotrop. Ichthyol*., **9**, 683-688.

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.

Duboie, M.G., Gilles, K.A, Hamilton, J.K., Roberts, P. A. & Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem*., **28**, 350-358.

European Pharmacopoeia (2007) 6 ed., European Directorate for the Quality of Medicines, Strassbourg.

Evans, D.H., Piermarini, P.M. & Choe, K.P. (2005) The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev*., **85**, 97-177.

Furukawa F., Watanabe S., Kaneko T. & Uchida K. (2010) Changes in gene expression levels of somatolactin in the pituitary and morphology of gill mitochondria-rich cells in Mozambique tilapia after transfer to acidic freshwater (pH 3.5).*Gen. Comp. Endocr*., **166**, 549-55.

Gharaei, A., Ghaffari, M., Keyvanshokooh, S. & Akrami, R. (2011) Changes in metabolic enzymes, cortisol and glucose concentrations of Beluga (*Huso huso*) exposed to dietary methylmercury. *Fish Physiol. Biochem*., **37**, 485–493.

Gibbs, A.S. & Somero, G.N. (1989) Pressure adaptation of Na^+/K^+-ATPase in gills of marine teleosts. *J. Exp. Biol*., **143**, 475-492.

Gimeno, L. , Ferrando, M.D. , Sanchez, S. & Andreu, E. (1994) Endosulfan effects on liver and blood of the eel, Anguilla Anguilla . *Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocr.*, **108** , 343–348.

Ji, S.C. , Takaoka, O., Lee, S.W., Hwang, J.H., Kim, Y.S. & Ishimaru, K. (2009) Effect of dietary medicinal herbs on lipid metabolism and stress recovery in red sea bream Pagrus major. *Fish. Sci.*, **75** , 665–672

Handeland, S. O., Björnsson, Arnesen, A.M. & Stefansson, S.O. (2003) Seawater adaptation and growth of post-smolt Atlantic salmon (Salmo salar L.) of wild and farmed strains. *Aquaculture*, **220**, 367–384.

Harrower, J.R. & Brown, C.H. (1972) Blood lactic acid. A micromethod adapted to field collection of microliter samples. *J. Appl. Physiol.*, **32**, 224-228.

Kakizawa, S., Kaneko, T. & Hirano, T. (1996) Elevation of plasma somatolactin concentrations during acidosis in rainbow trout. *J. Exp. Biol.* **199**, 1043–1051.

Kasper, R.S., Shved, N., Takahashi, A., Reinecke, M. & Eppler, E. (2006) A systematic immunehistochemical survey of the distribution patterns of GH, prolactin, somatolactin, beta-TSH, beta-FSH, beta-LH, ACTH, and alpha-MSH in the adenohypophysis of *Oreochromis niloticus*, the Nile tilapia. *Cell Tissue Res*. **325**, 303-13.

Kaneko, T. & Hirano, T. (1993) Role of prolactin and somatolactin in calcium regulation in fish. *J. Exp. Biol*., **184**, 31–45.

Laiz-Carrión, R., Fuentes, J., Redruello, B., Guzmán, J.M., Martín del Río, M.P., Power, D. Mancera, J.M.(2009). Expression of pituitary prolactin, growth hormone and somatolactin is modified in response to different stressors (salinity, crowding and food-deprivation) in gilthead sea bream *Sparus auratus*. *Gen. Comp. Endocrinol.* **162**, 293-300.

Lermen, C.L., Lappe, R., Crestani, M., Vieira, V.P., Gioda, C.R., Schetinger, M.R.C., Baldisserotto,B., Moraes, G. & Morsch, V.M. (2004) Effect of different temperature regimes on metabolic and blood parameters of silver catfish *Rhamdia quelen*, *Aquaculture,* **239**, 497-507.

Lazzari, R. , Neto, J.R., Veiverberg, C.A., Bergamin, G.T., Corrêia, V. & Pedron, F.A. (2007) Alimentação do jundiá (*Rhamdia quelen*, heptateridae) com ingredientes proteicos. *Arch. Zootec*., **56**, 116.

Leonardo, J.M.L.O., Vargas, L., Ribeiro, R.P., Moreira, H.L.M., Natali, M.R.M., Volski,T., Cavichiolo , F.(2001) Histologia das brânquias de larvas da tilápia do Nilo, *Oreochromis niloticus* (L.), de origem tailandesa, submetidas a diferentes níveis de vitamina C. *Acta Sci*., **23**, 863-70.

Lowry, O.H., Rosebrough, N.J., Farra, L. & Randall R.J. (1951) Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem*., **193**, 265-275.

McCormick, S. D. (1994) Ontogeny and evolution of salinity tolerance in anadromous salmonids–hormones and heterochrony. *Estuaries*, **17**, 26–33.

Morgan, R. (1997) Doença, aplicações, descrição e propriedades. In: Enciclopédia das Ervas e Plantas Medicinais. São Paulo, Brasil: Hemus.

National Research Council (NRC) (1993) Nutrient Requirements of Fish. Washington: National Academy Press.

Perdices, A., Bermingham, E., Montilla, A. & Doadrio, I. (2002). Evolutionary history of genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. *Mol. Phyl. Evol*., **25**, 172–189.

Pfaffl MW. (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29(9):e45. [online] Avaible: http://nar.oxfordjournals.org/content/29/9/e45.full . [accessed: November 2013]

Pretto, A., Loro, V.L., Morsch, V.M., Moraes, B.S., Menezes, C., Santi, A. & Toni, C. (2014) Alterations in carbohydrate and protein metabolism in silver catfish (*Rhamdia quelen*) exposed to cadmium. *Ecotox. Environ.Safe*., **100**, 188-192.

Reitman, S. & Frankel, S., (1957) Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol*., **28**, 53–56.

Saccol, E.M.H., Uczay, J., Pês, T., Finamor, I.A., Ourique, G.M., Riffel, A.P.K., Schmidt, D., Caron, B.O., Heinzmann, B.M., Llesuy, S.F., Lazzari,R., Baldisserotto, B. & Pavanato, M.A. (2013) Addition of Lippia alba (Mill) N. E. Brown essential oil to the diet of the silver catfish: An analysis of growth, metabolic and blood parameters and the antioxidant response. *Aquaculture,* **416–417**, 244–254.

Sakamoto, T. & McCormick, S.D. (2006) Prolactin and growth hormone in fish osmoregulation. *Gen. Comp. Endocrinol*. **47**, 24-30.

Sakamoto, T., Shepherd, B. S., Madsen, S.S., Nishioka, R.S., Siharath, K., Richman III, N. H., Bern, H.A. & Grau, E.G. (1997) Osmoregulatory actions of growth hormone and prolactin in an advanced teleost. *Gen. Comp. Endocr*., **106**, 95–101.

Sangiao-Alvarellos S., Arjona, F.J., Miguez, J.M., Del Rio, M.P.M., Soengas, J.L., Mancera, J.M. (2006) Growth hormone and prolactin actions on osmoregulation and energy metabolism of gilthead sea bream (*Sparus auratus*). *Comp. Biochem. Physiol. Part:A*, **144**, 491-500.

Shalaby, A. M., Khattab, Y. A. & Abdel Rahman, A. M. (2006) Effects of arlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*). *J. Venom. Anim. Toxins incl. Trop*. **12**, 172-201.

Shahsavan, D., Mohri, M. & Gholipour, K. H. (2010) Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas. *Fish Physiol. Biochem*., **36**, 39–43.

Sinha, A. K., Diricx, M., Chan, L. P., Liew, H. J., Kumar, V., Blust, R. & De Boeck, G. (2012) Expression pattern of potential biomarker genes related to growth, ion regulation and stress in response to ammonia exposure, food deprivation and exercise in common carp (*Cyprinus carpio*). *Aquatic Toxicol.*, **122-123**, 93-105.

Stagg, R.M., Rusin, J. & Brown, F. (1992) Na^+/K^+-ATPase activity in the gills of the flounder (*Platichthys flesus*) in a relation to mercury contamination in the Firth of Forth *Mar. Environ. Res*., **33**, 255–266.

Terblanche, F.C. & Kornelius, G. (1996) Essential oil constituents of the genus *Lippia* (Verbenaceae) - A literature review. *J. Essent. Oil Res*., **8**, 471–485.

Toni, C., Becker, A.G., Simões, L.N., Pinheiro, C.G., Silva, L., Heinzmann, B.M., Caron, B.O & Baldisserotto, B. (2014) Fish anesthesia: effects of the essential oils of *Hesperozygis ringens* and *Lippia alba* on the biochemistry and physiology of silver catfish (*Rhamdia quelen*). *Fish Physiol. Biochem*. **40**, 701-714.

Veeck, A.P.L., Klein, B., Ferreira, L.F., Becker, A.G., Heldwein, C.G., Heinzmann, B.M., Baldisserotto, B. & Emanuelli, T. (2013) Lipid stability during the frozen storage of fillets

from silver catfish exposed in vivo to the essential oil of *Lippia alba* (Mill.) NE Brown. *J. Sci. Food Agric*., **93**, 955–960.

Zall, D.M., Fisher, M.D. & Garner, Q.M. (1956) Photometric determination of chloride in water. *Anal. Chem*., **28**, 1665–1678.

Zheng, Z.L., Tan, J.Y.W., Liu, H.Y., Zhou, X.H., Xiang, X. & Wang, K.Y. (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**:(3-4), 214-218.

Figure Captions

FIGURE 1. Metabolic parameters in tissues of silver catfish fed with different concentrations of dietary essential oil of *Lippia alba* (EOLA). A) glycogen, B) glucose and C) lactate. Mean ± SEM.

FIGURE 2. Biochemical parameters in tissues of silver catfish fed with different concentrations of dietary essential oil of *Lippia alba* (EOLA). A) protein, B) lipids, C) AST and D) ALT. Mean ± SEM.

FIGURE 3. Osmoregulatory parameters of silver catfish fed with different concentrations of dietary essential oil of *Lippia alba* (EOLA). A) plasma ion levels and B) gill Na^+/K^+-ATPase and H^+/ATPase activities. Mean ± SEM.

FIGURE 4. Expression of pituitary hormones of silver catfish fed with different concentrations of dietary essential oil of *Lippia alba* (EOLA). A) growth hormone (GH), B) prolactin (PRL) and C) somatolactin (SL). Mean ± SEM.

FIGURE 5. Plasma cortisol of silver catfish fed with different concentrations of dietary essential oil of *Lippia alba* (EOLA). Mean ± SEM.

TABLE

Table 1

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		
Analysis of the feed (%)	0.00 mL kg^{-1}	0.25 mL kg^{-1}	0.50 mL kg^{-1}
Dry matter	95.43	95.48	95.56
Ashes	14.97	15.15	15.58
Crude protein	38.96	38.98	39.07
Fat	10.42	9.92	10.08

[a] Vitamin and mineral mixture (security levels per kilogram of product)—folic acid: 250 mg, pantothenic acid: 5000 mg, antioxidant: 060 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: 1000000 UI, vitamin B1: 1250 mg, vitamin B12: 3750 mcg, vitamin B2: 2500 mg, vitamin B6: 2485 mg, vitamin C: 28000 mg, vitamin D3: 500000 UI, vitamin E: 20000 UI, vitamin K: 500 mg, zinc: 17500 mg.

FIGURES

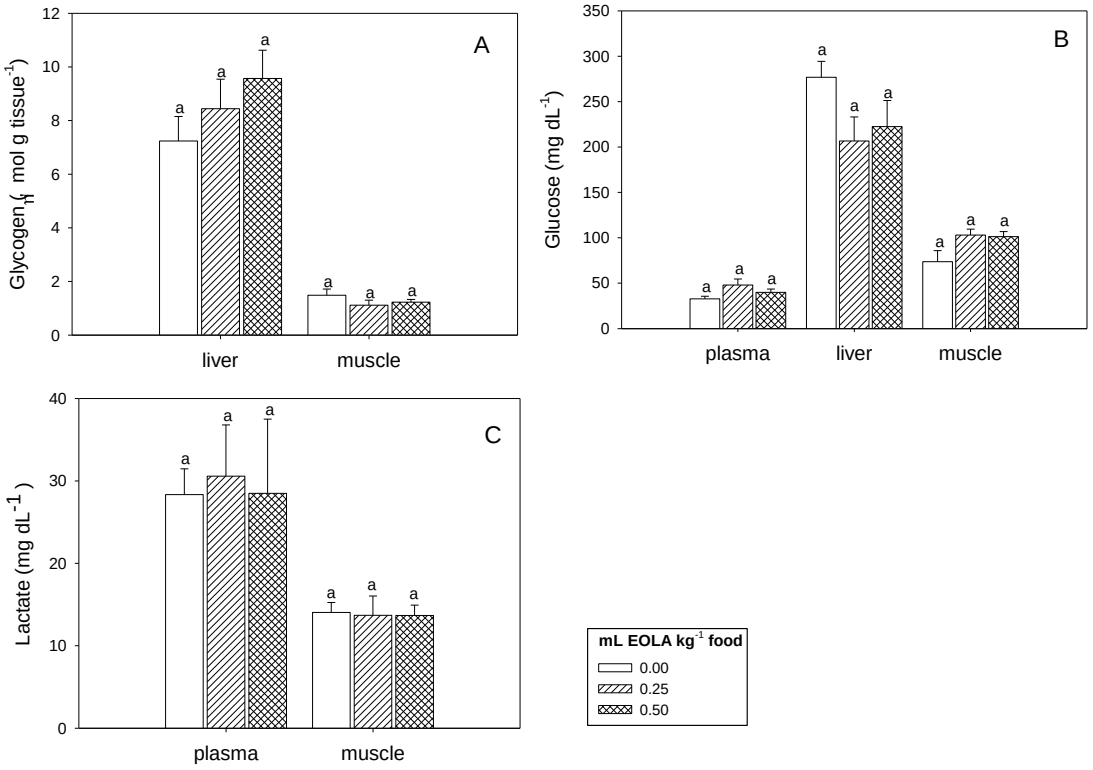

FIGURE 1

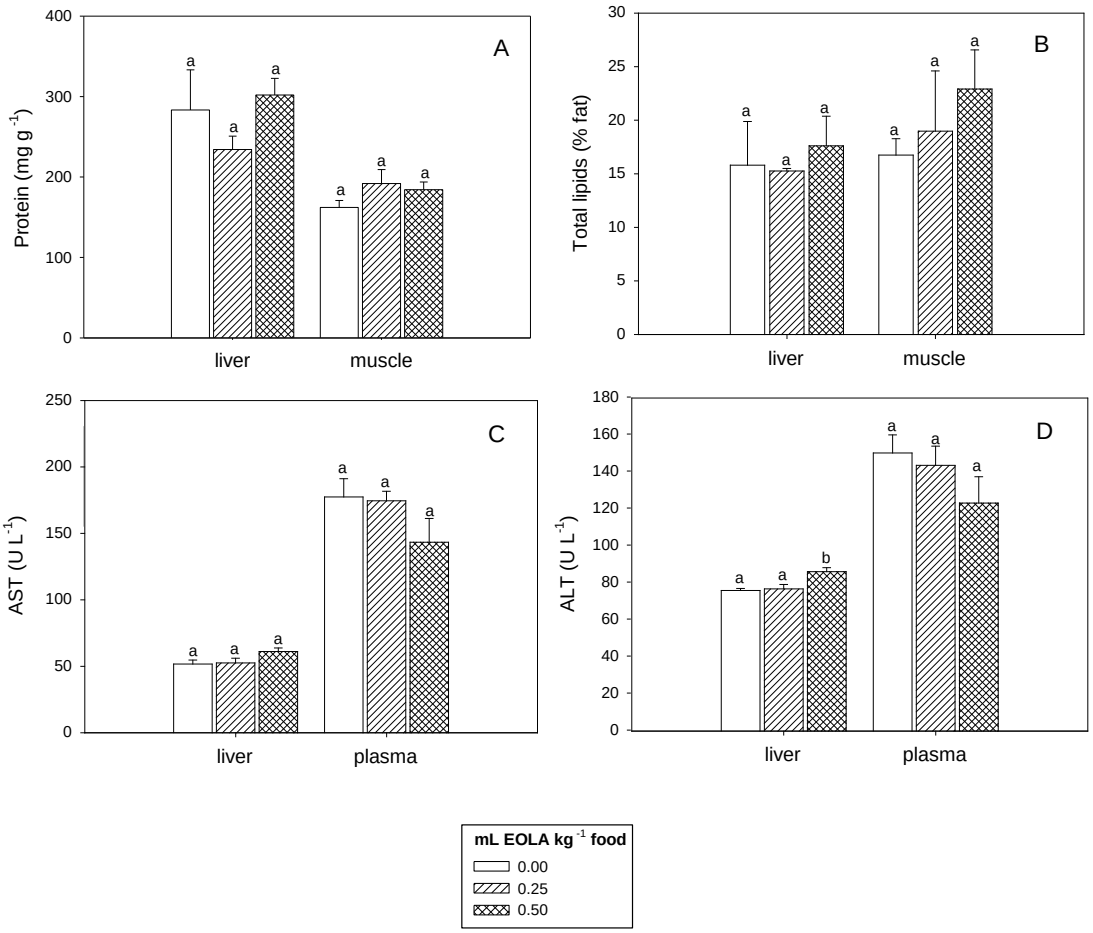
A
Protein (mg g -1)
400
300
200
100
0
liver
muscle
B
Total lipids (% fat)
30
25
20
15
10
5
0
liver
muscle
C
AST (U L -1)
250
200
150
100
50
0
liver
plasma
D
ALT (U L -1)
180
160
140
120
100
80
60
40
20
0
liver
plasma
mL EOLA kg -1 food
0.00
0.25
0.50

FIGURE 2

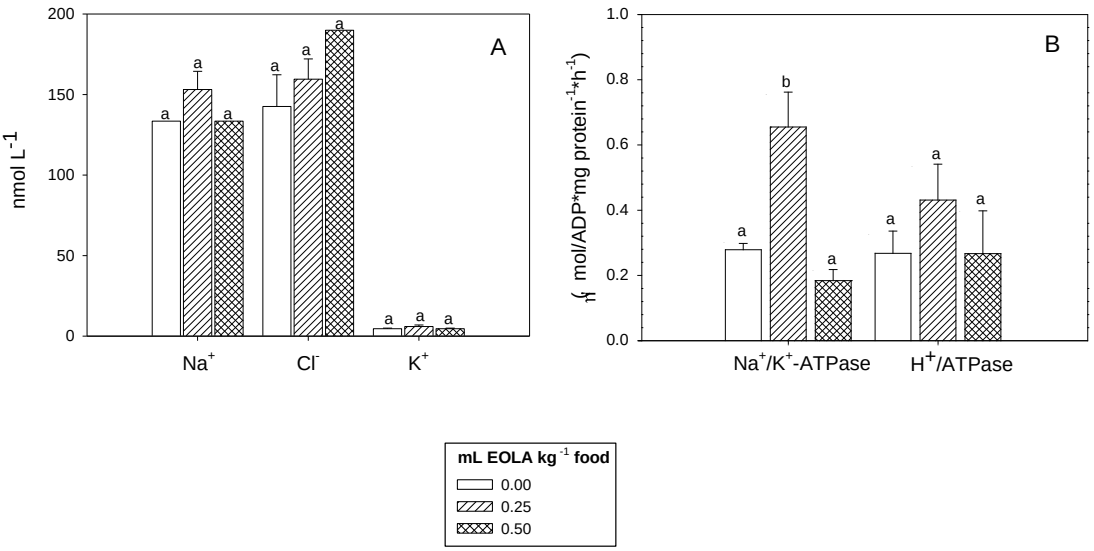
A
B
nmol L-1
µ mol/ADP*mg protein-1*h-1)
200
150
100
50
0
1.0
0.8
0.6
0.4
0.2
0.0
a
b
Na+
Cl-
K+
Na+/K+-ATPase
H+/ATPase
mL EOLA kg-1 food
0.00
0.25
0.50

FIGURE 3

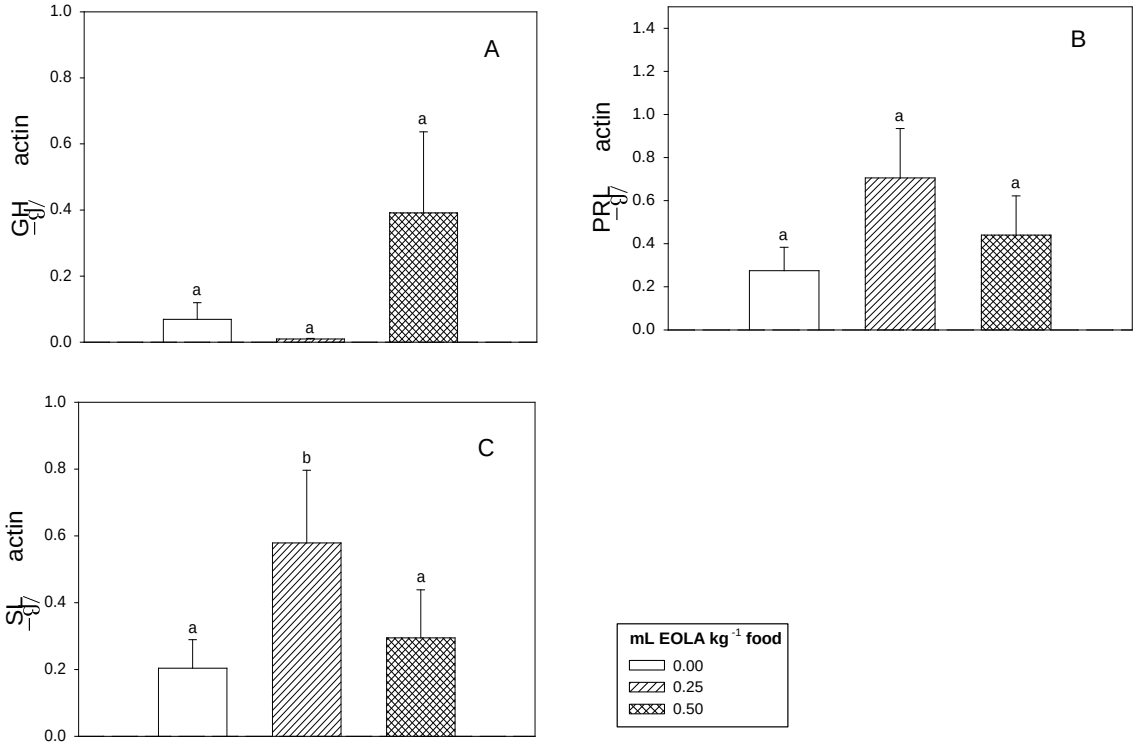
A
B
C
GH/β actin
PRL/β actin
SL/β actin
1.0
0.8
0.6
0.4
0.2
0.0
1.4
1.2
a
b
mL EOLA kg -1 food
0.00
0.25
0.50

FIGURE 4

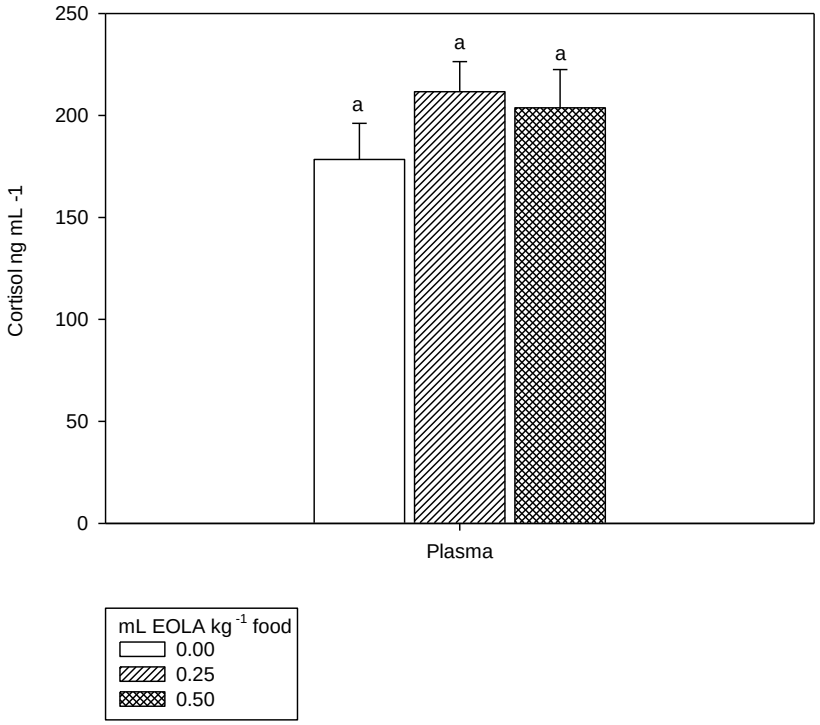
250
200
150
100
50
0
Cortisol ng mL -1
a
a
a
Plasma
mL EOLA kg -1 food
0.00
0.25
0.50

FIGURE 5

CONCLUSÕES FINAIS

- O óleo essencial de *L. alba* adicionado à ração não provoca alterações significativas no metabolismo do jundiá.

- O óleo essencial de *L. alba* adicionado à ração não provoca alteração no estresse nos jundiás.

- O óleo essencial de *L. alba* pode ser utilizado como aditivo em rações para jundiás, mas em concentrações iguais ou superiores a 0,50 mL kg^{-1} de ração é necessário um acompanhamento, pois aparentemente pode causar leves danos no fígado.